Advanced electrochemical oxidation of antibiotics in dairy slurry

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ABSTRACT

PURPOSE

To curtail the spread of antimicrobial resistance (AMR), considered one of the most pressing global threats to human health in the 21st century, we need to reduce the concentrations of antibiotics in environmental compartments. In this context, the management of slurries is particularly relevant due to the widespread use of antibiotics in livestock. There is a need for a cost-effective and sustainable technology that can degrade antibiotics from slurries and biosolids to eliminate or reduce their concentration considerably to allow a safe application into soil and minimise environmental impacts.

METHODS

We tested the bioelectrodialytic degradation of 14 antibiotics in the dairy slurry at constant current (20 mA) in a two-compartment electrodialytic cell. Solid-phase extraction (SPE) was used for clean-up and pre-concentration of the samples. The identification and quantification of antibiotics were performed in an Agilent 1100 coupled to an Applied Biosystems Q-Trap 5500 tandem mass spectrometer.

RESULTS

The bioelectrodialytic process had a COD removal of 85%. Antibiotic removals superior to 90% were obtained for cloxacillin (CLOX), cephalexin (CLX), cefquinome (CQM), ceftiofur (CFT) and novobiocin (NOV).

CONCLUSIONS

The results show potential to use electrochemical oxidation in slurry treatment to further remove antibiotics before application in soils and to replace sources of critical raw materials such as phosphate.

Keywords: antimicrobial resistance, slurry management, electrodialytic remediation, dairy farming waste

1. Introduction

Antimicrobial resistance (AMR) is a major global problem, due to the current and potential impacts on population health and costs to health care systems by reducing medical treatment options [1]. Wastewater treatment plant effluents, sludge and manure are key sources of antibiotics for environment compartments (soil, sediment, surface and groundwater) [2]. Veterinary antibiotics are extensively used to treat, protect and promote the growth of livestock, and are responsible for select and enrich antibiotic resistance [3,4]. Worldwide consumption of antibiotics in food animal production was estimated at 63,151 (±1,560) tons in 2010 and is projected to rise by 67%, to 105,596 (±3,605) tons, by 2030 [5]. On dairy farms, antibiotics are mainly used for treatment and prevention of mastitis (inflammation of the mammary gland and udder tissue) [6]. Antibiotics end up being excreted by the animals, and high concentrations of sulfonamides and tetracyclines (91 to 136 $mg\,kg^{-1}$ of dry matter) were found in manure [2]. Current waste management practices in farming include the application of liquid manure on agricultural soils as organic fertiliser. Predicted half-lives of antibiotics in soil can be 3.69 years [7], leading to soil accumulation with the average twice a year application of biosolids to soil. In this framework, veterinary medicinal products and antibiotics, in particular, are growing source of concern when processed manure or slurry is reused as fertiliser [8]. The recently published European Union Strategic Approach to Pharmaceuticals in the Environment [COM(2019) 128 final], highlights the need for "*cost-effective methods for reducing concentrations of pharmaceuticals including antimicrobials in slurry, manure and sewage sludge to enable their use in the circular economy"* [9].

Biological treatment processes such as composting manure and slurries can help reduce the antibiotics loads to the environment, but there are still knowledge gaps on the mechanisms for antibiotics and antibiotic resistance genes removal, as well as their interactions [2]. Advanced anaerobic digestion mostly remove antibiotics in the liquid fraction, and it was observed that tetracycline residues adsorb onto the solid fraction of manure [3]. Advanced oxidation processes based on electrochemical oxidation are gaining prevalence in the treatment of emerging pollutants, such as pharmaceuticals [10-13], but the majority of the studies are performed with deionised water instead of complex matrices such as slurry. Electrodialytic remediation was tested for emerging organic contaminants (caffeine, bisphenol A, estradiol, ethinyl estradiol and oxybenzone) in wastewaters [14], showing removals between 57–72%, largely through electrodegradation processes. Degradation of tetracyclines (oxytetracycline, chlortetracycline, and tetracycline) in soil by electrokinetic remediation showed antibiotic removals of 22 to 84% after 7 days [15]. To the best of our knowledge, electrodialytic remediation of antibiotics in slurry was never tested.

This work aimed to test the bioelectrodialytic degradation of a mix of 14 antibiotics typically used in dairy farming in real slurry samples. Simultaneously, we aim to differentiate the degradation mechanisms responsible for their degradation, as biological or electrochemical.

2. Materials and Methods

2.1. Standards and chemicals

Reference standards of penicillin (PEN), amoxicillin (AMX), ampicillin (AMP), cloxacillin (CLOX), cephalexin (CLX), cefoperazone (CPZ), cefquinome (CQM), ceftiofur (CFT), oxytetracycline (OTC), novobiocin (NOV), tylosin (TYL), lincomycin (LCM), sulfadiazine (SDZ), and trimethoprim (TMP) were obtained from Sigma Aldrich. High-performance liquid chromatography (HPLC)-grade methanol, acetonitrile, Milli-Q water, formic acid (99%), citric acid (99.5%), ethylenediaminetetraacetic acid disodium salt dehydrate (Na_2EDTA, 99%), sodium hydroxide (NaOH, 99%), sodium nitrate were purchased from Fisher Scientific (Leicestershire, UK). Ammonium acetate buffer (0.05 M) was prepared in water and acidified at pH 5 using hydrochloric acid (37%).

2.2. Sample collection and storage

Dairy slurry samples were collected from the 3000 m^3 slurry tank of a high input/high output dairy farm at the Centre for Dairy Science Innovation (CDSI), University of Nottingham Sutton Bonington campus. The CDSI is a £6M state-of-the-art dairy centre with 360 high-yielding Holstein cows, which operates commercially and offers the latest research technologies for studying a range of dairy-related topics, including wastewater treatment. The slurry tank collects and stores cattle manure, discarded milk from cows with mastitis containing antibiotics, and antimicrobial cattle foot wash. The samples were taken using autoclaved 2.5 L amber glass bottles, stored at 4ºC and immediately processed within transport time to the lab. To minimise variability in the feedstock, the slurry was frozen, and all experiments were performed with the same sample. Slurry for the biological control experiment was autoclaved at 124◦C and 2 bar (Astell SVS935D).

2.3. Sample extraction

Two ml of liquid slurry were placed into 50 mL centrifuge tubes with 10 mL of extraction solution (acetonitrile/EDTA/ammonium acetate 0.05 M pH5, 25:25:50, v/v). The samples were vortexed for 30 seconds and subsequently placed into an ultrasonic bath for 15 min and centrifuged at 4000 rpm for 15 min, at 4◦C. The supernatant was collected and diluted to 100 mL aliquots (solvent content <5%) with Milli-Q water before solid-phase extraction (SPE) using Oasis HLB (500 mg, 6cc, Waters Limited, Herts, UK) and SAX cartridges used (Agilent Technologies, Cheadle, UK).

The SPE cartridges (SAX: HLB) were conditioned with 10 mL of methanol, 5 mL of deionised water at pH 5, and 5 mL of 0.05M ammonium acetate buffer. The samples were passed through both cartridges at a flow of approximately 2 mL min^{-1}, then SAX cartridges were removed before washing with 5 mL of Milli-Q water, and, finally, HLB cartridges were dried under vacuum for 30 min. Elution was carried out from the HLB cartridges with 10 mL of methanol 0.1% formic acid. Samples were then evaporated to dryness under vacuum at room temperature using a Genevac EZ-2 series. Reconstitution was performed with 1 mL of acetonitrile:water 25:75 for HPLC LC-MS analysis.

2.4. Liquid chromatography-mass spectrometry

Antibiotic analyses were performed by high-performance liquid chromatography (HPLC) conducted with an Agilent 1100 HPLC coupled to an Applied Biosystems Q-Trap 5500 tandem mass spectrometer. The mobile phases consist of water with 1% ammonium acetate and 0.2% formic acid at pH 3 (A) and acetonitrile (B). The HPLC gradient run increased from 5% to 95% B in 5 min, then it was held constant for 3 min. Finally, the mobile phase was brought back to initial conditions in 1 min and held again for 3.5 min at a flow rate of 250 µl min^{-1} to the total run time that was 12.5 min including re-equilibration.The LC-MS/MS instrument was operated in positive ion mode (ESI+) for all analytes using the method of Baena Nogueras et al. [16].

2.5 Water quality analysis

Conductivity and pH were measured in samples using a Hach HQ40D meter. Water quality parameters such as Total suspended solids (TSS) and chemical oxygen demand (COD) were determined by using a Hach Spectrophotometer DR2800 (Hach Lange Ltd).

2.6 Electrodialytic experiments

The experiments were performed in triplicate, in the absence of light, using a triple output multi-range DC power supply (AIM & TTi) at constant current (20 mA), and a two-compartment electrodialytic cell. The total volume of the reactor was 120 mL. An anion exchange membrane (AMI-7001 S; Membranes International, Inc.) separated the two compartments. The slurry was placed in the anode compartment, with constant stirring at 100 rpm, after being spiked with the mixture of 14 antibiotics in a methanol solution at a concentration of 75 ng mL^{-1}. The catholyte was a solution 0.01 M $NaNO_3$. The electrodes used were Permascand type PSC101, with 0.5 cm width. Samples for antibiotic analysis and degradation kinetics were collected at 1, 2, 4.5, 8, 24, 30 and 48 h. Control experiments were performed with autoclaved slurry and without applying direct current to differentiate the biological and electrochemical degradation of antibiotics. Voltage drop between working electrodes was measured during the experiments with direct current.

2.6 Statistical analysis

Statistical analysis was carried out in RStudio [17] using “RVAideMemoire” package [18] to perform ANOVA and Tukey test.

3. Results and Discussion

The dairy slurry used in the experiments was collected weekly in the 3000 m^3 slurry tank of a high input/high output dairy farm at the Centre for Dairy Science Innovation (CDSI), University of Nottingham (Table 1). This feedstock shows high variability depending on the management practices and the climacteric conditions.

Table 1. Characterization of the dairy slurry (n = 10).

Determinant	Unit	Value (Average ± Standard deviation)
pH		7.67±0.17
Conductivity	µS/cm	12.70±1.36
Dissolved oxygen (DO)	mg L^{-1}	0.15±0.02
Chemical Oxygen Demand (COD)	mg L^{-1}	36552±11694
Total carbon (TC)	mg L^{-1}	7440±3820
Total Organic Carbon (TOC)	mg L^{-1}	11750±3456
Total Inorganic Carbon (TIC)	mg L^{-1}	2293±1000
Nitrate	mg L^{-1}	137.2±50
Nitrite	mg L^{-1}	16.5±4.7
Ammonium	mg L^{-1}	1057±255
Total-N	mg L^{-1}	2059±706
Phosphate	mg L^{-1}	467±215
Zinc	mg L^{-1}	26.8±9.8
Copper	mg L^{-1}	1057±39
Sulphate	mg L^{-1}	7022±2306
Total Suspended Solids (TSS)	mg L^{-1}	33343±14507

Higher removals of COD, total suspended solids (TSS) and antibiotics were obtained in the experiment with low level direct current and biological activity. Chemical oxygen demand removal was 85% in the bioelectrodialytic setup, 66% in the control experiment with autoclaved slurry, and 15% in the control without electric current. Similarly, for TSS, removals were 48% in the bioelectrodialytic setup, 28% in the control experiment with autoclaved slurry, and 35% in the control without electric current.

Recovery of antibiotics ranged between 76 and 118%, except penicillins (between 10 and 20%). Removal of antibiotics in slurry was mainly dominated by advanced oxidation processes (Figure 1), with similar removals for the electrodialytic setup and the control with the autoclaved slurry. Antibiotic removals superior to 90% were obtained for cloxacillin (CLOX), cephalexin (CLX), cefquinome (CQM), ceftiofur (CFT) and novobiocin (NOV). The highest differences between the two experimental conditions were

observed for cefoperazone (CPZ) with higher removals on the autoclaved slurry. On the other hand, both tylosin (TYL) and trimethoprim (TMP) showed greater removals in the electrodialytic setup. The statistical analysis showed that only TYL and SDZ were significantly different in the bioelectrodialytic setup and the autoclaved slurry ($p < 0.005$). The experiment without low level direct current, with only the biological activity of the microorganisms present in the slurry, showed lower removal percentages (<50%).

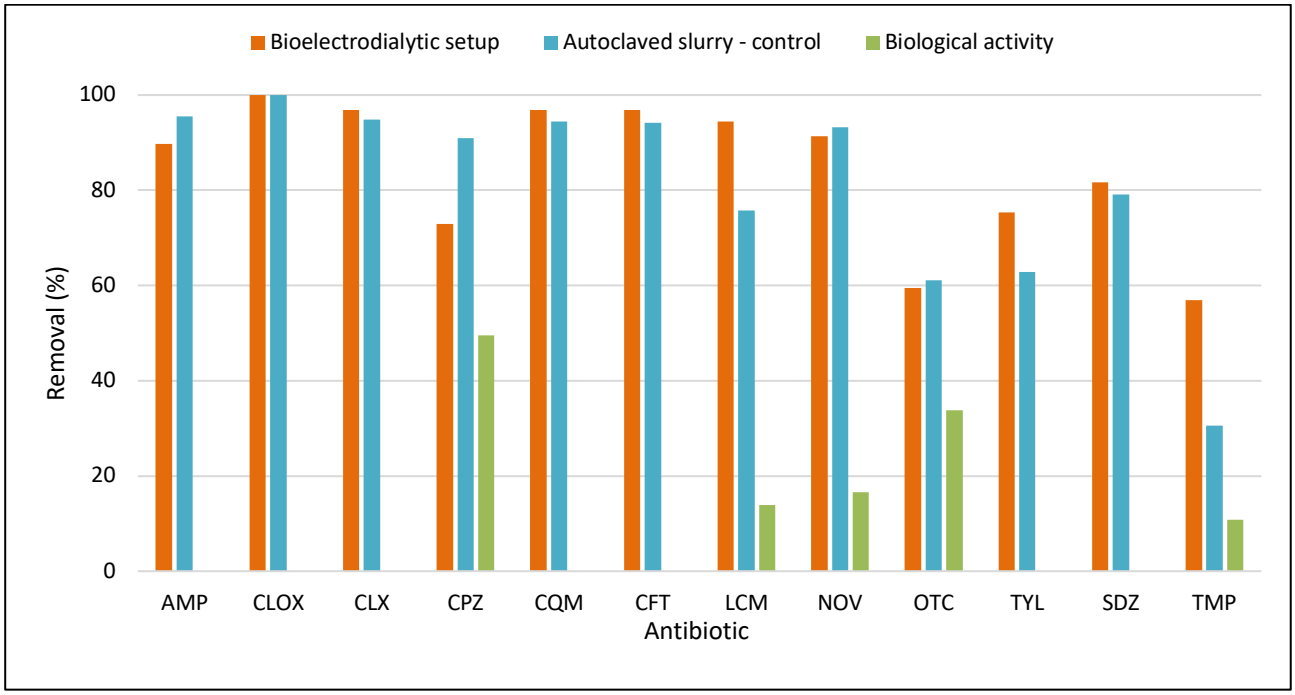

Figure 1. Comparison between percentage of antibiotic removal in the bioelectrodialytic setup and the control experiments with autoclaved slurry and biological activity.

4. Conclusion

The results show that electrodialytic treatment of dairy slurry can sucessfly remove antibiotics before application to soil. Antibiotics with higher removals are cloxacillin (CLOX), cephalexin (CLX), cefquinome (CQM), ceftiofur (CFT), novobiocin (NOV), and lincomycin (LCM). The degradation processes were dominated by advanced oxidation, with the biological activity removing less than 50% of the initial concentration of the antibiotic. Further research is needed to investigate if antimicrobial genes are also destoyed by the treatment to ensure minimal spread of antimicrobial resistance when using slurry as a fertiliser in agricultural soils.

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