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.

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POC2014011

Effective mass transfer of hydrogen into digester mixed liquor for biomethanisation of biogas CO_2

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 $k_L = 1.2 \times 10^{-4}$ 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 CO_2 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 H_2 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% H_2 and 20% CO_2, in continuously-stirred tank reactor digesters operating at mesophilic and at thermophilic temperatures. In terms of conversion to CH_4 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 % CH_4.

The mixed feed of H_2/CO_2 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".*

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POC2014012

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".*

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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.'*

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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.

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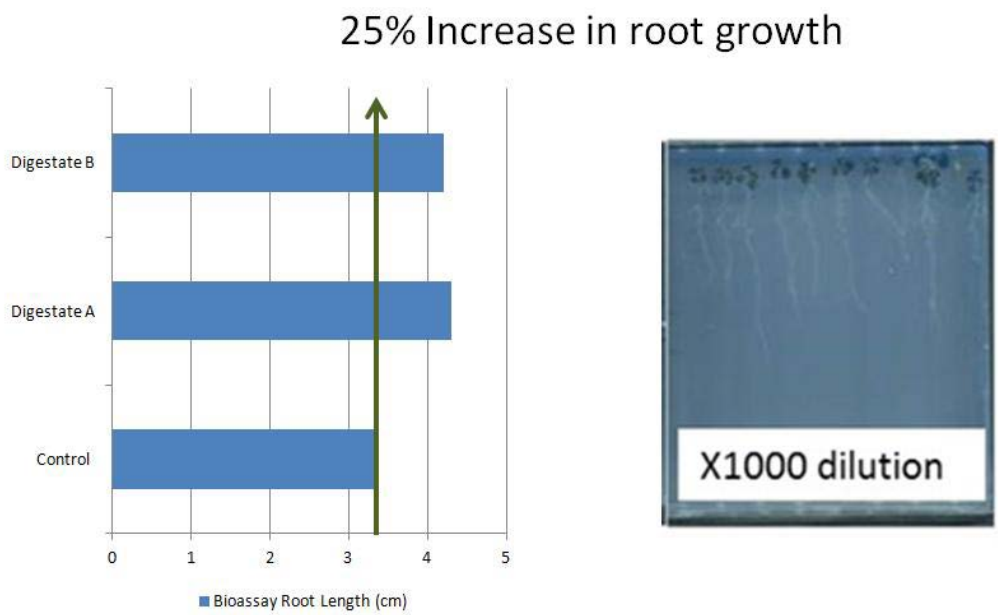
POC2015008

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.



Effect of Digestates on Root Growth Length in Bioassay

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