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Microbial Dynamics and Control in Shale Gas Production

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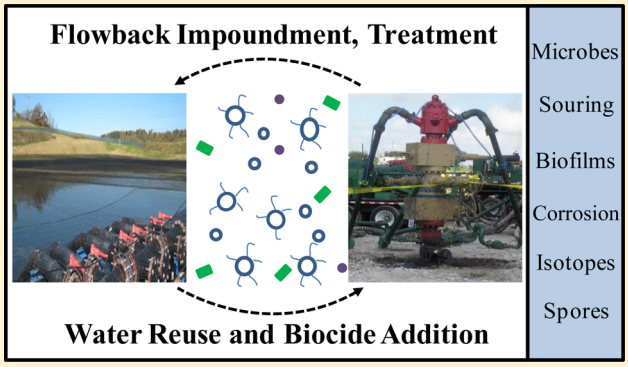
Supporting Information

ABSTRACT: Microorganisms can cause detrimental effects in shale gas production, such as reservoir souring, plugging, equipment corrosion, and a decrease in hydrocarbon production volume and quality, thus representing a multi-billion-dollar problem. Prefracturing fluids, drilling mud, and impoundment water likely introduce deleterious microorganisms into shale gas reservoirs. Conditions within the reservoir generally select for halotolerant anaerobic microorganisms. Microbial abundance and diversity in flowback waters decrease shortly after hydraulic fracturing, with Clostridia, a class that includes spore-forming microorganisms, becoming dominant. The rapid microbial community successions observed suggest biocides are not fully effective, and more targeted treatment strategies are needed. At the impoundment level, microbial control strategies should consider biocide rotation, seasonal loading adjustments, and biocide pulse dosing. In shale plays where souring is common, stable $^{34}S/^{32}S$ isotope analysis to identify abiotic H_2S is recommended to evaluate the merits of biocide application in treating reservoir souring. Overall, an improved understanding of the microbial ecology of shale gas reservoirs is needed to optimize microbial control, maximize well productivity, and reduce environmental and financial burdens associated with the *ad hoc* misuse and overuse of biocides.



INTRODUCTION

Microbial processes can profoundly impact shale gas well production, downstream processing, water quality, and the mobility of toxic metals and radionuclides.[1] Therefore, for human and environmental health, safety, and economic reasons, it is critical for operators to understand how decisions related to drilling, fracturing, water management, and well operation may affect microbial community dynamics and composition. The proliferation of bacteria in conventional oil and gas reservoirs has been linked to multi-billion-dollar problems such as reservoir souring (which refers to the production of H_2S, a corrosive and toxic gas that increases processing and refining costs),[2] reservoir plugging,[3,4] equipment and pipeline corrosion,[5−7] and lower product quality.[8] Additionally, sulfidogenic bacteria can consume short chain hydrocarbons such as propane and butane and potentially lower well productivity.[9] Conversely, some bacteria may contribute to beneficial outcomes such as paraffin removal[10] or biosurfactant production (enhancing oil recovery).[11−13]

While recent studies have helped clarify the role of various microbial populations in conventional oil reservoir production,[2,14−17] the broader implications for shale gas production are still a nascent area of research (Figure 1). There are very few published shale reservoir microbiology studies,[18−23] underscoring the need for novel insight into guiding practical strategies for mitigating undesirable microbial processes and enhancing positive outcomes.

Following hydraulic fracturing, indigenous or introduced microbes are flushed from the subsurface during the flowback period. The water that is produced at the surface is handled and temporarily stored onsite where microbial populations can contaminate production infrastructure such as surface separators, storage tanks, and flowlines.[24] In addition, malodorous and toxic compounds may be produced by anaerobic bacteria during storage of produced water.[21] Moreover, there is growing interest in reusing produced water (PW) for subsequent hydraulic fracturing, to minimize freshwater withdrawals and off-site disposal costs and potential liability. This is important, because the reuse of water may seed subsequent wells with a deleterious microbial community that may be preselected for resistance to the biocides that are commonly used to control microbial growth.[21,23,25]

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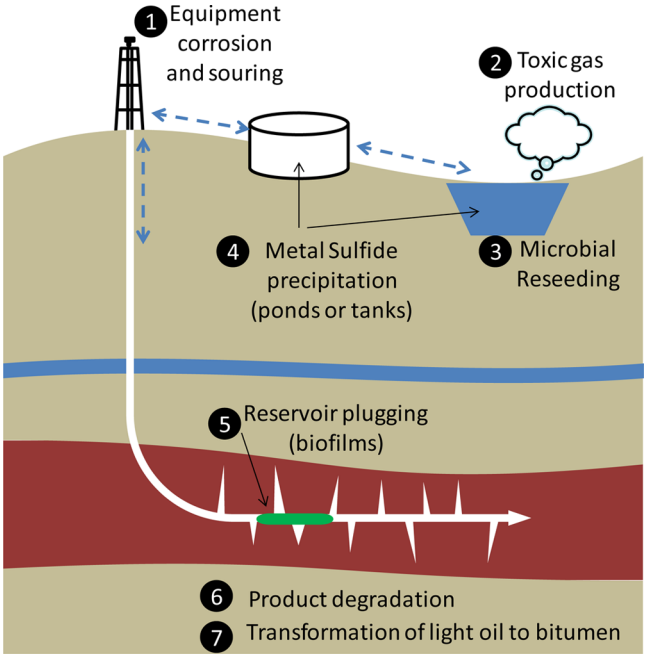

Figure 1. Microbial processes of concern in shale oil and gas production. (1) H_2S and acid production by reservoir microorganisms can accelerate equipment and flowline corrosion and increase refining costs. (2) The storage of impoundment water can lead to the production of toxic and odorous gases. (3) Water reuse may seed reservoirs with microorganisms preselected for reservoir conditions and biocide resistance. (4) Metal sulfide/sulfate precipitation. (5) Reservoir plugging caused by biofilm formation. (6) Product degradation (e.g., propane oxidation by SRBs). (7) Transformation of light oil to bitumen.

In this paper, we review the literature on the microbiology of unconventional shale gas development using hydraulic fracturing. We critique microbial assessment methodologies and discuss environmental factors and operational variables that influence microbial communities and biogeochemistry. Finally, we assess current strategies for microbial control, identify critical knowledge gaps, and offer alternative approaches to improving upon modern practices.

GEOCHEMICAL AND ENVIRONMENTAL FACTORS INFLUENCING MICROBIAL GROWTH AND CONTROL

Shale mineral composition varies widely at both local and regional scales.[26,27] Once shale gas production begins, changes in biogeochemical reactions, groundwater flow, gas desorption, and open degassing can change the microbial community.[28] While small pore size limits microbial activity in unfractured shale relative to other subsurface environments,[29] organic matter trapped in shale can serve as an abundant energy source for microbes.[30] Potential carbon sources or electron donors include CH_4, H_2, volatile fatty acids, and petroleum hydrocarbons. Moreover, sulfur-containing ores such as barite and gypsum common to shale provide a source of electron-accepting compounds. In general, redox potential is low in deep gas fields, and some electron acceptors (e.g., oxygen, nitrate, and ferric iron) may be absent.

Temperature is likely a limiting factor for microbial growth and survival in many shale reservoirs.[31] Hydrocarbon-bearing shales range in temperature between approximately 25 and 200 °C.[32,33] Wells with bottomhole temperatures above 120 °C would severely limit microbial abundance and diversity. High salinity values, typically ranging from 100000 to 200000 mg/L TDS in PW,[34] exert selective pressure for halotolerant microorganisms. The pressure within oil reservoirs (up to 500 atm) does not preclude some bacterial growth and proliferation, although it can influence their physiological or metabolic properties.[35]

Much of the focus on the intersection of shale geology and microbiology centers on understanding how sulfur-containing minerals may affect souring, which is perhaps the most problematic microbial process in terms of being least manageable and having a relatively large financial impact on production. Gas is considered sour and must be treated before industrial use when H_2S concentrations exceed 4 ppm_v.[36] Approximately 80% of conventional oil reservoirs are sour,[37] while 40% of known global gas reserves are estimated to be sour.[38,39]

Shale H_2S may arise from biogenic or thermogenic (abiotic) mechanisms.[40] If H_2S is predominantly thermogenic, using biocides to treat souring would be a considerable waste of material and financial resources. Current industry practice does not identify whether H_2S is biogenic, and biocides are universally added regardless of need. The unintended consequences of this practice (e.g., hindering some microbial ecosystem services or development of biocide resistance among bacteria) have not been systematically explored.

Biological sulfate reduction (BSR) may occur at temperatures up to 80 °C.[41] The observed upper temperature for life is 122 °C at 20 MPa.[42] Given reservoir temperatures vary from 25 to 200 °C, temperature may not be used exclusively to distinguish between biogenic and thermogenic gas. Instead, stable isotope analysis to quantify $^{34}S/^{32}S$ ratios should be considered. Lighter isotopes are preferentially processed through biogeochemical cycles over heavier isotopes, whereas no isotope fractionation results from abiotic processes. Accordingly, biogenic H_2S results in enrichment in lighter ^{32}S relative to the heavier ^{34}S fraction in the source sulfate,[41] while thermogenic H_2S routinely yields stable sulfur isotope ratios reflecting no enrichment or positive enrichment.[43,44] Isotopic analysis has been used successfully to discern biogenic and thermogenic H_2S in conventional oil and gas reservoirs in China.[45] Similar studies with H_2S in coal beds have shown that gas production from geologic means results in no enrichment.[46] Implementation of stable isotope analysis could provide a more accurate depiction of subsurface biogeochemistry[47] and may lead to substantial savings by avoiding unnecessary microbial control efforts in reservoirs where corrosion is attributable primarily to thermogenic H_2S and observed equipment fouling is minimal. Isotopic testing would enable the development of pad-specific microbial control practices versus the current one size fits all approach.

MICROBIAL COMMUNITIES ASSOCIATED WITH HYDRAULIC FRACTURING

The source of microorganisms in flowback and PW is poorly understood. Only a limited number of studies examined microbial communities associated with hydraulic fracturing, and these studies lack the deep sequencing and life cycle approach needed to unequivocally discern the microbial source. What is known may be cobbled together to conclude that microbial communities may arise from a variety of sources, including native populations from the fractured formation,

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drilling muds, hydraulic fracturing fluid, and infrastructure such as pipes and trucks that are used to transport, manage, and treat the water, or from imported surface sources such as air and soil.[20,21]

Drilling Muds. Drilling mud may serve as a source of microbes that are introduced into the reservoir during well development,[22] but this event is highly dependent on the makeup of the drilling fluid and its ability to sustain microbial life.[20] For example, the addition of drilling mud components to makeup water greatly reduced phylum-level bacterial diversity while increasing population numbers.[22] Within this complex community, the abundance of aerobic heterotrophs, acid producers, and sulfate reducers all increased. Firmicutes saw a large percentage increase, from an average of 7% in drilling waters to 55% in drilling muds. The combination of Firmicutes and Gammaproteobacteria represented 84−97% of all the sequences obtained in the drilling mud sets. Several of the lineages present in complex drilling mud were not detected in drilling makeup waters. These lineages included phylotypes most similar to sulfate-reducing Deltaproteobacteria and thermophiles from the order/phyla Thermales, Thermodesulfobacteria, and Thermotogae.[22] On the other hand, DNA analysis of synthetic drilling mud used in the Marcellus play suggested that drilling mud may not be a significant source of bacteria in produced water.[20] These studies imply that drilling fluid composition may be an important consideration for microbial control and raise the possibility that drilling fluids may be formulated without readily biodegradable hydrocarbons to minimize microbial growth.[20]

Flowback and Produced Waters. Microbial communities associated with shale gas production have only been examined at a few sites in the Marcellus,[20,21,48−50] Barnett,[18,22,23] Haynesville,[51] and Antrim[52] shale plays. One study examined microbial community changes in a Marcellus shale gas operation from drilling through hydraulic fracturing and production phases.[20] Source water, fracturing fluids, and early production phases contained microbial communities that were relatively unchanged and composed mostly of aerobic species within the classes Alphaproteobacteria and Gammaproteobacteria. However, over the course of production, the microbial community shifted toward one dominated by anaerobic halophiles. Specifically, after production for 187 days, both diversity and abundance had greatly decreased and the community was almost entirely (>99% pyrosequencing, 97% clone libraries) composed of a Firmicutes phylotype with a sequence that was >99% identical to that of *Halanaerobium congolense*. This species is an anaerobic, moderately halophilic, endospore-forming, thiosulfate- and sulfur-reducing bacterium, incapable of reducing sulfate, originally isolated from an offshore oil field in Congo.[53] Because phylogenetic association cannot reliably infer metabolic traits, future transcriptomic and metabolomic studies are needed to ascertain the role of *Halanaerobium* and/or similar microbes and discern whether sulfidogenesis from sulfur (rather than sulfate) reduction is an important contributor to reservoir souring.

A separate study of microbial community changes in two Marcellus shale gas wells also reported the eventual predominance of *Halanaerobium* species.[48] Similarly, Halanaerobiales was identified in samples from the Barnett shale along with other members of Firmicutes as well as Proteobacteria.[18] An independent analysis of flowback waters from two Barnett shale gas wells also reported that 75 and 98% of the 16S rRNA gene sequences recovered were affiliated with Firmicutes.[23] The emergence of phylotypes similar to known endospore-forming Firmicutes suggests a potential strategy for survival of the environmental stress induced through the hydraulic fracturing fluids, biocides, and the dynamic aqueous geochemistry.

The emergence of Acidobacterium species in one of three Haynesville shale wells tested is also noteworthy. In this study, 70% of PW sequences in one well were attributable to Acidobacterium, indicating fermentative, acid-producing bacteria (APB) may also thrive in shale plays.[51] APB such as acetate- and lactate-producing bacteria are known to contribute to pipeline corrosion in traditional oil operations. Most gas wells contain three phase (gas, oil, and water) separators at the well head that, if operating properly, should remove the majority of aqueous acid species before they enter transportation pipelines. Therefore, the effect of APB in shale is likely limited to fermentative hydrocarbon degradation within the play. Of the three wells tested, only one contained significant (11%) sequences that could be attributed to *H. congolense*. This discrepancy with previous studies reinforces the need for further characterization of the dynamic microbial ecology during hydraulic fracturing as variation is observed not only between different plays[20,21,48,49,51] but also locally within each play.[51]

Overall, these few studies reveal sharp decreases in diversity between makeup waters and produced water. The presence of acids and biocides in hydraulic fracturing fluid as well as high reservoir temperatures, salinity, pressure, and heavy metal and organic solvents exerts selective pressures for the poly extremophiles that emerge.[54,55] The decrease in microbial diversity over time suggests the need for more targeted (selective) microbial control strategies.

Impoundments. Flowback and produced water from hydraulic fracturing contain a mixture of fracturing fluid additives and dissolved constituents from the formation, including gaseous and liquid fractions that contain organics, metals, and microbes. Impoundments are used to store this wastewater prior to treatment and reuse, or disposal. Microbial communities present in flowback and PW water are introduced to impoundments where evolving biogeochemistry and microbiology drive water management and environmental control strategies. The principal microbial control strategies are biocide addition and mechanical aeration.

Despite the important role that microbes play in influencing the chemical characteristics of produced water impoundments, there are scant data about the geochemistry or microbiology of these unique environments. The single study that exists examined the communities and geochemistry of impoundments that were managed differently.[21] The surface of all impoundments was quite similar, despite different management strategies, dominated (80−91%) by phylotypes most similar to known aerobes and phototrophs in the Alphaproteobacterial clade. The striking dissimilarity was that the community profile was homogenized at all depths in the aerated impoundment and had greatly reduced diversity, while the untreated impoundment was stratified with phylotypes most similar to anoxic and strictly anaerobic bacteria at the middle and bottom clines, respectively. Absent from the aerated impoundment were phylotypes associated with the production of malodorous fermentation products and reduced sulfur, such as Clostridia and Deltaproteobacteria.[21] Conversely, Clostridia were present both in the middle and bottom samples of the untreated impoundment and at all depths in the biocide-amended impoundment.

Sulfidogenic and Fermentative Bacterial Diversity. Sulfate-reducing bacteria (SRB) play a pivotal role in microbially influenced corrosion (MIC) and souring of oil and gas.[56] Common mesophilic sulfidogens often belong to the *Desulfovibrio* genus.[57] However, not all biogenic H_2S is produced by SRB. Sulfidogenic taxa that are incapable of sulfate reduction but instead utilize sulfur, thiosulfate, or sulfite as electron acceptors likely contribute to corrosion.[20,50,53,58] This distinction is important because standard assays that test for the presence of SRB overlook these non-sulfate-reducing sulfidogens,[59] which include ribotypes similar to *H. congolense*, a dominant organism in several studies of produced water from hydraulic fracturing. Thus, SRB tests that are widely utilized in the industry may yield false negatives.

A variety of SRB and other sulfidogens may be present in shale reservoirs.[21,51] Many such bacteria (although not necessarily isolated from shale reservoirs) exhibit a broad substrate range and grow on a variety of carbon sources, such as sugars,[60] amino acids,[61] one-carbon compounds,[62] aromatic hydrocarbons,[63] alkenes,[64] and long chain alkanes.[65] However, most SRB cannot use polymeric organic compounds (e.g., starch and cellulose) directly[66] and normally require simple carbons. Some SRB using sulfate as a terminal electron acceptor may require only H_2 as the sole energy substrate.[66] Although fermentation products such as acetate, formate, and pyruvate have been detected in flowback waters,[67] the roles and effects of fermenters within shale gas reservoirs remain to be systematically explored.

MICROBIAL CONTROL

Microbial control in oil and gas production is commonly enacted by dogmatic practice, by observation, or in response to detection of undesirable microbes. In shale gas production, biocides are commonly added to fracturing fluids, injection water, or produced water during its storage or continuous injection into the vertical portion of the wellbore during production.

Detection and Identification. Commonly used methods of detecting microorganisms include culture-based techniques, biochemical assays,[68] cell activity assays,[69,70] genetic techniques, and microscopy.[58,71,72] The current practice is to utilize culture-based techniques, such as most probable number (MPN) counts. A major disadvantage of these culture-based techniques is that most microbes (>95%) are considered unculturable.[73] Organisms that do not grow are not counted and lead to biased results[73] (Figure 2). Additionally, current culturing procedures used in industry are performed in aerobic environments, while shale reservoirs and shale microbes are anoxic or anaerobic, highlighting the need for the development of an anoxic laboratory testing procedure to prevent further biases. Molecular approaches to examining microbial populations, such as the analysis of 16S rRNA genes, have not been widely adopted by industry and remain largely in the academic research community.[20,21,50] Most of these molecular techniques, including quantitative real-time polymerase chain reaction (PCR) and pyrosequencing, are performed in a culture-independent manner and avoid artifacts associated with culture bias.[74] However, several factors such as amplification bias and variance in 16S rRNA gene copies per genome limit the applicability of next-generation sequencing (NGS) methods in quantifying uncharacterized microorganisms.[75−77] Furthermore, data sets from clone libraries and NGS are not quantitative but qualitatively compositional in nature.[78] While quantitative PCR may accurately quantify individual species, it requires prior knowledge of the target and typically underestimates abundance if used for total 16S rRNA analysis.

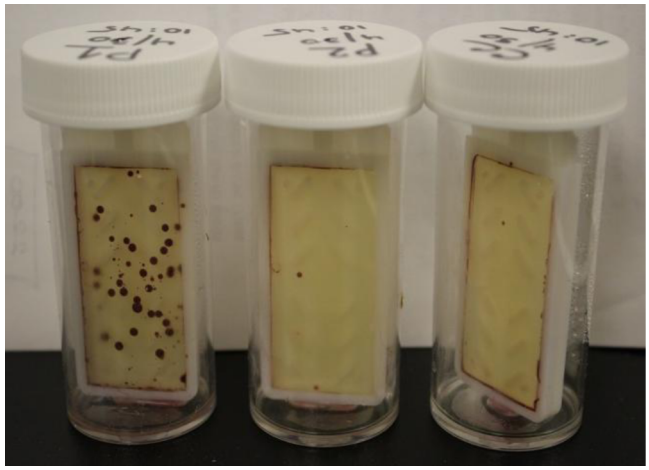

Figure 2. Culture-dependent detection of sulfidogens. Culture-dependent detection assays are often used to detect the presence of sulfidogens. Colony counts are used to determine relative abundances. The vast majority of microbes (>95%) are not culturable, resulting in significant experimental bias as well as a lack of detection of most sulfidogens present. Culture-independent techniques such as pyrosequencing avoid these pitfalls.

Sampling techniques may also create bias. Ideally, samples would be collected from within the reservoir using aseptic techniques. However, most samples are taken from production water at the well heads, separators, or storage tanks that can incur significant microbial contamination bias. The freezing of samples immediately after sampling has been found to preserve microbial diversity, while storage at room temperature hinders accurate community analysis.[79,80] Storage time for frozen samples does not appear to be particularly important;[81,82] however, sample perturbation does appear to be detrimental,[83,84] which has implications for biofilm community analysis. Additionally, for DNA-based methods of detection and enumeration, differences in extraction efficiencies can have significant impacts on results.[80,85]

Biocide Utilization. Biocides are broadly categorized as either oxidizing or nonoxidizing,[86] with nonoxidizing biocides being further classified as either electrophilic, lytic, or protonophoric (uncouplers), based on their mechanism of action.[87] Oxidants and electrophiles are reactive biocides that have multiple cellular targets, while lytic and protonophoric biocides target cellular membranes with the purpose of dissipating the proton motive force so that the cell cannot harvest energy. Each particular biocide class has specific advantages and disadvantages. In general, nonoxidizing biocides are more commonly used for shale reservoir microbial control than oxidizing biocides because of their higher stability and reduced reactivity with fracturing fluid components.[86] They are also less likely to cause equipment corrosion relative to oxidizing biocides. Oxidizing biocides are considered fast-kill, considerably more reactive, and less environmentally persistent. They are also less likely to have resistance to them develop and are effective against a wider spectrum of microorganisms.[86] These characteristics make them more suitable for application in stored water systems.[71,86] Additional information about biocide modes of action, potential environmental fates, and

effects on human health is provided in the Supporting Information.

Factors Affecting Biocide Efficacy. Traditionally, biocide efficacy has been assessed using pure cultures of microorganisms in a planktonic state.[88,89] However, it is now widely recognized that this method is not optimal for predicting biocide performance against sessile cells,[90] which represent the greater fraction of microbial populations in a reservoir. Therefore, although planktonic kill studies are still used by some,[24,91] biofilm monitoring should also be considered when performing biocide assessment.

Another consideration is that standard minimum inhibitory concentration tests, whether conducted using batch cultures or biofilms, test biocides only for their ability to inhibit growth and not for their potential to kill microorganisms.[89] However, this concern can be resolved through the use of "time-kill" tests, which require periodic sampling and enumeration of viable cells.[92] Organic loading was found to increase the minimum inhibitory concentration in all but one biocide (DDAC) tested of seven.[93] Geochemical conditions (e.g., pH, temperature, and O_2) also play a key role in biocide functionality.[94] One of the key factors affecting biocide efficacy is likely poor perfusion and distribution through the reservoir. Biocides are unlikely to reach dead pores or other areas not accessible to the injected fluids. Current biocide application methods during production typically deliver only biocide to the vertical area of the well, missing populations in the horizontal leg. In addition, sampling along the vertical portion of the well has shown significant bacterial counts,[51] suggesting biocide resistance or ineffective biocide delivery. Components of fracturing fluids as well as formation minerals may also influence biocide efficacy by reducing their solution concentration and bioavailability or changing their reactive properties. Therefore, it is recommended to check biocide compatibility with fracturing fluid additives and formation properties.[95]

Microbial Resistance to Biocide. Microorganisms can achieve resistance to biocides in several ways: (1) acquisition of resistance genes from the surroundings, (2) spore formation, (3) biocide inactivation via enzyme catalysis, (4) biocide efflux, (5) modification of phospholipids affecting membrane permeabilization or gene expression changes affecting expression of catalytic transmembrane proteins, and (6) biofilm and EPS growth (Figure 3).[96,97] Numerous studies have demonstrated that biocide efficacy against a single species varies substantially over time, depending on the growth state of the population.[93,98,99] Biocides have also been found to vary seasonally in efficacy, with poorer performance during the summer months.[24] Therefore, it may be worthwhile to increase impoundment biocide loading rates during warmer months.

Once established within the reservoir, as in natural environments, the majority of bacteria likely exist embedded in a biofilm or in microcolonies.[100,101] While the impact that biofilms may have on gas flow in shale reservoirs has not been empirically determined, mathematical models suggest that a biofilm pore volume of 10% would reduce gas flow by one-half.[102]

Biofilm formation is a major determinant of biocide resistance.[93,98,103,104] Some biofilm-associated microbes have been found to resist biocide concentrations approximately 10–20-fold higher than that used to kill planktonic populations.[93,98,105] Others have suggested biofilm resistance is increased up to 1000-fold.[106] There may be several reasons for this, including (1) higher cell densities, (2) the presence of

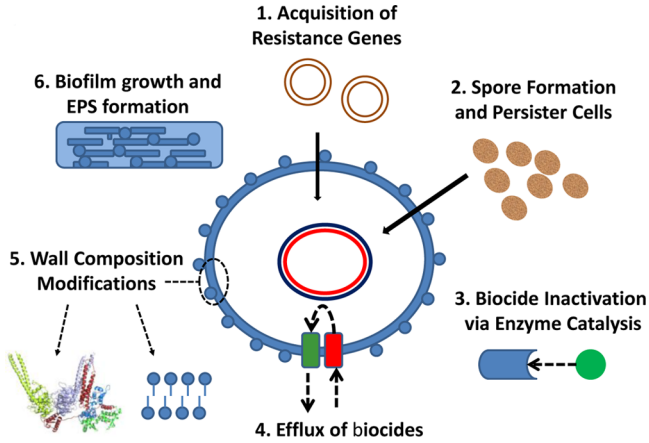

Figure 3. Microbial defense mechanisms against biocides. (1) Acquisition of resistance genes from the surroundings. (2) Spore formation. (3) Biocide inactivation. (4) Biocide efflux. (5) Modification of phospholipids affecting membrane permeabilization or gene expression changes affecting expression of transmembrane proteins. (6) Biofilm and EPS growth.

persister cells (i.e., dormant cells that resist biocides and other stressors), (3) reduced biocide diffusion due to cohesive interactions within the biofilm matrix,[107] (4) low growth rates, and (5) sequestration of positively charged biocides by negatively charged extracellular polymeric substances (EPS).[89,93,98,104] Biofilm heterogeneity also creates locally distinct microenvironments that may differentially affect biocide activity.[98,105,108] Therefore, minimum inhibitory concentration is much higher not only for biocides tested against biofilms (relative to planktonic populations) but also for most mixed cultures (either planktonic or biofilms).[93] Further exacerbating the difficulties of treating biofilms is the presence of persister cells.[104] Persister cells have greatly reduced susceptibility to biocides, although they do not grow or proliferate in their presence. Once the biocide is removed, persister cells may revert back to the nonpersister phenotype to begin rapid proliferation and biofilm deposition, which may explain the decreased recovery time needed for biofilms after biocide exposure,[109] especially when slug doses are utilized.[110] Biocide pulse dosing should be implemented in lieu of slug dosing to minimize persister cell enrichment.

Although we found no reports concerning biocide rotation practices in gas production, biocide rotation has been recommended to decrease the risk of acquisition of resistance in hospital settings,[111,112] and for the control of *Legionella* in cooling towers.[113,114] This strategy may therefore be relevant for controlling difficult-to-treat populations, such as persisters and spore formers, in shale gas production.

Competitive Exclusion for Microbial Control. Competitive exclusion dictates that two species that compete for the same nutrients cannot coexist in the same location when one has even a slight advantage. Aeration of produced water is a form of competitive exclusion in which the provision of oxygen as a more thermodynamically favorable electron acceptor precludes reduction of sulfate and sulfur. Addition of nitrate to injection water may also provide a thermodynamic competitive advantage to organisms that grow by denitrification or nitrate reduction, or to sulfide-oxidizing bacteria that outcompete sulfidogens. In laboratory studies, adding nitrate as a terminal electron acceptor reduced sulfide production as well as sulfidogen abundance.[115,116] In field studies, nitrate injection

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has seen mixed success,[117−125] and its benefits at the field scale are not always observed.[2]

Nitrite was an effective inhibitor of biogenic H_2S and SRB growth in model systems and some field studies.[116,126,127] All SRB use the enzyme DsrAB for dissimilatory sulfite reduction, which binds and is inhibited by nitrite.[126] Nitrite amendment was more effective than (the common biocide) glutaraldehyde at inhibiting souring in one study, and recovery of SRB was further delayed.[128] Nitrite has also been used as an H_2S scavenger. In one field study, oil production immediately increased following nitrite treatment.[123] This study also tested the efficacy of nitrite injection in a gas well that was producing 30−50 ppm of H_2S. Sampling immediately after a 36 h shut-in period detected no H_2S and no culturable sulfidogens in the produced water. The effect appears to be long-lasting, with the level of gas phase H_2S remaining below 5 ppm for 7 months and that of sulfidogens below the detection limit for 3 months. Nevertheless, some sulfidogens produce NrfHA, a cytochrome *c* nitrite reductase that endows resistance to nitrite inhibition up to millimolar concentrations.[126]

■ IMPLICATIONS

Environmental scientists and engineers are frequently tasked with controlling uncharacterized environments, such as those in shale gas production. At present, the few studies of the microbiology of hydraulically fractured environments point to a dynamic shifting community structure while also revealing shortcomings in microbial control. The emerging use of produced water as makeup for subsequent hydraulic fracturing fluid may inadvertently enrich resistant and well-adapted microbes, but limited information about these populations hinders the improvement of microbial control. Strategies for control should be made on a scientific basis, rather than a dogmatic one. The universal, heavy-handed application of biocides to all hydraulically fractured wells is one such area that should be reconsidered. An improved understanding of the microbial ecology of gas reservoirs, as well as isotopic H_2S source analyses, is needed to optimize microbial control and reduce environmental and financial burdens associated with *ad hoc* (and marginally effective) misuse and overuse of biocides. Accordingly, biocide rotation, pulse dosing, and seasonal loading adjustments should be considered to enhance microbial control efficacy.

■ ASSOCIATED CONTENT

Supporting Information

Expanded discussion of biocides commonly used in shale gas production. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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