Assessing the mechanisms of toxicity of oil sands process affected water by use of a genome wide live cell array reporter system

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INTRODUCTION

Oil Sands Process Affected Waters (OSPW) • OSPW is a by-product from the extraction and separation of bitumen in the Alberta Oil Sands [1], • OSPW is restricted to a zero-discharge policy and requires remediation before release [2], • OSPW has acute and chronic toxicity to a range of species [3, 4]. • Causative agents and mechanisms of toxicity are unknown but studies have shown that the dissolved organic fraction of OSPW is acutely toxic [5]. • Napthenic acids, class of cyclic and acyclic compounds with a -COOH functional group, have been proposed as the main toxic constituents of the organic fraction of OSPW. • Oxidative stress might contribute to the toxicity of OSPW [5, 6].

Escherichia coli K-12 MG-1655 gene reporter system • Open-format approaches for investigating the adverse effects of chemicals have gained popularity in recent years [7]. • The Live Cell Array (LCA) system facilitates measurement of promoter activity by use of transcriptionally fused fast-folding fluorescent proteins [8]. • E. coli MG-1655 system is composed of 1820 promoters in its genome.

OBJECTIVES
1. Perform an effects-directed analysis (EDA) of the organic fraction of OSPW to identify compounds responsible for acute toxicity.
2. Assess the molecular mechanisms of toxicity of fractions of organic compounds from OSPW by use of the E. coli MG1655 gene reporter system.
4. Combine acute toxicity data and gene enrichment profiles to compare molecular mechanisms of toxicity between toxic and non-toxic fractions. 5. Compare results with chemical profile of fractions to identify chemicals in OSPW that cause toxicity.

MATERIALS & METHODS

1. OSPW Fractionation and acute toxicity assays

Figure 1. Fractionation of the OSPW organisms. Round 1 fractionation was sequential solvent extraction at pH 7, 2 and 11. Round 2 fractionation was liquid-liquid washing at pH 2 and 12. Acutely toxic fractions were identified using A) The Microtox® system, B) 96-h growth and lethality test Chironomus dilutus and C) 96-h embryo toxicity test Pimephales promelas. The OSPW was collected from the Base mine lake and is the first end pit lake in the industry.

RESULTS

Figure 3. Clustering of the fraction- and time dependent expression of the 68 genes altered at least 2-fold change over background. The fold change of gene expression is indicated by the colour gradient, and time course expression changes from left to right.

Figure 4. Fraction induced response in the number of differentially expressed genes from three fractions of OSPW organics.

Table 1. Fraction induced response in the number of differentially expressed genes from three fractions of OSPW organics.

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Figure 5. Total abundance of species by heterotatom class based on the sum of the peak areas in the chromatograms of fractions of OSPW, by use of Orbitrap Mass spectrometry: A) NEF1 and NEF2 in ES+, B) NEF1 and NEF2 in ESI-.

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CONCLUSIONS

- An acutely toxic fraction (NEF2) of OSPW was identified using a suite of bioassays and contains the enriched chemical classes: ESI-, -O2-, -O, -O2-, -O3-, -O4-, O2N, -O2N.
- Gene enrichment analysis identified two processes that responded to the toxic fractions (NE, NEF2) of OSPW: Functioning of the electron transport chain (ETC; tric, atp6) • Stress response (clpX, dnaK, clpC, ssaB, mfd)
- Pathway analysis revealed significant responsiveness in the KEGG pathway (Kyoto Encyclopedia of Genes and genomes) ubiquinol and terpenoid biosynthesis: specifically affecting genes related to Oxidative Phosphorylation.
- Ub, UbiC > 2-fold down-regulation.
- Ionizable compounds are known to effect the electron transport chain... a role for naphthenic acids...would contribute to toxicity?

FUTURE WORK

- Continue EDA and fractionate NEF2 fraction.
- Screen fractions of NEF2 by use of LCA system.
- Investigate role of electron transport chain in toxicity of BML-OSPW.

REFERENCES


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