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RHODOLIVE
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# 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**
- Up to 24 g/L, mostly 1 to 10 g/L
- Mainly tyrosol, hydroxytyrosol, oleuropein

## PHENOLIC COMPOUNDS

**Antimicrobial properties**
- Disturbance in natural habitat/microbial equilibrium

**Avoid release** into environment

**Utilization** instead of simply removing (what happens afterwards?)

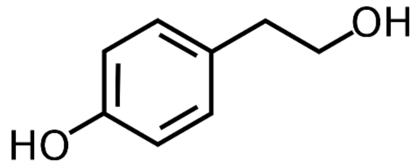
## LACK OF INFORMATION

**Removal** via microalgae discussed, but no exact mechanisms

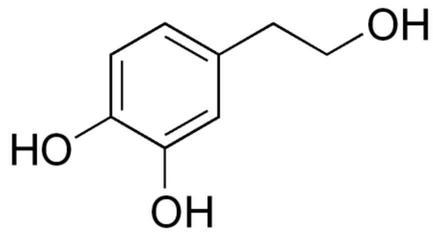
**Less focus on OMW-related** phenolic compounds – rather halogenated and added instead of „natural" origin

# Approach

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




Tyrosol

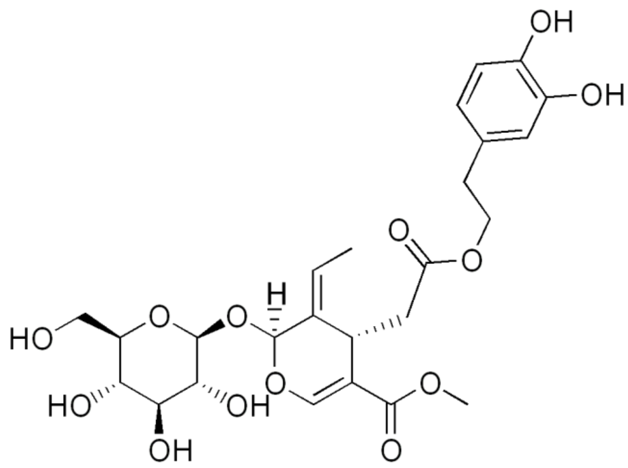



Hydroxytyrosol

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]

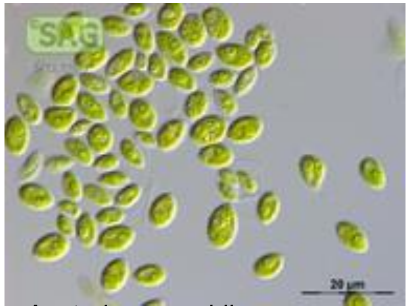



Oleuropein

[1]Ellis, B.E., *Degradation of phenolic compounds by fresh-water algae.* Plant Science Letters, 1977. **8**: p. 213-216.
[2]Pinto, G., Pollio, A., Previtera, L., Stanzione, M., Temussi, F., *Removal of low molecular weight phenols from olive oil mill wastewater using microalgae.* Biotechnology Letters, 2003. **25**(19): p. 1657-1659
[3]Papazi, A., Ioannou, A., *Bioenergetic strategy of microalgae for the biodegradation of tyrosol and hydroxytyrosol.* Zeitschrift für Naturforschung C, 2017. **72**(5-6): p. 227-236

# Cultivation in flasks and bioreactors

Microalgae: *Acutodesmus obliquus, Chlorella vulgaris, Monoraphidium braunii; soon: Desmodesmus communis, Dunaliella salina, Phaeodactylum tricornutum, Tetraselmis chuii*

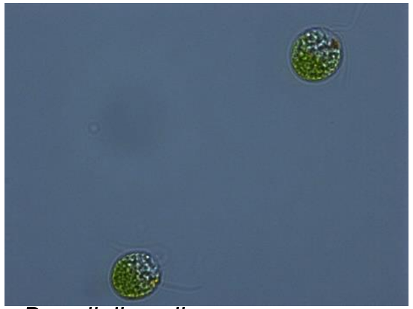



*Acutodesmus obliquus*

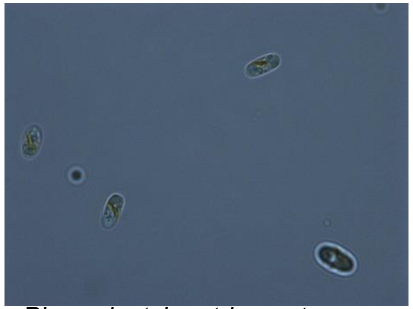

*Chlorella vulgaris*


*Monoraphidium braunii*

*Desmodesmus communis*



*Dunaliella salina*



*Phaeodactylum tricornutum*


*Tetraselmis chuii*

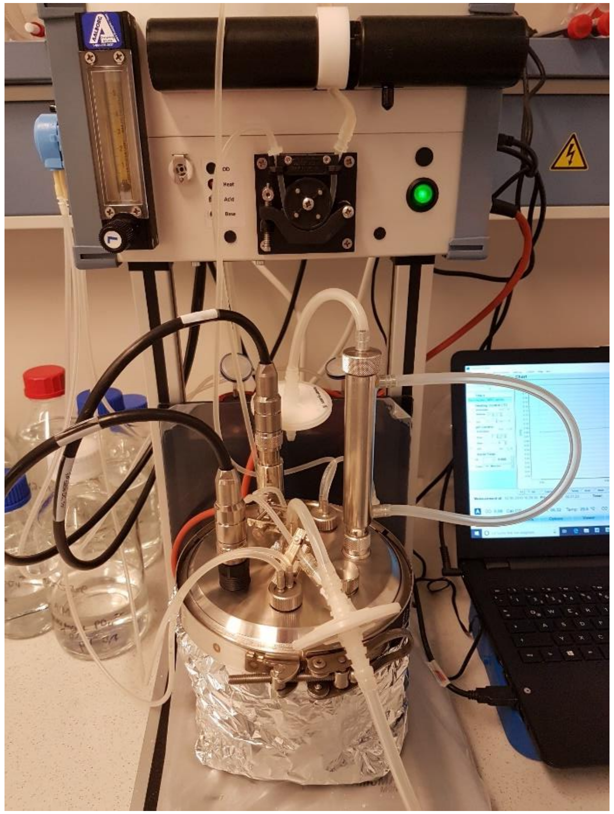
# Cultivation strategies

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:

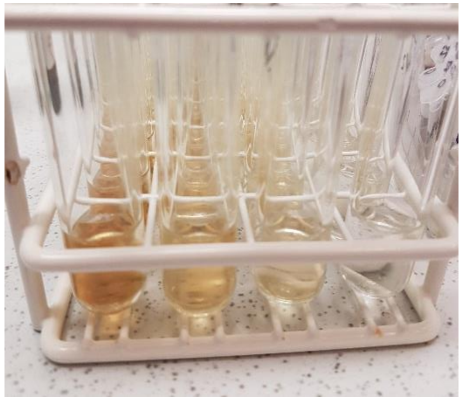
- 800 mL total volume, 1 % OMW, AF6 medium
- Dark, 1 g/L glucose
- 30 °C, aeration + magnetic stirring
- Controlled at pH 6.8
- Dissolved oxygen monitoring
- Continuous OD measurement

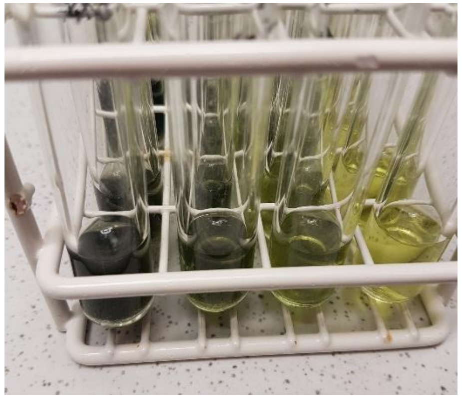


# Analysis

Phenolic compounds:

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

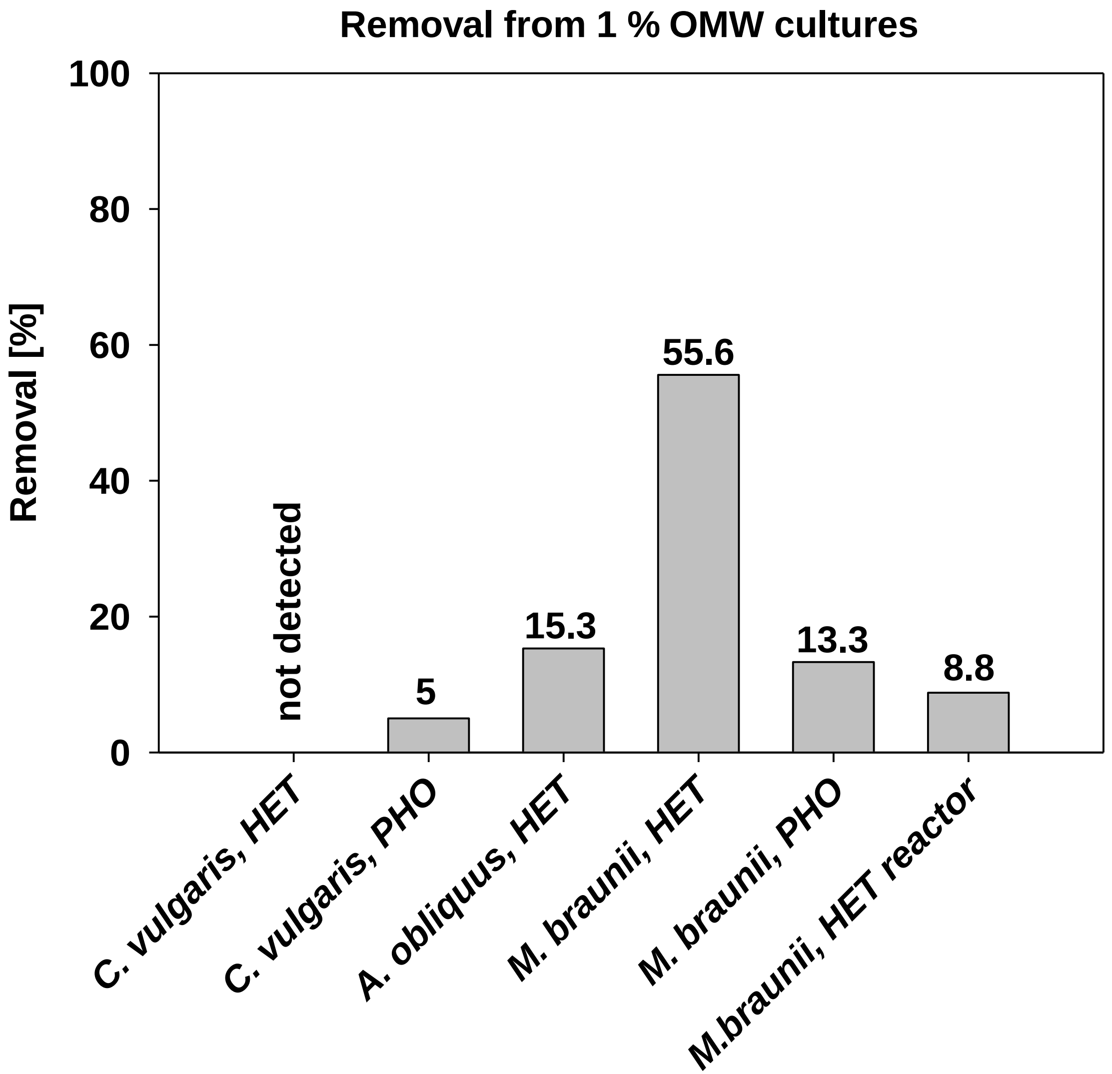




Cell growth:

- Optical density at 750 nm
- Correlation to cell density or biomass dry weight

# Removal from the supernatant after 10 days

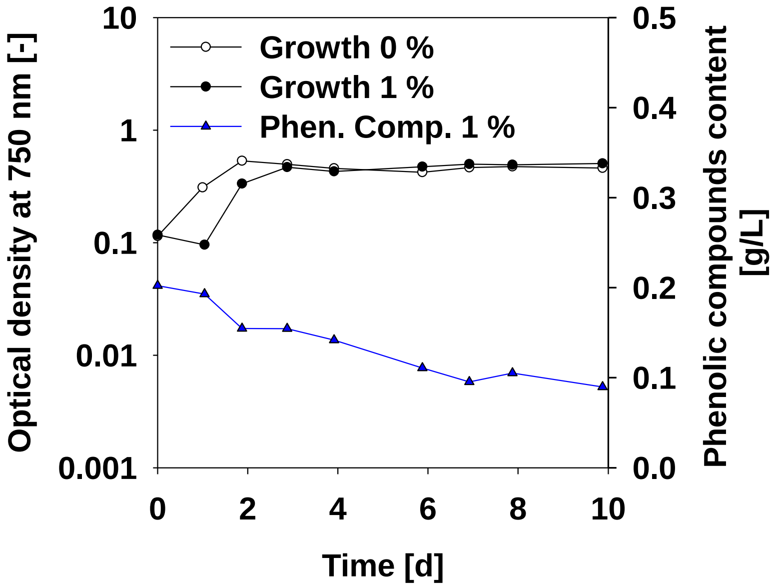


# *Monoraphidium braunii*

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

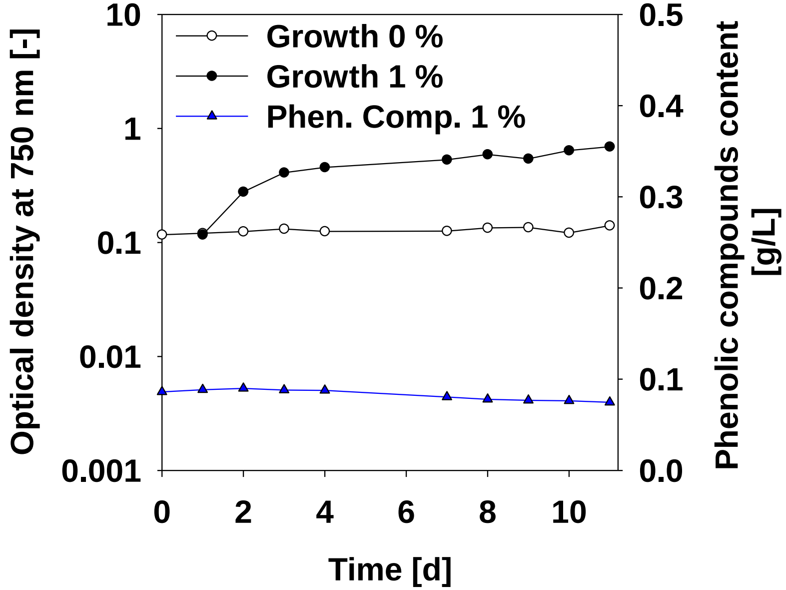
Better growth with 1 % OMW under light, 13 % removal of phenolic compounds in the supernatant – possibly utilized (therefore better growth).

***Monoraphidium braunii***
**0 and 1 % OMW heterotrophic**




| 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 % |

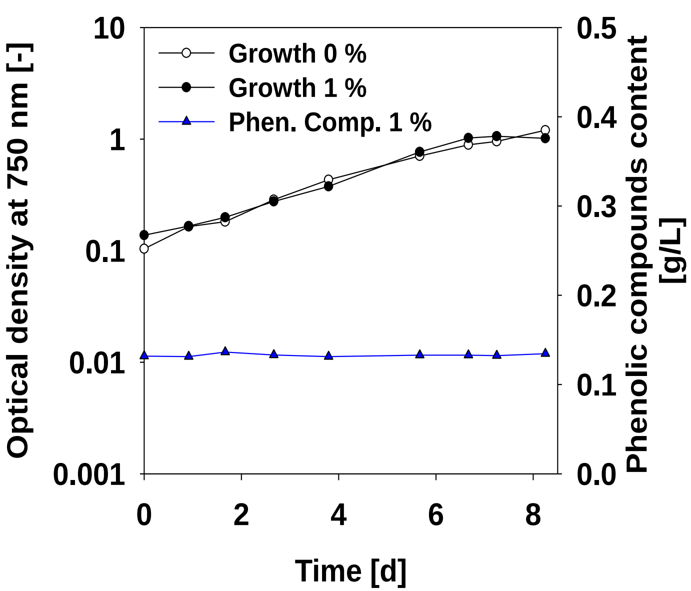
***Monoraphidium braunii***
**0 and 1 % OMW phototrophic**



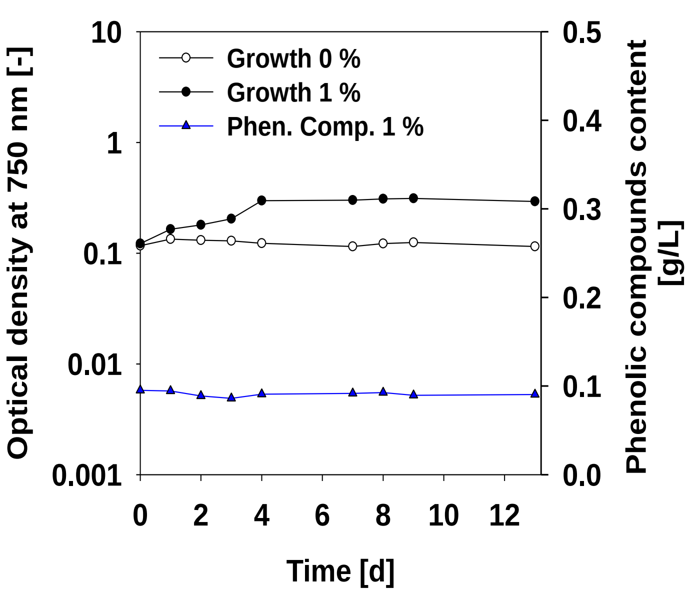
# *Chlorella vulgaris*

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 | |
|---|---|
| $\mu_{max\ 0\ \%}$ | 0.09 $d^{-1}$ |
| $\mu_{max\ 1\ \%}$ | 0.19 $d^{-1}$ |
| Removal | - |
| **Phototrophic** | |
| $\mu_{max\ 0\ \%}$ | - |
| $\mu_{max\ 1\ \%}$ | 0.044 $d^{-1}$ |
| Removal | 5 % |



# 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**

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