Science of the Total Environment 644 (2018) 183–192

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Succession of toxicity and microbiota in hydraulic fracturing flowback and produced water in the Denver–Julesburg Basin

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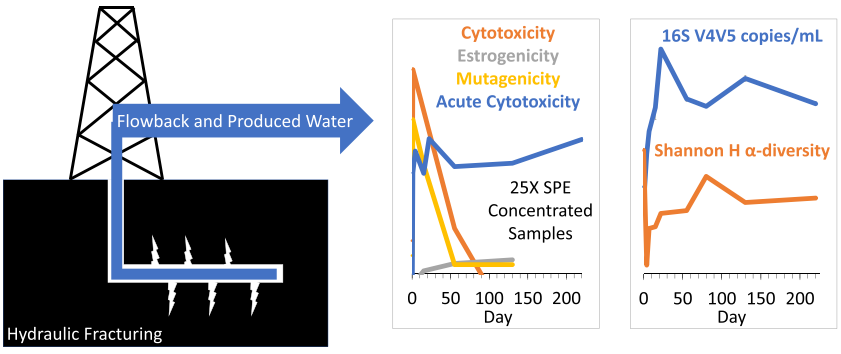
HIGHLIGHTS

- Horizontal drilling and hydraulic fracturing generate flowback and produced water (FPW).
- FPW toxicity and microbiota were characterized for 220 days in the Denver–Julesburg Basin.
- Temporal trends were similar between FPW toxicity and microbial communities.
- Fracking conditions are toxic and selective with long term ecological & industrial impacts.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 April 2018
Received in revised form 5 June 2018
Accepted 6 June 2018
Available online xxxx

Editor: Frederic Coulon

Keywords:
Fracking
In vitro bioassay
Flowback
Produced water
16S rRNA gene amplicon sequencing

ABSTRACT

Hydraulic fracturing flowback and produced water (FPW) samples were analyzed for toxicity and microbiome characterization over 220 days for a horizontally drilled well in the Denver-Julesberg (DJ) Basin in Colorado. Cytotoxicity, mutagenicity, and estrogenicity of FPW were measured via the BioLuminescence Inhibition Assay (BLIA), Ames II mutagenicity assay (AMES), and Yeast Estrogen Screen (YES). Raw FPW stimulated bacteria in BLIA, but were cytotoxic to yeast in YES. Filtered FPW stimulated cell growth in both BLIA and YES. Concentrating 25× by solid phase extraction (SPE) revealed significant toxicity throughout well production by BLIA, toxicity during the first 55 days of flowback by YES, and mutagenicity by AMES. The selective pressures of fracturing conditions (including toxicity) affected bacterial and archaeal communities, which were characterized by 16S rRNA gene V4V5 region sequencing. Conditions selected for thermophilic, anaerobic, halophilic bacteria and methanogenic archaea from the groundwater used for fracturing fluid, and from the native shale community. Trends in toxicity echoed the microbial community, which indicated distinct stages of early flowback water, a transition stage, and produced water. Biota in another sampled DJ Basin horizontal well resembled similarly aged samples from this well. However, microbial signatures were unique compared to samples from DJ Basin vertical wells, and wells from other basins. These data can inform treatability, reuse, and management decisions specific to the DJ Basin to minimize adverse environmental health and well production outcomes.

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1. Introduction

Technologies for horizontal drilling and hydraulic fracturing have enabled access to previously cost-prohibitive shale deposits, leading to

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https://doi.org/10.1016/j.scitotenv.2018.06.067
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rapid expansion of the oil and gas industry in the past 20 years (Norris et al., 2016; Vidic et al., 2013). Fracturing fluids utilize 10 to 15 million liters of source water (~90%), sand (~9%) and additives (~1% including surfactants, biocides, friction reducers, gelling agents, etc.) to initiate, elongate, and prop open shale fissures for the extraction of oil and/or gas (Stringfellow et al., 2014; Malakoff, 2014; Goodwin et al., 2014; Lipus et al., 2017; Mohan et al., 2014; Vengosh et al., 2014; Mouser et al., 2016a). Some of the injected fracturing fluid initially returns to the surface as flowback, along with shale formation brine containing oil and gas. Over time, flowback transitions to produced water which closely resembles formation brine. Wells produce 1.7–14.3 million liters of flowback and produced water (FPW) over the first 5–10 years, where the majority originates from the shale formation and half the volume is produced in the first 6 months (Kondash et al., 2017). These large volumes of FPW are complex and difficult to treat wastewaters, and have been the focus of many recent characterization and treatability investigations (Stringfellow et al., 2014; Lester et al., 2015; Rosenblum et al., 2017a; Camarillo et al., 2016; Stringfellow et al., 2017; Sutra et al., 2017; Rosenblum et al., 2016; He et al., 2017a; Mouser et al., 2016b; Thurman et al., 2014; Rosenblum et al., 2017b). The potential FPW environmental health impacts, including toxicity and impacts on microbial ecology, must be understood and mitigated to promote sustainable management, treatment, and reuse.

The potential routes of exposure and impacts of hydraulic fracturing on human health and the environment have recently been reviewed (Shrestha et al., 2017; Mayer, 2016; Kassotis et al., 2015; Annevelink et al., 2016; Torres et al., 2016; Kahrilas et al., 2015; McLaughlin et al., 2016; Chen, 2016; Chen and Carter, 2017; Konkel, 2016). The greatest risks have been summarized as those to water quantity, and both water and air quality (Vengosh et al., 2014; Awal and Fares, 2016). Many in silico toxicity evaluations conclude that most fracturing fluid and FPW chemicals lack toxicity data (Stringfellow et al., 2017; Elliott et al., 2017; Michalski and Ficek, 2016; Gordalla et al., 2013; Webb et al., 2014), while chemicals that have data are known to cause reproductive, developmental, and chronic oral toxicity (Elliott et al., 2017; Yost et al., 2016). Epidemiological studies associate proximity to drilling operations with adverse reproductive and developmental outcomes (Konkel, 2016; McKenzie et al., 2014). In vitro studies using bacteria, rat, zebrafish, and human cells have demonstrated stress responses at sub-toxic doses, cytotoxicity, and endocrine disruption caused by fracturing fluid chemicals (including biocides and synergistic toxicity of their mixtures), fracturing fluid, FPW, and surface water collected near an injection well disposal facility (Christen et al., 2017; Ishikawa et al., 2016; Payne et al., 2015; Chen et al., 2017; Kassotis et al., 2014; Kassotis et al., 2016a). In vivo studies using fish, crustaceans, and mice have demonstrated sublethal toxicity, developmental toxicity, reproductive effects, endocrine disruption, oxidative stress, changes in metabolic activity, and changes in gill morphology caused by fracturing fluid chemicals (including biocides and synergistic toxicity of their mixtures) at concentrations reported in drinking water sources (He et al., 2017a; Christen et al., 2017; Blewett et al., 2017; Kassotis et al., 2016b; He et al., 2017b).

Several recent studies have used nucleic acid sequencing to study microbial communities in fracturing fluids, FPW, impoundments, and in environmental matrices impacted by spills or disposal of wastewaters in the Marcellus, Antrim, Barnett, and Utica shales (Lipus et al., 2017; Mohan et al., 2014; Fahrenfeld et al., 2017; Lipus et al., 2016; Wuchter et al., 2013; Daly et al., 2016; Gaspar et al., 2014; Osborn et al., 2011; Liang et al., 2016; Mohan et al., 2013a; Mohan et al., 2013b; Cluff et al., 2014; Struchtemeyer and Elshahed, 2012; Struchtemeyer et al., 2011). Many of these have been reviewed (Gaspar et al., 2014) and incorporated into a meta-analysis of fracturing and the microbiota of the deep subsurface (Mouser et al., 2016a). Emerging generalities for other shale plays indicate rapid succession of high-diversity source water communities into low-diversity, halotolerant, thermotolerant, anaerobic FPW communities indicated to originate from both shale formation and source waters. These communities can play a role in adverse microbial outcomes including souring, plugging, and corrosion that can decrease well production and profitability. Genera of the Class Clostridia capable of sulfide production (souring) have been most commonly observed, including *Halanerobium* and *Thermoanaerobacter* (Lipus et al., 2017; Daly et al., 2016; Liang et al., 2016; Mohan et al., 2013a; Mohan et al., 2013b; Cluff et al., 2014). Shotgun metagenomic analyses of *Halanaerobium* spp. from one well revealed capabilities such as acid production, thiosulfate reduction, and biofilm formation that could contribute to corrosion and souring (Lipus et al., 2017). Further, *Halanaerobium* spp. can degrade guar gum to acetate and sulfide (Liang et al., 2016). In addition to genes for methanogenesis that could enhance well production, stress response genes that could allow organisms to survive and thrive including those for osmoprotection, dormancy, and sporulation have also been observed in FPW (Mohan et al., 2014; Fahrenfeld et al., 2017; Lipus et al., 2016; Daly et al., 2016).

Although these studies and reviews have made significant advancements, no long-term toxicity and sequence-based microbiology study has been reported for FPW of a hydraulically fractured well of the Niobrara formation in the DJ Basin (NE Colorado). Only one short-term (64 day) microbial characterization has been reported for a well in this basin (Oetjen et al., 2018). This presents a significant knowledge gap because FPW geochemistry in the DJ basin is much different than other shale basins, with lower concentrations of dissolved solids, ions, and metals but greater concentrations of dissolved organics (Lester et al., 2015; Osborn et al., 2011; Cluff et al., 2014; Warner et al., 2013). Our study coupled a long-term (220 day) time series characterization of bacterial and archaeal communities with in vitro toxicity bioassays to determine the potential ecological and environmental health impacts of these DJ Basin FPWs. These data were analyzed in the context of organic and inorganic characteristics previously described for this well (Rosenblum et al., 2017a; Rosenblum et al., 2017b), and compared to microbial communities in single samples collected anonymously from other wells (both horizontally and vertically drilled) at various stages of production in the DJ Basin. This information can be used to guide industrial production, treatment, and reuse practices in the DJ Basin and others.

2. Methods

2.1. Sample collection and processing

Time-series samples were collected from a single horizontally-drilled well employing gel-based hydraulic fracturing fluid in the Niobrara shale of the Wattenberg field in Weld County, CO (DJ Basin). Sample collection procedures and temporal characterization of organic and inorganic fractions of these samples have been described previously (Rosenblum et al., 2017a; Rosenblum et al., 2017b). Samples were collected from the groundwater used to generate fracturing fluid (Day 0) without any chemical additions, and longitudinally (on Days 1, 4, 7, 15, 22, 55, 80, 130, and 220) from the first day flowback began in January 2015 (Day 1) until September 2015 (Day 220). Individual samples described previously (Rosenblum et al., 2016) were also collected in the DJ Basin from other well pads at various stages. Samples VF1, VF2, and VF3 were collected from vertically drilled wells that were ≥2 years old (VF1, VF2) or refractured >100 days prior to sampling (VF3). Sample HF was collected from a horizontally drilled well approximately 30 days after flowback began. Samples were collected in burned amber bottles for toxicity assays or in sterile 1L HDPE bottles (Nalgene) for microbial community analyses from the on-site separator, except for Day 1 which was collected directly from a flowback tank, and stored at 4 °C with no headspace until analyses.

2.2. Toxicity assays

Samples and their dilutions were assayed for toxicity as received, after 0.45 μm filtration (Supor, Acrodisc), and after concentration by

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