Comparison of ozonation and reclamation pond biodegradation as treatments to eliminate oil sands process-affected water toxicity in *Chironomus dilutus*


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Overview

• Background
  — Alberta oil sands
  — Oil sands process-affected water
  — Biodegradation
  — Ozonation

• Study objectives

• Methods

• Results

• Discussion

• Future work
Background

• Alberta oil sands
  - Represented over 50% of Canadian crude oil production in 2010 (CAPP, 2011)
  - 1.5 million barrels of bitumen produced per day in 2009
    (Government of Alberta, 2011)

• Oil sands process-affected water (OSPW)
  - Produced during extraction of bitumen from oil sands
    $\Rightarrow \sim 1$ billion $m^3$ currently in active settling basins (Han et al., 2008)
Background

- **Oil sands process-affected water**
  - Major constituents:
    - Salts
    - Metals – Al, As, Cu, Fe, Ni, Pb, V, Zn, others
    - Ammonia
    - Naphthenic acids (NAs) – complex mixture of carboxylic acids found at elevated levels in OSPW

- **NAs**
  - Naturally occur in bitumen; solubilized and concentrated by extraction process and water recycling
  - Believed to be responsible for majority of OSPW toxicity to aquatic organisms → **targets for treatment efforts**
Background

• Biodegradation of OSPW
  — Indigenous microbial populations have the ability to degrade NAs to some extent
  — Method currently in use while OSPW is stored in active settling basins
  — Toxicity of OSPW shown to persist following aging
    • Impaired reproduction in fathead minnows (Kavanagh et al., 2011)
Background

• Ozonation
  – Popular for water treatment – disinfecting properties, precipitation of heavy metals/metal complexes, oxidizes ammonia, eliminates toxic organics

  – OSPW found to be non-toxic (using Microtox®) after 50 minutes and 70% NA reduction (to 20mg/L) (Scott et al., 2008; Gamal El-Din et al., 2011)

  – Greater MW NAs removed by ozonation – targets persistent NA fractions → increases microbial degradation (Martin et al., 2010; Gamal El-Din et al., 2011; Perez-Estrada et al., 2011)
Overall objectives

• 1. To characterize the effects of exposure to untreated OSPW in a benthic invertebrate model, *Chironomus dilutus*
  — “Fresh” OSPW from West In-Pit settling basin

• 2. To determine whether treatment of OSPW using ozonation or biodegradation ("aging") effectively reduces its toxicity to *C. dilutus* larvae
Study 1: Acute toxicity of OSPW
Study objectives

1. To characterize the effects of short-term (10 d) exposure to OSPW in *Chironomus dilutus* larvae

2. To determine whether treatment of OSPW using aging or ozonation effectively reduces its toxicity to *C. dilutus*
   - Assessed in terms of survival and growth (as fresh mass)
Methods

• *Chironomus dilutus* larvae – 8-9 days old
• 10-day exposure - survival and measurement of mean fresh mass
• Daily feeding and 50% water change on alternating days
• Waters: Freshwater control, saltwater control, untreated OSPW, ozonated OSPW, aged OSPW
  – OSPW collected from Syncrude West In-Pit settling pond in (A) 2009 or (B) 2010
  – Aged water from Big Pit, FE5, and TPW
• 2 degrees of ozonation – (A) 30 mg/L or (B) 80 mg/L applied to WIP-OSPW
Treatment waters

• WIP-OSPW:
  - Total [NAs] – 70-72 mg/L in both WIP-OSPW-A and WIP-OSPW-B (as measured by FTIR)

• Ozonated-OSPW:
  - Total [NAs] – 16 mg/L in OSPW-80

• Aged waters:
  - Big Pit – mature fine tailings capped with freshwater in 1993; [NAs] – 23 mg/L
  - FE5 – mature fine tailings capped with OSPW in 1989; [NAs] – 13 mg/L
  - TPW- OSPW aging since 1993; [NAs] – 35 mg/L
Results - Survival

- Significantly less survival in WIP-OSPW-A (p<0.05)

- Less survival in WIP-OSPW-B (non-significant trend)

- No differences among ozonated or aged OSPW and controls

Significant differences from the freshwater control were determined using a one-way ANOVA followed by Tukey's HSD post-hoc test (n=4, $\alpha = 0.05$).
**Results - Growth**

- WIP-OSPW-A-exposed larvae had 64% less fresh mass than freshwater controls.

- WIP-OSPW-B-exposed larvae had 79% less fresh mass than freshwater controls.

- Exposure to ozonated OSPW – significantly attenuated growth inhibition effects, but lesser masses than controls (22% and 32% less mass).

- No effects on fresh mass following aging of OSPW.

Significant differences from the freshwater control were determined by one-way ANOVA followed by Tukey’s HSD post-hoc test ($n=4, \alpha =0.05$).
Study 2: Chronic toxicity of OSPW
Study Objectives

• 1. To characterize the effects of long-term exposure to OSPW in *Chironomus dilutus* larvae

• 2. To determine whether treatment of OSPW using aging or ozonation effectively reduces its toxicity to *C. dilutus*

  — Assessed in terms of pupation, emergence, sex ratio
Methods

- Followed same exposure methodology as previous study, except activated charcoal-treated FE5 and WIP-OSPW-A

- Collected adults and recorded sex and day of emergence

- Noted larval and pupal deaths and recorded time to pupation

- Allowed all individuals from beaker to emerge or die before takedown
## Results – Pupation

- Significantly less pupation in WIP-OSPW-B vs. freshwater controls ($p<0.05$)

- Significantly less pupation in TPW (but not other aged waters) vs. controls

- Pupation in ozonated-OSPW no different from controls

### Mean pupation (%)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Pupation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshwater control</td>
<td>80</td>
</tr>
<tr>
<td>AC-WIP</td>
<td>60</td>
</tr>
<tr>
<td>AC-FES</td>
<td>60</td>
</tr>
<tr>
<td>WIP-OSPW-A</td>
<td>40</td>
</tr>
<tr>
<td>OSPW-30</td>
<td>20</td>
</tr>
<tr>
<td>WIP-OSPW-B</td>
<td>20</td>
</tr>
<tr>
<td>OSPW-80</td>
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</tr>
<tr>
<td>Big Pit</td>
<td>20</td>
</tr>
<tr>
<td>FES</td>
<td>20</td>
</tr>
<tr>
<td>TPW</td>
<td>20</td>
</tr>
</tbody>
</table>

Significant differences from the freshwater controls were determined by one-way ANOVA followed by Tukey's HSD post-hoc test ($n= 4$ or $8$, $\alpha =0.05$).
Results-Emergence

- Significantly less adult emergence in both WIP-OSPW-A and WIP-OSPW-B ($p<0.001$)
- Generally fewer emerging adults in aged-OSPW, statistically fewer in TPW vs. controls
- Ozonation attenuated effects on emergence
- Sex ratios no different from 1:1 in any treatment

Significant differences from the freshwater controls were determined by one-way ANOVA followed by Tukey's HSD post-hoc test ($n= 4$ or $8$, $\alpha = 0.05$).
Discussion

Based on studies 1 and 2, exposure of *C. dilutus* larvae to untreated OSPW may cause:

- Some reduced survival
- Significant growth inhibition
- Reduced pupation
- Severely reduced emergence of adults
Discussion

• Biodegradation of OSPW:
  – Results in *lesser concentrations of NAs* (vs. fresh WIP-OSPW)
  – Eliminated effects on growth and survival of larvae
  – Failed to eliminate effects on pupation and emergence – both were less than controls, especially in TPW (greatest concentration of NAs) – *chronic toxicity remains* ➔ *active treatment required*
Discussion

• Ozonation of OSPW has potential to:
  – Attenuate growth inhibition effects
  – Eliminate any reductions in survival
  – Improve pupation and emergence success
  – Minimize toxicity on a much shorter time scale than required by biodegradation
Conclusions

• Untreated OSPW resulted in toxicity in a benthic invertebrate model following both short-term and long-term exposures

• Toxicity manifested mainly as growth inhibition and impaired adult emergence

• Biodegradation improved survival and growth, but did not eliminate impairment of pupation and emergence

• Ozonation of OSPW attenuated the observed acute and chronic toxicity
Future work

• Field surveys - Implications of growth and emergence inhibition at the community level
  • Are effects occurring on-site?

• Elucidate mechanism of toxicity
  • Expression of metabolic, endocrine-related, and ribosomal protein genes
  • Any risk of new toxicity from ozonation by-products?

• Improve fractionation abilities to identify specific NAs of interest
  • Bioassays with different fractions since NA profiles varied in current tests
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Thanks for your attention!

Questions?