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Oil and Gas 2019- Novel Phenazine enhances the bioconversion of coal, prior to methanogenesis- Priyanka Srivastava- University of New South Wales

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Abstract

Artificially-stimulated, high-yield microbial production of methane from coal is a challenging problem that continues to generate research interest. Decomposition of organic matter and production of methane from coal are the results of multiple redox reactions carried out by different communities of bacteria and archaea. Recent work by our group (Beckmenn et.al.,2015) demonstrated that the presence of the redox-mediating molecule neutral red, in its crystalline form on a coal surface, can increase methane production, however essential precursor steps for methane production by archaea, are the hydrolysis and the acetogenesis of the coal. Acetogenesis is the preparation phase of methanogenesis because methanogens can assimilate acetate; CO_2 and H_2 among the products formed during this process. In the present study, the surface chemical analysis of neutral red treated coal, using attenuated total reflectance- fourier transform infrared (ATR-FTIR) and X-ray photoelectron spectroscopy (XPS) demonstrate the production of acetate occur at the nanoscale (<5 nm). We observed that in the presence of 250 µM crystalline form of neutral red and groundwater microbes, acetate signals in coal surface chemistry increased. This is the first evidence suggesting that neutral red enhances the biological deconstruction of coal, consistent with its stimulation of methanogenesis. Neutral red crystals were observed to co-localise with cells at the surface of coal in groundwater. This is consistent with neutral red crystals (NRCs) serving as a redox hub, concentrating and distributing reducing equivalents amongst the microbial community.

Different mechanism of extracellular electron transfer (EET) has been proposed for acetogens. Direct EET involves outer-membrane bound cytochromes, transporting extracellular electrons from outside to inside of the cells and the other way around. This mechanism is well studied in Geobacter sp. Some acetogens have cytochromes but most other acetogens are lacking them, few acetogens use flavins and phenazines to mediate the transport of electrons, another possibility is an indirect electron exchange based on the evolution of H_2 and electron transfer mediated by dissolved redox mediators, and neutral red (proposed hypothesis). Acetogens, accepted these electrons from NRCs by consuming it as a phenazine molecule, and may have used these electrons, in the conversion of CO_2 and H_2, by entering into Wood-Ljungdahl pathway for acetyl-CoA production. These electrons may have transported inside the cells through various channels, present in the cell membrane or by following indirect interspecies electron exchange.

NRCs accepts electrons more efficiently because of the midpoint potential of +444mV, more positive at +69, than the soluble neutral red of -375mV. This makes the crystalline form more amenable to attract electron from organic and inorganic electron carriers present in environment. In the present study, we have found out that acetogens are taking benefits from the 250µM of NRCs. The rate of conversion of coal into acetate was found more in NR treatments in comparison to the controls (without NR). The acetate analysis by GC, indicated the production of acetate, was double in the presence of neutral red, then the cultures without neutral red. This indicates that fermentative microbial communities involved in the production of H2 and CO_2 and acetogens are taking benefits from NRCs, by accepting the electrons, instead of just acetoclastic methanogens. More work is needed in this area to find out the exact mechanism behind the acetogens, accepting electrons from the neutral red, or external electron mediators. With the XPS studies, it was confirmed that, acetogens have changed the top 5nm of the coal surface, this indicates that surface area plays a major role here, the more surface area exposed to microbes, the acetate production will be more. Although, Fourier- transform infrared spectroscopy (FTIR), couldn't detect this change on the surface of coal. However, the scanning electron microscopy, showed mineral deposition on NRCs, and also microbial cells attached to the NRCs. The cells were also observed inside the natural fractures of coal. We have also used scanning electron microscopy (SEM) to show that microfluidics is an effective tool to coat the fracture surfaces of coal with crystalline neutral red. Overall, results suggested that, neutral red not only can benefit acetoclastic methanogens, but also the fermentative and acetogenic bacteria involved in generating acetate.

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