

Enhancement of Acetoclastic Methanogenesis during *In Situ* Biostimulation of Coalbed Methane Generation

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Many laboratory studies indicate acetoclastic methanogenesis predominates during enhanced methane production, yet major active *in situ* methanogenic pathways in the subsurface are largely unknown. Here, we use downhole monitoring methods in combination with deuterated water (D₂O) and a 200-liter injection of 0.1% yeast extract to stimulate, and isotopically label newly generated methane in a coal bed at a U.S. Geological Survey field site in the Powder River Basin, USA. An *in situ* enrichment device called a 'subsurface environmental sampler' was used to collect samples for metagenomic and metatranscriptomic analysis from three hydrologically connected wells at the field site before and after the injection. Initial analyses indicate the microbial community shifted following the injection with increased expression of genes related to a specific acetoclastic methanogen (*Methanothrix*). Specific hydrocarbon degradation and acetate formation metabolic pathways were investigated and correlated with *in situ* methane and acetate concentrations. Results provide insight into dominant active metabolic strategies used by subsurface microorganisms *in situ* during enhanced coalbed methane production. These results have broad implications for understanding ecologically relevant microbial populations and metabolic pathways involved in enhanced methane production in the subsurface.