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**NBIC WORKSHOP**

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The understanding of biofilms is key to discovering, controlling and directing the behaviour of microbial communities for sustainable environmental, engineering, public health and medical applications.

The 4 interventional strategies being explored by NBIC and its partners are the prevention, detection, management and engineering of biofilms. This workshop was aimed at exploring unmet needs in biofilm detection. 14 NBIC partner organisations shared their unmet detection needs (see Appendix 1) and 9 of these led syndicates to discuss the key challenges and way forward. There were 39 attendees from industry and 26 from universities/research institutions.

A wide number of common themes emerged. Problem owners usually wanted to understand:

- Is there a biofilm present?
- Where exactly is it? (e.g. location in a wound, a water system or industrial pipework)
- What can you tell me about it? (e.g. composition, characterisation and impact)

A wide range of possible detection approaches exist and were reviewed (from spectroscopic to biological techniques) and some novel ones were proposed. A key challenge is to adapt these to be usable in an in-situ, point of care context in the industrial or human/medical setting and not just for research, lab or product development investigations.

Across all the medical applications where detection was critical e.g. wounds, orthopaedics etc. there was a recognised need for the requirement to be able to detect and confirm the presence of a biofilm in a standardised reproducible manner, using approved protocols that would gain clinical and regulatory acceptance for both primary clinical diagnostic use, and use in controlled trials of anti- or biofilm-promoting interventions. In some settings (medical and otherwise) there was also a coupling between prevention and detection, in that detection becomes a method for assessing the effectiveness of prevention strategies. Additionally, there is a specific need to be able to identify or detect a “healthy” as opposed to an “unhealthy” or disease-causing biofilm, for example, the oral cavity, which was a recurring theme.

In industrial applications such as water and filtration systems, detection poses significant challenges relating to access to possibly remote surfaces down or outside pipework in order to locate a biofilm even if in-line sensing is able to detect the presence of one somewhere in the system and that damage may be occurring.

In consumer applications around the home then the ability to detect a biofilm in-situ on a surface is a key need. Whilst a number of techniques have the potential to achieve this the key challenge is the creation of easy to use approaches that could be used and interpreted by the consumer.

Finally, there was recurring need for wider engagement with consumers, regulators and other stakeholders in the need for both better definition of standards and policy development in the field of biofilms and biofilm detection.

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Background: National Biofilms Innovation Centre (NBIC)

AN INNOVATION KNOWLEDGE CENTRE (IKC)

NBIC was formed in December 2017 as an Innovation Knowledge Centre (IKC) funded by BBSRC, Innovate UK and the Hartree Centre.

NBIC’s mission is to harness the UK's industrial and academic strength in biofilms.

NBIC aims to be the recognised UK hub for accessing biofilm expertise, capability, science and innovation capacity. We exist to catalyse the growth in the UK's scientific, technological and industrial expertise in biofilms with the goal of delivering:

- World class science and scientists
- Breakthrough innovations
- Economic and societal value

It is working to create a network and community of researchers and industrial/commercial partners across the UK and internationally to progress all these elements.

We are working to create a network and community of researchers and industrial/ commercial partners across the UK and internationally to progress all these elements.

This and future workshops represent one key dimension of achieving these goals in creating a forum where academic experts and industrial practitioners can meet to explore solving unmet needs.

Biofilms in Context

Microbial biofilms and communities collectively represent the largest biomass and activity centre on the planet playing a major role in the biology of the environment (both natural and engineered) and in maintaining public health. Therefore, the understanding of biofilms is key to discovering, controlling and directing the behaviour of microbial communities for sustainable environmental, engineering, public health and medical applications.

Biofilms are central to some of the most urgent global challenges and exert considerable economic impact across industry sectors. Biofilm management is essential to deliver clean and globally sustainable drinking water and food safety and security. Contamination, fouling, and energy losses by biofilms impact on the £70 billion (UK) foods industry, the US$2.8 trillion consumer products sector, and US$117 billion global coatings industry. They are a leading cause of antimicrobial resistance (AMR), forecast to cost US$100 trillion in world GDP and 10 million deaths by 2050. Biofilms are also a major cause of chronic infections, costing the NHS £2 billion per annum (NBIC Market Report 2017).

In trying to both tackle and utilise biofilms the industrial and research community (led by BBSRC /Innovate UK) have defined 4 key interventional strategies:

Prevention: Limiting or preventing the early stage microbial adhesion and colonisation events at surfaces. This could employ the use of advanced techniques to create the knowledge-based design of next-generation surfaces.

Detection: Accurate, quantitative biofilm detection and metrology across multiple scales through innovative sensing, tracking and diagnostic technologies.

Manage: To destroy, remove or control established biofilms by understanding and exploiting their life cycle dynamics and development across a range of environments and levels of complexity.

Engineer: Harness the benefits of complex microbial consortia from knowledge of their composition, function, ecology and evolution. Exploit biofilm understanding at the interface with engineering and process applications. Improve engineered platforms and solutions e.g. wastewater, biotechnology, resource recovery from wastewater, microbial fuel cells, aerobic and anaerobic biorefinery. The scope for this theme also includes precision tools for microbial community engineering using synthetic biology.

NBIC’s Industrial and Academic Engagement Strategy

A key element of the engagement strategy of NBIC with its industrial and academic community is the exploration of the current unmet needs in each sectors context and markets and defining what the current state of scientific and technological knowledge is in relation to addressing these needs. Developing this understanding is to allow NBIC to better direct its research and translational strategy, as well as facilitating its industrial/academic engagement. It is NBIC’s intention to hold workshops and scientific fora around these 4 themes on a rotating basis to deepen the overall understanding around each theme and influence scientific and translational activity.

The KTN (Innovate UK) held a workshop in York on the 27th February 2018 entitled ‘Identifying and Prioritising Industrial Challenges and Potential Solutions for the Prevention, Detection, Management and Engineering of Biofilms’. In this report2 one of the conclusions was that “The biggest need identified via voting (of participants)... was the effective detection and characterisation of biofilms.” With this in mind NBIC decided to hold its first academic/industrial engagement workshop on the subject of Biofilm Detection.

Biofilm Detection Workshop

1.1 SETTING AND AIMS

The workshop was held in Birmingham on September 24th 2018 and started at 10:00am and finished at 16:00pm. The stated goals of the workshop were:

- To understand and describe the unmet needs in various settings for biofilm detection
- Identify possible solutions and gaps in current research (with respect to industry and academic or sector partner needs)
- To create new contacts and relationships
- To identify possible projects and collaborations
- To guide NBIC in the direction of its research and translational activity

The meeting was open to all NBIC industry partners and Research Institutions, with 39 attendees from industry representing 21 companies, and 26 attendees from research institutions representing 18 organisations. These numbers had to be limited to fit the room capacity.

To provide inputs to the meeting NBIC industrial partners were approached to share their needs using the format beneath. These were received from 14 partner organisations, but only 9 were able to attend the meeting and lead syndicates.

These were collated and sent to all participants (see Appendix 1), asking them to consider these needs and then capture their thoughts using the format beneath:

<table>
<thead>
<tr>
<th>Industry issues for Biofilm Detection</th>
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<tr>
<td>What are the current problems you face with respect to biofilm detection?</td>
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<td>What do we really need to know/ detect and where?</td>
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<th>Expert input</th>
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<td>Do you see immediate solution approaches to unmet needs?</td>
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<td>Are there limits on potential solutions and what are the gaps (e.g. evidence, scale up)</td>
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The intent was to ensure attendees arrived with an understanding of the range of unmet needs in the detection of biofilms and had given these some thought prior to the session.

There was an initial plenary session led by NBIC summarising and discussing an outline scope of the needs, problems and opportunities in detection followed by a number of cross industry/academia group sessions. The intended outputs of the day were:

- New connections and new collaboration opportunities
- Additions to the evolving NBIC map of scientific/needs landscape
- Agreed priorities and opportunities for translational projects and research investment to influence funders and drive NBIC activities

At the event, 9 problem owners led syndicate sessions whereby participants were allocated to a particular problem area and asked firstly to individually make notes and then provide feedback to the problem owner.

Discussion questions were:

- What are the key biofilm challenges that emerge from this topic?
- What are the headline areas for addressing this?

The groups were then rotated to a new need area with the problem owner remaining to lead the next group.

Finally, all the output was posted on the walls and all delegates had a chance to post input to problems they had not yet had the chance to review.

This was all then collected in a flipchart format as below:

![Flipchart Format](image)

The NBIC team collected and organised all the output and held follow up feedback discussions after the meeting with the syndicate leads to ensure there was context and additional input to each area.
1.2 SYNDICATE OUTPUT

1. Chelsea Technologies Group – John Attridge

What are the current problems you face with respect to biofilm detection?

• We are an in-situ optical sensor manufacturer looking for a solution to biofouling.

• As a number of our systems monitor the health of algae in the environment, we cannot use solutions that potentially affect the measurements we are trying to make.

• Detection of when biofilm formation is affecting the measurement would be useful.

What current ‘solutions’ or options do you have?

• Currently we use physical wiper systems to protect our sensors, but these are expensive.

• We have looked at coatings, but getting long-term reliability underwater is an issue.

• We have the capability of developing a number of optical techniques to detect biofilm formation in collaboration with a group with microbiology expertise.

What do we really need to know/detect and where?

• For us it would be biofilm formation rate, optical properties, on glass/optical surfaces.

What would be the value of better detection?

• Very difficult to assess at this stage.

Summary

Optically based sensors are used in marine/freshwater locations (sometimes remote) and (a network of them) are used to detect algae CO2 consumption. Sensors need to be deployed, cleaned and maintained and if their functionality is lost due to fouling then they will need to be replaced. Detecting when this fouling is occurring is the key problem (i.e. knowing when functionality is compromised). Prevention of fouling is also a key need. To extend the life of the sensor, even a few months, can reduce cost significantly. These antifouling approaches (e.g. novel coatings and surfaces) need to be nontoxic. There is also the need for a model system for in order to screen options. It was felt a wide range of surface and detection options may exist and an applied approach was needed to investigate their applicability for this context.

Syndicate discussion for detection focused around a range of optical techniques such as using intrinsic tryptophan fluorescence.

Next steps

• A collaborative activity possibly as a Proof of Concept project between CTG and an academic group looking at fluorescence/optical based detection.

• This was also felt to be a possible cross sector problem and maybe other industrial partners in, for example the water sector, may have similar issues.
2. Akzo Nobel - Marie Dale

What are the current problems you face with respect to biofilm detection?

- In-situ detection (underwater, on a vessel, remote) in terms of both characterisation, morphology and impact (relation to drag).
- Non-destructive detection, particularly of bacterial biofilms.
- Linking biofilm characteristics/properties to the resultant drag impact.
- Integrating the relative importance of different characterisation methods alongside and against one another.

What current ‘solutions’ or options do you have?

- Optical coherence tomography.
- Metagenomics.
- HPLC.
- Spectral methods.
- Microscopy.
- Visual methods (camera/staining).

What do we really need to know/detect and where?

- Presence of and characteristics of marine biofilms on hulls of marine vessels in-situ.
- Biofilm physical properties under high shear conditions.
- Automated detection of biofilm presence.

What would be the value of better detection?

- Ability to clearly defined biofilm metrics as robust predictors of biofilm associated drag penalty.
- Development of antifouling coatings (or other prevention techniques; new technology development, high throughput screening).

Summary

The key challenge in this area/setting was felt to be the identification of new methods for detection and characterisation (note parallels with the CTG problem area). These approaches would ideally be real time, in-situ and non-destructive.

The lead solution areas identified by the syndicates were:

- Exploiting existing features – using the surface as an anode and employing electrochemical detection of the biofilm.
- Following up crossovers with the nuclear, oil and gas industries where similar needs or sensors may exist. This would need to be remote (and automated) sensor technology e.g. employing Raman.
- The use of smart coatings such as bacteria detecting polymers with intrinsic sensing ability or capable of responding to vibration and ultrasound detection was also proposed.
- The use of organic or inorganic mapping of surfaces.
- Discussion also occurred around approaches for prevention, once again we see these two control strategies portrayed as linked in an application for water/marine sensing i.e. how can we first aim to first prevent biofilms and then detect their presence.

Next steps

- Some simple POC projects to explore available options in this setting (e.g. sensing polymers).
- There are some underlying and cross sector requirements for sensors capable of detecting biofilm formation:
  - A more focussed cross-sector session on common needs for remote sensing with academic input is possibly required.
  - Also keeping sensors clear of fouling is an unmet need.
3. Biocomposites - Craig Delury

What are the current problems you face with respect to biofilm detection?

- In periprosthetic joint infection, lab results will often come back clear even though further (research) investigation will determine biofilm presence. As such, the current detection methods are poor or not sensitive enough. This issue is further complicated because we cannot “see” the biofilm.

What current ‘solutions’ or options do you have?

- Delivery of antibiotics prophylactically.
- Broad spectrum delivery of antibiotics, delivered locally.

What do we really need to know/detect and where?

- Bacterial Species (Gram +ve/Gram –ve/ multi species).
- Where the biofilm is (Soft tissue? Hardware? Bone?)
- Susceptibility of biofilms to local release of antibiotics.

What would be the value of better detection?

- More targeted therapy.
- Good antibiotic stewardship.
- Ability to conduct clinical trials of Biocomposite products.

Summary

The key challenges emerging from this medical context where the early detection of bacteria/biofilms at the site of surgery, understanding which bacteria are causing the problem and when the biofilms/bacteria are have become a problem to the patient.

The lead solution areas identified by the syndicates were:

- A “revealing” spray or material to detect where the biofilm may be.
- The use of spectroscopy to look for specific signatures of bacteria.
- Identification of indicator biomarkers of biofilms/bacteria. These could be antibody (or fragments) related. The use of the MinION and PacBio technologies for third generation sequencing was also suggested for identification approaches.
- Optical related detection of biofilms e.g. Moleculight (fluorescence).
- Combinations of biomarkers and external scanning/visualisation could offer benefits.

Next steps

- Research is needed to understand how the biofilm first forms on the implant (during or post-surgery?), and therefore explore the link between prevention and early detection.
- An accurate test is needed to first detect (ex vivo and ultimately in vivo and/or point of care) and confirm the presence of biofilms and collaborative or Proof of Concept projects would be the way forward for some of the technologies referred to above and with overlaps in other medical settings (e.g. wounds).
4. Smith & Nephew - Emma Woodmansey

What are the current problems you face with respect to biofilm detection?

• Recognition/understanding of biofilms as a clinical issue in routine practice (are biofilms responsible for wound non-healing and could biofilm detection lead to interventions of clinical trials or better targeted treatments?).
• Cost of detection.
• Reproducibility of methods.
• Lack of simplicity (ease of use/no expensive equipment).
• Detection spectrum (multi species needed).
• Depth of detection (in granulation tissue as well as wound surface).

What current ‘solutions’ or options do you have?

• Presumption that most chronic wounds contain a biofilm.
• Signs and symptoms of biofilm (covert) infection.

What do we really need to know/detect and where?

• Biofilm location in the wound (heterogeneity in distribution).
• Quantification – aids understanding of any “threshold levels” (when is the biofilm leading to an infection for example).
• In-situ monitoring/standardized detection methods to understand success of interventions (e.g. topical/systemic antimicrobials, non-antibiotic therapies).

What would be the value of better detection?

• Targeted treatment: earlier aggressive (biofilm based) treatment intervention – to improve healing and reduce chance of infection.
• Biofilm not acute infection: differentiation from other microbial issues.
• Stimulates more focused development of effective anti-biofilm technologies.

Summary

The key challenge emerging from this context was:

• The lack of a bedside (in-situ/point of care) biofilm and infection detection approach for wounds - must be easy to use and interpret.

• What to measure? EPS/bacterial numbers/DNA/RNA.
• When is a biofilm really a biofilm (and not cellular debris for example).
• When is the biofilm pathogenic as opposed to passive.
• Where is it! “location, location, location!”
• Lack of education of clinicians on biofilms:
  - biofilm science fact and fiction including role in AMR;
  - sampling.
• Lack of standard wound models for development of tests and understanding.
• We don’t really have good biomarkers and tools for detection.
• Improved methods of data analysis and simplification of interpretation.

The key challenge emerging from this context was:

• Engagement with stakeholders in wound care to understand needs.
• Improved multidisciplinary education.
• Need handheld POC devices (e.g. MinION - Multiplex PCR, Moleculight - Auto fluorescence).
• Agreed protocols for sampling and assessment (both clinic and lab) need to be developed in conjunction and engagement with regulatory bodies who will define approvable criteria.
• Data needs to be linked to treatment outcomes using defined protocols to validate detection methods in this context (with link also to infection).
• Data interpretation needs to be simplified to enable Yes/No style basic information for the generalist.

Next steps

• A high degree of collaboration across all stakeholders in this field is needed. Researchers, clinicians and companies to achieve the data sets and education needed to better understand how to detect biofilms in wounds and link this to treatment outcomes.
• Some promising technologies are emerging for sequencing near to POC and imaging wounds.
5. University of Southampton/Southampton University Hospital (clinical biofilms) - Saul Faust

What are the current problems you face with respect to biofilm detection?

- No definitive biomarkers/diagnostic “is it biofilm” (directly – e.g. on tissue/material; or indirectly e.g. in blood, cf. industrial pipe fluids, any secondary medium).
- Sampling issues e.g. culturability, access to tissue (e.g. biopsies) in standardized ways, no mechanism to visualize in-vivo in humans.
- And/or to assess the implication of the stage of the biofilm (e.g. quiescent or pathogenic, cf ship hull – when is biofilm thick enough to cause drag?).
- No standardization of diagnostic technologies – which assays should be used for which type of biofilms and how can these be standardized across sectors (and for trials of anti-biofilm or biofilm promoting interventions?).

What current ‘solutions’ or options do you have?

- Standard microbiology but no agreed standardization for diagnosis or trials (see ECSMID consensus opinion, Clin Microbiol Infect 2015; 21: S1–S25 or http://dx.doi.org/10.1016/j.cmi.2014.10.024)
- Indirect methods (signs of things going wrong) e.g. chronic infection (signs and symptoms), cf pressure drop/loss of heat transfer capacity, corrosion, product spoilage in industry.
- Novel methods of detection that are currently experimental/research (e.g. new diagnostic technologies or imaging such as micro-CT, new microscopy techniques e.g. label-free imaging).

What do we really need to know/detect and where?

- How to take samples – culturability/standard microbiology.
- When are biofilms problematic or beneficial (is a biofilm pathogenic or destructive, what pathogens does it contain, species composition).
- How to test interventions (e.g. does rifampicin work as anti-biofilm Ab in-vivo).
- Ability to grade biofilms (e.g. traffic light system quiescent/pathogenic or cf grading ship hull biofilms).
- Understand quality of the data – e.g. reproducibility, sensitivity/specificity and positive/negative predictive values for pathogens in biofilms.

What would be the value of better detection?

- Ability to design and test interventions for market.
- Ability to detect early to intervene earlier – to inform more strategic management.
- Ability to compare products across a market.
- Operator independent cheap diagnostic platforms (across sectors).

Summary

The key challenges emerging from this context were:

- The lack of clear definition of biofilms and definitive markers.
- Large variability in samples (in-vitro standardization is easier, but what are we standardizing against!).
- Lack of validated sampling techniques and technologies.

The lead solution areas identified by the syndicates for this problem were:

- Use of animal/lab models for studies and method development.
- ‘Omics approaches to collect large data sets to assess samples.
- Imaging techniques (preferably label free) for visualising samples and rapid analysis. Studying host/system response.
- Data analytics to link data to outcome/biofilm presence.

Next steps

- Could NBIC provide the lead in developing standard approaches to biofilm sampling and setting of “standards” and methodologies through collaborations?
6. Pall Corporation - Kevin Charman

What are the current problems you face with respect to biofilm detection?

• Non-invasive/non-contact/‘occult’ detection of biofilm in water systems – e.g. in pipework (i.e. no direct visibility of biofilm).
• Real-time detection of biofilm.
• Visualising biofilm in-situ at customer site.
• Sensitivity (not sensitive enough) – detect early establishment for more rapid response.

What current ‘solutions’ or options do you have?

• Removal of pipework sections/destructive analysis to assess biofilm.
• Staining.

What do we really need to know/detect and where?

• Biofilm thickness and viability.
• Composition (presence of pathogens).
• Detection through opaque materials and in potentially difficult to access (internally and externally) pipework/places.

What would be the value of better detection?

• Demonstration of biofilm presence and extent to customers – hence determine need for any remedial action.
• Identify locations harbouring significant biofilm load to monitor disinfection treatment efficacy.
• Evidence-directed control/maintenance.

Summary

The key challenge emerging from this context was the detection of biofilm formation in hospital water distribution systems (also relevant to other sectors).

The lead solution areas identified by the syndicates for this problem were:

• Modelling – metagenomics data from water samples during waterborne infection outbreaks – this could provide the data to develop an early warning system of problems (collaborative opportunity).
• Biomarkers – detection of specific markers for biofilms in water which could also be tailored to organisms of interest (pathogens). Impedance measurements across the filter was also proposed as a possible route. These approaches could also be used to make point of use filters “smart” and capable of real time sensing monitoring and alarming (research focused activity).
• Filter analysis – if POU filters cannot be made “smart” then these could be used as collection devices for further analysis e.g. Quartz Crystal Microscopy, spectroscopy, image analysis, in-situ sequencing (collaborative and near-term opportunity).
• Acoustic Ultrasound – signal can be sent down a pipe and analysed to look for corrosion or biofilm deposition (collaborative opportunity).

Next steps

• A number of immediate collaborative approaches are possible to explore the use of modelling (based on collecting in use data), interrogation of PU filters and incorporation of sensors into these filters as a detection methodology for problem organisms.
• A number of parallels exist with other sectors sharing the requirement for real time in-situ sensing of biofilm formation in a water environment.
7. Severn Trent Water – Karen Heaton

What are the current problems you face with respect to biofilm detection?

- Our problematic biofilms are inside taps and pipes. An area for concern is in sample lines – which they harbour organisms that may slough off into regulatory samples which would give a false impression on the quality of the water being produced. Is there an optimum flow regime that maintains biofilms stability – continuously running taps? Periodic flushing? (we also have to ensure that there is no sediment build up in pipes that could result in a mis-representative turbidity result).

What current ‘solutions’ or options do you have?
- We currently don’t monitor in any way.

What do we really need to know/detect and where?

- Effect of changes in shear on biofilm stability.
- Materials that resist biofilm formation (must be Water Regulations Advisory Service approved).
- How substantial are biofilms in our sample lines?

What would be the value of better detection?
- Assurance that regulatory sampling results are indicative of the real water quality and not influenced by the pipework/taps required to facilitate sampling of the bulk water.

Summary

The key challenges emerging from this context were:

- The detection of biofilms in water pipes to assess if regulatory sampling of water supply is producing false negatives.

The lead solution areas identified by the syndicates for this problem were:

- Application of solutions from other areas e.g. in line sensors possible linked to detection of markers from key organisms (coliforms) and biofilms in the water.
- Catty out research on prevalence of biofilms in these systems and assess if modelling can be predictive of formation.

Next steps

- The opportunity exists to set up a parallel sampling system to the one used for regulatory samples which would allow creation of a clean starting point for studying and assessment of biofilm formation.
8. P&G - Andrew Graydon

What are the current problems you face with respect to biofilm detection?

• Detection methods often limited to the laboratory and not suitable for use “at source”. That is, current detection methods are not portable and require specialist training, such as fluorescence microscopy.

• Extensive research on single species biofilm is available. However, reproducible production of multi-species biofilm is not so well documented, especially those related to biofilms found on fabrics from sweat, sebum, detergent and inorganic salts.

What current ‘solutions’ or options do you have?

• No portable methods exist for “at source” detection of biofilms (e.g. at consumer home, at manufacturing facility, at hospital).

• Empirical: malodour testing or bacterial count from extracted solutions.

What do we really need to know/detect and where?

• Is there a biofilm (yes or no)?

• Example locations would be in kitchens, bathrooms, on appliances, in hospitals, on clothing/textiles, at liquid manufacturing sites.

• Biofilms in both hydrated and dehydrated states.

• Study effect of key technologies on hydrated biofilms and link it back to re-bloom of malodour. Response of multi species biofilms to nutrients and technologies is poorly understood and we need better methods here.

What would be the value of better detection?

• Ability to design and test interventions for market.

• Ability to detect early to intervene earlier – to inform more strategic management.

• Ability to compare products across a market.

• Operator independent cheap diagnostic platforms (across sectors).

Summary

The key challenge emerging from this context was:

• The in-situ detection of biofilms in a domestic and plant (production) environment in-situ.

The lead solution areas identified by the syndicates for this problem were:

• The potential to apply methods already available perhaps in combination:
  • Real time portable PCR (e.g. MinION)
  • Embedded sensors to measure change in impedance as biofilms form
  • ATP bioluminescence
  • LPS fluorescence
  • Live / Dead stains e.g. Baclight kit
  • Refractive Index change assessment
  • Raman spectroscopy (handheld instruments available)
  • Sampling coupons in the production equipment

• Use of microfluidic techniques with Raman, proteomics, MS for a deeper understanding of what to measure.

Next steps

• A number of currently available approaches and techniques offer the potential for assessment in the home and production environment. Perhaps most challenging is the creation of easy to use approaches in the home/domestic environment which are simple to use and easy to interpret to aid the consumer in understanding whether removal (cleaning) has been successful.
9. GSK – Rob Howlin

What are the current problems you face with respect to biofilm detection?

• Consumer engagement with biofilm detection and understanding.
• Harmonisation of methods to detect biofilms and demonstrate anti-biofilm efficacy.
• Detection and understanding of mechanisms behind transition of “healthy” biofilms to “unhealthy/disease-causing biofilms”. Detection of key species in disease and their role.

What current ‘solutions’ or options do you have?

• Engagement with academic partners and internal work in biofilm research (microbiome studies etc.).
• GSK methods based on relevant industry standards and various imaging platforms (CSLM, SEM, epifluorescence).
• Microbiome studies.

What do we really need to know/detect and where?

• Biofilms in the context of oral health, gut, skin and respiratory/nasal health.
• Role of biofilms in health and disease, what are key species relevant to pathogenesis and interaction with host.

What would be the value of better detection?

• Better understanding of role of biofilm in disease or conditions related to consumer health and development of intervention strategies.
• Public engagement in biofilms to empower consumer to take control of their health.

Summary

The key challenges emerging from this context were:

• What are the mechanisms behind the transition from a “healthy” biofilm to a disease or “unhealthy” one?
• Lack of availability of standard methods for sampling and assessing biofilms.
• Consumer/public/patient lack of understanding of biofilms (what they are, their role and their importance).

The lead solution areas and next steps identified by the syndicates for this problem were:

• Large multidisciplinary data sets are needed between academia and industry to understand transition from healthy to disease state.
• Cross industry collaboration and engagement in policy development (disease and public health).
Discussion and Conclusions

In considering the detection of biofilms then it is clear that usually our problem owners across a range of sectors want to know the answer to one or more of three key questions:

- Is there a biofilm present?
- Where exactly is it? (e.g. location in a wound or a water system or industrial pipework)
- What can you tell me about it? (e.g. composition, characterisation and impact)

Across the syndicates a wide range of detection approaches, many currently already available and some in the early stages of research application were discussed including: fluorescence, ultrasound, Raman spectroscopy and molecular tools. Each of these (and others) may have particular benefits for a specific context. Novel approaches for detection were also discussed and considered e.g. smart coatings and electrochemical detection (see Akzo Nobel syndicate for example). In most cases the problem-owners ideal solution for detection would be an approach that was real time, in-situ and non-destructive. In medical applications this need can sometimes also be for point of care detection. Whilst some detection techniques may be suitable for research applications the challenge for wider adoption is to identify how a technique can be used directly and robustly in the wide range of settings described. There is also a common theme linking prevention to detection in that identifying if a biofilm is present is a way of understanding if prevention strategies being deployed are actually working (e.g. Chelsea Technologies marine sensors) and to monitor or test new interventions.

In the medical/human applications (GSK, Smith and Nephew, Biocomposites and University of Southampton/NHS) some common themes emerge:

- The requirement to be able to detect and confirm the presence of a biofilm in a standardised reproducible manner using approved protocols that would be acceptable to regulatory agencies, that would tie in with clinical definitions of disease and infection and be able to validate the efficacy of interventions (the prevention or management of biofilms and being able to claim a biofilm has been prevented or removed).

- In the case where a biofilm is part of normal organism (human or animal, e.g. oral, gut, skin, respiratory tissue) then the need becomes one of being able to detect/characterise when a biofilm transitions from a “healthy” to “unhealthy or pathogenic” state. There was a recognition of the need for large multidisciplinary data sets and collaborative activity for this to be achieved.

In industrial applications such as water and filtration systems (Pall, Severn Trent) then detection poses significant challenges relating to access to possibly remote surfaces in order to locate a biofilm. It may be possible to detect a biofilm exists somewhere in the system via the use of in line sensing (e.g. biomarkers of biofilm presence) but locating it precisely may not always be feasible. Some novel approaches such as acoustic ultrasound offer potential here for development of location pinpointing and detection.

In consumer applications around the home then the ability to detect a biofilm in-situ on a surface is a key need and whilst a number of techniques have the potential to achieve this and may be of research use for product development activities the key challenge is the creation of easy to use and interpret approaches that could be used by the consumer to assess the need for, or the impact of, cleaning or hygiene interventions.

Finally, there was recurring need for wider engagement with consumers, regulators and other stakeholders in the need for both better definition of standards and policy development in the field of biofilms and biofilm detection. In this role then NBIC has a role to play in helping to develop a consensus view, influencing regulators and encouraging and facilitating cross-sector collaboration.
Appendix 1: Unmet Needs

NBIC DETECT WORKSHOP

Compilation of Unmet Needs in the detection of biofilms kindly supplied by partners.
(Note that some of these are also referred to in detail in the main text of the report as syndicate sessions).

Companies and Sectors

Moy Park
FOOD

Biocomposites
MEDICAL/ORTHOPEDIC

Smith and Nephew
MEDICAL/WOUND

Jaguar Landrover
AUTOMOTIVE

Chelsea Technologies
SENSOR Sector – Sensors & systems for the maritime, marine science, water environmental, defence & process control markets

Unilever
HOME MANUFACTURING HYGIENE and PERSONAL CARE

Pall Corporation
FILTRATION - filtration, separation and purification products

Akzo Nobel
COATINGS

BP
OIL/GAS

GSK
CONSUMER Healthcare-Oral health, Pain relief, Respiratory, Nutrition/gastro-intestinal and Skin health categories.

Severn Trent/Anglian
WATER

Genesis Biosciences
BIOSCIENCES – environmentally-responsible products and solutions in cleaning, waste water treatment, feminine hygiene, fish farming and marine applications.

University of Southampton/Southampton General Hospital team
Clinical detection but with cross sector impact

P&G
HYGIENE
What are the Current problems you face with respect to biofilm detection?

- Currently we don’t know where we have biofilms – we have assumptions but no definitive proof. Normal methods of swabbing post hygiene are probably not detecting organisms embedded within biofilm matrices.
- In factories we have fixed pieces of equipment with moving parts plus we have pieces of equipment (e.g. chains) which are a mile long and travel through our plant.
- In first instance the food/product can be the vector to move contamination from one biofilm hotspot to another. In the second it is the equipment itself which is the vector.
- Similarly in farms (e.g. drinker lines, general environment) we may not be detecting biofilms containing pathogens/non-pathogens which could be a source of reoccurring contamination.

What Current ‘solutions’ or options do you have?

- We assume that our current hygiene regimes and monitoring of cleanliness via swabbing is sufficient to remove any residual microbial contamination.
- Outside of this there are few other options available to detect biofilms which are feasible for use on farms or factories.
- Removal of biofilms could be done through sonication, for example, but the application in farms and factories, in all areas is not feasible.

What do we really need to know/detect and where?

- Need to be able to ensure that we have removed all biofilms/contamination hotspots during our hygiene process.
- Need to be able to design equipment and facilities which prevent biofilm build-up in the first place.

What would be the Value of better detection?

- Allow for targeted prevention and reduction strategies
### What are the Current problems you face with respect to biofilm detection?

- In Periprosthetic Joint Infection, lab results will often come back clear even though further investigation will determine biofilm presence. As such, the current detection methods are poor or not sensitive enough. This issue is further complicated because we cannot “see” the biofilm.

### What Current ‘solutions’ or options do you have?

- Delivery of antibiotics prophylactically
- Broad spectrum delivery of antibiotics, delivered locally

### What do we really need to know/detect and where?

- Bacterial Species (Gram +ve/Gram –ve/ Multi species)
- Where the biofilm is (Soft tissue? Hardware? Bone?)
- Susceptibility of biofilms to local release of antibiotics

### What would be the Value of better detection?

- More targeted therapy
- Good antibiotic stewardship
# INDUSTRY ISSUES FOR BIOFILM DETECTION (S&N)

## What are the Current problems you face with respect to biofilm detection?

- Recognition/ understanding of biofilms as a clinical issue in routine practice
- Cost
- Reproducibility
- Simplicity (Ease of use/ no expensive equipment)
- Detection spectrum
- Depth of detection

## What Current ‘solutions’ or options do you have?

- Presumption that most chronic wounds contain a biofilm
- Signs and symptoms of biofilm (covert) infection

## What do we really need to know/detect and where?

- Biofilm location (heterogeneity in distribution)
- Quantification – aids understanding of any “threshold levels”
- In situ monitoring to understand success of interventions

## What would be the Value of better detection?

- **Targeted treatment**: Earlier aggressive (biofilm based) treatment intervention
- **Biofilm not acute infection**: Differentiation from other microbial issues
- Stimulates more focused development of effective anti-biofilm technologies.
### INDUSTRY ISSUES FOR BIOFILM DETECTION (JLR)

<table>
<thead>
<tr>
<th>What are the Current problems you face with respect to biofilm detection?</th>
</tr>
</thead>
<tbody>
<tr>
<td>• There is limited data available about what bacteria is present in an automotive interior</td>
</tr>
<tr>
<td>• Detecting the different types of bacteria in a global product is challenging</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What Current ‘solutions’ or options do you have?</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Current solution is a “forensic” investigation to determine the bacterial loading and locations</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>What do we really need to know/detect and where?</th>
</tr>
</thead>
<tbody>
<tr>
<td>• The key thing for us is to understand what bacteria is present in a vehicle interior, where it is present, how much is present and how dangerous it could potentially be to customers.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What would be the Value of better detection?</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Better detection would allow us to develop a cleaner &amp; healthier interior to protect our customer</td>
</tr>
</tbody>
</table>
# Industry Issues for Biofilm Detection (Chelsea Technologies Group)

**What are the Current problems you face with respect to biofilm detection?**

- We are an in situ optical sensor manufacturer looking for a solution to biofouling.
- As a number of our systems monitor the health of algae in the environment, we cannot use solutions that potentially affect the measurements we are trying to make.
- Detection of when biofilm formation is affecting the measurement would be useful.

**What Current ‘solutions’ or options do you have?**

- Currently we use physical wiper systems to protect our sensors, but they are expensive.
- We have looked at coatings, but getting long-term reliability underwater is an issue.
- We have the capability of developing a number of optical techniques to detect biofilm formation in collaboration with a group with microbiology expertise.

**What do we really need to know/detect and where?**

- For us it would be biofilm formation rate, optical properties, on glass/optical surface.

**What would be the Value of better detection?**

- Very difficult to assess at this stage.
### What are the Current problems you face with respect to biofilm detection?

- Compositional analysis (cells, eDNA, protein, EPS, etc.)
- Distinguishing between biofilm “phases”... early attachment V's microcolonies V's “true” biofilm
- Detecting in-situ – rather than extracting and TVC's
- Defining MoA of actives – gross effects only
- Quantitative measurement (incl. visualisation & image analysis)
- Standardisation (in-vitro)
- Striated conditions within a biofilm (e.g. base = anoxic/dead; outer layer = metabolically active)

### What Current ‘solutions’ or options do you have?

- Cell counts
- Fluorescent staining (live/dead/EPS)
- Some (limited) microscopy
- Transcriptomics/qPCR – biofilm markers
- Metataxonomic studies

### What do we really need to know/detect and where?

- Whether dealing with clumps/microcolonies of closely related (near homogenous) species V's biofilm (when do they become “true” biofilms)? Defining the cell products or genetic regulation across relevant pathways.
- Spatial arrangement/location in-situ (incl. associations with other soils/substrates)
- Once have taxonomic data, require community functional information (translating the genomic data into functional data).

### What would be the Value of better detection?

- Greater understanding of the problem.
- Target approaches for intervention or treatment.
- Generation of *in vitro* models
  - standardization
  - testing
  - quantitative data
**INDUSTRY ISSUES FOR BIOFILM DETECTION – PALL**

**What are the Current problems you face with respect to biofilm detection?**

- Non-invasive/non-contact/’occult’ detection of biofilm – e.g. in pipework (i.e. no direct visibility of biofilm).
- Real-time detection of biofilm.
- Visualising biofilm in-situ at customer site.
- Sensitivity (not sensitive enough) – detect early establishment for more rapid response.

**What Current ‘solutions’ or options do you have?**

- Removal of pipework sections/destructive analysis to assess biofilm.
- Staining.

**What do we really need to know/detect and where?**

- Biofilm thickness and viability.
- Composition (presence of pathogens).
- Detection through opaque materials and in potentially difficult to access (internally and externally) pipework/places.

**What would be the Value of better detection?**

- Demonstration of biofilm presence and extent to customers – hence determine need for any remedial action.
- Identify locations harbouring significant biofilm load to monitor disinfection treatment efficacy.
- Evidence-directed control/maintenance.
## INDUSTRY ISSUES FOR BIOFILM DETECTION – AKZO NOBEL

### What are the Current problems you face with respect to biofilm detection?

- In-situ detection (underwater, on a vessel, remote) in terms of both characterisation, morphology and impact (relation to drag).
- Non-destructive detection, particularly of bacterial biofilms.
- Linking biofilm characteristics/properties to the resultant drag impact.
- Integrating the relative importance of different characterisation methods alongside and against one another.

### What Current ‘solutions’ or options do you have?

- Optical coherence tomography
- Metagenomics
- HPLC
- Spectral methods
- Microscopy
- Visual methods (camera / staining)

### What do we really need to know/detect and where?

- Presence of and characteristics of marine biofilms on hulls of marine vessels in-situ.
- Biofilm physical properties under high shear conditions.
- Automated detection of biofilm presence.

### What would be the Value of better detection?

- Ability to clearly defined biofilm metrics as robust predictors of biofilm associated drag penalty.
- Development of antifouling coatings (or other prevention techniques; new technology development, high throughput screening).
**INDUSTRY ISSUES FOR BIOFILM DETECTION – BP**

**What are the Current problems you face with respect to biofilm detection?**

**Occurrence of MIC**

Need to use indirect methods:
- Unknown/no correlation between planktonic & sessile populations.
- Lack of proxies for presence/extent of biofilm.
- Inability to interpolate across network/between sample points.

**What Current ‘solutions’ or options do you have?**

- Microbial count techniques – trending only – ATP, MPN, 16s qPCR.
- Retrievable coupon inserts or side streams with retrievable studs (Robbins device).

**What do we really need to know/detect and where?**

Monitor for (& ideally quantify) biofilm presence & activity:
- Primarily, in dead legs (many locations, but are known and discrete).
- Secondly, localize the occurrence biofilm in wider pipeline network.

**What would be the Value of better detection?**

- More effective/targeted use of biocide/clean up treatments – ability to measure performance.
- Fewer LOPC (loss of primary containment).
- Reduced risk – different monitoring methods, more predictable risk.
What are the Current problems you face with respect to biofilm detection?

- Consumer engagement with biofilm detection and understanding.
- Harmonisation of methods to detect biofilms and demonstrate anti-biofilm efficacy.
- Detection and understanding of mechanisms behind transition of “healthy” biofilms to “unhealthy/disease-causing biofilms”. Detection of key species in disease & their role.

What Current ‘solutions’ or options do you have?

- Engagement with academic partners and internal work in biofilm research (microbiome studies etc.).
- GSK methods based on relevant industry standards and various imaging platforms (CSLM, SEM, epifluorescence).
- Microbiome studies.

What do we really need to know/detect and where?

- Biofilms in the context of oral health, gut, skin and respiratory/nasal health.
- Role of biofilms in health and disease, what are key species relevant to pathogenesis and interaction with host.

What would be the Value of better detection?

- Better understanding of role of biofilm in disease or conditions related to consumer health and development of intervention strategies.
- Public engagement in biofilms to empower consumer to take control of their health.
## INDUSTRY ISSUES FOR BIOFILM DETECTION – SEVERN TRENT

### What are the Current problems you face with respect to biofilm detection?

- Our problematic biofilms are inside taps and pipes. An area for concern is in sample lines - which they harbour organisms that may slough off into regulatory samples which would give a false impression on the quality of the water being produced.
- Is there an optimum flow regime that maintains biofilms stability – continuously running taps? Periodic flushing? (we also have to ensure that there is no sediment build up in pipes that could result in a miss-representative turbidity result).

### What Current ‘solutions’ or options do you have?

- We currently don't monitor in any way.

### What do we really need to know/detect and where?

- Effect of changes in shear on biofilm stability
- Materials that resist biofilm formation (must be WRAS approved)
- How substantial are biofilms in our sample lines?

### What would be the Value of better detection?

- Assurance that regulatory sampling results are indicative of the real water quality and not influenced by the pipework/taps required to facilitate sampling of the bulk water.
## INDUSTRY ISSUES FOR BIOFILM DETECTION – ANGLIAN WATER

<table>
<thead>
<tr>
<th>What are the Current problems you face with respect to biofilm detection?</th>
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<tbody>
<tr>
<td>• Access to mains to test biofilm, distinguishing between bulk water and biofilm growth in regulatory samples. Reliable and repeatable techniques.</td>
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<tr>
<th>What Current ‘solutions’ or options do you have?</th>
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<tbody>
<tr>
<td>• Currently no options in the network. We use rigs to simulate networks and us coupons to test biofilm growth.</td>
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<tr>
<th>What would be the Value of better detection?</th>
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<tbody>
<tr>
<td>• To be able to assess the impact of changing water sources on the main wall. To assess the impact of altering doses to control water stability. Whether pathogens are likely to cause water quality issues. To assess the stability of biofilm and iron/manganese incorporated in it to inform planned cleaning.</td>
</tr>
</tbody>
</table>
**INDUSTRY ISSUES FOR BIOFILM DETECTION – GENESIS BIOSCIENCES**

<table>
<thead>
<tr>
<th>What are the Current problems you face with respect to biofilm detection?</th>
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<tbody>
<tr>
<td>• Rapid and simple quantification and identification of biofilms on carrier materials such as organics (bran) inorganics (zeolites) and hard surfaces (clinical setting).</td>
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</table>

<table>
<thead>
<tr>
<th>What Current ‘solutions’ or options do you have?</th>
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<tr>
<td>• We have employed the crystal violet method for some surfaces and coupon work. This is fine if the surface material is inert and amendable to the process, the challenge comes from materials that are not.</td>
</tr>
<tr>
<td>• Methods such as CLSM, SEM although effective consume man hours and require the use of external facilities.</td>
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<tr>
<th>What do we really need to know/detect and where?</th>
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<tbody>
<tr>
<td>• For us we wish to know how our bio augmentation method are working. So if the bacteria are colonizing the materials we are delivering to system.</td>
</tr>
<tr>
<td>• If our antimicrobial/anti-biofilm treatments are working (simple biofilm quantification).</td>
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<tr>
<th>What would be the Value of better detection?</th>
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</thead>
<tbody>
<tr>
<td>• A simple, quick and cheap visual method would help to save man hours and enable us to do conduct research on a broader range of surfaces and systems.</td>
</tr>
</tbody>
</table>
### What are the Current problems you face with respect to biofilm detection?

- No definitive biomarkers – diagnostic “is it biofilm” (directly – e.g. on tissue/material; or indirectly e.g. in blood, cf. industrial pipe fluids, any secondary medium).
- Sampling issues e.g... culturability, access to tissue (e.g. biopsies) in standardized ways, no mechanism to visualize in-vivo in humans.
- And/or to assess the implication of the stage of the biofilm (e.g. quiescent or pathogenic, cf ship hull – when is biofilm thick enough to cause drag?).
- No standardization of diagnostic technologies – which assays should be used for which type of biofilms and how can these be standardized across sectors?

### What Current ‘solutions’ or options do you have?

- Standard microbiology but no agreed standardization for diagnosis or trials (see ECCSMID consensus opinion).
- Indirect methods (signs of things going wrong) e.g. chronic infection (signs and symptoms), cf pressure drop/loss of heat transfer capacity, corrosion, product spoilage in industry.
- Novel methods of detection that are currently experimental/research (e.g. new diagnostic technologies or imaging such as micro-CT, new microscopy techniques).

### What do we really need to know/detect and where?

- How to take samples – culturability/standard microbiology.
- When are biofilms problematic or beneficial (is a biofilm pathogenic or destructive, what pathogens does it contain, species composition).
- How to test interventions (e.g. does rifampicin work as anti-biofilm Ab in-vivo).
- Ability to grade biofilms (e.g. traffic light system quiescent/pathogenic or cf grading ship hull biofilms).
- Understand quality of the data – e.g. reproducibility, sensitivity/specificity and positive/negative predictive values for pathogens in biofilms.

### What would be the Value of better detection?

- Ability to design and test interventions for market.
- Ability to detect early to intervene earlier – to inform more strategic management.
- Ability to compare products across a market.
- Operator independent cheap diagnostic platforms (across sectors).
What are the Current problems you face with respect to biofilm detection?

- Detection methods often limited to the laboratory and not suitable for use “at source”. That is, current detection methods are not portable and require specialist training, such as fluorescence microscopy.
- Extensive research on single species biofilm is available. However, reproducible production of multi species Biofilm is not so well documented, especially those related to biofilms found on fabrics from sweat, sebum, detergent and inorganic salts.

What Current ‘solutions’ or options do you have?

- No portable methods exist for “at source” detection of biofilms (e.g. at consumer home, at manufacturing facility, at hospital).
- Empirical: malodour testing or bacterial count from extracted solutions.

What do we really need to know/detect and where?

- Is there a biofilm (yes or no)?
- Example locations would be in kitchens, bathrooms, on appliances, in hospitals, on clothing/textiles, at liquid manufacturing sites.
- Biofilms in both hydrated and dehydrated states.
- Study effect of key technologies on hydrated biofilms and link it back to re-bloom malodour. Response of multi species biofilms to nutrients and technologies is poorly understood and we need better methods here.

What would be the Value of better detection?

- Better problem definition and targeted research into dispersal solutions (i.e. where are biofilms actually an issue).
- Malodour generation on fabrics happen when moisture is present (either from sweat or re-wetting of fabrics) when Biofilm is re-hydrated (re-bloom malodour).
Sainsbury’s

The biggest current problem I face (and I imagine this is common to many in our industry) is the ‘you don’t know what you don’t know’ question. So, one challenge for your group would be to develop methodologies and approaches to help businesses establish how to determine where biofilms may be presenting problems for them i.e. a toolkit for biofilm detection and quantification of impact. If you can understand where they are and what impact they are having one can then start to develop sound commercial business cases for investment in interventions. Similarly, this could be applied to the food safety and spoilage impact of biofilms albeit less of a commercial case and more of a reputational one. So that clearly then runs into the need for the development of cost-effective interventions to remove/prevent the formation of biofilms.

- ALEC KYRIAKIDES,
  HEAD OF CENTRAL TECHNICAL OPERATION
Appendix 2: Companies and Research Institutions registered at the workshop

Aston University
Liverpool University
Sheffield University
Perfectus Biomed
Birmingham University
Neem Biotech
Southampton University
Nottingham University
5D Healthcare Protection Group
Smith and Nephew
Anglian Water
St Andrews University
Edinburgh University
British Geological Survey
Sheffield University
Pennotec
Cardiff University
Knowledge Transfer Network
Manchester University
York University
Cranfield University
Liverpool University
Ozo Innovations
Oscar Mayer
Bath University

Birmingham University
Greenwich University
Zeiss
Biocomposites
Manchester Metropolitan University
Sheffield University
Ohio State University
Centre for Process Innovation
Portsmouth University
Nu-Angle
Chelsea Technology
Pall
Akzo Nobel
Biocomposites
Procter and Gamble
Severn Trent Water
GSK
Moy Park
Unilever
Southampton General Hospital
Jaguar Landrover
Shimyatech
Thank you

For further information please contact nbic@biofilms.ac.uk