Molecular Methods

Oilfield Microbiology

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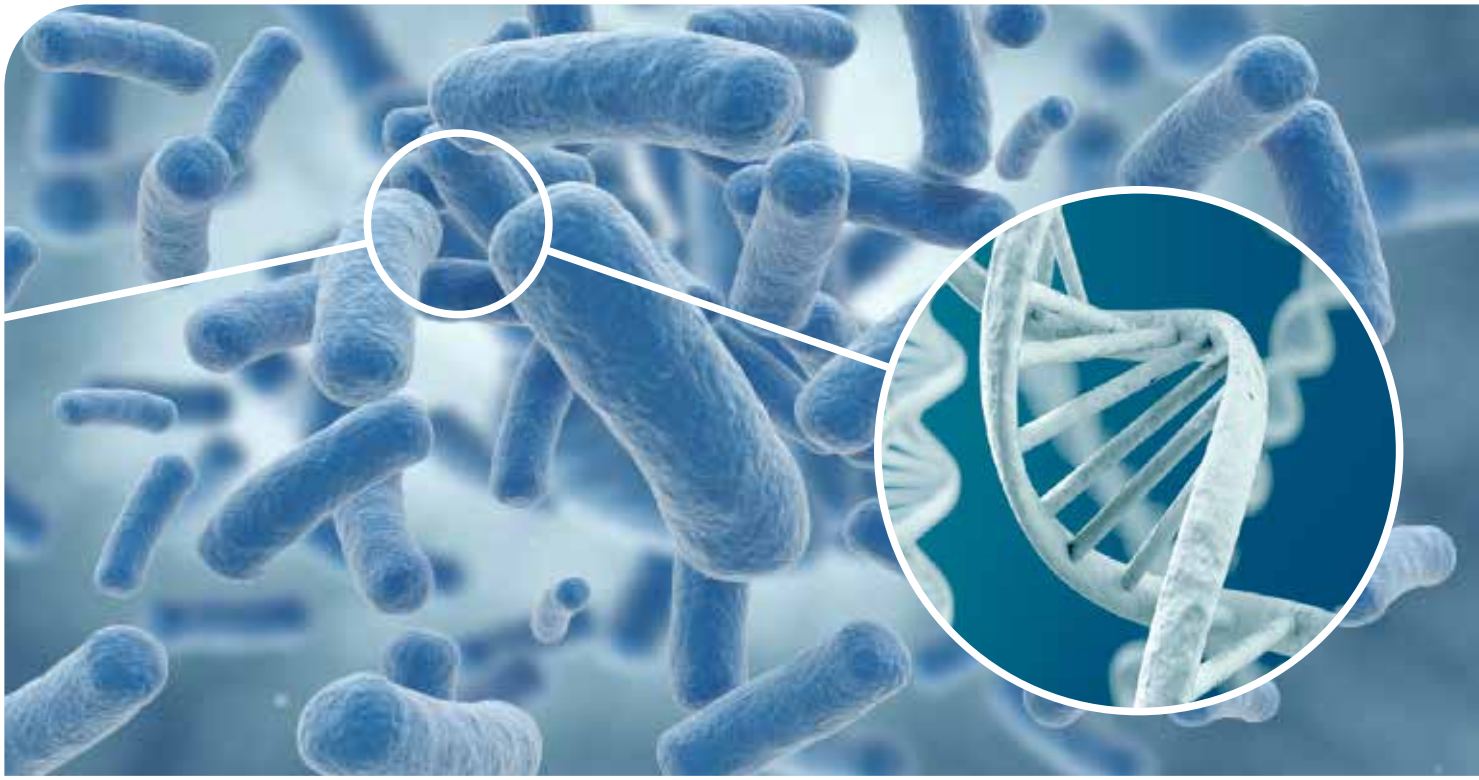

Monitoring microbial contamination is essential for the integrity of oil and gas installations and systems.

At Intertek, our oilfield microbiology team has extensive experience in researching, developing and working practically with more novel molecular methods to approach and evaluate industry issues.

Traditionally in the industry, microbiological cultivation and biochemical applications have been applied alongside one another in the analysis and solving of common problems.

However, these methods are not always sufficient, as the majority of microorganisms cannot grow in artificial media within a laboratory environment.

In situations where traditional culturing methods do not provide sufficient information, our teams work to apply these techniques to oilfield microbiological investigations.

Benefits

- Modern and innovative methods applied to microbial challenges.
- New approaches can be beneficial where traditional methods have proved insufficient.
- An experienced and knowledgable team working to protect your business.

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Intertek Molecular Microbiological Methods

At Intertek, we can adopt the following methods for monitoring microbial contamination.

Fluorescence *in situ* Hybridisation (FISH)

A rapid quantification method beased on detecting ribosomal ribonucleic acid (rRNA) in microorganisms, using artificial DNA probes labelled with fluorescent dye. The test can target specific microorganisms from an order down to genus level.

FISH analysis..

- **Is quicker to detect and enumerate microbial numbers.**
- **Gives a more sensitive result.**
- **Is more specific than most probable number (MPN) counts.**

How does it work?

FISH is a culture-independent fluorescence microscopy technique. The basis of FISH analysis is the perfect match of the probe with the rRNA present in the target microbial cells. Probes are artificially manufactured DNA strands labelled with a fluorescent dye. These will bind (hybridise) to the target rRNA within the microbial cell. The result is a fluorescent microbial cell, which can be visualised under a fluorescent microscope and further analysed.

Our Oilfield Microbiology Team

Intertek's oilfield microbiology team is made up of chemists, microbiologists and technicians with a wide range of specialisms, including micobiologically influenced corrosion, potable water, microbial monitoring software and legionella analysis.

We also offer scheduled or bespoke training in oilfield microbiology, which can be delivered on your own premises or from our own purpose-built centres of excellence.

Quantitative Polymerase Chain Reaction (qPCR)

This is a DNA-based approach is used to quantify specific microorganisms. Examples of targets are SRB, nitrate-reducing bacteria (NRB), sulphate-reducing archaea (SRA) and others.

qPCR...

- **Targets specific genes (e.g. dsrAB = SRB , etc.)**
- **Provides a faster result (detection and enumeration) compared to MPN counts.**
- **Gives more sensitive results than MPN counts.**

How does it work?

The general aim of PCR technology is to specifically increase a target (gene) from an undetectable amount of starting material. As with most DNA-based molecular methods, the first step is the extraction of the DNA from the samples, which will be subsequently subjected to the qPCR technology. During qPCR, gene copies are made during thermocycling and a fluorescent marker accumulates. This can be used to quantify the starting number of the target gene. The gene copied during the process depends on the primer used and can be tailored to specifically quantify certain microbial groups.

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Denaturing Gradient Gel Electrophoresis (DGGE)

This DNA-based approach produces a community composition/microbial population 'fingerprint'. This fingerprint of different sample points can provide an insight into differences or changes in the microbial communities by location or by response to a particular treatment.

DGGE analysis can..

- **Give an overview, or 'fingerprint' of microbial populations.**
- **Reveal the source of contamination in a network of pipelines.**

How does it work?

The first step is the extraction of DNA (or RNA if looking at a functional gene) from samples. Then multiple copies of the DNA are made (amplified) using PCR. These DNA fragments are separated on a denaturing gradient gel based on their DNA sequence. DNA contains four nucleotide bases which bond across the two strands of the molecule. The extent to which the DNA strand will denature depends on its nucleotide sequence composition.

Partial denaturation instigates a decrease in mobility through the gel. Therefore, fragments with different sequences, albeit of the same length, will migrate different distances through the gel when exposed to a gradient of denaturing conditions. This results in a distinct 'community' fingerprint, with bands at different positions in the gel. Each band represents a different microbial population and some idea of their respective dominance can be ascertained from the intensity of the band. These fingerprints can then be used to charactierise microbial diversity in a particular environment or sample.

Our Oiflield Microbiology Services

Contact us to find out more about the services we provide, including:

- Potable water testing,
- Legionella risk assessments,
- Sidestream monitoring,
- Biocide and chemical testing,
- Offshore water management,
- Legionella management,
- Microbiological surveys,
- Water system disinfection,
- Training - e.g. Oilfield microbiology and legionella awareness.

Next-Generation Sequencing (NGS)

This is a DNA-based sequencing method. Its primary advantage is the ability to identify the microorganisms present in samples down to the genus level for a more detailed assessment of the microbial communities.

NGS can..

- **Describe the complete community in a sample.**
- **Compare changes in communities due to treatments or other operational effects.**
- **Give an indication of predominant microbes.**

How does it work?

There are various different platforms available, but we use 454 pyrosequencing. This can read up to 20 million bases per run and give a deep insight into microbial communities in certain samples. While all platforms are unique, they follow a similar process flow.

As with PCR technology, DNA is extracted from the samples and amplified. With NGS, they are sorted into a library of small DNA segments prior to amplification. During sequencing, each DNA fragment is sequentially identified from light signals omitted by comparing their sequence with a DNA library. As only a small portion of the microbial load is culturable, this method can reveal unknown microorganisms (archaea or bacteria). The ratio of different microbes in certain samples can be detected and highlights microbes which are more abundant compared to the whole population.

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Intertek Exploration and Production Services

Oilfield Microbiology forms part of our Exploration and Production business, which offers services across the entire oil and gas supply chain - from reservoir to refinery.

With laboratories and offices worldwide, our global reach means we can offer services where they are needed most, while our regional presence allows us to bring local guidance, reduce turnaround times and ultimately add value for your business.

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