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PROMISING PERSPECTIVES ON THE EXPLOITATION OF GREEN ALGAE AND DUCKWEED FOR DAIRY EFFLUENT TREATMENT

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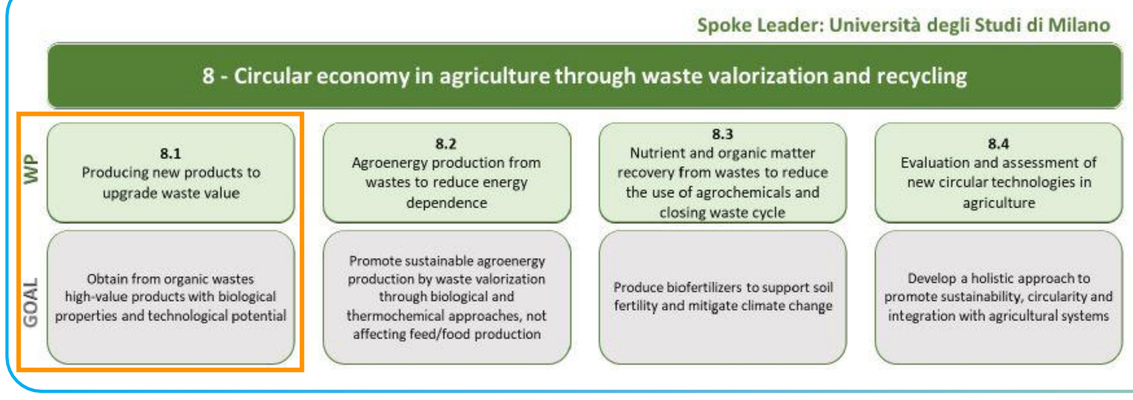
SPOKE, WP AND TASK

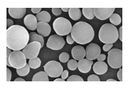


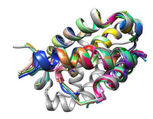
- Spoke 8
- WP 1
- **Task 8.1.2**

LCA assessment in collaboration with **WP 8.4** (**ENEA**)

Development, optimization and **scale-up** (that will be assessed at **PoliTO**) of biotechnological processes based on photosynthetic microorganisms (microalgae) and plants for the treatments of wastewaters from dairy industry to produce valuable biomass that can be exploited into high added-value compounds such as:


Pigments


Starch


Protein


Soil biostimulants and fertilizers

INTRODUCTION

Due to the increasing global water shortage and the growing amount of wastes and effluents released into the environment by anthropic activities, there is an urgent need to develop alternative waste treatment technologies that are low-carbon and high resource recycling, consume less energy, and promote the concepts of biorefinery and circular economy [1].
Among biotechnological processes for treating wastewaters (WWs) of various origin, particularly promising are those based on the use of microalgae and some aquatic plant species, such as duckweed. Both of these photosynthetic organisms have been shown to have an excellent nutrient removal capacity (particularly nitrogen and phosphorus) and accumulate a high amount of biomass, which can be valorised for a wide range of applications, such as protein source for feed, biofuel production, extraction of pigments, starch or compounds with pharmaceutical properties or as soil amendments/biostimulants.
Although the potential use of microalgae and duckweed for bioremediation dates back to the 1950s, to date several challenges remain to be overcome to make this technology feasible and economically viable (e.g. balancing problems in nutrient uptake, contamination, light availability, insufficient biomass accumulation). The use of microalgae and duckweeds for treatment of some types of WWs has been already demonstrated in some large-scale pilot plants, although other targeted studies need to be performed to implement remediation performance.

EXPERIMENTAL DESIGN

Growth Box 10x10 *cm*
***Lemna minor* on two seasonally distinct WW samplings**

L. minor (5500) • *December - 2023* • *May - 2024*[2] → *TQ*, *FL*, *Ctrl*[1] → 7 DAYS

Measured Parameters (at T0 – T7)

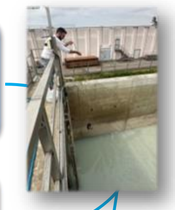
- *Area*
- *N of fronds*
- *Fresh weight*
- *Dry weight*
- *Pigments*
- *WWs Chemical composition*

L. minor (5500)
L. gibba (9598)
L. × mediterranea (LER0021)
L. × mediterranea (9562)
• *May-2024* → *TQ*, *Ctrl*[1] → 7 DAYS

Different Lemna species on same WW

Legend:
1. *Ctrl*: synthetic growth medium, SH formula + 2% sucrose
2. *May WW sample was diluted 1:2*

Wastewater Sampling



Pre-treatment optimization

Duckweed Growth Test

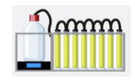
Growth assays
- RAW (TQ)
- FILTERED (FL)

Metabarcoding characterization

Microalgae Growth Test

6-wells plate ***Flasks*** ***Multicultivator***

C. Sorokiniana ccap 211/8k → *TQ*, *FL** x3, *Ctrl*



• *November-WW*
• *May-WW*
7 DAYS

* Tested concentrations: 75% and 100%

Parameters

- *Daily Measurement of Optical Density (OD) Measurement of the depletion of nitrate and phosphate (only for multicultivator experiment)*

Isolation of adapted strain from waste by agar Petri dish technique → *Characterization by 16S RNA sequencing* → *Parachlorella kessleri sp.*

• *May-WW*
7 DAYS

6-wells plate ***Flasks***

TQ, *Ctrl* x3

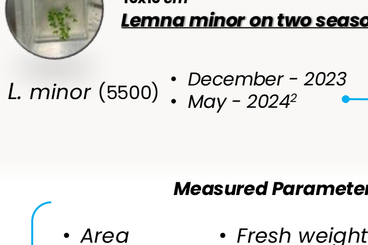
RESULTS

DUCKWEED

1. L. minor 5500 on two seasonally distinct WW samplings

Treated vs. raw WW

a)

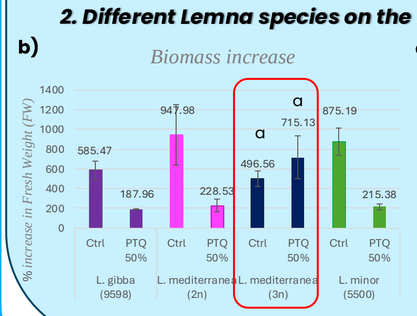


Graph Legends and Results

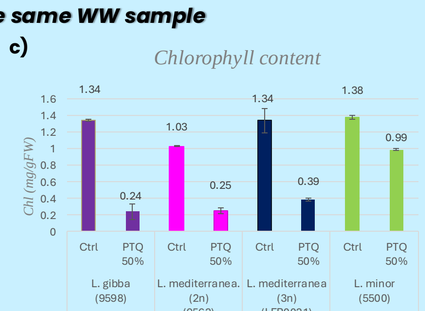
a) *L. minor* biomass increase on both WW samplings is significantly higher in raw vs. filtered WW. Dilution rate has to be adjusted case by case due to different starting concentration.
b) Among tested *Lemna* species, the hybrid *L. × mediterranea* (LER0021) exhibits the best growth performance in raw WW, with a sevenfold biomass increase over seven days, with the same growth rate as in the control growth medium (SH) supplemented with sucrose.
c) Chlorophyll content decreases in all genotype after one week, with *L. minor* (5500) showing the lowest decrease. Pigment reduction is likely compensated for by the myxotrophic growth conditions.
d) Removal efficiency range was 0-30% for TP, 10-37% for TP and 70-80% for COD over the 1 week growth test

2. Different Lemna species on the same WW sample

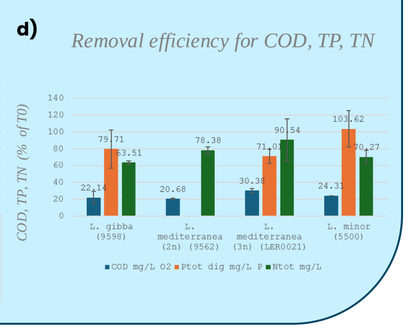
b)



c)

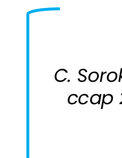


d)



MICROALGAE

Isolation of adapted strain from dairy effluents



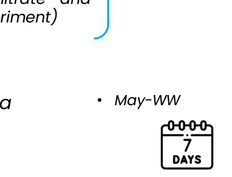
DNA EXTRACTION → 16S rRNA SEQUENCING → *Parachlorella kessleri* → WHOLE GENOME SEQUENCING

In progress

Growth assays

	Growth conditions	Species	WW concentrations	Control
6-wells plates	25° C, continuous white light, 20 µmol photons $m^{-2} s^{-1}$, 250 rpm	*Chlorella sorokiniana* ccap 211/8k	100% - 75% - 50% filtered	Mixotrophy
		Parachlorella kessleri	100% - 75%	Mixotrophy and Photoautotrophy
Flasks	25° C, continuous white light, 20 µmol photons $m^{-2} s^{-1}$, 55 rpm	*Chlorella sorokiniana* ccap 211/8k	Raw, 100% - 75% filtered	Mixotrophy
		Parachlorella kessleri	Raw	Mixotrophy
Multi-cultivator		*Chlorella sorokiniana* ccap 211/8k	Raw	Photoautotrophy

From the growth assays performed in 6-well plates and in flasks, 75% dilution of filtered effluent in water was found to be the best effluent concentration to support the growth of *C. sorokiniana*.
In addition, preliminary results of growth tests conducted in flasks show that the isolated putative *Parachlorella kessleri* strain grows better on raw effluent than the control on synthetic medium (TAP) in mixotrophy. Cultivation assays of *P. kessleri* with treated effluent are underway.



Chlorella sorokiniana

Chlorella sorokiniana in multicultivator photobioreactor

REFERENCES

[1] Srimongkol P. (2022). Frontiers in Bioengineering and Biotechnology. DOI 10.3389/fbioe.2022.904046

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