OKLAHOMA EPSCoR UPDATE

Promoting Innovative Research

OK NSF Established Program to Stimulate Competitive Research | May 2021

Salt Loving Microorganisms Can Help Cleanup Salty Produced Water

The oil and gas (O&G) industry produces large volumes of salty wastewater or produced water (PW) during the extraction, transportation, and storage of crude oil. PW is a complex mixture of organic and inorganic compounds and often contains a high-level of salt, reaching > 30% salinity. Depending on the age of the oil well, some estimates indicate that roughly 9 barrels of PW are generated per barrel of oil; this amounts to roughly 21 billion barrels of PW per year in the USA alone, representing a significant quantity of a useful resource for water-stressed regions in USA and the world if treated cost-effectively. As water resources are dwindling, water-users such as industry, agriculture, and households are competing more and more for access to clean water. Added to these stresses, freshwater use by the O&G industry is expected to further intensify competition and conflicts between water users and unconventional O&G operations.

O&G production is critical to the economies of many countries and states, including Oklahoma. However, the vast amounts of PW handling represent one of the more significant environmental challenges to the oil industry, not only from an environmental point of view, but also from a technical and cost view. The most economical option for disposal of this large volume of wastewater has been deep-well injection. While this form of disposal has been viable historically, increased earthquake events and environmental damage have occurred in recent years due to increased injection of PW. Hence, there is a need for cost-effective cleanup technology to allow PW to be used for beneficial purposes.

Dr. Babu Fathepure, Professor of Microbiology and Molecular Genetics at Oklahoma State University (OSU) collaborated with Drs. Mark Krzmarzick (OSU), Robert Murdoch (Battelle Memorial Institute-Ohio), and Frank Loeffler (University of Tennessee-Knoxville) as well as William Marsh and Brenden Heise (students at OSU) to explore an integrated microbes-and-membrane approach to clean up toxic saline PW for beneficial uses. The research team aims to study differ-ent membrane systems for cost-effective desalination of bioremediated PW.

Upcoming Event: **OK NSF EPSCoR Professional Development Seminar Series**
July 26, 2021 @ 12 noon

"We have been investigating the hydrocarbon degradation ability of salt-loving (halophiles) microorganisms from various petroleum-impacted high-salinity environments." Fathepure said. "We have enriched and isolated several pure cultures of bacteria that degrade aliphatic, aromatic, and other organics typically found in PW at high salinity ranging from 3-30% NaCl," Fathepure added.

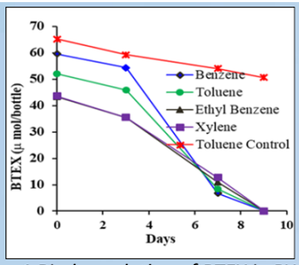

Figure 1 Biodegradation of BTEX in PW from Payne Country by Kuwait culture.

The team analyzed the genomics and metagenomics of hydrocarbon-degrading cultures to gain greater insight into their ecology, physiology, and mechanism of hydrocarbon degradation under high-salinity conditions. "The uniqueness of our approach lies in the application of these specialized halophilic microbes to remove toxic organics in PW, as no other microorganisms can survive extreme salinities," Fathepure said.

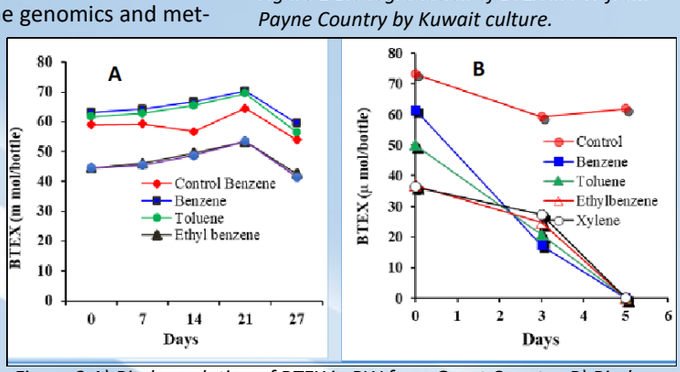

Figure 2 A) Biodegradation of BTEX in PW from Grant County. B) Biodegradation of BTEX in PW mixed with wastewater (50% PW + 50% WW).

The clean bioremediated saline PW, assuming that it meets environmental standards, can be re-used in the O&G industry or for other beneficial uses where salinity is not an issue. Alternatively, it can be desalinated using a membrane system for irrigation and other uses.

The team have evaluated the ability of a microbial culture enriched from a briny oil-field soil sample from Kuwait to degrade hydrocarbons in PW obtained from the Wilcox formation, Payne County, OK and the Mississippian formation, Grant County, OK and have set up laboratory-scale bioreactors with PW and inoculated with the Kuwait culture.

"Our results showed complete degradation of benzene, toluene, ethylbenzene, and xylenes (BTEX) in PW from Payne County in 9 days (Figure 1), suggesting the culture's potential to remediate the PW. On the other hand, degradation of the same compounds in PW from Grant County did not occur even after 4 weeks of incubation by the same bacterial culture (Figure 2)", Fathepure said. "We do not know the exact reason for this inhibition, but our analysis of the PW showed very high levels of total dissolved solids (TDS), NaCl, and heavy metals in this PW compared to PW from Payne County," Fathepure added.

The team further investigated the effect of dilution of the toxic PW on BTEX degradation by the culture. The team mixed the toxic PW (Grant County) with raw wastewater (WW) from Stillwater municipal wastewater treatment plant at various proportions. "Surprisingly, our results showed a complete degradation of BTEX within 5 days. These results are very encouraging because bioremediation of extremely toxic PW can still be accomplished simply by mixing with municipal wastewaters or other wastewaters and we have shown that our strategy can clean up two different wastewaters," Fathepure said. However, initial screening is needed to determine the toxicity of PW. A similar strategy is already in use in Brazil, where PW is mixed with municipal wastewater to clean up toxic PW biologically. The research group have also isolated a pure culture of a halophilic bacterium that degrades both aromatic and aliphatic hydrocarbons in the presence of salt ranging from 5 – 24% NaCl from the Payne County PW. "This organism has been designated as *Modicisalibacter* sp. strain Wilcox and is a close relative of *Modicisalibacter tunisiensis* isolated from an oil-field water in Tunisia and we have sequenced strain Wilcox' genome," Fathepure said. Genome analysis predicted the presence of several genes for the degradation of a variety of aliphatic and aromatic hydrocarbons as well as genes that confer resistance to a variety of heavy metals. This work has been recently published in Scientific reports journal. Please refer to *Scientific Reports. 2021. 11(1):6943.*

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