

RESEARCH ARTICLE

Biomolecular Engineering, Bioengineering, Biochemicals, Biofuels, and Food

Top-down enrichment of oil field microbiomes to limit souring and control oil composition during extraction operations

Ethan T. Hillman^{1,2}  | Louis Edwards Caceres-Martinez³  | Gozdem Kilaz³  | Kevin V. Solomon^{1,2,4} 

¹Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, Indiana, USA

²Purdue University Interdisciplinary Life Sciences, Purdue University, West Lafayette, Indiana, USA

³School of Engineering Technology, Fuel Laboratory of Renewable Energy (FLORE), Purdue University, West Lafayette, Indiana, USA

⁴Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, Delaware, USA

Correspondence

Kevin V. Solomon, Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, DE 19716, USA.
Email: kvs@udel.edu

Present address

Ethan T. Hillman, Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109, USA.

Funding information

Pioneer Oil; Purdue Enhanced Oil Recovery Lab

Abstract

Microbial processes sour oil, corrode equipment, and degrade hydrocarbons at an annual global cost to the oil and gas industry of nearly \$2 billion. However, top-down control of these microbial processes can reduce their damage and enhance oil recovery. Here, we screened microbial communities from five oil wells in the Illinois basin and evaluated nutrient injection strategies to control metabolism and community composition. Molasses with molybdate supplementation stimulated gas and organic acid production while suppressing corrosive H₂S formation in samples from two wells. These changes were accompanied with significant reshaping of the microbiome community. Simulations of field operations via a lab-scale mini-coreflood validated that oil well microbiomes can be engineered to inhibit deleterious H₂S and shape oil hydrocarbon composition *in situ*. These pilot studies validate the economic potential and sustainability of top-down approaches for microbiome engineering to control microbes in oil extraction and enhance the economic viability of oil recovery.

KEYWORDS

comprehensive 2D-gas chromatography, cultivation screening, microbial enhanced oil recovery, microbiome engineering, miniature coreflood, top-down design

1 | BACKGROUND

Fossil fuels are likely to remain a significant energy source for at least the next 30 years¹ as economies transition globally toward more renewable energy sources.² Extracting trapped oil from aging wells can not only increase the overall production of existing wells but also minimize the need to drill new wells and, ultimately, decrease the cost

of extracting oil from existing reservoirs.³ Expensive polymer and/or surfactant/polymer formulations that require large amounts of funding and research effort are used to extract oil not recovered by primary extraction techniques.⁴ Additionally, native and exogenous microbes have been harnessed as a more cost-effective⁵ alternative for secondary and tertiary oil recovery—an approach coined microbial enhanced oil recovery (MEOR).⁶ However, microbial activity in oil wells, can also be disruptive to oil recovery and/or alter its composition.⁶ For example, microbes can degrade and sour oil, and produce metabolites that corrode the well casing, flowlines, and pipelines.⁷ Globally, pipeline corrosion alone can result in nearly \$2 billion (USD)

Abbreviations: FID, flame ionization detector; GC × GC, comprehensive two-dimensional gas chromatography; H₂S, hydrogen sulfide; MCF, mini-coreflood; MEOR, microbial enhanced oil recovery; OTU, operational taxonomic unit; PCoA, principal coordinate analysis; PCR, polymerase chain reaction.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](https://creativecommons.org/licenses/by-nc/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2022 The Authors. *AIChE Journal* published by Wiley Periodicals LLC on behalf of American Institute of Chemical Engineers.

of damage and loss each year.⁸ This corrosion is due primarily to the oxidation of microbially produced hydrogen sulfide (H₂S) to sulfuric acid.⁹ Thus, engineering the composition and metabolism of oil well microbial communities promises to enhance the productivity and economic viability of oil extraction operations.

The microbial community composition of oil wells is generally quite variable from well to well, even when the wells are geographically close. These communities typically consist of *Bacillota* (Firmicutes), *Bacteroidota* (Bacteroides), *Pseudomonadota* (Proteobacteria), *Actinomycetota* (Actinomycetes), *Thermotogota*, *Campylobacterota*, and bacteria belonging to other phyla as well as archaea.^{10–12} The exact parameters that determine the community composition are not known but temperature, pH, salinity, and nutrient availability are known to have significant influences on the microbes that thrive in a given oil well.^{13,14} While oil wells vary greatly in microbial composition, the members of oil wells tend to have similar genomic capabilities. Specifically, oil well communities tend to have the pathways for methanogenesis, acetogenesis, sulf oxidation, denitrification, hydrocarbon degradation, sulfate reduction, nitrate reduction, and biosurfactant production among others.^{11,15} While the metabolic activity of microbes likely has the largest effect on oil recovery, biomass formation can plug pores in/between the rock formation of petroleum reservoirs to increase the well pressure-enhancing recovery.¹⁶ Trapped oil can be liberated by microbial metabolites: gases that displace immobile oil, organic acids that dissolve carbonaceous deposits and increase well permeability, solvents that dissolve and mobilize large hydrocarbons from the pores, and biosurfactants that act as emulsifiers.^{6,17} While some hydrocarbon degradation, such as the cleavage of sidechains, can be advantageous for some extraction processes, other oil recovery may target long chain hydrocarbons and, thus, may seek to inhibit any degradation.^{13,14} Similarly, the production of gas leads to an increased pressure that can force more oil out of the wells. However, if the produced gas is hydrogen sulfide, it can actually lead to less recovery of usable oil because extra processing is required to clean up this soured oil. Therefore, the ideal MEOR formulation would enrich for microbes that maximize oil produced through the production of acids and gases while inhibiting sulfate-reducing bacteria to minimize H₂S production and control the degradation of hydrocarbons by various microbes.

Microbiome engineering has emerged as a sustainable approach to control microbial processes for a number of industries from health and nutrition¹⁸ to agriculture¹⁹ and fuels.²⁰ To engineer microbial communities, there are two common general approaches: bottom-up and top-down.²¹ Generally, bottom-up design in microbiome engineering pertains to constructing communities of specific microbial species and strains with desired attributes and synergies to carry out a task or set of tasks. In MEOR, bottom-up approaches often focus on biofilm or surfactant production via communities centered around natural and/or engineered strains of *Pseudomonas*, *Bacillus*, and *Enterobacter* which are able to extract up to 26% of the additional trapped oil.^{6,22–25} However, the ecology of engineering communities using bottom-up designs is very complex and developing stable communities that colonize a natural, fluid ecosystem, like that of the oil well

microbiome, is exceedingly difficult.²⁶ Strains may fail to colonize because they do not fill a particular ecological niche in the community and can change the native microbiome composition in unpredicted and uncontrollable ways.²⁷ Emerging bottom-up strategies to overcome this challenge such as artificial syntrophy where microbes exchange metabolites for mutual survival²⁸ are challenging to develop in sustainable ways and can fail catastrophically if a single species is lost due to unanticipated competition with native microbes.²⁹ Lastly, strains used in bottom-up approaches are often genetically engineered, which raises both ecological concerns and creates regulatory burden related to the introduction of genetically modified organisms in the environment.^{19,30}

In contrast, top-down strategies for microbiome engineering manipulate environmental factors such as nutrients, pH, temperature, and ionic strength to tailor a native community for a desired outcome or task.²¹ This strategy does not require any bacterial species to be introduced into the community or colonize a new environment but instead leverages the present microbes.^{26,31} Tuning environmental factors is typically more cost-effective and is easily testable in controlled parallel experiments.³² Many micronutrients and carbon sources used in top-down approaches in MEOR are often much more economical than the synthetic polymers used for standard secondary and tertiary oil recovery.^{33,34} However, not all microbiomes will respond to changes in these environmental factors and are likely to have different responses depending on the composition of microbes, their viability, and the surrounding environmental factors.³⁵ Minerals such as chloride, ammonium, phosphate, nitrate, and molybdate have been used in MEOR trials to adjust their salinity,³⁶ provide essential CHNOPS elements,^{37,38} enrich for nitrate-reducing microbes,⁹ or inhibit sulfate-reducing microbes,³⁹ respectively. Up to 89% of MEOR trials have successfully produced additional oil but to varying degrees.^{35,40} Little, however, is known about the ecological progression through these enrichments and how they affect the composition of the recovered oil. Therefore, conditions for top-down approaches to microbiome engineering must be rapidly screened *in vitro* in a high-throughput, cost-effective manner to identify both candidate oil well communities and optimal MEOR intervention conditions. Taken together, the facts that these top-down approaches are cost-effective, can be rapidly screened, and do not require scientific ecological barriers suggest that this is an attractive strategy for developing MEOR formulations for field trials.

In this study, we use top-down strategies to screen wells and optimize oil extraction operations for the Illinois basin via MEOR. First, we evaluated nutrient sources that would stimulate the growth of microbes native to the oil field through *in vitro* cultivation, in order to identify suitable conditions and responsive wells with viable microbial populations for MEOR intervention. Next, we used top-down designs to evaluate molasses formulations coupled with inorganic salt solutions that prohibit or stimulate microbial activity to find one that reduced H₂S production and stimulated desired gas or organic acid production. Simulation of field recovery operations via a miniature coreflood experiment confirmed the ability of these interventions to reduce oil souring and also modify the specific hydrocarbon

TABLE 1 Characteristics of candidate wells from the Illinois basin

Well ID	State	Depth (ft)	Temp (°C)	pH
B	IL	1597–1626	27	7
C	IL	1585–1590	27	6.5
D	IL	1336–1343	27	7
G	IL	1620–1626	27	6
S	IN	2226–2258	27	6.5

composition of produced oil. Ultimately, this work demonstrates that top-down approaches to microbiome engineering can significantly benefit oil recovery operations while improving economic and environmental sustainability.

2 | RESULTS AND DISCUSSION

2.1 | Wells within the same basin have unique properties and microbial communities

Wells at mesophilic temperatures and moderate depths (1500–3000 ft, 457–914 m) are promising sites for successful MEOR applications^{35,40,41} within the Illinois basin. We selected five such wells from this basin that were all approximately 27°C (80°F) in order to hold the temperature constant between trials and simplify cross-well comparisons (Table 1). The wells had depths of 1336–2258 ft (407–688 m) while the pH remained fairly neutral (pH 6–7). DNA was extracted from the produced water (effluent) of each well to assess the native microbial community composition via 16 S rRNA gene profiling (Figure S1). We found that each well had a distinct microbial composition with various sulfur-reducing (or H₂S-producing) bacteria as well as other archaea and bacteria found in previous MEOR studies.^{17,41} Common bacteria phyla include Proteobacteria, Bacteroidota, Firmicutes, Synergistota, Spirochaetota, Verrucomicrobiota, Desulfobacterota, and Campylobacterota, of which the latter three are prominent sulfur-metabolizing bacteria.^{42–45} These populations were distinct from others in the literature,^{13,37} a finding that is not unexpected given that each community is driven by an environmental conditions such as temperature, salinity, pH, nutrient availability, and oil gravity among other factors.²⁵ However, there is no known correlation between native microbial community and MEOR success. Therefore, we pursued *in vitro* functional screening to evaluate candidate wells for MEOR microbiome engineering with top-down designs.

2.2 | *In vitro* screening identifies candidate wells and nutrient profiles for MEOR

In order to evaluate which candidate wells would respond to nutrient supplementation, *in vitro* microcosms were created in an anaerobic environment using a mixture of nine-parts oil well effluent and one-part crude nutrient source stock. We evaluated molasses and corn

syrup as carbon sources as they are common choices for MEOR,³⁸ are economical byproducts of local industry that can be readily sourced from the region, and may contain minerals and other vitamins needed to support microbial growth.⁴⁶ Target outputs for this top-down approach were increased signs of metabolic activity relative to unsupplemented controls that may enhance oil recovery. Specifically, we monitored over the course of a week the microbial production of gases (increased headspace pressure) that can help mobilize oil deposits and organic acids (pH reduction) that can dissolve carbonaceous deposits/widen pore throats to facilitate oil flow (Figure 1). Microbiomes from wells D and G responded strongly to both molasses and corn syrup supplementation. However, molasses supplementation was more robust for communities from both wells with up to 90 kPa of generated pressure and 2 pH-unit decreases in culture pH for those from Well G. Communities from Well S also responded favorably to carbon supplementation; however, the pressure generation response was noisy. Communities from Wells B and C did not respond to any nutrient supplementation and may indicate that the environments of these wells are deficient in some other nutrient or need different cultivation parameters. These findings are consistent with other biostimulation³⁷ results and further support the notion that not all wells respond to nutrient supplementation and may respond in different ways (e.g., more acids than gases or vice versa). Overall, the cultures grown on molasses, a commonly used nutrient source in MEOR,¹⁷ produced more pressure and had larger pH drops suggesting that it had stronger microbe-activating potential for MEOR. Therefore, molasses was chosen as the crude nutrient source for our subsequent screening efforts. Bacteria from the orders Lactobacillales, Enterobacterales, Bacteroidales, and Campylobacterales dominated the molasses microcosms from Wells D and G comprising ≥96% of the total reads (Figure S2). These bacteria are collectively known to produce CO₂ and H₂ gases as well as acetic, lactic, butyric, and propionic acids that have roles in MEOR.^{17,25,41} The increasing pressure and drop in pH imply that the production of some or all of these was stimulated by nutrient supplementation. However, production of H₂S by Campylobacteria, such as *Sulfurospirillum* and *Malaciobacter*,^{43,47} may have also been stimulated. While our results indicated at least two responsive wells for MEOR intervention, successful MEOR biostimulation requires limited production of corrosive H₂S while also increasing the gas, acid, and/or solvent production for oil recovery.

2.3 | Mineral supplements control metabolism and shape microbiome composition *in vitro*

To investigate if minerals can modulate the microbiome composition of the microcosms in MEOR top-down designs, our *in vitro* screening system was used to track metabolism and community composition under various mineral supplements. *In vitro* screening focused on Well D because it was one of the two most responsive wells. Moreover, Well G was heavily dominated by one genus (*Malaciobacter*, class Campylobacteria) (Figure S1), with less microbial diversity to adapt to the micronutrient conditions. In the second top-down evaluation, Well

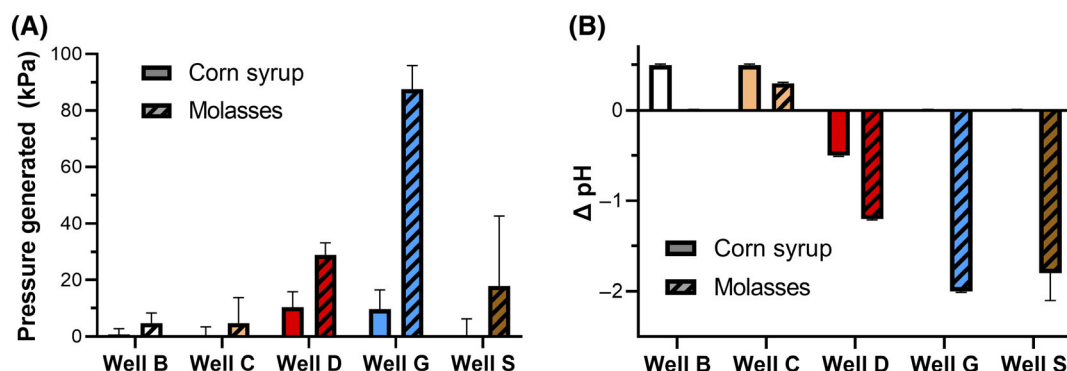


FIGURE 1 Carbon source screening of well candidates. (A) Accumulated pressure generated from microcosms over the 7-day span. (B) Change in pH in microcosms over the 7-day experiment. Carbon sources screens: corn syrup (solid bars) and molasses (stripped bars). Error bars represent standard deviation, $n = 3$.

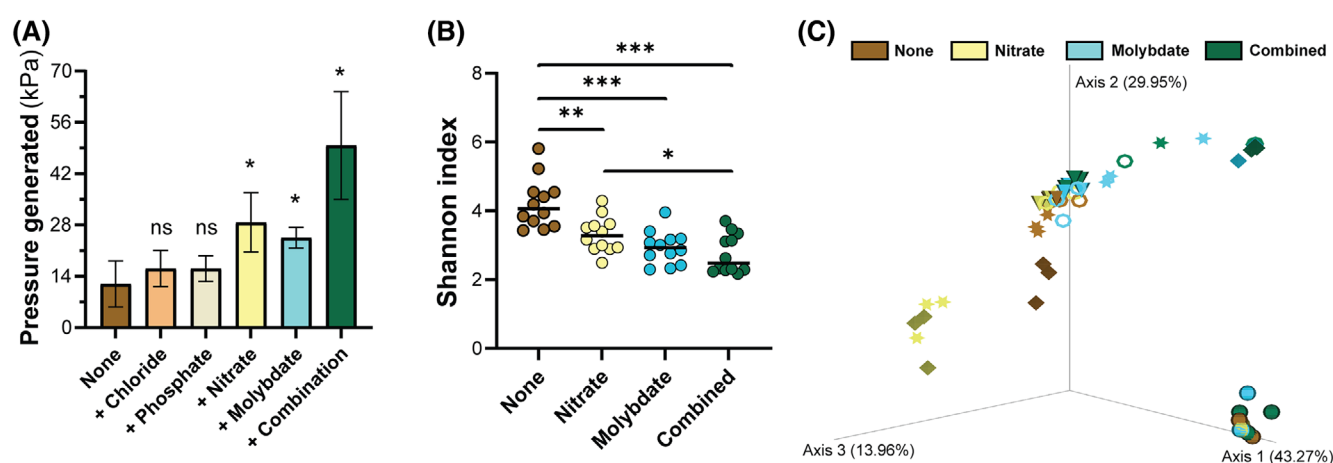


FIGURE 2 Non-essential nutrients stimulate metabolism and manipulate the microbiome composition. (A) Pressure generated in microcosms from Well D when treated with various nutrient supplements; T-tests performed with respect to none. (B) Diversity score (Pielou's evenness) of microcosms after treatment with minerals (Days 3, 4, 5, and 7). (C) Unweighted Unifrac PCoA plot of Well D microcosms after mineral supplementation; shapes represent days, colors indicate treatment group: filled circles = Day 1, diamonds = Day 3, star = Day 4, rings = Day 5, triangles = Day 7. Error bars represent standard deviation, $n = 3$; * $p < 0.05$, ** $p < 0.005$, and *** $p < 0.001$.

D was stimulated with molasses input supplemented with minerals including the salts chloride (NaCl and KCl), phosphate (K_2HPO_3 and KH_2PO_3), nitrate (KNO_3), molybdate (Na_2MoO_4), and a combination of both nitrate and molybdate. Chloride salts are not essential nor do they stimulate any specific microbial activity; it was evaluated as a negative (ionic strength) control. Microbial growth is frequently phosphorus-limited as one of the essential CHNOPS elements and can be supplemented to enhance growth and MEOR outcomes.^{37,38,41} To inhibit sulfate-reducing activity, nitrate and molybdate salts were tested. Nitrate is a competing electron acceptor in place of sulfate in several sulfur-reducing bacteria,^{9,48} while molybdate has been shown to inhibit H_2S generation and sulfate-reducing bacteria in different aqueous and marine contexts.^{39,49,50} Target outputs included increased headspace pressure, decreased pH, decreased headspace H_2S and suppression of growth of sulfur-reducing bacteria as measured by microbiome composition analysis.

Gas and organic acid production varied strongly as a function of mineral supplementation (Figure 2A, Figure S3). Nitrate- and molybdate-supplemented communities from Well D produced significant amounts of pressure. The cumulative effect of combination treatment was additive, resulting in the most observed pressure generation. Similarly, nitrate-, molybdate- and combination-supplemented communities have the largest decreases in pH relative to unsupplemented controls (Figure S3B). Surprisingly, nitrate supplementation reduced culture pH the most (by over 3 pH units); nitrate reduction or denitrification consumes protons and is expected to increase culture pH. However, the vigorous growth of denitrifiers may stimulate the growth of other microbes that enhance their organic acid production to offsets this pH increase. Under these conditions, chloride and phosphorous do not alter microbial metabolism in the microcosms while nitrate best stimulated the production of gases and acids for MEOR. There was some additional benefit to adding molybdate in combination with nitrate as it generated the most gas and one of the larger pH

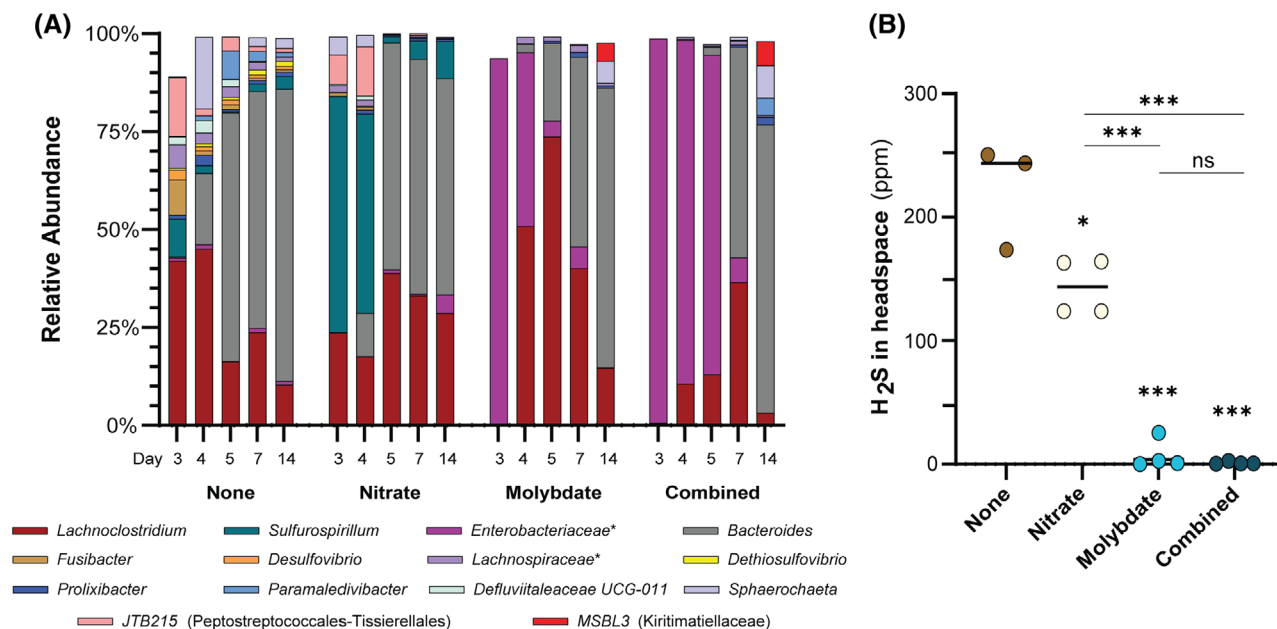


FIGURE 3 Top-down design of nutrient treatments inhibit and enrich specific microbes and their metabolism. (A) Microbiome composition of microcosms with and without treatment across days 3–14. (B) H₂S headspace measurements of microcosms after 14 days of treatment, T-tests performed with respect to none except where indicated. Error bars represent standard deviation, $n = 4$ (except for “None”, $n = 3$); * $p < 0.05$, ** $p < 0.005$, and *** $p < 0.001$. *Enterobacteriaceae** and *Lachnospiraceae** indicate unspecified genera within these two families.

changes. Because only select microbes can convert nitrate into N₂ and/or ammonia, or use it as a nitrogen source for the synthesis of DNA and amino acids, supplemented nitrate will confer a competitive advantage to specific microbes in an otherwise nitrogen-limited environment such as an oil well. In turn, we expect that the differences in the metabolic outputs (Figure 2A) are reflective of the differences created in the microbial community composition as a result of the added compounds.

While the fate of nitrate is ultimately determined by the microbial community, we found that the addition of nitrate and molybdate shifted the microbial community composition. Nitrate, molybdate, and the nitrate-molybdate combination treatments provide exogenous pressures that select for or enrich specific species. All three of these treatments were found to reduce the number and types of species (i.e., the alpha diversity as measured by the Shannon index) in the microcosms from Well D with the combination having the lowest alpha diversity score (Figure 2B). However, the treatments do not enrich or select against the same species, which is evident by the differences in their beta diversity scores, a metric of microbial diversity that is shared between communities, as depicted on the principal coordinate analysis (PCoA) plot of the 16S rRNA gene composition (Figure 2C). The PCoA plot reveals that on Days 3 and 4 the nitrate communities cluster along Axis 3 while the molybdate communities cluster along Axis 1, thus indicating that the nitrate and molybdate treatments select for different populations of microbes. Interestingly, the combination treatment communities are more similar to the molybdate treatment suggesting that the selection/enrichment power of molybdate is greater than that of nitrate (Figure 2C). This finding that the communities are more sensitive to molybdate than nitrate is

in agreement with the literature where hydrogen sulfide production is more inhibited by molybdate than nitrate,^{49,50} adding further support for the strong selective pressure of molybdate. Moreover, these communities diverge over the first 3 days (filled circles to diamonds) as a result of their different selective pressures but ultimately converge by the last time point (triangles) likely as a result of nutrient depletion that would select for efficient scavenging organisms regardless of pre-existing selection conditions.

To determine which taxa were specifically selected against and enriched in the stimulated microcosms, we evaluated the 16S rRNA gene composition of the microbial community. Interestingly, we found that after Day 1 of cultivation, there was little effect across the treatments with only a slight increase in the *Enterobacteriaceae* in the molybdate and combined treatments (Figure S4). After Day 3, however, the treatments varied noticeably from the untreated microcosms (Figure 3A). For example, nitrate microcosms saw a bloom of nitrate-reducing *Sulfurospirillum* on Days 3 and 4, while the molybdate and combined treatments each saw a bloom in *Enterobacteriaceae* sp. and *Lachnoclostridium* sp., which produce acids and gases relevant to enhancing oil recovery.^{8,17,37} *Sulfurospirillum* are sulfur-oxidizing bacteria that can outcompete sulfate-reducing bacteria for sulfate in the presence of nitrate, thereby reducing H₂S production.^{9,46} Similarly, a few *Enterobacteriaceae* have been found to reduce molybdate,^{51,52} which may have provided a similar competitive advantage to these microbes. At the same time, molybdate is structurally similar to sulfate and inhibits the growth of H₂S producers,⁵⁰ which may account for the absence of *Desulfovibrio*, *Dethiosulfobivrio*, *Sulfurospirillum*, and other sulfate-reducing bacteria in the molybdate and combined treatment microcosms. After 7 days, the cultures primarily consisted of

members of *Bacteroides* and *Lachnospirillum* regardless of treatment suggesting that the community may have shifted to scavenge or use other nutrient sources to survive. Similar progressions of microcosms toward primarily Bacteroidetes and Clostridia have been seen in previous studies³⁷ suggesting these groups of bacteria have the advantage over other organisms in anaerobic consortia when the initial nutrients have been depleted. Additionally, no known sulfate-reducing bacteria were detected with molybdate and combination treatments suggesting that the effects of the molybdate were effective over the whole time-course of the cultivation. In contrast, *Sulfurospirillum* species were present only at several timepoints in the nitrate microcosms. In a previous H₂S inhibition study, molybdate was also found to be the strongest inhibitor of sulfate-reducing bacteria⁵⁰ from marine enrichment cultures. Where previous findings suggested that the addition of these inhibitors had no effect on the composition of the microbial community,⁴⁹ we find these treatments can drastically shape the community composition and metabolism. However, our findings that Well D and G (Figure 2, Figure S3) respond differently to the nitrate and molybdate inhibitors further support previous assertions that the efficiency of H₂S inhibitors depends on the composition of the microbiome.^{49,50} While changes in microbial community composition indicate a response to our top-down strategies, changes in microbial abundances do not necessarily indicate their metabolic outputs, which must be characterized directly.

2.4 | Hydrogen sulfide production is reduced or suppressed by nitrate and molybdate treatments

To assess if these supplements resulted in metabolic changes that reduce or inhibit H₂S production, we measured H₂S in the headspace of the *in vitro* microcosms from Well D. Untreated microcosms from Well D generated, on average, approximately 220 ppm (±35) of headspace H₂S after 10 days of cultivation (Figure 3B). Nitrate-treated microcosms reduced the amount of H₂S to ~150 ppm on average, while the molybdate and combined treatments limited the H₂S to approximately 2 ppm or less. Taken together with the sequencing data, this suggests that the nitrate treatment here likely allowed for competitive metabolism of nitrate over sulfate as has been reported to decrease H₂S production.^{9,50,53} Biostimulation with nitrate alone, however, was not able to completely inhibit H₂S production and enriched for specific organisms that can utilize both nitrate and sulfate,⁴⁵ like *Sulfurospirillum*.^{9,54} We hypothesize that these taxa only inhibited sulfate reduction until the nitrate was consumed, resulting in modest decreases in H₂S production. In the presence of nitrate, non-sulfur-reducing bacteria such as *Desulfovibrio* and *Dethiosulfovibrio* species present in our control samples are outcompeted although their ability to produce H₂S is not inhibited. On the other hand, molybdate proved very effective at inhibiting H₂S generation both on its own and in combination with nitrate. Molybdate has been proposed to inhibit H₂S generation by binding to the sulfate adenylyl-transferase (Sat, ATP sulfurylase) complex and blocking the generation of ammonium persulfate precursor to sulfide while also

depleting the ATP pools of the cell.⁵⁰ We believe that this form of selection explains why the molybdate effectively inhibits H₂S formation while also limiting the number of H₂S generating organisms in the community, consistent with our decreased alpha diversity finding (Figure 2). Molybdate strongly inhibits the sat complex of sulfate-reducers, which allows other microbes to ferment the molasses into organic acids and gases for MEOR.

To evaluate how persistent the impacts of these treatments were, we passaged the communities into media that contained the same, different, or no supplements. After 3 days of growth, microcosms were passaged three separate times into fresh media with and without supplements. Subcultures of untreated communities with either no supplement or only nitrate produced moderate amounts of H₂S although lower than the untreated parent culture, while molybdate treatment of untreated subcultures matched that of the initial molybdate-treated cultures with little to no H₂S formation after the passages (≤7 ppm H₂S) (Figure 4, Figure S5A). When cultures were passaged from nitrate treatments and selection pressure was removed or kept the same (Figure 4B; no supplement or NO₃⁻¹ only, respectively), the H₂S production resumed and remained a little lower than the level of the parent culture (~100 ppm). This suggests that the nitrate treatment may have enriched sulfate-reducing bacteria or at best suppressed their metabolism initially. However, if nitrate selection is applied to molybdate cultures, H₂S levels remained below that of nitrate treatment of the native microbiome. Therefore, we suspect that the reduced alpha diversity of molybdate treatment (Figure 2) reflects a direct inhibition or loss of many of the sulfur-reducing species, which are either temporarily limited or possibly enriched by the nitrate treatment.

As before, molybdate was able to limit the H₂S production of the sulfate-reducing communities to very low levels (Figure 4B), further demonstrating that it was effective even against actively H₂S-generating communities like those from the nitrate-treated group that otherwise produce H₂S (Figure 4C). Molybdate-treated subcultures, regardless of parent culture, generated less H₂S (Figure S5A,D). Interestingly, when molybdate microcosms were subcultured and had their selection pressure removed (Figure 4C, Figure S5B,C), H₂S was produced although at low levels (<25 ppm) with each sequential passage producing more H₂S demonstrating that continuous application of this selection pressure is needed to permanently suppress sulfate reduction. This suggests that H₂S generation is inhibited by molybdate, but the associated microbes may still be present at low levels.

Lastly, subcultures of microcosms generated from combination treatment (Figure 4D) behaved like molybdate subcultures for combined supplement and molybdate passages. However, after three passages, the H₂S generation of these communities treated with nitrate began to return to higher levels. This suggests that the supplemented nitrate substrate in the combination treatment may have continued enriching for nitrate consumers, specifically, some that harbor both nitrate and sulfate-reducing pathways, so that sulfate reduction resumed once the nitrate was consumed. Throughout the subculture cultivations, the microbial community compositions were similar to their respective nutrient-screening microcosms which were

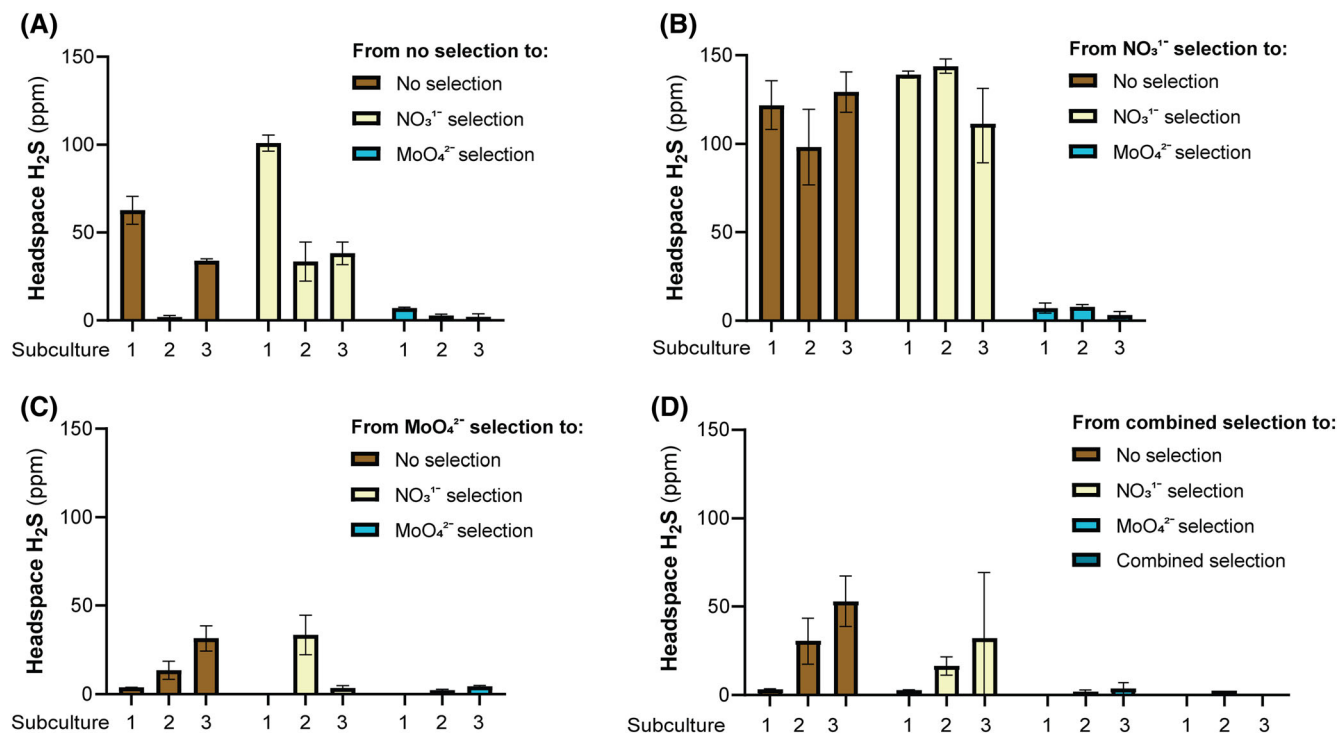


FIGURE 4 Continued application of H₂S inhibitors effectively limits H₂S production in microcosms cultures. Headspace H₂S from microcosms 7 days after subculture from parent cultures with (A) no, (B) nitrate, (C) molybdate, or (D) combined supplements and subsequent treatment with no (brown), nitrate (yellow), molybdate (cyan), or combined (teal) supplementation. Error bars represent standard deviation, $n = 3$. Additional comparisons and statistical analysis are shown in Figure S5.

dominated by *Enterobacteriaceae*, *Bacteroides*, and *Lachnospirillum* (Figure 3, Figure S6). Ultimately, the combination of molybdate and nitrate best limits H₂S (Figure S5E) and provides some redundancy of inhibitors in the case one of the selection pressures is resisted or lost; however, molybdate is the most important for effectively limiting H₂S production from these oil well communities (Figure S5D). From this *in vitro* screening system, we showed that oil well microbial communities can be engineered and controlled from the top down using nitrate and molybdate, structural analogs of sulfate, to inhibit sulfate-reducing bacteria and H₂S generation.

2.5 | Mini-coreflood experiments validate *in vitro* community control

To evaluate the potential of this approach for oil recovery, we applied the most effective treatments identified from the *in vitro* top-down evaluations to a miniature coreflood system. The mini-coreflood (MCF) system (Figure 5A–C) was used to simulate microbial-enhanced secondary oil recovery from representative core material to evaluate the effects of the carbon and mineral supplements in parallel. In addition to the pressure generated by the microbes in the MCF, we evaluated the composition of the microbial community and the produced oil. After initial oil recovery from brine flooding was completed, 9:1 mixtures of effluent and molasses-nutrient supplements were injected into the MCFs and allowed to sit for 10 days in an anaerobic

environment. Similar to our *in vitro* microcosms, we found that molasses was able to induce microbial growth and activity, generating up to 180 kPa. Nitrate-treated floods generated the most pressure while the molybdate and combination treatment generated less pressure (Figure 5D). We suspect that the suppression of H₂S and the limitation of sulfate-reducing bacteria account for the differences in pressure generation between the molybdate-treated cultures and the untreated or nitrate-treated cultures. Because increased pressure applies more force on the trapped oil deposits, the amounts of oil recovered trended with the pressure produced (Figure 5D/F).

We also found that the microbial community in the MCF was distinct from our *in vitro* screening, reflecting the contribution of the high petroleum hydrocarbon concentrations in these samples. MCF microbial communities drastically shifted toward *Bacteroides* under the molybdate and combination treatment whereas the untreated and nitrate-treated MCFs were dominated by *Lachnospirillum* (Figure 5G). Similar to the end of the *in vitro* microcosms, both *Bacteroides* and *Lachnospirillum* were prominent members of the final MCF microbiome (Figures 4 and 5G). Microbes belonging to the *Lachnospirillum* group degrade some hydrocarbons and reduce sulfate to H₂S.^{55–57}

Similarly, we observed increases in the abundance of organisms belonging to the genus *Pseudomonas* in the molybdate and combination MCFs (Figure 5G). This enrichment is associated with a decrease of n-paraffin in produced oil from ~35% w/w to ~25% w/w (Figure 5E), which is consistent with known degradation of

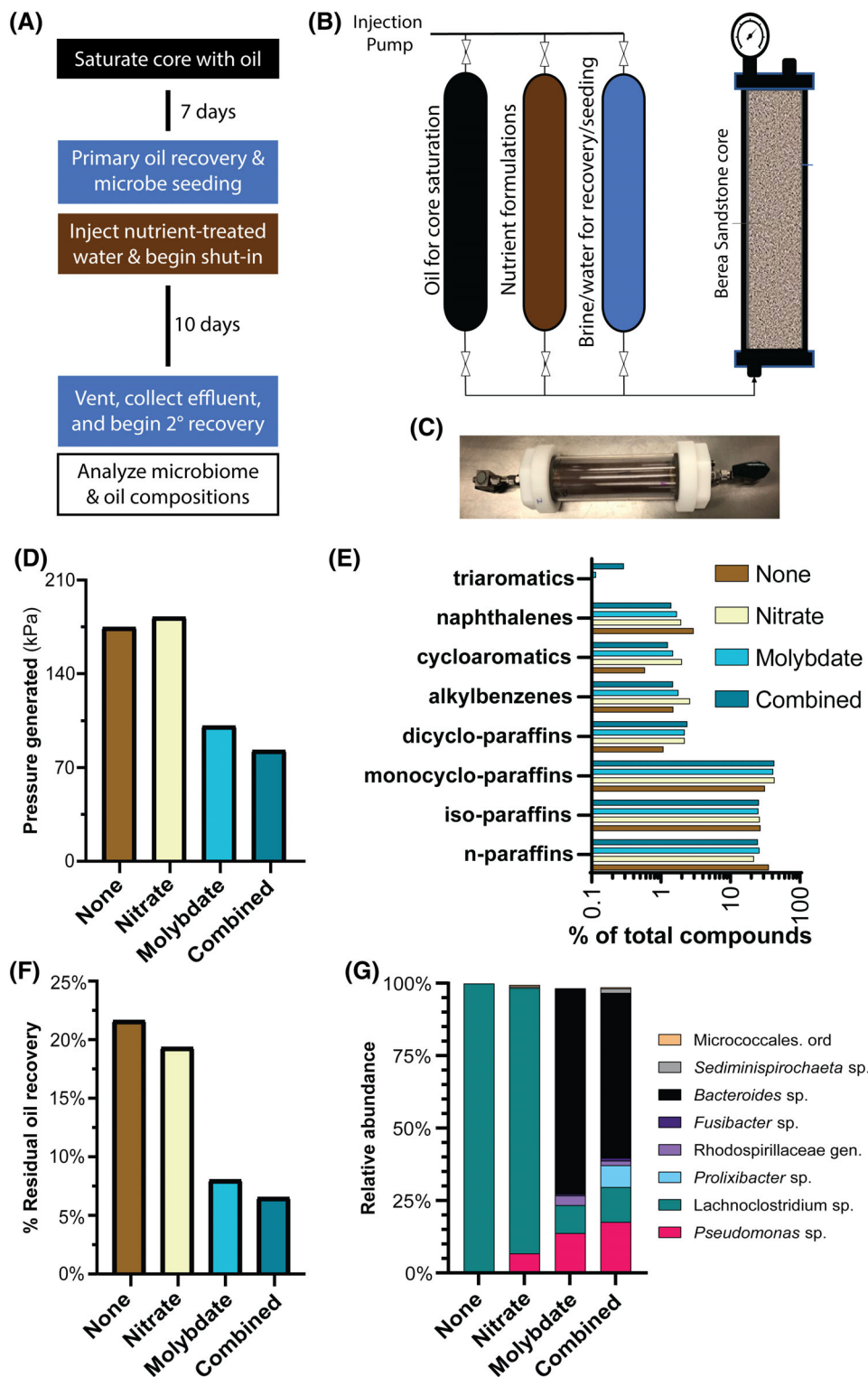


FIGURE 5 Nutrient formulations control microbial metabolism in ways that affect both oil yield and composition. (A) Overview of mini-coreflood (MCF) experimental design where cores are saturated with oil, accessible oil (primary recovery) is extracted with brine and microbes are seeded with produced water, nutrients are subsequently injected, and reactors are shut-in for 10 days before they are vented and flooded with brine to recover any of the oil liberated by the microbial processes (i.e., secondary recovery). (B) MCF experimental setup showing the pump which injects either oil, sterile brine, well water for seeding, or nutrient supplements with the MCF canisters encasing the core material and (C) an actual image of assembled MCF apparatus. (D) Pressure generated during 10-day mini-coreflood by various supplements. (E) Chemical composition of produced oil after microbial mini-coreflood. (F) Fraction of residual oil in place that was recovered after 10-day microbial shut-in period. (G) Composition of microbes in effluent from the microbial mini-coreflood after 10-day shut-in period. This data represents one trial which is the most data complete, a replicate performed at a different time from different produced water is presented in the supplement (Figure S8).

naphthalene,⁵⁸ normal paraffin,⁵⁹ and polycyclic hydrocarbons^{60,61} by *Pseudomonas*. Our data suggest that *Pseudomonas* sp. degraded around 30% of the long chain n-paraffins (C14–C29) in a period of 14 days (Figure S7A, Table S1). Although we were unable to resolve specific species here, *Pseudomonas* species, such as *P. proteolytica*, *P. xanthomarina*, and *P. aeruginosa*, are expected to induce n-paraffin degradation starting from n-tetradecane (C14) by alkane hydroxylases

systems.^{61–63} Certainly, biodegradation of long chain n-paraffins is recognized to have a positive influence on oil recovery processes by means of reducing the viscosity of the crude oil while increasing its fluidity.⁶⁴ The relative abundance of monocyclo-paraffins (C9–C17) increased from ~30% w/w to ~40% w/w for all treatments (Figure S7B, Table S1), likely due to the degradation of the n-paraffins. However, no specific trend was observed for the abundance of

iso-paraffins. Aromatics such as naphthalene also decreased in abundance with treatment and the presence of *Pseudomonas* sp. (Figure 5E/G). Species such as *P. mendocina*, *P. putida*, *P. fluorescens*, *P. paucimobilis*, *P. vesicularis*, *P. cepacia*, *P. testosteroni*, *P. aeruginosa*, and *P. stutzeri* have been reported to induce naphthalene biodegradation.^{60,65,66} In contrast, the triaromatic fraction (C17) increased as a response to the supplements here applied. The relative abundance of dicyclo- and monocyclo-paraffins increased when these treatments were applied, likely because of n-paraffin degradation, and thus, changed the overall composition of the produced oil (Figure 5E, Table S1). While the results presented in Figure 5 represent one trial of these treatments, an additional replicate treatment at a different time (Figure S8A–C) showed n-paraffin degradation for all treatments. However, the same trend for the aromatic polycyclic hydrocarbon fraction is not seen in the replicate likely because the microbiomes of the produced water used to seed these wells were different (Figure S8D). Regardless of these specific differences, we observed reproducible control of the oil well microbiome as a function of nutrient supplementation to alter produced oil paraffin profiles while limiting H₂S production and pressurizing the reserve.

The MCF results demonstrate a clear correlation between pressure generation and oil recovery in MEOR processes. While the strongest H₂S-limiting treatments did not substantially enhance pressure generation in the presence of hydrocarbons and confined geometry, we were able to validate the role that molybdate plays in controlling souring and corrosion by oil well microbes. However, field trials and economic analysis are needed to determine the viability of this approach relative to current surfactant-based oil recovery processes. The modest improvements in oil recovery may be offset by the increased operating costs needed for corrosion maintenance, surfactant production, and microbial control via more expensive ammonium quaternary disinfectants currently used.²⁵ Moreover, we demonstrated that the specific oil compositions were a strong function of microbial activity, which could be controlled with top-down designs. Further optimization of the nutrient formulation, such as increased amounts of molasses or other nutrients, may enhance pressure generation or alter the microbial dynamics so that more oil can be recovered, and specific hydrocarbon compounds can be enriched or depleted. In agreement with the *in vitro* findings, these results suggested that we can control the microbial population in small-scale oil recovery settings and can use that to concurrently modulate oil composition.

3 | CONCLUSIONS

Microbial activity of oil wells has a significant impact on oil recovery efficiencies, the souring of produced oil or H₂S generation, and final oil composition. Top-down approaches via direct nutrient injection and mineral supplementation can be used to shape community structure and control metabolism in MEOR. We validated the ability of molasses supplemented with nitrate and/or molybdate to apply different selective pressures which were either competitive or inhibitory of

H₂S production, respectively. Molybdate supplementation in particular provided strong selective pressure whose effects persisted over multiple generations or passages even once removed, demonstrating a strong capacity to shape community composition and function. While the H₂S-limiting treatments did not enhance oil recovery over the no supplement control in our coreflood experiments under the conditions tested, these treatments had a demonstrable impact on the composition of oil recovered. Our work highlights the power of microbiome engineering for the improvement of oil recovery operations and develops a workflow for the rapid screening and evaluation of candidate oil wells.

4 | METHODS

4.1 | Cultivation

Effluent oil well water was collected from several wells around the Illinois oil basin and transported to the laboratory (West Lafayette, IN, USA) the same day. For cultivation of the oil well microbiome, 9 ml of effluent water was mixed in Hungate tubes with either 1 ml of sterile 40% (w/v) corn syrup or 1 ml of sterile 40% (w/v) molasses in an anaerobic chamber (PLOS, Grand Rapids, MI, USA) with an atmosphere of 85% N₂, 10% CO₂, and 5% H₂. Cultures that were treated with additional non-essential mineral supplements (final concentration of 0.5 g/L potassium nitrate, 0.5 g/L sodium molybdate, 0.25 g/L potassium monophosphate, and 0.25 g/L potassium diphosphate, or 0.25 g/L sodium chloride and 0.25 g/L potassium chloride)—solutions were sterile filtered and equilibrated overnight in the anaerobic chamber. After nutrient solutions were added, cultures were thoroughly mixed and incubated at 27°C. Pressure measurements were taken every 24 h with a pressure gauge (APG, Logan, UT, USA).⁶⁷ 1 ml of culture was removed for microbiome analysis and pH monitoring by pH test strips (pH 2–8, MilliporeSigma, St. Louis, MO, USA). After sampling, the cultures were then vented to a gauge pressure of 0 and replaced in the incubator; cultures were monitored and sampled for 7–10 days.

4.2 | DNA extraction, PCR, sequencing and analysis

To evaluate the composition of the native oil well, ~1 L of effluent water was prefiltered with Glass Fiber Filters (1.2 µm pore, MilliporeSigma) and then biomass was concentrated on Express™ PLUS Membrane Filters (0.22 µm pore, MilliporeSigma) similar to previous work.¹² DNA was then extracted from the microbial biomass on the second filter using the DNeasy PowerFecal Kit (QIAGEN, Germantown, MD, USA) along with the FastPrep 24 5G homogenizer (MP Biomedicals, Santa Ana, CA, USA) as per instructed by the manufacturers. All DNA was stored at –20°C until further analysis. DNA concentration and quality were assessed on a NanoPhotometer (Implen NP80, Los Angeles, CA, USA). The microbial community

composition of oil well microcosms was assessed by DNA extracted from a 1-ml aliquot of the cultures as mentioned above. The 1 ml aliquots were centrifuged at 10,000g for 7 min to pellet the cells. After removing the supernatant, the microbial DNA was extracted with the same DNeasy PowerFecal kit as noted above.

As in previous microcosm studies,⁶⁸ the microbial community was then assessed by amplifying and sequencing the V4-V5 region of the 16 S rRNA gene from the extracted DNA (primers: 515f—forward, 5' GTGYCAGCMGCCGCGGTAA 3'; and 926r—reverse 5' CCGYCAATTYMTTTRAGTTT 3').⁶⁹ 16 S rRNA gene PCRs were set up using Phusion Polymerase MasterMix (Thermo Fisher Scientific, Waltham, MA, USA) as follows PCR-grade water (22 µl), Phusion mastermix (25 µl), forward primer (10 µM, 1.0 µl), reverse primer (10 µM, 1.0 µl), and template DNA (1.0 µl) in a total reaction volume of 50 µl; for samples that had very low template concentration up to 5 µl of DNA template was used and the water volume reduced to 17 µl. PCR amplification conditions were set to 98°C for 0.5 min; 35 cycles of 98°C for 15 s, 50°C for 30 s, 72°C for 60 s; and 72°C for 10 min, and then a 4°C hold. The resulting amplicons were confirmed by gel electrophoresis, purified using the Clean and Concentrator kit (Zymo Research Irvine, CA, USA), and then labeled using unique 8-bp tagged i5 (i509-i516) index forward primers and 8-bp tagged i7 (i713-i724) index reverse primers as suggested by the manufacturer (Illumina San Diego, CA, USA). Pooled amplicons were then multiplexed and sequenced via 2 × 250-bp paired reads on an Illumina MiSeq at the Purdue Genomics facilities. Sequences were analyzed using the QIIME2 pipeline⁷⁰ which quality-filtered, joined paired reads, checked for chimeras, and denoised the data (via DADA2⁷¹). The operational taxonomic units (OTUs) were assigned using the SILVA SSU database (SILVA 138)⁷² for assigning taxonomy to the representative OTUs. All alpha and beta diversity metrics were calculated using QIIME's built-in analysis tools.

4.3 | Micro GC H₂S quantification

An Agilent 490 Micro GC (Agilent Technologies, Palo Alto, CA, USA) equipped with parallel Molsieve 5A and PoraPLOT U columns and a thermal conductivity detector was used for H₂S analysis with helium carrier gas (15 PSI). The column and injector temperatures were isothermally set to 60°C for the duration of the 120 s run. The injector was set to split mode with an injection time of 40 ms and a back-flush time of 10 s. H₂S standards were prepared as previously described⁷³ where, briefly, H₂S was generated by reacting Na₂S (Sigma-Aldrich, Saint Louis, MO, USA) with excess HCl (Fisher) in a 20-ml Hungate bottle with a butyl rubber stopper in the anaerobic chamber. Six working calibration standards at concentrations corresponding to 25, 50, 100, 250, 750, and 1500 ppm of H₂S were prepared by diluting from a 5 µmol/mL stock. Either a 2-ml headspace of standard or the microcosm samples was withdrawn and injected into the instrument with a gas syringe. Standards were run in triplicate to create a calibration curve while the biological samples were each run once.

4.4 | Mini-coreflood set up

These studies used high-permeability (>400 mD), 1" diameter by 6" long Berea Sanstone™ cores (Cleveland Quarries, Vermilion, OH, USA) that were dried to completeness in an oven at 110°C overnight. Once dried, the cores were saturated with a synthetic brine comprising a salinity (9400 mg/L and a hardness of 250 mg/L) closely resembling fluids from the representative wells to determine pore volumes (PV) by the mass of brine that was retained in the core. Following brine saturation, mini-coreflood reactors were assembled in the anaerobic chamber and consisted of a PVC sleeve (1" inner diameter, McMaster-Carr, Cleveland, OH, USA) that surrounded the core and a plastic canister to hold the core in place (built by Purdue University's Chemistry Precision Machine Shop, West Lafayette, IN, USA). The plastic canisters were then filled with DI water that was pressurized to 80 PSI to apply pressure on the sleeve and keep it tightly fit to the core. Once mini-coreflood reactors were assembled, dead oil from the representative wells was processed and injected into the core at a rate of 0.5 ml/min until the core was saturated with oil; the amount of brine displaced represented the volume of original oil in place (OOIP) before the primary water flood. The primary flood was carried out by injecting two PV of effluent oil well water at a rate of 1 ml/min to displace the oil for primary recovery and simultaneously seed microbes into the core. The secondary flood was carried out with 1 PV of a mixture of nine parts of well water to one part of the molasses solution with supplements as indicated in the "Cultivation" methods section above. The flow rate of the secondary flood was 1 ml/min, and no additional oil was recovered during this nutrient injection step. Mini-corefloods were shut-in for 10 days inside the anaerobic chamber (27°C) replicating ground conditions. The pressure generated over the 10-day period was measured with an in-line pressure gauge. Oil generated by the microbial pressure was collected and then an additional 2 PV of brine was used as a final water flood to recover oil displaced by the microbial activity. The collected brine flood volumes were centrifuged, and DNA was extracted from the pellet; V4-V5 16 S rRNA gene regions were amplified and sequenced as described above (see Section 4.2).

4.5 | Chemical quantitation via GC × GC-FID

The quantitative chemical characterization of the oil samples was carried out using a comprehensive two-dimensional gas chromatography system Agilent 7890B GC (GC × GC) coupled to a flame ionization detector (FID). A thermal modulator cooled with liquid nitrogen (LECO Corporation, Saint Joseph, MI), an Agilent 7683B series injector, and an HP 7683 series autosampler were also used. A reversed-phase column configuration was selected having a primary mid-polar column DB-17 ms (30 m × 0.25 mm × 0.25 µm) and a secondary nonpolar column DB-1 ms (0.8 m × 0.25 mm × 0.25 µm). Both columns were provided by Agilent (Santa Clara, CA). Ultrahigh purity helium (99.9999%) was used as the carrier gas at a constant flow rate of 1.5 ml/min, and a front inlet septum purge flow of 3 ml/min and a front inlet gas saver flow of 20 ml/min were set in the GC × GC

system. A split inlet liner with glass wool suitable for low-pressure drop provided by Agilent (Santa Clara, CA) was used to protect the system due to the nature of the samples under study. The temperature of the front inlet, initial oven temperature, and the target oven temperature was set at 280°C, 40°C (hold time of 0.2 min), and 260°C (hold time of 5 min), respectively. An oven temperature ramp rate of 3°C/min was considered for a total GC method time of analysis of 4712 s (78.5 min) per sample. Secondary oven and modulator temperature offsets were set at 50 and 15°C, respectively. A modulation period of 2.5 s was used to avoid wraparound. Samples of 0.5 µl were injected into the system using a manual dilution factor of 100 (10 µl of sample in 1 ml of pentane) and an automatic split ratio of 20:1. The development and refinement of the GC × GC-FID classification map (Figure S9) were made based on the methodology described in a previous study.⁷⁴ Thus, the chemical characterization encompassed nine hydrocarbon groups and carbon numbers as follows: n-paraffins (C6-C32), iso-paraffins (C6-C32), monocyclo- (C6-C30), dicyclo- (C8-C20), and tricyclo-paraffins (C10-C16), alkylbenzenes (C6-C20), cycloaromatics (C9-C18), naphthalenes (C10-C19), and triaromatics (C14-C19). Data were processed in ChromaTOF software version 4.71.0.0 optimized for GC × GC-FID (LECO Corporation, Saint Joseph, MI) with a signal-to-noise ratio of 50. Weight percentage (wt. %) relative to each hydrocarbon class and carbon number was calculated via normalizing the peak area by integration of the GC × GC chromatograms extracting solvent and column bleed peaks using Microsoft Excel 365.

4.6 | Statistical methods

T-tests were performed using GraphPad Prism's in-software analysis tools (https://www.graphpad.com/guides/prism/latest/statistics/stat_key_concepts_ttests.htm). Relative abundance, alpha diversity, and beta diversity metrics were calculated in QIIME2⁷⁰ and the results (such as PCoA) were visualized using QIIME2 view (<https://view.qiime2.org/>).

AUTHOR CONTRIBUTIONS

Ethan T. Hillman: Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); project administration (equal); supervision (equal); validation (equal); visualization (lead); writing – original draft (lead). **Louis Edwards Caceres-Martinez:** Data curation (supporting); formal analysis (supporting); investigation (supporting); methodology (supporting); writing – original draft (supporting). **Gozdem Kilaz:** Funding acquisition (supporting); project administration (supporting); resources (supporting); supervision (supporting); writing – review and editing (lead). **Kevin V. Solomon:** Conceptualization (lead); funding acquisition (lead); project administration (lead); resources (lead); supervision (lead); writing – review and editing (lead).

ACKNOWLEDGMENTS

We would like to thank the members of Purdue's Enhance Oil Recovery Lab, specifically Dr. Nate Schultheiss, Dr. Thomas

Everett, Dr. Jeremy Holtsclaw, Dr. Katherine Wehde, and Timothy Henderson, who made this work possible by coordinating well samplings, provided experimental feedback, and supplying technical supported and insight for our work. We are also grateful to the Purdue University Genomics Core for their role in sequencing the 16 S rRNA gene amplicons and the Purdue Institute of Inflammation, Immunology, and Infectious Disease for access to the Bell computing cluster which allowed us to analyze the sequencing reads. Additionally, we are thankful to both Bindley Bioscience Center and the Fuel Laboratory of Renewable Energy at Purdue University for the research space and support staff where this research was carried out.

FUNDING INFORMATION

This project was financially supported by the Pioneer Oil Company (Vincennes, IN) and the Purdue Enhanced Oil Recovery Lab.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

DATA AVAILABILITY STATEMENT

The sequencing datasets supporting the conclusions of this article are available under the NCBI SRA: SUB11545621. Additional Data including GCxGC data can be found in Supplemental Material file.

ORCID

Ethan T. Hillman  <https://orcid.org/0000-0002-3846-1692>

Louis Edwards Caceres-Martinez  <https://orcid.org/0000-0001-8096-5125>

Gozdem Kilaz  <https://orcid.org/0000-0002-0302-6527>

Kevin V. Solomon  <https://orcid.org/0000-0003-2904-9118>

REFERENCES

1. DNV AS. Energy Transition Outlook 2021 Executive Summary. A Global and Regional Forecast to 2050. 2021;1–282. <https://eto.dnv.com/2021>
2. Raffa P. Where is research on fossil fuels going in times of climate change? A perspective on chemical enhanced oil recovery. *MRS Commun.* 2021;11(6):716–725. doi:10.1557/s43579-021-00131-y
3. Farajzadeh R, Kahrobaei S, Eftekhari AA, Mjeni RA, Boersma D, Bruining J. Chemical enhanced oil recovery and the dilemma of more and cleaner energy. *Sci Rep.* 2021;11(1):829. doi:10.1038/s41598-020-80369-z
4. Mohsenatabar Firozjahi A, Saghafi HR. Review on chemical enhanced oil recovery using polymer flooding: fundamentals, experimental and numerical simulation. *Petroleum.* 2020;6(2):115–122. doi:10.1016/j.petlm.2019.09.003
5. She H, Kong D, Li Y, Hu Z, Guo H. Recent advance of microbial enhanced oil recovery (MEOR) in China. *Geofluids.* 2019;2019:1–16. doi:10.1155/2019/1871392
6. Nikolova C, Gutierrez T. Use of microorganisms in the recovery of oil from recalcitrant oil reservoirs: current state of knowledge, technological advances and future perspectives. *Front Microbiol.* 2020; 10(2996):1–18. doi:10.3389/fmicb.2019.02996

7. Youssef N, Elshahed MS, McInerney MJ. Chapter 6 Microbial processes in oil fields. Culprits, problems, and opportunities. *Advances in Applied Microbiology*. Vol 66. 1st ed. Elsevier Inc; 2009. doi:[10.1016/S0065-2164\(08\)00806-X](https://doi.org/10.1016/S0065-2164(08)00806-X)
8. Bermont-Bouis D, Janvier M, Grimont PAD, Dupont I, Vallaes T. Both sulfate-reducing bacteria and Enterobacteriaceae take part in marine biocorrosion of carbon steel. *J Appl Microbiol*. 2007;102(1):161-168. doi:[10.1111/j.1365-2672.2006.03053.x](https://doi.org/10.1111/j.1365-2672.2006.03053.x)
9. Hubert C, Voordouw G. Oil field souring control by nitrate-reducing *Sulfurospirillum* spp. that outcompete sulfate-reducing bacteria for organic electron donors. *Appl Environ Microbiol*. 2007;73(8):2644-2652. doi:[10.1128/AEM.02332-06](https://doi.org/10.1128/AEM.02332-06)
10. Nazina TN, Sokolova DS, Babich TL, et al. Microorganisms of low-temperature heavy oil reservoirs (Russia) and their possible application for enhanced oil recovery. *Microbiology*. 2017;86(6):773-785. doi:[10.1134/S0026261717060121](https://doi.org/10.1134/S0026261717060121)
11. Vigneron A, Alsop EB, Lomans BP, Kyrpides NC, Head IM, Tsesmetzis N. Succession in the petroleum reservoir microbiome through an oil field production lifecycle. *ISME J*. 2017;11(9):2141-2154. doi:[10.1038/ismej.2017.78](https://doi.org/10.1038/ismej.2017.78)
12. Daly RA, Borton MA, Wilkins MJ, et al. Microbial metabolisms in a 2.5-km-deep ecosystem created by hydraulic fracturing in shales. *Nat Microbiol*. 2016;1:1-9. doi:[10.1038/nmicrobiol.2016.146](https://doi.org/10.1038/nmicrobiol.2016.146)
13. Nazina T, Sokolova D, Grouzdev D, et al. The potential application of microorganisms for sustainable petroleum recovery from heavy oil reservoirs. *Sustainability (Switzerland)*. 2020;12(15):1-23. doi:[10.3390/SU12010015](https://doi.org/10.3390/SU12010015)
14. Head IM, Jones DM, Larter SR. Biological activity in the deep subsurface and the origin of heavy oil. *Nature*. 2003;426(6964):344-352. doi:[10.1038/nature02134](https://doi.org/10.1038/nature02134)
15. van Hamme JD, Singh A, Ward OP. Recent advances in petroleum microbiology. *Microbiol Mol Biol Rev*. 2003;67(4):503-549. doi:[10.1128/mmr.67.4.503-549.2003](https://doi.org/10.1128/mmr.67.4.503-549.2003)
16. Klueglein N, Kögler F, Adaktylou IJ, et al. Understanding selective plugging and biofilm formation of a halophilic bacterial community for MEOR application. In: *Society of Petroleum Engineers Improved Oil Recovery Conference*. Vol All Days. SPE Improved Oil Recovery Conference. 2016. [10.2118/179620-MS](https://doi.org/10.2118/179620-MS)
17. Lazar I, Petrisor IG, Yen TF. Microbial enhanced oil recovery. *Pet Sci Technol*. 2007;25:1353-1366. doi:[10.1016/S0376-7361\(09\)70098-6](https://doi.org/10.1016/S0376-7361(09)70098-6)
18. Foo JL, Ling H, Lee YS, Chang MW. Microbiome engineering: current applications and its future. *Biotechnol J*. 2017;12(3):1-9. doi:[10.1002/biot.201600099](https://doi.org/10.1002/biot.201600099)
19. Ke J, Wang B, Yoshikuni Y. Microbiome engineering: synthetic biology of plant-associated microbiomes in sustainable agriculture. *Trends Biotechnol*. 2021;39(3):244-261. doi:[10.1016/j.tibtech.2020.07.008](https://doi.org/10.1016/j.tibtech.2020.07.008)
20. Lee ED, Aurand ER, Friedman DC, Group EBRMRW. Engineering microbiomes—looking ahead. *ACS Synth Biol*. 2020;9(12):3181-3183.
21. Lawson CE, Harcombe WR, Hatzepichler R, et al. Common principles and best practices for engineering microbiomes. *Nat Rev Microbiol*. 2019;17(12):725-741. doi:[10.1038/s41579-019-0255-9](https://doi.org/10.1038/s41579-019-0255-9)
22. Youssef N, Simpson DR, Duncan KE, et al. In situ biosurfactant production by *Bacillus* strains injected into a limestone petroleum reservoir. *Appl Environ Microbiol*. 2007;73(4):1239-1247. doi:[10.1128/AEM.02264-06](https://doi.org/10.1128/AEM.02264-06)
23. Yue M, Zhu W, Song Z, Long Y, Song H. Study on distribution of reservoir endogenous microbe and oil displacement mechanism. *Saudi J Biol Sci*. 2017;24(2):263-267. doi:[10.1016/j.sjbs.2016.09.014](https://doi.org/10.1016/j.sjbs.2016.09.014)
24. Lin X, Zheng X, Liu R, et al. Extracellular polymeric substances production by ZL-02 for microbial enhanced oil recovery. *Ind Eng Chem Res*. 2021;60(2):842-850. doi:[10.1021/acs.iecr.0c05130](https://doi.org/10.1021/acs.iecr.0c05130)
25. Quraishi M, Bhatia SK, Pandit S, et al. Exploiting microbes in the petroleum field: analyzing the credibility of microbial enhanced oil recovery (MEOR). *Energies (Basel)*. 2021;14(4684):1-30.
26. Diaz-Colunga J, Lu N, Sanchez-Gorostiaga A, et al. Top-down and bottom-up cohesiveness in microbial community coalescence. *Proc Natl Acad Sci U S A*. 2022;119(6):1-11. doi:[10.1073/pnas.2111261119](https://doi.org/10.1073/pnas.2111261119)
27. Walter J, Maldonado-Gómez MX, Martínez I. To engraft or not to engraft: an ecological framework for gut microbiome modulation with live microbes. *Curr Opin Biotechnol*. 2018;49:129-139. doi:[10.1016/j.copbio.2017.08.008](https://doi.org/10.1016/j.copbio.2017.08.008)
28. Amor DR, Bello MD. Bottom-up approaches to synthetic cooperation in microbial communities. *Life*. 2019;9(22):1-17. doi:[10.3390/life9010022](https://doi.org/10.3390/life9010022)
29. Lindemann SR, Bernstein HC, Song HS, et al. Engineering microbial consortia for controllable outputs. *ISME J*. 2016;10(9):2077-2084. doi:[10.1038/ismej.2016.26](https://doi.org/10.1038/ismej.2016.26)
30. Rottinghaus AG, Ferreira A, Fishbein SRS, Dantas G, Moon TS. Genetically stable CRISPR-based kill switches for engineered microbes. *Nat Commun*. 2022;13(1):1-17. doi:[10.1038/s41467-022-28163-5](https://doi.org/10.1038/s41467-022-28163-5)
31. Gilmore SP, Lankiewicz TS, Wilken SE, et al. Top-down enrichment guides in formation of synthetic microbial consortia for biomass degradation. *ACS Synth Biol*. 2019;8(9):2174-2185. doi:[10.1021/acssynbio.9b00271](https://doi.org/10.1021/acssynbio.9b00271)
32. Pacheco AR, Osborne ML, Segrè D. Non-additive microbial community responses to environmental complexity. *Nat Commun*. 2021;12(1):1-11. doi:[10.1038/s41467-021-22426-3](https://doi.org/10.1038/s41467-021-22426-3)
33. Portwood JT. A commercial microbial enhanced oil recovery technology: evaluation of 322 projects. In: *Society of Petroleum Engineers Production Operations Symposium*. SPE Oklahoma City Oil and Gas Symposium/Production and Operations Symposium. 1995. [10.2118/29518-MS](https://doi.org/10.2118/29518-MS)
34. Nikolova C, Gutierrez T. Marine hydrocarbon-degrading bacteria: their role and application in oil-spill response and enhanced oil recovery. *Microbial Biodegradation and Bioremediation*. Springer; 2022. doi:[10.1016/b978-0-323-85455-9.00005-9](https://doi.org/10.1016/b978-0-323-85455-9.00005-9)
35. Zahner RLL, Tapper SJJ, Marcotte BWG, Govreau BRR. Lessons learned from applications of a new organic-oil-recovery method that activates resident microbes. *SPE Reservoir Eval Eng*. 2012;15(6):688-694.
36. Chai LJ, Zhang F, She YH, Banat IM, Hou DJ. Impact of a microbial-enhanced oil recovery field trial on microbial communities in a low-temperature heavy oil reservoir. *Nat Environ Pollut Technol*. 2015;14(3):455-462.
37. Zhan Y, Wang Q, Chen C, et al. Potential of wheat bran to promote indigenous microbial enhanced oil recovery. *J Ind Microbiol Biotechnol*. 2017;44(6):845-855. doi:[10.1007/s10295-017-1909-0](https://doi.org/10.1007/s10295-017-1909-0)
38. Yao CJ, Lei GL, Ma JY, Zhao FM, Cao GZ. Experiment and simulation of indigenous microbial enhanced oil recovery (IMEOR). *International Petroleum Technology Conference, IPTC*. 2011:1-12.
39. Kögler F, Hartmann FSF, Schulze-Makuch D, Herold A, Alkan H, Dopff N. Inhibition of microbial souring with molybdate and its application under reservoir conditions. *Int Biodeterior Biodegrad*. 2021;157:105158. doi:[10.1016/j.ibiod.2020.105158](https://doi.org/10.1016/j.ibiod.2020.105158)
40. Sen R. Biotechnology in petroleum recovery: the microbial EOR. *Prog Energy Combust Sci*. 2008;34(6):714-724. doi:[10.1016/j.peccs.2008.05.001](https://doi.org/10.1016/j.peccs.2008.05.001)
41. Safdel M, Anbaz MA, Daryasafar A, Jamialahmadi M. Microbial enhanced oil recovery, a critical review on worldwide implemented field trials in different countries. *Renew Sustain Energy Rev*. 2017;74:159-172. doi:[10.1016/j.rser.2017.02.045](https://doi.org/10.1016/j.rser.2017.02.045)
42. Anantharaman K, Hausmann B, Jungbluth SP, et al. Expanded diversity of microbial groups that shape the dissimilatory sulfur cycle. *ISME J*. 2018;12(7):1715-1728. doi:[10.1038/s41396-018-0078-0](https://doi.org/10.1038/s41396-018-0078-0)
43. Rojas CA, de Santiago TA, Park S, Bosak T, Klepac-Ceraj V. Organic electron donors and terminal electron acceptors structure anaerobic microbial communities and interactions in a permanently stratified sulfidic lake. *Front Microbiol*. 2021;12:1-19. doi:[10.3389/fmicb.2021.620424](https://doi.org/10.3389/fmicb.2021.620424)

44. Hahn CR, Farag IF, Murphy CL, Podar M, Elshahed MS, Youssef NH. Microbial diversity and sulfur cycling in an early earth analogue: from ancient novelty to modern commonality. *mBio*. 2022;13(2):1-16. doi:[10.1128/mbio.00016-22](https://doi.org/10.1128/mbio.00016-22)
45. Begmatov S, Savvichev AS, Kadnikov V, et al. Microbial communities involved in methane, sulfur, and nitrogen cycling in the sediments of the barents sea. *Microorganisms*. 2021;9(2362):1-21. doi:[10.3390/microorganisms9112362](https://doi.org/10.3390/microorganisms9112362)
46. Gassara F, Suri N, Voordouw G. Nitrate-mediated microbially enhanced oil recovery (N-MEOR) from model upflow bioreactors. *J Hazard Mater*. 2017;324:94-99. doi:[10.1016/j.jhazmat.2015.12.039](https://doi.org/10.1016/j.jhazmat.2015.12.039)
47. Iwanowicz DD, Jonas RB, Schill WB, Marano-Briggs K. Novel microbiome dominated by Arcobacter during anoxic excurrent flow from an ocean blue hole in Andros Island, the Bahamas. *PLoS One*. 2021;16:1-16. doi:[10.1371/journal.pone.0256305](https://doi.org/10.1371/journal.pone.0256305)
48. Varjani SJ, Gnansounou E. Microbial dynamics in petroleum oilfields and their relationship with physiological properties of petroleum oil reservoirs. *Bioresour Technol*. 2017;245:1258-1265. doi:[10.1016/j.biortech.2017.08.028](https://doi.org/10.1016/j.biortech.2017.08.028)
49. Nemati M, Mazutinec TJ, Jenneman GE, Voordouw G. Control of biogenic h₂s production with nitrite and molybdate. *J Ind Microbiol Biotechnol*. 2001;26(6):350-355. doi:[10.1038/sj.jim.7000142](https://doi.org/10.1038/sj.jim.7000142)
50. Stoeva MK, Coates JD. Specific inhibitors of respiratory sulfate reduction: towards a mechanistic understanding. *Microbiology*. 2019;165(3):254-269. doi:[10.1099/mic.0.000750](https://doi.org/10.1099/mic.0.000750)
51. Saeed AM, el Shatoury E, Hadid R. Production of molybdenum blue by two novel molybdate-reducing bacteria belonging to the genus *Raoultella* isolated from Egypt and Iraq. *J Appl Microbiol*. 2019;126(6):1722-1728. doi:[10.1111/jam.14254](https://doi.org/10.1111/jam.14254)
52. Rahman MF, Rusnam M, Gusmanizar N, et al. Molybdate-reducing and SDS-degrading *Enterobacter* sp. strain Neni-13. *Nova Biotechnol Chim*. 2016;15(2):166-181. doi:[10.1515/nbec-2016-0017](https://doi.org/10.1515/nbec-2016-0017)
53. de Jesus EB, de Andrade Lima LRP, Bernardez LA, Almeida PF. Inhibition of microbial sulfate reduction by molybdate. *Braz J Petrol Gas*. 2015;9(3):95-106. doi:[10.5419/bjppg2015-0010](https://doi.org/10.5419/bjppg2015-0010)
54. Lohmayer R, Kappler A, Lösekann-Behrens T, Planer-Friedrich B. Sulfur species as redox partners and electron shuttles for ferrihydrite reduction by *Sulfurospirillum deleyianum*. *Appl Environ Microbiol*. 2014;80(10):3141-3149. doi:[10.1128/AEM.04220-13](https://doi.org/10.1128/AEM.04220-13)
55. Yutin N, Galperin MY. A genomic update on clostridial phylogeny: gram-negative spore formers and other misplaced clostridia. *Environ Microbiol*. 2013;15(10):2631-2641. doi:[10.1111/1462-2920.12173](https://doi.org/10.1111/1462-2920.12173)
56. El-Sayed WS, Al-Senani SR, Elbahloul Y. Diversity of dehalorespiring bacteria and selective enrichment of aryl halides-dechlorinating consortium from sedimentary environment near an oil refinery. *J Taibah Univ Sci*. 2018;12(6):711-722. doi:[10.1080/16583655.2018.1495869](https://doi.org/10.1080/16583655.2018.1495869)
57. Sallam A, Steinbüchel A. Clostridium sulfidigenes sp. nov., a mesophilic, proteolytic, thiosulfate- and sulfur-reducing bacterium isolated from pond sediment. *Int J Syst Evol Microbiol*. 2009;59(7):1661-1665. doi:[10.1099/ijs.0.004986-0](https://doi.org/10.1099/ijs.0.004986-0)
58. Dennis JJ, Zylstra GJ. Complete sequence and genetic organization of pDTG1, the 83 kilobase naphthalene degradation plasmid from *Pseudomonas putida* strain NCIB 9816-4. *J Mol Biol*. 2004;341(3):753-768. doi:[10.1016/j.jmb.2004.06.034](https://doi.org/10.1016/j.jmb.2004.06.034)
59. Okoye AU, Chikere CB, Okpokwasili GC. Characterization of potential paraffin wax removing bacteria for sustainable biotechnological application. In: *SPE Nigeria Annual International Conference and Exhibition*. Vol Day 3 Wed. 2019. [10.2118/198799-MS](https://doi.org/10.2118/198799-MS)
60. Speight JG, El-Gendy NS. Chapter 9 – Chemistry of biotransformation. *Introduction to Petroleum Biotechnology*. Gulf Professional Publishing; 2018:287-359.
61. Alseabri H, Hamad AA, Hassam MM. Biodegradation of petroleum hydrocarbons using indigenous bacterial and actinomycetes cultures. *Pak J Biol Sci*. 2020;23(6):726-734.
62. Das K, Mukherjee AK. Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from north-east India. *Bioresour Technol*. 2007;98(7):1339-1345. doi:[10.1016/j.biortech.2006.05.032](https://doi.org/10.1016/j.biortech.2006.05.032)
63. Li YP, Pan JC, Ma YL. Elucidation of multiple alkane hydroxylase systems in biodegradation of crude oil n-alkane pollution by *Pseudomonas aeruginosa* DN1. *J Appl Microbiol*. 2020;128(1):151-160. doi:[10.1111/jam.14470](https://doi.org/10.1111/jam.14470)
64. Lan G, Fan Q, Liu Y, et al. Effects of the addition of waste cooking oil on heavy crude oil biodegradation and microbial enhanced oil recovery using *Pseudomonas* sp. SWP- 4. *Biochem Eng J*. 2015;103:219-226. doi:[10.1016/j.bej.2015.08.004](https://doi.org/10.1016/j.bej.2015.08.004)
65. Barman SR, Banerjee P, Mukhopadhyay A, Das P. Biodegradation of acenaphthene and naphthalene by *Pseudomonas mendocina*: process optimization, and toxicity evaluation. *J Environ Chem Eng*. 2017;5(5):4803-4812. doi:[10.1016/j.jece.2017.09.012](https://doi.org/10.1016/j.jece.2017.09.012)
66. Samanta SK, Singh O v, Jain RK. Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation. *Trends Biotechnol*. 2002;20(6):243-248. doi:[10.1016/s0167-7799\(02\)01943-1](https://doi.org/10.1016/s0167-7799(02)01943-1)
67. Hillman ET, Li M, Hooker CA, Englaender JA, Wheeldon I, Solomon K v. Hydrolysis of lignocellulose by anaerobic fungi produces free sugars and organic acids for two-stage fine chemical production with *Kluyveromyces marxianus*. *Biotechnol Prog*. 2021;37(5):e3172. doi:[10.1002/btpr.3172](https://doi.org/10.1002/btpr.3172)
68. RedCorn RM, Hillman ET, Solomon KV, Engelberth AS. Xanthobacter-dominated biofilm as a novel source for high-value rhamnase. *Appl Microbiol Biotechnol*. 2019;103(11):4525-4538.
69. Walters W, Hyde ER, Berg-lyons D, et al. Improved bacterial 16 S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community analysis. *mSystems*. 2016;1(1):e0009-e0015. doi:[10.1128/mSystems.00009-15.Editor](https://doi.org/10.1128/mSystems.00009-15.Editor)
70. Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol*. 2019;37(8):852-857. doi:[10.1038/s41587-019-0209-9](https://doi.org/10.1038/s41587-019-0209-9)
71. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods*. 2016;13(7):581-583. doi:[10.1038/nmeth.3869](https://doi.org/10.1038/nmeth.3869)
72. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res*. 2013;41(D1):590-596. doi:[10.1093/nar/gks1219](https://doi.org/10.1093/nar/gks1219)
73. Varlet V, Giuliani N, Palmiere C, Maujean G, Augsburger M. Hydrogen sulfide measurement by headspace-gas chromatography-mass spectrometry (HS-GC-MS): application to gaseous samples and gas dissolved in muscle. *J Anal Toxicol*. 2015;39(1):52-57. doi:[10.1093/jat/bku114](https://doi.org/10.1093/jat/bku114)
74. Vozka P, Kilaz G. How to obtain a detailed chemical composition for middle distillates via GC × GC-FID without the need of GC × GC-TOF/MS. *Fuel*. 2019;247:368-377. doi:[10.1016/j.fuel.2019.03.009](https://doi.org/10.1016/j.fuel.2019.03.009)

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Hillman ET, Caceres-Martinez LE, Kilaz G, Solomon KV. Top-down enrichment of oil field microbiomes to limit souring and control oil composition during extraction operations. *AIChE J*. 2022;68(12):e17927. doi:[10.1002/aic.17927](https://doi.org/10.1002/aic.17927)