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Biofilm development on fractured rock surface under oligotrophic groundwater environment: an experimental study using in-situ reactors

KANGHYUN PARK[1], KUE-YOUNG KIM[2] AND MAN JAE KWON[1]

[1]Korea University

[2]Korea Institute of Geoscience and Mineral Resources

Presenting Author: parkkh831@korea.ac.kr

Biofilm in the subsurface environment develops with the complex interaction between indigenous microbes and various characteristics of surrounding system. Growth cycle of biofilm in long-term can also affect the hydrological characteristics of both fractured and porous media. However, unlike many recent studies focused on the formation under static and eutrophic conditions, formation of the biofilm in deep subsurface environment could reveal different features due to lack of energy and nutrient sources. Therefore, oligotrophic and continuous flow conditions should be considered to simulate formation of the biofilm at deep subsurface and investigate the contributing factors.

In this study, we conducted a long-term in-situ reactor experiment to reproduce the biofilm formation in deep fractured rock media. The morphology and microbial community of the biofilm were compared along with rock types and different geochemical conditions. Two replicates of biofilm reactors were constructed using fractured gneiss and sandstone cores. Each reactor was placed in two adjacent groundwater wells with different depth: 1) bedrock groundwater (depth= 100 m) in anaerobic condition (dissolved oxygen (DO) < 0.6 mg/L) and 2) alluvial groundwater (depth= 30 m) in aerobic condition (DO= ~5.6 mg/L). Both groundwater wells indicated neutral pH (6.7 and 6.3, respectively). Samples were taken after 11 and 19 months from the deployment, accompanied with monitoring of groundwater geochemistry and planktonic microbial community. Microbial community analysis using Illumina MiSeq sequencing of 16S rRNA genes revealed distinct clusters of biofilm community composition along groundwater environment. Generally, biofilm microbial communities showed consistency throughout sampling time compared planktonic microbial communities varying over seasons. Bedrock biofilms indicated similar community compositions with planktonic microbial community, dominance of anaerobic denitrifiers utilizing NO_3^- rich in groundwater. Meanwhile, alluvial biofilms showed more diverse microbes mainly composed of organotrophic aerobes. Visualization and biomass quantification of the biofilm using confocal laser scanning microscopy (CLSM) revealed distinct morphology and development process of biofilm along rock types and oxygen levels. Biofilm on the gneiss formed a thin layered structure with 10-20 µm thick, compared to sandstone with randomly distributed pattern, implying that the morphology of biofilm was governed by rock types with different permeability and surface structure.