Production optimization and characterization of trehalose lipid-based biodispersants for oil spill response

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Introduction

- **Biodispersants**
  - Use biosurfactants produced by microorganisms to replace the chemically synthetic surfactants in dispersant formula
  - Advantages of biosurfactants (Muthusamy et al., 2008)
    - Low toxicity • High Biodegradability
    - More effective and stable even at extreme conditions
    - Can be produced from various sources (e.g., industrial wastes)
    - Simple and inexpensive procedures

- **Trehalose lipid-based biodispersants**
  - Trehalose lipids produced by *Rhodococcus erythropolis* were found with superior oil dispersion efficiency in our previous study (Cai et al., 2016) when compared with some prevalent biosurfactants (e.g., rhamnolipid, surfactin, and elsanol).

- **Challenges and opportunities**
  - Bottleneck of biosurfactant application • High production cost and low yields.
  - In our previous study, mutagenesis was applied to enhance productivity • We generated mutant M36, which has more effective and stable even at extreme conditions biosurfactants (Franzetti et al., 2010)

Objectives

- To optimize the fermentation conditions for the production of trehalose lipids using *Rhodococcus erythropolis* mutant M36
- To investigate the effect of activated carbon (AC) as the solid carrier in assisting the production
- To evaluate the performance of the optimized product • Critical micelle concentration (CMC), dispersion effectiveness, toxicity, and biodegradability

Methodology

- **Minimum resolution V design**
  - Design Type: Minimum Run Resolution V
  - Runs: 42
  - Center Points: 4

<table>
<thead>
<tr>
<th>Factor</th>
<th>Name</th>
<th>Units</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>Std. Dev.</th>
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<tbody>
<tr>
<td>A</td>
<td>AC</td>
<td>g/L</td>
<td>1</td>
<td>19</td>
<td>10</td>
<td>8.56</td>
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<tr>
<td>B</td>
<td>Salinity</td>
<td>g/L</td>
<td>10</td>
<td>30</td>
<td>20</td>
<td>9.51</td>
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<tr>
<td>C</td>
<td>pH</td>
<td>1</td>
<td>5</td>
<td>8</td>
<td>6.5</td>
<td>1.43</td>
</tr>
<tr>
<td>D</td>
<td>Carbon</td>
<td>%</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>0.95</td>
</tr>
<tr>
<td>E</td>
<td>Nitrogen</td>
<td>g/L</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>0.95</td>
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<tr>
<td>F</td>
<td>Mg</td>
<td>g/L</td>
<td>0.1</td>
<td>0.3</td>
<td>0.2</td>
<td>0.10</td>
</tr>
<tr>
<td>G</td>
<td>Ca</td>
<td>g/L</td>
<td>0.01</td>
<td>0.09</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>H</td>
<td>Fe</td>
<td>g/L</td>
<td>0.01</td>
<td>0.09</td>
<td>0.05</td>
<td>0.04</td>
</tr>
</tbody>
</table>

  Response: Units
  - Total carbohydrate: mg

- **Product extraction**
  - Extracted with 75% formic acid: • Neutralizing and washing with water phase
  - Extracted with 1 M NaOH solution: • Rotary evaporation

- **Performance evaluation**
  - To evaluate the performance of the optimized product • Critical micelle concentration (CMC), dispersion effectiveness, toxicity, and biodegradability

Results

- **Production optimization**
  - ANOVA for selected factorial model
  - Analysis of variance table (Partial sum of squares - Type III)

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F Value</th>
<th>p-value</th>
<th>Prob &gt; F</th>
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<tr>
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<td>54.00</td>
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<td>25.35</td>
<td>18.04</td>
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<tr>
<td>F-Mg</td>
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<td>15.25</td>
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<td>0.0024</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>G-Ca</td>
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<td>1</td>
<td>15.17</td>
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<td>0.0024</td>
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<tr>
<td>H-Fe</td>
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<td>1</td>
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<td>0.0001</td>
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<tr>
<td>FG</td>
<td>14.91</td>
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<td>14.91</td>
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<tr>
<td>FH</td>
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<tr>
<td>Cor Total</td>
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<td>41</td>
<td>5.40</td>
<td>4.26</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
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</table>

  - **Dispersion effectiveness**
    - CMC < 0.215 g/L
    - Weathered ANS crude oil
    - Both dispersants used at 2.5 CMC

  - **Toxicity**
    - Microtox® Toxicity test
    - Microtox® Toxicity test

Conclusions

- Concentrations of AC, carbon source, Mg2+, Ca2+, and Fe2+ were significant factors affecting the productivity, while salinity (1%-3%), pH (5-8) and concentration of nitrogen source (1-3 g/L) were insignificant factors.
- AC was found to have negative impact on the production.
- Production conditions for trehalose-lipid was optimized using response surface methodology.
- Trehalose-lipid based biosurfactants performed comparable with Corexit 9500A in terms of dispersion effectiveness, while having lower toxicity and higher biodegradability.

References


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