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The Effect of Anaerobic Pig Slurry Redox Potentials on the Degradation of Veterinary Medicines

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ABSTRACT

Veterinary medicines are frequently used within intensive livestock husbandry and there has been a growing interest regarding their environmental fate following manure application to land. However, research has seldom assessed the influence of pig slurry properties on the fate of veterinary medicines even though such an understanding is essential for a more robust environmental risk assessment. Changes within manure degradation rates have the potential to alter the concentration of antibiotics applied to land, and the outcome of the risk assessment. The aim of this work was to investigate whether commonly reported redox potentials affect the degradation rates of acetyl-salicylic acid, ceftiofur, florfenicol, oxytetracycline, sulfamethoxazole, and tylosin. The employed redox potentials were -100mV (reduced), -250mV (anaerobic) and -400mV (very anaerobic). A compound specific relationship was observed where the degradation of ceftiofur, florfenicol, oxytetracycline and sulfamethoxazole was inhibited under reduced conditions over that of very anaerobic; the respective DT_{50} values were 0.7-1.84 h, 1.35 h-3.61 h, 22.2 -49.8 h, 131-211 h and 35.4-94 h. In contrast, tylosin was found to degrade faster at reduced conditions over very anaerobic (DT_{50} 6.88 to 19.4 h). The presented research demonstrates the importance of redox potential on degradation rates and suggests we need stringent and harmonized redox control to improve the environmental risk assessment of veterinary medicines.

Environmental relevance and significance: Given the significant effect of anaerobic redox potentials on veterinary medicine fate tighter regulation is required in manure degradation trials.

INTRODUCTION

Veterinary medicines are routinely used within animal husbandry to improve/protect animal health, moreover, in some regions of the world veterinary antibiotics are also used to promote the growth of livestock (Patel *et al.,* 2020). Often high percentages of administered veterinary medicines and their metabolites are excreted resulting in high concentrations of biologically active chemicals being detected within animal manures and urine (Halling-Sørensen *et al.,* 2001; and Sukul *et al.,* 2009). Typically, animal manures are used as organic fertilizers to enrich soils, improve nutrient contents/cycling as well as this being a suitable method for waste disposal. A consequence of doing so, however, is the potential spread of veterinary medicines into the environment (Kutcha *et al.,* 2009; Potter *et al.,* 2010; Lee, 2010; Kim *et al.,* 2011; Bogaard *et al.,* 2013; Balzer *et al.,* 2016; and Martínez *et al.,* 2019). This is of concern given societal impacts such as antimicrobial resistance as well as impacts on terrestrial and aquatic ecosystems (Thiele-Bruhn *et al.,* 2005; Liu *et al.,* 2009; and Joy *et al.,* 2014). Due to the aforementioned environmental risk of veterinary medicines, the environmental risk assessment was implemented under directive 2004/28/EC (EMEA, 1997; VICH, 2000; VICH 2002; and EMA 2016). Laboratory manure degradation trials are often conducted to assess the degradability of veterinary medicines during on-farm storage (CVMP, 2016). Such assessments are essential to understand the concentrations of veterinary medicines applied to land; during storage veterinary medicines are subject to varying dissipative processes such as microbial mineralization, sorption, and hydrolysis which have the potential to reduce the parent compound concentration (Lamshöft *et al.,* 2010).

Currently, there is variability in reported veterinary medicine degradation rates within manures; this is most likely attributed to differences in slurry properties and the unknown effect this has on degradation rates (Kreuzig, 2010; CVMP, 2011; and Whode *et al.,* 2016). For example,

45 degradation rates (DT_{50}) ranging between <2 – 45d have been reported for tylosin in pig manures
46 (Loke *et al.,* 2000; and Berendsen *et al.,* 2018). Despite reported uncertainty and variability within
47 the literature, the use of just one manure per animal type is permitted within the risk assessment.
48 As a consequence, such assessments may result in bias and poor environmental representativeness.
49 We speculate that variability in properties such as redox potential is driving this variability.
50 Manure properties are highly heterogenic due to differences in storage conditions, water
51 properties, animal feed, age, usage of biocides as well as physical manure amendments (Cahn *et*
52 *al.,* 1998a; Cahn *et al.,* 1988b; Deng *et al.,* 2007; Kreuzig *et al.,* 2010; and Weinfurtner *et al.,*
53 2011). The redox potential of animal manures is highly heterogenic due to differences in microbial
54 processes (methanogenic vs aerobic) and the oxidative-reductive state is governed and correlated
55 with pH, temperature, moisture, and manure age (Singh, 2001). For example, Park *et al.,* (2006)
56 investigated the influence of temperature and moisture on redox and reported lower redox values
57 within summer (-333 mV) over winter (-232 mV) as a result of increased microbial activity.

58 Redox potentials of pig slurries are seldom reported in the scientific literature, despite it being a
59 requirement within manure degradation trials under the risk assessment (-250 mV to -400 mV)
60 (CVMP, 2011; and Whode *et al.,* 2016). When redox potentials are reported they range from
61 slightly aerobic-reduced values (Kolz *et al.,* 2005; Kreuzig. 2010; Widyasari-Mehta *et al.,* 2016)
62 to more commonly reported anaerobic conditions (Lamshöft *et al.,* 2010; Richter *et al.,* 2016; and
63 Junker *et al.,* 2020).

64 Redox potential of environmental matrices/wastes can be controlled to eliminate contaminants
65 such as nutrients, metals and organic matter (Charpentier *et al.,* 1987; Charpentier *et al.,* 1998; and
66 Dusing *et al.,* 1992). For example, redox potential control is often utilized to treat wastewaters,
67 with a series of anaerobic-aerobic phases being employed to establish biological nutrient removal

(BNR) (nitrification-denitrification), a reduction in organic matter, bacterial disinfection, and removal of emerging contaminants such as endocrine disrupters, phenolic compounds, and industrial chemicals (Goncharuk *et al.,* 2010; Erontistis *et al.,* 2011; and Ghernaout, and Elboughdiri, 2020). Moreover, aeration of wastewaters is often utilized within membrane bioreactors to increase the transformation of emerging contaminants (Yoon *et al.,* 2004 and Sun *et al.,* 2016). Given the benefits of redox control, scientific interest has also considered its applicability for nutrient and odor control within animal manures (Pain *et al.,* 1990; Burton 1992; and Béline *et al.,* 2004). Limited studies have considered aerobic redox conditions and veterinary medicine fate, although Ali *et al.,* (2013) demonstrated that the removal of tylosin in dairy lagoon sediment was increased under aerobic (+350mV) compared to reduced (-100mV) conditions and Bachmann *et al.,* (1987) reported that oxic conditions promoted degradation of A/B-Hexachlorocyclohexane. These findings demonstrate the influence of redox potential on veterinary medicine fate, however, very little is known with regards to the effect of a range of anaerobic redox potentials in animal slurries (Whode *et al.,* 2016). It is essential to understand this relationship to harmonise laboratory assessments and reduce variability within the risk assessment; such an understanding would contribute to more accurate assessments providing better environmental representativeness.

Until now the influence of anaerobic pig slurry redox potentials on veterinary medicine degradation was largely unknown; this work aims to bridge this knowledge gap and improve our understanding of variability within manure degradation trials. Controlling the redox potentials of wastes and other environmental parameters has been troublesome for researchers for some time. Previous redox control methods are available within the literature; however, these methods are

often costly, time-consuming, and complex (Patrick *et al.,* 1973; Chuan *et al.,* 1996), here we present a pragmatic and cost-effective means for redox control.

METHODOLOGY

CHEMICALS

All chemicals and solvents were of the highest available purity (94-98%). NaOH, Na_2EDTA, citric acid and di-sodium hydrogen orthophosphate were purchased from Fischer Scientific (UK). A broad range of veterinary medicines were selected for use within the experiment (SI Table.1), Florfenicol and sulfamethoxazole were purchased from VWR (UK), whilst acetyl-salicylic acid (ASA), oxytetracycline (OTC), ceftiofur (CFT) and tylosin tartrate (TYL) were purchased from SLS (UK). 0.1M Na_2EDTA-Mcilvaine buffer 50:50 (pH4) was prepared by mixing 614.5ml of 0.1M citric acid, 385.5ml of 0.2M disodium orthophosphate and 500ml 0.1M Na_2EDTA. Redox probes were calibrated using a +220mV checking solution from Mettler-Toledo (Sigma-Aldrich, UK). Matrix matched standards were used to quantify the analytes; these were prepared on the day of the extraction.

MANURE SAMPLING AND PROPERTIES

Fresh pig manure was sampled from fattening pigs at a farm in Welburn, York (54°05'31.2"N 0°53'03.0"W), pig manure was collected two weeks prior and stored at 3ºC. The pigs had not received any of the selected veterinary medicines used within this study. The manure was homogenised and moisture was corrected to a dry weight of 5% (CVMP, 2011).

In order to characterize the dissolved fraction of the slurry it was centrifuged at 3,250rpm for 2h and decanted, before the supernatant was collected and sequentially filtered through varying filter grades. The filtering sequence was as follows; G/F Whatman, 1Ps filter paper, 20µm GF syringe

filter then a 0.45µm nylon syringe filter. Dissolved carbon and nutrients were then analysed using a Analytik Jena Multi NC2100 (carbon) and Autoanalyzer (nutrients). Manure was sampled twice in order to repeat the experiment (replication), (see Table.2 for reported manure characteristics). The properties of the manures utilized within the studies were in-line with one another, an exception of this is redox potential although this is unimportant given that redox was specifically manipulated within the study.

Table 2: *Pig slurry property data for manures collected for both replicates of the study, the data displayed demonstrates the dissolved available fraction of the slurry.*

Slurry Sample	**Starting redox (mV)**	**pH**	**DOC, DIC, DC (mg/l)**	**NH_4^{-1}, NO_2 (mg/l)**	**PO_4 (mg/l)**
14/01/2021	-278	7.3	2578.71±8.05, 656.92±0.68, 3235.64±8.74	276.44±1.7, 1.27	19.95±0.14
20/02/2021	-412.7	7.4	2838.36±13.41, 267.36±1.68, 3105.37±14.05	523.34±8.1, 0.40±0.033	12.39±0.027

BIOREACTOR AND REDOX CONTROL

Bioreactor vessels were designed using 2000ml wide neck Erlenmeyer flasks which were sealed using 62mm rubber bungs (Fig.1 and SI FIG.1). The bung contained four holes made from food grade Nalgene tubing in the top to facilitate aeration, nitrogen, outlet, and sample retrieval. A OCS.tec GmbH & Co. KG ORP controller containing a platinum electrode was used to maintain the redox values, the specifications of this controller were -1000mV to +1000±0.2mV. The ORP controlling system was connected to an air pump and when the redox potential value dropped below the desired ORP value (mV) the pump automatically turned on aerating the vessels. The electrodes were installed into the system at 8cm depth. Within the duplicate vessel a WTW xylem platinum redox potential probe was utilized to ensure redox potentials were uniform between the replicates. An aquatic airflow manifold was used to evenly distribute the rate of aeration between both vessels (Fig.1). The system was set up on a rotary bed shaker that was used to homogenize the sample and distribute oxygen throughout the entire sample. The rotary bed shaker was set to 30rpm throughout the study duration, although prior to sample retrieval the sample was homogenized at 120rpm for 30 seconds.

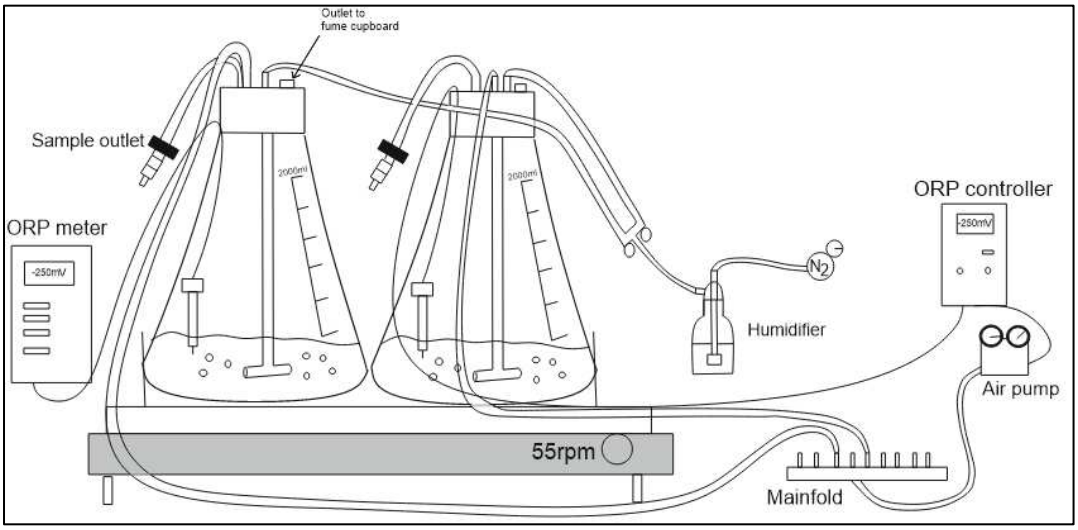


Figure 1: *Schematic drawing of the experimental rig used to control redox potential. Three treatments of this design were simulated in tandem.*

EXPERIMENTAL CONDITIONS

Three treatments of pig slurry were assessed with the selected redox potentials within the anaerobic range defined by OECD 307 and CVMP (2016): -100mV (reduced), -250mV (anaerobic) and -400mV (very anaerobic). The tolerance of these redox potentials was ±50mV throughout the duration of the study. The treatments were achieved using the following; reduced (-100mV) was achieved with intermittent aeration, anaerobic (-250mV) incorporated nitrogen at 8cc's with a similar aeration system as reduced, and very anaerobic (-400mV) utilized nitrogen (10cc's) to purge the system of oxygen. The bioreactors were maintained at the desired redox potentials for two weeks prior to dosing with antibiotics; this acclimation period allowed the microorganisms present to adjust to the conditions. The moisture content of the slurries were adjusted every 2 days to ensure that the treatments were consistent. The experiment was kept in the dark (foil coating) at 23^{o}C for the duration of the study and repeated twice in order to obtain sufficient replicates for statistical comparisons (n=4).

Veterinary medicine dosage concentrations were derived using the Spaepen (1997) model. The dosage concentrations selected were at 1/5th of the calculated Predicted Environmental Concentration (PEC) to avoid any significant inhibition of the microbial populations within the pig slurry (SI Table.2). Moreover, CFT was dosed at 2/5th of the PEC due to sensitivity issues of this analyte. The study was conducted over 14d and the given timepoints were 0h, 2h, 6h, 12h, 24h, 48h, 3d, 7d and 14d.

SAMPLE EXTRACTION AND ANALYTICAL TECHNIQUE

At the given timepoint 12.5ml of slurry was retrieved from the bioreactor using a pre-installed outlet which was connected to a vacuum pump (Fig.1). After each timepoint 5ml of deionized water was used to clean the tubing to remove any contaminants. 12.5ml of 0.1M Na_2EDTA-Mcilvaine buffer was added to the slurry sample and shaken using a rotary bed shaker at 250rpm for 20 minutes, the sample was then centrifuged at 3,250rpm for 20 minutes at 4°C. The extraction was repeated twice, and the supernatants were combined. Samples were then filtered to 0.2µm and stored at -20°C prior to analysis. Extraction efficiencies of the six analytes at 1%, 10% and 100% of the dosage concentration were calculated (SI Table.3). The majority of the compounds met the SANCO 3029 recovery criteria (<70%) at the 100% dose, an exception to this was CFT which did not meet the requirements at any dosage level. Due to sensitivity issues, TYL failed the criteria at 1%, however still met the 10% criteria.

Veterinary medicines were analysed using a SCIEX Triple Quad 5500+ LC-MS/MS System. The analytical method comprised of using a Phenomenex Kinetics XB-C18 column (50x2.1mm) at a set temperature of 40°C and the mobile phases consisted of 0.1% formic acid (aqueous) and 0.1% formic acid in methanol. The method had a 30µl injection volume, the flow rate was set to 0.4ml/min, and the chromatographic duration was 11 minutes. The gradient was a reversed phase and consisted the following organic gradient percentages, 0mins (0%), 3mins (90%), 8mins (90%), 8.1mins (10%) 11mins (0%). Please see the SI Table.4 for additional mass spectrometer details. Analyst 1.6 was utilized to process the data as well as to quantify the concentrations based on a calibration curve (≤95%).

DEGRADATION KINETICS AND STATISTICAL ANALYSES

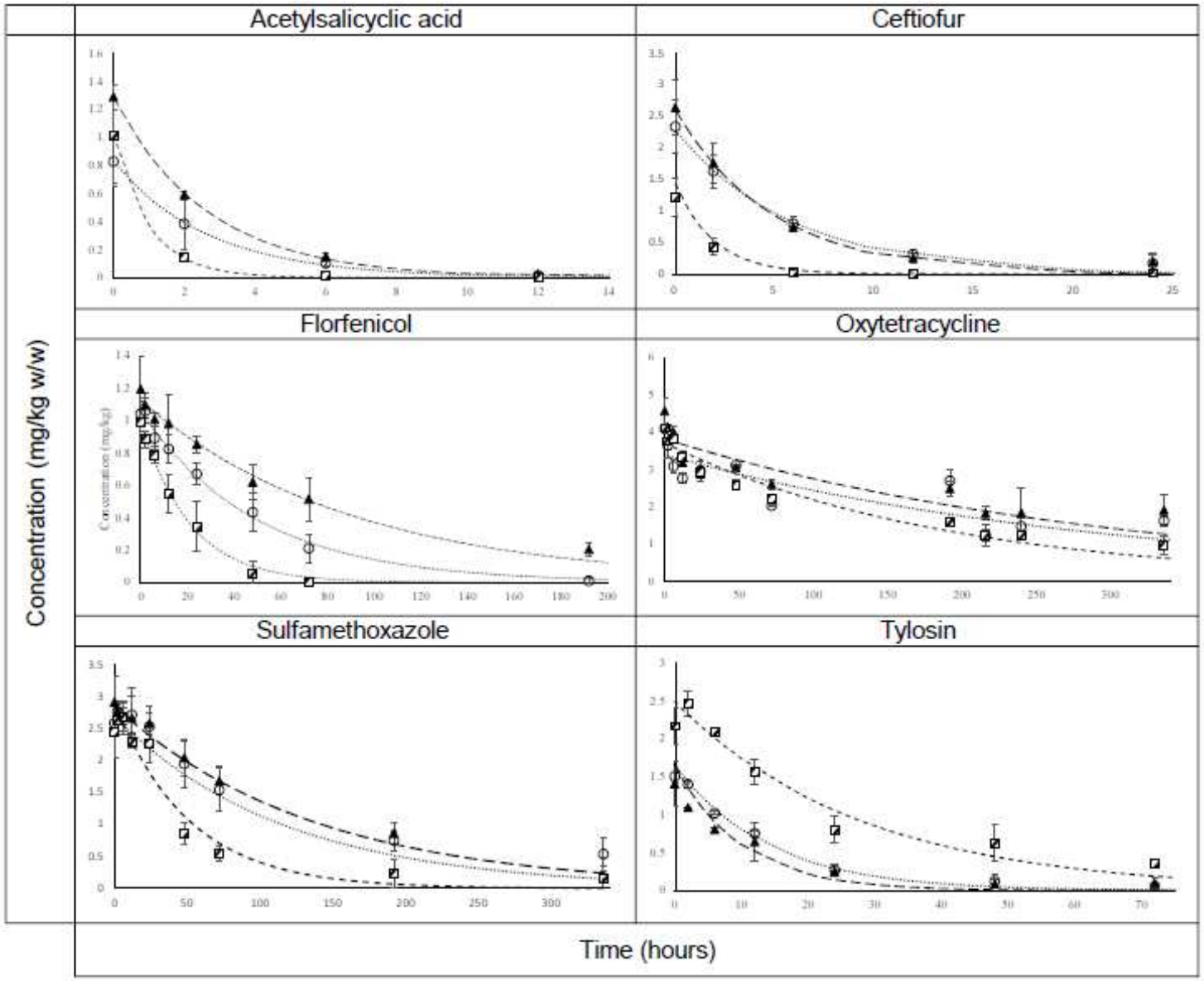
The degradation kinetics modelling suite CAKE (v3.3) was utilized to plot the kinetics profiles of the studied analytes within various redox treatments (Tessella, 2021). The most appropriate fit was selected based of a chi-squared of <15%, the assessed models were Single First Order, First Order Multi Compartmental and Hockeystick. Statistical analysis was performed using Minitab 18, an analysis of variance (ANOVA) (two-way) was used to statistically compare between redox potential treatments and concentration over time (concentration=time*redox). Tukey post hoc comparisons were then employed to distinguish differences between the redox treatments. Statistical significance was reported at the 95% confidence level ($p = <0.05$).

RESULTS

The majority of the assessed veterinary medicines (ASA, CFT, FLO, SMX and TYL) were found to be impersistent within pig slurries (Hollis, 1991). An exception was OTC which was found to be persistent, and the study duration was too short to fully assess the degradability of OTC under varying redox potentials. All of the degradation kinetics were found to be of Single First Order. The influence of redox potential on the degradation rates of selected veterinary medicines was found to be compound specific (Fig.2 and Table.3). Reduced conditions (-100 mV) were found to inhibit the degradation of CFT, FLO, OTC and SMX over that of both anaerobic and very anaerobic (-400 mV) ($p=<0.05$) (Fig.2). Inhibited FLO degradation under reduced conditions resulted in a calculated DT_{50} two times that of very anaerobic, the DT_{50} values being 22.22h at -400 mV and 49.8h at -100 mV. Similarly, SMX degradation rate was stunted under reduced conditions over that of very anaerobic; the degradation rate constant (k) was found to be over 2 times larger (i.e. $k\ 0$.007377 at 100 mV and 0.01956 at -400 mV). Such inhibition drove differences within the calculated DT_{50} values, these were 35.4h and 94h ($p=<0.05$). Moreover, a significant

effect between very anaerobic and reduced conditions was also observed for CFT and for OTC (Fig.2 and Table.3) ($p=<0.05$). Only a minor difference was observed between the degradation rates of OTC, which would suggest that a longer study duration is required to assess this relationship (*k* 0.005303 at -400 mV and 0.003306 at -250 mV).

Comparatively, TYL degradation rate was stunted by a factor of 2.8 under very anerobic conditions over reduced, this resulted in DT_{50} values of 6.88h at -100 mV and 19.4h at -400 mV ($p=<0.05$). No significant differences were observed between the anaerobic-reduced treatments for the majority of the assessed veterinary medicines, an exception of this was FLO for which, under reduced conditions, a DT_{50} of 49.8h was reported compared to 29.6h under anaerobic ($p=<0.05$). The degradation rate of ASA was unaffected by redox potential and the persistence of this compound was short lived (Fig.2).



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223 ***Figure 2:*** *Degradation of veterinary medicines under varying anaerobic redox potentials over time.*

Key: -100 mV -▲- -250mV ···◯··· -400mV-▨-

Table 3: *Collated degradation parameters of veterinary medicines within pig slurry under varying anaerobic redox potentials.*

Degradation Parameter	Redox potential	Acetylsalicylic acid	Ceftiofur	Florfenicol	Sulfamethoxazole	Tylosin	Oxytetracycline
DT_{50} (hours)	-100 mV (reduced)	1.84	3.61	49.8	94	6.88	211
	-250 mV (anaerobic)	1.87	4.28	29.6	80.5	9.58	210
	-400 mV (very anaerobic)	0.71	1.35	22.22	35.4	19.4	131
k degradation rate constant	-100 mV (reduced)	0.3767	0.2071	0.01392	0.007377	0.1007	0.003288
	-250 mV (anaerobic)	0.3706	0.1717	0.02338	0.008615	0.07233	0.003306
	-400 mV (very anaerobic)	0.9812	0.5132	0.03128	0.01956	0.03574	0.005303

DISCUSSION

REDOX POTENTIAL CONTROL

The test system showed appropriate control throughout the duration of the study as a tolerance of ±50mV was achieved (28d) (SI Fig.2); this was also achieved within the replicate study (total duration was 56d). There are publications detailing experimental details for redox control of environmental matrices, however, these typically utilize a methodology that was devised via Patrick *et al*., (1973). This system has proved to work and has been adopted for a range of environmental matrices (Willis *et al*, 1974; Chuan *et al*, 1995; Carbonell-Barrachina *et al*, 2000;

Lissner *et al*, 2003; Hjorth *et al*, 2012; and Ali *et al*, 2013), however this design is arguably outdated, expensive and requires sufficient knowledge in electrical rewiring. Moreover, there is no evidence within the literature to suggest redox control has been achieved on pig slurries; there is also no defined methodology that would be deemed suitable to control redox potentials of slurries during degradation assessments that meet the CVMP, (2016) criteria.

Here we have demonstrated a cheap and effective means to control the redox potential of pig slurries; the presented system design could also be adapted for use in other environmental matrices such as water, wastewater, sludges, and soil solutions. The system was maintained at the desired redox potential throughout the duration of the study, although, to do so it is paramount to take care in the construction and development of the system to obtain an experimental apparatus that remains functional and within tolerance for the duration of the experiment. For example, to replicate redox conditions in two vessels, it was critical to evenly distribute the airflow between the vessels; this was shown to work well using the aquatic air manifold. One of the major issues with the system was the durability of the redox probes, during the development of this system it was apparent false readings can result in excess aeration. To overcome this issue, we would suggest a more robust redox probe that could connect to the controller, which could be achieved using a BNC adapter, or alternatively spare probes and a thorough cleaning process (i.e. fine grain sandpaper and acetone). The position of the ORP probes was at 8cm depth and secured to the side of each vessel and it was paramount to ensure these positions were the same given what is known regarding the influence of depth on ORP readings (Yu and Bishop, 2001). Moreover, the positioning of the aeration inlet was centered within the vessel, ensuring that aeration did not affect the readings.

VETERINARY MEDICINE FATE

The degradation rates of SMX, TYL and CFT under very anaerobic conditions were found to be in line with previous assessments within pig manures and pig slurries, for example the following DT_{50} values have been reported, SMX 2.6d, TYL <2d and CFT ~2h (Gilbertson *et al.,* 1990; Loke *et al.,* 2000; and Berendsen *et al.,* 2018). The degradation rates of OTC and FLO under all redox treatments were reported to deviate from previous assessments (Blackwell *et al.,* 2005; and Junker *et al.,* 2020). We speculate this to be related to the unique experimental design. For example, the constant addition of water to wash the sample extraction line will increase the moisture content; in addition, aeration of the vessels resulted in solid particulates sticking to the side of the vessel. OTC is well known to be hydrolytically unstable whilst FLO is stable within aqueous solutions; therefore, an increase in moisture and a reduction in particulates would decrease OTC sorption and promote its hydrolysis, whilst increasing the stability of FLO (Xuan *et al.,* 2009; Mitchell *et al.,* 2015; and He *et al.,* 2020).

REDOX POTENTIAL AND DEGRADATION RATE

Based on the current literature we can speculate that a number of processes could be contributing to the differences in degradation rates observed under varying redox potentials. The pH values at each redox treatment were similar (8.3-8.5), therefore differences in degradation rates are unlikely to be related to adsorption via ionic charge and the pKa of the chemical. There is however compelling evidence of increased adsorption rates of 2,4,6-Trinitrotoluene and pentachlorophenol to soil-sediment suspensions under oxidized conditions (Gambrell and Patrick. 1988; and Pennington *et al.,* 1990). The reasoning for this is generally unknown and suggests further research is required to understand this relationship (Price *et al.,* 2001; and Dorival-García *et al.,* 2013).

There is a possibility that an increase within the oxygen contents of sludges, wastes, and slurries may increase the Chemical Oxygen Demand (COD), indicating greater oxygen consumption and organic matter/carbon degradation (Zhang *et al.,* 2006; Moura *et al.,* 2012; and Barana *et al.,* 2013). This suggests that higher oxygen contents would reduce adsorption to OM and OC (Luo *et al.,* 2002), which could have reduced the adsorption for CFT, FLO, and SMX. However, biotic processes are the predominant degradation processes for these analytes, therefore it is unlikely that adsorption mechanisms resulted in the variation of degradation rates within differing redox potentials (Liu *et al.,* 2010; and Fan et al, 2019). Future work could be aimed at conducting abiotic assessments to ascertain the influence of microbial mineralization on the degradation of veterinary medicines under varying redox potentials.

Ali *et al.,* (2013) reported a similar effect of redox potential on the degradation of TYL within dairy lagoon sediment, for example, they found TYL to fully degrade in 4d under aerobic conditions (+350 mV), whereas under reduced (-100 mV) it took 20d. Moreover, similar trends have been reported in Loke *et al.,* (2000), Kolz *et al.,* (2005), and Seo *et al.,* (2018), where aerobic conditions promoted TYL degradation in pig slurries. Microbial transformation has been suggested to be the predominant process which affects TYL degradation under varying oxygen levels (Ali *et al.,* 2013; and Loke *et al.,* 2000). However, Kolz *et al.,* (2005) stated that adsorption mechanisms may have also contributed. It remains unclear whether adsorption mechanisms contribute towards these differences. Sodium azide was used as a sterilant within these studies although its effectiveness is questionable given the loss of potency that was observed via Ali *et al.,* (2013) when using sodium azide to conduct abiotic-biotic degradation assessments.

From the available literature, it is clear that biotransformation of SMX is complex under anaerobic and aerobic conditions and varying results have been published, although it is hard to directly

compare these to this study given differences in matrices and scientific scope. Aerobic/oxic conditions have been reported to increase the biodegradation of SMX within granular/suspended activated sludge, wastewater, and soils, findings which oppose those presented here (Liu *et al.,* 2010; Poirier-Larabie *et al.,* 2016; and Kang *et al.,* 2018). Conversely and in line with the presented findings, Ouyang *et al.,* (2021) reported increases in SMX degradation under anaerobic sludge conditions over that of aerobic (nitrate-reducing conditions), which was attributed to the presence of sulfate-reducing-bacteria (Desulfovibrio Vulgaris). This bacterial genus is considered an SMX degrader and is also abundant within pig slurries (Cook *et al.,* 2008; and Karnachuk *et al.,* 2021). Moreover, similar findings were reported via Jia *et al.,* (2017) who investigated the degradation of SMX within an SRB sludge reactor and stated removal rates were enhanced via the presence of Clostiridum sp. These findings are further supported via a comprehensive assessment presented via Alvarino *et al.,* (2016), who investigated ^{14}C-SMX degradation within sludges at varying redox potentials. The authors concluded under anaerobic conditions microbial mineralization and adsorption were greater than that of aerobic nitrifying conditions, the respective $K_{bio\text{-}l}$ and K_d values were 0.08L/gvss d and 40l/kg under anaerobic conditions and 0.01L/gvss d and 7L/kg under aerobic conditions. Although a difference was observed in the K_d value of aerobic-anaerobic sludges, the authors concluded that adsorption was negligible for SMX (Alvarino *et al.,* 2016).

It is evident from the dataset and the available literature that redox potential has a compound-specific effect on contaminant fate within the environment. For example, de Souza Santos *et al.,* (2014) found anaerobic activated sludge biomass to promote the degradation of norfloxacin over that of aerobic due to mineralization rates. To further this point, DDT is known to degrade faster under anaerobic conditions whereas kepone and permethrin degrade faster under aerobic/reduced conditions (Gambrell and Patrick 1988). Given what we know regarding degradation processes of

the assessed analytes we consider it sensible to speculate that increases in degradation rates are attributed to the greater mineralization efficiency of methanogenic bacteria. We suggest that reduced conditions have greater microbial diversity (aerobic and anaerobic) but overall reduced microbial abundance of specific degraders, thus resulting in poor removal rates for SMX, CFT, and FLO.

IMPLICATION OF SCIENTIFIC FINDINGS

The results clearly demonstrate that redox potential has a significant effect on the degradation rates of veterinary medicines. A compound-specific effect was observed, suggesting that aeration of slurries for odor and nutrient control need to be considered in regards to pharmaceutical fate. Moreover, it is clear from the dataset that there were differences in the degradation rates of FLO across a range of redox potentials that are deemed acceptable under the current manure degradation guidance document (CVMP, 2011) (-100 – 400 mV). Thereof presenting the requirement for more stringent redox control during manure degradation assessments. Moreover, OECD 307/308 states anaerobic conditions are achieved at redox potentials of <-100mV, which highlights the implications of greater variability. The consequence of utilizing such a wide range of redox potentials within manure degradation assessments means that such laboratory assessments may inadequately predict the concentrations applied to land.

Differences in degradation rates of FLO and SMX under varying anaerobic redox potentials result in differences in the risk assessment of these compounds (Table.4). The environmental risk assessment for veterinary medicines utilizes a Risk Quotation (RQ) approach (RQ=PEC/PNEC) with an RQ > 1 suggesting there is an environmental risk. The differences observed between anaerobic redox potentials and subsequent degradation rates result in a range of refined PEC values

and thus the outcome of the risk assessment. For example, under extremely anaerobic conditions the RQ for both antibiotics is <1, indicating no environmental risk, however, under both anaerobic and reduced conditions the risk assessment the RQ was > 1 and thus suggested a risk.

Within the reduced treatment the SMX $PEC_{refined}$ surface water was 17 times that of very anaerobic, the calculated $PEC_{refined}$ were 2.83 µg/L and 49.58 µg/L respectively. As a result of the inhibited degradation and elevated PECs the calculated RQ was ≥1 for *Chlorella vulgaris* and *Danio rerio* under both the reduced and anaerobic scenarios. Similarly, under reduced conditions, the refined PEC_{soil} was 16 times that of the very anaerobic treatment; despite this difference no risk was identified as SMX has been shown not to be toxic to terrestrial plants (Liu *et al.,* 2009). Comparatively, FLO is extremely toxic to terrestrial plants even at low concentrations (Richter *et al.,* 2016; and Simon *et al.,* 2015) and moderately toxic in the aquatic environment (Farrelly, 1999 and, Gray, 2007). Based on the calculated refined PEC_{soil} for both the reduced and anaerobic conditions (0.06124 - 0.00702 mg/kg) this would indicate risks towards both *Lepidium Sativum* and *Brassica Oleracea var. Capitata* (RQ>1) that would have been missed following assessments under very aerobic conditions.

Due to seasonal variations within redox potentials and pig slurries, it is a possibility that degradation rates would differ between the summer and winter months (Park *et al.,* 2006). For example, reduced methanogenic activity (i.e. elevated redox potential) during the winter months would inhibit FLO and SMX degradation; ultimately this would result in a greater environmental exposure and risk. Furthermore, the winter manure application timing is arguably the most important, increased volume of manures are often reported due to the closed application period (October-January), increased housing, as well as increase precipitation for open lagoons, for example storage overflow contributed towards 24% of manure spills within Iowa (1992-2002)

(Burkholder *et al.,* 2007; and Armstrong *et al.,* 2010). This phenomenon could result in excess environmental exposure during this application event (DEFRA, 2010). This is of course a concern regarding the reduced microbial activity within soils during these months, which would result in greater persistence and environmental concern (Srinivasan and Sarmah, 2014; and Bansal 2014).

Table 4: *Environmental risk assessment risk quotations using refined PECs that were generated using manure degradation data for SMX and FLO within pig slurries.*

Veterinary Medicine	Ecosystem	Redox Potential (mV)	$PEC_{Soil\ Refined}$ (mg/kg), $PEC_{Surface\ water\ refined}$ (mg/l)	Ecotoxicological data	Target Species and Endpoint	Effect	PNEC	RQ -100 mV	RQ -250 mV	RQ -400 mV	Reference
SMX	Terrestrial	-100	0.1642 mg/kg	38mg/l Rice	Rice EC50	Growth	3.8	0.0432	0.0323	0.00266	Liu *et al.,* (2009)
		-250	0.12382 mg/kg	30mg/l Oat	Oat EC50	Growth	3	0.0547	0.041	0.0034	Liu *et al.,* (2009)
		-400	0.01012 mg/kg	>300mg/l Cucumber	Cucumber EC50	Growth	30	0.00547	0.0041	0.00033	Liu *et al.,* (2009)
	Aquatic	-100	0.04958 mg/L	1.51 mg/L Green algae	Chlorella vulgaris EC50	Growth inhibition	0.0151	**3.28**	**2.33**	0.19	Borecka *et al.,* (2016)
		-250	0.03513 mg/L	75 mg/L Crustacean	Daphnia Magna EC50	Acute immobilisation test OECD 202	0.075	0.66	0.47	0.04	NOTOX (1996)
		-400	0.00283 mg/L	8 mg/L Zebrafish	Danio rerio EC50	Fish embryo toxicity test	0.008	**6.20**	**4.39**	0.35	Ferrari *et al.,* (2004)
FLO	Terrestrial	-100	0.06124 mg/kg	0.5mg/kg Cress	Lepidium sativum EC50	weight	0.0055	**11.13**	**1.28**	0.00	Farrelly, 1999
		-250	0.00702 mg/kg	6.7mg/kg Wheat	Triticum aestivum EC50	weight	0.067	0.91	0.10	0.00	Farrelly, 1999
		-400	2.76E-65 mg/kg	0.859mg/kg Cabbage	Brassica oleracea var. capitata EC50	weight	0.009	**6.80**	**0.78**	0.00	Gray, 2007
	Aquatic	-100	0.01734 mg/L	1mg/L Microlagae	Pseudokirchneriella subcapitata EC50	weight	0.1	0.1734	0	0	Hoberg, 1991
		-250	0 mg/L	>330mg/L Crustacian	Daphnia Magna EC50	weight	3.3	0.0052	0	0	LeLievre, 1991
		-400	0 mg/L	>780mg/L Rainbow trout	Oncorhynchus mykiss LC50	weight	7.8	0.0022	0	0	LeLievre, 1991

Footnote: *Bold = RQ > 1. An assessment factor of ten was applied to the EC_{50} data to obtain a PNEC as per the regulatory guidance.*

CONCLUSION

The presented study demonstrates a cost-effective means of controlling redox potentials within laboratory scale experiments. Improved accessibility for redox control could result in further research and understanding regarding the influence of redox potentials on contaminant fate. The derived data regarding the fate of veterinary medicines under anaerobic redox potentials was significant and compound specific. Given what we now know regarding anaerobic redox potentials and veterinary medicine fate it is clear that, in order to have uniform assessments, tighter redox potential control is required. Under the currently available manure degradation guidance and OECD documents the acceptable range of anaerobic conditions is230mV to -400mV and >-100mV respectively, here we demonstrate such a range can drive differences within the outlined risks towards aquatic and terrestrial organisms. Therefore, it is critical to harmonise manure degradation protocols at an EU or OECD level, during the development of such guidance we would suggest the usage of a range of slurries and redox potentials. Not doing so will continue to contribute to inaccurate predictions of environmental exposure. Furthermore, until now the influence of aerating pig slurries on the degradation of a broad range of veterinary medicines was unknown. This work highlights that such manure processing techniques could in fact reduce the degradation of veterinary medicines which would in return increase the exposure of the environment.

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ABBREVIATIONS.

Liquid Chromatography Mass Spectrometry (LC-MS), below detectable limits (bdl), organic matter (OM), organic carbon (OC), acetylsalicylic acid (ASA), ceftiofur (CFT), florfenicol (FLO), tylosin (TYL), sulfamethoxazole (SMX) and oxytetracycline (OTC).

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