VALORIZATION OF OLIVE MILL WASTEWATER VIA MICROALGAE

ASTRID LINDNER
PHD STUDENT
SUSTAINABLE CHEMISTRY (RESOURCE EFFICIENCY)
Problem

MASS PRODUCTION OLIVE OIL

Olive mill wastewater (OMW)
- 45 billion L annually

PHENOLIC COMPOUNDS

Phenolic compounds
- Up to 24 g/L, mostly 1 to 10 g/L
- Mainly tyrosol, hydroxytyrosol, oleuropein

Antimicrobial properties
- Disturbance in natural habitat/microbial equilibrium
- Avoid release into environment
- Utilization instead of simply removing (what happens afterwards?)

LACK OF INFORMATION

Removal via microalgae discussed, but no exact mechanisms
Less focus on OMW-related phenolic compounds – rather halogenated and added instead of „natural“ origin
Approach

Search for microorganisms able to degrade these structures & transform into value-added products

Microalgae as advantageous organisms

Reduction of BOD and COD, while producing proteins, lipids, carbohydrates

Previous research shows possible ring-fission and mineralization\(^1\) as well as transformation\(^2,3\)

\(^1\)Ellis, B.E., *Degradation of phenolic compounds by fresh-water algae*. Plant Science Letters, 1977. 8: p. 213-216.


Cultivation in flasks and bioreactors

Cultivation strategies

Flasks: Screening with OMW (1, 6, 12, 25 %, control)
In dark and under light (50 µmol/m²s) conditions,
30 °C, shaken 90-110 rpm, AF6 medium, pH 6 - 7
dark: 1 g/L glucose
Cultivation strategies

Reactor:
• 800 mL total volume, 1 % OMW, AF6 medium
• Dark, 1 g/L glucose
• 30 °C, aeration + magnetic stirring
• Controlled at pH 6.8
• Dissolved oxygen monitoring
• Continuous OD measurement
Analysis

Phenolic compounds:
Folin-Ciocalteu reagent – detection in the supernatant (tyrosol equivalent)

Cell growth:
– Optical density at 750 nm
– Correlation to cell density or biomass dry weight
Removal from the supernatant after 10 days

Removal from 1 % OMW cultures

- C. vulgaris, HET
- C. vulgaris, PHO
- A. obliquus, HET
- M. braunii, HET
- M. braunii, HET reactor

Removal [%]

<table>
<thead>
<tr>
<th>Sample</th>
<th>Removal [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. vulgaris, HET</td>
<td>not detected</td>
</tr>
<tr>
<td>C. vulgaris, PHO</td>
<td>5</td>
</tr>
<tr>
<td>A. obliquus, HET</td>
<td>15.3</td>
</tr>
<tr>
<td>M. braunii, HET</td>
<td>55.6</td>
</tr>
<tr>
<td>M. braunii, PHO</td>
<td>8.8</td>
</tr>
<tr>
<td>M. braunii, HET reactor</td>
<td>13.3</td>
</tr>
</tbody>
</table>
**Monoraphidium braunii**

Growth in dark conditions with 0 and 1 % OMW + decrease in phenolic compounds content by 55 %.

Better growth with 1 % OMW under light, 13 % removal of phenolic compounds in the supernatant – possibly utilized (therefore better growth).

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**Heterotrophic**

- $\mu_{max} 0 \%$: 0.21 d$^{-1}$
- $\mu_{max} 1 \%$: 0.11 d$^{-1}$
- Removal: 55.6 %

**Phototrophic**

- $\mu_{max} 0 \%$: -
- $\mu_{max} 1 \%$: 0.16 d$^{-1}$
- Removal: 13.3 %
**Chlorella vulgaris**

Better growth in dark conditions: probably due to addition of glucose (no removal of phenolic compounds).

Better growth with 1 % OMW under light, 5 % removal of phenolic compounds in the supernatant – possibly utilized (therefore better growth); in the dark no removal.

### Heterotrophic

<table>
<thead>
<tr>
<th>Phen. Comp. 1 %</th>
<th>( \mu_{\text{max}0 %} )</th>
<th>( \mu_{\text{max}1 %} )</th>
<th>Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.09 d(^{-1})</td>
<td>0.19 d(^{-1})</td>
<td>-</td>
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### Phototrophic

<table>
<thead>
<tr>
<th>Phen. Comp. 1 %</th>
<th>( \mu_{\text{max}0 %} )</th>
<th>( \mu_{\text{max}1 %} )</th>
<th>Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>0.044 d(^{-1})</td>
<td>5 %</td>
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</table>
Conclusions

Growth and phenolic compounds removal depend on microalgal strain and culture conditions.

Promising candidate for heterotrophic and phototrophic cultivation:

*Monoraphidium braunii*

Further analysis with HPLC, TOC, GC to identify mechanisms of transformation, utilization etc.

Optimization of culture conditions (pH control, temperature, …)
Contact

Leuphana University of Lüneburg
Institute for Sustainable and Environmental Chemistry
Astrid Lindner, M. Sc.
Universitätsallee 1
21335 Lüneburg

Fon +49 4131 677−1968
astrid.lindner@leuphana.de
» www.leuphana.de