CULTIVATION OF CHLOROMONAS ON MEDIUM & REGENERATION WATER FROM A DEMINERALISATION UNIT: IMPACT ON ALGAE BIOMASS

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Introduction & aim

- Algae biomass cultivation is a process that requires water and nitrogen, besides other nutrients like (P, Fe, Zn, etc.), CO_2 and light.
- Aim of the study = to evaluate the possibility of growing *Chloromonas typhlos* as a unialgal strain on (MAF-permeate of) N-containing regeneration water from a demineralization unit.
- Lab-scale and longer-term pilot-scale trials were performed.

Conclusions

- Chloromonas cultivation in permeate of N-rich process water showed occurrence of stressed algae in different ways:
  - During MAF harvesting: foam formation & formation of lipid-like globules were observed (Fig. 1) causing flux reductions,
  - Less efficient harvesting via centrifugation
  - Different cell morphologies – lipids globules in the cell (Fig. 2)
- Lipid levels for algae grown on permeate-based medium tended to be slightly higher than those grown on regular medium, but differences were small. Possibly, lipids were released in the medium and got lost via the centrate.
- The accumulation of lipids in *Chloromonas* biomass might be caused by stress like exposure to high salt concentrations (Hounslaw et al., 2016 & Bazzani et al., 2021).
- Further research is required to unravel the biochemical processes and link them to the biomass composition.

Characteristics of process water

- The process water had a low organic carbon content (< 0.9%, w/v), an elevated salt level (EC 9-11 mS/cm) and contained nitrogen (up to 50 mg N/L), mainly present as nitrate.
- The composition of the process water before and after MAF-filtration is given in Table 1.
  - The concentration of Ca, Cu, Mn, Sr and Zn in the process water and permeate is much higher compared to normal medium while Mg, P and S is much higher in regular medium.
  - Most elements pass the membrane during filtration, only a few elements like iron are retained.
  - The electric conductivity of the process water is higher compared to that of the regular medium (Table 2).

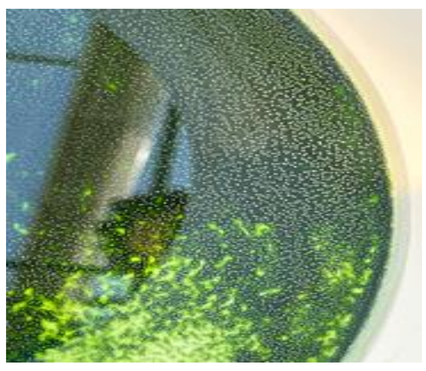


Figure 1: MAF concentrate with lipid-like globules.

Figure 2: Lipid globules (circles inside the cell) visible in *Chloromonas* grown in permeate of process water.

Table 1: Comparison of the Normal Growth medium, process water (= regeneration water) and MAF-permeate of the process water.

	µg/L												
	Ca	**Co**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Mo**	**Sb**	**Sr**	**Zn**	**P**	**S**
medium TM	113755	NA	1.07E-05	188.40	86394	310565	2.62	2.01	NA	NA	1.12	3413	411049
process water	616000	<5	17	290	75000	50000	5,2	20	<10	2200	15	<75	150000
permeate of process water	603000	<5	10	11	67000	48000	14	17	<10	2000	67	<75	150000

Table 2: Electric conductivity and pH of the *Chloromanas* culture during cultivation in MAF- permeate of the process water and in normal medium.

date	1500 L PBR permeate process water		300L PBR permeate process water		300L PBR normal medium	
	conductivity (ms/cm)	pH	conductivity (ms/cm)	pH	conductivity (ms/cm)	pH
27/02/2023	empty	empty	9.83	7.95	1.975	8.25
29/03/2023	9.64	7.106	10.97	7.581	1.131	7.736
05/04/2023	8.72	7.252	11.02	8.01	0.927	8.13
13/04/2023	9.15	7.144	9.75	7.363	-	-

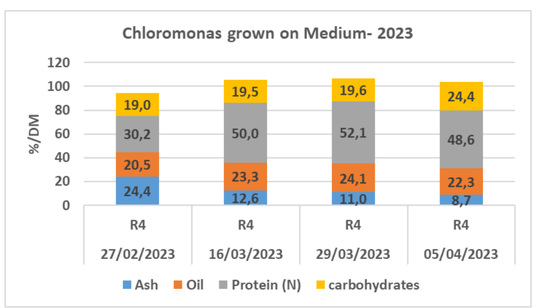
Impact on algae harvesting

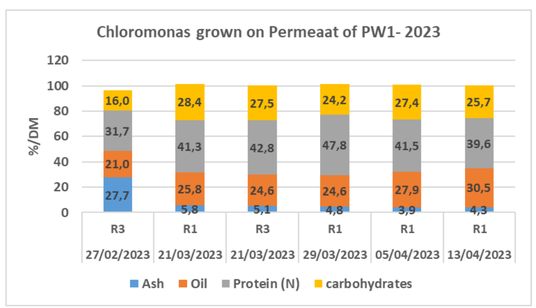
- In February 2021, *Chloromonas* grown on normal medium was harvested successfully for 1 month using the membrane-based MAF-technology (= continuous harvesting), with medium recycling.
- In November 2022, drastically reduced fluxes were observed during MAF-harvesting of *Chloromonas* grown on the permeate of N-rich process water. The appearance of the culture was very different, with large lipid-like globules in the MAF concentrate (Fig. 1) and excessive foaming, possibly caused by cell lysis.

- Microscopy analysis revealed a different cell morphology: intense cell division and large intracellular globules, possibly lipid globules (Fig. 2).
- In February 2023, the cultivation of *Chloromonas* on normal medium and on permeate of N-rich process water was compared. Again, MAF membranes clogged for *Chloromonas* grown on permeate, while harvest for algae grown on normal medium was successful. When the permeate-based culture was centrifuged, relatively low biomass amounts were observed in the pellet.

Impact on algae biomass composition

The lipid level of algae grown on medium and on process water was determined for multiple harvests.





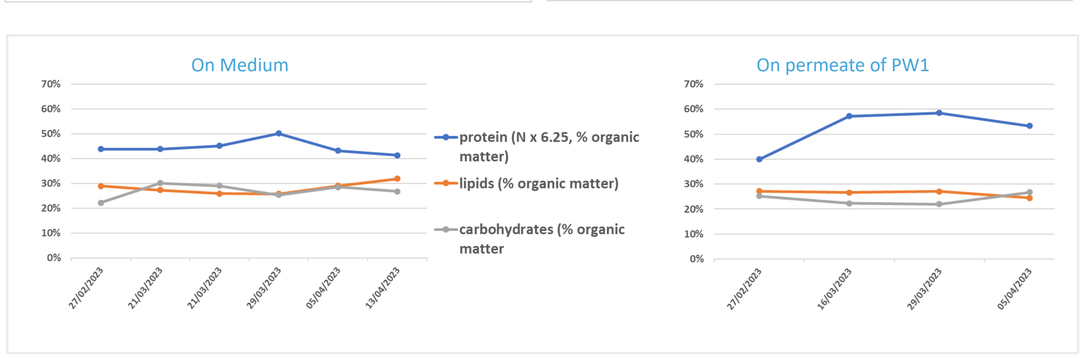


Figure 3: Chemical composition of *Chloromonas* biomass. Upper graphs: Proximate analyses on dry matter basis, where slightly increased lipid concentrations were observed for *Chloromonas* grown on permeate of the process water. Lower graphs: this increase in lipid concentration is less pronounced when plotting data on organic matter basis.

References: Hounslaw et al., 2016 (Thesis submitted for the degree of Doctor of Philosophy) & Bazzani et al., 2021 (doi.org/10.3390/jmse9111242)

IDEA - Implementation and development of economic viable algae-based value chains (NWE639)

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