

Metagenomic analysis confirms high acetate accumulation pathways and potential microbial interactions with hydrogen gas in natural gas wells targeted for subsurface hydrogen storage

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Underground storage of hydrogen gas presents a means of large capacity and low-cost storage; it has attracted worldwide attention in the last decade. However, it has been reported that subsurface microbes cause up to 17% hydrogen gas loss and exacerbate metal corrosion risks for pipes. In this study, we aimed to identify key metabolic pathways of indigenous microorganisms with the potential for hydrogen consumption in prospective gas wells under operation to compare subsurface chemistries and microbial compositions. Samples were collected from natural gas wells located in an inland Site 1 (S1) and coastal Site 2 (S2) in California during the spring of 2023. The chemical properties of the samples were analyzed for gas composition, cation, anion, and organic concentrations. Shotgun metagenomic sequencing was performed for microbiological characterization. Kraken2 and eggNOG-mapper were used for metagenome taxonomic and functional metabolic pathway analysis, respectively. The gas composition from both wells consisted of more than 80% methane, and we measured organic acid content, which indicated high acetate concentrations. Site samples from S1 were dominated by potential nitrate, sulfate, and metal-reducing bacteria, including *Citrobacter*, *Halomonas*, and *Shewanella*. On the contrary, the S2 was dominated by methanogens such as *Methanobacterium* and *Methanosarcina*. These contrasting sites presented unique microbial metabolic features. Microorganisms found at S1 were observed to be associated with acetate-dependent methanogenesis, nitrate, and sulfate reduction pathways. Whereas microorganisms at S2 were more associated with carbon dioxide, acetate, and methanol-dependent methanogenesis metabolic pathways. Our metabolic analysis ultimately explained high acetate concentrations and potentially other high amounts of organic acid accumulation. In summary, our analysis of microbial metabolism highlights opportunities and risks associated with hydrogen storage in subsurface environments.