



Understanding risks and optimising anaerobic digestion to minimise pathogen and antimicrobial resistance genes entering the environment

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Anaerobic digestion (AD) utilises organic materials to produce energy via biogas while also producing nutrient-rich digestate ideal for land application as fertiliser. However, there may be a risk to human (and livestock) health through transmission of pathogens to land and uptake into the food chain, direct environmental contact or run-off into water courses¹. This is compounded by concerns over antibiotic-resistant bacteria (ARBs) entering the environment². Resistance genes (ARGs) can be transferred widely within the bioreactor and soil microbiome, including to and from pathogens. Pathogens of concern include Clostridia, which can proliferate during digestion and resist pasteurisation³. The key challenge is to optimise AD processes to minimise risks associated with application of AD to land. Lack of data has precluded evaluation of risk from pathogens entering the environment through AD and information on manipulating AD to reduce pathogens and resistance genes without compromising gas generation are scarce. Furthermore, there is little clarity on how feedstocks might be ranked in terms of their pathogen or ARG content, nor how that feeds through into the fate of pathogens/ARGs once applied to land.

This understanding is critical because process optimisation and post-digestion sanitisation that do not require the energy inputs of high temperature pasteurisation (which is also not practical in some circumstances e.g. small-scale systems, developing countries) could make energy generation through AD more sustainable and more widely viable.

The project aims to:

- 1) evaluate pathogen/ARG content of common AD feedstocks
- 2) understand the role of feedstock type and process conditions on pathogen/ARG persistence
- 3) compare persistence of pathogens/ARG in traditional organic waste-amended soils vs. digestate-amended soils.

The aims will be addressed through a series of experimental and literature-based steps:

1. Pathogen/ARG content of feedstocks

The student will undertake a literature review including a synthesis of data on pathogen/ARG presence and concentrations in different feedstocks for AD. This will be supplemented with experimental work in which the student will sample and enumerate a suite of pathogens/ARGs in feedstocks from a range of digesters with which the supervisors have existing links.

2. Role of feedstock and process conditions on pathogen/ARG persistence during AD

The student will establish laboratory scale digesters through which to undertake experiments to manipulate process conditions (e.g. loading rates/retention time, water content, temperature) within industry-relevant operating envelopes as identified during the literature review and through discussion with site operators during (1) above. These will exploit the opportunity to control feedstocks and conditions carefully to define optimal envelopes of operation for both gas production and purity, pathogen/ARG reduction and production of an

agriculturally useful digestate. This will be supplemented by sampling digestate corresponding to feedstock samples in (1) above, with process conditions recorded.

3. Pathogen/ARG persistence in amended soils

The student will establish field experiments in which digestate will be applied to experimental grass/crop plots in a factorial experiment also incorporating livestock manure/slurry and an inorganic fertiliser as treatments, alongside untreated control plots. Plots will be sampled over the period of a year and pathogens/ARGs, soil nutrients/physico-chemical characteristics and soil microbiome analyses will be undertaken.

Throughout, PCR/multiplex PCR and culture will be used for sample screening for pathogens/ARGs followed by q-PCR for quantification.

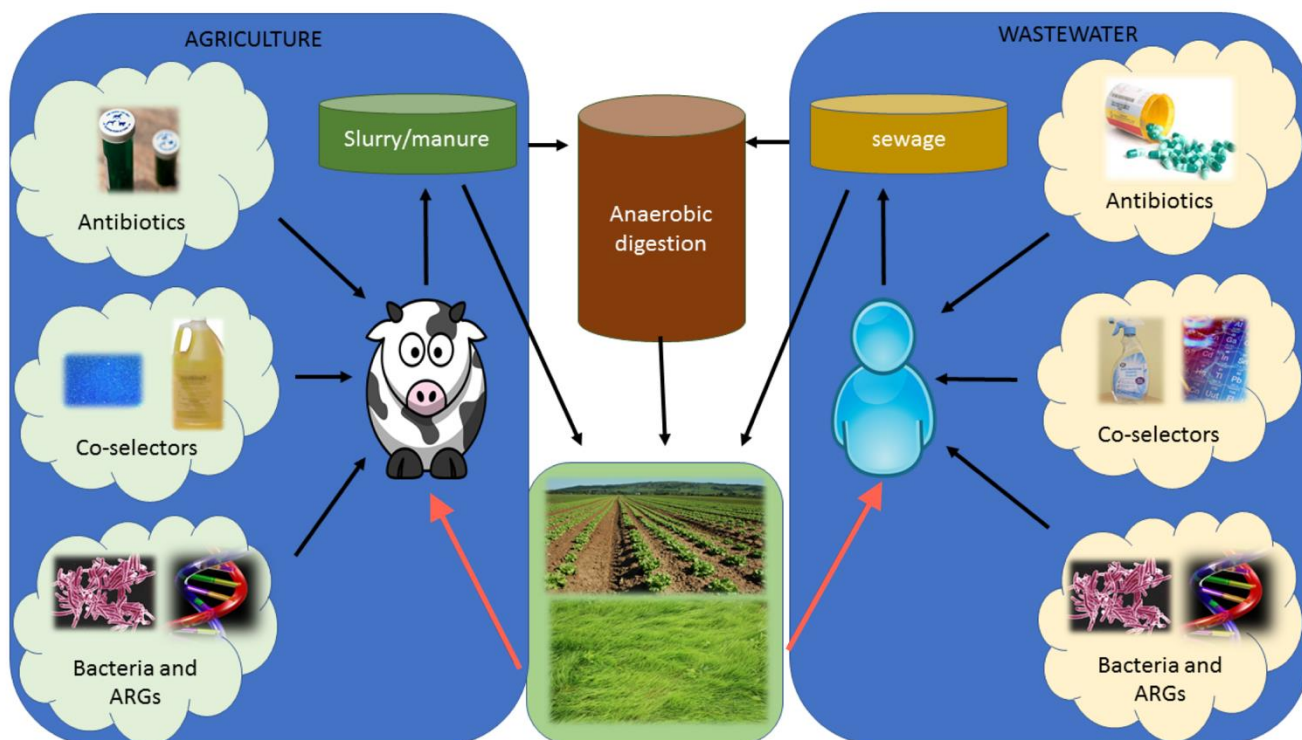


Figure 1. Drivers of AMR entering the environment via organic amendments to land, with and without AD treatment.

Training opportunities:

The proposal provides structural guidance and relevant support to ensure students can succeed and generate novel data to present at institutional, national and international level conferences and high-quality journal papers. The student will have access to expertise on environmental engineering (Surrey), facilities to establish mesocosm scale anaerobic digesters (Hutton) access to a state-of-the-art microbiology laboratory with expertise in the pathogen-related microbiology of AD (Avery) and to expertise in environmental risk assessment (Hough). The supervisory team has existing links with AD operators at different scales and utilising different feedstocks, therefore access to sampling locations is readily available.

The University of Surrey's Doctoral College supports the academic and professional development of postgraduate researchers. The Doctoral College will facilitate excellent supervision, deliver training, and enhance postgraduate researchers' professional skills. The University of Surrey offers training and support via the Researcher Development Programme (RDP), providing development opportunities for postgraduate research students across the University. The RDP offers workshops, tailored events, one-to-one coaching and other personal development opportunities to all the postgraduate researchers.

Students at the James Hutton Institute have access to statistics courses through BiOSS and are part of a lively and vibrant graduate school which hosts an annual symposium and a number of other events and courses. The

student will have the opportunity to learn molecular microbiology, field sampling skills, environmental microbiology and risk assessment skills.

The James Hutton Institute has strong links with Scottish Water (e.g. Deerdykes AD) farm scale AD operators (e.g. Gask Farm, Turriff) and our intention is to arrange for the student to spend time with these organisations as part of their training. Conversations have been initiated with potential partners to this effect.

Student profile:

Candidates will ideally have a Masters' Degree in biological sciences, environmental science, environmental engineering or a related topic or a good (2.1 or 1st class) honours degree in the above subject areas. They will show an aptitude for understanding process control and working in a precise manner. They should be a self-starter with the confidence to interact with a wide range of contacts from academic to laboratory staff and industrial contacts and they must demonstrate enthusiasm and willingness to learn. Experience in microbiology, molecular biology or environmental engineering would be advantageous.

References: (optional)

¹Avery et al. (2014) Biomass Bioenergy 70, 112-24.

²Xu et al. (2019) Bioresour. Technol. 282, 179-88.

³Pulvirenti et al. (2015) Biomass Bioenergy 81, 479-82.

<https://research.reading.ac.uk/scenario/>