Water Research 88 (2016) 83–92

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Evaluation of a sequencing batch reactor sewage treatment rig for investigating the fate of radioactively labelled pharmaceuticals: Case study of propranolol

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ARTICLE INFO

Article history:
Received 11 December 2014
Received in revised form
15 September 2015
Accepted 17 September 2015
Available online 21 September 2015

Keywords:
Propranolol
Radiolabelled compounds
Sewage treatment plant
Sequencing batch reactors
Laboratory simulation

ABSTRACT

Pharmaceuticals are frequently detected in the aquatic environment, and have potentially damaging effects. Effluents from sewage treatment plants (STPs) are major sources of these substances. The use of sequencing batch reactor (SBR) STPs, involving cycling between aerobic and anoxic conditions to promote nitrification and denitrification, is increasing but these have yet to be understood in terms of removal of pharmaceutical residues. This study reports on the development of a laboratory rig to simulate a SBR. The rig was used to investigate the fate of radiolabelled propranolol. This is a commonly prescribed beta blocker, but with unresolved fate in STPs.

The SBR rig (4.5 L) was operated on an 8 h batch cycle with settled sewage. Effective treatment was demonstrated, with clearly distinct treatment phases and evidence of nitrogen removal. Radiolabelled ^{14}C-propranolol was dosed into both single (closed) and continuous (flow-through) simulations over 13 SBR cycles. Radioactivity in CO_2 off-gas, biomass and liquid was monitored, along with the characteristics of the sewage. This allowed apparent rate constants and coefficients for biodegradation and solid:water partitioning to be determined.

Extrapolation from off-gas radioactivity measurements in the single dose 4-d study suggested that propranolol fell outside the definitions of being readily biodegradable (DegT50 = 9.1 d; 60% biodegradation at 12.0 d). During continuous dosing, 63–72% of propranolol was removed in the rig, but less than 4% of dose recovered as $^{14}CO_2$, suggesting that biodegradation was a minor process ($K_{biol(M)}$ L kg d^{-1} = 22–49) and that adsorption onto solids dominated, giving rise to accumulations within biomass during the 17 d solid retention time in the SBR. Estimations of adsorption isotherm coefficients were different depending on which of three generally accepted denominators representing sorption sites was used (mixed liquor suspended solids, reactor COD or mass of waste activated sludge).

With further development and evaluation, the rig developed for simulating SBR processes has potential to be used for informing better environmental risk assessments for those pharmaceuticals showing ambiguous results in field fate studies.

1. Introduction

The occurrence of active pharmaceutical ingredients (APIs) in wastewater is the result of excretion by patients and improper disposal of medicines. This situation is likely to worsen due to growing populations and increasingly ageing demographics, leading to increased usage of these chemicals (Arnold et al., 2013; Daughton and Ternes, 1999). Sewage treatment plants (STPs) generally have a sequence of discrete unit processes with different

Abbreviations: API, active pharmaceutical ingredient; CAS, conventional activated sludge; DegT$_{50}$, time taken for 50% removal (or half-life); DOC, dissolved organic carbon; HRT, hydraulic retention time; LSC, liquid scintillation counting; MLSS, mixed liquor suspended solids; PNEC, predicted no effects concentration; SBR, sequencing batch reactor; SPE, solid-phase extraction; SRT, solid retention time; SS, suspended solids; STP, sewage treatment plant; WAS, waste activated sludge.

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http://dx.doi.org/10.1016/j.watres.2015.09.033

treatment mechanisms, typically combining solids separation and biological oxidation. Removal of APIs in STPs is therefore determined by the treatment processes utilized and the physicochemical properties and biodegradability of the chemicals themselves (Drewes, 2007). However, STPs are dynamic systems, receiving variable wastewater loads and are affected by environmental conditions, such as temperature. Perhaps as a consequence, reported removal rates for APIs in STPs range from complete to almost negligible (Gros et al., 2010; Kostrich et al., 2014) and consequently many of these substances are detected in environmental waters (Verlicchi et al., 2012; Vulliet and Cren-Olivé, 2011). Although APIs generally occur at low concentrations (ng L^{-1} to μg L^{-1}) in the environment (Kosma et al., 2010), their bioactive nature makes them potentially harmful to aquatic organisms (Williams, 2005).

The most widely used biological sewage treatment process is conventional activated sludge (CAS) (Metcalf and Eddy, 2003) in continuous flow reactors. Various CAS operating conditions have been shown to influence the removal of APIs, including mixed liquor suspended solids (MLSS) concentration, solid retention time (SRT) and hydraulic retention time (HRT) (Barbieri et al., 2011; Suárez et al., 2012; Verlicchi et al., 2012). The Urban Waste Water Treatment Directive of the European Union 91/271/EEC (1991) requires STPs discharging to environmentally-sensitive areas to remove nitrogen and/or phosphorus. Consequently, CAS is being replaced at many locations by variants capable of biological nutrient removal (BNR). BNR plants incorporate aerobic, anoxic and anaerobic zones into the treatment system to act as selectors, 'switching on' certain groups of bacteria, enabling them to undertake nitrification, denitrification and/or phosphorus removal (Barker and Dold, 1997). Switching between conditions has been shown to promote the removal of APIs in laboratory and field studies (Hu et al., 2005; Lubliner et al., 2010; Okuda et al., 2008; Suárez et al., 2012; Tran et al., 2009; Wick et al., 2009).

Sequencing batch reactors (SBRs) are a type of activated sludge system operating in batch mode which are capable of BNR. Conditions alternate between aerobic and anoxic during the batch treatment cycle due to intermittent aeration (Seviour and Nielsen, 2010; Shaw et al., 2009). SBRs offer a high level of operational control and generally have higher SRTs than CAS, enabling stable microbial populations to become established (Hu et al., 2005; Sirianuntapiboon and Ungkaprasatcha, 2007). The use of SBRs has therefore become an attractive alternative for the removal of various xenobiotic compounds from wastewater (Celis et al., 2008). SBRs are being installed at large STPs (Puig et al., 2007), such as the twenty-four SBRs at Ringsend, Dublin (Celtic Anglian Water Ltd.) treating waste from a population equivalent of 1.7 million.

Studies into the fate of APIs in STPs involve mainly field monitoring of parent compounds, often with a 'black box' experimental design (Suárez et al., 2012). Sewage treatment processes present complex and variable conditions for chemical extraction, making monitoring difficult. This may contribute to the variability in the range of reported removal efficiencies and estimates of kinetic rate constants for specific APIs in STPs (Teerlink et al., 2012). Laboratory simulations offer a more controllable setting for investigating the fate of APIs, but lack field variability and their small-scale gives less dilution and buffering capacity. OECD guidelines describe two CAS laboratory systems for studying the fate of organic chemicals. OECD 303A (OECD, 2001) is a continuous flow test based on either the traditional "Husmann Unit" or "Porous Pot" CAS simulations, while OECD 314 B is a less complex and cheaper batch mode test (OECD, 2008). The 303A CAS flow through simulation recommends treating carbon amended primary settled sewage in 3 L aerated reactors with a HRT of 6 h, a SRT of 6–10 d and a MLSS of 2500 mg L^{-1} (OECD, 2001); this requires a high level of intervention and control to maintain these conditions. Consequently, SBRs have been used in many laboratory simulation studies as their operating conditions and microbial populations are easier to maintain compared to flow through systems (Seviour and Nielsen, 2010). An advantage of controlled laboratory studies is that radiolabelled compounds can be used to investigate the fate of chemicals. Radiolabelled ^{14}C studies enable the fate of organic compounds to be established with some confidence, but the carbon compounds associated with the radioactivity (apart from CO_2) are not resolved, unless specifically developed analytical methods are used (Federle and Itrich, 1997). Some radiolabelled studies have been undertaken on laboratory scale CAS systems (Bouju et al., 2011; Cirja et al., 2007; Junker et al., 2006), but to our knowledge there are no studies on the fate of radiolabelled APIs in a model SBR system. The study outlined in this paper aimed to develop a laboratory simulation of a SBR plant, and undertake a ^{14}C-labelled investigation to establish the fate of propranolol. This compound was selected as it a widely used API, whose reported removal efficiency in STPs has been very varied and typically low (Verlicchi et al., 2012). Propranolol is also included in the Chemicals Investigation Programme being undertaken by the UK water industry, where concentrations of propranolol in many UK STP effluents were found to be above the predicted no effects concentration (PNEC) of 0.01 μg L^{-1}; even after assuming an environmental dilution of 1:10 (Gardner et al., 2012).

Developing an understanding of the fate of APIs, such as propranolol in a range of sewage treatment systems is therefore important to inform better environmental risk assessments of medicinal products. Use of standard laboratory simulations for investigating treatment technologies may also offer opportunities for selecting conditions for the most effective removal of these chemicals.

2. Materials and methods

All work was carried out at the AstraZeneca Brixham Environmental Laboratory, Devon, UK. Radiolabelled ^{14}C-propranolol was supplied by AstraZeneca UK Ltd. and was stored at −20 °C, its chemical structure and purity is shown in the Supplementary Material (SM) (Figs. S1 and S2). Primary settled sewage and MLSS biomass were obtained, courtesy of South West Water Ltd., from Totnes STP, Devon, UK, which is a nitrifying CAS plant treating mainly domestic sewage. Water obtained by reverse osmosis was used in all experiments.

2.1. Description of rig and operating conditions

A schematic diagram of the laboratory-scale SBR rig is shown in Fig. 1; further details are given in the SM (Fig. S3). The reaction vessel was a sealed 4.5 L glass flask inoculated with nitrifying MLSS

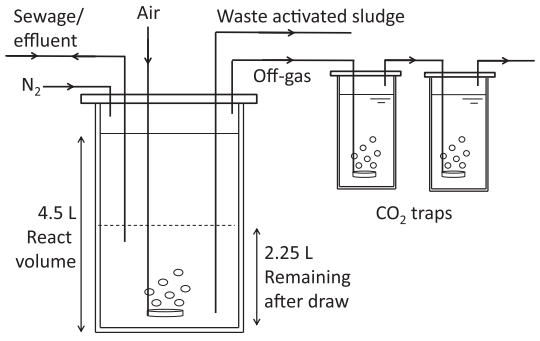

Fig. 1. Schematic diagram of the laboratory-scale sequencing batch reactor showing the reaction vessel, CO_2 traps and main flow paths of gas and liquid.

adjusted to a concentration of 3000 mg L^{-1} (dry weight) with primary settled sewage. It was fed with primary settled sewage, however, as this sewage was relatively weak in terms of carbonaceous material (Table 1) it was amended with sodium acetate to increase the available chemical oxygen demand (COD) so as to enhance anoxic conditions and support denitrification. Many full-scale STP plants in the UK receive similar "weak" inputs from combined sewerage and require an additional carbon source to achieve N removal consents e.g. Budds Farm STP, Havant, UK operated by Southern Water PLC. The 8 h SBR cycle consisted of 10 min fill, 170 min anaerobic/anoxic conditions, 240 min aerobic conditions, 45 min settle and 10 min effluent removal (draw). A programmable logic controller system operated the pumps, stirrers, N_2 and compressed air flow during the experiments.

Peristaltic pumps were used to add influent and remove effluent. Waste activated sludge (WAS) was also removed at the end of the aerobic phase once every 24 h. Compressed air was bubbled (0.5 L min^{-1}) through a glass sinter into the SBR during the aerobic phase. N_2 was delivered (1 L min^{-1}) to the headspace of the reactor at the beginning of the anaerobic/anoxic phase to displace air and exclude O_2. Temperature, pH, dissolved oxygen (DO) and Eh (mV) were monitored continuously. $^{14}CO_2$ in the off-gas was captured in two traps containing 130 mL of NaOH (2 M). In batch radiolabelled biodegradability studies simulating environmental conditions, with relatively low rates of carbon cycling, it is necessary to acidify flasks to directly or indirectly recover ^{14}C present as bicarbonate. This can be a significant portion of the ^{14}C mass balance. However, in an 'open' system with a large throughput of CO_2 from sewage biodegradation, which is being continually stripped during aeration, bicarbonate will be a very small fraction of the overall carbon mass balance. Unpublished studies of CAS simulation systems by AstraZeneca Ltd. have shown this to be an insignificant percentage of carbon mass balance. There are also practical difficulties in acidifying the effluents from continually running processes such as in this rig. Therefore, acidification was not undertaken to recover ^{14}C as bicarbonate.

Prior to each dosing experiment, fresh MLSS was collected to seed the SBR. OECD 303A (2001) recommends a stabilisation period prior to dosing to establish a consistent treatment performance (although it also allows dosing from the start). In our study, the SBR rig was operated for two weeks to acclimatise the CAS population to the selective pressures in the SBR and to test operational performance prior to dosing with radioactive propranolol. It should be noted that effective carbon removal was observed from the start-up of the rig.

2.2. Dosing

Two dosing strategies for ^{14}C-propranolol were applied to the SBR rig:

Batch loading: The dosing solution was spiked into the reactor as a single pulse consisting of 22.5 μg of ^{14}C-propranolol dissolved in autoclaved reverse osmosis water (total volume 10 mL), supplying 0.037 MBq of radioactivity. The rig was then run for 13 SBR cycles, each of which was 8 h with no influent addition or effluent removal.

Semi-continuous loading: A fresh stock of ^{14}C-propranolol was prepared and dosed directly into the reaction vessel by a peristaltic pump at the start of each 8 h SBR cycle over 5 days. Each dose supplied 0.032 MBq of radioactivity, equivalent to 19.7 μg of ^{14}C-propranolol.

2.3. Sampling and analytical methods

2.3.1. Rig samples

The radioactivity of samples from the two CO_2 traps was analysed at the end of the 8-h day time SBR cycles (i.e. 8, 24, 32, 48, 56, 72, 80, 96 and 104 h) to determine evolved $^{14}CO_2$. The trap contents were emptied into pre-weighed Nalgene bottles, which were then re-weighed. At 24, 48, 72 and 96 h both CO_2 traps were emptied into two separate Nalgene bottles, whereas at 32, 56, 80 and 104 h only the first trap was emptied, since at the latter time points there was minimal radioactivity breakthrough into the second trap.

During semi-continuous loading, traps were sampled at the same time as during single pulse dosing (8, 24, 32, 48, 56, 72, 80, 96 and 104 h). The effluent was collected at the end of the cycle at 8, 32, 56, 80 and 104 h for liquid scintillation counting and at 32, 56, 80 and 104 h the concentration of propranolol in the effluent was measured using a solid-phase extraction method followed by radio-HPLC (see Section 2.3.3.). WAS was collected (at 7, 31, 55, 79 and 103 h) to determine the proportion of radioactivity in the liquid- and solid-phases. The liquid- and solid-phases were separated immediately by centrifuging an aliquot of the WAS. The liquid-phase was decanted, sub-sampled and transferred to liquid scintillation counting (LSC) vials for analysis of radioactivity. The weight of the remaining solid pellet, after centrifugation, was determined, and then three sub-samples weighing ~0.1 g were transferred into combustion cones. The cones were stored in a freezer prior to combustion using a Packard 307 sample oxidiser (Perkin Elmer Inc., Cambridge, UK) to quantify the amount of radioactivity in the solid-phase by oxidising the ^{14}C compounds to $^{14}CO_2$. The mean of the three sub-samples was then used to assess the radioactivity in the solid-phase; these measurements showed good precision, apart from one low reading on day 3 which has been kept in the data set (see Section 4.1 in the SM).

2.3.2. Liquid scintillation counting

All aqueous samples taken directly from the rig (effluent, aqueous WAS and CO_2 trap samples) were split into sub-samples (3×5 mL) by pipetting into glass scintillation vials. Gold Star multi-purpose liquid scintillation cocktail (Meridian Biotechnologies Ltd., Surrey, UK) was added to samples until 20 mL LSC vials were completely filled the contents were then mixed vigorously.

Solid samples were combusted, converting any of the ^{14}C present into $^{14}CO_2$, which was trapped subsequently by a CO_2 absorbent (CarbonTrap, Meridian Biotechnologies Ltd., Surrey, UK). The

Table 1
Characteristics of the influent and effluent streams of the SBR rig, all values in mg L^{-1} apart from pH.

	TSS	BOD_5	COD	DOC	TP	TN	NH_4^+–N	NO_3^-–N	PO_4^{3-}–P	pH
Influent										
Mean	138.6	101.7	280.8	40.1	6.1	46.9	23.4	3.4	3.9	7.64
SD	92.2	29.9	176.1	22.2	2.6	17.9	11.7	4.0	2.0	0.15
N	35	5	41	40	24	23	41	41	43	3
Effluent										
Mean	13.1	3.2	39.1	10.1	4.4	21.6	2.0	15.4	3.8	7.66
SD	7.9	2.3	30.0	6.2	1.7	11.6	3.8	11.0	1.8	0.10
N	33	5	41	39	24	24	42	41	43	4

trapped $^{14}CO_2$ was then mixed with liquid scintillation cocktail (CarbonCount, Meridian Biotechnologies Ltd., Surrey, UK), so that there was a 50:50 v/v ratio of the two scintillation cocktails. The radioactivity contained in the NaOH solution in the traps, liquid and solid samples was then counted in a Tri-carb 2900TR spectrometer (Perkin Elmer Inc., USA). Each group of samples for LSC was preceded by one background measurement (necessary to subtract background radioactivity). Samples were counted for 20 min, or until a two sigma value of less than two was obtained, which ever was sooner.

2.3.3. Solid-phase extraction and radio-HPLC

Effluent samples were stored in the fridge and then extracted within 24 h. Aliquots (3 × 100 mL) were filtered (0.7 μm Whatman GF/F paper) and then extracted using Oasis MCX solid-phase extraction cartridges (6 mL, 150 mg packing, from Waters Ltd., Elstree, UK). Cartridges were pre-conditioned with MeOH (6 mL) followed by water (6 mL) at 5 mL min^{-1} and then the sample (100 mL). The cartridges were then eluted with a 2% formic acid and 5% MeOH (5 mL) solution, and dried under vacuum. The first elution step involved extractions using MeOH (2 × 4 mL), followed by a second elution step with 6% NH_4OH in MeOH (2 × 3 mL), both eluates were eluted into the same vial. Samples were dried under a gentle stream of N_2 to near dryness and then reconstituted in 1 mL of prepared H_2O and 0.1% acetic acid (95% v/v) and MeOH and acetonitrile (5% v/v) (i.e. the mobile phase starting gradient used in HPLC analysis).

HPLC analysis of the above eluates was performed using an Agilent 1200 series instrument (Agilent Technologies, California, USA) connected to a Mirastar model radio-detector MIRA star radio-detector (raytest Isotopenmessgeraete GmbH, Baden-Württemberg, Germany). Separation was carried out on a C_{18} column (Gemini-NX 50 cm × 3.0 mm from Phenomenex, Macclesfield, UK). Mobile phase A consisted of H_2O with 0.1% acetic acid and 100 mM ammonium acetate. Mobile phase B consisted of MeOH and acetonitrile (60:40 *v/v*). Mobile phase was pumped through the system at 0.25 mL min^{-1}. The mobile phase gradient started at 5% B and remained there for 2 min, then % B increased steadily until 100% over 11 min, where it remained for 2 min. The % B then returned to the starting conditions (5%) at 13.1 min. The column was equilibrated under these conditions until the method cycle ended (17 min). Sample injection volume was 100 μL and the column temperature was maintained at 40 °C.

2.3.4. Analysis of wastewater

The performance of the SBR rig was assessed by measuring biochemical oxygen demand (BOD_5), dissolved organic carbon (DOC), COD, total suspended solids (TSS), ammonium (NH_4^+–N), nitrate (NO_3^-–N), orthophosphate (PO_4^{3-}–P), total nitrogen (TN) and total phosphorus (TP) in the influent and effluent. Samples were also taken during the SBR cycles to investigate the conditions during the different phases. Analysis of DOC, NH_4^+–N, NO_3^-–N and PO_4^{3-}–P was carried out on filtered (0.45 μm syringe filters) samples. Details of these methods are given in the SM.

2.3.5. Data analysis

The data were analysed to assess the performance of the rig, the partitioning of radioactivity between the liquid- and solid-phases and the rate of biodegradation, as indicated by the release of $^{14}CO_2$.

2.3.5.1. Sorption. There are several sorption isotherms that are used to assess the partitioning behaviour of APIs between liquid- and solid-phases within a STP. Commonly used models are the linear sorption isotherm described by the solid-water distribution coefficient (K_d) and the non-linear Freundlich isotherm (based on the adsorption capacity, K_f and adsorption intensity, $1/n$ coefficients). K_d is estimated as the partitioning of an API between the solid- and liquid-phases, expressed as a concentration, divided by the availability of sorption sites, often taken to be MLSS concentration in CAS (Cantrell et al., 2002; Ternes and Joss, 2006; Joss et al., 2004; Wick et al., 2009). However, the use of MLSS as a descriptor of available sorption sites has been questioned due to the presence in MLSS of inorganic and stabilized material. Other studies have, therefore, suggested that reactor COD concentration or WAS (as kg MLSS per L, – as a measure of the production of new sludge) are better representations of available sorption sites when reporting K_d values in CAS (Ternes et al., 2004; Ternes and Joss, 2006). This gives the following equation for calculating K_d (L kg^{-1}):

$$K_d = \frac{C_S}{X_{SS} \times C_L} \quad (1)$$

Where:

C_S = concentration of API in the solid-phase (μ L^{-1})
C_L = concentration of API in the liquid-phase (μ L^{-1})
X_{ss} = sorption sites in the reactor (kg_{MLSS} L^{-1}, or kg_{COD} L^{-1}, or kg_{WAS} L^{-1})

Most studies estimate these parameters from direct measurements of the partitioning behaviour of parent compounds, however, in radiolabelled studies it has been suggested that 'apparent' values (e.g. $K_{d(app)}$, $K_{f(app)}$ and $1/n_{(app)}$) can be estimated from the partitioning of the radioactive ^{14}C label (Shimp and Larson, 1996). This approach means that radioactivity in breakdown products and microbial biomass is included in the calculation, so this needs to be considered in presenting results. However, many breakdown products of APIs are also of environmental concern and this method does overcome issues in extraction and recovery of parent compounds from sewage and sludge. $K_{d(app)}$ was therefore calculated for the five day time cycles as this was when MLSS and WAS were monitored. The equation for calculating $K_{d(app)}$ (L kg^{-1}) was:

$$K_{d(app)} = \frac{D_S}{X_{SS} \times D_L} \quad (2)$$

Where:

D_S = radioactivity in the solid-phase (as either activity, fraction or % kg^{-1})
D_L = radioactivity in the liquid-phase per litre of reactor (activity, fraction or % L^{-1})
X_{ss} = sorption sites in the reactor (kg_{MLSS} L^{-1}, or kg_{COD} L^{-1}, or kg_{WAS} L^{-1})

The Freundlich isotherm describes the non-linear isothermal equilibrium (concentration dependent), between a solute on the surface of an adsorbent and the concentration of the solute in the contacting liquid. The parameters K_f and $1/n$ are the Freundlich sorption coefficient and the linearity coefficient, respectively. Radiolabelled studies allow apparent constants to be estimated $K_{f(app)}$ and $n_{(app)}$ in a similar manner to $K_{d(app)}$, giving the Freundlich isotherm as described by Equation (3):

$$\frac{x}{m} = K_{f(app)} C_e^{\frac{1}{n(app)}} \quad (3)$$

Where:

x = mass of adsorbed compound (radioactivity L^{-1} converted to kg L^{-1})
m = mass of adsorbent (kg_{SS} L^{-1})
C_e = dissolved concentration at equilibrium (μg L^{-1}) (note here the system is assumed to be in equilibrium at the end of a cycle)
$K_{f(app)}$ and $1/n_{(app)}$ = coefficients

Equation (3) can be solved for the two coefficients by taking the logarithms and fitting a linear regression line, where $K_{f(app)}$ is the intercept and $1/n_{(app)}$ the gradient of the fitted line. The five day time cycles were used to determine apparent Freundlich isotherm coefficients for the same reasons as used for the estimation of $K_{d(app)}$.

2.3.5.2. Biodegradation. Kinetic constants, describing biodegradation of particular compounds under specific conditions, include $DegT_{50,}$ (time taken for 50% removal, or half-life) and $K_{biol,}$ the first order rate constant for microbial removal of an API in a CAS reactor, expressed per unit of biomass, in this case MLSS (Ternes and Joss, 2006). These values are usually estimated by the rate of removal of parent compounds. In studies using radioactive compounds, biodegradation (to H_2O and CO_2) or "mineralization" rate constants ($DegT_{50(M)}$ and $K_{biol(M)}$) can be estimated from the radioactivity contained in the off-gas from the reactor. For the ^{14}C-propranolol used in this study, mineralisation would require cleavage of at least one ring structure due to the location of the labelled carbon (Fig. S1). Rearranging the K_{biol} equation (as given in Ternes et al. (2004) and Ternes and Joss (2006)) to include mineralisation instead of compound concentration, gives Equation (4):

$$K_{biol(M)} = \frac{-D_D}{X_{SS} \times S} \quad (4)$$

$K_{biol(M)}$ = reaction rate constant (L kg_{MLSS} d^{-1})
D_D = sum of radioactivity as CO_2 in the off-gas, per day (converted to μg L^{-1} equivalent of the API)
X_{ss} = concentration of suspended sludge in the reactor (kg_{MLSS} L^{-1})
S = concentration of soluble compound (μg L^{-1})

$K_{biol(M)}$ was calculated for the continuous dosing study over the four complete daily cycles after cycle 1, as these had direct measurements of MLSS. Radioactivity found in the off-gas was, therefore, summed over cycles 2 to 4, 5 to 7, 8 to 10 and 11 to 13 to calculate values of D_D.

3. Results and discussion

3.1. Sewage treatment processes in the rig

The water quality data for the influent and effluent during semi-continuous loading operation are shown in Table 1, as the mean, standard deviation and number of samples. As the rig was fed with a real sewage matrix there is inherent variation in data between samples. The data in Table 1 includes the rig operating conditions recorded throughout the entire SBR operation, including during ^{14}C-propranolol dosing and the establishment phases. The mean MLSS in the reactor during the aerobic (therefore mixed) phases of the monitored cycles (1, 4, 7, 10 and 13) was 2100 mg L^{-1}, which when divided by the mean SS losses in the effluent and WAS gave an estimated SRT of 17 d, which is approximately twice as long as standard flow through CAS simulations (OECD, 2001).

The SBR rig received a relatively 'weak' sewage with an average BOD_5 and TSS of approximately 100 mg L^{-1} and 140 mg L^{-1}, respectively. The average removal of both parameters was high (96% BOD_5 and 87% TSS). Although effective nitrification was suggested by NH_4^+–N falling from an average of 23 to 2 mg L^{-1}, a removal efficiency of 93%, NO_3^-–N increased from 3 to 15 mg L^{-1}. There was an overall removal of >50% of TN, suggesting that partial denitrification was occurring. Low removal of TP (28%) was observed, which is to be expected as conditions in the reactor, i.e. anaerobic with a readily available carbon source, were not present in the reactor (Gebremariam et al., 2012). Many sewage treatment systems operating in the UK now use chemical dosing to achieve low TP consents (Manyumba et al., 2009) as biological removals can be inconsistent.

3.1.1. Characterisation of treatment phase

The SBR phases were well differentiated with distinctly different Eh conditions (Fig. 2a), in the anoxic (62 ± 116 mV, SD) and aerobic (302 ± 79 mV, SD) phases. Fig. 2b shows how the DO concentration was differentiated in the anoxic (median 0.1 mg L^{-1}) and aerobic (median 6.5 mg L^{-1}) phases. The developed rig, therefore, demonstrated cycling between distinct anoxic and aerobic conditions. Overall pH in the reactor (Table S1 in SM) was slightly alkaline (7.86 ± 0.29, SD) and did not vary significantly between the operational phases. The concentrations of NH_4^+–N and NO_3^-–N during the SBR phases are shown as a box plot in Fig. 3 (a and b). Decreases in the concentration of NH_4^+–N were seen during the aerobic phase, whilst NO_3^-–N shows a decrease in concentration after the anoxic phase, indicating denitrification, and an increase during the aerobic phase, indicating nitrification of ammonia carried forward from the anoxic phase. The rig was therefore generally representative of a nitrifying SBR, with evidence of denitrification, but with insufficient N removal to achieve the 10 mg L^{-1} total N required under the EU Urban Waste Water Directive (EU, 1991).

3.2. Radiolabelled propranolol fate studies

3.2.1. Single pulse loading

Overall during the single pulse loading study, 29% of the applied radioactivity was recovered from the NaOH solution contained in traps. Fig. 4 shows the percentage of radioactivity remaining in the rig with time (d), calculated by subtracting the radioactivity evolved as $^{14}CO_2$ from the initial dose. An exponential decay has then been fitted to the data and extrapolated beyond the 4-d test, the parameters estimated from the fit therefore have large potential errors due to the extrapolation, so have to be considered as

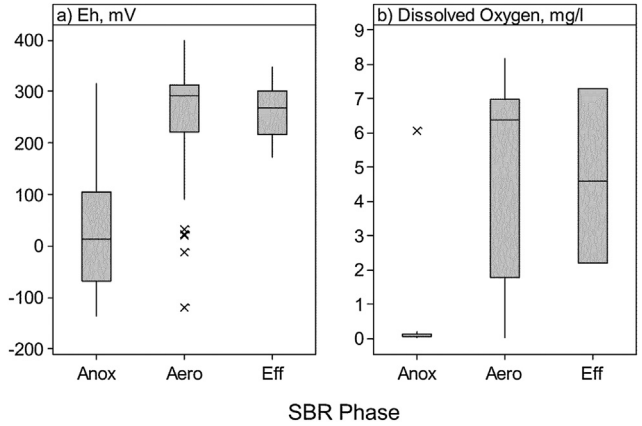

Fig. 2. Box plot of (a) Eh and (b) Dissolved oxygen (DO) during the phases of sequencing batch reactor operation (Anox – anaerobic/anoxic phase, Aero – aerobic phase and Eff – effluent). The cross bar shows the median and whiskers show the inter-quartile range. The shaded boxes show the central 50% of values and the crosses are outliers.

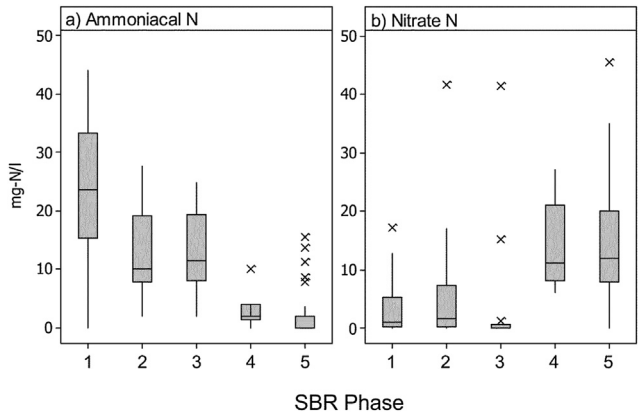

Fig. 3. Box plot of (a) NH_4^+–N and (b) NO_3^-–N during the phases of sequencing batch reactor operation (1 – influent, 2 – start of anaerobic/anoxic phase, 3 – end of anaerobic/anoxic phase, 4 – end of aerobic phase and 5 – effluent). The cross bar shows the median and whiskers show the inter-quartile range. The shaded boxes show the central 50% of values and the crosses are outliers.

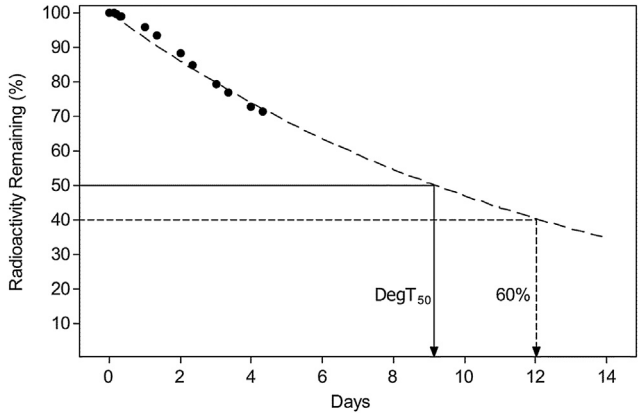

Fig. 4. Plot of activity remaining in the SBR simulation versus time after pulse loading. The exponential function ($y = 100.0e^{-0.076x}$, $r^2 = 0.98$) fitted to this decay is shown, by the dashed line. The $DegT_{50}$ value is shown by the solid line and the 60% pass rate within a 10 d window, as quote in the OECD guidelines (1992), is shown by the dotted line.

indicative only. This suggested that a $DegT_{50}$ would be achieved in approximately 9.1 d, and 60% degradation in 12.0 d. This is outside the 10 d limit for 60% biodegradation required for a compound to be classed as readily biodegradable under OECD Ready Biodegradability 301 guidelines (OECD, 1992); however, errors in extrapolating the exponential fit mean there are uncertainties in these overall estimates. However, these values are similar to the observations of Ribeiro et al. (2013) who observed ~65% removal of propranolol over 15 d in small scale batch systems seeded with activated sludge and dosed at 1 and 10 μg L^{-1} concentrations of the API. AstraZeneca Ltd. (Brixham Laboratory) has undertaken two unpublished, but publicly available, studies into biodegradation of ^{14}C-propranolol in batch CAS systems at 3000 mg L^{-1} MLSS. The first gave $DegT_{50}$ of between 4.9 and 6.8 d for propranolol at concentrations between 0.001 and 100 mg L^{-1}, and the second study produced $DegT_{50}$ of 14.6, 14.8 and 16.5 d at respective propranolol concentrations of 100, 0.1 and 0.01 mg L^{-1} (AstraZeneca, 2010) A $DegT_{50}$ of 9.1 d seen in this study is, therefore, within a similar range to other batch studies using radiolabelled propranolol (with the ^{14}C label in the same position see Fig. S1). This variability in the half-live of propranolol found in the different laboratory studies is also reflected in the range of values reported in field studies (e.g. Alder et al., 2010; Gabet-Giraud et al., 2010; Sun et al., 2014).

3.2.2. Semi-continuous loading

The overall recovery of radioactivity found in the semi-continuous propranolol dosing study was estimated to be 85.6%. This required interpolation of the activity in the overnight phase effluents and ignored radioactivity lost during the sampling for water quality measurements. The partitioning and fate of the radioactivity in the system is shown in the SM using cycle 7 as an example (Fig. S4 in the SM) and a pie chart of the overall distribution of radioactivity over all cycles (Fig. S5). However, as the maximum adsorptive capacity had not been reached, these values may be different than those attained at final equilibrium. Similar recoveries have been reported for other compounds in flow-through CAS simulations and slightly higher recoveries seen in single dose batch tests (Federle and Itrich, 1997). Flow-through systems, however, are more challenging in terms of capturing the radioactivity contained in the labelled analyte distributed across the different phases and streams. Losses in the system could also have occurred due to adhesion of compound to glassware and pipework in the rig, and possible statistical errors could result from the extrapolation of data from small sub-samples.

The radioactivity (as $^{14}CO_2$) found during semi-continuous dosing in the NaOH solution contained in the traps is shown in Fig. 5. Overall, 3.8% of the total radioactivity introduced into the system, was detected as $^{14}CO_2$ and production of radiolabelled CO_2 increased almost linearly up to cycle 7 (56 h), after which there is a decline in the apparent rate of mineralisation. A lag-phase detected during the first 8 h suggests the microorganisms needed a short acclimation period before they were capable of mineralising propranolol.

Table 2 shows the concentration of propranolol found in the influent and effluent streams associated with the rig. The influent concentration is derived from the dose applied divided by influent volume and the effluent concentration is from specific analysis using radio-HPLC.

The concentration of propranolol in the influent during the semi-continuous dosing was 8.7 μg L^{-1} (discarding purity errors), which is typically higher than values reported in STPs, however, concentrations were selected to meet analytical instrument limits of detection (Gabet-Giraud et al., 2010; Gardner et al., 2013). Table 2 shows the removal efficiency of propranolol from the liquid-phase was initially 73% (cycle 1) and this fell subsequently to 63% (cycle 13).

The fraction of radioactivity as ^{14}C-propranolol was assessed in SBR effluent of cycles 4, 7, 10 and 13. Three replicate samples were

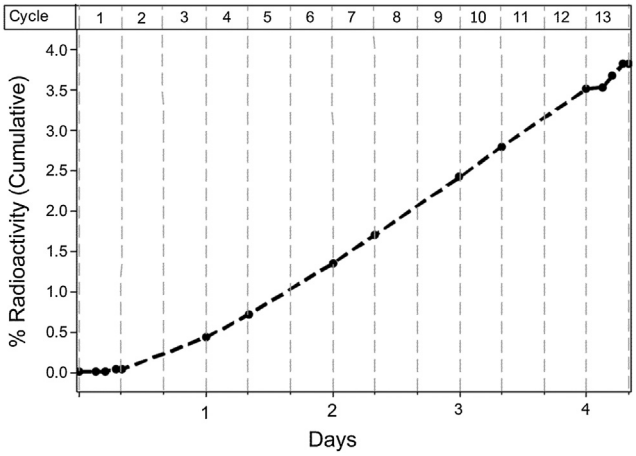

Fig. 5. Cumulative accumulation of $^{14}CO_2$ in the NaOH solution contained in the traps during semi-continuous dosing of ^{14}C-propranolol over thirteen SBR cycles, expressed as a % of the total radioactivity introduced into the rig.

Table 2
Concentration of ^{14}C-propranolol in the influent and effluent streams of the SBR. Concentrations in the effluent were measured using SPE and radio-HPLC analysis. The % of the radioactivity in the effluent, present as ^{14}C-propranolol is shown as the average of three replicate samples.

Cycle	Influent (μg L^{-1})	Effluent (μg L^{-1})	Removal (%)	Effluent radioactivity as propranolol (%), mean (±SD)
4	8.7	2.4	72.5	69.6 (±5.6)
7	8.7	2.6	70.0	63.9 (±7.3)
10	8.7	2.6	69.9	58.2 (±8.0)
13	8.7	3.3	62.8	65.3 (±7.9)

extracted and analysed by radio-HPLC. A typical chromatogram is shown in the SM (as Fig. S2b). Table 2 shows that the proportion of radioactivity in the effluent as ^{14}C-propranolol, decreased from 70% of total radioactivity in SBR cycle 4–58% in cycle 10, after which the percentage increased slightly to 65%. This suggested that breakdown products of propranolol were accounting for about 30–40% of the radioactivity in the effluent.

Fig. 6 shows the increase in radioactivity in the reactor, and the partitioning between the MLSS and liquid-phase, as a percentage of the total radioactivity introduced into the rig. Over the 13 cycles, the activity in the reactor increased to 5 times that in a single dose, and the majority of this activity was associated with the MLSS. The higher SRTs typically found in SBR systems give opportunities for sorbed material to accumulate in the system. It is difficult to differentiate between sorption of the parent compound and sequestration of ^{14}C into the microbial biomass components without further investigation (Federle and Itrich, 1997). However, radioactivity in the solid- and liquid-phases had a significant linear association (solid = 3.85(liquid) − 1.40, $r^2 = 0.97$, n = 5, $p < 0.000$), suggesting that, although the adsorption sites on the solids were not yet saturated, an adsorption equilibrium was established between the adsorbed and soluble radioactive compounds during the treatment phases.

3.2.2.1. Apparent kinetic rate constants. Table 3 shows the values of $K_{d(app)}$ and $K_{biol(M)}$, found during the semi-continuous dosing of the SBR rig. Equation (1) was used to calculate $K_{d(app)}$ by dividing by either the mass of WAS per litre of wastewater treated, or MLSS in the reactor. There was a strong linear association between COD and TSS in the reactor (COD = 1.82(TSS) + 23.4, $r^2 = 0.899$, n = 68, $p < 0.001$), which also allows $K_{d(app)}$ values to be approximated as per mass of COD. The estimates for $K_{biol(M)}$ in the monitored phases were also calculated as per Equation (4). The accumulation and partitioning of radioactivity in the rig gives progressively increasing estimates of the $K_{d(app)}$ values, whereas $K_{biol(M)}$ increases from cycle

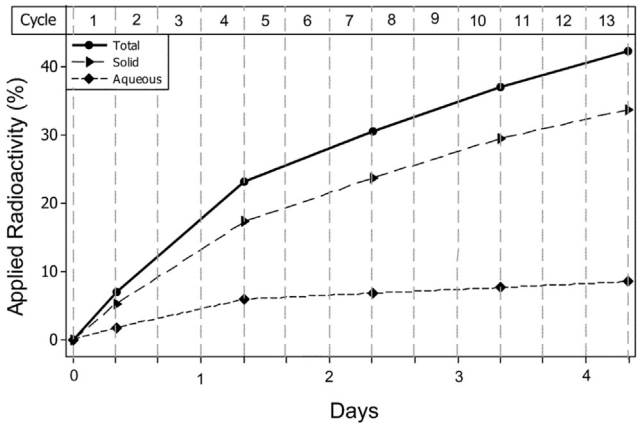

Fig. 6. Accumulation of radioactivity and the partitioning of activity between the solid- and liquid-phases in the SBR rig over the different cycles monitored.

Table 3
Apparent sorption coefficients for the linear sorption isotherm ($K_{d\ (app)}$) and biodegradation ($K_{biol\ (M)}$) models in the SBR rig.

Cycle	$K_{d(app)}$ (L kg_{WAS}^{-1})	$K_{d(app)}$ (L kg_{MLSS}^{-1})	$K_{d(app)}$ (L kg_{COD}^{-1})	Cycle	$K_{biol(M)}$ (L kg_{MLSS} d^{-1})
1	41,612	1387	758		
4	40,491	1349	737	2–4	49.2
7	51,770	1725	942	5–7	25.9
10	56,714	1890	1032	8–10	26.7
13	59,879	1995	1090	11–13	22.1

2–4 before decreasing between cycles 5–7, and remaining at this rate until cycles 11–13, when the rate decreases slightly again.

Table 3 highlights the disparity in K_d values depending on which system characteristic (COD, MLSS or WAS) was used to represent available sorption sites. Although the partitioning ratio between solid and aqueous phases remain the same the estimate using COD produces the lowest K_d values, followed by the MLSS and then the WAS. The WAS K_d values are one to two orders of magnitude greater than those found using COD or MLSS, SBRs may be particularly sensitive to this method of calculation as they generally have longer SRTs and lower sludge production compared to CAS. Overall, the K_d values found in our work are higher than those reported in the literature for secondary sludge. Martín et al. (2012) calculated a K_d for propranolol between 25 and 155 L kg^{-1}, and likewise, Radjenovic et al. (2009) reported a K_d for propranolol of 366 L kg^{-1} and Wick et al. (2009) a value of 343 L kg^{-1}. The reasons for the higher values in our study could be because of the use of apparent $K_{d(app)}$ values or, as K_d values are specific to the measurement conditions, differences in the characteristics of the systems. This could either be due to longer SRTs allowing compounds with high sorptive capacities to increase in concentration in the system, as reported for metals (Ke et al., 2012), or may be due to the floc structure as the anaerobic and granular flocs often found in SBRs have been shown to have higher adsorption capacity compared with CAS sludge (Drillia et al., 2005; Wang et al., 2010). Accumulation of APIs could, potentially be a problem for disposal of WAS from SBRs and additional advanced treatment prior to disposal may be required. It is therefore recommended that sorption studies are undertaken to assess these potential risks. The sorption of APIs to biomass and the longer SRT in SBRs, however, may offer possibilities for bioaugmentation to increase the degradation of recalcitrant APIs. Duque et al. (2011) found that their laboratory SBR successfully retained a strain of augmented bacteria that was capable of degrading 2-fluorophenol, a highly recalcitrant compound.

The accumulation of radioactivity in the solid- and liquid-phases of the reactor was plotted according to the linear (K_d) and Freundlich isotherms (Fig. 7a–b). Fig. 7(a) shows the linear relationship between radioactivity associated to each unit mass of MLSS (Bq kg^{-1}), plotted against the radioactivity in the liquid (Bq L^{-1}). Fig. 7(b) shows the Freundlich isotherm using the same data as for the linear plot, but values on both axes are plotted on $\log_{10}$ scales, to allow the apparent Freundlich coefficients to be estimated

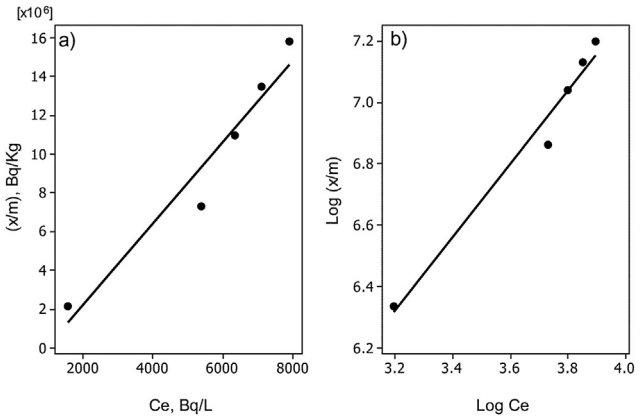

Fig. 7. Plot of (a) radioactivity per unit mass of MLSS (Bq kg^{-1}) against the radioactivity in the liquid-phase (Bq L^{-1}) (linear regression) (b) radioactivity per unit mass of MLSS (Log(x/m) against equilibrium radioactivity in the liquid-phase in the SBR reactor (Log C_e) (Freundlich isotherm). The data points represent the sequencing batch reactor cycle number (1, 4, 7, 10 and 13).

as per the linearised version of Equation (3). The fit to the linear isotherm gives $r^2 = 0.94$, n = 5, $p = 0.01$. The Freundlich regression fit to the data gives estimates of $1/n_{(app)} = 1.19$ and $K_{f(app)} = 324$ ($r^2 = 0.97$, n = 5, $p < 0.01$). Both the linear and Freundlich isotherms provided a good fit to the data; however, the Freundlich isotherm achieved a slightly better fit. A $1/n$ coefficient of 1.19 was obtained from the linear regression equation and indicated the data fits an S-type isotherm, since a value of $1/n > 1$ represents an isotherm where the sorption energy increases with increasing surface energy. Hence, as the concentration of propranolol increased within the system, which had a relatively fixed amount of biomass, the total percentage of propranolol removed by sorption increased.

Although biodegradation is generally thought to be a minor process for the removal of propranolol, there are limited K_{biol} values for propranolol from CAS simulations in the literature for comparison. Wick et al. (2009) reported K_{biol} values an order of magnitude higher than this study, i.e. 360 and 460 L kg^{-1} d^{-1}, but this was in smaller reactors that were continually aerated. CO_2 evolution studies often give more conservative removal efficiencies (Federle and Itrich, 1997) as they measure ultimate fate of carbon, rather than initial transformations of the parent compound; but the magnitude of difference in these values suggests that the systems are not comparable.

3.3. Strengths and limitations of the rig

The rig provided a robust model of SBR sewage treatment and the automation of the cycles worked well. Further development, however, is required before this can be recommended as a standard test. There is potential to modify the treatment phase sequence and duration to promote biological nutrient removal processes and investigate removals of APIs under various SBR operation regimes. The environmental realism of laboratory biodegradability and sewage treatment simulations has been questioned (Kowalczyk et al., 2015). Conditions such as temperature, concentrations of test substance and microbial ecology can all affect the operation of the rig and the estimated rate constants.

3.3.1. Operating conditions

Field CAS aeration tanks are reported to operate in the temperature range 4–32 °C (Beychok, 1971), but in the South of England a narrower temperature range of 11–15 °C is typically encountered (Widdows, 2015). In the rig, temperature was held constant at 20 °C in line with the OECD (2001) guidelines for CAS simulation. This will potentially affect wastewater treatment, especially N-cycling, microbial ecology and the rates of API removal. The K_{biol} has been generally shown to increase with temperature in biodegradability testing and CAS models, however, the increase in rates varies significantly between compounds and is also influenced by other environmental conditions (Thompson et al., 2011; Kowalczyk et al., 2015). In field-studies, Gabet-Giraud et al. (2010), found that CAS aeration tank temperatures did not influence removal rate efficiency of most beta blockers, including propranolol. However, in CAS simulations at room temperature (ranging from 14 to 23 °C), Suárez et al. (2012) found that temperature was a significant factor influencing biodegradation of some API and personal care compounds; and this appeared to be inversely proportional to K_{biol}. Therefore, although any standardised fate test will require fixed temperatures, it would be useful to operate the rig at a range of temperatures to ensure that the predictions are environmentally relevant, especially for compounds with a relatively low K_{biol} like propranolol.

One of the common criticisms of simulation tests is that they use higher concentrations of test compounds than are environmentally relevant, to allow the fate of dosed analytes to be observed (Kowalczyk et al., 2015; Xue et al., 2010). OECD fate tests, such as Ready Biodegradability No. 301 (1992), typically use dosing at concentrations in the range mg L^{-1} which are well above environmental values. The effect of concentration on biodegradation rates is complex, involving changes to biodegradation pathways and eventually inhibition (Kowalczyk et al., 2015). The concentrations of propranolol used in this study were based upon the limits of detection for the analytical equipment. They are higher than those generally encountered in the field, although similar peak concentrations have been detected (Verlicchi et al., 2012). Few simulation studies have examined the effect of applying different concentrations of propranolol: Ribeiro et al. (2013) reported similar patterns of behaviour for the degradation of propranolol in a simulation with CAS inoculum at both 1 and 10 μg/L but they did not present the comparative data. Propranolol has also been reported to have no effect on nitrite oxidation at up to 10 mg L^{-1} (Dokianakis et al., 2004), however, another study showed an effect on anaerobic digestion rates at an inhibition constant concentration of 40 μg L^{-1} (Fountoulakis et al., 2008). This was ascribed to the surfactant properties of the molecule. High radioactivity accumulations in SBR flocs (e.g. at day 4 this was the equivalent of 9.6 mg kg^{-1} of propranolol) would be in direct contact with bacteria so could potentially have a higher risk of inhibiting sewage treatment processes. There is, therefore, potential for further testing at different concentrations to assess if accumulations of APIs with high sorption coefficients do reach inhibitory levels.

Microbial ecology is perhaps the biggest concern in relating simulation studies to field scale. The microbial populations in simulation rigs have different selective pressures to that in STPs. In the standard OECD tests (1992; 2001), these are assessed only indirectly by their treatment performance. A recent review of biodegradation tests (Kowalczyk et al., 2015) has explored the use of molecular ecology methods (i.e. omics) to modernise the standard OECD tests and this would appear to be a priority research area for establishing how the overall microbial community profiles in simulation tests compare with operational STPs. It is even more difficult to draw conclusions regarding specific degrading populations for APIs, as they are probably small and their composition is not well defined. In our study the lag-phase (Fig. 5) only appeared to last for 1 SBR cycle (not used in estimates of kinetic constants), so the microbial population in the SBR rig appeared to be able to break-down the propranolol dose applied. However, the population was also continually supplemented with microorganisms in the

sewage feed, so making conclusions about their stability and adaption difficult.

3.3.2. Radiolabelled studies

The SBR simulation rig, in tandem with the use of radiolabelled APIs, allowed a high degree of resolution to determine the fate of ^{14}C labelled carbon. It was possible to use the data to provide apparent kinetic rate constants and sorption coefficients for use subsequently in environmental risk assessments. However, care needs to be taken in reporting and interpreting these constants due to their "apparent" nature.

Monitoring $^{14}CO_2$ in the off-gas is a measure of mineralisation of the radiolabelled carbon. In this case the propranolol used in this study, was labelled in the number 1 carbon atom where the side group attaches (Fig. S1 in SM). The degradation pathways for propranolol are complex (Wilde et al., 2013), but this C atom would appear to be mineralised in the latter stages of the breakdown of the molecule. Some ^{14}C will also be incorporated into microbial biomass, soluble organic compounds and a small proportion into inorganic carbon compounds in the reactor (Shimp and Larson, 1996). The $K_{biol(M)}$ will, therefore, generally underestimate the rate of biodegradation compared to direct measurements of the removal of parent compounds or by respirometry, especially in the early stages. Making direct measurements of parent compounds in sewage can be difficult and recoveries varied, and also potentially damaging breakdown products are not considered. Gejlsbjerg et al. (2003) found that $^{14}CO_2$ apparent biodegradation rates for surfactants in soil tended to be lower or the same as rates estimated from respirometry, but that radiolabelling did overcome difficulties in detecting small increases in CO_2 in systems with high rates of aerobic respiration.

The use of radioactivity partitioning to estimate sorption coefficients also has limitations as ^{14}C in propranolol breakdown products, microbial biomass and soluble C compounds would all contribute to the radioactivity in each phase. In addition, there is a potential for error in extrapolation from small samples of WAS. Previous estimates of $K_{d(app)}$ for surfactants have given comparable values to other methods in CAS (Shimp and Larson, 1996), the higher estimates for propranolol in this study may relate to the conditions in the SBR, particularly the longer SRTs typical of this technology. Further investigations with other compounds in the rig will enable this to be clarified.

Using radiolabelled compounds overcomes the limitations of extracting APIs from sewage and sludges, but needs to be combined with specific compound analysis to provide a more complete overview of the fate of parent compounds. The approach does require laboratories able to safely handle and dispose of labelled isotopes and is also limited by the relatively small number of radiolabelled APIs readily available. It is possible to synthesise a wider range of radiolabelled compounds, but this would incur additional expense. The use of radiolabelled APIs in laboratory rigs is a valuable tool in environmental risk assessments for APIs, providing unique insights into various fate processes. It is recommended that standardised laboratory simulation rigs are developed for all widely used wastewater treatment technologies building on the CAS guidance of OECD 303A and 314b (OECD, 2001, 2008) and developments in molecular biology. Preserving and enhancing the capability to undertake sewage simulation tests, particularly radiolabelled studies, in laboratories is therefore an investment priority for regulators and the pharmaceutical industry to enable robust risk assessments to be undertaken.

4. Conclusions

The SBR rig was efficient in treating sewage and was capable of similar efficiencies as full-scale STPs in current use in the UK. Distinct aerobic and anoxic phases were created during the SBR cycles. Dosing with ^{14}C-radiolabelled propranolol allowed the fate of the ^{14}C in the compound to be ascertained within the system. The release of $^{14}CO_2$ was evidence of limited propranolol mineralization in the system. However, the largest proportion of the radioactivity was found in the effluent, and a significant fraction of this was present as propranolol. The in-vessel solids made up the second largest fraction of radioactivity and this could be a result of the physicochemical properties of propranolol and the SBR sludge and operational characteristics. As a result both the liquid and solid waste from SBRs needs to be considered in any environmental risk assessments.

The overall fate of propranolol has been uncertain in many laboratory studies using unlabelled dosing and field monitoring has given very varied rates of removal. The combination of laboratory simulation with a radiolabelled API provided a direct and unique assessment of the various fate processes, but one inherently focused on the location of the labelled C atom in the parent compound. Further specific analysis of the parent compound in such studies would give more information about fate processes and also greater comparison with unlabelled laboratory and field studies, but also would incur a greater cost.

This SBR rig should be used to investigate the fate of other recalcitrant APIs. With further validation experiments, the rig could be the basis for a standardised laboratory-based test, used to inform the environmental risk assessments of pharmaceuticals in SBRs. Further development of standard laboratory simulation rigs, for use with both labelled and unlabelled compounds, in other emerging sewage treatment processes, would also allow for a controlled comparison of the performance of the technologies and inform the best process selection to address the growing concern over the impact of APIs in the environment.

Acknowledgements

This work was funded by the Engineering and Physical Sciences Research Council (UK) (Grant Number EP/G501688/1), AstraZeneca and the University of Portsmouth under an Industrial CASE award. The authors acknowledge the assistance of Dr Catherine Mant (University of Portsmouth) in wastewater analysis and the staff at the AstraZeneca Environmental Laboratories, Brixham, UK who facilitated aspects of this work, particularly Dr Gary Roberts who kindly advised on some technical aspects of the manuscript.

Appendix A. Supplementary material

Supplementary material related to this article can be found at http://dx.doi.org/10.1016/j.watres.2015.09.033.

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