Applied Microbiology and Molecular Biology in Oilfield Systems
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Preface

This book provides a combined theoretical and practical approach to molecular microbiology studies in oil reservoirs and related downstream processing plants and is written by some of the world’s leading authorities in this area from both academia and the oil and petroleum industry. The book is based on specific case studies and encapsulates the ethos of the ‘International Symposium on Applied Microbiology and Molecular Biology in Oil Systems (ISMOS)’. The text explains how each method works and the type of information that can be obtained. This is of direct relevance to reservoir engineers, production chemists, corrosion managers, field technicians and microbiologists, who are working in the oil and petroleum industry. The text begins with Chapter 2, which addresses practical issues relating to sampling from oilfield systems and the types of samples to be collected. Specifically, the focus is on the importance of correct sampling techniques, how to maintain sample integrity, for example, from offshore facilities, until the samples are processed in the laboratory. Details are also given on methodologies relating to DNA/RNA extraction and PCR amplification of nucleic acids from field samples.

Chapters 3–11 introduce each molecular approach in turn and explain the type of information that can be obtained, depending on the specific microbiological or technical questions that are being addressed. For example, if the question is ‘which microbial community is present in a sample?’ then possible molecular approaches which could be applied include PCR, DNA fingerprinting, fluorescent in situ hybridisation (FISH) and cloning and sequencing. If the question is ‘how many microorganisms are present in a sample?’ then quantitative PCR (qPCR) that targets a specific gene may be applied. In order to address the question of ‘which microorganisms are active in a sample?’ then approaches such as the use of RNA analyses targeting functional genes may be applied. This categorisation of each technique is rather an oversimplification and indeed information from one method may address more than one of these microbiological questions. For example, while FISH enables the identification and localisation of microbes within their environment, quantitative data on their relative abundance can also be obtained, if specific probes are applied. Details are also given on the recent developments of next-generation sequencing methods and microarrays which enable vast amounts of data to be generated very quickly and will allow us to significantly advance our knowledge of oilfield microbiology in the future. However, the production of such large data sets
requires more sophisticated bioinformatics software and user expertise in order to process it, understand it and apply it, in order to benefit the industry.

Chapters 12–19 give details of the problems caused by microbes in oilfield systems such as biocorrosion (MIC) and reservoir souring. The text focuses on how molecular microbiological methods (MMM) offer a novel approach for monitoring the in situ microbial communities in order to mitigate potential problems caused by microbes in the future, as well as to determine the efficacy of possible treatment strategies (e.g. nitrate, biocides, scavenging of H\textsubscript{2}S, pigging/cleaning and corrosion/scale inhibitors). In addition to the problems caused by microbes, Chapters 20–25 consider the beneficial implications of specific microbial processes and how MMM may be used to further exploit these. This includes the use of microorganisms in bioremediation and biorefining and bioprocessing for petroleum oil upgrade, the exploitation of microorganisms in oil recovery, i.e. microbial-enhanced oil recovery (MEOR) and using anaerobic microbial processes for obtaining methane from oil.

Chapters 26–28 describe novel fuels for the future, including the development of biofuels and the removal of naphthenic acids for both heavy oil upgrade and the bioremediation of contaminated tailing pond waters. Finally, in the Appendix, details are provided for the methodologies used by the contributing authors as guidelines for non-specialist users working in the oil and petroleum industry. Currently, there are other methods, such as stable isotopes, proteomics and metabolomics, which may also have the potential to further advance our understanding of microbial ecology and their processes in the sub-surface. Thus, in complex oil systems, where many physicochemical parameters are driving the microbial communities and their activities, it is important to adopt a polyphasic approach which combines molecular microbiological studies, with physiological and biochemical analyses of the microorganisms, alongside a detailed characterisation of their environment. Such a combined approach of analytical technologies will enhance oil production, extend life time of aging equipment/facilities, increase personnel safety and more efficiently protect the environment. Therefore, the energy sector will greatly benefit from the widespread implementation of MMM in the near future.
Acknowledgements

We would like to thank all of the contributors to this book and also to the reviewers for their useful comments and suggestions.

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Part I

Introduction, Sampling and Procedures
Microorganisms are ubiquitous in the environment and are fundamental to the biogeochemical cycling of nutrients in marine, freshwater and terrestrial ecosystems. In the deep biosphere, microbial-mediated transformations are responsible for shaping our natural resources over geological time. However, for many years our knowledge and understanding of microorganisms in sub-surface environments, like oil reservoirs, have been based primarily on culture-based approaches such as plate counts or most probable numbers (MPNs). MPN involves performing serial dilutions of a bacterial culture or an environmental sample into media until the sample is diluted to extinction (Fig. 1.1). Cultures are incubated and assessed by either eye for growth based on turbidity, media colour change or colony/microscopic counting.

There are several major weaknesses associated with MPN including the need for large numbers of replicates at the appropriate dilution to narrow the confidence intervals. Also media biases may select for the fastest growing or most abundant microorganisms which may not represent those species mediating specific catabolic processes in the sub-surface environment. Furthermore, since many microorganisms are difficult to culture in the laboratory, these approaches may lead to a gross underrepresentation of the in situ microbial communities present. It is estimated that between 0.1 and 10% of the total communities are culturable (Amann et al., 1995). Also MPN cannot be used to enumerate the microorganisms found in solid samples. Besides the technological constraints with the MPN method it also requires long incubation times (e.g. up to 28 days for some microbes). The MPN approach is laborious for the personnel performing these methods in the field, as several hours may be spent performing the serial dilutions, and if done incorrectly (for instance, by using the same syringe between dilutions) the reported numbers will be inaccurate and of limited value to the operator.

Over the last 25 years the development of molecular microbiological methods (MMM) has completely revolutionised microbial ecology research and the ways microbes are enumerated in technical systems. These methods are based on the
direct extraction of nucleic acids from natural samples, e.g. water, soil and sediments (both organic and inorganic materials). These approaches have enabled the previously unculturable microbial groups to be studied and enumerated in a rapid and reproducible manner.

Analysis of nucleic acids is driven by Polymerase Chain Reaction (PCR) approaches which amplify specific gene sequences from target microorganisms. Of particular importance are the macromolecules which form the ribosomes of microbial cells known as ribosomal RNAs (rRNAs). The rRNA has turned out to be an excellent target for determining the evolutionary relationships between microorganisms. Based on comparative rRNA sequence analysis three phylogenetically distinct lineages (or domains) of organisms have been identified which form the tree of life. These domains are the Bacteria and the Archaea (both consisting of prokaryotes) and the Eukarya (eukaryotes) (Fig. 1.2).

Sub-surface microbial community structure is highly diverse containing representatives from each domain. PCR can be applied to target which key functional microbial groups (e.g. sulphate-reducing bacteria) that are present under different environmental conditions and analyse specific metabolic processes that are occurring in the sub-surface environment. In oil reservoirs, microorganisms may readily catabolise the hydrocarbon compounds found in oil and this is exploited during the
Fig. 1.3 The ISMOS symposium series ethos aims at giving the oil, gas and petroleum industry combined and innovative solutions for problems caused by microorganisms.

Bioremediation of oil spills. Under certain conditions hydrocarbon biodegradation results in methane production which can be harnessed and this biogas may be used as a fuel source. Microorganisms may also be used to improve and upgrade oil productions, for example, by removing contaminants like heavy metals or sulphur, as well as reducing reservoir porosity and reducing oil viscosity. Some microorganisms can also produce hydrocarbons, e.g. the green microalga *Botryococcus braunii* is able to excrete long-chain (C\textsubscript{30}–C\textsubscript{36}) hydrocarbons. Not only are the activities of microorganisms beneficial, but they may also be a hindrance. For example, under some circumstances, such as in fuel storage tanks, microbial growth and hydrocarbon degradation can be problematic. In addition, specific microbial-mediated processes may lead to the production of toxic, corrosive gases like hydrogen sulphide which causes significant economic, safety, environmental and human health costs. Figure 1.3 summarises the processes involved in applying novel and innovative solutions to specific problems in the oil and petroleum industry. Thus, the energy sector will greatly benefit from the widespread implementation of MMM approaches in the future, in daily operations, in public Research and Development projects and new Joint Industry Projects (JIPs).

References


Chapter 2
Sampling and Nucleic Extraction Procedures from Oil Reservoir Samples

Geert M. van der Kraan, Maarten de Ridder, Bart P. Lomans, and Gerard Muyzer

Introduction

Today there is a renewed interest towards biological aspects in oil reservoir systems. This interest comes not only from academia but also from the petroleum industry. Fields of common interest are ‘microbial-enhanced oil recovery’ (MEOR), efforts to lower H₂S production and subsequently microbial corrosion (caused by sulphate-reducing microorganisms) and the analysis of microorganisms found in oil wells as additional information source for reservoir conditions.

In the past, the main focus of studies towards oilfield systems has been mostly on the isolation of microorganisms from these special ecosystems (see, for instance, Nazina et al., 2006; Magot et al., 2000). More recently, there has been an increase in the use of molecular microbiological methods (MMM), based on analysis of nucleic acid material (DNA and RNA). To perform such studies, various types of samples can be obtained from oilfield systems. Oilfield production waters (i.e. water produced at the well sites) have mostly been investigated, since produced water is relatively easy and cheap to sample. Still, due to the on average low cell densities in these production water samples (up to 10⁴ cells/ml, in best cases 10⁵ cells/ml) (Mueller and Nielsen, 1996; Nilsen et al., 1996) a filtration step is required to concentrate the cells before molecular analysis can be performed.

Since many microorganisms are attached to surfaces, the production water samples will give only part of the story, since only the suspended free cells are commonly produced along with production water. Also the risk of contamination by the drilling process itself and by the equipment is substantial, see for an example (Juck et al., 2005). Research on both oilfield core material and formation waters gives a more complete view of the indigenous organisms present in a subsurface environment (Vrionis et al., 2005). However, so far only a small number of cores have been analysed, due to the high costs of taking such as sample. In addition, the retrieval of a core sample usually involves the cooperation of an oil company.

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As a result of these difficulties, only a small number of articles can be found in the literature on the study of oil reservoir cores retrieved during the drilling of new exploration or production wells (Azadpour et al., 1996; Belyaev et al., 1983; Spark et al., 2000).

Despite this, it is important to learn more about oilfield ecosystems, since microbial activity not only causes issues like reservoir souring and oil pollution with H_2S, but potentially can also be used to our advantage, e.g. MEOR applications (Grigoryan and Voordouw, 2008; Sen, 2008). Therefore, a correct sampling strategy is a crucial factor for the study of down-hole communities. Here, we will present two case studies in which both sample pretreatment and nucleic extraction are addressed. These case studies demonstrate how to successfully retrieve the genetic material of two sample types: (i) production water and (ii) oilfield cores. The samples were taken from different locations. In addition, we will discuss some points of concern when sampling oilfield systems. Particular focus is given on the filtration of production water and the avoidance of filtration of the oil phase. Also, methods for treatment of cores and taking core fragments after retrieval of a core sample are addressed in detail.

Points of Concern When Sampling Oilfield Brine Waters

Various issues arise when taking brine water samples. Most obvious is usually the time in between the sampling and the actual analysis of the sample, especially with respect to samples taken from offshore locations, like drilling platforms. The arrival time in worst case scenario’s can be weeks. Commonly these samples are taken in anaerobic containers, because oxygen intrusion can change the predominance of species in such a community (Moura et al., 2009; van der Kraan et al., 2009). In addition, also the time interval (bringing the sample to the surface) can induce changes since the surface conditions hardly match the down-hole conditions (e.g. in situ pH and temperature). A rapid fixation or storage of the samples at low temperature is essential to avoid a change in the composition of the original community (Rochelle et al., 2006). The second issue is the presence of oil and usually the vast amount of dissolved salts in the water phase, which can go up to salt saturation. High salt concentrations and the presence of oil disturb the DNA extraction (see Table 2.1).

Case Study 1: Brine Water Samples from a Dutch Oilfield and Its Surface Facility Units

In this study, 10 l brine water wellhead samples were taken from various beam pumps and oil–water separator sampling points. Samples were collected in sterile jerry cans. Samples from the oil–water separators were taken from the water phase (close to the oil–water interphase). When sampling the beam pumps, the first water
fraction is discarded. This to exclude sampling of water that has been contained in
the upper part of the piping system, which might hold another community due to this
containment (Basso et al., 2005). The sampling location was an oilfield located in
the western part of the Netherlands. The jerry cans were filled to the top and sealed
with screw caps to avoid oxygen intrusion. Subsequently, the samples were taken to
the laboratory and filtered directly to minimise the chance of community changes.
For the collection and concentration of the cells, two times 4 l of brine water (in
duplicate) was filtered using hollow fibre filters (Fig. 2.1).

The lamellas of these filters contain pores with the size of 0.22 μm, which allow
the liquid to pass through and retain the bacterial cells, which usually have a size
between 1 and 2 μm. Hollow filters offer an advantage over traditional filters since
they allow a larger liquid volume to pass through before the filter gets clogged.
This is due to their large filter surface area, which allows a more representative fil-
tration (Somerville et al., 1989). During filtration procedures, filtration of the oil
phase must be avoided, because oil blocks the filters and disrupts the extraction of
DNA from the filtered cells. Avoidance of oil filtration can be achieved by allow-
ing a natural separation between the oil and the water phase by natural flotation
combined with a slow filtration by pumping the water phase from the bottom of
the jerry can. Optionally in some studies a better release between the oil phase and
cells that are attached to the oil phase is enforced by the addition of a surfactant
(e.g. Tween).

### Table 2.1 DNA extraction from different filtered production water fractions

<table>
<thead>
<tr>
<th>Sample (name)</th>
<th>DNA amount after extraction (ng/μl)</th>
<th>Wavelength 260/280</th>
<th>PCR result b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filter, released water</td>
<td>nd c</td>
<td>nd</td>
<td>–</td>
</tr>
<tr>
<td>Cut filter pieces (much)</td>
<td>0.8</td>
<td>0.35</td>
<td>–</td>
</tr>
<tr>
<td>Cut filter pieces (less)</td>
<td>0.7</td>
<td>3.91</td>
<td>–</td>
</tr>
<tr>
<td>Water from filter</td>
<td>2.1</td>
<td>6.44</td>
<td>–</td>
</tr>
<tr>
<td><strong>Centrifuged water:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pellet (10×)</td>
<td>20.1</td>
<td>1.76</td>
<td>+</td>
</tr>
<tr>
<td>Supernatant</td>
<td>1.42</td>
<td>nd</td>
<td>–</td>
</tr>
<tr>
<td><strong>Centrifuged backwashed water:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pellet (10×)</td>
<td>17.3</td>
<td>1.8</td>
<td>+</td>
</tr>
<tr>
<td>Supernatant</td>
<td>nd</td>
<td>nd</td>
<td>–</td>
</tr>
</tbody>
</table>

a The quality of the genetic material is determined by the absorption ratio at 260 and 280 nm. For DNA/RNA the value should be between 1.8 and 2. Other values indicate that pollution is disturbing the measurement.
b PCR is an amplification reaction, described in more detail later in this book. If the PCR reaction is positive, a final indication is given that the DNA extraction worked well and that it is pure enough to allow amplification.
c nd value is not realistic.
Fig. 2.1  Hollow fibre filter (e.g. from Spectrumlabs, Mediakap-5). The filter lamella is clearly visible in the cartridge. This filter is suitable to filter larger volumes of water than traditional filtering approaches and can stored without touching the filter, thus reducing the chance of contamination with allochthonous microorganisms

Pretreatment of Filters After Filtration with Brine Water Samples

After filtration a few choices can be made regarding the DNA extraction. The main purpose is to release the cells from the filter, which can be done in various ways. First, the filter cartridge, in particular the filter lamella, retains some liquid after filtration, which might have a high cell density. Second, the filter can be back-washed with a sterile buffer solution to remove retained cells from the filter. Third, the filter with cells attached can also be analysed.

If the production water contains a lot of salt (e.g. >50 g/l) the released cell suspension can be centrifuged to remove most of the salt and traces of oil, which might disturb the DNA extraction. One filter of each of the earlier described sampled environments was taken, while the second filter was kept frozen as a backup. The filters used in this study released about 3 ml of suspension after opening. Opening of the hollow fibre filter cartridges was performed in a fume hood under a flame to avoid contamination from the air. The 3 ml of liquid was divided over three sterile Eppendorf tubes. Also the filter lamellas were back-washed with sterile buffer releasing even more cells. Finally, the filter lamellas were cut into small pieces using sterile scissors. Liquid samples were centrifuged for 1 min at 13,200 rpm. Subsequently, 90% of the supernatant was removed, thereby achieving a 10 times concentration of the biomass and a removal of potential PCR inhibitors. The cell pellet was resuspended into the remainder of the supernatant. Four types of material now served as a basis for DNA extraction: (1) released suspension, (2) back-washed suspension, (3) centrifuged cell/particle pellets and (4) small cut filter pieces.
Alternative Sampling Using Specific Commercial Concentrator Kits

If the sample location is too far from the laboratory, then an on-location filtration can be applied. This can be performed with specific commercial kits. One type of kit has proven itself to be specifically convenient for sampling brine waters. The kit contains filters that hold chemicals to lyse cells and to denature proteins. The water is filtered and the cells that the water contains fall apart when they come in contact with the filter due to the present compounds. The DNA is preserved in the filter and proteins are denatured. Subsequently, the filter can be frozen and taken back to the laboratory for further analysis. An example of such a kit is the FTA Concentrator Kit produced by Whatmann\textsuperscript{TM}.

Furthermore, DNA preservation is prolonged extensively if kept below 0\textdegree C. Pieces of these filters can be used directly for microbial community analysis using molecular techniques. Since such a procedure was not necessary here, this sampling route has not been applied. The major concern with this approach is that lysis of cells is not a constant factor. The cells of various species vary in their resistance towards cell lysis and cells of some species might not lyse and therefore will not be detected in the molecular analysis. This is due to differences in their cell wall composition (Gram negative or Gram positive). Extraction methods may therefore give different results (Hong et al., 2009). Another alternative that can be considered is bringing the filter set-up (pump, hoses, filters, etc.) to the sample location. This might involve also a power generator to supply the electrical pump with energy.

Taking Oilfield Core Fragments Without Contamination

Often researchers do not always have influence on the retrieval of the core sample which is commonly performed by the oil company. It is important that contamination during retrieval of the core remains limited and with respect to the preservation of the genetic content should be frozen as quickly as possible. Various methods exist to estimate core sample contamination. To discuss these in detail would be beyond the scope of this chapter. Mostly they involve chemical tracers indicating drilling mud intrusion. Core samples are after retrieval checked for the intrusion of drilling mud in the core itself using a microscope. Also microbial tracers can be used exposing a core to ‘positive control’ microorganisms (for more information on contamination checks, see Colwell et al., 1992; Zhang et al., 2005).

During the transport of a core sample, it is advised to keep the temperature below 0\textdegree C. This can be achieved by storing the core in dry ice (solid CO\textsubscript{2}). To be sure that the sample that will be analysed has not been subjected to any kind of laboratory contamination, the core itself can be cut in a fume hood or a sterile cabinet using a sterilised chisel. It is also possible to cut the core while keeping an active gas burner next to it; this provides an air updraft, preventing contamination from the air. Pieces of the inner part of the core can now be obtained that have not been
in contact with the outside atmosphere or drilling mud. It is important to keep the core at a temperature below 0°C. This can be performed by leaving the core itself contained in solid CO₂. Also some laboratories are equipped with a cold-room. The obtained core samples now have to be crushed; this is done with a sterile pestle and mortar. The subsamples are kept below 0°C in liquid nitrogen (N₂) during the grinding (Zhang et al., 2005); the obtained sand-like material is now ready for DNA extraction using commercial extraction kits.

Case Study 2: Sampling and Treatment of Different Core Samples

Core 1: Unconsolidated Sandstone from an Oilfield in Gabon (Africa)

Our laboratory received a frozen core sample from an oilfield in Africa. The core consisted of unconsolidated sandstone and was obtained from a depth of 1,154 m. The in situ temperature and pressure of the oilfield were 43°C and 120 bar, respectively. The porosity of the core was 24–32% with a permeability of 1.0–3.5 Darcy. The core was salt saturated. Small pieces of core were taken from four different spatial positions. Also a piece of the outer core was taken as a control, this to check if the outer area of the core had a different species composition indicating contamination. Core pieces were crushed under liquid nitrogen using a sterile pestle and mortar (Fig. 2.2). The pestle and mortar were sterilised before usage by autoclaving.

![Image of a core sample](image1.png)  ![Spatial sampling of the core](image2.png)

Fig. 2.2 (a) Image of a core sample, (b) Spatial sampling of the core (schematic)
Sampling and Nucleic Extraction Procedures from Oil Reservoir Samples

at 160°C for 4 h. DNA extraction on the crushed material was performed using a commercial DNA extraction kit (MoBio soil DNA extraction kit).

Core 2: Sandstone from an Oilfield in Rotterdam (The Netherlands)

Our laboratory received two core samples from an oilfield located in the western part of the Netherlands. Both cores were retrieved from separate sample locations coming from a depth of around 2,000 m. The core material resembled coal ashes; it held a very loose structure. In this case grinding was not needed. Subsamples were taken out of three different areas in the cores using a sterile spatula. The upper layer of the material was scraped off before taking the 1 g samples, to rule out contamination from the air. During these procedures, the core was kept below 0°C.

The first attempts to retrieve DNA from these core samples using the standard protocol provided by the manufacturer of the DNA extract kits were unsuccessful. We assumed that the released DNA could adsorb strongly to the core material which resembled a kind of active carbon, which is known as an effective sorbent. We therefore modified the standard protocol and in addition tried different DNA extraction kits (see Appendix).

Standard DNA Extraction Method (Brine Water Samples and Core 1)

Nowadays commercial DNA extraction kits are widely available. Usually they are based on a series of extractions and removal of substances that can inhibit future DNA amplification reactions. In most cases there is also a protein removal step included. To lyse the cells bead beating is used. The starting material containing the cells is placed in a tube containing beads and is subsequently subjected to heavy shear stress thereby breaking the cells (beating). The commercial kits commonly involve the following steps:

- A cell lysis step in which the cells are disrupted releasing the genetic content into the solution
- A removal step in which PCR inhibitors are removed from the solution
- A protein degradation step
- Several cleaning steps
- A step in which the genetic material (DNA, RNA) is concentrated

All the above-described samples in the brine water study and the first core were prepared and subjected to DNA extraction. In these two particular studies DNA was extracted using the soil DNA extraction kit (Mo Bio Laboratories Inc, Carlsbad)
according to the manufacturer’s protocol. This kit was chosen since the crushed core sample resembles sandy soil and the brine water samples contained particles.

Subsequently the amount of DNA from all extractions was quantified using a Nanodrop 1,000 Spectrophotometer (see Table 2.1 for an example of a performed DNA extraction). The obtained DNA was then used in further studies to analyse the microbial community.

**Alternative and Improved DNA Extraction Methods**

Usually the soil DNA extraction kit (MoBio) is sufficient to obtain a good DNA extractions from various samples (see Table 2.1). However, for some samples, such as the core samples from the Rotterdam oilfield, a pretreatment, or the use of a different extraction kit is necessary. In this case the Powersoil DNA extraction kit (Mobio)™ was tried. However, this kit does not result in the desirable extraction efficiencies. To improve the DNA extraction procedure using the Powersoil extraction kit, various pretreatment steps were tried. Addition of “so-called” chaperone compounds to the soil sample prevents the genomic DNA from absorbing to the core material by shielding charges. Two effective substances are skimmed milk (a concentrated mixture of proteins) and poly-deoxyinosinic-deoxycytidylic acid (poly-dIdC) which competes with the DNA for adsorption. In our case study, two of the three pretreatments proved successful, i.e. (i) use of different washing steps and (ii) the addition of skimmed milk. To cover all different DNA extraction methods would be beyond the scope of this chapter, but various other DNA extraction methods are available (see for an example Pel et al., 2009), in which separation is performed based on the nonlinear response of long, charged polymers like DNA. Another example is van Doorn et al., (2009) in which internal amplification controls are used.

**Conclusions**

Overall conclusions that can be drawn are (1) Samples need to be preserved either by fixation or by freezing as soon as possible to prevent community changes; (2) production water can best be filtered over hollow fibre filters to avoid rapid clogging of the filter and contamination with allochthonous microorganisms. The overall conclusion with respect to the two case studies presented is that most effort should be put in to cleaning the samples thoroughly to get rid of various extraction and PCR inhibitors, before a DNA extraction is started. If samples from different environments have to be compared, a consistent DNA extraction protocol is required to be able to draw sound conclusions from the comparison.

The applied pretreatments can be different in any case when samples from oilfields are taken. In the case of the brine water samples, the centrifuged cell pellets gave the best result with respect to the DNA yield and the amplification step. Dependent on the type of core material, a choice should be made, as to which additional pretreatment is selected if a direct extraction is not successful.

In the case
of the Dutch core samples, sample washing and addition of skimmed milk proved to be the most effective for DNA extraction (Fig. 2.3). Eventually in both case studies the DNA was successfully extracted and studied.

References


