**The Role of** **Standards in Biofilm Research and Industry Innovation**

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**ABSTRACT**

Biofilms are widely recognised as a contributing factor in significant problems currently facing human health and industry. The following paper summarises a round table forum held at the 2021 International Biodeterioration and Biodegradation Symposium which discussed the potential role of standards in biofilm research and industry innovation. Standards and other forms of best-practice guidance are reviewed in an academic research context as well as in relation to industry impacts and product development. The understanding of fundamental aspects of biofilms is rapidly evolving, driven in part by new analytical methods. However, the complex and multidisciplinary nature of biofilm-associated problems and typically limited training available for industry personnel tackling the associated issues often reduces the ability to provide best-practice solutions. As such it is argued that more effort needs made by both academia and industry experts to provide consensus and associated documentation on standard test methods or guidance documents related to studying and combating biofilms.

1. **Introduction**

The term *biofilm* is used to describe self-organised single or multispecies communities of microorganisms generally associated with a surface. There is a rich published history of biofilm research and today there are numerous research institutes and collaborative networks at national and international levels dedicated to this topic (Fields et al., 2020; Flemming et al., 2021; NBIC 2018a). Biofilms are of major importance, and have significant health and economic impacts, across a broad spectrum of areas such as medicine, marine vessels and structures, oil and gas production and storage as well as water supply and treatment facilities (Cámara et al., 2022). Because of this, there is a need for antibiotics, antimicrobials, biocides, and clean-in-place practices that target biofilm control.

Research, testing and the understanding of biofilms has advanced drastically since the early observations of van Leeuwenhoek in the late 17th century (van Leeuwenhoek, 1677). The field encompasses a wide range of scientific and engineering disciplines, with cutting edge research being performed to solve biofilm associated problems. Biofilm research and monitoring can be highly specialised, and there is often a need for testing to be performed in conditions with limited infrastructure. This issue is compounded by a limited number of cross-disciplinary guidelines that provide instructions for decision making by the plant operator, field engineer, and corrosion specialist, for instance. Hence there is a clear requirement, in both research and industry, for guidance on methods and reporting guidelines as well as the development of standards for biofilm testing.

The issue of standards in reporting and test methods is a key issue for all fields of research. There are numerous discussions in relation to reproducibility in scientific studies, such as a common problem of a third party not being able replicate a study result (Baker, 2016; Ioannidis, 2005; National Academies of Sciences, Engineering, and Medicine, 2019). In the biofilm research community, efforts to address this reproducibility “crisis” have included the publication of Minimum Information Guidelines which specify the information an author should include so that a research experiment may be replicated in a third party laboratory (Allkja et al., 2019; Lourenco et al., 2014). This paper will explore similar efforts to develop guidelines and methods in the energy sector, however further efforts in this space are drastically needed. Conversely, the development and use of confidential test methods by industry that are viewed as ‘trade secrets’, and can be seen as providing a competitive advantage, can result in limiting the sharing of important knowledge.

This paper summarises discussions held during a round table panel session entitled “Closing the gap: the role of regulatory standards in biofilm research and industry innovation” presented as part of the 18th International Biodeterioration and Biodegradation Symposium held in July 2021. The panel (see Table 1) consisted of 10 speakers and included multiple presentations and discussions on industrial and academic perspectives on the importance of standard biofilm test methods in relation to medicine, oil and gas and corrosion. The discussions included important comments from audience members and such relevant points have been incorporated into the text where appropriate. Clearly, the perspective presented from an industrial vantage point will not necessarily overlap with those of academic laboratories. In fact, while industry may be best served by standard test methods or best practices, academia may benefit more from minimum information guidelines for publications. Interestingly though, by the end of the panel session what was clear is that both industry and academia have the same need for consensus.

The paper has been broken up into the main topics of discussion included in the panel session, with content provided by presenters and additional comments as appropriate. At this stage the authors would like to acknowledge the many pioneers of biofilm research for their hard work starting from scratch, which included developing many of the test methods that are routinely used today but were completely novel at the time. One of the aims of this paper is to help build on their efforts, rather than reinventing the wheel or repeating past mistakes. As the quote often associated with Isaac Newton states “*If I have seen further it is by standing on the shoulders of Giants*”. Finally, to paraphrase a senior biofilm researcher from a talk discussion during the IBBS18 Symposium “*If I was starting in this field from scratch, what would I like to know so that I didn’t make silly mistakes and focussed instead on research that was useful*.”

1. **Why are standards needed?**

*2.1 Are academic research and standardisation clashing paradigms?*

The benefit of using a standard test method in fundamental academic research is often lost amid the pressure to publish a significant scientific breakthrough in a high impact journal. Clarifying what defines a standard test method, how standard methods are used in industry and regulatory decision making, and how a research laboratory may therefore benefit may entice an academic to consider the utility of incorporating standard methods into their list of go to options when designing experiments that answer those significant research questions.

In a standard test method, every step of the process is exactly defined with tolerances added to parameters that are more challenging to control, for instance incubation temperature for a bacterial culture. A standard test method is reviewed by a consensus organisation that includes representation from a body of stakeholders. This is done so that the method does not favor one sector of the community that will use the data the method generates. For instance, it is important that a method does not show more efficacy for a particular class of biocides or process. In this way, standard test methods level the playing field between the various stakeholders by identifying the common goals they all must achieve. One of the most favorable traits of a standard test method is that the statistical attributes of the method are clearly defined. This is accomplished in an interlaboratory study (also known as collaborative studies, round robin tests, or ring trails), where the method is performed in multiple labs (often more than six) which enable for the calculation of the expected within experiment, among technician, within laboratory, and among laboratory variability, captured in the repeatability and reproducibility standard deviations (Hamilton et al., 2013). In addition, standard methods must produce data that is responsive (for instance the method is able to distinguish between biocides of low and high efficacy) and rugged (small changes in the operational settings lead to a minimal change in the data of interest). Statistically defined standard test methods enable regulatory decision making (Parker et al., 2014) and are referenced in guidance documents that define the pathway for companies wanting to make label claims on their product. While this clearly helps understand how industry and regulators benefit from the development and validation of standard test methods, how does this benefit the academic community?

Standard test methods are great teaching tools. Typically, a research laboratory writes a Standard Operating Procedure based upon the steps defined in the standard test method that is very specific to the equipment and methods of operation (for instance biosafety protocols) in their laboratory. When a student starts in a laboratory, they often have no idea how to conduct research. Providing a student with a standard to follow is no different than giving someone a detailed recipe when they are learning how to cook. A well written method will have all the unfamiliar terms defined for them, most likely include schematics and even videos on how to do some of the more complicated steps and alert them to any potential bias or interference. In addition, the professor will often have years of data and know instantly when they see the results if the method was followed correctly and how precise the student is in their data collection, thereby serving as a laboratory quality control check. Standards enable professors to track data trends over time, noting for instance if a cell culture line is evolving. Standard test methods improve communication within a lab and across laboratories. Everyone is on the same page as to how the data was collected, which removes ambiguity in its interpretation. This is particularly helpful if the research laboratory is large, or if multiple laboratories within a single center or department are contributing data for a single project. Because standard methods have been statistically defined, using them allows for a more optimal experimental design and higher degree of statistical confidence in the data saving both time and money. And, to go back to the recipe metaphor, the student and professor can use the standard method as starting point to create something that is unique to their research question of interest and publish or standardise this new approach for others to follow. Standard test methods provide another tool in the toolbox, and as the saying goes, one always needs to use the best tool for the job at hand.

*2.2 Academic need for standards in microbiologically-influenced corrosion*

Microbiologically-influenced corrosion (MIC) can cause rapid and unexpected corrosion failures of critical structures and is a major problem in many industries (Little et al., 2020). MIC can be defined as the changes to corrosion that can occur due to the presence and/or activities of microorganisms (often in the form of biofilms). The understanding that microorganisms can affect corrosion has been known for over a century (Gaines, 1910) and while there was consistent research on the topic over the years, it remained a relatively niche area of interest until recently where research and general interest on this topic has increased significantly (Hashemi et al., 2018; Lekbach et al., 2021).

Like many other areas of biofilm research, MIC studies are inherently multidisciplinary in nature. This causes problems for researchers who need to be an expert across the multiple disciplines relevant to MIC, especially as there are limited to no real guidelines on how to conduct MIC research studies. Indeed, there are relatively few groups that have worked on MIC that could legitimately claim to have performed fundamental studies across the disciplines of metallurgy, microbiology and test media. In addition, there are many aspects of MIC, including fundamental mechanisms, that are not well understood. The testing conditions used in MIC studies, for example, can have a major impact on the test outcome (Javed et al., 2014; Wade et al., 2017; Chen et al., 2014; Xu & Gu, 2014; Li et al., 2015). A combination of these factors can lead to issues with test design and reporting as well as reviewing of publications (see Fig. 1 for further discussion).

In terms of standards related to MIC testing, there have been notable efforts by various professional societies, institutes, standards organisations, and researchers to provide guidelines on how best to diagnose MIC. In addition to the NACE (now merged into AMPP, www.ampp.org) standards discussed in other sections of this paper, there are numerous guides that can be consulted (e.g. Little et al., 2006; Eckert & Skovhus, 2022; Korbin, 1993; Licina, 1988; Pope, 1986; Gas Research Institute, 1990; Skovhus, 2017; Thompson, 2022). Overall, there is a growing understanding that multiple sources of evidence (metallurgical, microbiological, environmental) and appropriate storage and handling of samples are key to an accurate diagnosis, and that the mere presence of certain microorganisms and pitting morphologies; alone should not be taken as conclusive evidence (Little et al., 1997). It is also worth mentioning that the fundamental mechanisms involved in MIC are still an active area of research and so standards will need to evolve to keep pace with this improved understanding.

With respect to academic research on MIC there are relatively few useful standards currently available. While this might be understandable to a certain extent, as it could be argued that the novelty of research means that testing should be novel, the lack of use of standard test methods and reporting causes difficulty in replication and trust in reported outcomes. Unfortunately, the current environment of rush to publish means that there can be reduced efforts to validate novel test methods. There are, however, some guidelines / recommendations that should be considered, such as:

* Cleaning effects: information of recommended methods to remove corrosion and biofilm products from the surfaces of test samples and the effect of cleaning methods can be found in the following references (ASTM G1, 2003; Wade et al., 2015; Javed et al., 2021)
* Electrochemical testing methods: electrochemistry is a powerful tool that can be used to help understand MIC processes, but certain test techniques can modify biofilms and/or cause corrosion which can affect the test outcome and interpretation of the results. Helpful information on MIC and electrochemical test methods can be found in the following references (Zhao, 2017; Mansfield, 1991; and Dexter, 1991).

Finally, it is worth discussing areas of interest that might assist to overcome some of the current challenges in MIC research (see Fig. 2 for more details). The hope is that by improving the understanding in these areas it might help to ensure that the efforts undertaken provide longstanding benefits, as opposed to just ticking the box of having another paper published.

***Summary of Section 2***

In a standard test method, every step of the protocol is defined which enables for a statistical assessment of the repeatability, reproducibility, ruggedness, and responsiveness. Once standardised and statistically assessed, regulatory bodies or industry groups may use this information to establish guidelines and pathways for product develop. Academics may use the methods as teaching tools, to address the reproducibility crisis in science, to improve reporting, and enable for better communication.

It is important to note that the recommendation of the development and use of standards should not be seen as an attempt to stifle academic creativity. Rather it should be viewed as providing a set of validated tools that can be used as part of the experimental process to help address the questions academics are exploring. It is expected that new test methods will continuously be developed and some of these may themselves become new standard test methods in the future. In addition, academia would benefit from generating a set of minimum information guidelines that supports the material to include in a publication so that their research may be reproduced in an independent laboratory.

1. **What does industry need in a standard test method?**

*3.1 The role of standards in driving innovation*

Appropriate regulation and standards can be a vital part of the infrastructure required to support industrial growth. As a key example, without the relevant standards it is not possible to make product claims for innovative biofilm technologies. Standards also help de-risk research and development for companies, promoting the benefits that innovation can bring whilst reducing the scope for its misuse.

A recent international academic-industry road-mapping carried out by the co-authors has consistently identified standards in biofilms as an urgent priority need. For example, the requirement to be able to detect and confirm the presence of a biofilm in new, standardised and reproducible ways using approved protocols that would be acceptable to regulatory agencies, and that would allow the assessment of the efficacy of interventions (e.g. the prevention or management of biofilms and being able to claim a biofilm has been prevented or removed) (NBIC, 2020). Similarly, there is a requirement for models and methods for characterisation relevant to the industrial context and standardised and accessible to industry and academia (NBIC, 2019a).

Building on the need for standard models is the requirement for truly cross-disciplinary ‘all party’ engagement (access, buy-in and funding) for collaborations at a cross-industry level and also with the regulators in creating and validating standards and models. This will allow products to be approved, claims to be made and the science to move forward in a way relevant to industry needs. There is also a strong resounding call from the industry community for improving both the clarity and ease of navigating for the regulatory environment (NBIC 2018a, b, 2019a, b, 2020).

In summary, a major unmet industrial need for innovation, technology and product development for biofilms is the ability to demonstrate alignment to biofilm-relevant standards and a clear regulatory framework.

*3.2 Historical perspective and evolution of need for methods*

The NACE standard TM0194, Field Monitoring of Bacterial Growth in Oil and Gas Systems, was originally written in 1994. The standard was revised in 2004 and most recently in 2014. This standard has been the primary culture testing reference used by the oil and gas industry since 1994, and is used in certified training courses, company standards, government regulations, and is often referred to in litigation involving pipeline corrosion. A portion of the standard is devoted to biocide testing/screening using planktonic samples and liquid culture methods and has been used by chemical vendors and pipeline operators for decades as the primary means of biocide and dose selection, although it is now well known that planktonic microbiological characteristics do not accurately reflect sessile populations, nor do they have a direct correlation with MIC (Little & Wagner, 1997; Zintel, 2003; Wrangham, 2013). This fact was also clearly pointed out in TM0194:

*“The tests described here are only for planktonic organisms. The ability of biocides to control sessile bacteria in the system cannot be determined by this technique…”,*

and,

*“…biocides are much less effective against sessile bacteria than against planktonic bacteria.”*

Yet regardless of this warning, biocide selection has largely continued to be performed using planktonic samples and culture media, with the expectation that demonstrating reductions in planktonic bacteria levels would provide a direct indicator of reducing MIC (which years of field data has shown it does not).

In the last 15 years or so, an increased emphasis on biocide testing using biofilms has been developing in the oil and gas industry. A few chemical vendors now offer some form of biocide screening using biofilms, yet there are no standard methods or published protocols for this testing and no way to compare test results between different labs or vendors. Operators or end users also have no way to assess the veracity of the biofilm test results. Approximately two years ago, NACE began work on developing a standard for laboratory testing using biofilms, AMPP TM21495. The standard is still being drafted as of June 2022. This case provides a good example of how the need for new standards does not necessarily drive the timely development of such standards, particularly where transdisciplinary subjects (corrosion and microbiology) are not aligned or do not fully exist within standards development bodies. As a result, corrosion management and MIC mitigation is nowhere near as technically robust as would be possible if a biofilm testing standard was published a decade ago. This example just reinforces the fact that standards are an important means of translating new knowledge and technologies to end users, thereby improving sustainability and reducing operating risk.

Fig. 3 illustrates some of the key areas identified by industry as being important to include in biofilm standards (and standards in general), based on the roundtable panel session. First, industry looks to standards to provide consistent, reliable, reproducible methodologies, including requirements for test equipment, materials, methods, controls, replicates, data collection and guidance on avoiding common problems or errors. Industry also expects that standards will demonstrate expert consensus on best practices, so that they can in turn inspire confidence in stakeholders and regulators. Often, companies within an industry will not have internal resources with the level of expertise and knowledge represented by those who work to compile the standards. Standards also help govern relationships between companies in the same or in different industries, for example between chemical vendors and operators, or in which industries a manufacturer decides to place a new product. Another benefit of standards is that they provide companies the ability to specify test conditions and/or criteria for product selection or procurement, and it is beneficial when these criteria originate from an industry consensus standard, rather than from the company itself. Finally, standards help industry have a deeper understanding of the meaning of the test data and how it can be interpreted and applied, and also any limitations on how the test results can be translated into action. In terms of testing using biofilms, the oil and gas industry presently has significant gaps in the standards available to help navigate this subject.

*3.3 Examples of relevant standards in the oil and gas sector*

Technical standards are formal documents used in the oil and gas industry to establish uniform engineering criteria, processes, and practices. They are often employed during the planning of major projects where errors in engineering design and judgement can result in significant financial and/or human losses, future liabilities, and risk of reputational harm. Major projects in the oil and gas sector include design and construction of new offshore platforms, pipelines, gas plants, and decommissioning activities to name a few. These projects can be very complex and the responsibility of various internal technical experts not only from the operating company but from project partners and various external contractors. These technical standards provide established requirements for technical tasks performed during the project to reduce safety and environmental risks, as well as improve reliability and integrity of equipment following commissioning. Technical standards employed include those approved by various organisations such as ASTM, ANSI, ASME, AMPP/NACE and ISO. In addition, it is not unusual for companies to develop their own internal standards based on these industry standards. Standards typically employ tried and tested practices (i.e., uniform and well accepted), are prescriptive (i.e., not optional), and reproducible (Yates & Aniftos, 1997). Whereas best practices are defined as procedures proven by research and experience to produce optimal results that may become standards (Definition of Best Practice, 2022). Technical standards are voluntary and approved by a consensus of technical experts, unlike regulatory standards which are compliance driven and typically issued by government agencies.

An important microbiological role in the oil and gas industry is the design and approval of procedures for prevention of biofilms that could form during hydrotesting. Hydrotesting is an essential practice often employed in major projects during pre-commissioning of pressure vessels and pipelines to ensure their longer-term integrity. Hydrotesting involves the introduction of a fixed volume of water to a vessel that is subsequently pressurised above its maximum allowable operating pressure (MAOP) to test for leaks. Industry standards exist for the pressure requirements and allowable times for the hydrotest which are typically 125% of MAOP at less than 10 hours, but they do not include potential treatment of this water (e.g., seawater and brackish waters) which can remain in contact with the metal for weeks to months prior to and even after the test is completed. During this wet parking, or layup period, the presence of oxygen, bacteria, and other suspended solids pose the greatest threat for corrosion and cases of MIC have been reported due to corrosive biofilms formed during wet parking (Darwin et al., 2010; Machuca, 2017; Salgar-Chaparro et al., 2020; Machuca et al., 2021). Therefore, the proper sourcing and treatment of hydrotest waters are important concerns to prevent biofilms. Chemical treatments include the addition of oxygen scavengers, biocides, and corrosion inhibitors. However, the variability in water quality employed (e.g. levels and types of nutrients present), the unpredictability of exposure times for wet parking, the many types of chemistries to be considered, and the variability of environmental regulations governing discharge of treated waters make it very difficult to standardise on a chemical treatment. Darwin et al. (Darwin et al., 2010) proposed a method to assess the probability that biofilms and MIC would occur during hydrotest/wet parking using factors such as water source, solids content, temperature, and operating conditions which in turn were used to assess the probability that water treatment would be needed to prevent significant MIC. A process such as this could be employed as the basis for an industry standard to assess the probability of biofilms forming and the need for hydrotest biocides. However, to get this accepted as an industry standard would require extensive testing, vetting and review to validate such an approach and achieve consensus approval from industry experts. Therefore, development of industry standards can be a long term, contentious and expensive process. For this reason, guidelines and less prescriptive best practices that are recommendations designed to streamline processes and make them more predictable may be a better choice, but situations may arise where these best practices become requirements resulting in their standardisation.

*3.4 Important considerations for biofilm standards development*

The development of biofilm standards is a non-trivial exercise as it needs to balance between serving industry needs, while recognising, not all testing scenarios can be represented in a single test. Hence, a key part of the process of establishing a standard method is to first consider the most important outputs required for a particular method. With respect to MIC, there is a need to separate the specific role of microorganisms from chemical or abiotic corrosion processes as it is understood that biotic and abiotic corrosion processes are not equal. Current estimates are that MIC contributes to approximately 20% of total corrosion, but this figure has not been experimentally validated, but is rather more of a guestimate (Flemming, 1996). Therefore, a standard method for MIC should include a sterile, abiotic control for comparison to corrosion in the presence of the test organisms. This is important in particular for testing product streams where knowing if the problem is biological or not informs on control strategies.

Additional considerations would revolve around how the method will deal with different metals, such as copper, aluminium, or zinc-based materials, where corrosion mechanisms and MIC organisms may be very different from steel/iron. When testing new compounds for potential activity as antibiotics, the timeframe for testing is relatively short, e.g. 24-48 hours and is hence not an issue for testing. In contrast, MIC and general corrosion is a slow process, often measured as 10-100’s of μm/year, a time frame that is far too long for most researchers or businesses to undertake. Therefore, an MIC standard test procedure would ideally be performed under accelerated testing conditions, as is done for other industries. However, the accelerated conditions must be verified to give an accurate measure of MIC under operating conditions. While most antibiotic testing is based on a single time point to show efficacy, resistance studies are more dynamic, requiring several weeks of culture to determine if resistance is relatively easily developed. For MIC testing, this question of single time point measurement or dynamic testing is also important. Dynamic testing would give the most robust results, especially in cases were corrosion rates may accelerate exponentially once a threshold corrosion is reached, although this complicates both the test method as well as the analysis of the data. Ultimately, whether the method is to be used to evaluate biocides and the specific end-goals need to be clearly defined. For example, for MIC, biocides should be qualified based on their ability to decrease corrosion rates. For control of biofouling, biocides should be qualified based on their ability to decrease biofilm. Similarly, the testing of coatings for the control of biofilms and MIC need to be clearly defined. Some examples of relevant antimicrobial standards related to surfaces include ISO 20743, ISO 22196, JIS L 1902 and JIS Z 2801 (ISO 20743; ISO 22196; JIS L 1902; JIS Z 2801).

*3.4 General comments*

The following is a summary of some additional points on the topic of what industry needs in a standard test method, made by attendees and/or speakers at the panel session.

* Standards need to be tried and tested for industry to accept
* Standards need to be easy to understand and interpret, for example not all users are trained microbiologists.

Regardless, for the method to be translatable it must consider and incorporate key microbiological parameters such as monitoring of the nutrient state, environmental conditions, and the resulting microbial ecology.

Finally, biofilms will likely always be present so methods that enable us to understand how to control their impact on the system, and what that means, are necessary so as to mitigate their negative impact.

***Summary of Section 3***

* Test methods and understanding of processes change over time, so standards need to evolve.
* Care needs to be taken to ensure standards are used for their intended purposes.
* It is expensive and resource intensive to develop standard test methods, therefore guidelines and less constrictive ‘best practices’ may be a more efficient approach in some cases.
1. **The pros and cons of standards**

*4.1 Standards related to microbiologically influenced corrosion*

In relation to biofilms and MIC, a number of relevant historical standards are available, while several new/updated standards are currently being developed (see Table 2 for examples). As described above, the historical standards have been somewhat limited in detail and often relied on culture-based test methods to detect microorganisms. Over the last few decades there have been rapid advances in test methods and associated technologies, reductions in costs, as well as increased understanding of processes that are driving the development of new standards. For example, many of these newer standards are starting to include molecular microbiological methods (MMM), which can provide additional, and arguably better, information about the microbial ecology of an environment compared to culture methods (Eckert, 2018; Muyzer & Marty, 2014). This change to providing a more holistic perspective for microbial monitoring is underway in a variety of industries, not only in oil and gas. An excellent example is the work performed by Health Canada to update its guidelines for drinking water quality. While the previous guidelines (Health Canada, 2012) were largely focussed on the use of heterotrophic plate counts (HPC), the new guidelines include comprehensive information on monitoring methods (including molecular methods), monitoring programs, management strategies and water quality targets (Health Canada, 2022).

How companies see the use and development of standards depends on their own technological capabilities. A couple of examples in which standards may be seen as a threat are:

* A company that has been selling culture tests for decades to test for SRB growth (as per the NACE TM0194 standard) would potentially see the implementation of molecular microbiological methods (MMM) (such as included in new AMPP TM0212, TM0106 standards) as a threat to their business. Such a business may be reluctant to go down this new path and educate clients on the new methods.

*Alternatively*, however, such a company could:

* + Develop guidelines showing specifically how / when / where culture tests may still be useful,
	+ Develop capabilities for testing, training, and/or analysis facilities based on MMM.
* A company that has already developed high quality internal standards of advanced microbiological monitoring (based on MMM), could see this as a competitive advantage (e.g. being able to detect MIC/leaks early) compared to other companies that rely on culture tests. The company would then likely be reluctant to share business critical knowledge and develop MMM standards for its competitors.

However, things don’t typically stay the same forever and unless ongoing improvements are made by the aforesaid company current competitive advantages will be overtaken by future advances.

Another example of the development of a new standard in this field that builds on recent technological advances is AMPP TM21465 (Molecular microbiological methods – Sample handling and laboratory processing). A workgroup for this standard was initiated in 2019 when companies realised that they needed to get together to share knowledge as they could see a consensus was being formed around these new methods. Companies that embrace and take part in standards development such as this can take advantage of the combined knowledge of experts from academia and industry and gain an understanding on the most recent updates on the topic, rather than trying to do it all on their own.

*4.2 Standard test methods in a broader industry context*

Although there is not universal agreement on the best way to monitor and control biofilms and MIC in oil and gas, there at least is a significant body of available information on best practices and some effort to promote these through industry standards. The practitioner responsible for controlling materials degradation in oil and gas can therefore access many publications through NACE and other forums to formulate a plan of biofilm sampling and mitigation. Many other industries also suffer from materials or product degradation from biofilms and/or MIC, but they may not have the benefit of significant research expenditures to help inform decision making or best practices, let alone industry standards. Many industries, such as consumer products manufacturing, do not have a cooperative industry group that promotes either standards or best practices for controlling biofilms in the manufacturing process. Many companies have therefore been left to devise their own protocols for biofilm sampling and control, with variable results. Furthermore, best practices with regard to monitoring and prevention of materials biodegradation may be industry specific, particularly when considering the options available for the choice of remedial measures. Antimicrobials or biofilm control measures used in oil and gas are typically not be applicable in more sensitive manufacturing processes due to toxicity or incompatibility with construction materials.

Even within a particular industry, problematic biofilms leading to product or materials degradation may vary significantly. Best practices or standards therefore need to consider the variability in microorganism types and consortia, growth conditions, and in-plant variables across different production facilities (process type, geographic region, biocide use, etc.) It is therefore important that companies maintain a staff of professionals who understand that best practices for microbial sampling and control in one industry may help them design a materials protection program, but they may have to make changes for their particular situation. Even with these variables, professionals charged with maintaining the microbiological integrity of facilities benefit from published industry standards and/or best practices. Unfortunately, easy access to published information isn’t always possible, either because the information is held within a company as a trade secret or because it is published in an industry-specific journal that isn’t widely accessed by professionals from another industry.

The existence of best practices and standards is also industry-specific in the sense that different industries vary as to their understanding of biofilm and the issues it can cause. Considering how a particular practice, whether it’s monitoring, remediation or reporting-related is first proposed as a best practice and subsequently as a standard necessarily involves consideration of the maturity of the industry relative to biodeterioration. For industries relatively new to understanding the role of microorganisms in process or product deterioration, there may not be well-established best practices, and no real standards. As this understanding within an industry progresses, there is an evolution of best practices, which may or may not be communicated between companies sharing the same problem. Over time, these best practices may be promoted into standards, and may see regulatory enforcement, depending on the industry and potential for health or safety issues. In some cases, these standards may carry regulatory consequences if not followed.

One of the big challenges for practitioners in industries without a strong understanding of biodeterioration is effective communication between academia and industry, within an industry, and between regulatory authorities and industry. In this case, the existence of published best practices can greatly assist moving the industry in the right direction, and these might evolve into standards.

In some cases, a company that has developed in-house best practices or standards may decide to treat these as a trade secret and purposely not publish them, since development of these practices may represent a considerable investment of time and resources. While this may seem a wise way to maintain a competitive edge, the risk is that another entity (company or academic) may publish a different standard, which, if adopted industry-wide, would require the original company to follow a standard they did not create. It therefore may behove a company to publish their best practices such that they become the standard.

To conclude, it is difficult to say whether a standard or best practice is more useful without considering the particular situation. But it is apparent that communication within an industry as to what constitutes a best practice for controlling biofilm growth or MIC is key to moving the industry forward in this area.

*4.3 General comments*

The following is a summary of some additional points on the pros and cons of standards, made by attendees and/or speakers at the panel session.

* Different industries, or companies might have different environmental conditions that the standards will need to represent
* What microorganisms to use? Does this represent the real world? Should single microbial strains or consortia sampled from the field be used?

***Summary of Section 4***

Industry is not in complete agreement that standard test methods are actually what is necessary. In certain situations, best practice or more general guidelines will provide the decision-making guidance that is needed for innovation without inhibiting a marketing or knowhow commercial edge.

1. **Current biofilm standard development**

As previously discussed, MIC and biofouling impact assets across various industries. Effective management and control of MIC and biofouling requires careful selection of biocides that are most effective before they are applied. However, there are currently no industry standards that describe the method to select biocides by evaluating their impact on biofilm prevention and mitigation for these applications. NACE/AMPP standard TM0194 focuses on biocide selection using culture-based testing of planktonic samples and not biofilms. The absence of standards to guide the selection of the most effective biocides leads to safety concerns and increased costs.

The AMPP TM21495 committee was formed in late 2020 to develop a standard test method for laboratory evaluation of the effect of biocides for industrial systems. This standard will be useful for industry operators, service providers, third party labs, and biocide manufacturers to provide guidance on how to select biocides for biofilm mitigation to control MIC and biofouling for a specific asset. This standard will present test methods to establish and test biofilms in the laboratory with guidance on test conditions and experimental design, microbial community considerations for inoculum, chemical composition of the fluids, and selection of an appropriate experimental setup. This standard will also present the biofilm and planktonic microbiology information that should be collected during these biofilm tests along with the analytical methods that can be utilised to obtain this information. Finally, this standard will include a methodology for interpretation of biocide efficacy accounting for the different types of microbiological information (microbiological activity, abundance, and community composition) and the analytical method used to obtain the information, along with application-specific information for MIC and biofouling applications (corrosion rate, pressure drop, etc.).

As of September 2022, TM21495 was in the draft stage with three subgroups working to prepare a first draft. It is anticipated that the standard will be available in mid-2023. Once it is published, the AMPP TM21495 standard will guide asset operators to make better informed decisions on biocide selection specific to their concern, which is key to prevent future asset failures and manage costs. Since biocide testing using biofilms is an emerging topic with an increasing number of operators steering away from planktonic-only testing, a prescriptive standard will prevent the wide adoption and acceptance of biofilm testing. Hence, the AMPP TM21495 standard is not intended to be a prescriptive standard with strict guidelines on the test design, experimental setup, and analytical methods used, but rather a standard with step-by-step guidance on best practices for laboratory evaluation of biocides in the laboratory using biofilms along with minimum requirements to standardise this process.

***Summary of Section 5***

Regardless of whether the efforts are fully supported by all industry sectors or not, companies are working together to develop standard biofilm test methods.

1. **IBSTG and biofilm center perspectives and outlook**

The needs and problems already described with respect to establishing biofilm standards are ones that exist globally and therefore opportunities certainly exist to learn from best practice and to ensure coordination of activities. With this in mind, the International Biofilm Standards Tasks group (IBSTG) comprising members from the Center for Biofilm Engineering, National Biofilms Innovation Centre, Singapore National Biofilms Centre and an EU Cooperation in Science and Technology (COST) action group (CA20130), was formed in October 2021 called “European MIC Network – New paths for science, sustainability and standards (Euro-MIC)”. Its aim is to support the drive towards the international development and acceptance of standardised biofilm test methods in health care, the built environment and industrial systems. An important goal is to enable informed and consistent decision making on the international regulation of anti-biofilm products. It seeks to achieve this by working in partnership internally and externally and ensuring there is collaboration and coordination across our different regions. This international group has access to a network of academics, industrial partners, health care professionals, engineers, economists, regulators, and the standards community. These stakeholders all have a clear need for evidence-based data to establish international standards and regulations to aid in the development and testing of products to control or exploit biofilms. Furthermore, they recognise the importance of using standard test methods when validating product efficacy.

It is the groups primary intent that science and evidence-based data should always support the use of a product in a particular application. All participants agree that their Industry partners have a need for a defined regulatory pathway to create successful innovation in the marketplace and the adoption of new approaches to tackling biofilms. We collectively believe there is value in this independent global task force being able to represent the current state of the science and the needs of our stakeholders in relation to biofilm methods in their own regions and on international bodies.

1. **Discussion**

Overall, the panel agreed that there is a need for guidance documentation (regardless of whether it is in the form of standards, best practices, guidelines, etc) and for discussions about these documents (e.g. workshops, conferences, collaborations, etc.) to educate industry and academia on biofilms, associated biologically induced degradation and approaches for studying these topics in the laboratory and field. These documents offer ethical guidance via a series of checks and balances that level the playing field and provide general confidence that outcomes are relevant and reproducible. The path forward on how best to address this need is dependant upon the stakeholder.

It is clear that industry wants and needs *consensus* from a range of relevant experts on the best way to approach studying these challenging problems and ensuring proposed solutions actually work. This consensus will lead to a toolbox of methods by which they can support label claims, such as product effectiveness. Standard test methods are critically important for industrial stakeholders if their product label will be registered with a regulatory body. For this to happen, however, companies need to agree to be actively involved in this process and to consider funding research which assists in the development of standard test methods. Industrial stakeholders need to understand the extended period of effort required by researchers to develop what become test standards and the benefit of academic independence from commercial conflicts of interest. And, there needs to be a commitment to revisit ‘older’ test methods and revise them to include the latest knowledge and know how to ensure that they remain relevant and useful.

Academic stakeholders, on the other hand, may benefit most from accessible guidance documents that reference, commonly used methods that may be incorporated as useful tools into their research workflow. Such documents help to ensure these methods are performed correctly and provide someone new to the field an understanding of their associated interferences, advantages, and limitations. Further work on providing guidelines for the minimum reporting information to be included in publications would also be of assistance to academic researchers, both in terms of guidance in the preparation of experimental designs and manuscripts but also to assist with paper reviewing process. Such guidelines are especially important in the multidisciplinary field of biofilm research. Academic stakeholders may also benefit from consulting with their industrial colleagues to understand their needs, so that their research is translatable to real world challenges.

In conclusion, the exact format of documentation proving advice on biofilm testing (e.g. standards vs. best practices vs guidelines) is stakeholder dependant. By the end of the panel discussion the fundamental need identified was for guidance based on consensus. Panel discussions, such as the one documented here, help begin this consensus process. This work needs the participation and continuing support from a combination of industry, academia, and government/regulatory agencies, so if you want to make a concrete difference make sure you get involved!

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**Author contributions**

Scott Wade – co-organised the workshop on which this collaboration has been assembled, drafted the structure of the paper, contributed to the Introduction, the Standards in microbiologically influenced corrosion and the Discussion sections, handled submission, serves as the corresponding author and edited the manuscript together with DG and JW.

Darla Goeres – co-organised the workshop on which this collaboration has been assembled, contributed to the Introduction, the “Are academic research and standardisation clashing paradigms?” and the Discussion sections, edited the manuscript together with SAW and JW.

Jeremy Webb – co-organised the workshop on which this collaboration has been assembled, contributed to “The role of standards in driving innovation” section, edited the manuscript together with SAW and DG.

Rick Eckert – contributed the “Historical perspective and evolution of need for methods” section.

Gary Jenneman – contributed the “Examples of relevant standards in the oil and gas sector” section.

Scott Rice – contributed the “Important considerations for biofilm standards development” section.

Torben Lund Skovhus – contributed the “Standards related to microbial corrosion” section.

Paul Sturman – contributed the “Standard test methods in a broader industry context” section.

Susmitha Purnima Kotu – contributed the “Current biofilm standard development” section.

Mark Richardson – contributed the “IBSTG and biofilm center perspectives and outlook” section.

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Table 1

List of panel speakers and topics discussed during “Closing the gap: the role of regulatory standards in biofilm research and industry innovation”.

|  |  |
| --- | --- |
| Topic | Speakers |
| Are academic research and standardisation clashing paradigms? | Darla Goeres, Scott Wade |
| What does industry need in a standard test method | Rick Eckert, Scott Rice |
| Controversial Pro’s/Cons, Standards vs. best practice | Torben Lund Skovhus, Paul Sturman,Gary Jenneman |
| AMPP TM21495 Biofilms Methods exemplar | Susmitha Purnima Kotu |
| IBSTG and biofilm center perspectives and outlook | Mark Richardson |
| General discussion | Jeremy Webb |

Table 2

Examples of current and developing standards related to biofilms and MIC in engineering systems.

|  |  |  |  |
| --- | --- | --- | --- |
| Standard/Guidance Document | Society/Institute | Information Provided | Status |
| TM0106-2016, Detection, Testing, and Evaluation of Microbiologically Influenced Corrosion (MIC) on External Surfaces of Buried Pipelines | NACE Int. | Types of microorganisms and mechanisms by which MIC occurs on external surfaces of buried, ferrous-based metal pipelines. Testing for the presence of bacteria, research results, and interpretation. | *Available at ampp.org/nacestandards* |
| TM0194-2014 Field Monitoring of Bacterial Growth in Oil and Gas Systems | NACE Int. | Describes field methods for estimating bacterial populations found in oilfield systems. Sampling methods and culture media for enumerating bacteria are described. | *Available ampp.org/nacestandards*  |
| TM0212-2018, Detection, Testing, and Evaluation of Microbiologically Influenced Corrosion on Internal Surfaces of Pipelines | NACE Int. | Standard test method for MIC on internal surfaces of pipelines. Types of microorganisms, MIC mechanisms, sampling and testing. Research results, and interpretation of test. | *Available ampp.org/nacestandards*  |
| TM21465, Molecular Microbiological Methods – Sample Handling and Laboratory Processing | AMPP (formerly NACE) | Standard test method that may be used to perform DNA-based microbiological analysis of samples collected for corrosion monitoring and control. | Under development – estimated publication 2022 |
| TM21495, Laboratory Evaluation of the Effect of Biocides on Biofilms | AMPP (formerly NACE) | Standard test method that can be used to evaluate the effects of biocides and other production chemicals on biofilms and MIC before field application for effective MIC prevention and mitigation. | Under development – estimated publication 2023 |
| D8412-21, Quantification of Microbial Contamination in Liquid Fuels and Fuel-Associated Water by Quantitative Polymerase Chain Reaction (qPCR) | ASTM | Procedures for using quantitative polymerase chain reaction (qPCR), a genomic tool, to detect, characterise and quantify nucleic acids associated with microbial DNA present in liquid fuels and fuel-associated water samples. | *Available at ASTM.com* |
| Guidance on the use of Biocides in the Oil Industry | Energy Institute | Provides a review of the types of biocides used in the oil industry and discusses the scope and impact of pertinent regulations.  | *Available at EnegyInsitute.org*  |
| Guidelines on Managing Microbiologically Influenced Corrosion (MIC) in Water Injection Systems | Energy Institute | Provides a description and quantification of MIC threats associated with typical water injection process equipment, considering the materials in current use, biocide treatments, the presence of other production chemicals and process conditions. | *Available at EnegyInsitute.org*  |
| Guidance on selection, applicability and use of molecular microbiological methods (MMM) in the Upstream and Downstream oil industry | Energy Institute | Aims to provide guidance on the strengths and limitations of MMM, where they should be applied and how the results should be analysed in a manner that supports decision making for asset management. | Under development – estimated publictaion 2022 |



Fig. 1. Discussion of some of the current challenges facing MIC researchers where guidance documents or standards could be of assistance.



Fig. 2. Examples of areas where future efforts may help overcome some of the current challenges in MIC research.

Consistent, reliable,

reproducible methodology

Improved ability to use data from multiple providers / labs

Promotes understanding of how the data can be used and interpretation challenges

Quality control built in,
current best practices,
represents consensus



Fig. 3. Requirements for biofilm standards from an industry perspective.