

Advancing Gas Fermentation Technologies:

A multi-disciplinary challenge

Report of the joint EBNet / Carbon Recycling Network workshop on microbial systems with gaseous feedstocks

Hosted by the Environmental Biotechnology Network and the Carbon Recycling Network

Shringley Hall, Cheshire 27-28 March 2024

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Biotechnology and
Biological Sciences
Research Council



Engineering and
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Advancing Gas Fermentation Technologies: A multi-disciplinary challenge

Executive summary

Gas fermentation technologies have the potential to revolutionise sustainable bioproduction by enabling carbon capture and utilisation (CCU), but key research issues and implementation challenges need to be addressed. In the field of microbiology, these include: improved understanding of systems biology; exploration of a wider range of species (i.e. non-model organisms) and of mixed cultures; and development of associated tools for genetic characterisation and manipulation. Better insights on how microbial metabolism and spatial and community structures are influenced by the engineering envelope will open new opportunities for process development and optimisation.

Enhanced understanding of gas-liquid transfer processes and the hydrodynamic behaviour of complex multi-phase fermentation liquors is fundamental to effective system design. New multi-scale modelling approaches that integrate biokinetics, thermo- and hydrodynamics will be needed to support these advances. Scale-up is a particularly critical area, due to the significance of scale effects for mixing and mass transfer, and thus for microbial performance. Easier access to scale-up facilities is essential to progress the development of cost-effective bioreactor designs.

Feedstock, process and product selection are vital links in the chain to widespread technology implementation. Open discussions supported by techno-economic and whole-life sustainability assessments are needed to determine which bioprocesses and products to focus on. Consideration must be given to the impact of gas quality, purification requirements, and intermittent patterns of renewable energy production. Product recovery methods and integration with upstream, downstream and sidestream processes all have key roles to play. Modelling, including AI and machine learning, can aid in both design and operational decisions.

Two-stage processes, where gases are converted by chemical catalysis into soluble feedstocks (e.g. formate, methanol), also merit attention as they eliminate some difficulties associated with gaseous substrates. Tackling the R&D issues identified above will additionally benefit such processes, as well as a broader range of industrial biotechnologies.

Implementing gas fermentation technologies requires multi-disciplinary perspectives. Better understanding and communication across specialisms is vital to create a new generation capable of rapidly advancing this field. Talent acquisition and retention can be facilitated through interdisciplinary work and training opportunities. Cross-remit funding and support for industrial engagement are crucial for effective technology progression.

Addressing these key R&D issues will unlock the full potential of gas fermentation technologies and allow them to contribute to meeting national and international net-zero and sustainability targets.

Advancing Gas Fermentation Technologies: A multi-disciplinary challenge

1 Introduction

This report presents the results of a workshop on microbial systems with gaseous feedstocks run jointly by the Environmental Biotechnology Network (EBNet, www.ebnet.ac.uk) and the Carbon Recycling Network (<https://carbonrecycling.net>). The goal was to identify key questions and knowledge gaps, R&D needs for technology progression and transfer, and actions that should be undertaken to promote progress and alleviate any obstacles. The outcomes are summarised in Section 3 and the main output, a position statement supported by 28 participating experts, is presented in Appendix 1.

Gas fermentations are central to the Carbon Recycling Network's remit. EBNet covers anaerobic digestion and its sister technology of CO₂ biomethanisation, a specific example of gas fermentation. EBNet's proposal for a joint workshop arose from its Engineering/Biology theme. This considers the interactions between microbial systems and the envelope of conditions within which they operate; conditions which in many cases can be adjusted by simple engineering-scale interventions.

The workshop covered a wide range of aspects of gas fermentation technology, but did not include bioelectrochemically-enhanced systems as these were felt to merit separate discussion.

2 Workshop Process

The workshop took place over two half-days on 27-28 April 2024 at Shrigley Hall in Cheshire, UK, following immediately on from the Carbon Recycling Network's annual conference.

Experts were invited to participate based on discussions and recommendations from the two Network teams. Some who were unable to attend were invited to submit brief comments in the form of bullet points, and to review the final output. A list of participants is given in Appendix 1.

The event started with a joint lunch with attendees at the Carbon Recycling Network conference, followed by a brief welcome from Profs Nigel Minton (Carbon Recycling Network director) and Sonia Heaven (EBNet director).

The organising team was aware that the workshop participants came from a wide range of specialisms, and did not all know one another. The afternoon session therefore began with each participant giving a short pre-prepared overview covering two key points:

- **who are you, what areas do you work on, and why do you think we are so keen to have you at this workshop?**
- **from your own viewpoint, what are some key questions, knowledge gaps and issues in this area?**

These overviews, including details presented on the day for some of those who could not attend, are given in Appendix 2.

This session was followed by invited presentations from Prof Raul Munoz, Prof Sandra Esteves, and Prof Will Zimmerman: see Appendix 3.

Participants were then given time to talk informally in the evening and over dinner.

Based on preliminary examination by the workshop team of the points originally submitted, it was decided to structure the following day's discussions into three broad categories: Microbial, Engineering Envelope and Other.

In the first session next morning, participants were asked to review the original bullet points from the initial round, which were not grouped or clustered at this stage. The majority of these were printed on slips of paper; a few later submissions were handwritten by the organising team, and a small number were unintentionally omitted from this process. Participants were invited to flag up any topics that they felt were particularly important by marking the relevant slip with a self-adhesive coloured dot. Red dots were also available to indicate that more information was needed.

The results of this ranking and the grouped and clustered points are shown in Appendix 4.

In the following session participants were asked to spend a short period considering the original bullet points and the information in the previous day's flash and extended presentations; then, working individually, to write new bullets focusing on research-related aspects and to post them on flipchart boards under the headings 'Microbial', 'Engineering Envelope' and 'Other'. The participants were then split into 3 groups, each specified to include a mixture of backgrounds and disciplines, and were invited to discuss, cluster / prioritise, and summarise the key issues under the Microbial heading. Each group was supported by one member of the workshop team (Charles Banks, Louise Byfield, Angela Bywater) to assist with questions, time keeping and note taking. In this and following sessions the groups were also invited to add bullet points from original set if they wished, and some did so.

The same pattern was repeated for the Engineering Envelope and Other headings in the following sessions. For each session, membership of the groups was re-arranged to ensure a different mixture of individuals.

After a coffee break and an invited presentation by Kristi Potter, the participants were then asked to write new bullet points on potential obstacles to progress and the actions needed to overcome them ('Actions'). The same process of discussing, clustering and summarising was carried out. There was no plenary feedback from these sessions but photographs were taken of the flipchart boards with individual bullet points. These are presented in Appendix 5 and 6. The summary notes provided by the groups are shown in Appendix 7.

The morning ended with a brief feedback session on the operation of the workshop, followed by more informal individual discussion over lunch. The invited participants were then free to go.

Initial notes were completed that afternoon by the workshop team. They were then written up as a draft overall summary and circulated both to the workshop participants and to the wider group involved in the preliminary stage, for amendment and approval.

3 Workshop Outcomes

The following main research and implementation issues were identified:

Microbial: Our understanding of fundamental systems biology in this area lags behind that in other fields, with some major knowledge gaps to be addressed.

There is significant untapped potential for the use of non-model organisms: very few strains have been investigated or had cultivation protocols developed, and much of the prokaryotic tree of life is unexplored. As an example, the entire domain of *Archaea* is under-represented in gas fermentations and in industrial biotechnology generally.

The potential of mixed cultures and microbial communities warrants more extensive investigation. Key questions include, what opportunities can they offer and when is the added complexity inherent in such systems of value? What are the trade-offs (ecological, technological, economic, regulatory) between synthetic biology and wild-type organisms, and between open and closed systems.

To unlock these opportunities will require the development of new tools for genetic characterisation and manipulation of non-model organisms and for mixed culture/community engineering.

Metabolic: Primary needs include a better overall grasp on the impact of external conditions on microbial metabolism, and on community structure where relevant. Key metabolic aspects include the role of electron transfer, electron donor selection, and electron bifurcation systems; the effect of microbial metabolites; and the prevention or mitigation of inhibition. More work is needed to explore spatial structure in microbial cultures, and how to manipulate and exploit it. Development and maintenance of biofilms is particularly relevant for many gas fermentations, given potential gains in mass transfer and volumetric throughput.

Mass transfer and hydrodynamics: Limitations in gas-liquid mass transfer are a critical factor in the design of most gas fermentation systems, and work is needed both to improve fundamental understanding of relevant factors and to develop better hydrodynamic models and design tools.

Experimental assessment is needed to clarify how fermentation broth properties (viscosity, surface tension etc) affect gas-liquid transfer and hydrodynamic behaviour. Better understanding of the rheological characteristics of these complex liquids is essential as a basis for engineering solutions with improved mixing and distributed biokinetics.

Improved insights into gas-liquid-biomass interactions in these complex multi-phase systems will elucidate how system design and operation can be used to modify the local micro-environment, and will also enable better design of scale-down experiments, allowing targeted investigation before transition to more expensive pilot-scale studies.

Process monitoring and control: Monitoring of fermentation parameters is vital for effective operation, and further advances in development of sensors and monitoring tools are needed to support this, with real-time in situ measurement of dissolved gas concentrations a particular priority.

Modelling: Multi-scale mechanistic modelling approaches have a critical role in this field. Simulation of bioreactors with integrated biokinetics offers a powerful tool for elucidation of microbe-microbe and microbe-environment interaction. The task is to bring together all levels from genetic, cellular and community through to bulk physical and chemical parameters. This will require liquid culture models covering cells and biofilms/flocs/granules and incorporating thermodynamics (metabolism) and hydrodynamics (flows and mass transfer) across scales relevant to the microbial environment.

Scale-up: One major topic requiring attention is scale-up, including the impact of scale effects on mixing and mass transfer and their repercussions for microbial metabolism and performance. To progress our understanding in this area will require both further development of open access facilities for gas fermentation, with investment in additional infrastructure; and more targeted support for scale-up and demonstration to move technology/integration readiness levels upwards.

Empirical and theoretical studies are needed to enable the development of high mass-transfer scalable gas phase bioreactors, and to allow understanding and exploitation of hydrodynamic and concentration gradients at full scale.

Other topics related to scale-up for technology progression include methods for hygienic operation of biofilm reactors; cost-reduction strategies where sterile or pure culture operation is required; and the development of cost-effective standard designs for gas fermentation reactors.

Feedstock, process and product: Several interlinked issues were identified concerning feedstock, process and product selection and diversification. There is a need for open and honest discussion of which bioprocesses/products to focus on. This could be supported by cost-benefit analysis of bioproduction methods for different classes of bioproducts e.g. bulk chemical, high-value and pharmaceutical.

Other factors to consider are the impact of gas quality and any purification requirements; and in the case of H₂ production, the need to accommodate intermittent renewable energy production while matching CO₂ supply conditions.

Significant work remains to be done on process selection for targeted products, and on recovery methods. One key aspect is recovery of non-volatile products from fermentation broths/liquors, and its effects on system biology, either directly via in situ extraction or in downstream processing and recycling. Technology innovations in this area must be closely linked to overall process optimisation, with tools for effective integration of up, down and sidestream processes a critical requirement.

Modelling, including AI and machine learning approaches, again has a key role to help answer 'what-if' questions in process control and operational decisions.

Mapping the location, scale and composition of gaseous and other feedstocks and linking this to logistics and markets is an essential step, both to identify specific process applications, and to assess the overall contribution of gas fermentation technologies to national and international net zero and sustainability targets.

Consideration should be given to the relative advantages of two-stage processes in which gaseous feedstocks are first converted into soluble form (e.g. formate, methanol) by physico-chemical means, before microbially-mediated conversion. This idea is attractive as it can reduce or eliminate some of the difficulties associated with gaseous feedstocks, such as mass transfer limitations and safety (flammability risks etc); although fermentation of these liquid feeds also has its challenges. Many of the key issues identified in the workshop - from systems biology to mixing and mass transfer, and from scale-up to process optimisation and investor confidence - also apply to systems of this type, however; and indeed are relevant to the development of a much wider range of industrial biotechnologies.

Actions for implementation

The multi-disciplinary nature of the subject has led to a perceived lack of holistic overview and knowledge integration in this area. Better understanding and communication is vital to create a generation that can engage effectively across disciplines and specialisms.

Staff recruitment can also be problematic, with current UK policies limiting access to the global talent pool. Talent acquisition and retention will be facilitated by initiatives to promote trans-disciplinary work and training.

Funding is key to progressing this area, via targeted cross-disciplinary, cross-sectoral funding opportunities. Open competitive challenges are an effective way to ensure progress, as is support for collaborative projects between industry and academia. Industrial engagement is also essential to enable informed appraisal and techno-economic assessment.

Many of the R&D needs identified above are also directly relevant to other microbially-mediated systems, and will offer performance benefits in a wide range of industrial biotechnology: progress in these areas thus adds value across the whole sector. Funding can be fragmented or subject to cross-Council remit issues, however, so targeted support is needed focusing on the interactions between physico-chemical, biological and engineering factors and on scale effects.

There is a clear need for dedicated funding streams to support scale-up, and for improved mechanisms to access such facilities. R&D and demonstration funding with a longer horizon for planned returns is also needed, to ensure the UK's place as an innovation leader rather than a follower.

Transparent reporting that facilitates comparison and sharing of data, models and practices is essential for rapid progress. There is a need to develop and promote agreed formats that allow 'anonymised' results to be collated for process data-mining on a wide variety of fermentations. Recent moves requiring accessibility of data and other outputs have been effective, and should be continued and strengthened.

Access to equipment and instrumentation at laboratory level is dispersed across Universities nationally. Safe working is essential even at small and pilot scale, and further initiatives to share expertise in H&S, HazID and HazOps should be promoted.

There is a lack of agility in contracts procedure and IP management in Universities. IP arrangements at University level could and should be simplified by development and sharing of sample agreements and templates.

Lack of trust by investors is an issue for all new technologies, perhaps especially in this area due to its relative novelty. Increased investor confidence could be promoted by identifying or creating new business cases with real positive societal, environmental and economic impact; as well as by raising the profile of gas fermentation technologies in general and by more focused support for technology translation and commercialisation.

For similar reasons the policy framework and investment climate are not fully supportive in this area. Initiatives are needed to inform policymakers and regulators on the potential contributions of these technologies, to promote their inclusion in broader policy assessments, and to facilitate the development of appropriate regulatory environments.

4 Summary of key R&D priorities

The overall goal is to develop our understanding of gas fermentation systems to allow optimisation of operational strategies and conversion efficiencies. Key areas for R&D to achieve this are:

Microbial

- Exploration of more diverse (i.e. non-model) microbial species and communities, including those able to produce novel products, deal better with contaminants, or work under more extreme environmental conditions
- Development of new tools for genetic characterisation and manipulation of non-model organisms
- Systems biology of unique microbes and microbial communities, and metabolic responses to their environment
- Gas-liquid-biomass interactions and microbial inhibition mechanisms during gas fermentation
- Data collection for development of multi-scale mechanistic and predictive modelling tools

Engineering Envelope

- Rheological properties of complex multi-phase liquids and their influence on gas and mass transfer
- Hydrodynamics of bioreactor mixing and mass transfer for process intensification
- Process monitoring tools (e.g. for measurement of dissolved gases and concentration gradients) as a basis for control and optimisation
- Multi-scale mechanistic models, incorporating metabolic and hydrodynamic aspects, to de-risk scale up
- Effective designs for bioreactor manufacture and operation

Other

- Scale-up studies, including the influence of scale effects and the development of reliable scale-down models
- Feedstock mapping and characterisation of production facilities
- Product selection and diversification
- Process integration and optimisation with upstream, downstream and sidestream components, including coordinating supply and demand
- Predictive modelling tools leveraging AI, machine learning and big data
- Support for economic and business models to improve investor confidence

Actions needed include:

- Cross-sector and cross-remit funding enabling collaboration between disciplines
- Sharing infrastructure, knowledge, facilities, data. 'Fair' data practices and methods for sharing anonymised data. Improved discoverability (e.g. searchable databases). Enable inter-institutional access to facilities, lab equipment, etc.
- Increased support for scale-up (construction of and access to facilities) to allow quicker iteration between research and pilot-scale implementation
- More agile university/industry collaboration arrangements (including resolution of tensions between academic publication and IP protection).

Gaseous feedstocks can present safety and operational challenges, and two-stage processes based on pre-conversion to liquid substrates (e.g. methanol, formate) also warrant attention. Many of the R&D issues identified here also apply to these processes, and addressing them would benefit a much wider range of industrial biotechnologies.

Follow-up is needed on the challenges and gaps identified: these should be re-assessed after 2 years to determine progress and further actions needed.



(See also Appendix 8 for versions of this and other visualisations of relationships between key points)

Advancing Gas Fermentation Technologies: A multi-disciplinary challenge

Gas fermentation technologies have the potential to revolutionise sustainable bioproduction, providing effective routes to carbon capture and utilisation and a transformative contribution towards net zero. Some pioneering examples are already at commercial scale, but to deliver their promise in full several key research and implementation issues need to be addressed. These challenges include interactions between complex microbial and engineering factors, as well as the importance of scale-up and technology transfer.

In the field of microbiology, there is a pressing need to enhance our understanding of fundamental systems biology, with significant knowledge gaps to be filled. Non-model organisms in particular offer massive untapped potential; but the necessary cultivation protocols and genetic tools are poorly developed. The potential of mixed cultures and microbial communities also warrants more extended investigation, including understanding the trade-offs between synthetic biology and wild-type organisms.

Metabolic aspects play a crucial role in gas fermentations. More detailed understanding of inhibition is needed, and of the effects of mixing and external conditions on microbial metabolism and community structure. Key metabolic factors include electron transfer, electron donor selection, and electron bifurcation systems. Exploring spatial structure in microbial cultures, including biofilm development and maintenance, is also essential to optimise these processes.

Limitations in gas-liquid mass transfer pose significant design challenges, necessitating better understanding of such processes. Improved insights will enable more efficient design of scale-down experiments and better prediction of performance at larger scales. Additionally, the impact of fermentation broth properties, such as viscosity and surface tension, on gas-liquid-biomass transfer and hydrodynamic characteristics needs to be experimentally assessed.

Multi-scale modelling approaches are needed that can simulate bioreactors with integrated biokinetics in order to elucidate microbe-microbe and microbe-environment interactions. These models must cover genetic, cellular and community levels, as well as incorporating thermodynamics and hydrodynamics across relevant scales. Such approaches, supported by advances in real-time monitoring, will aid in design and operational decision-making, allowing 'what-if' scenarios to be explored.

Scale-up is a major challenge limiting development in the field. Scale effects can significantly influence mixing and mass transfer, and thus microbial metabolism and system performance. To address this knowledge gap, better access to gas fermentation scale-up facilities is required, along with targeted funding. Empirical and theoretical studies are needed to support cost-effective designs for high mass-transfer bioreactors. Improved methods for hygienic operation of biofilm reactors must be developed, as well as cost-reduction strategies for sterile or pure culture operation where required.

Development and diversification of feedstock, process and product choices are interconnected issues. Open and honest discussions are needed to determine which bioprocesses and products to focus on. Techno-economic assessment and carbon footprinting of bioproduction methods for different classes of bioproducts are vital to support informed decision-making. Consideration must be given to the impact of gas quality, purification requirements, and intermittent renewable energy patterns. Process selection for target products and recovery methods requires further work, particularly in the recovery of non-volatile products from fermentation broths. Modelling, including AI and machine learning approaches, can aid in process optimisation and control.

Two-stage processes using gases converted by chemical catalysis into soluble form (e.g. formate, methanol) also merit attention as they eliminate some difficulties associated with gaseous substrates. Many of the key issues identified above also apply to these processes, and will benefit a much wider range of industrial biotechnologies.

To implement these advances, multi-disciplinary approaches are essential. Better understanding and communication across specialisms and disciplines is vital to support a new generation capable of engaging effectively in this field. Talent acquisition and retention can be facilitated through promotion of interdisciplinary work and training. Funding plays a crucial role in progressing gas fermentation technologies, and targeted cross-disciplinary, cross-sector opportunities are needed. Support for industry/ academic collaboration is essential, as is industrial engagement for informed appraisal and techno-economic assessment.

As experts, we believe that addressing these key research and development issues will unlock the full potential of gas fermentation to contribute to national and international sustainability and net-zero targets.

Workshop participants

Reuben Carr, Ingenza

James Chong, University of York

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This position statement was produced as part of a workshop on microbial systems with gaseous feedstocks run jointly by the Environmental Biotechnology Network (EBNet, www.ebnet.ac.uk) and the Carbon Recycling Network (<https://carbonrecycling.net>) on 27-28 March 2024. The goal was to identify key questions and knowledge gaps, R&D needs for technology progression and transfer, and actions that should be undertaken to promote progress and alleviate any obstacles.

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Appendix 2 Short Presentations

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For in-depth presentations see Appendix 3

Joint workshop on microbial systems with gaseous feedstocks – short presentations

Hated by the Environmental Biotechnology Network and the Carbon Recycling Network
Shringley Hall, Cheshire 27-28 March 2024



Workshop aims

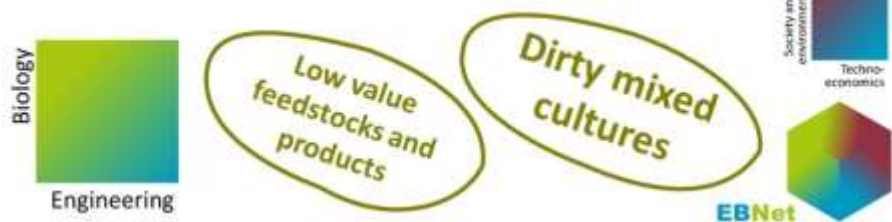
- We hope to (encourage you to) identify (some)
 - key research questions and knowledge gaps
 - R&D needs for technology progression and transfer
 - actions to promote progress and alleviate any obstacles
- Outcome(s) will take the form of a position statement or strategy document for circulation to relevant bodies and individuals (e.g. funding agencies and government and regulatory bodies)
 - And any clever spin-offs

Introduction - Sonia Heaven, EBNet / University of Southampton

Introduction - slide 2

EBNet strategic aim

- To bring together natural and social scientists and engineers to move discovery science towards practical application in creating and optimising **engineered microbial systems** for environmental protection, bioremediation and resource recovery

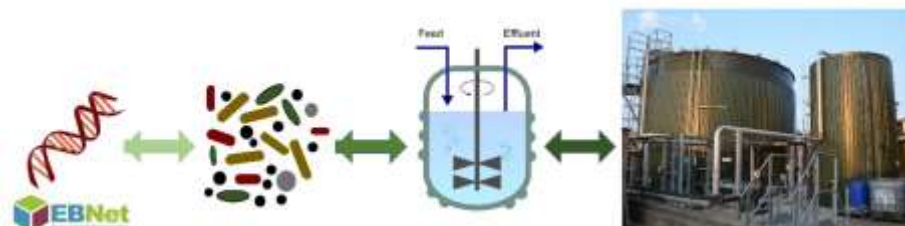


Introduction - slide 3

Opportunity

- Revolution in biosciences and analytical science
 - genetic to community level
- Accessed through engineering and technology
- Collaboration vital to move these through to higher TRL

Highly interactive



Introduction - slide 4



Workshop activities

Today

- Introductions
- **Coffee**
- Presentations



Follow-up

- Collate, circulate, amend, credit, *utilise*

Tomorrow morning

- **Presentation**
- **Ideas sessions**
 - Identify
 - Group
 - Prioritise
 - Summarise
- **Lunch and go**

Coffee

Metaplan?



People

- **EBNet:** Sonia Heaven, Network Managers Angie Bywater and Louise Byfield
- **Carbon Recycling Network:** Nigel Minton, Alan Burbidge, Loretta Waddon
- Charles Banks – ADNet director, CJC Labs



Introduction - slide 5

Introduction - slide 6

Claudio Avignone Rossa, University of Surrey



Bioresources research at CWSI

Sustainable treatment of biomass while maximising resource recovery

Dr. Yadira Bajón-Fernandez

Senior Lecturer in Bioresources Science and Engineering

Yadira Bajon-Fernandez, Cranfield University

Hi everyone!

Sorry that I cannot be there today, but thanks for the chance to introduce myself. I am Yadira, a senior lecturer at Cranfield University, where I lead the Bioresources research within the Water Science Institute and the Bioresource Technology Lab.



My passion is on bridging the gap between scientific discovery and full-scale implementation, for which I work very closely with industry and government. My research focusses on asset resilience and resource recovery from sludge and wastes through implementation of biorefinery concepts. In practice this means technology development and optimization on anaerobic digestion, dark fermentation, methanation, GHG & air pollutants abatement, solid/liquid separation, and others. Easier to see the next slide for current projects within the Bioresources Team ☺

I am currently seconded to the Department of Energy Security and Net Zero (DESNZ) within the UK government, supporting policy development to mitigate methane losses from AD sites. The ultimate aim is to ensure sustainability of biogas and biomethane generation.

CWSI Bioresources Team - current



Nasreen Nasar
AD Pre-treatments



Nnenna Chukwuekezie
Dry waste AD optimisation



James Manu
Sludge biorefineries / dark fermentation



Siqi Xu
Sludge pyrolysis



Muna Hassan
Mitigating methane emissions



Tracy Mupinga
Sludge dewatering



Steven Bungay
N & CH₄ from digestate



Chimamaka Amala
Wetlands intensification (inc. decentralised sludge)

Y Bajon-Fernandez - slide 2

Engineering biology – by design or operation

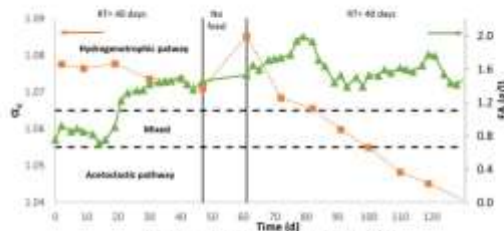


Fig. 4. Design of metabolic pathways and FA profiles for AD.

- We can control metabolic pathway of Methanosarcina by playing with HRT. It links to controlling the wash-out of SAOBs at high levels of inhibitors, which is strongly linked to H₂ supersaturation on the liquid
- This project has enabled Thalia Waste management to increase their biogas production in full-scale dry ADs

Y Bajon-Fernandez - slide 4

Y Bajon-Fernandez - slide 3



John Bridgeman & Davide Dapelo
Department of Civil and Environmental Engineering

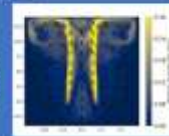
john.bridgeman@liverpool.ac.uk
D.Dapelo@liverpool.ac.uk

What we do


- Modelling anaerobic digestion processes
- Multiphase computational fluid dynamics and lattice Boltzmann modelling (LBM) of wastewater sludge flow and gas mixing in anaerobic digesters to optimise treatment by reducing energy and chemical usage

Key questions and knowledge gaps


- Can we use neural networks to improve gas mixing of microbial systems?
- Resolve reaction and flow patterns inside a vessel at mixing initiation via innovative LBM and pass resolved flow to a combination of deep, convolutional and recurrent neural networks to predict mixing performance over much longer, process-relevant time scales.



John Bridgeman/Davide Dapelo, University of Liverpool




CEAD
Centre of Excellence for Anaerobic Digestion




UNIVERSITY
of York

MY QUESTIONS, KNOWLEDGE GAPS AND
ISSUES



UNIVERSITY
of York



Professor
JAMES
CHONG

MULTI-OMICS

Genomics, transcriptomics, proteomics, metabolomics, lipidomics, and metabolite flux


SYSTEM-60

60 x 50
m² x 50
m² x 50 m²

BESPOKE PIPELINES

Analysis of multi-omics datasets (including metagenomics and metabolomics)


cead.york.ac.uk



What should we be making? From what?
What are the opportunities for mixed microbial communities?
How do we separate products from liquors?

Scale up (inc. mixing and safety)
Gas quality variability
Genetic tools for non-model organisms

Industrial engagement / funding
Trans- / inter-disciplinary working



James Chong, University of York

J Chong - slide 2

Dr. Christian Fink

Head of Synthetic Biology at Arkeon GmbH

- PhD in Methanogen Genetics at University of Tuebingen
- Master of Science at Archaea Center of University of Regensburg

What areas do I work in?

- Genetic engineering of biocatalysts for Gasfermentation processes
- Optimization- and development of genetic tools for gas fermenting Archaea
- We turn gas fermenting Archaea into cell factories for amino acids (or any other platform chemicals)

Key questions and knowledge gaps

- Can enough carbon dioxide be fixed as biomass in relation to ethanol/methane via Wood Ljungdahl Pathway to generate biomass derived platform chemicals in a commercially feasible way.
- Enhance electron bifurcation systems with genetic engineering?



CASE Group: Cellular Adventures in [Simulated/Scaled] Environments Delft University of Technology, the Netherlands

Pt. Dr. ir. Cees Haringa (Assistant Professor bioprocess engineering)

Group description: Transport limitations will lead to heterogeneous environments in industrial bioreactors, that may impact cellular metabolism and thereby process performance. This is often named as a cause of 'the valley of death' upon scale-up. We use computational fluid dynamics simulations with integrated biokinetics to study the impact of process conditions on cell metabolism, how lab experiments can be designed to study these phenomena, and how bioprocess design can be optimized

Research themes:

- **Bioreactor hydrodynamics:** Experimental assessment of gas-liquid hydrodynamics in fermentation broths
- **CFD simulation:** Simulation of bioreactors with integrated biokinetics to study cell-environment interaction
- **Reduced order models:** Coarse models for rapid assessment of heterogeneity & design optimization
- **Scale-down:** Design of lab-scale setups to study impact of heterogeneous conditions on cells
- **Applications:** (Syn)gas fermentation, precision fermentation, cellular agriculture, biopharmaceutical processes

Christian Fink, Arkeon Ltd

Cees Haringa, Delft University of Technology

Questions in gas fermentation

- Impact of conditions on rates and product spectrum:
How do (local) concentrations of dissolved gases affect product spectrum, production rates, e.g. $pCO \rightarrow$ acetate/ethanol ratio in syngas ferm. [1]
- Mechanisms behind the above [1]
How to control conditions to direct maximum flux to certain products
- Impact of broth composition on hydrodynamics [2,3,4]
How do components in the broth affect bubble size, mass transfer rates.
- Downstream processing
Impact of hard-to-remove byproducts, product titer, etc. on purification [5]

[1] Pulman et al., *Biochem. Eng. J., letter review*, [2] Pulman et al., [10.1016/j.bej.2022.108555](https://doi.org/10.1016/j.bej.2022.108555), [3] Volger et al., [10.1016/j.bej.2023.109124](https://doi.org/10.1016/j.bej.2023.109124), [4] Wang et al., [10.1002/aic.18291](https://doi.org/10.1002/aic.18291), [5] Jankovic et al., [10.1016/j.seppur.2023.124320](https://doi.org/10.1016/j.seppur.2023.124320) & [10.1002/jctb.7578](https://doi.org/10.1002/jctb.7578)

C Haringa - slide 2

Who I am?

- Klaas J. Hellingwerf, PhD in (bio)Chemistry, 1979
- Prof. emeritus in 'General microbiology', University of Amsterdam & 'Photophysics' at Free University Amsterdam
- Scientific Advisor of Photanol BV
- Consultant in Translational Biotechnology

Klaas Hellingwerf, University of Amsterdam

Why am I here?

- Since my retirement (2015) I have been lecturing at various public and academic events about the global carbon cycle and 'sustainability', with (artificial) photosynthesis as the starting point.
- Gradually, while preparing such lectures, the numbers from the IPCC report about the global carbon cycle, have made me convinced that anaerobic digestion of 'waste biomass' deserves a more prominent role in these discussions.
- Also, in the board of CCNet, at occasions, I expressed this view.

Some key questions, knowledge gaps and issues in this area:

- How to operate a thin film reactor hygienically?
- Systems biology of aerobic vs anaerobic gas fermentation
- Growth coupling of product formation in aerobic gas fermentation
- Systems biology, electron bifurcation, and enzyme specificity
- Process economy (and feedstock supply) in gas fermentation

K Hellingwerf - slide 2

K Hellingwerf - slide 3

Brief Introduction About Me and My Research

Ahsan Islam, PhD
Senior Lecturer in Biochemical Engineering
Loughborough University, UK
March 26, 2024

Ahsan Islam, Loughborough University

My Background

Bangladesh University of Engineering and Technology (BUET)
Process simulation with Aspen HYSIS

Imperial College London
CFD modelling of Haemolytic Sickle Cell Anaemia

Bachelor Research
MIT Massachusetts Institute of Technology
Engineering and analysis of cell metabolism

Master's Research
UNIVERSITY OF TORONTO
Engineering microbial community for bioremediation

Postdoctoral Research
Doctoral Research

A Islam - slide 2

Myself and My Research

- A biochemical engineer by training
- Research areas: metabolic engineering, systems biology, synthetic biology, bioinformatics, anaerobic microbiology, environmental biotechnology
- Apply both computational and experimental approaches in the mentioned research areas to solve important societal challenges regarding sustainability, environment, and human health

A Islam - slide 3



AARHUS UNIVERSITY

Michael Vedel Wegener Kofoed, Aarhus University

Who are we?
Dept. of Biological & Chemical Engineering, Aarhus University (AU BCE)
Research Group: Microbial Conversion Technologies

Our work within microbial gas fermentation aims to understand the intricate biological processes and their effects on upscaling in different technology pathways such as:

In situ biomethanation of:

- Biogas

Ex situ biomethanation of:

- Biogas
- Flue gas
- Syngas

Acetate production



What are the key knowledge gaps?

The **key knowledge gaps** within the development of microbial gas fermentation relate to both biological, physiochemical, and system bottlenecks.

The **role of biology** in gas fermentation is currently regarded as a black box with significant gaps in understanding the microbial dynamics that e.g. leads to side products such as acids and heat generation.

The **physiochemical barriers** related to working with H_2 have been identified as the main rate-limiting factors due to the poor solubility and gas-liquid mass transfer of H_2 .

The **gas fermentation system** should enable a flexible operation capable of accommodating intermittent energy patterns while complying to various CO_2 supplies. Additionally, utilizing the side streams to enhance the overall system efficiency.

By applying a holistic approach, we at AU aim to acquire an in-depth understanding of the principles of gas fermentation to develop bio-reactor systems that facilitate gas fermentation, which we scale to be directly be applied in a realistic environment.

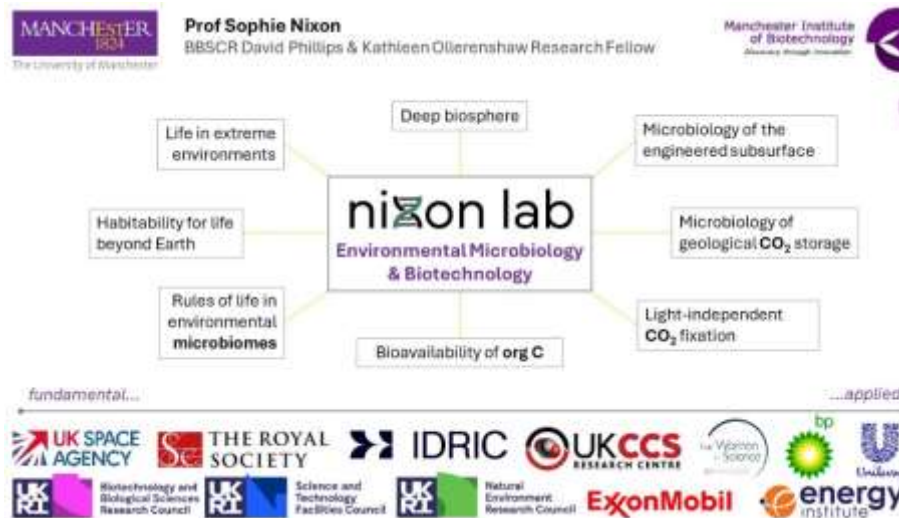
Example of ex situ biomethanation:



Example of in situ biomethanation:



M V W Kofoed - slide 2



Sophie Nixon, University of Manchester

M V W Kofoed - slide 3

Nature-based solutions to problematic carbon
Harnessing environmental microbiomes

Bioconversion of CO₂ emissions to useful products

How do **microbial communities** metabolise CO₂ in the absence of light?

What **knock-on metabolic processes** are triggered by CO₂ fixation?

How can we **engineer microbiomes** to yield value-added products from waste CO₂?

Biodegradation of polymeric organics

How do **microbial communities** degrade polymers?

What **knock-on metabolic processes** are triggered by polymer degradation?

Can we **harness enzymatic degradation pathways** from microbial communities to minimize environmental impact?

nixon lab

S Nixon - slide 2

Bart Pander, Frysland, Netherlands, Edinburgh Scotland, Father, Educator, Entrepreneur, Researcher

2013-2017 PhD on *Clostridium autoethanogenum*'s metabolomics and carbonic anhydrase

2017 Post doc on anaerobic digestion of high sulfate and high salt waste

2018-2021 Co-founded Deep Branch

2021-now Curriculum Development Gujarat Biotechnology University

2022-now Post-doc on Real time metabolomics for CHO cell bioprocess monitoring and industrial waste utilisation (side project)

2023-now University teacher Commercial aspects of Biotechnology

Broad knowledge of many aspects of biotech, ask critical questions and happy to share ideas, crazy, dumb and sometimes also useful.

THE UNIVERSITY OF EDINBURGH

Bart.Pander@ed.ac.uk

Bart Pander, University of Edinburgh

From your own perspective, what are some key challenges facing your research field?

- Holistic overview and integration of knowledge is lacking, key questions on towards commercialisation not asked
- Too few strains and their cultivation properly developed for actual industry
- Policy framework and investment climate not where it should be
- Fundamental systems biology still lagging behind compared to other biology

THE UNIVERSITY OF EDINBURGH

Bart.Pander@ed.ac.uk

Bart Pander - slide 2

Carbon Recycling Workshop

Marilene Pavan

LanzaTech Inc.

ABOUT ME

Marilene Pavan | from Brazil | living in Chicago

Biologist | 15+ experience | Synthetic Biology | Biomanufacturing | Metabolic Eng.

Monsanto | Braskem | Boston U. | Innovation Manager at LanzaTech (2019)

Technology Monitoring | Partnerships | Team Building | Early-Stage Evaluation | Landscape and Market Analysis (Technology, Competitors, New Markets...)

CARBON RECYCLING - KEY CHALLENGES

- 1) Scale-up investment and infrastructure
- 2) Development of genetic tools and characterization for non-model organisms
- 3) Broader policies that take all these technologies into consideration

LanzaTech

LanzaTech

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Marilene Pavan, LanzaTech

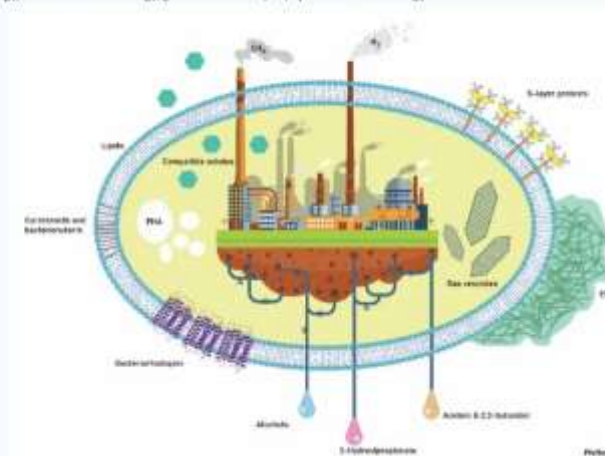
M Pavan - slide 2

Dr. Simon K.-M. R. Rittmann, Privatdoz.

Co-founder and Chief Scientist of Arkeon GmbH

PI and Head of Archaea Physiology & Biotechnology Group, Universität Wien

Archea physiology, archaea biotechnology, gas fermentation, C1, synthetic microbiology



Pfeifer et al. 2011, J. Bacteriol. Adv. Biosci. et al. 2011, Biotechnol.

26

Simon Rittmann, University of Vienna

S Rittmann - slide 2



Ioannis V. Skiadas
Associate Professor

DTU CHEMICAL ENGINEERING
Department of Chemical and
Biochemical Engineering
PILOT/PLANT
Danmarks Tekniske Universitet
Søtofts Plads
Building 228, Room 019
2800 Kgs. Lyngby



Phone: +45 45252729
Mobile: +45 21171915
Email: iusk@kt.dtu.dk
ORCID: 0000-0001-6183-4592
vCard: vCard

<https://netzerotube.com/videos/syngas-as-a-renewable-energy-source>

Research areas

- Syngas and CO₂ fermentation to methane, organic acids and alcohols focusing on the use of trickle bed reactors (TBR) and mixed microbial cultures (biofilm on the packing material) originating from anaerobic digestion
- During the last couple of years, a significant part of our research effort was to analyze mass transfer phenomena and we have recently developed a simulation tool that allows us to predict the kLa of different gasses as functions of the reactor and packing material geometry as well as liquid and gas flowrates.
- The tool can be used for the design and upscaling of efficient (from lab to pilot and to full scale) TBRs.

<https://doi.org/10.1016/j.ces.2023.145085>

Ioannis Skiadas/Antonio Grimalt-Alemany, Technical University of Denmark | Skiadas/A Grimalt-Alemany - slide 2

DTU Orbit

Home Profiles Research units Publications Activities Projects Prizes ...



Antonio Grimalt-Alemany

Postdoc, Department of Chemical and Biochemical Engineering
Bio Conversions
<https://orcid.org/0000-0001-7652-743X>

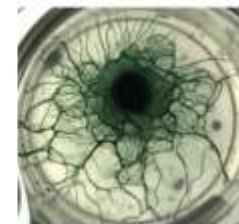
Phone:
50344426

Email:
anral@kt.dtu.dk

Website:
<http://www.kt.dtu.dk>

[View Scopus Profile](#)

Saltøffs Plads, 227, 037
2800 Kgs. Lyngby
Denmark



OSS LAB Orkun S. Soyer
O.Soyer@warwick.ac.uk

EBNet mini workshop, Macclesfield, March 2024

Community function and stability, spatial organization, (cyanobacterial) microbial communities, modelling, thermodynamics, resource-consumer models.



I Skiadas/A Grimalt-Alemany - slide 3

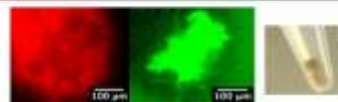
Orkun Soyer, University of Warwick

QGI in "mass transfer between gases and microbial cultures growing in a liquid phase or film":
Why (and how) spatial structure? How to exploit it? How to manipulate it?

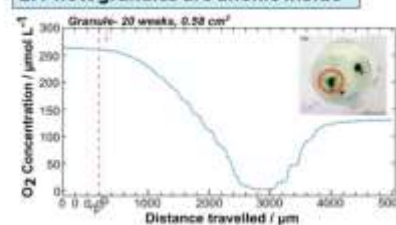
1. Spatial structures (mats and photogranules) are common!



3. Photogranules actively "forage" environmental minerals / particles!



2. Photogranules are anoxic inside



Some relevant literature, FYI:

Duxbury S et al., *Interface Focus* 13:2, (2023).
Duxbury S et al., *BioRxiv* (2023).
Trebuch, LM et al. *ISME J* 17 (2023).
Abouhend AS, et al. *Environ Sci Technol*, 54:1 (2020).

And a movie:

O Soyer - slide 2

O Soyer - slide 3

Adrie Straathof
a.j.j.straathof@tudelft.nl

Associate professor and Section leader
Bioprocess Engineering
Department of Biotechnology
Delft University of Technology
The Netherlands

CRNet/EBNet workshop on
Gas Fermentation
Chester, 27 March 2024

TU Delft



Adrie Straathof, Delft University of Technology

Principal investigators @ TU Delft involved in gas fermentation



 Henk Noorman Bioprocess design	 Ludovic Jourdin Microbial electrosynthesis	 Jean Marc Daran Genomics in MES
 Cees Haringa Computational fluid dynamics	 Robert Kleerebezem Mixed cultures	 Tony Kiss Bioproduct recovery
 John Posada Bioprocess assessment	 Adrie Straathof Bioprocess integration	

A Straathof - slide 2

Syngas fermentation

Key questions, knowledge gaps and issues

- On-line measurement of dissolved gas concentrations
- Predictive models for $k_L a$ (especially a) in microbial broth
- Concentration gradients in industrial-scale reactors, and how to mitigate or exploit them
- Qualitative understanding of microbial kinetics: Why 2,3-BDO formation?
- Product diversification; metabolic engineering; metabolic models
- Recycling microbial broth after downstream product removal
- Recovery of non-volatile products
- Integration with upstream processes; gas recycles; impact of gas impurities; gas purification

A Straathof - slide 3

Microbial electrosynthesis using biofilms

Key questions, knowledge gaps and issues

- Designing scalable/stackable reactors, and their cost-effective production
- Modelling flow and diffusion in reactors, through biofilm/electrode/membrane
- Understanding microbial kinetics, understanding electron transfer
- Measuring concentration gradients in biofilms, and how to mitigate or exploit them
- Identifying microbes in open cultures, using the best ones in defined cultures
- Product diversification
- Anodic reactions besides $H_2O \rightarrow O_2$
- Integration with upstream processes; gas recycles; impact of gas impurities; gas purification
- Recovery of products

A Straathof - slide 4

About me

General expertise

Process engineering of biochemical systems

Specific expertise

Anaerobic digestion, biogas, waste/wastewater

Main methods

Process modelling and systems assessments (TEA, LCA, CF)

Relevant experience

Mass transfer in gas-liquid reactors (for CO₂ biomethanation)



Dr Mark Walker
School of Engineering
UNIVERSITY of HULL

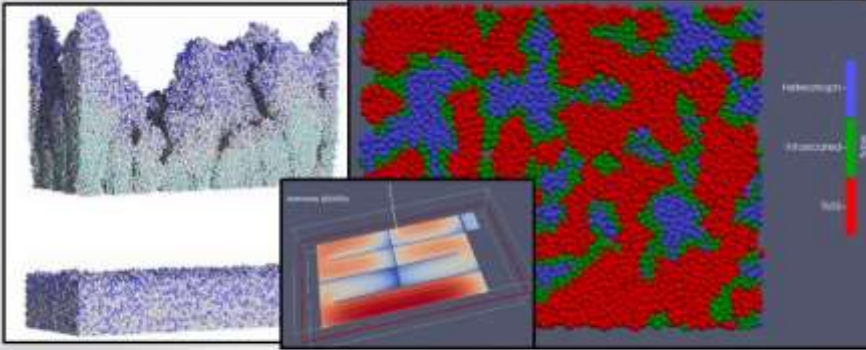
Questions

- How much should we care that we only measure bulk/macroscopic characteristics, but microorganisms predominantly influenced by highly localised environmental conditions?
- How do we know when mass transfer is limiting? (often difficult to measure intermediates)
- Relatedly: How do we predict (or even know definitively) when enough (mass transfer) is enough? How much does this depend on the application, organisms etc.?
- Are particular equipment/reactor designs suited to particular applications/organisms?
- What are critical design criteria for mass transfer/mixing systems? (given multiple process requirements).




Mark Walker, University of Hull

M Walker - slide 2



Joseph E. Weaver (he/him)
Joe.Weaver@newcastle.ac.uk
@joe.e.weaver
@joeweaver.bsky.social
joeweaver.github.io



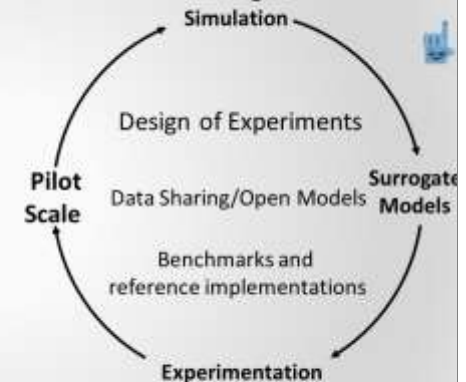
NSF Postdoctoral Fellow
Visiting Researcher
Newcastle University

Joe Weaver, Newcastle University

Questions and issues

- When is the complexity of a mixed community worth it?
- Trade-offs between synthetic biology and wildtype organisms
 - Technologically, economically, regulatory
 - Ecologically
- When is it useful (and how!) to switch between desired products
 - Or between varying feedstocks
- Product generation as main goal vs. 'augmented waste treatment'

The A way forward



How do we answer 'what-if' questions in design & operation decisions?

J Weaver - slide 2

Yue Zhang



❖ Water and Environmental Engineering Group, School of Engineering

➤ Bioprocesses for resource recovery from organic waste and wastewater

- Anaerobic digestion
 - ✓ In-situ biomethanisation of CO₂
- Bioelectrochemical processes
- Mixed-culture fermentation and chemical-free downstream processes

➤ Questions and issues

- Selection of electron donors, e.g. H₂ vs electron; one-stage vs two-stage
- Opportunities for detailed engineering appraisal and techno-economic assessment

51

Yue Zhang, University of Southampton

Why was I invited? Microbubble intensified/accelerated bioprocessing, e.g. Anaerobic Digestion, Microalgae, but also downstream and in situ separations



• Best R&D Innovation - Sponsored by Cooper Oxford Ltd

Winner: Perlemax Ltd and Partner Viridor. In situ Ammonia Removal in the Desai-Zimmerman Anaerobic Digester

The Award Ceremony - 2019

- Desai-Zimmerman AD fermenter now achieves up to 13-fold increase in biogas production rate due to microbubble intensification in wet food waste digesters over conventional unsparged AD. Less than 2 days to get all the biogas out, vs. 20-25 days conventionally (pilot scale with Viridor at Parkwood/Sheffield).

- Proposed mechanism is due to "Desai artificial lichen" – microbial consortium coordinated around microbubble aggregate produces hydrogen with acidogens / acetogens and transfers the hydrogen gas directly across the microbubble to the methanogens to make methane.

- In situ ammonia removal by hot microbubble stripping. Removes 95% of ammonia in 2 minutes contact time (industrial stripping: 100hrs!)



ICHEME Moulton Medal: Gas-lift loop bioreactor

W Zimmermann - slide 2

Will Zimmerman



Professor in Chemical, Material, and Biological Engineering at the University of Sheffield

Who am I?

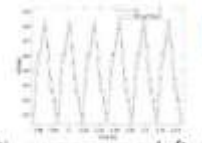
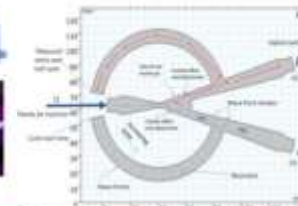
- Chemical Engineering graduate of Princeton (BSc, Eng), Stanford (MSc, PhD) with minors in pure and applied maths, respectively.
- Winner, Royal Society Innovation Award for (Energy Efficient) *microbubble* generation via fluidic oscillation.
- CEng, FICHEME, co-founder of one spinout (Perlemax) and two startups (Reepel, vertical farming + bioreactors; Matsya, aquaculture)

CCm Technologies (lead), Reepel and Perlemax hold current InnovateUK and DESNZ grants totaling £2.6m on CCU with biological and chemical approaches.



Will Zimmermann, University of Sheffield

We also make fluidic oscillators: DZFO



- Pressure traces on outlet leg show fluid kinetic energy conserved after 200 diameters (Re ~100000)!
- Microbubbles produced with ~100 millibar pressure drop across diffusers
- Hybrid synthetic **jet disrupts tangential boundary layer formation in pipes**
- With Rachel Rothman & Ann Call – showed **mass transfer limitation removed** with **impinging jets** in electrochemical reactions.
- Useful for cleaning and biofilm removal



World's largest fluidic oscillator:
Throughput ~2400 cubic metres / hr



Application to aeration of municipal wastewater

W Zimmermann - slide 3

From your own viewpoint, what are some key questions, knowledge gaps and issues in this area?



Developing new tools in bioprocessing ...

- What are the mechanisms for microbubble – microorganism interactions? How can they be tuned for symbiotic engineering?
- Can microbubble absorptive processes intensify metabolism? Facilitate downstream processing or *in situ* separations?
(Foaming or frothing with microbubbles attracting bioproducts in the plateau borders)
- Microbubble *reactive*-separations, say for removal of higher value added molecules? Similar notion to conventional reactive extraction in chemical processing.

W Zimmermann - slide 4

Appendix 3 In-depth presentations

Contents Appendix 3

Day 1

[Sandra Esteves, University of South Wales](#)

[Raul Muñoz, Universidad de Valladolid](#)

[Will Zimmermann, University of Sheffield](#)

Day 2

[Kristi Potter, Centre for Process Innovation](#)

R&D Project Examples - Gas Fermentations



Environmental Biotechnology Network and
Carbon Recycling Network Workshop
27-28th March 2024

Prof. Sandra Esteves and Dr. Savvas Savvas

© University of South Wales

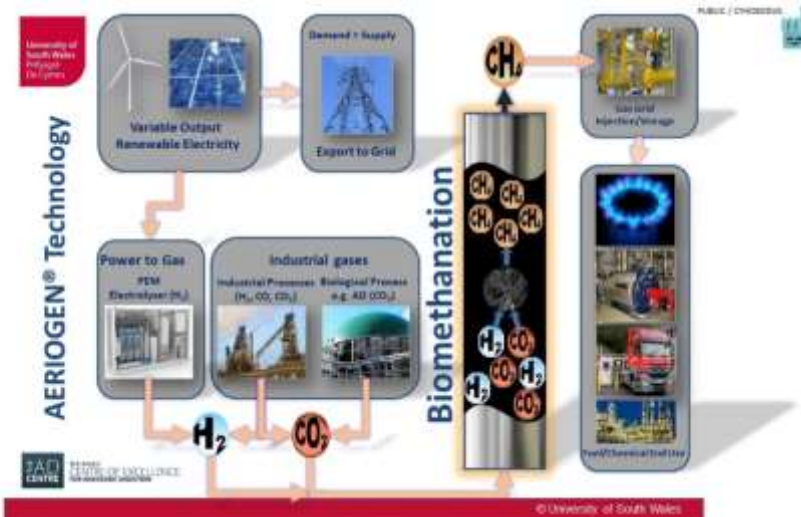
Gases Fermentation Lab Facilities



© University of South Wales

Sandra Esteves, University of South Wales

S Esteves - slide 2



© University of South Wales

S Esteves - slide 3

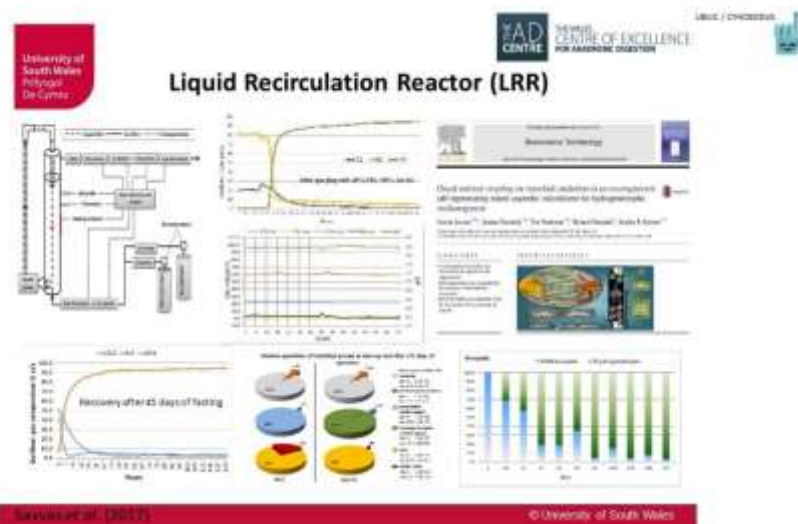
USW Biomethanation Process USPs

- Ex-situ process
- Process stability/robustness under varying conditions: mixed microbial culture
- Low nutrient input requirement: nutrient recycling & culture self sustenance
- Lower capital costs & improved returns: high conversion rates & reduced footprint
- Lower operating costs & improved returns: reduced energy input
- Flexibility to convert to carboxylic acids or methane: chemical and fuel vectors for multiple products

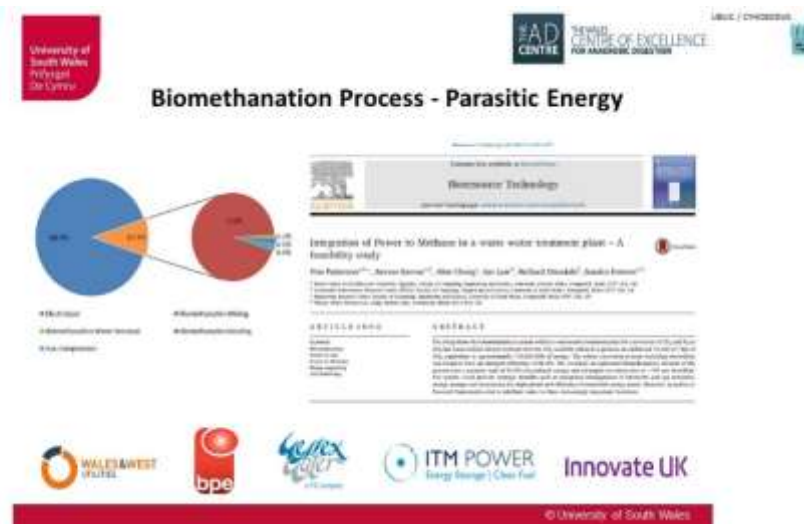


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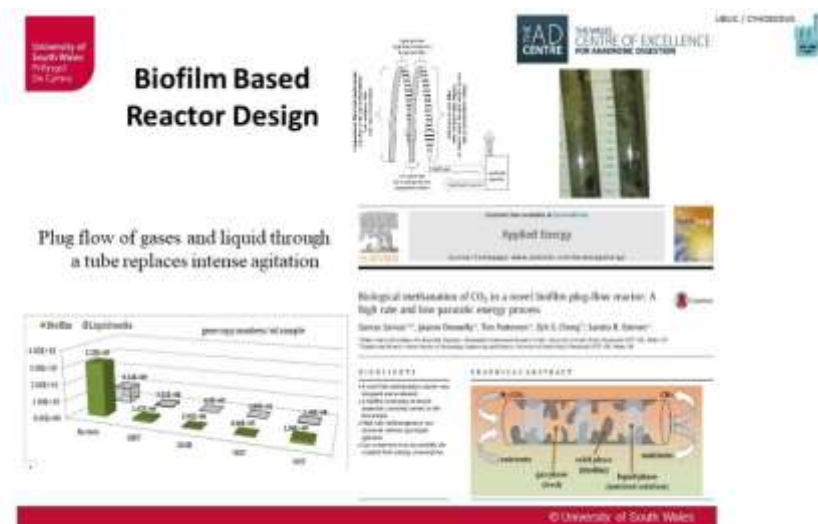
S Esteves - slide 4



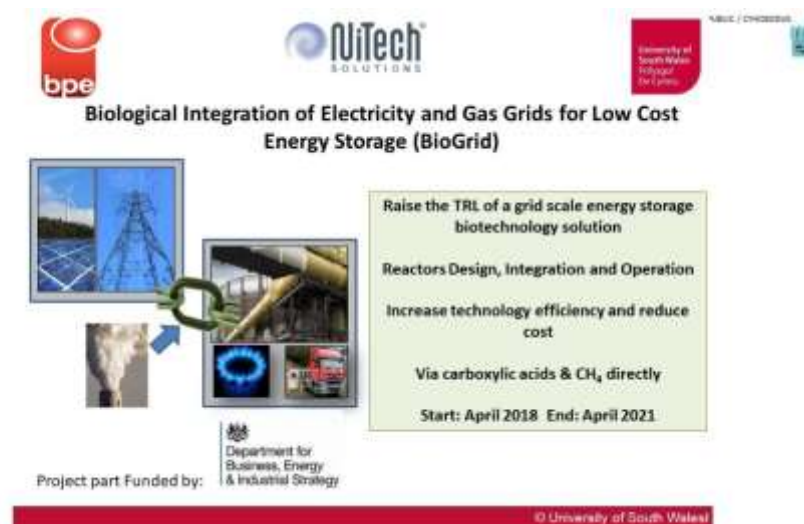
S Esteves - slide 5



S Esteves - slide 6



S Esteves - slide 7



S Esteves - slide 8

BioGrid Project Partners



Biomethanation
Carboxylic Acids

Expertise in Anaerobic Biotechnology & Ancillaries - IP related to Self-Sustaining Robust Mixed Culture, Nutrient Recycling for $H_2/CO_2/CO$ Conversions

Scientific Understanding, Commercialisation & Impact



IP in Novel Oscillatory/Tubular Baffled Reactor Technology - Expertise in Reactor Design and Manufacturing

Extend Tech Market



Expertise in Process Engineering Design, Integration, Instrumentation, Control, Safety; Process Development, Modeling, Scale-Up and Optimization

Extend Services to New Market





© University of South Wales

S Esteves - slide 9


Biogrid Project Dedicated Reactors



© University of South Wales


S Esteves - slide 10

Numerous Reactors Evaluated




Liquid Recirculation Reactor (LRR)

180 VVD
Extensive Foam; 215 VVD pump broke down, ~15% parasitic energy load



Oscillating Baffle Reactor (OBR)

>250 VVD
Foam and shear reduction, ~10% parasitic energy load, fast inoculation



Continuously stirred tank reactor (CSTR)

500 VVD
High shear, ~20% parasitic energy load

20% parasitic energy load; scalability issues, rapid inoculation not possible

Versus

Negligible parasitic load, 200 VVD, biofilms homoacetogenic route

Use to Gas Plug Flow Fixed Bed Reactors

Confidential

S Esteves - slide 11

SMARTExpertise: T Collaboration for an Industrial Resource uLar Economy (CIRCLE) Project Partners

Sept. 2017 – Oct 2022



















● Biomethanation / CO_2 Utilisation

● Heat Recovery

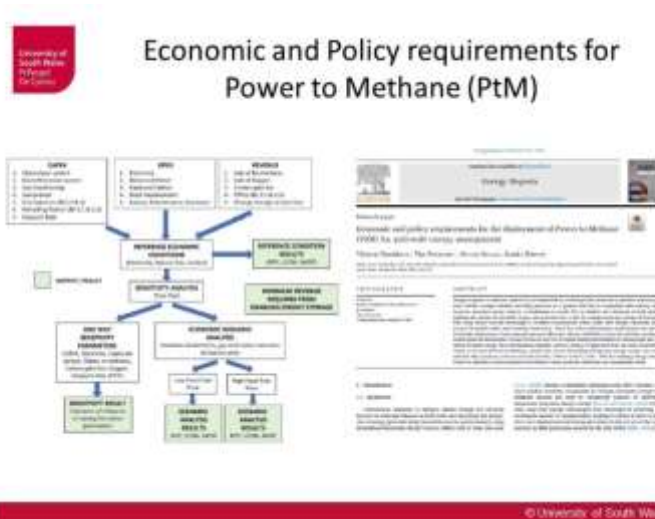
● Nutrient / Metals recovery

● Advanced Process Monitoring

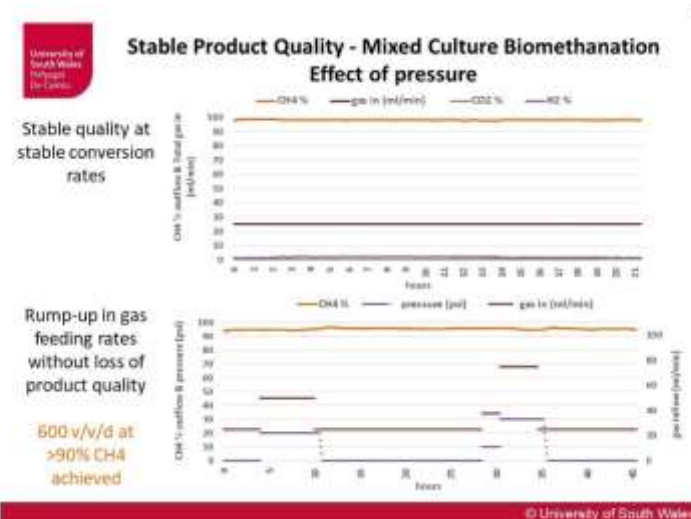
● High Value molecule recovery

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S Esteves - slide 12



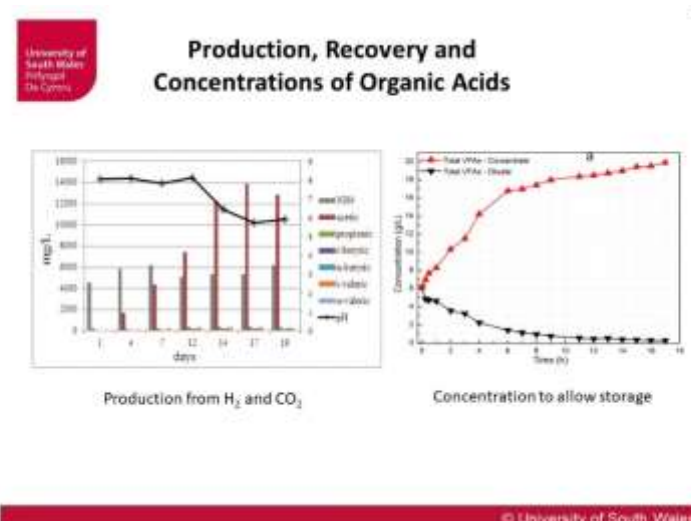
S Esteves - slide 13



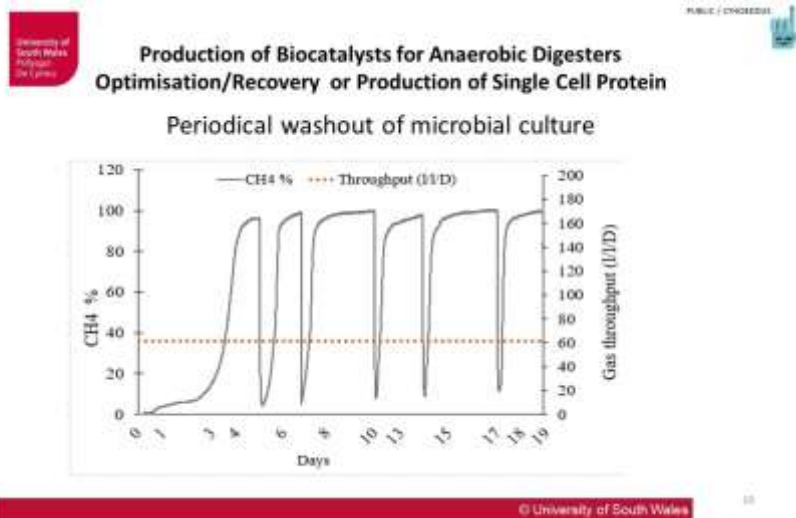
S Esteves - slide 14



S Esteves - slide 15



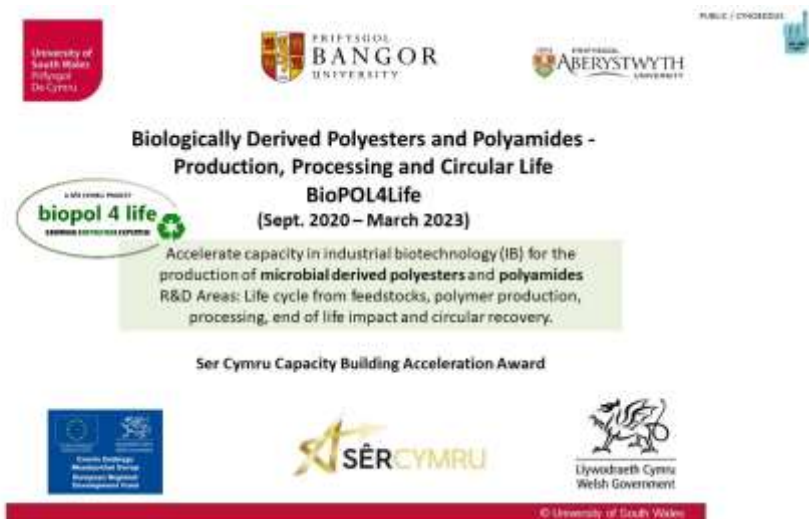
S Esteves - slide 16



S Esteves - slide 17



S Esteves - slide 18



S Esteves - slide 19



S Esteves - slide 20

University of South Wales
Cyfrithon

AD CENTRE
THE WELSH CENTRE OF EXCELLENCE
FOR ANAEROBIC DIGESTION

Public / Cyhoeddus

Production of Higher Chain Alkane Gases (C2-C4) From Anaerobic Biological Processes

- Higher chain alkanes required to meet gas grid quality gas
- Increase the gaseous stream sustainability and further decarbonisation of the gas grid
- Dense and sustainable heat and transport fuels

Wales & West
Wales & West

kess

BEACON
from plants to products
© Hartington & Glynrhondda

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S Esteves - slide 21

Acknowledgments

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S Esteves - slide 22

ISP
Institute of Sustainable Processes
UNIVERSITY OF SOUTH WALES

EBNet
Environmental Biotechnology

INTRODUCTION AND BACKGROUND

Raul Muñoz Torre

Raul Muñoz, Universidad de Valladolid

What did I do with my life?

EBNet

ISP

Bachelor Degree: CHEMICAL ENGINEERING	
Institution: Universidad de Valladolid	
Date: 2001	
Erasmus Stay: 6 months at Lunds Universitet (Sweden)	
PhD Degree: PhD in Environmental Biotechnology	
Institution: Lunds Universitet (Sweden)	
Date: 2005	
Thesis: Algal-Bacterial Photobioreactors for the Degradation of Toxic Organic Pollutants	
Supervisors: Bo Mattiasson & Benoit Guieysse	

R Muñoz - slide 2

What did I do with my life?

Position: JUAN DE LA CIERVA Researcher

Institution: Universidad de Valladolid

Dates: November 2005-December 2007

Position: RAMÓN Y CAJAL Researcher

Institution: Universidad de Valladolid

Dates: January 2008-December 2012

Position: Associate Professor

Institution: Universidad de Valladolid

Dates: December 2012- November 2020

Position: Full Professor

Institution: Universidad de Valladolid

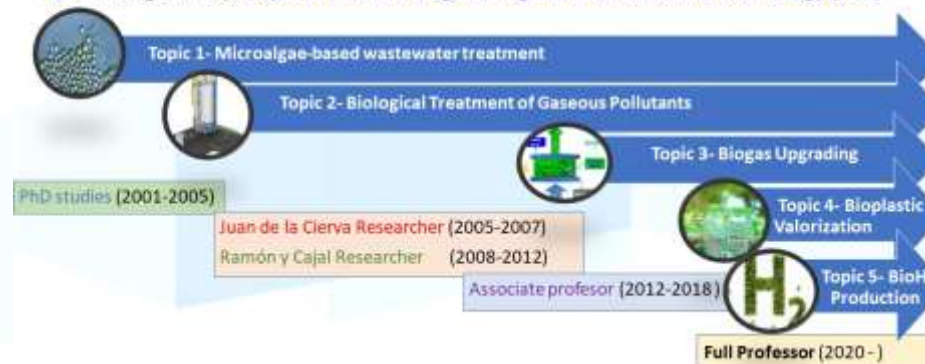
Dates: November 2020-To date



What did I do with my life?

Research activity: Institute of Sustainable Processes (Uva)

Teaching activity: Dept. of Chemical Engineering and Environmental Technology (Uva)

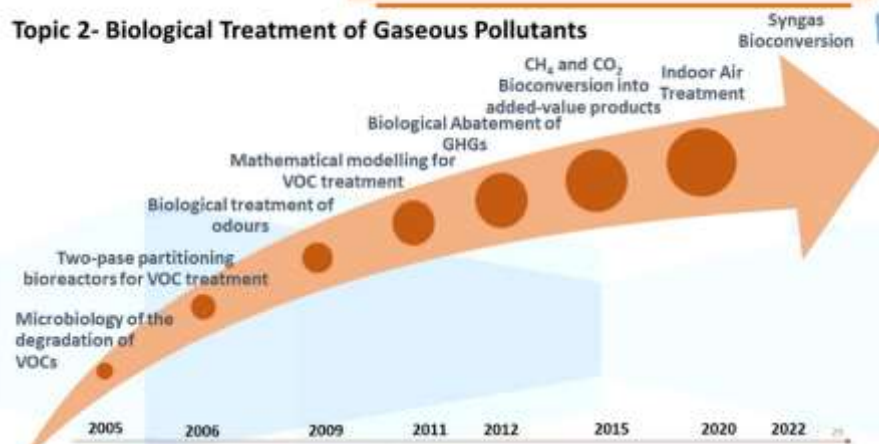


R Muñoz - slide 3

R Muñoz - slide 4

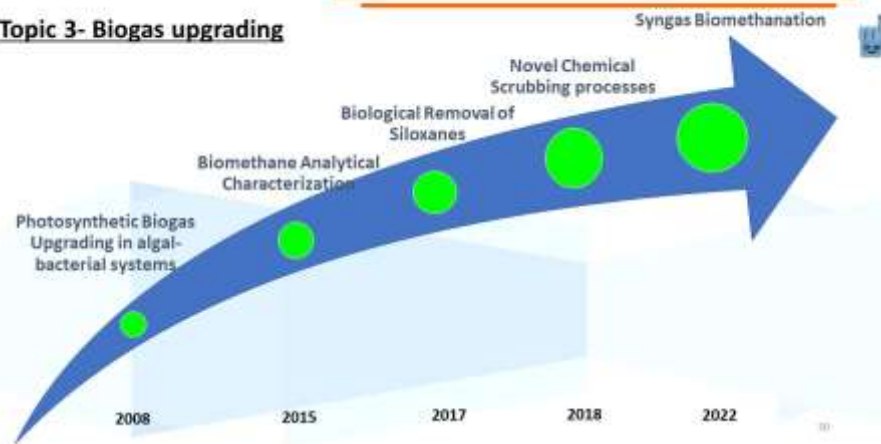
Core Research Lines

Topic 2- Biological Treatment of Gaseous Pollutants



Core Research Lines

Topic 3- Biogas upgrading



R Muñoz - slide 5

R Muñoz - slide 6

Research Niches in Biological Gas Treatment



Chemical &
Biological
Engineering



Environmental Problems Solved before XXI Century



Environmental Problems to be Solved in this XXI Century

- Odour Treatment
 - Lack of specific regulations
 - Limited performance of the hydrophobic VOC fraction
- CH₄ abatement
 - Limited gas-liquid transfer
 - Inhibition by metabolites
- N₂O abatement
 - Microbiological limitations
- CO bioconversion
 - Limited gas-liquid transfer
- H₂ assisted CO₂ bioconversion
 - Limited gas-liquid transfer



Microbubble Intensification of Anaerobic Digestion: The Role of Desai Artificial Lichen

Professor Will Zimmerman

BSc (Princeton) MSc PhD (Stanford) CEng FIChemE,
Chemical and Biological Engineering, University of Sheffield
Chairman & Founder, Perlemax Ltd.

Dr Pratik Desai, Perlemax. Winners: ADBA R&D Innovation of the Year, 2019.

Acknowledgments to S. Wilkinson, M. Al-Mashhadani, W. Nugroho, U. Haji-Hassan.
Thanks to InnovateUK and EPSRC.

R Muñoz - slide 7

Will Zimmermann, University of Sheffield

Outline

- How do we make the microbubbles?

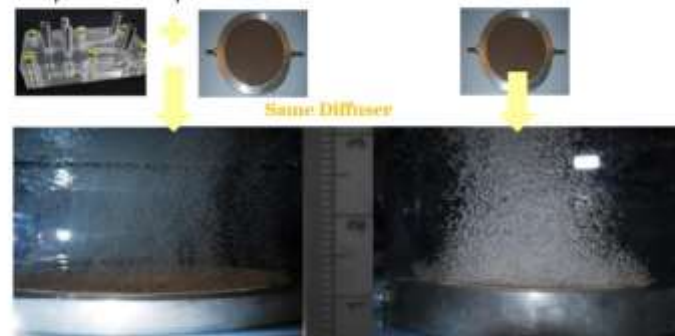
How could microbubbles intensify AD?

New horizon with microbubble distillation

- Extraction of ammonia from aqueous solution.

Evidence for direct microorganism—microbubble gas exchange.

Fluidic oscillator makes microbubbles! Royal Society Innovation Award 2010



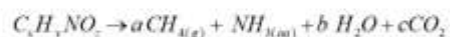
- 20 micron sized bubbles from 20 micron sized pores
- Rise / injection rates of 10⁻⁴ to 10⁻³ m/s without coalescence: uniform spacing/size

W Zimmermann - slide 2

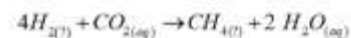
W Zimmermann - slide 3

Le Chatelier's "principle" adapted to anaerobic digestion.

In order to make biomethane, the bioculture mediates a reaction like:



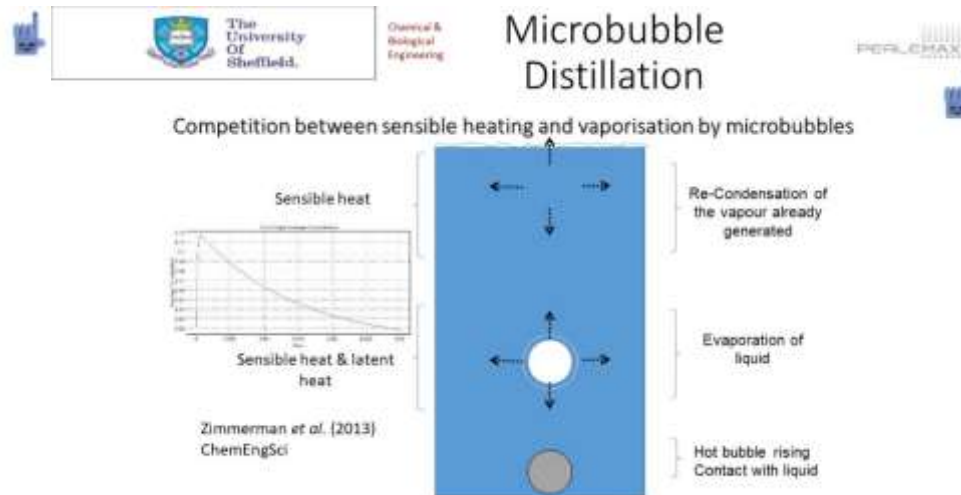
With the final reaction conducted by methanogens



Suppose these were equilibrium reactions. Le Chatelier's Principle says that we can expect more product (methane) if we

1. Remove methane = Le Chatelier's "pull" Al-Mashhadani et al, 2016
2. Push in more carbon dioxide = Le Chatelier's "push". Elise Cartmell / Cranfield
3. Push in more hydrogen = Le Chatelier's "push". H2AD / Southampton
4. Remove ammonia = Le Chatelier's "pull" U. Haji-Hassan + Perlemax

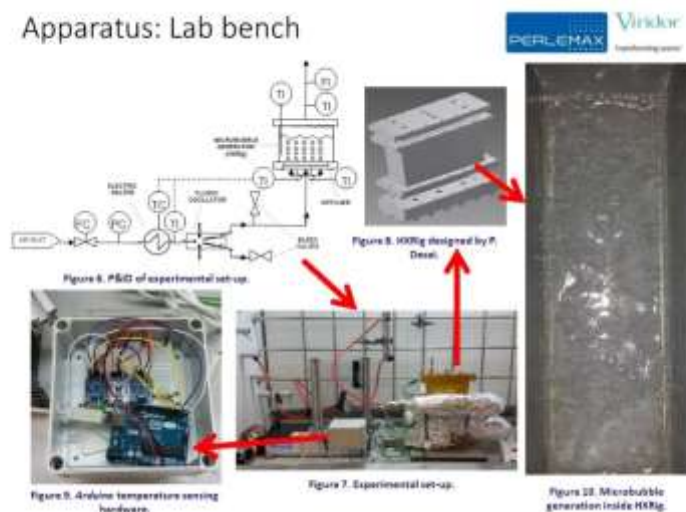
Does inhibition removal act like Le Chatelier's pull?



W Zimmermann - slide 4

W Zimmermann - slide 5

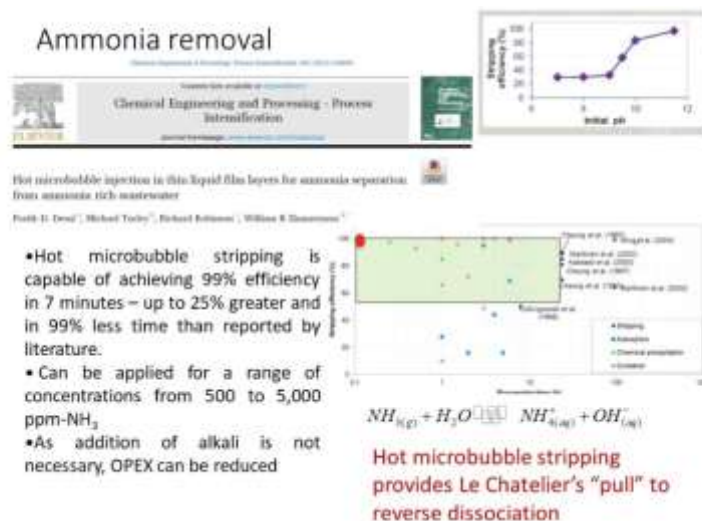
Apparatus: Lab bench



W Zimmermann - slide 6



W Zimmermann - slide 7



W Zimmermann - slide 8

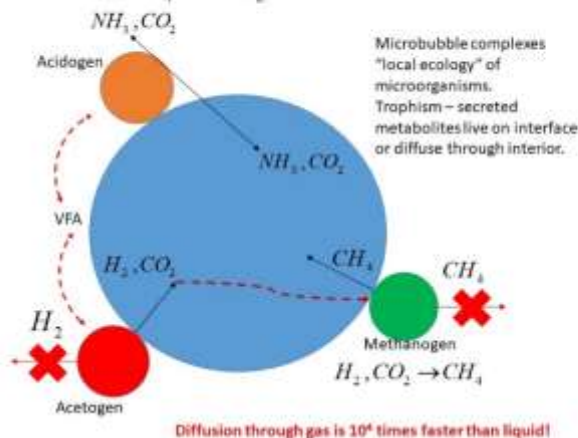
Bailey and Ollis popularized the two fluid theory for gas transfer in fermenters. But what about nearly insoluble gases such as methane and hydrogen?

A. Canonical two film theory: Dissolved gas transport is liquid mediated.



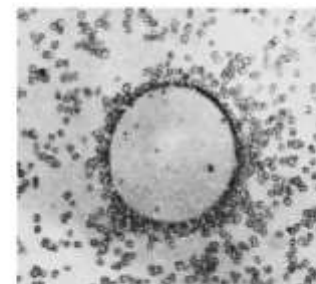
W Zimmermann - slide 9

Big problem with conventional theory of bubble mass transfer – CH₄ and H₂ do not swim!



W Zimmermann - slide 10

Do DALs happen in real life?



Affinity of yeast to a coarse bubble, from Ouchi and Akiyama (1971). Yeast are well known to be oxygen starved.

Examples with larger bubbles

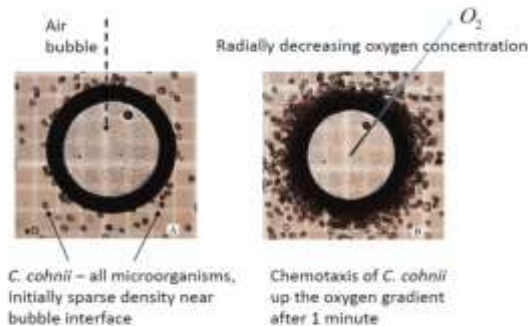
Samra FA, Wardner JS, Nelson GL, Pech-Arce L, Weber PG, Mayal V, Attachment between heterotrophic bacteria and microalgae in fluorescent symbiotic microcapsule interactions, *Environmental Microbiology*, 2018, doi.org/10.1111/1365-2656.13157

Kakama S, Czeckow H, Van Nguyen TT, Croft MT, Shenwood S, Sasse S, Imboden SC, Warren M, Smith AG, Alkhalaf H, Interactions between vitamin B12-dependent algae and heterotrophic bacteria exhibit regulation, *Environmental Microbiology* (2022) 1495, 1489–1470.

Ouchi Y, Akiyama M (1971) Non-Floating Mutants of *Sake Yeasts*, *Agricultural and Biological Chemistry*, 35:7, 2024–2032.

W Zimmermann - slide 11

Chemotaxis to large bubbles. Why not small?



Photographs of an air bubble trapped between a hemocytometer and the cover slip, the air bubble being surrounded by a suspension of motile *Cryptocodinium cohnii* microalgal cells. B is taken 1 minute after A. From Hu et al. (2010).

So how would you know if direct microbubble-microorganism gas exchange were occurring?

One answer: Lower dissolved oxygen levels for microbubbles than for conventional in municipal wastewater treatment.



Two Tesar-Zimmerman (Tesar et al. 2006) fluidic oscillators (two inch diameter connections) feeding one of two sequencing batch reactors (SBR) on a municipal wastewater treatment works. The control SBR was fed air from a bank of blowers ducted into the same header, hence the same pressure source to both SBRs, outfitted with industry standard membrane slit diffusers. Both SBRs were fed activated sludge from the same source tank.

Old TZFO: 35-50% kLa improvement in clear water, but lower DO in WW.
Yet SBR batch done 40% faster.
New DZFO: 90% higher kLa in clear water, ~25% increase in DO over steady flow.

W Zimmermann - slide 12

W Zimmermann - slide 13

Faster AD with pure CO2 microbubbles



- Desai-Zimmerman AD fermenter now achieves up to 13-fold increase in biogas production rate due to microbubble intensification in wet food waste digesters over conventional unsparged AD. Less than **4 days** to get all the biogas out, vs. **20-25 days** conventionally.
- Proposed mechanism is due to "artificial lichen" – microbial consortium coordinated around microbubble aggregate produces hydrogen and CO_2 with acidogens / acetogens and transfers the hydrogen gas directly across the microbubble to the methanogens to make methane.
- In situ* ammonia removal by hot microbubble stripping.

Please read the seminal paper on DALs – feature article in JMAT TechRev in special edition on bioprocessing

Desai PD, Zimmerman WB. Microbubble Intensification of Bioprocessing: The role of direct microorganism and bubble interactions. Johnson Matthey Technology Review. 2023. DOI:10.1595/205651323X16778518231554



Thirteen sample vials, taken each minute, from a DZ flotation column. Removing microalgae from an oil-rich wastewater – in 12 minutes!



W Zimmermann - slide 14

W Zimmermann - slide 15

Modelling & simulation for biological processes

Kristi Potter

Process Engineer, Biotechnology



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We help companies to develop, prove, scale-up and commercialise new products and processes



cpi

Kristi Potter, Centre for Process Innovation

K Potter - Slide 2

We help deliver, de-risk and accelerate...



...your concepts into successful products



Biotechnology team assets and resources

CPI has designed and operates four facilities to deliver our Industrial Bioprocessing services (operating to ISO9001 standards):

- **Fermentation laboratory** 1ml to 10L scale down facilities for microbial strain characterisation and bioprocess development. CI gas enabled fermenters (CO_2 , CH_4 , CO , H_2)
- **Comprehensive analytical suite** for complete for method development and plant analysis
- **USP and DSP capability** scale down facilities for the development of scalable USP, fermentation and DSP bioprocesses. Skid mounted rigs for pilot and demonstration processing.
- **Pilot facility** 10/20/50/750L fermenters, associated downstream processing. Flexible plug & play configuration.
- **Demonstration facility** 10,000L fermenter; Flexible upstream and downstream processing. Flexible plug & play configuration.



FIGURE 1: Bioprocess Development Laboratories



FIGURE 2: NBBF Pilot Facility

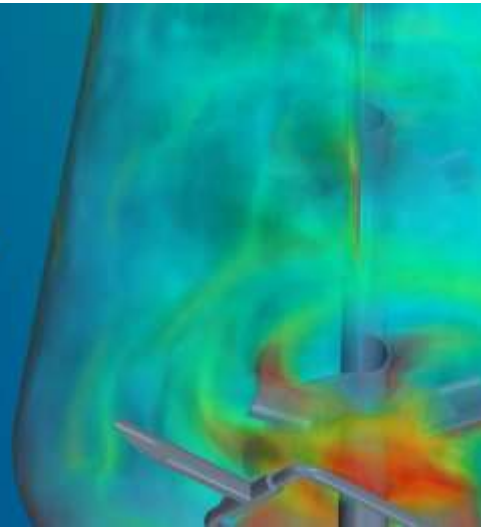


FIGURE 3: NBBF Demonstration Facility

K Potter - Slide 3

K Potter - Slide 4

Intro to some modelling techniques...



K Potter - Slide 5

CFD

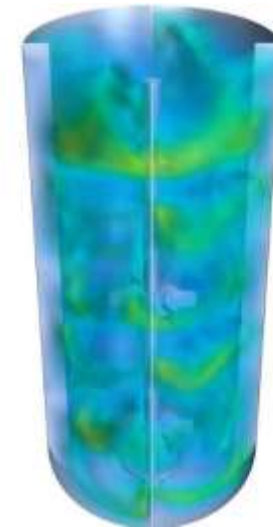
CFD (Computational Fluid Dynamics) is a software-based simulation of fluid mechanics.

An engineering tool, helping to understand your process and create an optimal environment in which your process can run.

Not necessarily modelling the process itself, but the physical conditions in which the process will run.

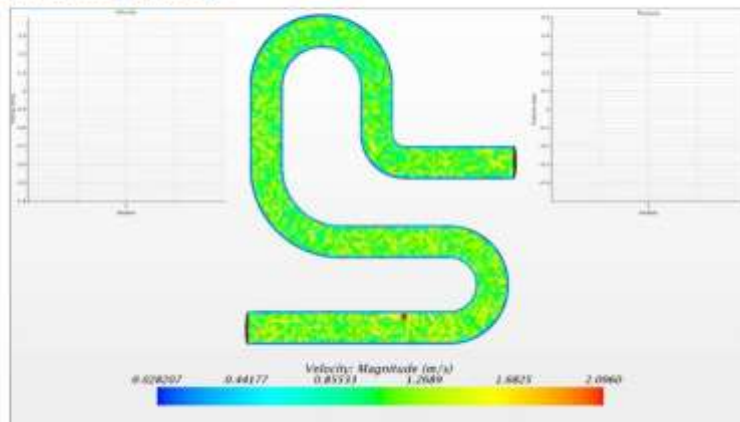
Simulation of the movement and interactions of materials and energy - gas, liquid and solid. Effects of flow, pressure, temperature & other operating conditions.

Creating a visual representation and a detailed numerical analysis of the behaviour of material within a system.



K Potter - Slide 6

How does it work?

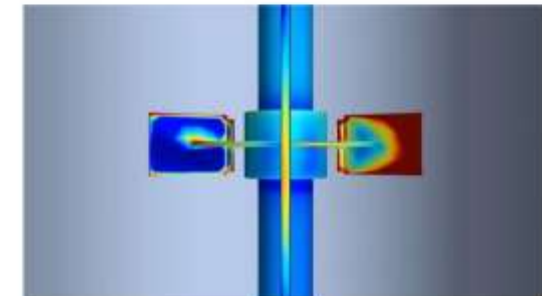


K Potter - Slide 7

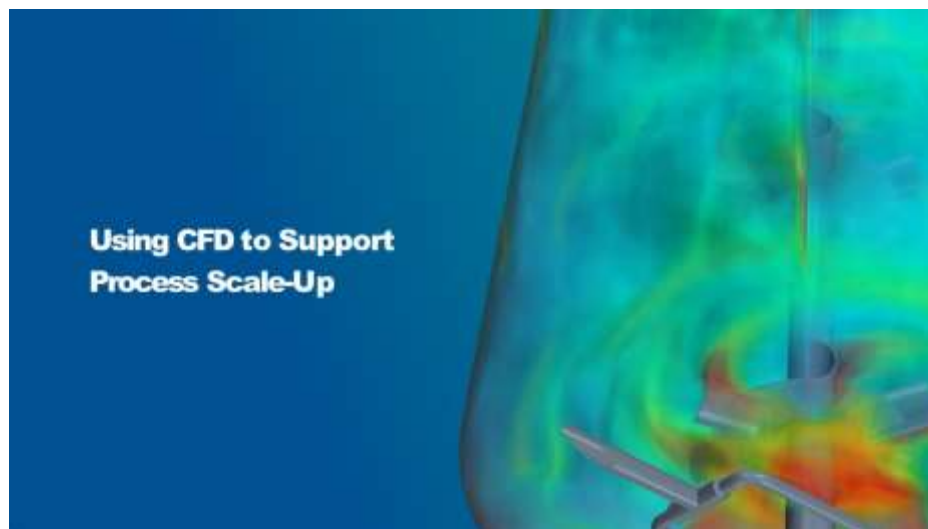
What do we do with it?

Gaining a better understanding of process design, and increased confidence in design & operational decision making

- Troubleshooting
- Process characterisation & optimisation
- Process scaling
- Design exploration



K Potter - Slide 8



K Potter - Slide 9

Process Scaling and CFD

When scaling up a process, there are some traditional "rules" which tend to be applied, both to the design of equipment and to its operation.

For example from an operational point of view:

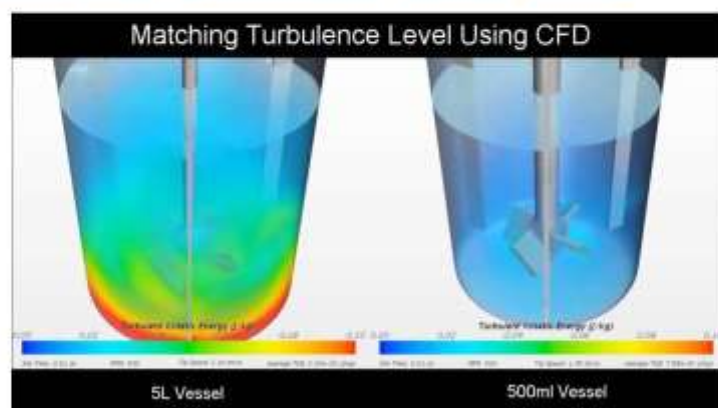
- To match mixing performance, you should match the impeller tip speed.
- To match solids distribution performance, you should match energy input.
- To match heat transfer, you should match Reynolds Number
- Etc...

These rules provide a sound guide, and a solid starting point. But how much further can we go by exploring around the edges of these guides with CFD?



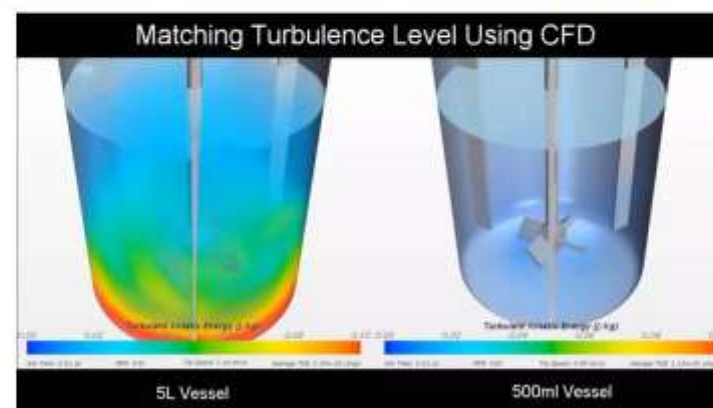
K Potter - Slide 10

Process Scaling – Geometrically Similar Vessels



K Potter - Slide 11

Process Scaling – Geometrically Dissimilar Vessels

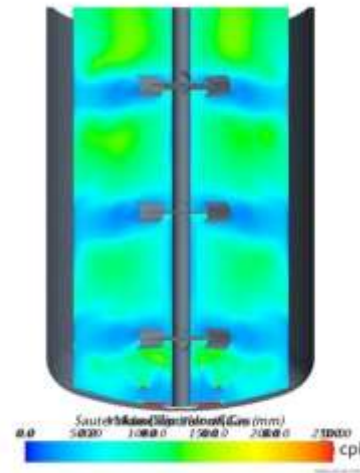


K Potter - Slide 12

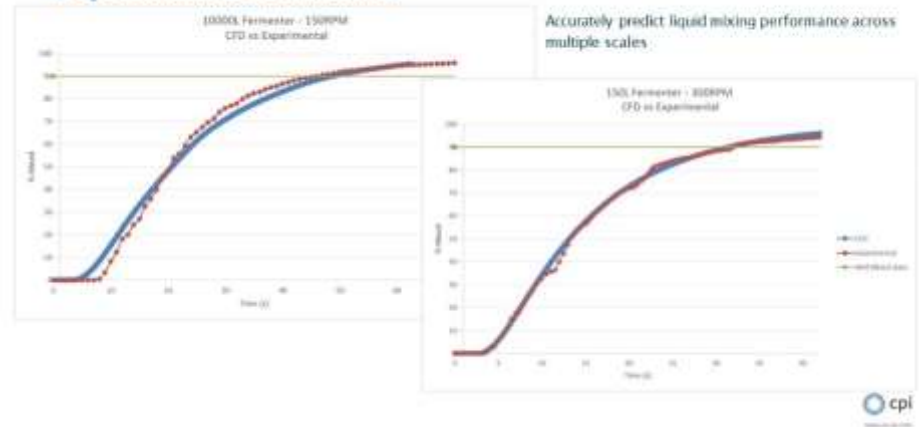
How have we applied this at CPI?

We have developed a suite of CFD models to assess the expected performance of a fermentation process through CFD, by assessing key performance affecting criteria and how these change with scale

- Liquid mixing
- Interphase mass transfer (k_{La})
- Gas holdup
- Bubble breakup and coalescence
- Power input
- Turbulence, shear, velocity profiles, etc....



Experimental Validation



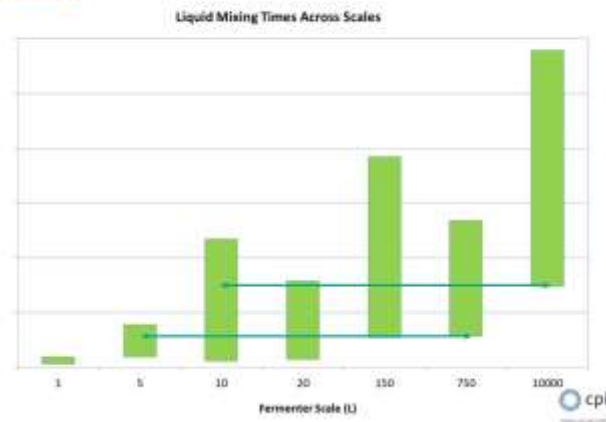
K Potter - Slide 13

K Potter - Slide 14

Scale-Down Operation

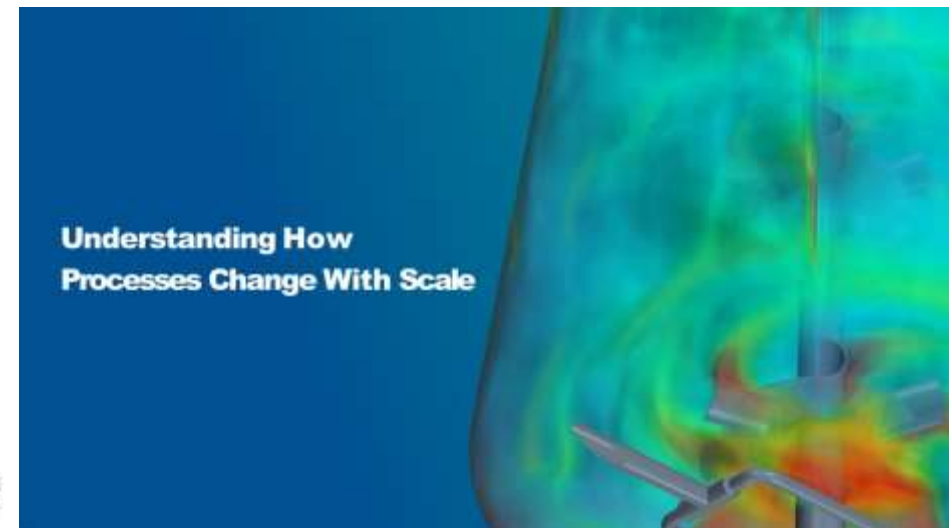
Understanding the operational range of each of our vessels allows us to establish the most effective routes to scale-up.

Identifying overlaps in performance between vessels allows us to scale-down larger vessels to mimic performance at smaller scale



K Potter - Slide 15

Understanding How Processes Change With Scale



K Potter - Slide 16

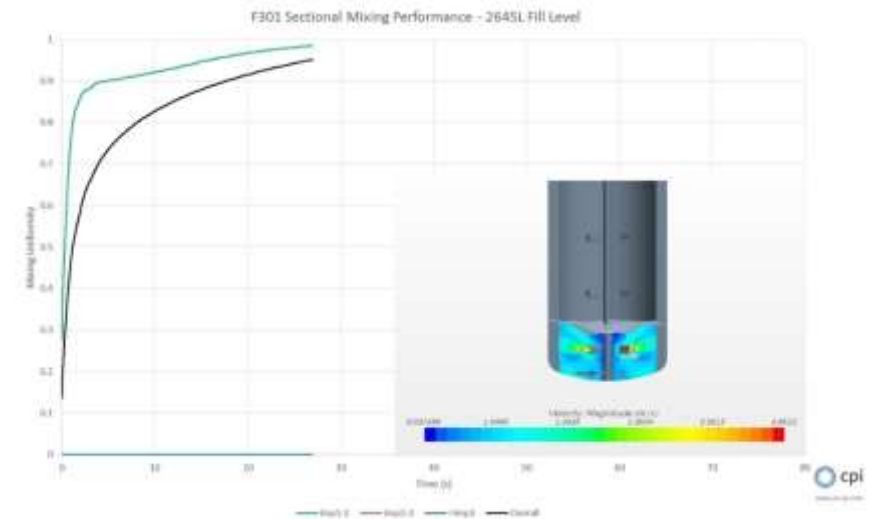
Detailed Analysis of Mixing in a Large Scale Stirred Tank

An exercise has been carried out in order to assess the mixing performance of our largest fermenter vessel and how this changes with fill level. This helps us to understand the optimal fill level to use from a mixing point of view.

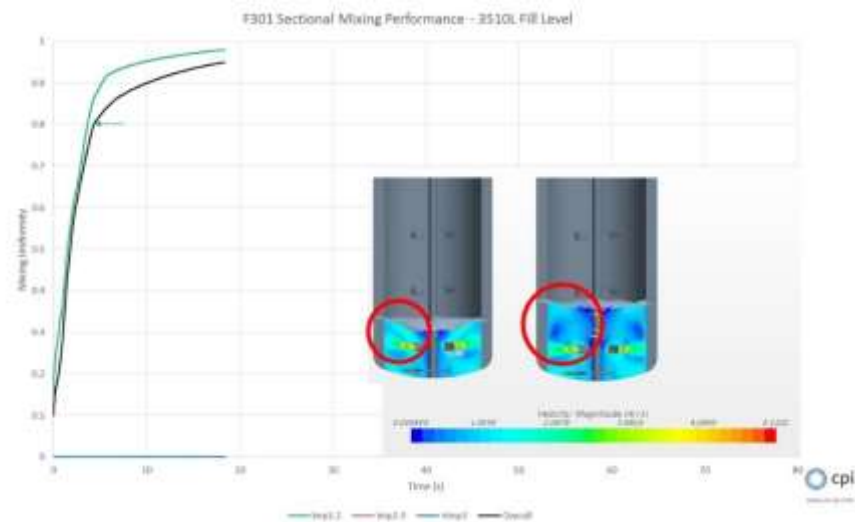
The following plots show the volume uniformity of an added tracer against time, within four different mixing zones:

- Between impellers 1 & 2
- Between impellers 2 & 3
- Above impeller 3
- Overall

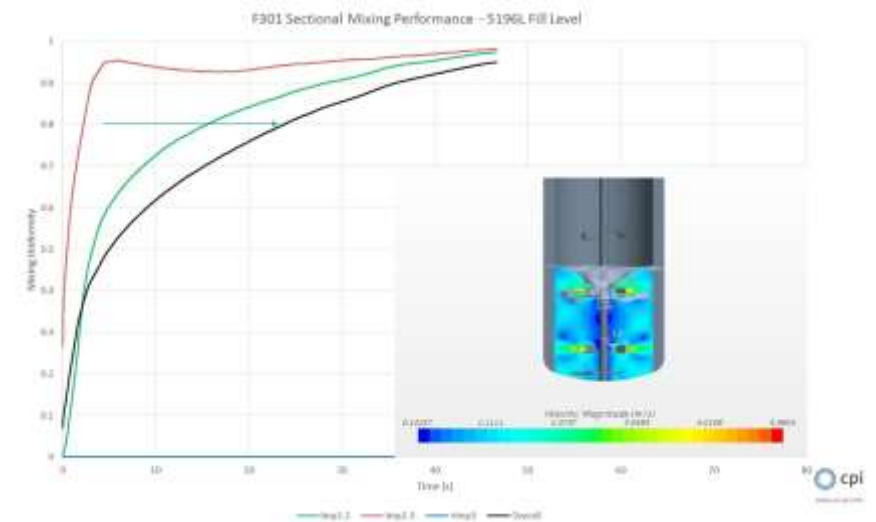
As can be seen, each mixing zone becomes uniformly mixed at different rates, depending on fill level.



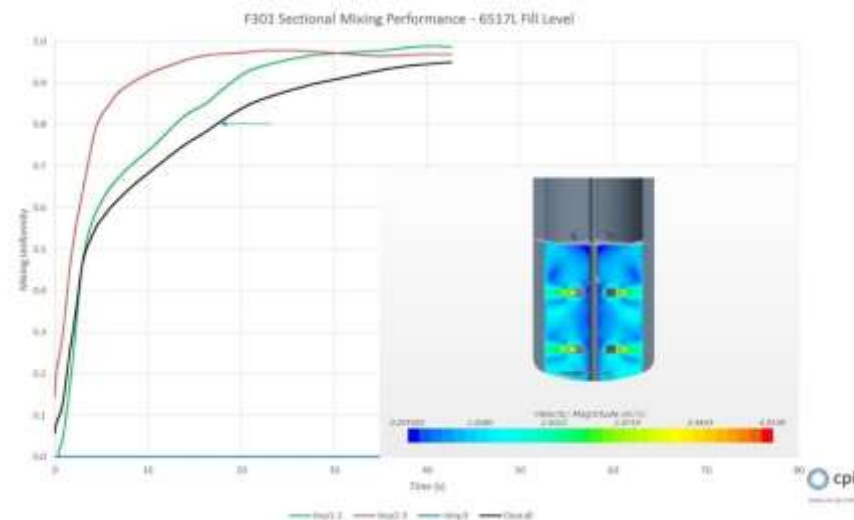
K Potter - Slide 18



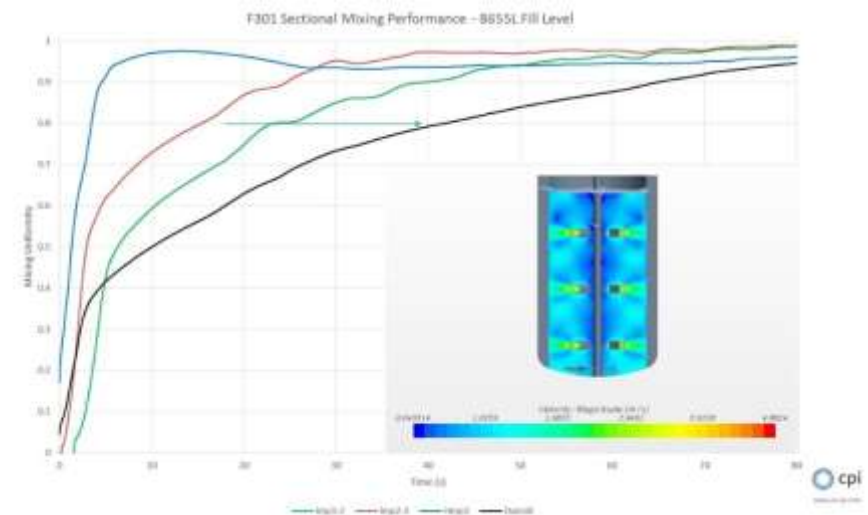
K Potter - Slide 19



K Potter - Slide 20



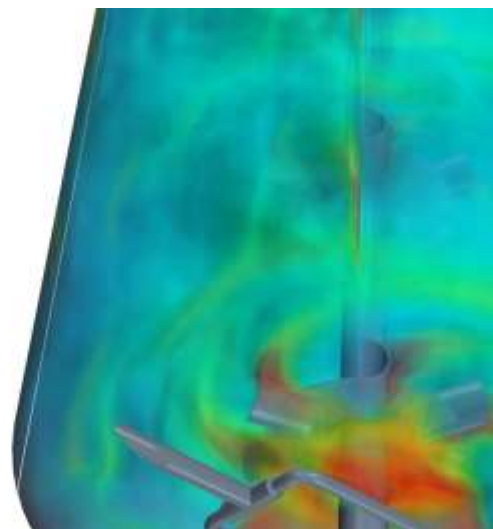
K Potter - Slide 21



K Potter - Slide 22

Summary

- CPI - who are we and what do we do?
- Challenges of process scale-up
- Intro to CFD
- Understanding the key performance differences between small and large scale process equipment, and accounting for that in lab scale experimentation
- Understanding the specific limitations of large scale equipment and accounting for them in the design of process control loops.



K Potter - Slide 23

Thank you

For more information visit www.uk-cpi.com

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Appendix 4 Original bullet points

A4.1 Original bullet points grouped and clustered, with summary scores

Note: This clustering was not available to workshop participants during scoring session. Items indicated by * were described in the short presentations but not included in the flag-up session.

Original bullet points by topic	Flags
Microbiology	28
<i>Microbial</i>	<i>11</i>
Fundamental systems biology still lagging behind compared to other biology	1
The role of biology in gas fermentation is currently regarded as a black box with significant gaps in understanding the microbial dynamics that e.g. leads to side products such as acids and heat generation.	-
How can we engineer microbiomes to yield value-added products from waste CO ₂ ?	-
What are the opportunities for mixed microbial communities?	1
When is the complexity of a mixed community worth it?	1
Synbio should include microbial community engineering - not just genetic engineering	1
Trade-offs between synthetic biology and wildtype organisms (Technological, economic, regulatory? Ecological?)	2
Genetic tools for non-model organisms	1
Development of genetic tools and characterization for non-model organisms	1
Identifying microbes in open cultures, using the best ones in defined cultures	1
Too few strains and their cultivation properly developed for actual industry	1
Archaea are under-represented in biotech / gas fermentation	-
N ₂ O abatement: Microbiological limitations	-
Systems biology of aerobic vs anaerobic gas fermentation	1
Microbial cultures: why (and how) spatial structure? How to exploit it? How to manipulate it?	-
<i>Metabolic</i>	<i>17</i>
Better grasp on effects of mixing on microbial metabolism (and community structure)	2
Understanding microbial kinetics, understanding electron transfer	1
Selection of electron donors, e.g. H ₂ vs electron; one-stage vs two-stage	4
Systems biology, electron bifurcation, and enzyme specificity	-
Enhance electron bifurcation systems with genetic engineering?	1
Growth coupling of product formation in aerobic gas fermentation	1
Significant gaps in understanding microbial dynamics that e.g. leads to side products such as acids and heat generation	4
What knock-on metabolic processes are triggered by CO ₂ fixation?	1
How can microbubble-microbe interactions be tuned for symbiotic engineering?	1
Can microbubble absorptive processes intensify metabolism? Facilitate downstream processing or <i>in situ</i> separations?	1
Qualitative understanding of microbial kinetics: Why 2,3-BDO formation?	-
How do microbial communities metabolise CO ₂ in the absence of light?	-
CFD simulation of bioreactors with integrated biokinetics to study cell-environment interaction	1
Engineering envelope	26
<i>Mass transfer</i>	<i>13</i>
H ₂ assisted CO ₂ bioconversion: Limited gas-liquid transfer	4

Original bullet points by topic	Flags
Mass transfer between gases and microbial cultures growing in a liquid phase or film	3
Physico-chemical barriers - poor solubility and gas-liquid mass transfer of H ₂	-
Physico-chemical barriers related to working with H ₂ have been identified as the main rate-limiting factors due to the poor solubility and gas-liquid mass transfer of H ₂ .	-
Better tools for prediction and analysis of mass transfer	-
CH ₄ abatement: Limited gas-liquid transfer, inhibition by metabolites	-
CO bioconversion: Limited gas-liquid transfer	-
How do fermentation broth properties (viscosity, surface tension etc) affect gas transfer characteristics?	-
What are the mechanisms for microbubble – microorganism interactions?	-
Can reactor design for gas transfer move beyond empirically-based approaches?	1
On-line measurement of dissolved gas concentrations	5
Measuring concentration gradients in biofilms, and how to mitigate or exploit them	-
How much should we care that we only measure bulk/macroscopic characteristics, but microorganisms predominantly influenced by highly localised environmental conditions?	*
How do we know when mass transfer is limiting? (often difficult to measure intermediates)	*
How do we predict (or even know definitively) when enough (mass transfer) is enough?	*
Are particular equipment/reactor designs suited to particular applications/organisms?	
What are critical design criteria for mass transfer/mixing systems? (given multiple process requirements)	*
Impact of conditions on rates and product spectrum: how do (local) concentrations of dissolved gases affect product spectrum, production rates, e.g. pCO ₂ -> acetate/ethanol ratio in syngas fermentation	*
How to control conditions to direct maximum flux to certain products	*
<i>Hydrodynamics</i>	2
Can CFD work across scales relevant to microbial environments?	1
Bioreactor hydrodynamics: Experimental assessment of gas-liquid hydrodynamics in fermentation broths	-
Impact of broth composition on hydrodynamics: How do components in the broth affect bubble size, mass transfer rates?	*
Predictive models for k_La (especially a) in microbial broth	-
Modelling flow and diffusion in reactors, through biofilm/electrode/membrane	-
Can we use neural networks to improve gas mixing of microbial systems?	-
Concentration gradients in industrial-scale reactors, and how to mitigate or exploit them	1
Reduced order models: Coarse models for rapid assessment of heterogeneity & design optimization	-
<i>Scale effects and scale-up</i>	11
Scale-up	3
Scale effects on gas transfer (and on microbial metabolism / performance)	2
Scale-up investment and infrastructure	2
Scale-up (inc. mixing and safety)	1
Scale-up is limiting advancement particularly in 1-10 litre range.	1
Working at small pilot scale with flammable/ explosive gases	2
Scale-down: Design of lab-scale setups to study impact of heterogeneous conditions on cells	-
Different bioprocess conditions must be considered if infrastructure for gas fermentation in developed	-

Original bullet points by topic	Flags
Designing scalable/stackable reactors, and their cost-effective production	-
How to operate a thin film reactor hygienically?	-
Other	31
<i>Feedstocks & Products</i>	5
Gaseous feedstocks - mapping composition, scale, location	1
Accommodating intermittent energy patterns while complying with various CO ₂ supplies.	-
Gas quality variability	-
Process economy (and feedstock supply) in gas fermentation	2
When is it useful (and how!) to switch between desired products, or between varying feedstocks?	*
Product diversification	-
Product diversification; metabolic engineering; metabolic models	1
What should we be making? From what?	1
Product generation as main goal vs. 'augmented waste treatment'	-
<i>Process design and integration</i>	14
How do we separate products from liquors?	4
Recovery of non-volatile products	3
Microbubble <i>reactive</i> -separations, say for removal of higher value added molecules?	-
Downstream processing: impact of hard-to-remove byproducts, product titre, etc on purification	*
Recycling microbial broth after downstream product removal	1
Integration with upstream processes	2
Gas recycles; impact of gas impurities; gas purification	1
Gaseous feedstocks versus soluble (e.g. formate, methanol)	2
Need to utilise side-streams in gas fermentation	1
Utilizing side streams to enhance overall system efficiency	-
How do we answer ' what-if ' questions in design & operation decisions?	-
<i>Economics, Policy, Implementation</i>	12
C1 products need to be competitive against existing industry	3
Key questions on towards commercialisation not asked	2
Can enough CO ₂ be fixed as biomass in relation to ethanol/methane via Wood Ljungdahl	-
Pathway to generate biomass-derived platform chemicals in a commercially feasible way?	-
Opportunities for detailed engineering appraisal and techno-economic assessment	-
Industrial engagement / funding	4
Holistic overview and integration of knowledge is lacking	1
Field is diverse with regard to industrially relevant organisms + processes: pressure, shear forces, growth media, productivity yields	-
Identify key components in gas fermentation knowledge gaps - physiology, synbio methods, bioreactors, bioprocesses	-
Trans- / inter-disciplinary working	-
Policy framework and investment climate not where it should be	1
Broader policies that take all these technologies into consideration	1

Appendix 5 Flipchart images

Contents Appendix 5

[Microbiology topic flipcharts](#)

[Engineering envelope topic flipcharts](#)

[Other topic flipcharts](#)

[Actions/obstacles topic flipcharts](#)

Microbiology



Group 1a

Microbiology



Group 1b

Microbiology



Group 1c

Engineering envelope



Group 2a

Handwritten notes on sticky paper:

- Envelope** (Pink note)
- Mass Transfer** (Yellow note)
- Heat Transfer** (Blue note)
- Fluid Mechanics** (Green note)
- Mass Transfer** (Blue note)
- Heat Transfer** (Yellow note)
- Fluid Mechanics** (Green note)
- Mass Transfer** (Yellow note)
- Heat Transfer** (Blue note)
- Fluid Mechanics** (Green note)

Hand-drawn Venn diagram with three overlapping circles labeled:

- Mass Transfer
- Heat Transfer
- Fluid Mechanics



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EVALUATING EMERGE

- Scale effects on gas transfer (and on microbial metabolism/performance)
- Designing stable/microbial reactors, and their cost-effective production

MASS TRANSFER

- Mass transfer between gases and microbial cultures growing in a liquid phase or film
- H_2 dissolved CO_2 bioregeneration: limited gas-liquid transfer
- CO_2 bioregeneration: limited gas-liquid transfer
- Physico-chemical barriers (pH, solubility and gas-liquid mass transfer g/L)
- Convergence: products to be produced to some reaction, and how to integrate or exploit them
- What are the mechanisms for dissolution - microorganism dependent?
- CO_2 utilization: limited gas-liquid transfer, inhibition by metabolites
- Can we use neural networks to estimate gas usage of microbial systems?
- Production models for heterotrophic growth

REACTOR DESIGN

- Can reactor design for gas transfer wave beyond empirically-based approaches?

MEASUREMENT TOOLS + DATA COLLECTION

- Small reactors: bubbles $H_2O \rightarrow O_2$
- Measuring concentration gradients in biofilms, and how to integrate or exploit them
- On-line measurement of dissolved gas concentrations
- Modeling flow and diffusion in reactors, through wall bioreactors/ membranes

MODELING

- Can CFD work (and scales, relevant to microbial environments)?
- How do fermentation broth properties (viscosity, surface tension etc) affect gas transfer characteristics?
- Can membrane adsorption processes intensify mass transfer? Facilitate downstream processing or in situ separation?

PRODUCTION

- Can we use neural networks to estimate gas usage of microbial systems?
- Production models for heterotrophic growth
- Can membrane adsorption processes intensify mass transfer? Facilitate downstream processing or in situ separation?

Flowchart:

```

graph TD
    A[Reaction Design + Transfer] --> B[Process Design]
    B --> C[Production]
    A --> C
  
```

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[illegible]

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[illegible]

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Optimization

- Other**
 - Change process feedback loops to become positive to make new optimal system
 - Process selection for targeted products
- Policy & Regulatory Change**
- Integration with upstream processes**
- Lifting side streams to enhance overall system efficiency**
- Opportunities for process engineering appraisal and techno-economic assessment**
- Knowledge**
 - How to measure the impact of process changes on the overall system performance
 - How to measure the impact of process changes on the overall system performance
 - How to measure the impact of process changes on the overall system performance
- Costs**
- Externalities**
 - eg. emissions, land use, water consumption, etc.
- Cost benefit analysis of the production activities for different classes of products**
- Process to conduct such an analysis**
 - Requires to assess trade-offs between different objectives to be economically viable



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[illegible]

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Other



Group 4b

Actions/obstacles



Group 4c

Appendix 6 Bullet points from workshop sessions

Key to areas: FPP - Feedstock, process, product; KT - Knowledge transfer; M&M - Mixing and mass transfer; Metab - Metabolic; Micro - Microbiological; Mod - Modelling; P&I - Policy and implementation; PM&C - Process monitoring and control; S-U - Scale-up

Bullet points from workshop sessions by topic	Area
Microbiology	
Microbial community understanding	Micro
Microbial knowledge gap: Significant potential to use non-model organisms has yet to be realised.	Micro
- Much of the prokaryotic tree of life ignored.	
- Rich diversity in relevant samples should be directly tapped (e.g. enrichment for new strains/communities).	
Microbial knowledge gap: Significant potential for microbial communities/mixed cultures to perform gas fermentations (and other biotech processes), but knowledge gaps remain as to their stability/engineerability (environmental & genetic) and optimal or pre-requisite complexity	Micro
Symbiotic engineering of mixed culture consortia	Micro
Understanding (i.e. predictive modelling) microbial interactions among themselves and with the environment i.e. what determines microbe-environment and microbe-microbe interactions?	Micro
Microbial knowledge gap: Tools for mixed culture / community genetic engineering are immature/lacking. Warrants targeted development	Micro
More genetic tools for non-model microbes	Micro
Liquid/gas interactions with microbes	Micro
Developing and maintaining biofilms	Micro
Make research into microbiology both fundamentally interesting and makes a positive impact on the world	Micro
Complexity/links genetic - community behaviour/outcome - physical engineering design	Mod
Modelling	Mod
Predictive models	Mod
Understanding (i.e predictive modelling) metabolic network dynamics within cells i.e. what determines cellular metabolic fluxes	Metab
Microbial metabolites and their impact	Metab
Understanding microbial inhibition during gas fermentation and designing mitigation strategies	Metab
Using AI/Machine learning to gain understanding of the organisms' metabolome / genome (need big datasets!)	Metab
Don't ferment gases: pre-process into liquid feed then ferment	Metab
Engineering Envelope	Metab
Gas transfer between gas - liquid - biomass	M&M
Mass transfer	M&M
Fundamental understanding/prediction of kLa values	M&M
Engineering for better mixing and distributed kinetics (CFD-like modelling)	M&M
New reactor designs (for gas - liquid - bio)	M&M
Development of high mass-transfer scalable gas phase bioreactors	M&M
Optimised reactor design to enhance substrate availability	M&M
Complexity/links genetic - community behaviour/outcome - physical engineering design	Mod

Bullet points from workshop sessions by topic	Area
Predictive models	Mod
Multi-scale models of liquid cultures, covering cells, biofilms/granules and the bulk that incorporate thermodynamics (metabolism) and hydrodynamics (flows and mass transfer)	Mod
Development of better scale-driven models	Mod
Fermenter monitoring and operating strategies	PM&C
In situ measurement of gas compositions in the aqueous phase	PM&C
How to monitor, control and optimise the integration of the full production process towards more efficiency, sustainability and economy	PM&C
Exploit best of chemical (methanol) and biology (methylophony) as two stage processing	PM&C
Other	
Feedstock and product diversification	FPP
Optimisation of feedstocks and recovery methods for selected bioproducts	FPP
Cost-benefit analysis of bioproduction methods for different classes of bioproducts e.g. bulk chemicals, high value, pharma	FPP
Process selection for targetted products	FPP
Integration up/downstream and supply chains	FPP
Use of neural networks for optimisation of biomethanisation	FPP
Comparison of AD and gas fermentation for various feedstocks	FPP
How to stop making methane and start making more valuable and sustainable products from waste or air capture CO ₂	FPP
Change biomass feedstock fermentation to biomass and H ₂ to make more efficient and faster	FPP
Economic comparison of various waste feedstocks by AD and the combination of syngas fermentation	FPP
Look into dissolved forms of H ₂ , CO ₂ (formate, methanol)	FPP
Funding for R&D and demo which has a longer horizon for planned returns -> UK as an innovation leader rather than follower	S-U
Funding streams to support scaling-up	S-U
Scale-up / demonstration facilities	S-U
Developing new tools for the design of gas fermenters	S-U
How to reduce costs of sterile systems to allow lower value products to be economically viable	S-U
Gas transfer mechanisms and repercussions for H ₂ , and CH ₄	S-U
Optimisation of biofilms through CFD	S-U
'External' stuff (but not really) e.g. economics, funding, strategic interests, upstream	P&I
Cross engagements / education across disciplines / approaches	P&I
How can we develop tools to model complex systems?	P&I
Knowledge centralisation for competencies, progression-check, capabilities and opportunities	P&I
Policy and Regulation (HSE) e.g. Clostridia	P&I
The discussions about IP are the most complicated when establishing partnerships. More generalizable agreements would be nice to have	P&I
Follow up is needed for the challenges and gaps we identify today. We should, 2 years from now, re-assess them to check for their progress	P&I
Actions	
Communication and understanding across disciplines (barrier)	KT

Bullet points from workshop sessions by topic	Area
Hyper-specialisation super-incentivised. Knowledge silos. We need generalists who speak engineering, biology, programming and economics.	KT
Transparent reporting that facilitates comparison and sharing of data/practice (Action)	KT
Develop a dataset format that can allow 'anonymised' results to be collated for data mining on process data from a wide variety of fermentations	KT
Too many papers "I've a great model" but no implementation shared to play with	KT
Mapping of feedstock availability, composition, quality	FPP
More coordination between feedstock suppliers	FPP
Honest/open discussion of what bioprocess/product to focus on	FPP
Markets and logistics	FPP
Sustainability and environmental impacts	FPP
Obstacle: instrumentation/equipment access at laboratory level dispersed across Universities nationally	S-U
<u>Safe</u> and cheap relevant research and development facilities	S-U
Expert H&S / Hazop / HazID support	S-U
More targeted funding support to move technological/Integration readiness levels upwards	S-U
Funding support for scale-up	S-U
Further development/investment in open access scale-up facilities dedicated to gas fermentation	S-U
More accurate and improved scale-down models	S-U
More targeted cross-disciplinary cross-sector funding opportunities	P&I
For progress: open competitive challenges, e.g. in the same spirit of 'Protein structure prediction' competition	P&I
Promoting collaborative projects between industry and academia	P&I
Lack of agility in contracts / IP management in Universities	P&I
Lack of trust by investors in new technologies	P&I
Obstacle: Niche venture capital with whole sector 'ecology' experience	P&I
Identify or create real business cases with real positive societal, environmental and economic impact	P&I
Talent - acquisition / retention	P&I
High cost of immigration to UK limiting global talent pool	P&I

Appendix 7 Summary of priorities from participants' in-session notes

A7.1 Microbial theme

Group 1a

1. Physical interaction and microbial inhibitions mechanisms during gas fermentation
2. Genetic tools for non-model organisms
3. Systems biology of unique microbes and microbial communities and contextual responses to their environment
4. Data collection for predictive modelling tools leveraging AI, machine learning and big data

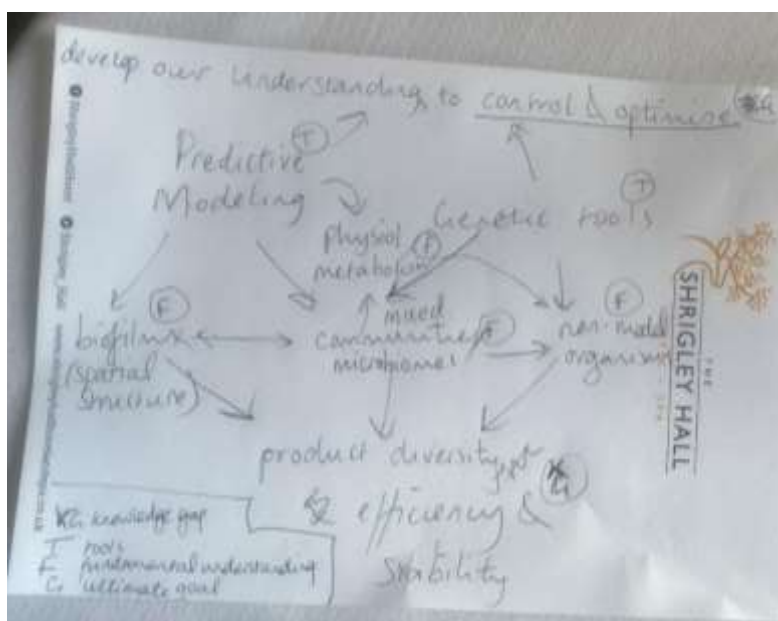
Group 1b



- Predictive Models – Pool large datasets and predict AI aided optimised mixed cultures and optimised pure strains
- Liquid gas interactions – uncertainty of exact composition of the aqueous phase limits the development of processes
- Metabolic fluxes – targeted products. Optimise the operational strategies and conversion efficiencies

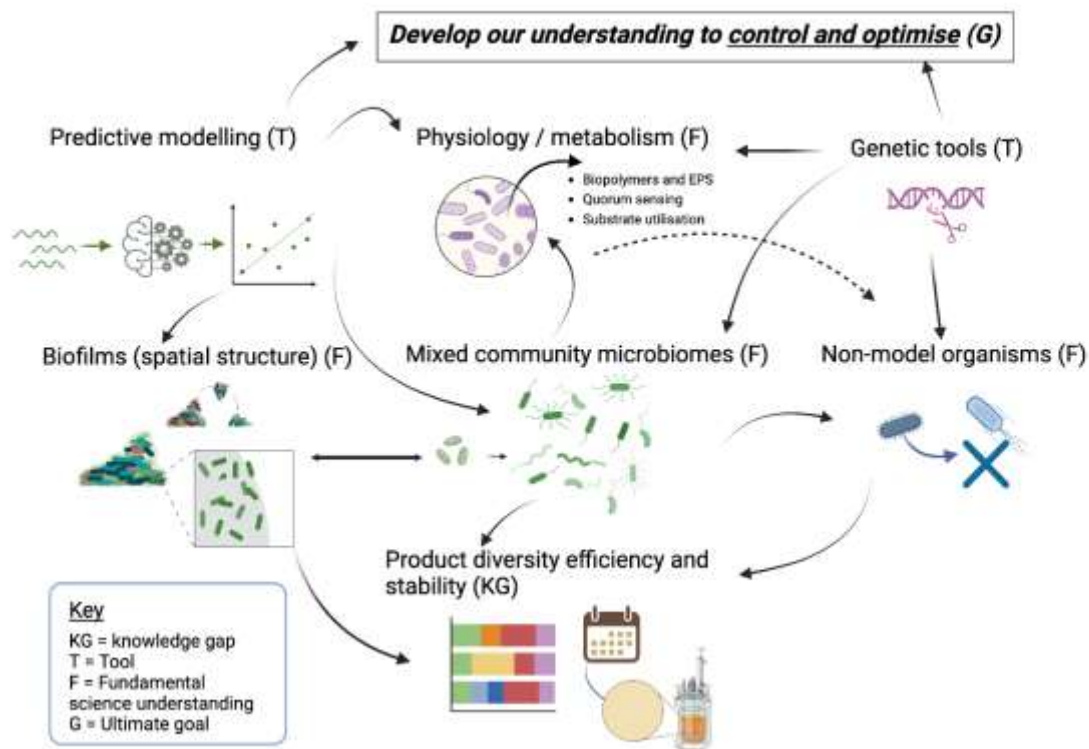
Group 1c

Goal – Develop our understanding to control and optimise microbial systems



Sketch diagram from session - Group 1c

Advancing Gas Fermentation Technologies: microbial aspects



Drawn version of diagram from session - Group 1c

A7.2 Engineering Envelope

Group 2a

- Optimising mass transfer in gas-phase bioreactors
- Control, optimisation and monitoring of gas fermentation
- Optimising the modelling tools at macro and microscopic scale

Group 2b (NB Equally important)



1a. Optimising reactor design for mass transfer to support process intensification and integration. Considerations include temperature and pressure (safety issues) and energy optimisation.

1b. Process measuring and monitoring. Required to inform and integrate modelling.

1c. Predictive modelling to de-risk scale up. Accurate models required to translate optimised lab experiments/results to account for differences encountered at scale.

Group 2c

- Predictive models. Measurement includes tools, data collection (e.g. concentration gradients and dissolved gas), TEA, LCA.
- Mass transfer, balanced inputs and product removal (Operational and design parameters).
Balanced inputs include pressure, light heat, mixing and heat, growth, micro- and macro-nutrients, chemical redox.
- Reactor design including variants for reactor type and purpose.

A7.3 Other

Group 3a

- Assessing the economic availability and suitability of feedstocks for gas fermentation and specific target products.
- Developing innovative products and associated downstream processes
- Scalability of gas fermentation processes
- Safety considerations for explosive gas mixtures.

Group 3b

Product and feedstock diversification and identification. Feedstock mapping and characterisation of the different production facilities. Coordinating supply and demand

Scaling up. How to reduce costs. More reliable scale-down models in order to prevent high scale-up costs. Funding for scaling-up.

Economics and business models. Tension between academic publications and business IP protection. Economic viability (does it make sense to do this from an economic point of views).

Group 3c



- Integration and optimisation regarding upstream processes, biomass utilisation as feedstock and TEA.
- Cost/benefit analysis and cost reduction.
- Knowledge transfer and centralisation, cross engagement, and reassessment of all of these bullet points.

A7.4 Actions

Group 4a

- Map and interpret feedstock characteristics - improve coordination
- Promote transparent reporting and open data formats
- Target funding support on scale-up, demo and TRL progression to support investor confidence
- Training and information exchange, including H&S expertise

Group 4b



1. Facilitate multi-disciplinary working. Cross sector funding calls promoting collaboration between disciplines. Disrupt silos and avoid “empire building”. Actively promote examples of cross-disciplinary working.
2. Sharing infrastructure, knowledge, facilities, data. “Fair” data practices. Develop methods for sharing anonymised data. Enable inter-institutional access to facilities (HPC, lab equipment, etc). Improve discoverability (eg searchable database).

Group 4c

- Feedstock
- Funding
- Markets and logistics
- Sustainability and environmental impact
- Talent acquisition and retention

Appendix 8 Visualisations

This appendix contains some visual representations of relationships between the bullet points listed in Appendices 4 and 6. These were produced after the workshop, and are examples only: each could be re-drawn in many different ways, as the relationships themselves are multi-dimensional.

Larger versions in editable format are available from EBNNet ebnet@ebnet.ac.uk

[Pre-workshop - Microbiological aspects](#)

[Pre-workshop - Engineering envelope](#)

[Pre-workshop - Other](#)

[Workshop - Microbiological aspects](#)

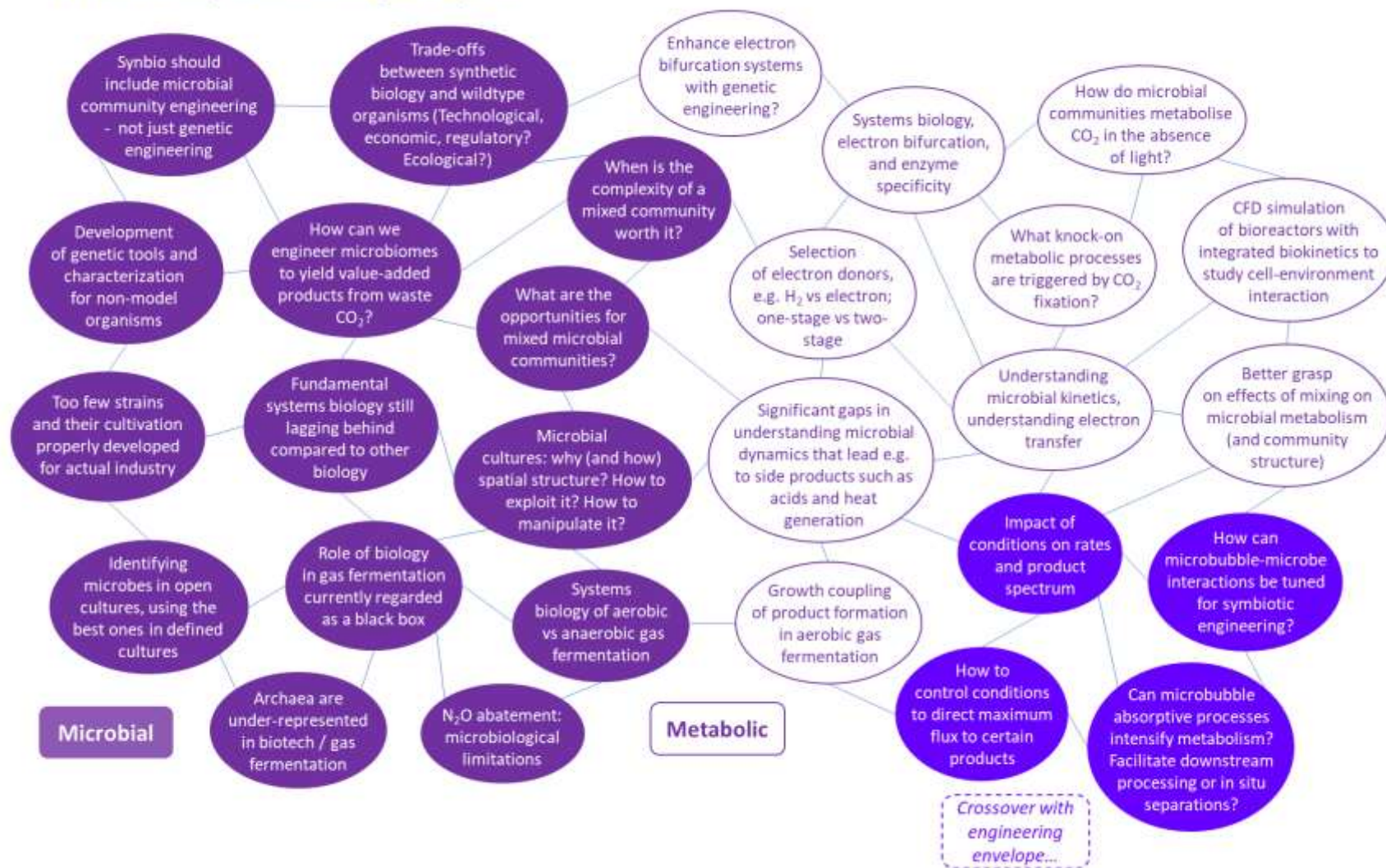
[Workshop - Engineering envelope](#)

[Workshop - Other](#)

[Workshop - Actions and obstacles](#)

[Summary of key R&D priorities](#)

Pre-workshop – Microbiological aspects



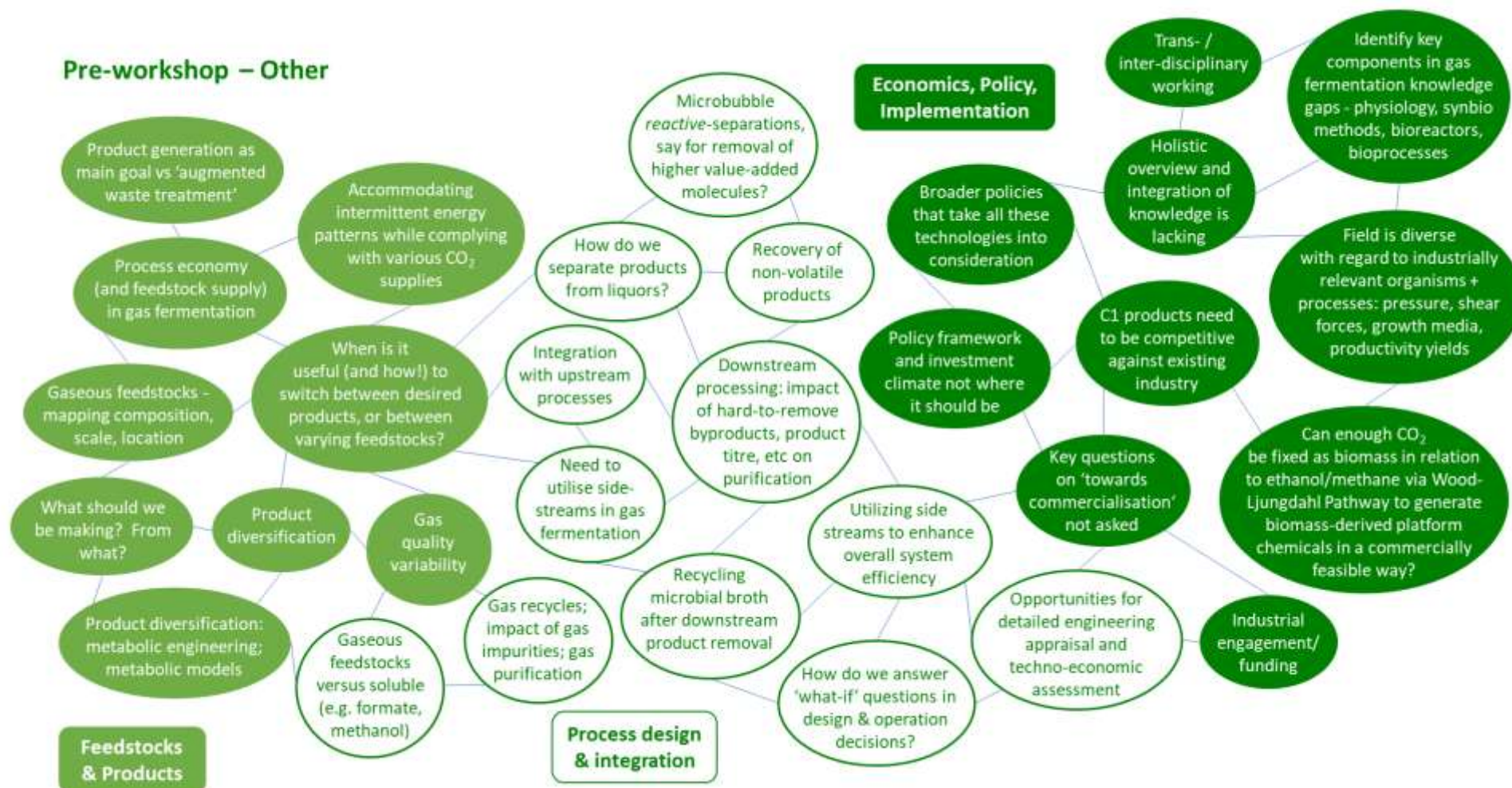
Pre-workshop - Microbiological aspects

Pre-workshop – Engineering envelope



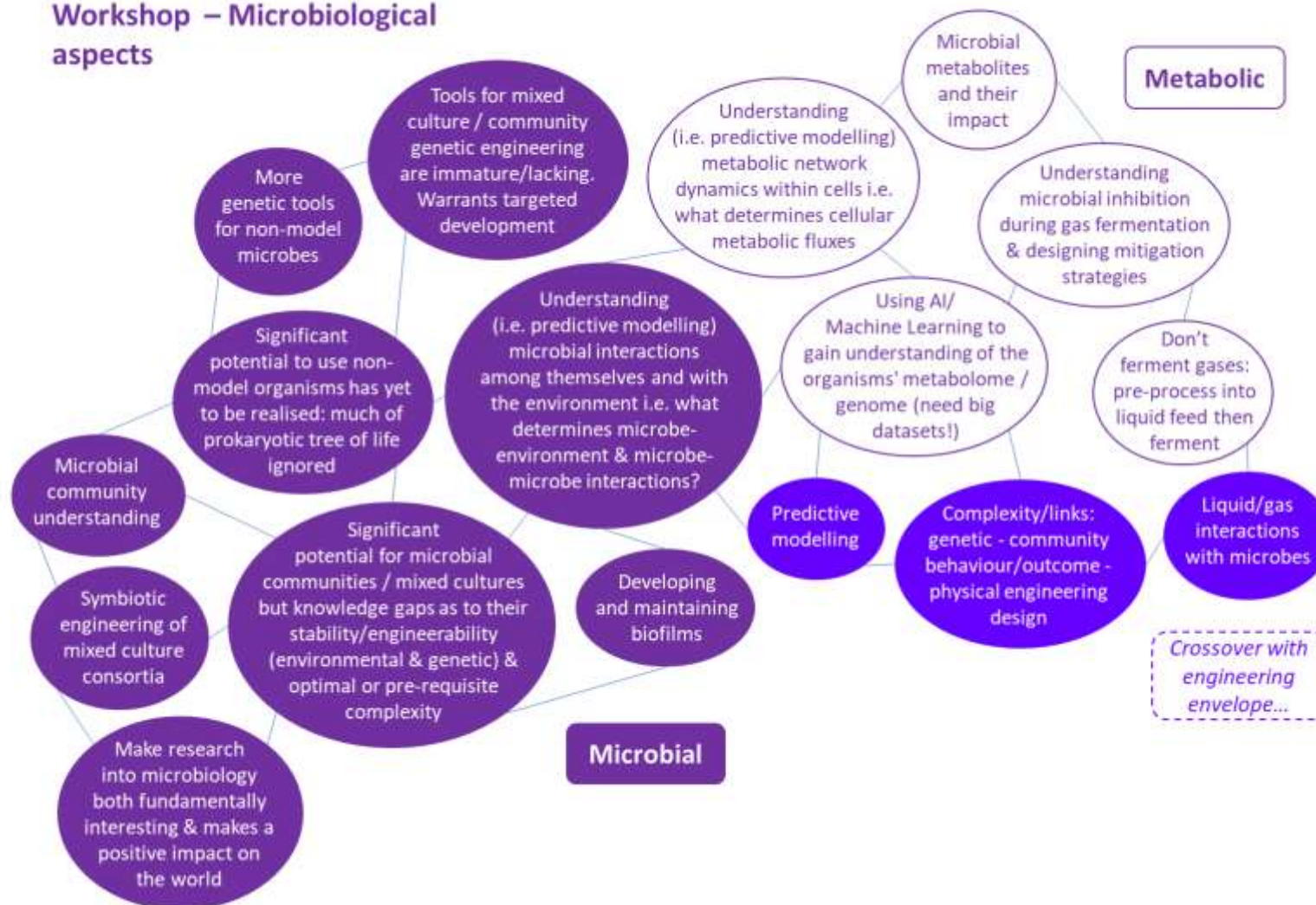
Pre-workshop - Engineering envelope

Pre-workshop – Other



Pre-workshop - Other

Workshop – Microbiological aspects



Workshop - Microbiological aspects

Workshop – Engineering envelope

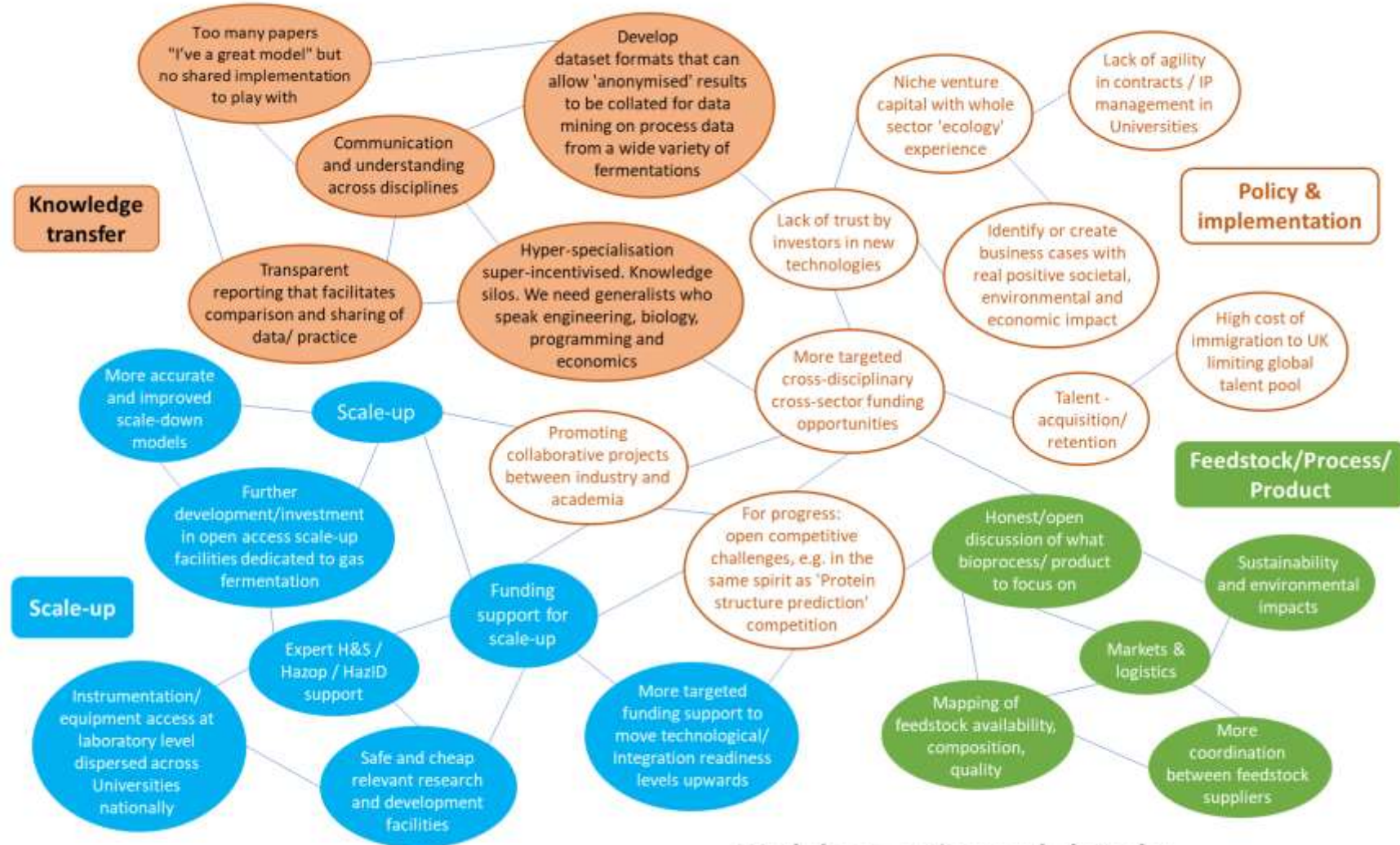


Workshop - Engineering envelope

Workshop – Other



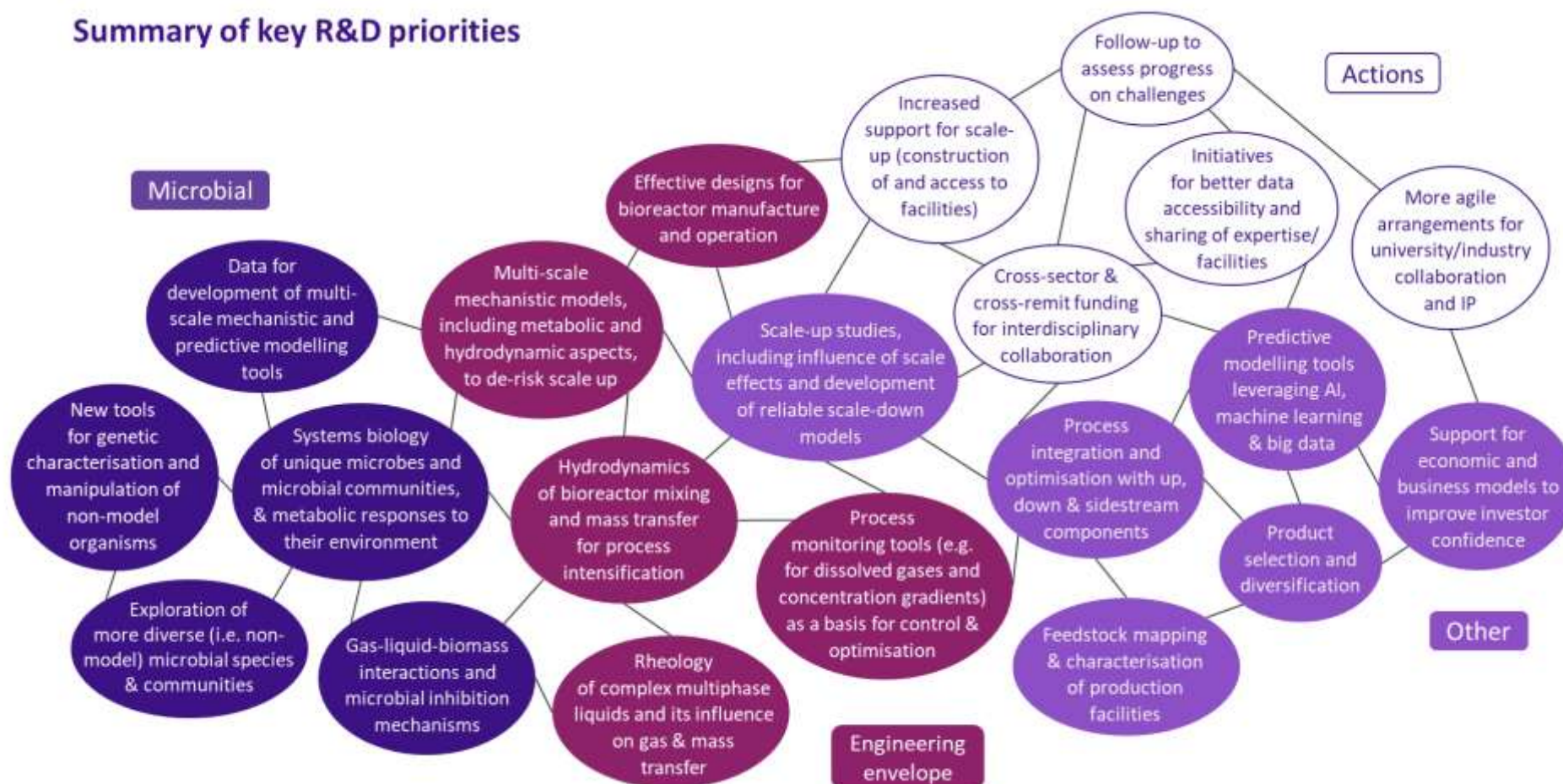
Workshop - Other



Workshop – actions and obstacles

Workshop - Actions and obstacles

Summary of key R&D priorities



Summary of key R&D priorities