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BIOTRANSFORMATION OF AROMATIC COMPOUNDS IN OIL SANDS PROCESS-AFFECTED WATER BY PSEUDOMONADS

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The potential environmental impact of oil sands process-affected water (OSPW), contained in storage ponds until eventual release after treatment, has received extensive attention in recent years. Biological treatments, which exploit degradation by microorganisms, have been used successfully for wastewater reclamation in various industries. However, previous work has demonstrated the recalcitrance of OSPW to biological treatment. Aromatic naphthenic acids (NAs) in OSPW have been proved to cause more toxicity of OSPW than classic NAs. No study has yet been conducted for the biodegradation of aromatic compounds in OSPW, although synthetic aromatic alkanoic NAs have been used to investigate the mechanisms involved in aromatic NA degradation. *Pseudomonas fluorescens* and *Pseudomonas putida* have been demonstrated to have the capacity to degrade commercial NAs; however, no classical NAs in OSPW could be degraded. As Pseudomonads have showed the capability to biodegrade aromatic compounds, those two Pseudomonas species were used to treat aromatic compounds in OSPW in this study. Furthermore, biotransformation by Pseudomonads was also employed to remove the residual toxicity after ozonation. The objective of the current study was to determine the potential biodegradation of aromatic compounds in OSPW by *P. fluorescens* and *P. putida*. This degradation was monitored by assessing the synchronous fluorescence spectroscopy (SFS) spectrum before and after treatment to determine the biodegradation of aromatic compounds as described previously. Due to the recalcitrance of organic compounds in OSPW, an external carbon source was added to provide adequate bacterial nutrition. In addition, various concentrations of this external carbon source, and an external iron source, on biodegradation were evaluated. Furthermore, the role of the Pseudomonads in removing the residual toxicity of ozonated OSPW was also investigated.

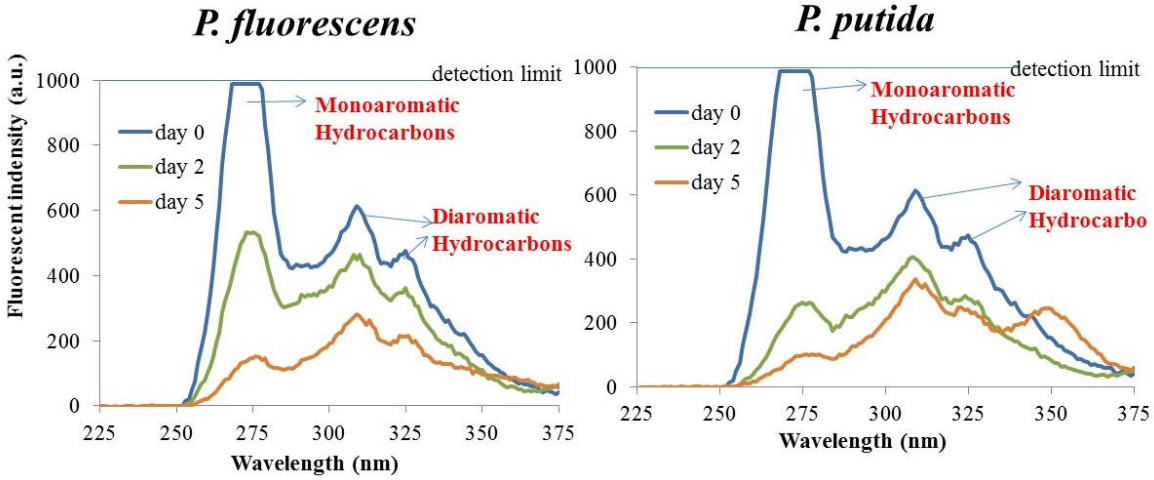

Fig. 1. The degradation of aromatic compounds after exposure to OSPW as indicated by the changes of SFS spectrum without the addition of $FeCl_3$ [1]

After exposure to raw OSPW, significant bacterial growth was observed for both *P. putida* and *P. fluorescens*. In the presence of $FeCl_3$, more biomass was produced as compared to those without $FeCl_3$ treatments. The OSPW SFS spectrum (Figure 1) revealed a maximum peak at 273 nm (one aromatic ring) and other peaks at 310 nm and 324 nm (two aromatic rings), which is consistent with previous OSPW work. Rowland et al. [2] indicated that these peaks around 282 nm and 320 nm coincided with dehydroabioatic acid (a specific monoaromatic compound) and 1,2,3,4-tetrahydrophenanthrene 1,2-dicarboxylic acid, dimethyl ester (a specific diaromatic acid), suggesting that those peaks could be attributed to mono and diaromatic compounds, respectively. The SFS spectra changed over the 5-day treatment period indicating the biodegradation of aromatic compounds (Figure 1). The intensity of the monoaromatics peak at 273 nm declined more significantly than the multiple-rings aromatics peaks at 310 nm and 324 nm. This indicates that the monoaromatics are more rapidly biodegraded which is in agreement with a previous study. For the killed controls, the SFS spectra did not change, indicating that aromatic compounds were not being removed due to biomass adsorption. To further confirm the degradation of aromatic compounds, GC-MS was applied to determine the BTEX, PAHs and PAH and petroleum hydrocarbon (PHC) fractions after treatment. After biodegradation, PHC fraction F2 (C10-C16) became undetectable after biodegradation that indicates the effectiveness of Pseudomonads degradation of this fraction.

Results of the Microtox® *V. fischeri* assay (Figure 2) showed that all Pseudomonads treatments have significantly reduced toxicity of raw OSPW (P value <0.05). This was unexpected as there was no significant reduction in the NAs after biodegradation of raw OSPW without $FeCl_3$ addition, which suggests that the toxicity reduction must be due to the degradation of other compounds. As indicated previously, biodegradation reduced the overall aromatics as indicated by SFS and FT-IR results. In addition, this reduced toxicity may be the result of the biotransformation, versus complete biodegradation, of the NAs to less toxic compounds in these treatments. The addition of $FeCl_3$ improved the toxicity reduction for both Pseudomonads for both raw and ozonated OSPW treatments. Although an ozonation dose of 80 mg/L reduced the classical NAs concentrations in OSPW by more than 90%, the toxicity of ozonated OSPW was still significant (16.7% effect to the luminescence of *V. fischeri*). This result further indicates that classical NAs may contribute a smaller portion of the OSPW toxicity than previously considered, making the toxicity reduction performance of ozonation more limited. However, the biodegradation of the ozonated OSPW showed promising results with decreases in the toxicity by both Pseudomonads with and without $FeCl_3$ addition (P value < 0.02). Of note is the elimination of toxicity after biodegradation for both species in the presence of $FeCl_3$. This indicates that biodegradation is an excellent candidate for removing the residual toxicity after ozonation.

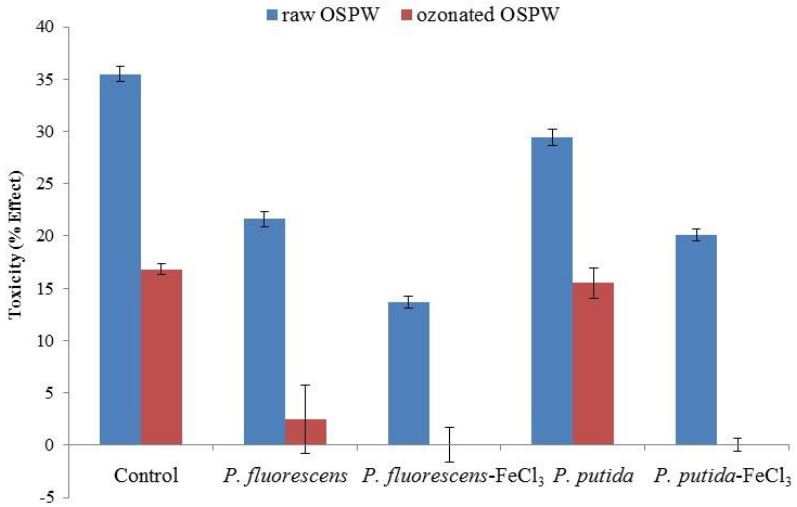

Fig. 2. Microtox® toxicity after exposure to raw and ozonated OSPW with and without $FeCl_3$ addition on Day 2. [1]

A limitation of recalcitrant compounds' biodegradation may be attributed to the lack of available carbon for nutrition. To investigate this limitation, the SFS peaks including 273, 310 and 324 nm (discussed previously) of various sodium acetate concentrations were considered for the Pseudomonads over a 4-day duration for treatments. When the concentration of sodium acetate was less than 0.5 g/L, only limited biodegradation was observed indicating that the external carbon source played an important role in mitigating ring cleavage. When the concentration of sodium acetate was higher than 1 g/L, biodegradation of aromatic compounds started to increase with increasing acetate concentration and the highest reduction of peak intensities were observed when the acetate concentration was 4 to 5

g/L. Interestingly, the results of toxicity tests showed that the addition of acetate greater than 1 g/L did not further reduce toxicity despite the continued reduction in peak intensities.

In summary, *P. fluorescens* and *P. putida* showed their capability in the biodegradation of OSPW aromatic compounds, especially for the petroleum hydrocarbons (C10-C16). Although no significant NAs were degraded during this process, the toxicity of OSPW was reduced significantly after biodegradation. The external carbon source played an important role in ring cleavage. The addition of iron could accelerate the growth of biomass and reduction of toxicity. Our study indicates biotransformation by Pseudomonads is a complementary approach to ozonation to eliminate the residual toxicity of ozonated OSPW.

Reference

1. Zhang, Y., K.N. McPhedran, and M.G. El-Din, *Pseudomonads biodegradation of aromatic compounds in oil sands process-affected water.* Science of the Total Environment, 2015. **521**: p. 59-67.
2. Rowland, S.J., et al., *Steroidal aromatic Naphthenic Acids in oil sands process-affected water: Structural comparisons with environmental estrogens.* Environmental Science and Technology, 2011. **45**(22): p. 9806-9815.