



ENVIRONMENTAL BIOTECHNOLOGY NETWORK

Anaerobic Fermentation WG



Anaerobic Fermentation (AF WG)



Led by <u>Dr Yue Zhang</u>, University of Southampton and <u>Dr Luca Alibardi</u>, Cranfield University with a core team of <u>Prof Raffaella Villa</u> (De Montfort), Dr <u>Jaime</u> <u>Massanet-Nicolau</u> (South Wales) and Dr <u>Özge Eyice-Broadbent</u> (Queen Mary University of London).

Mixed culture anaerobic fermentation (AF) is a relatively new area compared to anaerobic digestion (AD), although they are linked in terms of process and biochemistry. Recovery of organic products including fatty acids is the core task for AF, which also offers potential for plant nutrient recovery.

One of the key goals to unlock the potential of AF for the circular bioeconomy is direct product recovery from fermentation broth. This is important for steady-state continuous operation, in contrast to over-explored batch experiments. It is also an example of the interactions between a biological system and its engineering envelope, a key area of interest for EBNet. Product-induced feedback inhibition can be explored to produce longer chain acids which are more valuable and easier to recover. Feedstock characteristics also affect the product composition, and the topic has strong links to our sister NIBB <u>BBNet</u>. AF thus has upstream, central and downstream aspects: for it to be adopted, all these components must work together.



This WG therefore aims to promote the integration of the different elements within the anaerobic fermentation process and system in order to maximise resource recovery and at the same time minimise the use of external inputs and the generation of waste materials.

Anaerobic fermentations are an essential aspect of many successful Environmental Biotechnologies, and are a recurring topic at EBNet's annual ECR conference, with 30 presentations on topics such as resource recovery in products like H₂ or Volatile Fatty Acids (VFAs).





ACTIVITY SYNOPSIS

Industry and other interactions abound, including site meetings with companies interested in applications of AF, and a webinar on <u>Mixed-Culture Anaerobic Fermentation in the EU</u>: <u>Updates</u> <u>from HORIZON 2020 projects on the biowaste-derived volatile fatty acid platform</u> in 2025.

The group has carried out desk-based feasibility analysis and comparison of proposed approaches, supported by small-scale testing, to gather data needed to support technoeconomic assessments (TEA). It has also supported joint work on the implications of emerging biotechnology processes for materials and corrosion with EBNet's <u>Bioelectrochemical Systems</u> <u>WG</u>. For further information on outputs from these activities see <u>Resources</u>.

EBNet has funded one Business Interaction Voucher (BIV) project in this area, on <u>Sludge</u> <u>dewaterability: improved tools for the emerging biotech industries</u>, with a joint journal paper in preparation. It also provided an ECR <u>travel bursary</u> to for a <u>presentation</u> at the IWA Congress 2024 in Toronto.

Outreach activities include the production of a short <u>animation</u> explaining the concept of AF for the public and non-specialists.



In collaboration with three other WGs the group co-hosted the *New Biomethane* workshop. In January 2025 the WG organised an *Anaerobic Fermentation* workshop, supported jointly by EBNet and the Biomass Biorefinery Network (<u>BBNet</u>) to gather top UK and international experts together to discuss the state-of-the-art in AF. Reports from these events are available under <u>Resources</u>. The WG members have also applied for a dedicated follow-on BBSRC Network on AF.



WG Publication: Bourgade, B. and Islam, M.A., 2024. <u>Progresses and challenges of engineering thermophilic</u> <u>acetogenic cell factories</u>. Frontiers in Microbiology, 15, p.1476253.





PROJECT PARTNERS: Dr Yue Zhang, University of Southampton & Triton Electronics Ltd BIV202102



Sludge dewaterability: Improved tools for the emerging biotech industries

"Very pleased to have gained some experience with our new CST device. Further, to have looked at dewaterability tests beyond the main market of sewage sludge dewatering. We are very keen to build on this project and have many ideas about how to exploit the excellent early results". - Julian Tapp, Triton Electronics Ltd

THE PROJECT

Processing of wastewater biosolids is a major issue for the water industry, typically accounting for half the cost of wastewater treatment. This is due to both the volume and the nature of the material: sludges are complex biological substances capable of holding large amounts of water via different mechanisms.

The capillary suction time (CST) is one key parameter used to assess sludge dewaterability. Separation of liquids and concentration of the solid fraction can have very significant benefits both on transport costs and impacts, and on the organic loading rates that can be applied to digestion plant and infrastructure.

Other bioprocesses undergoing rapid development, such as anaerobic digestion for the circular bioeconomy, offer new opportunities and challenges for dewaterability analysis. This is due to the introduction of new feedstocks such as food and agro-wastes, and of new treatments including low-temperature anaerobic processes and biorefinery fermentations.

In collaboration with Triton Electronics, the international market leader on CST apparatus for decades, this project analysed a range of samples from wastewater treatment works, commercial anaerobic digestion plants and laboratory-based bioreactors. Factors affecting CST test repeatability included alternative test apparatus models and parts, test conditions and physicochemical and biological parameters of samples. Its comparability with other dewaterability test methods was also investigated (mainly with respect to frozen image centrifugation and specific resistance to dewatering).





The results demonstrated that most commercial anaerobic digestate samples showed very different properties from the familiar waste activated sludge produced from biological treatment processes in wastewater treatment works, and different testing protocols need to be investigated for future real-world applications.

"It has been a great pleasure working with Triton on this project to explore the challenges and R&D needs for the dewaterability test methods on digestate and other emerging materials. The results obtained and the discussions carried out have formed a solid basis for future collaboration with Triton".



Digestate from wastewater biosolids (left) has a relatively low solids content. Other digestates from food and agro-wastes (right) have higher solids content and different rheological properties which make dewatering more challenging



TEAM LEAD Dr Yue Zhang Faculty of Engineering and Physical Sciences, University of Southampton, Southampton, SO17 1BJ, UK



AF WG Small-scale test-the-concept studies

AIMS

Mixed-culture fermentation is a powerful enabling industrial biotechnology for a circular bioeconomy especially for unavoidable and putrescible heterogeneous organic wastes.

Preliminary desk-based and small-scale test-theconcept laboratory studies on integrated anaerobic fermentation and product recovery were conducted between October 2024 and January 2025.

The laboratory study focused on the feasibility of chemical-free selective recovery of volatile fatty acids (VF) and ammonia from fermentation broth, and major operating factors affecting it.



The desk-based study investigated the integration options between the main fermentation process and downstream product recovery steps based on the laboratory results, and the potential of the selective recovery and its feedback loop to be used to manipulate microbial communities of the fermentation to enhance preferred metabolic output by redirecting metabolic fluxes.



RESULTS

Preliminary comparisons were conducted between the tested concept and available recovery options with estimation of the benefits of chemical avoidance. While more comprehensive techno-economic analysis and life cycle assessment is required to verify its potential advantages, the work has contributed valuable data for this purpose.



The outputs of the studies provide essential evidence for more comprehensive investigation in this research line, and will contribute to a journal publication and research applications on mixed-culture fermentation with integrated chemical-free product recovery.

The study findings were incorporated in a presentation given to the EBNet & BBNet joint Anaerobic Fermentation Workshop in January 2025. The EBNet AF WG has also organised a webinar on this theme of VFA recovery.



Implications of Emerging Biotechnologies for Bioreactor Materials



Report prepared by Dr Maria Ramos Suarez, University of Southampton

Supported by EBNet AF and BES Working Groups

The need for this study arose from a discussion early on in the Network between EBNet academic and industry members about the implications of emerging industrial and environmental biotechnologies for producers and users of industrial bioreactors.

This topic is relevant both to EBNet's Anaerobic Fermentation (AF) WG and to the Bioelectrochemical Systems (BES) WG because of the relevance of Microbially-Induced Corrosion (MIC) in this context. Further discussions led to a joint-funded PhD and an extensive desk-based study, carried out by postdoctoral researcher Maria Ramos Suarez in conjunction with experts from industry and the University of Southampton. The full report covers multiple scenarios and consists of 40 pages incorporating 217 references.

Report

The report examines major emerging bioproduction processes in industrial and environmental biotech, based on the top 10 bio-based products - as selected by market size and research importance. It looks both at the conditions likely to occur during production and their significance for different bioreactor materials. Finally, it also looks briefly at the ranges of conditions likely to be in use as a result of extremophile exploitation, and at the significance of MIC.

As few studies have performed immersion tests of metal and polymer specimens within a bioreactor environment, and the information on corrosion and abrasion mechanisms is still limited, it is timely to identify any areas of inadequacy in the light of such emerging processes & conditions. The IB sector is expanding rapidly, necessitating such advancements in bioreactor materials to withstand corrosion and wear.

The full report is currently embargoed whilst a journal paper is in preparation but, once the paper is published, the report will be made freely available on the EBNet website.

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Top 10 bio- based products	Biomethane
	Biohydrogen
	Bioethanol
	Biobutanol
	Lactic acid
	Volatile fatty Acids (VFA)
	Succinic acid
	Biosurfactants
	Polyhydroxyalkanoates (PHA)
	Enzymes

For further information on outputs from this activity see Resources. <u>https://ebnet.ac.uk/resources</u>









Anaerobic Fermentation Workshop

Event Summary

Joint EBNet / BBNet workshop on the Role of Anaerobic Fermentation in the Circular Bio-economy

Hosted by the Environmental Biotechnology Network and the Biomass Biorefinery Network

Birmingham 23-24 January 2025

Workshop participants

Luca Alibardi, Cranfield University Yadira Bajon-Fernandez, Cranfield University Gabriel Capson Tojo, INRA Narbonne, France Zeynep Cetecioglu, KTH, Sweden Des Devlin, Dwr Cymru Welsh Water Richard Dinsdale, University of South Wales Ozge Eyice-Broadbent, University of Birmingham Lindsey Gove, Anglian Water Adrian Higson, NNFCC Marie Kirby, Harper Adams University Sigrid Kusch, Ulm University of Applied Sciences, Germany

Also contributing

Paul Lavender, HaskoningDHV UK Ltd, a company of Royal HaskoningDHV Thomas Dietrich, Tecnalia, Spain Lily Lee, Imperial College University Piet Lens, IHE Delft, Netherlands Jaime Massanet-Nicolau, University of South Wales Deborah Rathbone, University of York Alba Serna-Maza, VORN Bioenergy, Spain Tom Taylor, Yorkshire Water Helen Theaker, Alps EcoSciences Pete Vale, Severn Trent Raffaella Villa, De Montfort University Yue Zhang, University of Southampton

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Joint EBNet / BBNet workshop on the Role of Anaerobic Fermentation in the Circular Bio-economy

Summary of event

With administrative and financial support from the Biomass Biorefinery Network (BBNet) and Environmental Biotechnology Network (EBNet), EBNet's <u>Anaerobic Fermentation Working Group</u> (WG) hosted a workshop in January 2025. The workshop aimed at bringing together key individuals in anaerobic fermentation (AF) and related areas, from both academia and industry, to address research and development gaps and needs for this industrial biotechnology route. The workshop was oriented to produce a final document highlighting the potential for AF to contribute to a low carbon society and possible ways to make this biotechnological route a reality. The event took place over two days (see Appendix 1 of main report: AF Workshop Agenda) and was attended by 23 individuals with a range of backgrounds and expertise.

The workshop opened with introductions from the co-Leads of the AF WG and Network Managers of both BBNet and EBNet, and slides prepared by BBSRC were also presented by the BBNet manager (see Appendix 2 of main report: AF Workshop introduction and activities). The participants were invited to give short presentations covering two points: 1. the areas you work on, your experience in the field of Anaerobic Fermentation and your view of the potential for Anaerobic Fermentation in the context of a circular bio-economy; and 2. from your viewpoint, what are some key questions, knowledge gaps and issues in this area? All slides were made available to all participants after the presentation sessions to support the group activity on the following day, and to form the basis for compiling a final document as one of the outcomes of the workshop (see Appendix 3 of main report: Participant presentation slides). The Mentimeter voting app was also used to capture the participants' opinions on this industrial biotechnology (see Appendix 4 of main report: Mentimeter results and open floor discussion notes).

The second day was dedicated to group activities aimed at defining possibilities to advance the knowledge on anaerobic fermentation focusing on real case studies and establishing actions to move the field towards wider implementation. Three key application areas of AF were discussed based on the sources of organic residues and wastes, including biosolids produced from wastewater treatment works, organic fraction of municipal solid wastes (OFMSW), and agro-industrial wastes and residues. Participants were organised into three groups, A (including subgroups a1 & a2) for biosolids, B (b1&b2) for OFMSW and C (c1&c2) for agro-industrial wastes, with three pairs of facilitators. The subgroups moved between tables (with post-it notes & dot stickers) for different sessions, but the facilitators stayed with a specific topic area for the day (Jaime Massanet-Nicolau & Louise Byfield on biosolids; Raffaella Villa & Luca Alibardi on OFMSW; and Özge Eyice-Broadbent & Lucy Booth on agro-industrial materials).

Session 1 covered all aspects of AF systems, from defining the potential, opportunity and application to proposing a way forward (see Appendix 2 of main report). Facilitators made sure key points were noted on large flipchart sheet using post-it notes. Subgroups swapped for session 2, i.e. biosolids (b1, c1), OFMSW (a1, c2) and agro-industrial wastes (a2, b2). The facilitators explained at the start of the session about what had been achieved by the previous group, and the newcomers voted for what they think the most important aspects and added more aspects as necessary. Session 3 took place with participants swapped again, i.e. biosolids (b2, c2), OFMSW (a2, c1) and agro- industrial wastes (a1, b1). This arrangement was made to maximise the opportunities for participants to talk to each other and to express their opinions. The final 30 minutes of the morning sessions were used for the facilitators to summarise the discussions carried out on each waste and residue streams, and opportunities for participants to add additional comments. The summary for AF of each waste stream can be found in Appendix 5 of the main report.

The workshop closed with a general discussion from participants of the proposals and next steps of AF technologies and assessment of the effectiveness of the workshop.



Mixed-Culture Anaerobic Fermentation in the EU Updates from HORIZON 2020 projects on the biowaste-derived volatile fatty acid platform

Webinar Tue 4 March 13-14:00 UK time

Keeping you up-to-date with pioneering work in Europe, these speakers will cover the HORIZON 2020 projects VOLATILE/CAFIPLA, developments in Standardisation, and insights from Pilot Scale research. Find the TEAMs link below or register via Eventbrite now.

Talks

Volatile Fatty Acid Platform – from biowaste treatment towards upcycling (Thomas Dietrich, TECNALIA).

CEN Workshop agreement CWA 17484: 2020 – Anaerobic digestion plants – Feasibility assessment methodology for integrating a Volatile Fatty Acid Platform Technology (Dr Jochen Michels, DECHEMA).

Experiences in Operating a Volatile Fatty Acid Pilot from the industry perspective (Dr Marie-Aline Pierrard, IDELUX)

Joining information

TEAMS meeting link



Meeting ID 326 290 566 521, Passcode hA37xa2H Dial in by phone: +44 20 3794 0272,,786972790# London UK, or Find a local number. Phone conference ID 786 972 790# or via EBNet AF Working Group











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