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Protein-rich microalgae biomass cultivated with liquid fraction of digestates for animal-feed formulation

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Introduction

- In **biogas plants** organic waste is valorised to produce biogas.
- Biogas can be used for electricity, heat, and biomethane production.

- **Carbon dioxide (CO_2)** is the byproduct obtained from biogas upgrading process to produce biomethane.

- **Digestate** is the byproduct obtained from anaerobic digestion with high nutrient content.

Introduction

PROBLEM

CO_2 valorisation

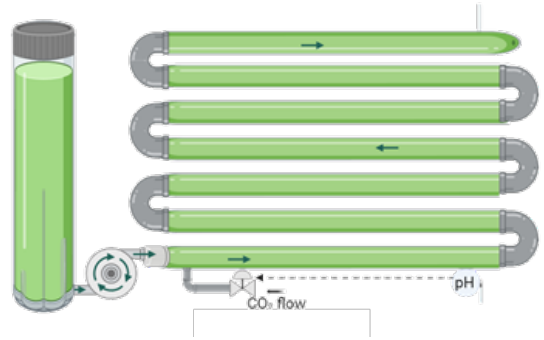
× Necessity to valorise **CO_2** from biogas streams to reduce GHG emissions

Digestate treatment

× Necessity to treat **digestate** from biogas plants in zones with nutrient excess to avoid environmental problems.

OPPORTUNITY

✓ **Microalgae cultivation** using digestate and CO_2 from biogas plants could be used as an advanced technology to produce valuable biomass.



Objective

The present work aims to valorize **liquid fractions of digestate** and **CO_2** for **microalgae cultivation** with high-protein content and good amino acid score for animal feed.

Experimental design

2 Liquid Fraction of Digestates

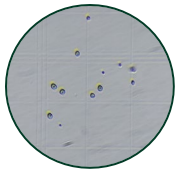
➢ **Permeates obtained after an ultrafiltration membrane process**

- Digestate from Cheese-whey valorisation **(CWD)**

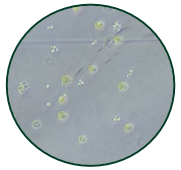
- Agri-food waste digestate **(AFWD)**

Parameters	Mineral media	CWD permeate	AFWD permeate
pH	5.70 ± 0.01	8.45 ± 0.05	8.35 ± 0.05
EC (mS cm^{-1})	0.93 ± 0.05	10.67 ± 0.05	25.41 ± 0.07
Turbidity (NTU)	0.83 ± 0.02	34 ± 0.01	72 ± 0.05
COD (mg L^{-1})	<5	132 ± 1	1275 ±7
TKN (mg L^{-1})	-	647 ± 10	3108 ± 1
NH_4^+-N (mg L^{-1})	100 ± 1	595 ± 28	3033 ± 2
TP (mg L^{-1})	9.05 ± 0.83	99.40 ± 5.56	6.81 ± 0.32
Dilution (%)	**-**	**16.7**	**3.3**

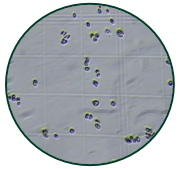
3 microalgae strains



***Chlorella vulgaris* ACOI 879-I**



***Parachlorella kessleri* ACOI 2928**



Tetradesmus obliquus

Operational conditions

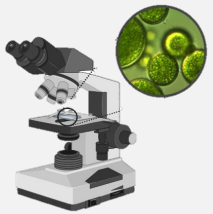
Reactor volume: 0.5 L
pH: 8.0 ± 0.1
Light intensity: 100 µmol m^{-2} s^{-1}
Temperature: 26 ± 3 °C
Aeration rate: 0.3 vvm
CO_2: 1.62 %
Control: Mineral Media
100 mg NH_4^+-N L^{-1}
Cultivation time: 10 days

Analytical methods

Biomass growth

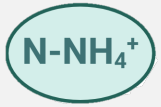
Optical Density

Dry Weight



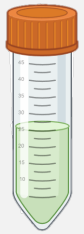
Cell count

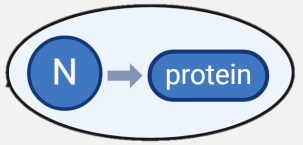
Nutrient uptake



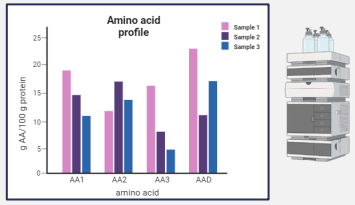
Ammonium analysis

Biomass composition





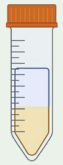
Protein content (TKN x 4.78)



Amino acid profile (HPLC)

Centrifugation
(4200 rpm, 30 min)

Hydrolysis
(6N HCl)

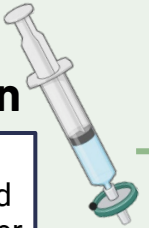


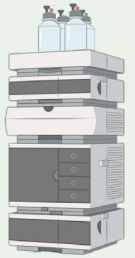
Neutralisation
(6N NaOH)

Centrifugation
(3500 rpm, 10 min)

Filtration

0.22 µm RC (regenerated cellulose) filter



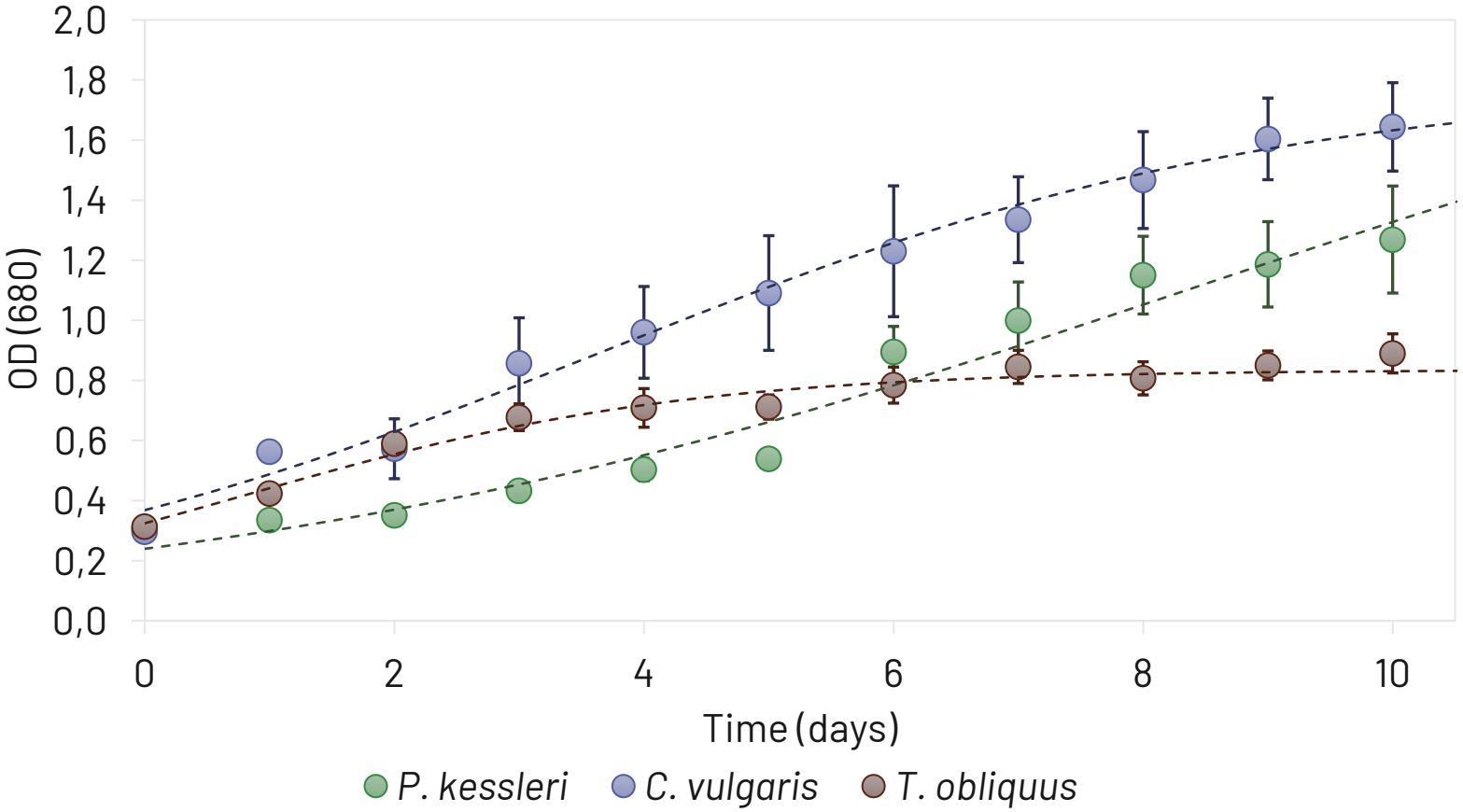


HPLC

Amino acid profile (HPLC sample pretreatment)

Results

Cheese whey digestate (CWD) growth curve

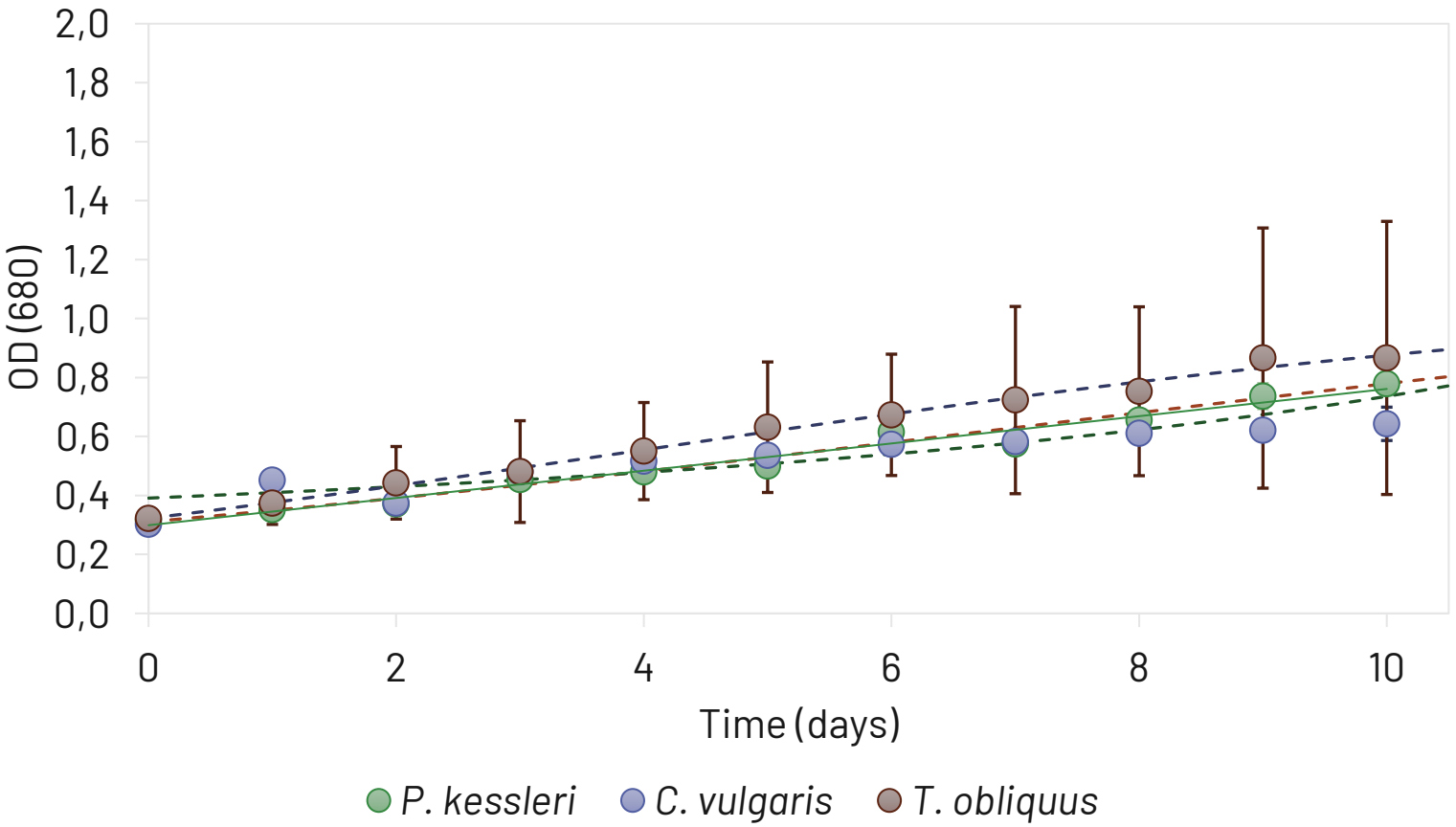


Key results

- ✓ ***C. vulgaris*** achieved the highest OD value (**1.640 ± 0.147**) followed by *P. kessleri* (**1.270 ± 0.178**).
- ✓ Similar **growth performances** (μ_{max}, d^{-1}) were obtained for ***P. kessleri*** (0.23 ± 0.02 d^{-1}) and *T. obliquus* (0.24 ± 0.01 d^{-1}), followed by *C. vulgaris* (0.16 ± 0.02).

Results

Agri-food waste digestate (AFWD) growth curve

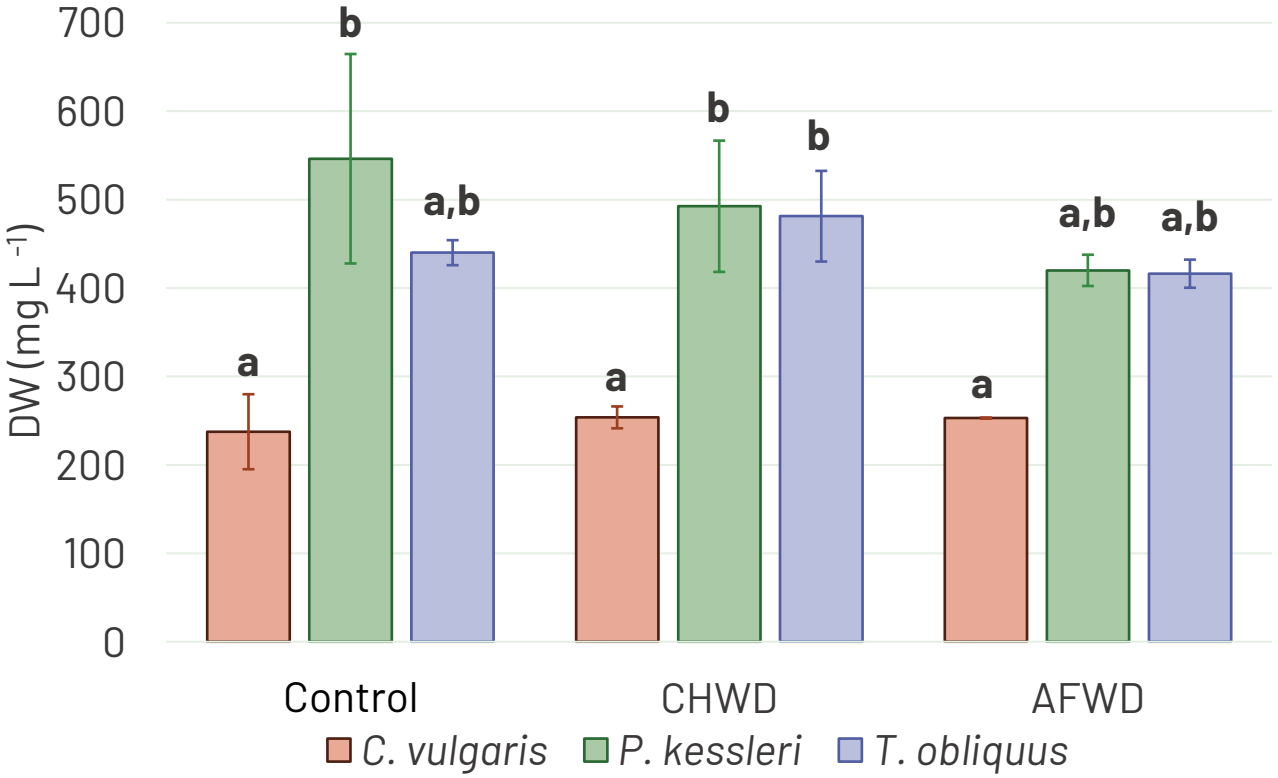


Key results

- ✓ With **AFWD**, the three strains achieved similar OD values (0.6 – 0.8).
- ✓ **Growth** for AFWD (μ_{max}, d^{-1}) is lower than CWD, and **comparable** between the **strains**: 0.11 ± 0.02, 0.10 ± 0.01, 0.14 ± 0.02 for *C. vulgaris*, *P. kessleri* and *T. obliquus*, respectively.

Results

Biomass production: Dry weight (DW) content



Data was checked for normality (Shapiro–Wilk test) and homogeneity (Levene test) in SPSS Software. One-way analysis of variance (ANOVA) was applied to assess statistically significant differences. Post-hoc Tukey test was used when statistical significance was found, represented with different letters ($p < 0.05$).

Key results

- ***C. vulgaris*** biomass production is similar between the treatments **(240-250 mg L^{-1},** *$p<0.05$*), but significantly lower to the other strains in CWD (*$p>0.001$*).
- ***P. kessleri*** DW **(420-550 mg L^{-1})** higher than *C. vulgaris*. Biomass production between the treatments have no significative differences (*$p<0.05$*).
- ***T. obliquus*** *biomass* production and dry weight values are comparable to *P. kessleri* (*$p<0.05$*).

Results

Microalgae biomass composition produced with CWD

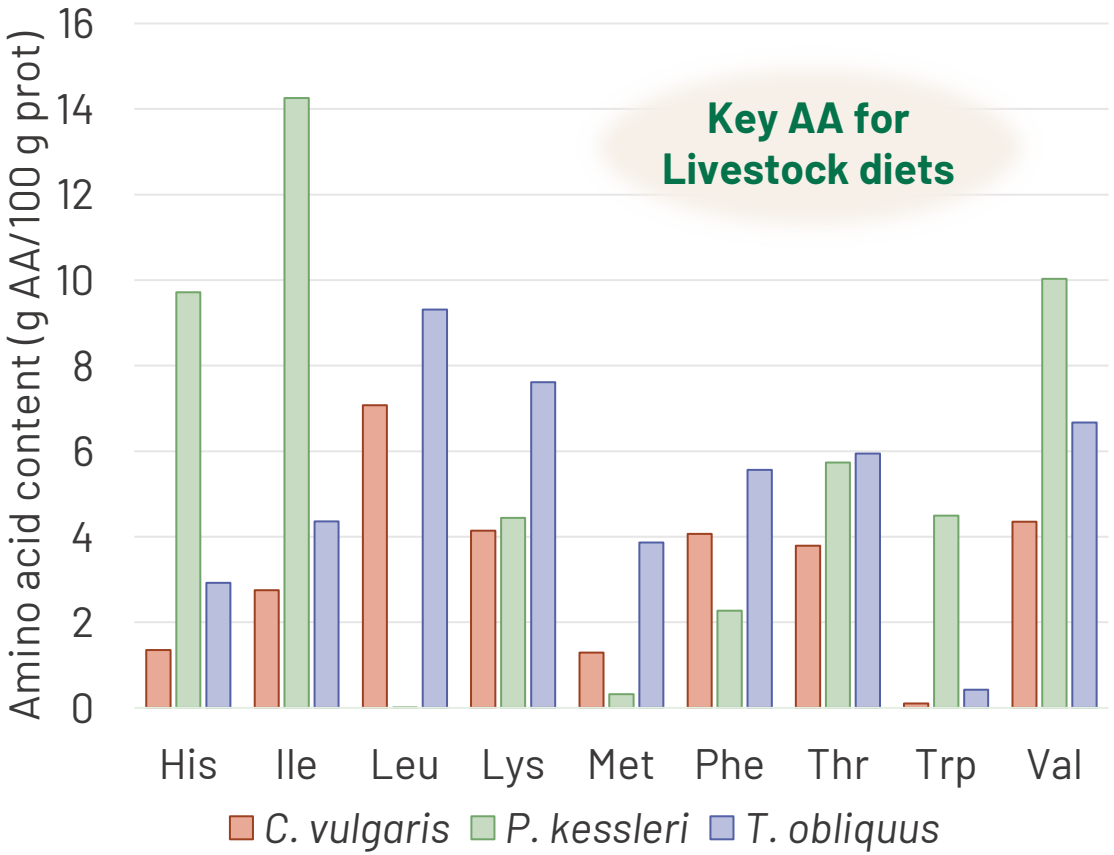
Protein content

Microalgae biomass (this study)

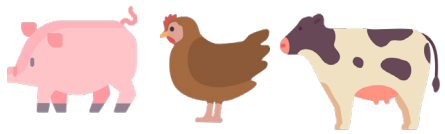
P. kessleri	41 ± 1 % dw
C. vulgaris	46 ± 16 % dw
T. obliquus	50 ± 12 % dw

Soybean 40-50 % dw

Sunflower Seed 20-30 % dw



Amino acid profile



Lysine (Lys)

Microalgae: 4-7
Soybean : 3-6
Sunflower: 1-3

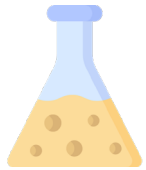
(g AA/100 g prot)

Methionine (Met)

Microalgae: 1-4
Soybean 0.5-1.5
Sunflower 0.5-2.5

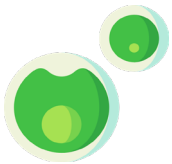
(g AA/100 g prot)

Take home messages



Digestate obtained from cheese whey valorisation has been identified as a suitable nutrient source for microalgae cultivation.

P. kessleri as the selected strain to be cultivated in the pilot scale photobioreactor.



Potential of microalgae biomass produced for animal-feed applications as alternative **protein** sources.

Work in progress

SEMPRE-BIO

Beta
Biodiversitat, Ecologia,
Tecnologia Ambiental i Alimentària

Thank you for your attention

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