Microbiology of Oil- and Natural Gas-Producing Shale Formations: An Overview

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Abstract

Shales are the most abundant type of sedimentary rock on Earth. Many shale formations contain high concentrations of organic matter and can serve as both sources and reservoirs of oil and natural gas. Oil- and natural gas-producing shale formations are characterized by very low permeability and extremely small pore throat sizes, which have traditionally made it very difficult to extract economic volumes of hydrocarbons. Recent advances associated with horizontal drilling and hydraulic fracturing have led to increased exploration and extraction of oil and natural gas in shale formations throughout the world. This increased activity in shale formations has been accompanied by a variety of microbial-related issues including reservoir plugging, reservoir souring, sulfidogenesis, and corrosion. Even though it is clear that microorganisms cause a wide variety of deleterious processes in shales, very little is known about their origins in these formations. There are several plausible sources of microorganisms in shale, including the formation itself and the fluids that are used during the drilling and hydraulic fracturing processes. This chapter contains an overview of what is currently known about the microbiological properties of shale formations and the fluids that are used during the drilling and hydraulic fracturing processes. The chapter also contains a brief description of the research issues that need to be addressed in future studies.

Contents

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1 Introduction

Since the early 2000s, rising oil and natural gas prices, along with increased confidence that oil and natural gas could be profitably produced from shales, led to the exploration of these formations as possible alternative sources of fuels (Kerr 2010; Wang et al. 2014). Technological advances associated with horizontal drilling and hydraulic fracturing have made it profitable to recover oil and natural gas from shale formations (Hu and Xu 2013; Vengosh et al. 2013, 2014). These technological advances have also led to sharp increases in oil and natural gas production in the United States (USA). The quantity of oil produced from fractured wells in the USA has increased from 102,000 barrels per day (equivalent to less than 2% of total US oil production) in 2000 to more than 4.3 million barrels per day (equivalent to approximately 50% of total US oil production) in 2015 (Cook and Perrin 2016). The majority of the new oil that has been produced over this 15-year period has originated from the Eagle Ford and Permian shale formations in Texas and the Bakken and Three Forks shale formations, which are located in Montana and North Dakota (Cook and Perrin 2016). Similar trends have been observed in US shale gas production rates, which have increased from 0.32 trillion cubic feet (tcf) in 2000 to 6.84 tcf in 2011 (Hu and Xu 2013). Current projections suggest that shale gas will account for approximately 50% of the total US natural gas supply in 2035 (Vengosh et al. 2013). It is also expected that the USA will become a net natural gas exporter by this time (Hu and Xu 2013). The increased production of oil and natural gas from US shale formations has led to global exploration of shale formations (Hu and Xu 2013; Vengosh et al. 2013, 2014). Efforts are currently underway to assess the feasibility of oil and gas production from shale formations in Canada, South America, Africa, Europe, China, New Zealand, and Australia (Hu and Xu 2013; Vengosh et al. 2013, 2014).

The increase in oil and natural gas production from US shale formations has been accompanied by a number of microbial-related issues. Microorganisms that are present in oil and natural gas reservoirs have been shown to cause corrosion of metal-containing production and transport equipment, reservoir plugging, reservoir souring due to sulfide production, and reduced product quality as a result of the degradation of reservoir hydrocarbons (Fichter et al. 2009; Kermani and Harrop 1996; Youssef et al. 2009). To date, very little is known about the origin of microorganisms in oil and natural gas reservoirs (Magot 2005). The majority of studies that have examined populations of microorganisms in these reservoirs have relied on the use of production water samples (Magot 2005). In these studies,

microorganisms were generally assumed to be indigenous to a given oil or natural reservoir if their optimum growth temperatures and physiological capabilities were consistent with the in situ conditions (Magot 2005). More recent work involving the study of conventional oil reservoirs that contain biodegraded hydrocarbons have provided insight on a number of factors, including temperature, salt concentrations, and nutrient availability, which likely control microbial growth in shale formations (Foght 2010; Head et al. 2010; Jones et al. 2008). However, these studies have not provided definitive proof for the existence of indigenous populations of microorganisms in shale formations. Analysis of the microbial properties of drilling and fracturing fluids has provided a better understanding of how the drilling and fracturing processes influence the microbiology of shale formations (Mohan et al. 2014; Murali Mohan et al. 2013; Struchtemeyer et al. 2011; Struchtemeyer and Elshahed 2012). This work has clearly shown that drilling and fracturing stimulate microorganisms that are capable of survival and contributing to detrimental processes (e.g., sulfidogenesis, microbiologically influenced corrosion, and biofilm formation) in shale formations (Mohan et al. 2014; Murali Mohan et al. 2013; Struchtemeyer et al. 2011; Struchtemeyer and Elshahed 2012). The recent insight gained from microbiological studies of shale, drilling muds, and fracturing fluids has led to enhanced efforts to control microbial growth and eliminate deleterious microbial processes in oil- and gas-producing shale formations.

2 The Microbiology of Oil- and Natural Gas-Producing Shale Formations

Shales are the most abundant type of sedimentary rock on earth and contain a variety of minerals including clays (primarily illite), calcite, dolomite, siderite, feldspars, pyrite, gypsum, and mica (Boyer et al. 2011; Bruner and Smosna 2011; Gaspar et al. 2014). The concentrations of these minerals tend to vary both locally and regionally in shale formations (Bruner and Smosna 2011; Gaspar et al. 2014). Shales are composed primarily of clay- and silt-sized particles, which tend to accumulate as muds in low-energy depositional environments (tidal ponds and deepwater basins) (Arthur et al. 2008). Several different types of organic matter including algae, plant stems, and leaves infiltrate these muds during the deposition process (Alexander et al. 2011; Arthur et al. 2008). Over time (millions of years), the muds are buried under other layers of rock and become highly compacted in layers (Alexander et al. 2011; Arthur et al. 2008; Bowker 2007; Riebeek 2011). These compacted sediments are then exposed to elevated temperatures and pressures, which results in the formation of oil- and gas-bearing shales (Alexander et al. 2011). These oil- and gas-bearing shale formations are typically characterized by low permeability, low porosity, extremely small pore throat sizes, and high concentrations of organic matter (Alexander et al. 2011; Arthur et al. 2008; Bowker 2007; Riebeek 2011).

Isotope fractionation has commonly been used to assess whether microbially induced processes are occurring in oil and natural gas reservoirs. This technique has been used to determine whether the natural gas in shale formations is generated

through biogenic, thermogenic, or a combination of biogenic and thermogenic processes (Curtis 2002; Martini et al. 1998, 2008; Schoell 1980). Isotopic fractionation has also been used frequently to evaluate the origins of a variety of gaseous waste products (H_2, CO_2, H_2S, and CH_4) that are generated as a result of the degradation of hydrocarbons in oil and natural gas reservoirs (Jones et al. 2008; Kleikemper et al. 2002; Meckenstock et al. 1999, 2004). The results of these isotopic studies have shown that microorganisms, abiotic processes, or a combination of these two factors are often responsible for hydrocarbon degradation in oil and natural gas reservoirs (Jones et al. 2008; Kleikemper et al. 2002; Meckenstock et al. 1999, 2004). Even though isotopic studies have clearly demonstrated that microbial processes are often responsible for hydrocarbon production and degradation in oil- and natural gas-producing reservoirs, they cannot be used to provide definitive proof for the existence of indigenous (native) microorganisms in these formations.

Some of the strongest evidence for the presence of indigenous microorganisms in shale formations comes from a large number of studies that have observed microbially degraded hydrocarbons in conventional oil reservoirs (Foght 2010; Head et al. 2010; Jones et al. 2008; Magot 2005; Youssef et al. 2009). The results of this work have provided significant insight on the factors that dictate whether or not a given shale formation possesses an indigenous population of microorganisms. Temperature is one of the most important factors that govern whether or not biodegraded hydrocarbons are present in conventional oil reservoirs. Biodegraded hydrocarbons have not been observed in conventional oil reservoirs when temperatures exceed 80 °C (Head et al. 2010; Magot 2005). Thus, it is generally assumed that microorganisms will not be present in shale formations that contain temperatures above 80 °C. In other cases biodegraded oils have not been observed in deeply buried reservoirs which experienced temperatures above 80 °C, but cooled to temperatures that are more suitable for microbial growth (Foght 2010). In these cases, it is generally hypothesized that the formations were sterilized by elevated temperatures, cooled by uplifting, but not repopulated by microorganisms (Röling et al. 2003; Wilhelms et al. 2001). This hypothesis is likely highly applicable to thermogenic shale formations, such as the Barnett shale, which have undergone similar heating and cooling events. Even though many thermogenic shales have cooled to temperatures which are suitable for microbial growth, the extremely low permeabilities and small pore throat sizes within these formations are thought to have prevented their repopulation by microorganisms (Bowker 2003). The high concentrations of organic matter in many thermogenic shales also suggest that these formations may lack an active population of microorganisms (Loucks and Ruppel 2007). Salinity has also been shown to be an important factor that often limits the biodegradation of hydrocarbons in conventional reservoirs (Foght 2010; Head et al. 2010). Reservoirs with highly saline waters often exhibit limited hydrocarbon biodegradation, and studies have shown that it was not possible to cultivate hydrocarbon degraders from oil reservoirs when salt concentrations exceeded 100 g/L (Foght 2010; Grassia et al. 1996; Head et al. 2010). The availability of nutrients (e.g., electron donors, electron acceptors, phosphorous, etc.) also plays a critical role in determining whether or not

hydrocarbons are degraded by microorganisms in conventional reservoirs (Foght 2010; Head et al. 2010).

Only a handful of studies have collected core samples to test for the presence of indigenous microorganisms in shale formations (Fredrickson et al. 1997; Krumholz et al. 1997; Onstott et al. 1998). Shallow core samples (collected less than 250 m below ground) from the Mancos shale formation in New Mexico generally lacked detectable levels of microbial activity (Fredrickson et al. 1997; Krumholz et al. 1997). However, low levels of $^{35}SO_4^{2-}$ reduction were detected in some shale cores and sulfate-reducing bacteria were also enriched from one shale sample that was collected (Fredrickson et al. 1997; Krumholz et al. 1997). The authors of this study noted that $^{35}SO_4^{2-}$ reduction and sulfate-reducing bacteria were only observed after extended (14 days or longer) incubation periods in laboratory experiments (Fredrickson et al. 1997; Krumholz et al. 1997). Therefore, the authors concluded that sulfate-reducing microorganisms were likely dormant in the shale and stimulated by nutrient amendments during laboratory experiments (Fredrickson et al. 1997; Krumholz et al. 1997). This conclusion was supported by diglyceride fatty acid (DGFA)/phospholipid-derived fatty acid (PLFA) ratios, which also indicated that significant levels of nonviable biomass were present in shale and in many cases exceeded the amount of living biomass (Fredrickson et al. 1997). The authors found that the levels of microbial activity were highly dependent on pore throat sizes in this formation (Fredrickson et al. 1997; Krumholz et al. 1997). The shale layers that were examined in this formation contained pore throat sizes that were less than 0.2 μm in diameter (Fredrickson et al. 1997; Krumholz et al. 1997). The authors concluded that the small pore throat sizes of the shale resulted in nutrient limitations and reduced microbial activity (Fredrickson et al. 1997; Krumholz et al. 1997).

Studies by Onstott et al. collected cores from a low-permeability zone within the Taylorsville Basin of Virginia and tested for the presence of indigenous microorganisms (Onstott et al. 1998). This low-permeability zone was located approximately 2,800 m below ground and was composed of organic-rich shale and siltstone, which was interbedded with more porous sandstone and high natural gas concentrations (Onstott et al. 1998). This particular area was chosen for sampling because it was hydrologically isolated, it contained relatively few boreholes, and it was thought that the low permeability of the formation would reduce the possibility that the core samples would become contaminated with drilling muds (Onstott et al. 1998). Saline-tolerant, thermophilic-fermenting, iron (III)-reducing, and sulfate-reducing microorganisms were recovered from these samples (Onstott et al. 1998). These microorganisms possessed physiological traits that were consistent with the temperatures (76 °C), pressures (32 MPa), and salt concentrations (≈0.8% wt. % NaCl equivalent) in the sampled portion of the reservoir (Onstott et al. 1998). Thus, it was assumed that these microorganisms were indigenous to the sampled portion of the formation (Onstott et al. 1998). However, subsequent studies revealed that this particular formation had been exposed to paleotemperatures ranging from 160 to 200 °C, which geologically sterilized the formation (Tseng and Onstott 1997). Modeling data suggests that the microorganisms in the sampled portion of the

formation were deposited from overlying strata by meteoric water during the basin's last major tectonic event (Tseng and Onstott 1997).

To date, our knowledge of the microbiology in shales remains limited, due to the small number of core samples that have been collected. Isotope work and studies of biodegraded oil reservoirs have provided indirect evidence for the presence of indigenous microorganisms in shale formations. This work has also provided insight on the factors that control the growth of microbial populations in shale formations. Even though a handful of studies have directly examined the microbiological properties of shale cores, they have not provided absolute proof for the existence of active indigenous microbial communities in shale formations. While it generally seems to be accepted that thermogenic shale formations lack indigenous populations of microorganisms, the situation is less clear in shale formations that have not been exposed to temperatures above 80 °C (Bowker 2003; Loucks and Ruppel 2007). The work that was done with shale cores from the Mancos formation in New Mexico, which never exceeded 65 °C, may provide the strongest evidence for the presence of indigenous microorganisms in non-thermogenic shale formations (Fredrickson et al. 1997; Krumholz et al. 1997). Previous work has clearly shown that this formation contains populations of microorganisms (Fredrickson et al. 1997; Krumholz et al. 1997). The fact that these microorganisms appear to be dormant in the shale but are stimulated upon the addition of exogenous nutrients seems to support the notion that they are indigenous to the formation (Fredrickson et al. 1997; Krumholz et al. 1997). It appears highly unlikely that these microorganisms are surface contaminants (Fredrickson et al. 1997; Krumholz et al. 1997). The extremely small pore throat sizes (less than 0.2 μm) that created nutrient-limiting conditions in this formation would also have likely prevented the migration of microorganisms from the surface into the shale (Fredrickson et al. 1997; Krumholz et al. 1997). The fact that these dormant microorganisms were stimulated by exogenous nutrients also has important implications from a well construction standpoint in oil- and gas-producing shale formations. During the drilling and fracturing processes, large volumes of nutrient-laden drilling and fracturing fluids are pumped into the formation (Struchtemeyer et al. 2011; Struchtemeyer and Elshahed 2012). Previous work has shown that significant volumes of these fluids are lost to the formation (Darley and Gray 1988; Veil 2010). Thus, it is likely that these lost fluids could stimulate the growth of any indigenous microorganisms that are present in oil- and gas-producing shale formations.

3 The Influence of Drilling Mud on the Microbiology of Oil- and Natural Gas-Producing Shale Formations

Drilling muds serve a number of important functions during the construction of oil and natural gas wells in shale formations. These muds transport shale cuttings to the surface, balance surface and formation pressures in order to prevent blowouts, cool and lubricate the drilling bit, and help preserve and protect the hole during the drilling process (Darley and Gray 1988). Water-based muds are most commonly

used to construct oil and natural gas wells in shale formations due to their low cost, readily disposable nature, and low ecological footprint (Burke and Veil 1995; Murali Mohan et al. 2013). However, in some settings such as reactive shale formations, deep wells, and horizontal/extended reach wells, water-based muds occasionally exhibit poor performance (Burke and Veil 1995). In these cases, synthetic drilling muds are often used in place of water-based drilling muds (Burke and Veil 1995). A wide array of chemicals including internal olefins, esters, linear alpha-olefins, poly alpha-olefins, and linear paraffins can serve as the basal fluid in synthetic drilling muds. Prior to their injection into shale formations, water-based and synthetic drilling muds are amended with a variety of prepackaged powdered components (Table 1). These components are added to the muds in order to adjust their density, viscosity, pH and filtration, circulation, and hole stabilization properties. Several of the components can also serve as nutrient sources for microorganisms (Table 1). Components that are used to add viscosity and control the filtration and circulation properties of mud often serve as sources of carbon for microorganisms (Table 1). Components that are used to add weight, reduce viscosity, and stabilize the hole can often serve as sources of sulfate or phosphate for microorganisms (Table 1). Water-based and synthetic muds are prepared and mixed at elevated temperatures in tanks that are not protected from their surroundings (Darley and Gray 1988; Struchtemeyer et al. 2011). Therefore, any microorganisms that are present in the mud components or introduced from the surrounding environment during the drilling process will likely have an opportunity to grow and proliferate before the mud is injected into the subsurface. Large volumes of drilling mud are also routinely lost in the formation during drilling, which will lead to the introduction of exogenous nutrients and microorganisms into oil- and natural gas-producing shales (Darley and Gray 1988).

Despite the fact that drilling muds are routinely lost in shale formations and contain a variety of nutrients, which are known to support microbial growth, very few studies have examined their potential influence on the microbiology of shale formations. The microbiological impacts of water-based drilling muds on shale formations were recently examined at seven newly constructed wells in the Barnett shale (Struchtemeyer et al. 2011). The results of quantitative real time PCR and most probable number dilutions from this study showed that the addition of powdered mud components to drilling water caused an increase in numbers of total bacteria, aerobic heterotrophic bacteria, acid-producing bacteria, and sulfate-reducing bacteria (Struchtemeyer et al. 2011). The microbial communities in drilling water and the final mud mixtures, which were pumped into the formation, were also compared at each of these seven newly constructed well sites using pyrosequencing-based surveys of 16S rRNA genes (Struchtemeyer et al. 2011). The results of this work showed that a substantial decrease in microbial diversity occurred as a result of the mud formulation process (Struchtemeyer et al. 2011). The microbial communities in drilling water were highly diverse and were dominated by 16S rRNA sequences that are typically observed in freshwater ecosystems including members of the phyla/classes *Actinobacteria*, *Bacteroidetes*, *Alphaproteobacteria*, *Betaproteobacteria*, *Firmicutes*, *Gammaproteobacteria*, *Verrucomicrobia*, *Cyanobacteria*, *Planctomycetes*, *Acidobacteria*, and *Gemmatimonadetes* (Struchtemeyer et al. 2011). The microbial

Table 1 Drilling mud additives that can serve as nutrient sources for microorganisms

Name of mud component[a]	Purpose in drilling mud[a]	Nutrient(s) supplied to microorganisms[a]
Barite ($BaSO_4$)	Weighing agent	Sulfur
Lignosulfonates	Thinning agent	Sulfur
Potassium sulfate (K_2SO_4)	Hole stabilizer	Potassium/sulfur
Polyphosphates	Thinning agent	Phosphorous
Cellulose	Increases mud viscosity	Carbon
Starch	Increases mud viscosity	Carbon
Xanthan gum	Increases mud viscosity	Carbon
Wood fiber blends	Lost circulation material	Carbon
Nut hulls	Lost circulation material	Carbon

[a]All of the names of mud components and the information regarding the purpose of individual mud components and the nutrients supplied to microorganisms by mud components were taken from previous studies that examined the chemical properties of drilling muds (Prasad and Katiyar 2010; Struchtemeyer et al. 2011)

communities in drilling muds were almost completely distinct from and much less diverse than the drilling water samples (Struchtemeyer et al. 2011). The microbial communities in six out of the seven mud samples were dominated by 16S rRNA sequences affiliated with *Firmicutes* and *Gammaproteobacteria* (Struchtemeyer et al. 2011). The microbial community in the seventh mud sample was dominated by 16S rRNA sequences that were affiliated with *Firmicutes* and *Betaproteobacteria* (Struchtemeyer et al. 2011).

The drastic shifts that were observed in the microbial communities from the water-based Barnett shale drilling mud samples appeared to reflect physical changes associated with the mud formulation process (Struchtemeyer et al. 2011). Many of the 16S rRNA sequences that were present at higher abundance in drilling mud than drilling water were affiliated with strict and facultative anaerobes (Struchtemeyer et al. 2011). This was likely attributable to the increased viscosity and lower oxygen concentrations associated with the drilling muds (Struchtemeyer et al. 2011). 16S rRNA sequences that were affiliated with polymer-degrading genera including *Acetovibrio*, *Ruminococcus*, *Eubacterium*, and *Clostridium* were also much more abundant in drilling mud than drilling water (Struchtemeyer et al. 2011). These genera of bacteria were likely stimulated by the large quantities of different polysaccharides (nut hulls, cedar fiber, and xanthan gum) that were used to make drilling muds at these sites (Struchtemeyer et al. 2011). A variety of sulfide-producing lineages including *Desulfotomaculum*, *Desulfovibrio*, *Desulfomicrobium*, *Desulfobacterium*, *Desulfitobacterium*, *Thermodesulfobacterium*, *Thermoanaerobacter*, and *Thermotoga* were also much more abundant in drilling mud than drilling water (Struchtemeyer et al. 2011). Several of the sulfide-producing lineages that were present at higher abundance in drilling mud than drilling water (*Desulfotomaculum*, *Thermodesulfobacterium*, *Thermoanaerobacter*, and *Thermotoga*) were also thermophilic (Struchtemeyer et al. 2011). These observations

coupled with microcosm studies, which showed that the addition of powdered mud components to drilling water stimulated sulfide producers at temperatures observed in Barnett shale natural gas wells, provided evidence that drilling mud can contribute to incidences of sulfidogenesis in shale formations (Struchtemeyer et al. 2011). In addition to thermophilic sulfide producers, several genera of non-sulfide-producing, thermophilic microorganisms (e.g., *Geobacillus* and *Petrobacter*) were also much more abundant in drilling mud than drilling water (Struchtemeyer et al. 2011). The increased abundance of thermophilic lineages in drilling mud relative to drilling water was attributed to the elevated temperatures (up to 50 °C) associated with the mud-making process (Struchtemeyer et al. 2011).

The results of the water-based drilling fluid studies in the Barnett shale clearly showed that the drilling mud formulation process stimulated microbial communities which are capable of surviving the anoxic conditions and elevated temperatures encountered in many shale formations (Struchtemeyer et al. 2011). The microbial communities stimulated in water-based drilling muds also appear to be capable of contributing to deleterious microbiologically related processes (e.g., sulfidogenesis and corrosion) that have been previously reported in oil- and natural gas-producing shale formations (Struchtemeyer et al. 2011). Several of the microbial lineages that were observed in the water-based mud samples from the Barnett shale, including *Desulfobacterium*, *Desulfovibrio*, *Thermotoga*, *Petrobacter*, *Thermodesulfobacterium*, and *Thermoanaerobacter*, have been previously observed in production water samples from high temperature oil and natural gas wells (Magot 2005; Salinas et al. 2004). In many cases these microorganisms were assumed to be indigenous to high temperature oil and natural gas wells since they had been routinely observed in geographically distinct reservoirs and possessed physiological properties (optimum growth temperature and salt tolerance range) that were consistent with in situ reservoir conditions (Magot 2005). However, the observation that these microorganisms were stimulated in water-based muds during the drilling mud formulation process in newly constructed Barnett shale natural gas wells, suggests that they may, in fact, be of exogenous origin (Struchtemeyer et al. 2011). Even though it is clear that water-based drilling muds impact the microbiology of oil- and natural gas-producing shale formations, the impact of synthetic drilling fluids on the microbiological properties of these formations is less clear. A recent study conducted in the Marcellus shale was unable to extract detectable levels of DNA or amplify 16S rRNA sequences from a synthetic oil-based drilling mud that was used to construct a new natural gas well in this formation (Murali Mohan et al. 2013). The authors performed a series of control experiments in which they spiked the synthetic oil-based drilling mud with *E. coli* DNA to ensure that the mud components did not contain inhibitors that would prevent the extraction of DNA and the amplification of 16S rRNA gene sequences (Murali Mohan et al. 2013). The authors were able to extract *E. coli* DNA and amplify its 16S rRNA gene sequence from this spiked synthetic oil-based drilling mud sample (Murali Mohan et al. 2013). This observation provided conclusive proof that the lack of DNA in this drilling mud sample was due to the absence of microorganisms rather than the presence of inhibitors (Murali Mohan et al. 2013). The authors were unable to conclude if the absence of

microorganisms in this synthetic oil-based drilling mud was attributable to the presence of long chain hydrocarbons, which are not readily degradable by microorganisms, or biocide treatments that occurred (Murali Mohan et al. 2013). Thus, it remains unclear if synthetic oil-based drilling fluids impact the microbiology of other oil- and natural gas-producing shale formations. However, it is important to note that a variety of studies have observed microbial degradation of synthetic oil-based drilling fluids (Benka-Coker and Olumagin 1996; Nweke and Okpokwasili 2003; Okpokwasili and Nnubia 1999). Therefore, it would seem likely that these synthetic oil-based drilling fluids could potentially harbor active populations of microorganisms, which would impact the microbiological properties of shale formations.

4 The Influence of Hydraulic Fracturing on the Microbiology of Oil- and Natural Gas-Producing Shale Formations

Since shale formations are extremely impermeable, drilling alone will not allow for the economic recovery of oil and natural gas (Arthur et al. 2008; Veil 2010). Therefore, newly constructed wells in shale formations must be hydraulically fractured (Arthur et al. 2008; Veil 2010). During the fracturing process, large volumes of fluid are pumped into a predrilled portion of the formation at high pressure, which creates fractures in the shale (Arthur et al. 2008; Veil 2010). These fractures increase the permeability of the shale and allow for the recovery of economic volumes of oil and natural gas (Arthur et al. 2008; Veil 2010). Water from municipal sources or wells, which contains low levels of bacteria, is the primary component that is used to make fracturing fluids (Fichter et al. 2009). This water is hauled to and stored in man-made ponds (Fig. 1) for prolonged periods of time prior to the start of the fracturing process (Fichter et al. 2009). During this storage period, the frac water is exposed to air, rainfall, and runoff, which often leads to high levels of microbial contamination (Fichter et al. 2009). Once the fracturing process starts, frac water is pumped from the pond into frac tanks (Fig. 1) where biocide is added to control microbial growth (Struchtemeyer and Elshahed 2012). After the frac water is treated with biocide, it is pumped to a blender (Fig. 1) where a mixture of chemicals and sand are added (Struchtemeyer and Elshahed 2012). Some of the notable chemicals that are added to the frac water in the blender include sugar-based polymers which increase the viscosity of the frac fluids (Arthur et al. 2008). These polymers can also serve as growth substrates for any microorganisms that survive biocide treatment (Arthur et al. 2008). The mixture of water (approximately 90.6%), chemicals (approximately 0.45%), and sand (approximately 8.95%) that leaves the blender (Fig. 1) represents the final frac fluid mixture (Arthur et al. 2008; Struchtemeyer and Elshahed 2012). These frac fluids are pumped from the blender into the formation at high pressure (Fig. 1), which creates fractures in the shale (Arthur et al. 2008).

After the fractures are created in the shale, the frac fluids are often left in the formation for a period of time ranging anywhere from 1 day to several months in length (Struchtemeyer and Elshahed 2012). Newly fractured wells then undergo a

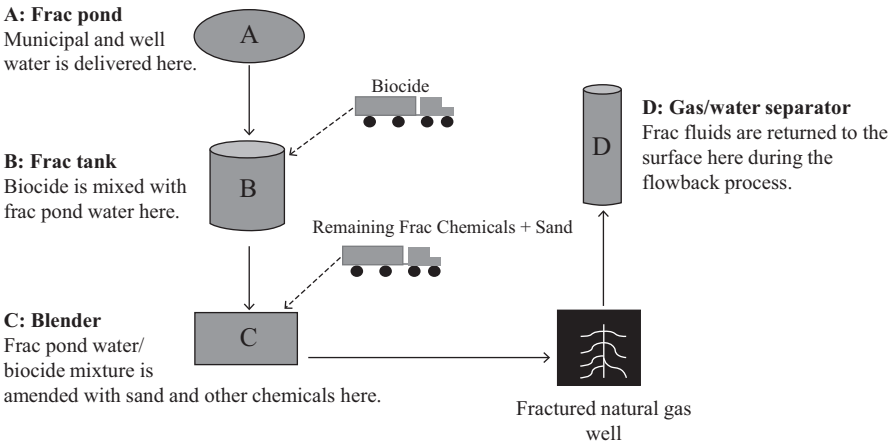

Fig. 1 Generalized overview of the events that occur during the hydraulic fracturing process in shale formations (Struchtemeyer and Elshahed 2012). The *solid arrows* represent the direction that fracturing fluids flow during the fracturing and flowback processes. The *dashed lines* are used to highlight points where biocides, sand, and other fracturing chemicals are introduced to the system during the process

process referred to as flowback (Fig. 1), which involves the removal of the liquid portion of the frac fluids from the formation (Struchtemeyer and Elshahed 2012). Previous work has shown that the flowback process only recovers between 30% and 70% of the fracturing fluids that were pumped into the formation (Veil 2010). The large volumes of frac fluids that are left behind in the formation are exposed to high temperatures, become anaerobic, and leach chlorides, iron, and organic matter from the shale (Blauch et al. 2009; Houston et al. 2009). These conditions will likely allow for the growth and proliferation of indigenous microorganisms that are present in the shale and any microorganisms in the frac fluids that survived biocide treatments (Blauch et al. 2009; Houston et al. 2009). The sand that was added to the frac fluids is also left behind in the shale and holds open the newly created fractures, which allows gas to flow to the wellbore (Arthur et al. 2008; Veil 2010).

In spite of the importance of hydraulic fracturing, the microbiological properties of frac fluids have only been investigated recently. Studies conducted at newly constructed natural gas wells in the Marcellus and Barnett shale formations have used 16S rRNA gene sequencing methodologies to examine the microbial communities in samples of preinjection frac fluids and flowback waters (Murali Mohan et al. 2013; Struchtemeyer and Elshahed 2012). The microbial communities in preinjection frac fluids from both shale formations were highly diverse and dominated by 16S rRNA sequences affiliated with aerobic *Actinobacteria*, *Bacteroidetes*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Firmicutes*, and *Cyanobacteria*, which have previously been observed in freshwater ecosystems (Murali Mohan et al. 2013; Struchtemeyer and Elshahed 2012; Wu et al. 2007; Zwart et al. 2002). These observations were not surprising since freshwater was the

primary component used to prepare fracturing fluids in these shale formations (Murali Mohan et al. 2013; Struchtemeyer and Elshahed 2012). The microbial communities in flowback water samples from these two shale formations were much less diverse than the microbial communities present in the preinjection frac fluids (Murali Mohan et al. 2013; Struchtemeyer and Elshahed 2012). The flowback water communities from these two shale formations also bore little, if any, resemblance to the preinjection frac fluid communities (Murali Mohan et al. 2013; Struchtemeyer and Elshahed 2012). As the flowback process progressed in each of these two shale formations, the microbial communities in flowback waters became dominated by 16S rRNA gene sequences that were affiliated with the phylum *Firmicutes* (Murali Mohan et al. 2013; Struchtemeyer and Elshahed 2012). Many of the 16S rRNA gene sequences that were more abundant in flowback waters than the preinjection frac fluids were affiliated with facultative/obligate anaerobes, endospore-forming microorganisms, thermophilic microorganisms, halophilic microorganisms, and sulfide-producing microorganisms (Murali Mohan et al. 2013; Struchtemeyer and Elshahed 2012). The increased abundance of these different groups of microorganisms in flowback waters was attributed to a variety of physical (e.g., increased temperature) and chemical (e.g., reduced oxygen concentrations, biocide exposure, increased salt concentrations, and increased sulfur concentrations) changes that these fluids were exposed to during the fracturing process (Murali Mohan et al. 2013; Struchtemeyer and Elshahed 2012).

One of the major drawbacks associated with 16S rRNA sequencing-based studies is their inability to provide insight on the metabolic capabilities of microorganisms within a specific microbial community (Mohan et al. 2014). Traditionally these studies have required the use of assumptions regarding the metabolic capabilities of specific microbial community members (Mohan et al. 2014). These assumptions are typically made based on the physiological properties of the closest cultured microorganisms that are identified in 16S rRNA sequence databases (Mohan et al. 2014). Recent advances in the field of metagenomics allow researchers to simultaneously gain insight on the taxonomic and metabolic profiles of microorganisms within a specific community. Metagenomic studies were recently conducted with the same preinjection frac fluids and flowback waters from the Marcellus shale that had previously been subjected to 16S rRNA sequence analyses (Mohan et al. 2014; Murali Mohan et al. 2013). The microbial communities that were detected in these fluids using metagenomics were consistent with those obtained using 16S rRNA gene-based surveys (Mohan et al. 2014; Murali Mohan et al. 2013). The metabolic profile from these samples demonstrated that increases in relative abundance and functional changes occurred for genes responsible for carbohydrate metabolism, respiration, sporulation and dormancy, iron acquisition and metabolism, stress response, and sulfur metabolism in flowback water relative to the preinjection frac fluids (Mohan et al. 2014). As was the case with 16S rRNA sequencing surveys, the results of this metagenomic study seemed to suggest that the populations of microorganisms in flowback water were adapted to survive the elevated temperatures, reduced oxygen concentrations, biocide amendments, and increased concentrations of salt, iron, and sulfur, which

are routinely encountered in shale formations (Mohan et al. 2014; Murali Mohan et al. 2013; Struchtemeyer and Elshahed 2012).

Even though 16S rRNA and metagenomic studies have provided strong evidence that fracturing stimulates populations of microorganisms which could theoretically survive in shale formations, it is important to point out that these cultivation-independent methodologies cannot be used to identify active microbial communities or processes. However a variety of cultivation-based studies, which were conducted at newly fractured well sites in shale formations, have produced results that are similar to what has been observed using these cultivation-independent approaches (Harrington 2015; Liang et al. 2016). A wide variety of bacterial isolates were recently obtained from pre-injection frac fluids and flowback waters from the Bakken shale formation in North Dakota (Harrington 2015). Taxonomic comparisons of the isolates from these water samples, which involved the use of 16S rRNA gene sequences, indicated that the microbial community in these fluids underwent drastic changes during the fracturing process and was accompanied by a significant decrease in microbial diversity (Harrington 2015). The 16S rRNA sequencing data also indicated that the majority of the isolates from the flowback water were affiliated with *Firmicutes* (Harrington 2015). All of these observations are consistent with what has been observed in other shale formations using cultivation-independent approaches (Harrington 2015; Mohan et al. 2014; Murali Mohan et al. 2013; Struchtemeyer and Elshahed 2012). Many bacterial isolates from flowback water collected in the Bakken and Niobrara shale formations possessed physiological properties, including the ability to grow at high temperatures under anaerobic conditions, produce spores, resist biocide treatments, and tolerate high salt concentrations, which are all consistent with genes that displayed increased abundance in metagenomic studies of flowback water (Harrington 2015; Mohan et al. 2014). The phylogeny of the majority of these bacterial isolates (e.g., *Bacilli* and *Gammaproteobacteria*) was also highly similar to 16S rRNA sequences that have been observed in other flowback water samples (Harrington 2015; Murali Mohan et al. 2013; Struchtemeyer and Elshahed 2012). An isolate from the genus *Halanaerobium*, which is one of the most commonly observed genera of halophilic bacteria in flowback waters from shale formations, was also recently recovered from a natural gas-producing field in the Barnett shale that underwent hydraulic fracturing (Liang et al. 2016; Murali Mohan et al. 2013; Struchtemeyer and Elshahed 2012). This microorganism coupled the degradation of guar gum, which is a polysaccharide commonly used to increase the viscosity of hydraulic fracturing fluids, to the production of sulfide when thiosulfate was present (Liang et al. 2016). The ability of this *Halanaerobium* sp. to degrade one of the major components in fracturing fluids, along with the fact that members of this genus have been repeatedly observed in flowback waters from multiple and distinct formations, suggests that these microorganisms are likely stimulated by and survive the hydraulic fracturing process. The production of sulfide by this *Halanaerobium* sp. is also consistent with metagenomic studies from flowback waters, which showed that sulfur-metabolizing genes were more abundant in flowback water relative to the preinjection frac fluids (Liang et al. 2016; Mohan et al. 2014).

The results of previous work have clearly shown that biocide treatments do not kill all of the microorganisms in frac fluids prior to their injection into shale formations (Struchtemeyer and Elshahed 2012). This observation is extremely concerning since large volumes of frac fluids are routinely lost to shale formations during the fracturing process (Veil 2010). The collective results of cultivation-independent and cultivation-based studies all seem to show that the hydraulic fracturing process stimulates populations of microorganisms, which appear to be adapted to survive the conditions that are encountered in shale formations (Harrington 2015; Mohan et al. 2014; Murali Mohan et al. 2013; Struchtemeyer and Elshahed 2012). Many of these microorganisms (e.g., sulfide-producing microorganisms) are known to contribute to deleterious processes (e.g., reservoir plugging/souring and corrosion) in oil and natural gas wells (Fichter et al. 2009; Kermani and Harrop 1996; Youssef et al. 2009). Thus it appears highly likely that the fracturing process, much like the drilling process, contributes to many of the deleterious microbially induced processes that have been observed in shale oil and natural gas wells.

5 Research Needs

Even though more recent studies have begun to shed light on the microbiology of oil- and natural gas-producing shale formations, additional research is needed to obtain a more complete understanding of these complex ecosystems. The microbiological properties of shale formations clearly need to be studied more extensively. However, the collection of shale samples can be quite challenging due to a variety of factors (e.g., the requirement for sophisticated drilling equipment to prevent outside microbial contamination), which have been documented previously (Magot 2005). Many of the factors that make the collection of shale samples difficult lead to cost, personnel, and time constraints (Magot 2005). These issues have prevented the oil and natural gas industries from using optimized drilling methods to collect shale samples that are free of drilling mud contamination (Magot 2005). The impacts of the drilling and hydraulic fracturing processes on the microbiological properties of shale formations also need to be evaluated further. To date, the microbiology of the fluids used during these two processes has primarily been characterized using 16S rRNA sequencing and metagenomic analyses (Mohan et al. 2014; Murali Mohan et al. 2013; Struchtemeyer et al. 2011; Struchtemeyer and Elshahed 2012). The results of this work have clearly shown that drastic shifts in microbial communities occur during these two processes. However, these types of studies provide very little insight on which populations of microorganisms are active in these fluids. Metatranscriptome analysis of drilling and fracturing fluids both pre- and postinjection would provide valuable insight on the active populations of microorganisms that are stimulated by these two processes.

Recent insight on the microbiology of oil- and natural gas-producing shale formations has led to a surge in efforts to control microbial growth in these ecosystems. Numerous studies have conducted laboratory-based experiments to

evaluate the effectiveness of chemical biocides against microorganisms that are commonly found in hydraulic fracturing fluids (Gaspar et al. 2014; Kahrilas et al. 2014; Struchtemeyer et al. 2012). The results of this work have shown that non-oxidizing biocides are more suitable for controlling microbial growth in shale formations than oxidizing biocides (Kahrilas et al. 2014). This has been attributed to the fact that nonoxidizing biocides are generally more stable and less likely to react with the components in fracturing fluids than oxidizing biocides (Kahrilas et al. 2014). The results of laboratory-based studies of chemical biocides have also provided valuable insight on factors that influence biocide efficacy and the mechanisms that microorganisms use to resist biocide treatments (Gaspar et al. 2014; Kahrilas et al. 2014; Struchtemeyer et al. 2012). However, very few studies have conducted long-term in situ examinations of the effects of biocide type, biocide concentration, and frequency of biocide applications on microbial growth in shale formations. This type of work would likely provide valuable insight on how to control microbial growth in oil- and natural gas-producing shale formations, which cannot be obtained through the use of laboratory-based studies alone. For example, recent studies showed that the practice of biocide rotation in hospitals and cooling towers led to decreased biocide resistance (Bentham and Broadbent 1995; Murtough et al. 2001, 2002; Rangel et al. 2011). In situ examinations of biocide rotations in shale formations may produce similar results and help control populations of microorganisms that are typically difficult to treat (e.g., spore formers) in shale formations (Gaspar et al. 2014). In addition to biocide treatments, many other operational practices should also be reevaluated in order to better control microbial growth in oil- and natural gas-producing shale formations. For example, the use of barite as a weighing agent in water-based drilling muds is problematic because it has been shown to stimulate the growth of sulfide-producing microorganisms (Struchtemeyer et al. 2011). Studies have shown that this issue can be avoided by using dolomite in place of barite in water-based drilling muds (Badrul et al. 2007). The amount of time that passes between the frac and flowback processes in newly constructed oil- and natural gas-producing wells in shale formations is also highly variable (Struchtemeyer et al. 2012). The extended incubation of frac fluids in shale formations appears to stimulate the growth and proliferation of microbial communities that are known to cause deleterious processes including sulfidogenesis and corrosion (Mohan et al. 2014; Murali Mohan et al. 2013; Struchtemeyer et al. 2012). This observation is problematic since recycled flowback waters are being used more frequently during the fracturing process at newly constructed oil- and gas-producing wells in shale formations (Arthur et al. 2008; Veil 2010). Additional well construction practices that contribute to microbial-related issues in shale formations need to be identified and studied using long-term, comprehensive, and statistical analyses to prevent their occurrence in newly constructed wells that are used for the production of oil and natural gas.

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