

BBSRC NIBB AD Network
Proof of Concept Awards: FINAL Report

2014 Proof of Concept Awards

POC2014001

Shotgun metabolomics in anaerobic digestion

We were interested in identifying key indicators of AD performance and health that in addition to helping operators maximise output from their systems can be used to populate computer-based models to improve our understanding of the AD process.

One such set of indicators that could be targeted for measurement is the complement of small molecules (metabolites) produced by AD. Although the utility of metabolomics in this field has been posited a number of times, this approach requires specialist equipment and knowledge in order to be effectively executed, and is likely to require the development of specific preparation protocols for successful deployment.

We used an untargeted metabolomics approach using a state-of-the-art, ultra high resolution instrument to determine the speed, magnitude and reproducibility with which soluble metabolites change in a model AD system with the view of generating preliminary data for a larger application making use of this technology in AD. We sampled three identical 30 L AD systems at 1 day intervals over a 3 day period, at 2 hour intervals over a 12 hour period in one of those days and at 10 minute intervals over a one hour period during one of these hours. Samples were collected using an in-line dewatering system based on tangential flow filtration and snap frozen in multiple aliquots before being stored at -80°C for subsequent analysis. To ensure detection of the full range of soluble metabolites, a subset was separated using reversed phase chromatography prior to analysis by mass spectrometry. Each sample was run in both positive and negative ion modes on a Bruker maXis ultra-high resolution instrument to provide comprehensive high resolution data. Technical replicates were separated into controlled groups before being analysed in random order using a column preconditioned by 20 injections of a sample pool, to minimise the background variation usually encountered in multiple sample analyses. Collected spectra were subjected to principle component analysis. We determined it was possible to identify a number of components via well-defined peaks. These components varied more between the biological replicates in different AD systems than they did over time that we sampled from a single vessel. Thus, we conclude that metabolomics measurements on the timescale of days can provide an accurate reflection of the state of an AD system.

Researchers:

PI: Dr James Chong

Department of Biology, University of York, Wentworth Way, Heslington, York YO10 5DD

CO-I

Prof Jane Thomas-Oates

Department of Chemistry, University of York, Heslington, York YO10 5DD

Dr Julie Wilson

Departments of Chemistry and Mathematics, University of York, Heslington, York YO10 5DD

POC2014011**Effective mass transfer of hydrogen into digester mixed liquor for biomethanisation of biogas CO₂**

Biomethanisation is attracting interest because of its potential both to maximise the yield of methane from anaerobic digestion, and to provide a large-scale energy storage solution to balance the electricity grid. The process involves the biochemical conversion of carbon dioxide and hydrogen into methane by hydrogenotrophic methanogenic microorganisms. A range of process configurations is possible, with carbon supplied from ex situ or in situ sources. In all cases, the gas-liquid mass transfer of hydrogen has been identified as a limiting factor, and presents a significant engineering challenge.

The mass transfer characteristics of different types of commercially available microporous hollow fibre membranes, including a polyvinylidene fluoride (PVDF) membrane, a polysulfone membrane and polypropylene membranes, were determined experimentally. The effect of gas and liquid flow rate, pressure, hollow fibre spacing and spatial configurations were evaluated as a basis for optimising hydrogen mass transfer within an anaerobic digester.

For hollow fibre membranes to provide sufficient hydrogen to meet the needs of the anaerobic microorganisms, a significant gas-liquid contact area is necessary. Based on a mass transfer coefficient of $k_L = 1.2 \times 10^{-4} \text{ m s}^{-1}$ obtained in the experimental work, a volumetric surface area of around 10 m^2 of membrane per m^3 of reactor is required even at relatively low CO₂ conversion rates. The small diameter of these membranes means this equates to a length of 10,000 m per m^3 of reactor. While this is feasible, in practice may present problems for cleaning and maintenance of flow rates.

The performance of the membranes operating as diffusers was compared with that for bubbled systems, based on literature data and correlations. Modelling of these showed that complete H₂ mass transfer could be achieved in a typical commercial-scale digester provided the initial bubble size is small enough.

Inoculum from a conventional anaerobic digestion plant treating municipal wastewater biosolids was successfully acclimatised to a feedstock consisting of 80% H₂ and 20% CO₂, in continuously-stirred tank reactor digesters operating at mesophilic and at thermophilic temperatures. In terms of conversion to CH₄ the thermophilic reactors performed better than the mesophilic, while higher input gas flow rates also improved the conversion. Output gaseous concentrations reached a maximum of 90 % CH₄.

The mixed feed of H₂/CO₂ was injected through a length of hollow fibre membrane around the bottom perimeter of the reactor, which was initially intended to act as a membrane diffuser. The membranes proved to be prone to leakage, however, and were effectively operating as bubbling systems; while in longer-term operation there were problems of fouling and severe reductions in the input gas flow rate, meaning gas transfer was again limiting.

Taken together, the results of the study suggested that while the use of membrane diffusers is feasible it is likely to present practical problems of fouling and cleaning in a real digester environment, especially for in situ systems; while offering no major advantages. Feed systems based on micro-bubbles appear more promising at full scale, and research into these is likely to offer a more effective way forward.

Professor Charles Banks said *"Suggestions in the literature about the benefits of membrane diffusers could not be shown experimentally in laboratory trials for any of the hollow fibre types tested. They do not appear to have any great advantage over micro-bubbles, and future work will concentrate on developing effective means of generating these in-situ in anaerobic reactors"*.

Researcher:

Prof Charles Banks
Water and Environmental
Engineering
University of Southampton
Southampton SO17 1BJ

Development of anaerobic biomass support particles for effective membrane cleaning

Membrane bioreactors are becoming an increasingly popular means of treating wastewater because of their ability to produce highly clarified effluents and to eliminate the need for sedimentation tanks for biomass removal and recovery. They are also opening up opportunities for the adoption of anaerobic processes which are energetically more favourable than the conventionally-used aerobic systems. One of the major issues affecting performance and uptake, however, is membrane fouling. In aerobic membrane systems this can be overcome by the use of bubble scouring in conjunction with the need to supply oxygen. Gas bubbling in anaerobic systems represents an energy loss, and more effective systems of membrane cleaning could give significant energy benefits. The current work follows on from some research into using activated carbon as a carrier particle which was also found to give improvements in membrane flux and fouling resistance. The concept investigated as part of this work was to use a lighter open-cell polyurethane material in a pseudo-fluidised bed, so that the soft particles could rub gently against the membrane surfaces to keep them clean. The research also investigated how these particles could be modified to change their density and surface properties for further optimisation of the cleaning process.

The results showed the technique could be very successful, with particles significantly improving the flow through the membrane even when used at low packing densities (bulk volume of particles per unit volume of reactor). The major limitation was the quantity of other material that could be incorporated into the polymer, which was insufficient to provide a wide range of bulk particle densities for full evaluation under different reactor configurations. Experiments were therefore carried out under uniform conditions to provide comparative data that would allow selection of the best particle types for later use in large-scale hydraulic experiments. The results of the work were sufficiently encouraging to justify its continuation, and the concept has been included in a successful application for funding under the ERA-Net BESTF2 programme, in which large-scale pilot studies will be conducted. The concept will also be taken forward as part of a Newton Institutional Link programme between the University of Southampton and the National University of Civil Engineering in Vietnam, with further experimental work planned on laboratory and pilot-scale systems. Full characterisation of the hybrid particles was not carried out within the current project, nor was it possible to explore the full range of minerals that could be immobilised in the polymer. It is likely that further improvements in cleaning efficiency are achievable, with the added benefit that in 'live' systems the particles will also become colonised by acclimated micro-organisms to enhance the treatment process and further reduce the required membrane area, as fouling is strongly related to the presence of suspended solids of the type found in dispersed growth systems.

Dr Sonia Heaven said: *"The work has clearly demonstrated the effectiveness of particle cleaning of membrane surfaces in anaerobic systems. Now that the POC has established the benefit of this approach, it is being taken forward for testing at a larger scale".*

Researcher:

Dr Sonia Heaven
Water and Environmental Engineering
University of Southampton
Southampton SO17 1BJ

POC2014016

Production and extraction of C3 and C4 aliphatic carboxylic acids from the anaerobic digestion of waste blood as a model substrate

One of the challenges of anaerobic digestion is to process organic wastes with high nitrogen content, such as slaughterhouse wastes. Due to the inhibitory effect of ammonia on anaerobic microbial community, the disturbance of the inner balance of the digestion system occurs and the intermediate products accumulate when this type of materials is fed in large quantities. Based on this recurring observation, this proof-of-concept project looked at the feasibility of production and harvesting of short chain carboxylic acids as intermediate bulk chemicals as an effort to diversify anaerobic digestion into the field of biorefinery. The specific aim of this research was to produce and recover high concentrations of volatile fatty acids (VFA) through anaerobic fermentation using blood as a model substrate, taking a non-sterile mixed-culture approach in which the microbial consortium was naturally selected by the reactor conditions.

VFA production rate, extent and profile in batch, fed-batch and semi-continuous reactors were evaluated in this study under a range of operational conditions and treatments. Two harvesting routes, esterification and pertraction, were then tested for their efficiency to extract VFA from the fermentation broth. Potential process flowsheets with integrated VFA production and recovery were also developed to look at the overall efficiency of the processes. While these results provided important information for the development of a carboxylate-platform biorefinery using high-protein wastes as substrate, further in-depth research will be needed before they can be translated into an industrial-scale process. This will involve interdisciplinary teams to explore the interface between production and extraction of the value-added products, supported by experts in systems biology to implement advanced process diagnostics: a collaborative proposal will therefore be prepared for larger grant funding.

Dr Yue Zhang – *‘This proof-of-concept project focused on diversification of anaerobic digestion into the field of industrial biotechnology through the production and harvesting of carboxylic acids as building block chemicals. It proved that by selection of reactor conditions and substrate type, anaerobic digestion can be directed towards specified intermediate products in concentrations suitable for extraction.’*

Dr Lina Chi – *‘It was a great pleasure to participate in this project. This is a very nice example that further demonstrates the synergy between membrane technologies and anaerobic technologies.’*

Researcher:

Dr Yue Zhang
Water and Environmental Engineering Group
Faculty of Engineering and the Environment
University of Southampton, Southampton,
SO17 1BJ

2015 Proof of Concept Awards

POC2015001

Redesigning hydrolysis reactors for the development of high power density advanced anaerobic digestion enabling containerised electricity production from agricultural residues.

This project has taken the first steps towards mimicking the rumen conditions within a cow to bridge the 30-fold gap in digestion between traditional reactors and a cow's natural digestive process.

A reactor design (see figure 1) has successfully been developed to digest maize silage and produce VFAs. The reactor design allows the liquid and the solid phase to have different residence times. It also recirculates the liquid phase whilst holding the solids in a bed, and has a feedback loop to actively maintain pH.

However, the experiments yielded no consistent stable results, and did not demonstrate accelerated AD. The experiments suggested that the rate of digestion was high – (feed rates were 50gms dry matter per litre reactor volume per day) and the failures were mechanical and to do with fluid flow rather than biochemical.

There was a problem with blockage of the lower filter – which occurred within days of starting the reactor. Various different filter plate geometries were designed and printed, but all failed ultimately. The problem appeared to be that digestion of the feedstock left fine fibres of recalcitrant material that were carried into the filter channels where they rapidly caused blockage. This was true even with the most sophisticated filter designs. This issue was finally resolved by replacing the filter with a simple sand bed that could be back-flushed.

The second problem with this reactor was removal of the digested material. This rose to the top of the reactor as planned, but formed such a thick and dense fibrous mat that it would not come out of the planned exit hole. Instead, liquid that was being recirculated flowed in at the top of the reactor, and instead of permeating the bed, had a tendency to flow out of the exit hole. The reactor was redesigned to removed spent solids by forced mechanical removal, by using a slide valve. A linear electrical ram was needed to provide enough traction to open the slide valve.

A feedback loop was developed to maintain the pH. The pH within the reactor was actively maintained by inline measurement of the pH, which activated the removal of the liquid phase from the reactor and replaced the liquid with buffered artificial saliva. The pH was held stably at 6.8. It was hypothesised that the continuous circulation through the reactor of an artificial saliva buffer prevents inhibition of the community from the digestion products.

The development of the pH maintenance system, coding the micro-computer, developing filtration and troubleshooting leakages and blockages has provided the basis for further development of AD reactors and subsequent research into optimising conditions using multiple reactors to maximise digestion rates.

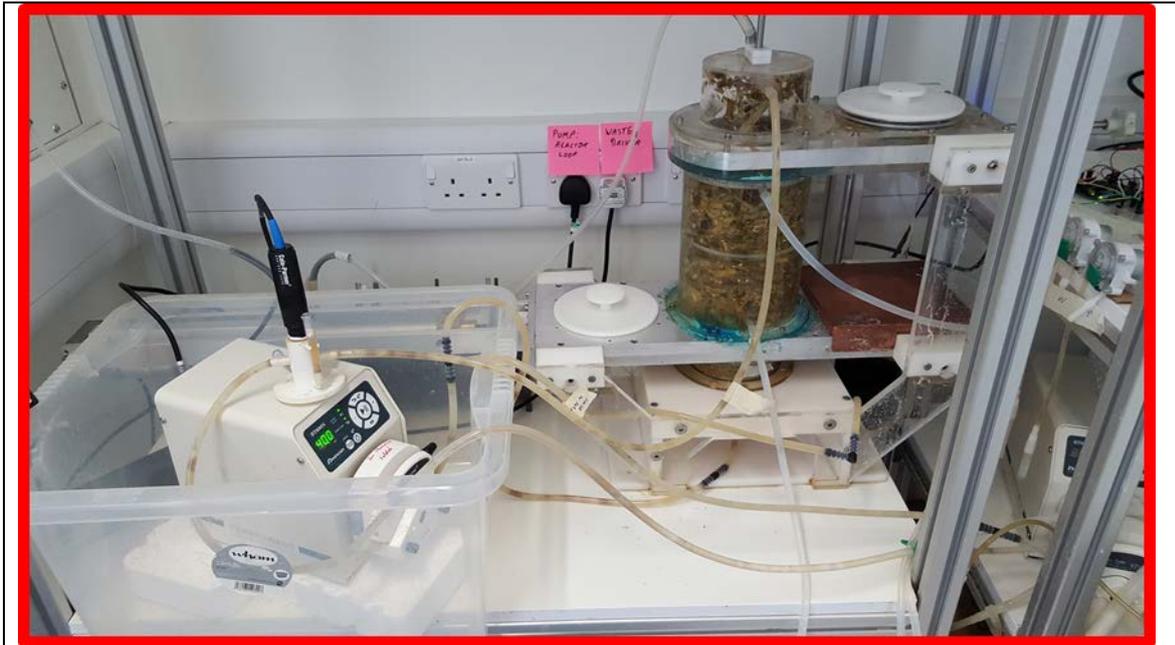


Figure 1: Advanced Anaerobic Digester including a pH feedback loop, and liquid recirculation.

Researcher:

Professor Ian Thompson
Department Of Engineering Science
University Of Oxford
Parks Road
OX1 3PJ

Recovery and purification fatty acids and nutrients from anaerobic digester fluids using integrated membrane freeze-thaw (MFT) processes

Anaerobic digestion as a commercial process is increasingly becoming problematic mainly due to the changing financial incentives and the many environmental and economic challenges faced by the use and disposal of spent digestate fluids. Although they represent a source of valuable nutrients for springtime application, they are difficult to handle and store being a major source of green gas emissions they represent substantial environmental hazard to both atmospheres and water environments. The cost effective concentrating these fluids would alleviate many of these problems while adding value and increasing its utility as a sources of nutrient, making it possible for reformulation and to use as nutrients in industrial Bioprocesses.

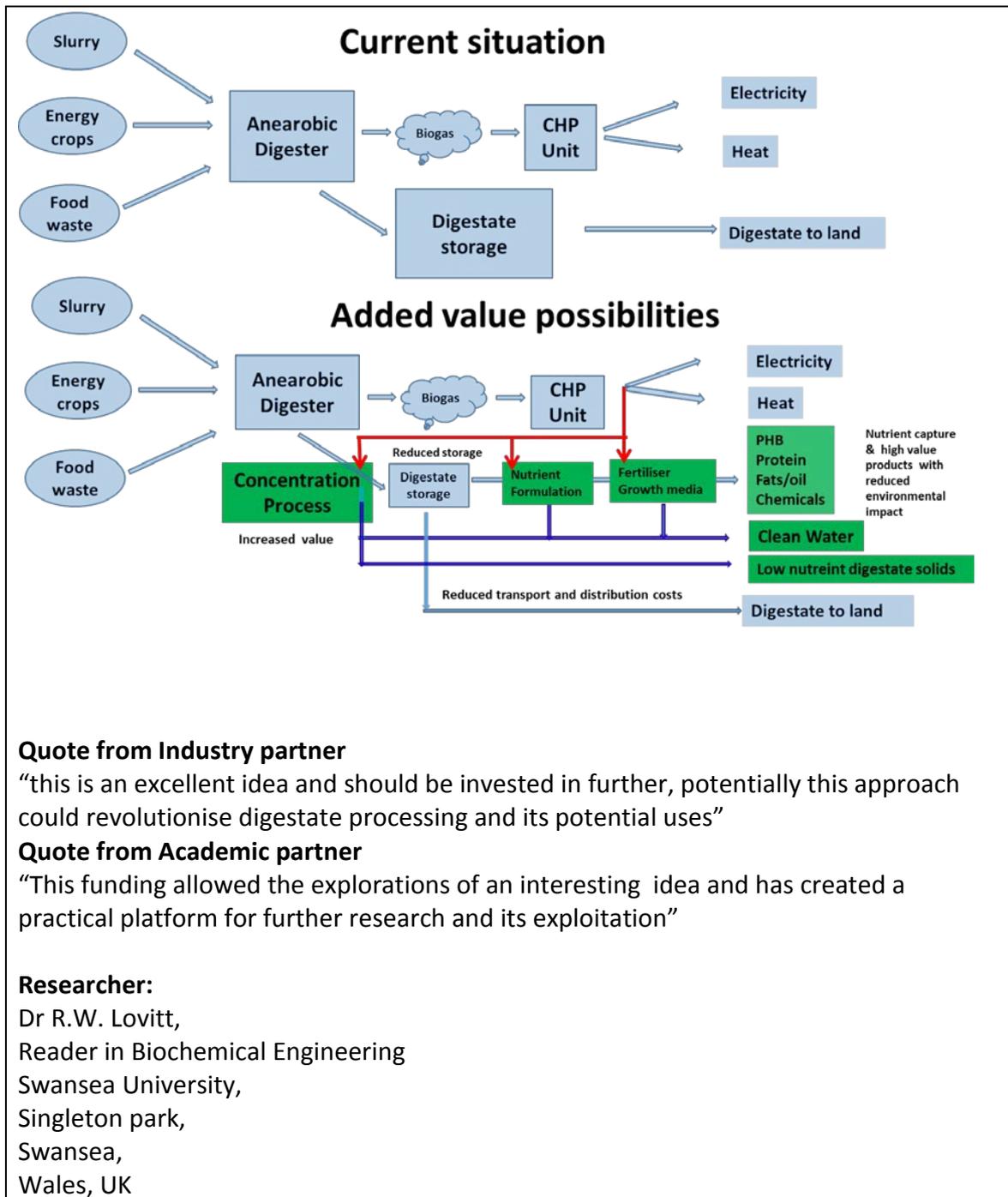
We have investigated a novel low-cost concentration process that combines the use of membrane technology with freezing to produce concentrated digestate fluids and nutrient replete organic rich solids fraction. Increasing the value of both fractions, the nutrient replete is a better soil conditioner with high organic matter with slow nutrient release. The concentrated wastes having more value as a fertilizer and therefore transportable with more utility.

Two major pieces of work were carried out. Practically freezing and filtration methods were investigated to determine the levels of concentration possible by these methods using defined mixtures and pre-treated digestates. Individually using either freezing or membrane a concentration by factor of 5 waste was possible and by combining these, a concentration factor of 20 was possible when the two operations were combined.

The second study involved the theoretical assessment of the amount of power required to carry this out. We used the heat and electrical energy data obtained from Fre energy food digester /CHP system. This work reveal that the power for the freezing process could be generated from the CHP system of the digester gas. The value of the digestate was enhanced considerable using this method. We estimate that this rises from about £3 per tonne to about £50 plus the recovery of about 80% of the water for reuse. This we suspect can be improved further with more optimisation and careful integration.

The concentrated digestate was able to support good growth of a variety of bacterial including acetate utilising PHB producing bacteria. The concentrate (around 3%N, 1.5 %P & 1.5 % K) also present a useful starting point for reformulation and recovery of nutrients (NPK).

We now wish to develop these concepts further by the use of process pilot plant (2.t digestate per day) to confirm and optimise this feasibility study and to investigate commercial aspects of the process, its potential limitations, its robustness (i.e. membrane and equipment longevity) and the potential of digesate reformulations for specific products.



Quote from Industry partner

“this is an excellent idea and should be invested in further, potentially this approach could revolutionise digestate processing and its potential uses”

Quote from Academic partner

“This funding allowed the explorations of an interesting idea and has created a practical platform for further research and its exploitation”

Researcher:

Dr R.W. Lovitt,
 Reader in Biochemical Engineering
 Swansea University,
 Singleton park,
 Swansea,
 Wales, UK

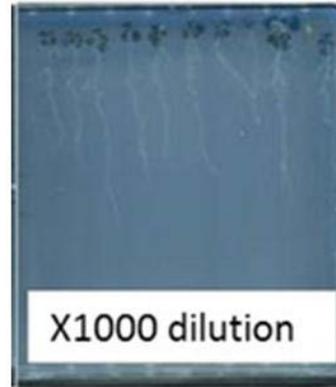
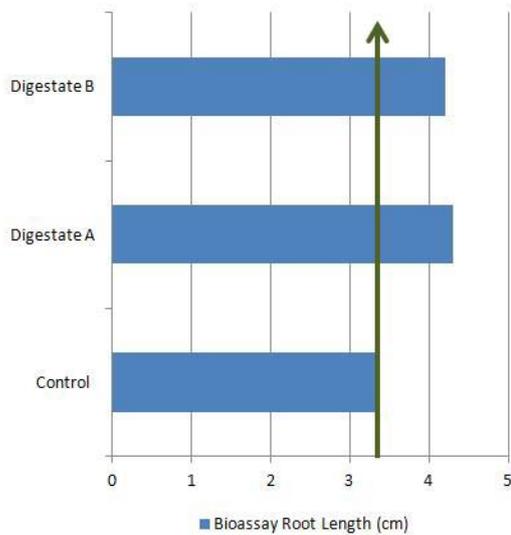
Microbial Enhancement of Phyto Active Compound in Digestate

The residue from anaerobic digestion (known as digestates) has often been reported to promote the growth of plants and therefore increase the potential yield of crops. The conventional reason proposed for this increased crop yield is that the digestate contains extra nutrients for plants such as nitrogen, phosphorous and potassium. However there are potentially other reasons for this improved crop yield. Anaerobic digesters have up to a 1000 different species of bacteria involved in the anaerobic digestion process. Do the bacteria involved in the anaerobic digestion process produce other products such as plant hormones which would increase plant growth, can bacteria which have been identified as been able to act as plant growth promoters grow in anaerobic digestate and does the operation and composition of the digester feedstock effect the presence of these plant growth promoting products?

To test these ideas, samples from a number of industrial anaerobic digesters treating a ranges of wastes such as sewage sludge and food waste as well as bioenergy crops were chemically analysed for the presence of plant hormones. Samples were also analysed using a plant based bioassay which would also confirm the presence of these plant growth promoting compounds. Both the chemical analysis and plant based bioassay showed that some anaerobic digestates did indeed have levels of plant growth promoting hormones at concentrations which would have significant effects on plant growth. To determine the potential mechanism of hormone production some samples were modified by the addition of a natural occurring compound which is a precursor of plant hormone formation. In the samples with this feed addition, the level of plant hormones did increase significantly. Sterile samples of digestate were also inoculated with plant growth promoting bacteria which both showed these bacteria were able to grow in the digestates and produce significant quantities of plant growth promoting hormones. Some of the bacteria used have also been associated with promoting greater plant disease resistance.

Our aim is to take these preliminary results forward by developing a greater understanding of the plant growth promoting activities of bacteria in anaerobic digesters. Can we control and manage their presence in industrial plants?; what will be the effects of the plant growth promoting properties on increasing the yields of field grown crops?; how do the hundreds of billions of bacteria in the digestates react with the equally huge number of bacteria present in the soil and the roots, stems and leaves of crops? If successful we could see significant extra benefits of anaerobic digestion used to support the enhanced growth of food crops with reduced use of fertilisers and pesticides as well a better financial return for the use of digestates for anaerobic plant operators.

25% Increase in root growth



Effect of Digestates on Root Growth Length in Bioassay

Researcher:

Professor Richard Dinsdale
The Sustainable Environment Research Centre,
Faculty of Computing, Engineering and Science,
University of South Wales,
Pontypridd.
CF37 1DL

2016 Proof of Concept Awards

POC2016002

Novel bioelectrodes for energy positive ammonia removal from municipal wastewater

The aim of the award was to provide proof of concept for a novel energy positive technology to remove ammonia from municipal wastewater. In a desk study on the thermodynamics of ammonia removal processes we had calculated that, analogous to the oxidation of organic compounds, it should also be possible to oxidize ammonia to nitrogen gas with bacteria that can use electrodes as electron acceptor. The challenge was to enrich the bacteria that can perform this reaction and demonstrate that the concept works.

We have set up, inoculated, run and analysed several series of microbial electrolysis cells poised at different electrode potentials, supplemented with known quantities of ammonia, plus appropriate non-inoculated controls, and controls run at open circuit potential. The reactors were inoculated with bacteria from aerobic and anaerobic wastewater treatment plants and Fe(III) containing sediment. The most promising results were obtained with electrolysis cells inoculated with Fe(III) containing sediment. Results were erratic though, and a stable ammonia-oxidizing anode has not (yet) been obtained. We will endeavour to continue this line of research, as the rewards of developing novel bioelectrodes for energy positive ammonia removal from municipal wastewater will be substantial.



The iron-rich sediment that provided the inoculum for our most promising experiments thus far on the development of novel bioelectrodes for energy positive ammonia removal from municipal wastewater.

Researcher:

Dr Jan Dolfing
School of Engineering
Newcastle University

Electrode interface to control and extend metabolic outputs of AD microbial communities

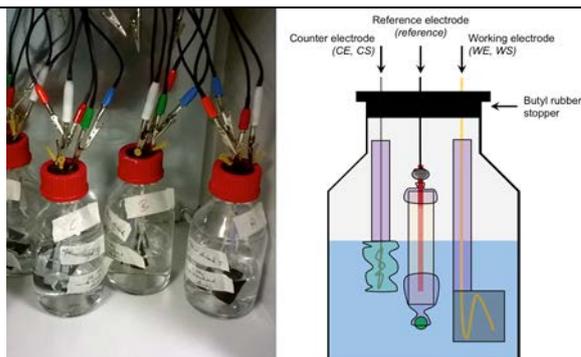
We hypothesized that the (methanogenic) Anaerobic Digestion (AD) process can be influenced by electrically interfacing the underpinning microbial community. Most particularly, we aimed at a) changing the microbial community composition by promoting particular respiratory pathways with electrodes as terminal electron acceptor (TEA), and b) using the electrochemical interface as switchable “valve” for the removal of organic acids by electrode-respiratory organic acid oxidation for non-fermentable organic acids (i.e. acetate).

Microbial metabolism is based on redox conversions that are only possible within the regime of negative free energy, i.e. within an appropriate redox gradient of reaction educts and products. Fermentation provides low potential difference and hence the reaction space is energetically limited. In AD communities, this limitation often leads to accumulation of organic acids (i.e. acetate) with associated acidification that can eventually lead to process-failure. An electro-interface hence offers the possibility to broaden the redox capabilities by simulating the presence of potent TEAs. While this non-natural (extracellular) TEA is different from native TEAs (which often are reduced intracellularly), hence providing different energies to metabolizing microbes, electric interfaces can be used to expand and control the redox regime for any organism that can interface with the electrode.

In this pilot project, we established appropriate bioelectrochemical cells (BEC) to study electrical interfacing of AD communities. Particularly, we developed a self-made BEC with low-cost components, that can readily allow experimental design with multiple replicates. The protocols for these BECs are provided through our group homepages.

The developed BECs are gas tight and hence allow the accumulation of pressure during the AD process with subsequent measurement of gas phase, and liquid phase composition. With this setup we found both the formation of hydrogen and methane, as expected, plus the generation of current via a conductive carbon felt electrode on which microbes can settle, depending on the set electrode voltage. This together confirms the functionality of our setup. Microbial community analysis of this pilot experiment is pending.

Having succeeded in the establishment of functional BECs in this pilot, we are equipped to further analyse the effects of the bioelectrochemical interfacing on the AD process and AD microbial communities. Due to the complexity of this process, type of sample and microbial community, careful experimental investigation is required and this will be pursued in our future work.



Photograph (left) and sketch (right) of our gas-tight bioelectrochemical cells (BEC). The protocols to build these BECs are made available on our homepage.

Prof. Orkun S. Soyer – ‘This work allowed us to establish easy-to-follow and clear protocols for bioelectrochemical cells, which has been a bottleneck in the field to develop multi-factorial experiments. We are now in a position to develop our work in this area further, towards electrical control of communities.’

Researcher:

Prof. Orkun S. Soyer,
School of Life Sciences,
University of Warwick

**Computational Methods for Anaerobic Digestion Optimization – Act 1
(CoMAnDO1)**

Effective mixing is crucial for anaerobic digestion but it is an energy-intensive operation. Despite its importance, research is lacking in this area. Traditional approaches to digester design are rooted in empiricism rather than science, and design standards focus on treated sludge quality, not gas yield and energy consumption.

The challenge is to improve digester performance and maximise biogas yield. An innovative solution is to simulate simultaneously the hydrodynamic and microbiological processes found in anaerobic digestion. An innovative solution is to simulate simultaneously the hydrodynamic and microbiological processes found in anaerobic digestion. We hypothesized there is a direct link between mixing-induced turbulence and biogas yield. Our focus for this CoMAnDO1 Proof of Concept project was to simulate these interrelationships in a complex multiphase, non-Newtonian fluid environment

Therefore, as part of the CoMAnDO1 project, we developed the first-ever hydrodynamic-biokinetic coupled modelling approach within the highly innovative Lattice-Boltzmann framework. An illustrative 2D model of a laboratory-scale digester has been modelled to identify the flow patterns arising from gas mixing and this is shown in Figure 1. The same figure also shows that instantaneous and cumulative biogas yield can be tracked using the model. In addition, each of the biokinetic species (different kinds of substrate and bacterial populations) behave as expected.

This work served as the basis for a recently-awarded three-year EPSRC grant (“CoMAnDO”), which will: (i) extend the model to 3D geometries; (ii) perform state-of-the-art experiments on laboratory and pilot-scale digesters treating municipal sewage sludge; (iii) fully validate the approach against hydrodynamic, biokinetic and coupled experiments; (iv) simulate a wide range of full scale scenarios and (v) produce design and operational guidance for industrial anaerobic digestion optimization.

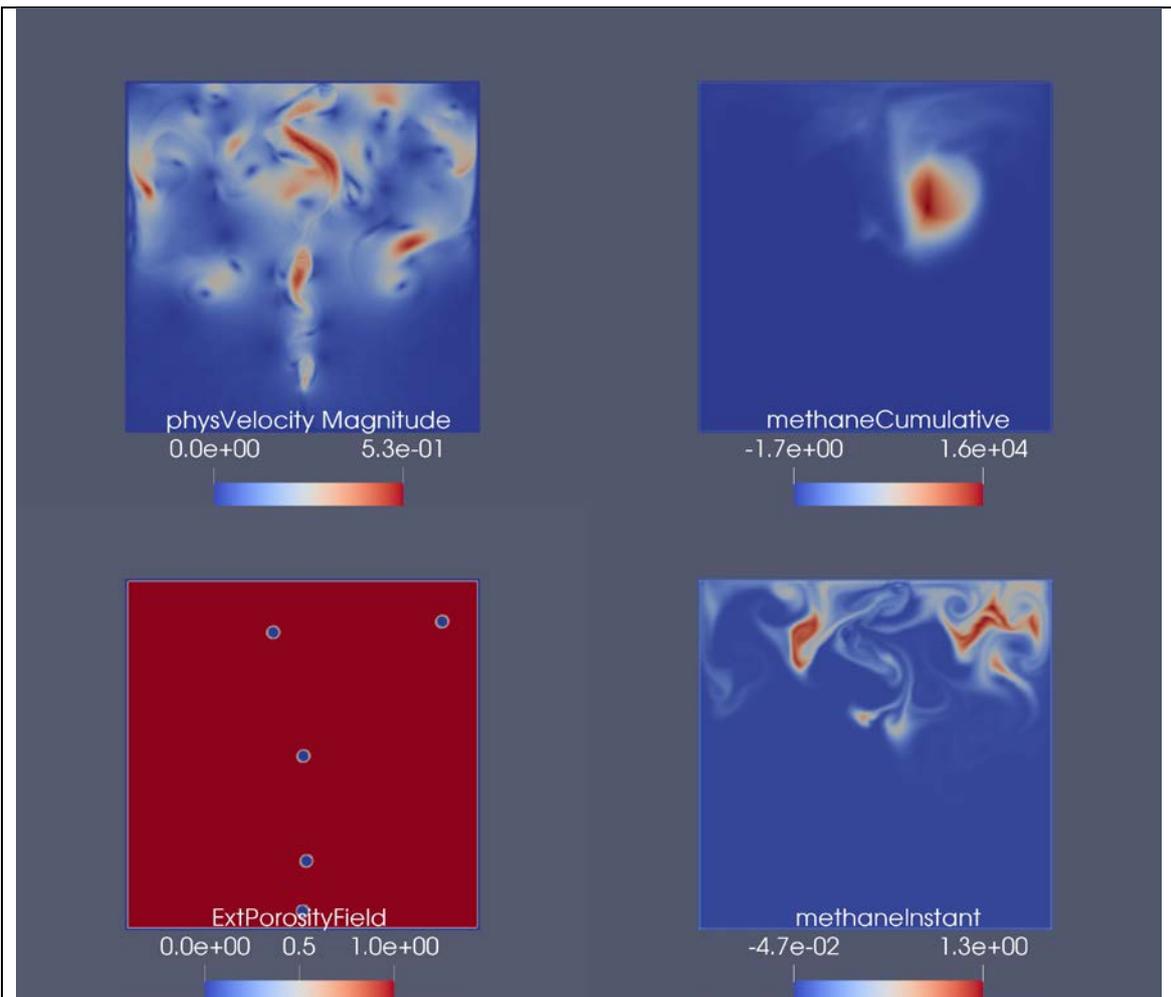


Figure 1: Top left: velocity patterns. Bottom left: injected gas bubbles. Top right: cumulative biogas yield. Bottom right: instant biogas yield.

NMCNomenca. - "We recognize the tremendous, untapped potential of renewable energy from liquid and solid waste streams, and see your research as providing valuable and much needed step in the right direction."

Researcher:

Prof. John Bridgeman
 (Formerly) School of Engineering
 University of Birmingham

Computational Methods for Anaerobic Digestion Optimization – Act 1**(CoMAnDO1)**

Anaerobic Digestion has been represented as a collection of macro-scale processes leading to the degradation and conversion of a complex feedstock into a mixture of products of anaerobic microbial metabolism. For simplicity and sometimes lack of information, AD has been modelled without including details of the metabolic processes led by microorganisms. New biomolecular tools and advances in computational biology provide now the possibility of including this micro-level in the analysis of AD processes. Our aim was to demonstrate the feasibility of analysing AD through a multi-scale approach, ranging from the metabolic micro-level to the kinetic macro-level, to simulate and generate predictions for the analysis, design and optimization of full-scale processes.

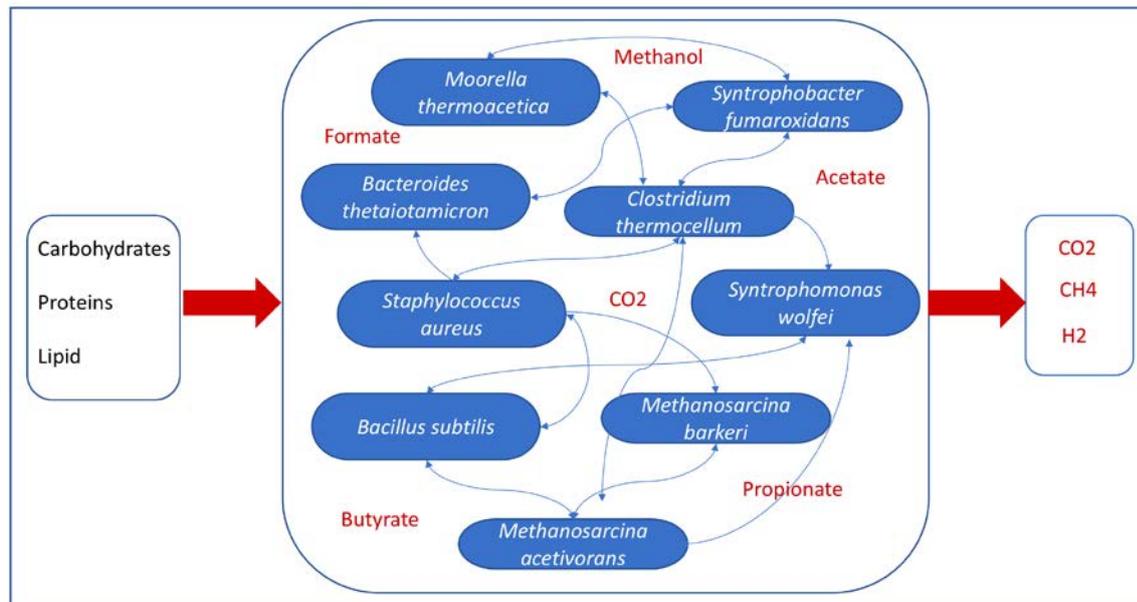
Based on sequence analysis, we constructed genome scale metabolic networks (GSMNs) for nine microbial species usually found in AD communities, presenting the metabolic capability to degrade complex macromolecules (proteins, fats, carbohydrates). The GSMNs were combined into a community model (c-GSMN) containing all nine microbial species. The in silico metabolic capability of the species was confirmed by simulations using bioinformatics tools such as Flux Balance Analysis and Dynamic Flux Balance Analysis, which allow for the identification of the metabolic pathways active under diverse conditions. The simulations showed degradation of the feedstock and production of CH₄, VFAs, CO₂ and H₂, and highlighted the metabolic interactions and cross-feeding among the species in the community. For example, in those simulations, metabolic products of some species (e.g. CH₃OH) are consumed by methanogenic species. Some of those species showed hydrogenotrophic methanogenesis consuming CO₂ and H₂ as its preferred substrates over acetate and methanol, while others showed acetoclastic methanogenesis by consuming acetate as its preferred substrate over CO₂ and H₂. The results are in line with various experimental. Other intermediates such as propionate are utilized by acetateproducing species. Our results confirm that the model is able to capture the crossfeeding existing between the different species in the community.

The multi-species metabolic model was linked to ADM1, a kinetic model for anaerobic digestion, which was adapted and converted into a Petri-Net and QSSPN format. The models are connected using components of the feedstock such as monosaccharides, amino acids and short chain fatty acids, all of them products of degradation of the complex macromolecules present in the feedstock. In our simulations, the growth of the community is the objective function to maximize in the FBA, and the calculated flux values of molecules of interest were used as the input for the kinetic model.

Exploratory experiments were performed to experimentally validate the model and its predictive power, and to confirm the results of our simulations, combining species to generate a minimal synthetic microbial community and fed with a synthetic feedstock.

The preliminary results obtained are in line with the results of the simulations.

The outcomes of this Proof of Concept are a collection of genome-scale metabolic models for species commonly found in anaerobic digestion processes; a community genome scale metabolic network able to represent the collective metabolism of an anaerobic digestion microbial community; a model of anaerobic digestion, combining an ordinary differential equation model (ADM1) with a stoichiometric constraint-based model. Such a hybrid-model exploits the strength of both approaches.



Schematic representation of the multi-species metabolic model

Researcher:

Dr Claudio Avignone Rossa
Reader in Systems Microbiology
University of Surrey