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CASE STUDY: EVALUATION OF PURE AND MIXED BACTERIAL CULTURES ON SULFOLANE BIODEGRADATION IN AQUEOUS MEDIA

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Abstract: The objective of this research is to evaluate the feasibility of using *P*seudomonas and *Archaea* to aerobically biodegrade sulfolane in aqueous medium. Their sulfolane degradation capacity was also compared with the performance of aquifer sediments contain mixed microbial populations indigenous to sulfolane contaminated sites. In addition, *Pseudomonas* and *Archaea* were amended with indigenous microbes to enhance their sulfolane degrading efficiency. Acclimatization strategy was also adopted to reduce the lag period exhibited in *Pseudomonas*' sulfolane biodegradation process. Experimental results show that sulfolane can be adequately degraded by both pure and mixed microbial cultures. *Pseudomonas* demonstrated a better sulfolane degrading capability among all three microbes. Adding indigenous microbes with *Pseudomonas* and *Archaea* resulted in accelerated sulfolane degradation.

Keywords: Sulfolane, biodegradation, *Pseudomonas*, *Archaea*

1 PROJECT OVERVIEW

Oil and gas industry is one of the major energy sectors in the Western Canada and bringing economic prosperity to society over the past half century. However, large amount of chemical wastes have been produced during the natural gas production process and disposed into the environment. In the early 1960s, Shell introduced the Sulfinol® process for the sweeting of sour gas which utilized a solvent named sulfolane ($C_4H_8SO_2$) to extract H_2S and other undesirable compounds. Despite the effective usage of sulfolane in treating high H_2S concentration sour gas, spills, disposal of sulfolane containing waste and leaching from process water storage ponds have resulted in sulfolane entering nearby groundwater and soil (Greene and Fedorak 2001). With high water miscibility and mobility, sulfolane contamination has spread off-site in many locations. According to toxicological studies, experimental animals exposed to sulfolane can experience leukopenia, convulsions, depression and hypoactivity (Andersen et al. 1977, Ruppert and Dyer 1985). Considering the widespread sulfolane contamination and potential health hazard toward humans, there is a need to develop an effective and environmentally friendly method to remediate sulfolane contaminated groundwater and soil.

Among the various water treatment technologies, bioremediation provides an efficient and economical solution in removing many contaminants from aqueous media. In most cases, bioremediation leads to complete degradation of pollutants into non-toxic compounds such as carbon dioxide and water (Fernández-Luqueño et al. 2011). Past research has shown sulfolane can be biologically degraded under aerobic conditions (Fedorak and Coy 1996, Greene et al. 1998). While research on aerobic degradation of sulfolane using indigenous microbes contain mixed microbial cultures are successful, there is limited study focused on the application of pure microbes. The use of mixed microbial cultures in bioremediation have

the advantage of synergistic interactions amongst various cultures (Ghazali 2004). However, in certain cases the use of pure microbial culture with desirable phenotypic characteristics can possibly expedite the biodegradation process. To this end, it is essential to explore the potential of pure microbe culture treatment of sulfolane in water.

In this project, the aerobic biodegradation capacity of sulfolane by pure microbial culture was evaluated and compared with the performance of mixed bacterial cultures. Two microbial strains namely *Pseudomonas* and *Archaea*, and aquifer sediments contain mixed microbes indigenous to sulfolane contaminated site were used. In addition, amendment and acclimatization strategy were used to enhance the efficacy of pure microbial cultures.

2 METHODOLOGY

Sulfolane biodegradation capability of *Pseudomonas*, *A*rchaea and aquifer sediments was evaluated through batch experiments. 0.5 g of biomass were added into 300 ml solution containing 100 ppm sulfolane. Each sample was supplemented with appropriate nutrients and aerated during the experiment. Water samples were collected periodically and filtered to remove the suspended biomass, then subjected for further sulfolane analysis.

Pure *Pseudomonas* and *Archaea* culture were also amended with aquifer sediments to enhance their sulfolane degrading efficiency. Experimental procedure followed the same step as described above, except for the introduction of additional 0.05 g aquifer sediments into each sample. Acclimatization technique was used to eliminate the potential lag period that exist in *Pseudomonas*' sulfolane degradation process. Acclimatized *Pseudomonas* was achieved by cultivating 50 g of *Pseudomonas* strains in 4 L solution containing 200 ppm sulfolane solution for a period of time. Upon the completion of acclimatization stage, the acclimatized *Pseudomonas* was collected, dried and stored for use of further biodegradation experiments.

3 INNOVATIONS AND FINDINGS

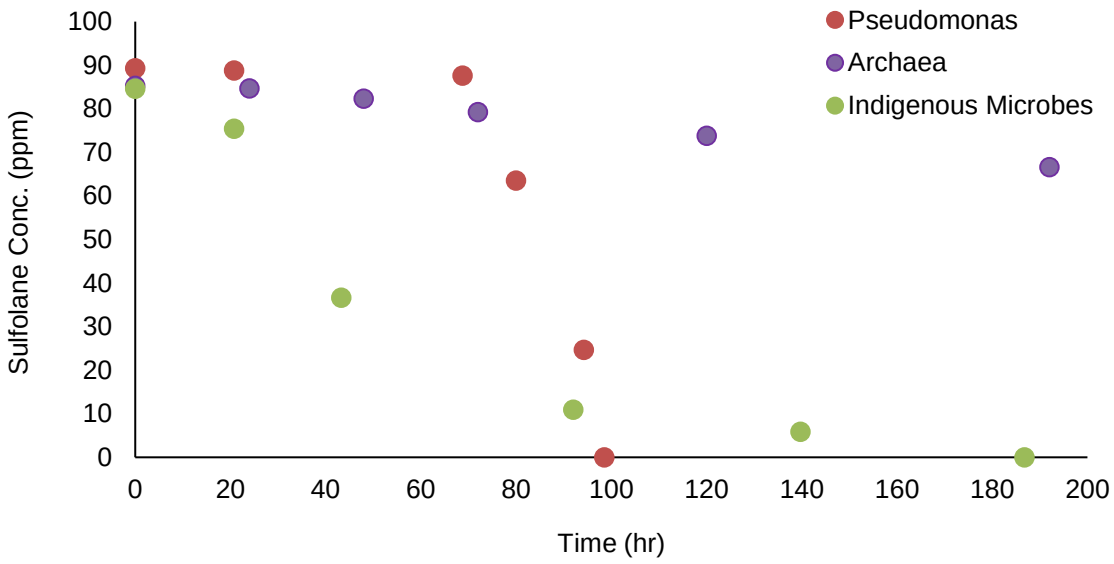

Figure 1. Sulfolane degradation performance of *Pseudomonas*, *Archaea* and indigenous microbes

Experimental results showing the degradation performance of *Pseudomonas*, *Archaea* and indigenous microbes are presented in Figure 1. All three microbes are effective on aerobically degrading sulfolane in an aqueous media. For *Pseudomonas,* sulfolane was degraded to below detectable concentration within

100 hr. It was also found that *Pseudomonas* have a lag period of about 70 hr before the initiation of sulfolane biodegradation. *Archaea* demonstrated a much slower degradation rate compare with *Pseudomonas*. Around 4.5% of sulfolane was removed within 48 hr which signifies the initiation of sulfolane biodegradation, and around 22% of sulfolane removal was achieved after 200 hr of incubation. Microbes indigenous to the sulfolane environment degraded the sulfolane fully within 190 hr.

The sulfolane degrading efficiency of pure *Pseudomonas* and *Archaea* culture were enhanced through the addition of amendments (aquifer sediments). Table 1 presents the percent sulfolane removed by amended *Pseudomonas* and *Archaea* at different time intervals. In comparison to the 4% and 15% sulfolane removal achieved by pure *Pseudomonas* and aquifer sediments within 25 hr, amended *Pseudomonas* have shown to degrade sulfolane fully within the same time period. The amended *Archaea* have also demonstrated an increased suflolane removal efficiency. After 100 hrs, the amended *Archaea* degraded 30% of sulfolane while pure *Archaea* only achieved 14% sulfolane removal. From the obtained results, it is evident that microbial diversification can improve pure culture microbes' sulfolane degradation performance.

Table 1. Percent sulfolane removed by *Pseudomonas* and *Archaea* amended with indigenous microbes

Time (hr)	Sulfolane Percentage Removal (%)				
	Aquifer Sediments	Pure *Pseudomonas*	Amended *Pseudomonas*	Pure *Archaea*	Amended *Archaea*
0	0	0	0	0	0
25	15	4	100	3.5	2.5
50	55	12	-	4	9.5
75	75	30	-	7	20
100	90	100	-	14	30

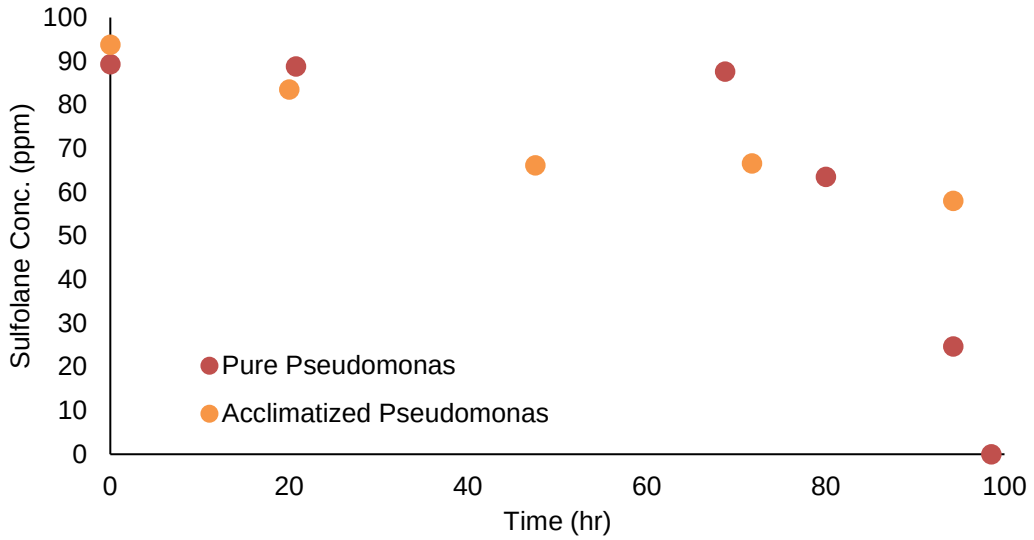

Figure 2. Comparison of sulfolane degradation capability of pure and acclimatized *Pseudomonas*

The sulfolane degrading efficiency of *Pseudomonas* can also be enhanced through acclimatization which helps to reduce the lag period exist in biodegradation process. Figure 2 compares the performance of pure and acclimatized *Pseudomonas*. It is noted there is no observable lag period exist in acclimatized

Pseudomonas, indicating the *Pseudomonas* had fully acclimatized to the sulfolane environment. However, only 40% of the sulfolane was degraded within 95 hr while pure *Pseudomonas* led to complete sulfolane removal, suggesting the acclimatized Pseudomonas lack of robustness in degrading sulfolane.

4 LESSONS LEARNED

Among three microbial cultures investigated (*Pseudomonas*, *Archaea* and indigenous microbes), Pseudomonas (even though it had a lag phase) demonstrated better sulfolande degradation capacity, indicating the effectiveness of utilizing pure cultured microbes in treating sulfolane contaminated waters. The expedited sulfolane biodegradation of *Pseudomonas* and *Archaea* amended with indigenous microbes suggested a possible synergistic interaction developed between the pure culture microbes and indigenous microbes. Acclimatization successfully reduced *Pseudomonas*' lag period in treating sulfolane, but also decreased its sulfolane degradation rate. The decreased degradation rate can be possibly attributed to the loss of metabolic activity during the drying process of acclimatized *Pseudomonas*. It is expected with adequate drying technique, acclimatized *Pseudomonas* with exceptional robustness in degrading sulfolane can be developed and used for rapid remediation of sulfolane in contaminated waters.

5 CONCLUSION

The following conclusions are drawn from this study:

- Both pure and mixed microbial culture can aerobically biodegrade sulfolane in aqueous media.
- Pure *Pseudomonas* (after an initial lag period) achieved better sulfolane degrading capability than *Archaea* and aquifer sediments contain indigenous microbes.
- *Pseudomonas* and *Archaea* amended with indigenous microbes demonstrate an increased sulfolane degrading efficiency.
- No lag period is observed on *Pseudomonas* acclimatized to the sufolane environment.

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