Molecular methods for oilfield microbial monitoring: qPCR

Unchecked microbial growth in an oilfield environment can have serious consequences, and so microbial monitoring is an essential activity. It is the first step towards assessing the risk of, and mitigating, microbially influenced corrosion (MIC), reservoir souring, and biofouling.

Oilfield microbiology presents a number of challenges, but advances in molecular biology have revolutionised the tools available for this kind of work. One of the techniques that is becoming increasingly popular for monitoring numbers of potentially problematic microbes is quantitative polymerase chain reaction (qPCR). In this article, NCIMB's analytical services manager, Michelle Robertson, takes a look at the science behind qPCR, and what it offers in comparison to the more traditional culture-based approach.

The qPCR technique is a method that can very rapidly detect the presence or absence of defined species or groups of microorganisms in a sample, and it has been widely applied outside of the oil and gas sector. It is commonly used in clinical microbiology for the rapid detection of infectious diseases, and can be a key tool in planning and managing the response to deadly epidemics as well as in monitoring the emergence of new strains of diseases.

It is also used extensively in forensic science and as a research tool in a range of different academic disciplines. For example, it has been used to amplify traces of ancient DNA from long-extinct animals, such as mammoths preserved in permafrost. At NCIMB we have also been called on to use qPCR to test for the presence of specific strains of bacteria in probiotic food products.

Nobel Prize winning technique

qPCR is a development of the Nobel Prize-winning polymerase chain reaction (PCR) technique. PCR is a way of making a large number of copies of a small amount of DNA in a short period of time. The development of this technique was a game-changing event in molecular biology, and its inventor, Kary B. Mullis, was awarded the Nobel Prize in Chemistry in 1993.

The process is outlined in ***Fig 1***. DNA occurs as a double stranded molecule. In PCR, heat is applied to separate the two strands of DNA: this is the denaturation stage shown in ***Fig 1A***.

The process of DNA replication is then initiated with the use of primers. This is the annealing step (***Fig 1B***). Primers are small pieces of DNA that match the ends of the DNA segment that is to be copied, and their selection is key to the specificity of the technique. In the third step shown in ***Fig 1C***, elongation, copies of the DNA strands are made by the addition of nucleotides.

This cycle can then be repeated using the two copies to make four, which are then replicated to make eight. In other words, through the repetition of this cycle, the quantity of DNA increases exponentially to give scientists amounts of DNA that they can work with and analyse, within a short space of time. This opened the door to all kinds of other developments.

qPCR

Quantitative polymerase chain reaction (qPCR) uses this same approach to quickly replicate DNA, within a method that can be used to quantify the numbers of particular types of organisms in a sample.

Dyes that fluoresce when bound to double stranded DNA, but emit low fluorescence in solution, are incorporated during the replication process. Measurement of fluorescence can be used together with the number of cycles undertaken, to calculate the number of copies of the target gene in the initial sample. This can in turn be used to quantify the number of microbes present. The process is illustrated in ***Fig 2***.

Application to the oil and gas sector

As qPCR technology has matured, it has transitioned from a specialist research tool to an everyday analytical technique. Interest in qPCR for monitoring of microbial numbers in oilfield settings has arisen because it has been seen as a way of overcoming a number of issues associated with the more traditionally used, culture-based most probable number (MPN) method.

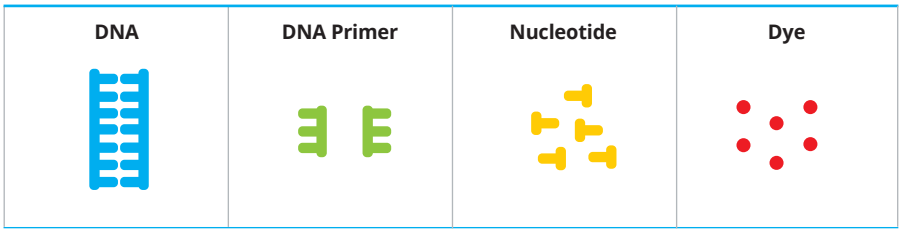

Figure 1: PCR Cycle

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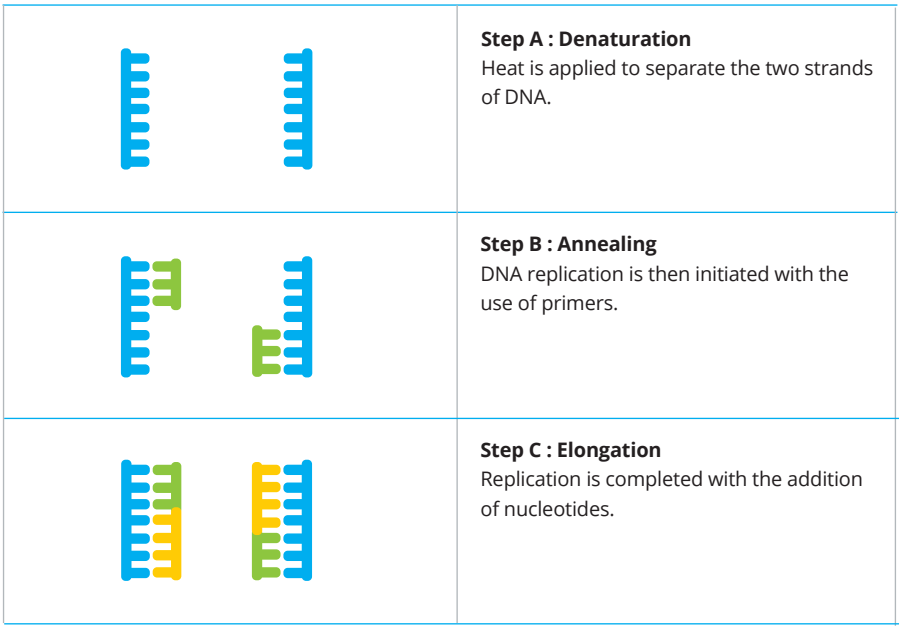

Figure 2: qPCR Cycle

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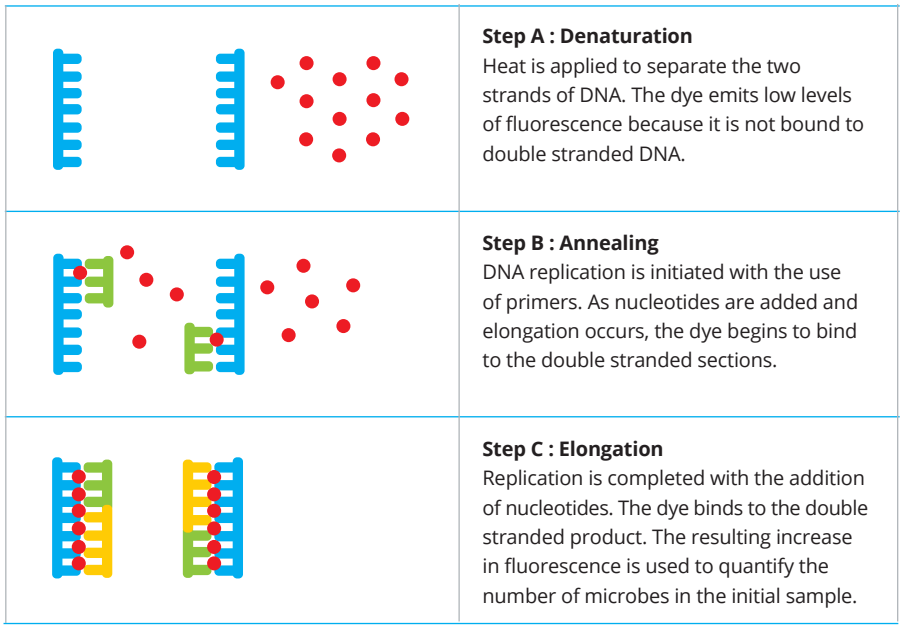

The MPN method estimates the numbers of different groups of organisms through serial dilution in selective media. In other words, the dilution at which no growth occurs following incubation in media that favours growth of the group of organisms of interest, e.g. sulphate reducing prokaryotes, is used to estimate the most probable number of microbes present in a sample.

For many years, culture-based approaches such as MPN were the only ones available for monitoring populations of oilfield bacteria. However, it is well established that not all microbes will grow under laboratory conditions, and those that don't will not be detected using the MPN technique. It can be difficult to recreate the exact conditions that exist within the oilfield environment in the laboratory, as the microbes that increase the risk of MIC often live in complex biofilm communities on the walls of pipes and vessels, or within the pores of reservoir rock. This increases the likelihood that problematic microbes may be present, which do not grow under the test conditions.

If large numbers of viable and active, but non-culturable microbes are present in oilfield environments, then there is a risk that reliance on culture-based enumeration will underestimate the risk of corrosion. Reports of instances where the MPN results have suggested low risk, yet corrosion characteristic of locations where there is microbial activity has still occurred, have led the industry to investigate alternative methods.

Benefits of qPCR

qPCR is one of a number of different molecular techniques that have been used in oilfield microbiology in an attempt to overcome this issue of the detection of active but non-culturable microbes, as no growth is required for its use. Selection of specific primers allows different groups of organisms such as sulphate reducing bacteria and archaea to be targeted, and the method also offers the advantage of faster turnaround than MPNs, which can require an incubation time of up to four weeks.

The qPCR technique does, however have some issues of its own. Because it is based on the measurement of DNA, it does not distinguish between active, viable but inactive, and dead cells, so where MPN could potentially be underestimating numbers, qPCR could potentially over estimate the level of risk. Additionally, the results obtained from this technique are highly dependent on the primers selected. Different groups of microbes such as sulphate reducing bacteria or nitrate reducing bacteria are enumerated by the use of primers that target functional genes associated with them. However, variation in the genetic code within the target group can result in numbers being underestimated.

Conclusions

In conclusion, neither MPN or qPCR in themselves provide a complete, 100% accurate and issue-free solution to the enumeration of microbes that can cause problems in oilfield environments, and consequently, the best results are likely to be obtained through the use of a combination of complementary techniques. It is also important to remember the role of historical data in the interpretation of results and the identification of trends.

Discussions are underway within the industry to develop the standards that are currently in place for biomonitoring for the purpose of microbially influenced corrosion risk assessment. Their progression could result in a new and more robust approach, that allows the oil and gas industry to benefit from application of the latest developments in molecular biology. Events such as SPE's Reservoir Microbiology Forum provide an excellent opportunity for industry experts from both operating companies and microbiology service providers, to discuss the issues and the potential improvements that can be made, while highlighting any practical issues that need to be overcome in transitioning to a new methodology.

While qPCR and MPN both have their pros and cons, one thing that is certain, is that the potentially catastrophic results of unchecked microbial growth make monitoring an essential activity. Action taken on the basis of results obtained using either of these techniques, can help avoid serious problems. At NCIMB we have extensive experience of applying both of these approaches and can offer advice on their use as well as interpretation of results.

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