



Scenario
DOCTORAL TRAINING PARTNERSHIP

NERC
SCIENCE OF THE
ENVIRONMENT

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

Lead Supervisor: Devendra Saroj, University of Surrey, Centre for Environmental and Health Engineering

Email: d.saroj@surrey.ac.uk

Co-supervisors: Lisa M Avery, James Hutton Institute Aberdeen; Rupert L Hough, James Hutton Institute, Aberdeen

Material that originates from the human or animal gut can contain pathogens and other organisms, any of which may be carrying genes that make them resistant to antibiotics. If we apply those to land, and allow them to enter the environment, they can remain in the soil or be washed into water bodies which may be used for irrigation of food crops, water supplies, recreation or shellfish production. From here, humans and animals can potentially consume pathogens/resistant organisms or come into direct contact with them in the environment. Given that earlier this year, the UK's outgoing chief medical officer said that "we are in an arms race against microbes" and that if no action is taken on antimicrobial resistance, 10 million people worldwide could die each year by 2050; we ought to understand exactly what is in the organic amendments we apply to land.

Anaerobic digestion (AD) utilises organic materials to produce energy *via* biogas while also producing nutrient-rich digestate ideal for application to land as a fertiliser. However, there may be a risk to human (and livestock) health if pathogens originating in the feedstock are transferred to land and potentially taken up into the food chain. This is compounded by concerns over antibiotic-resistant bacteria (ARBs) entering the environment. Resistance genes (ARGs) can be transferred widely within the soil microbiome, including to and from pathogens, and we do not know their fate during and post-anaerobic digestion. Organisms of particular concern include Clostridia, which, being anaerobes, can proliferate under the digester conditions and isn't always removed by pasteurisation. With little data on pathogen and ARB/ARG prevalence in feedstocks, persistence/proliferation through the AD process, we cannot determine risks associated with application of AD to land or how it compares to traditional organic amendments.

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 begin the PhD with a literature review capturing all relevant aspects of the project and this will include 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

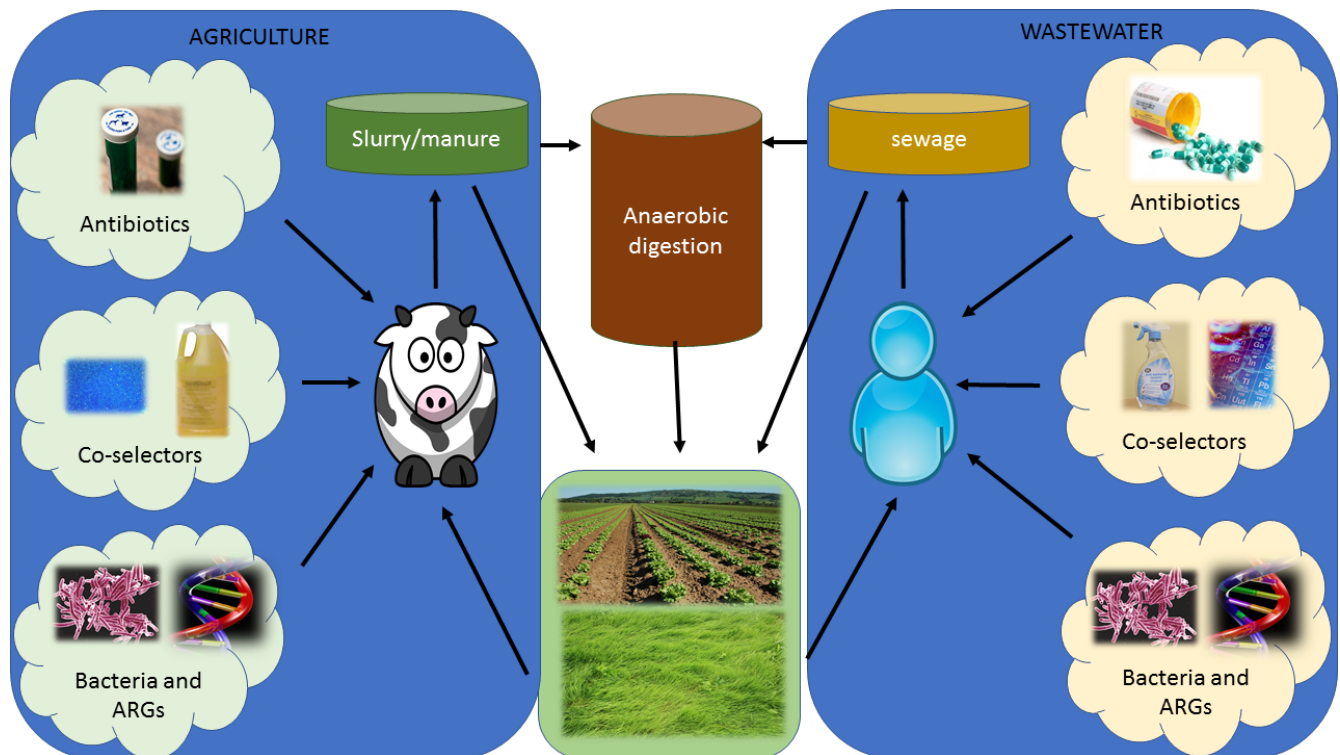
The student will establish laboratory scale digesters through which to undertake experiments to manipulate process conditions (e.g. loading rates/retention time, water content, temperature) within industry-relevant operating envelopes as identified during the literature review and through discussion 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, molecular biology techniques including PCR/multiplex PCR and culture will be used for sample screening for pathogens/ARGs followed by q-PCR and possibly a high-throughput q-PCR chip will be used for quantification.



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

Training opportunities:

The student will have the opportunity to develop links with anaerobic digestion operators, sampling feedstock and digestate and understanding how operators control the process. We hope to facilitate a placement for the student with one of the AD operators with whom the supervisory team have established links. This is in addition to the opportunities provided by the University of Surrey's Doctoral College which 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 (<https://www.bioss.ac.uk/>) 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 as well as developing a strength in environmental engineering and process control.

Student profile:

This project would be suitable for students with an honours degree (1st class or 2.1) or a Masters degree in biological sciences, environmental science, environmental engineering or a related topic.

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.