RECYCLING ORGANIC RESIDUES INTO VALUABLE BIOMASS THROUGH THE HETEROTROPHIC CULTURE OF *Chlorella protothecoides*

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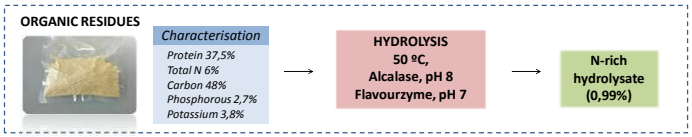
BACKGROUND

The heterotrophic culture of oleaginous microalgae allows improving productivity as well as scaling-up the culture by using conventional reactors. However, the main drawback is based on the higher economic cost of raw materials [1]. This problem can be solved by using organic residues treated by enzyme hydrolysis. These hydrolysates, rich in soluble nutrients, can be used in the growth process improving its sustainability and making possible reusing residues according to "zero waste concept".
With this idea in mind, the aim of this work is to evaluate the suitability of organic residues as nutrient source for the growing process of *C. protothecoides* as a valuable biomass for biodiesel production.

METHODOLOGY

- Enzymatic protein hydrolysis (EPH) of organic residues (*Schizochytrium limacinum* deffated biomass)



- *C. protothecoides* growth under controlled conditions: from flask to Biostat

- Hydrolysate composition

Hydrolysate Composition	
Total N (%)	0.99
Free Amino N (%)	5.50
Protein (%)	0.7
Carbon (%)	12.60
Phosphorus (%)	0.23
Potassium (%)	0.11

- Culture media composition

Composition	100 ml Erlenmeyer	5 L Biostat B+
Nitrogen (g/L)*		
Total N	0.2	0.3
Free amino N	1.11	1.8
Glucose (g/L)	10	30-40
Sea salts (g/L)	10	5

* N is provided by yeast extract (control) and hydrolysate

- Settings

flask
140 rpm
24°C

Biostat
250 rpm
24°C
pH 6
pO2 ≥25%

RESULTS

✓ Bench-scale (Erlenmeyer)

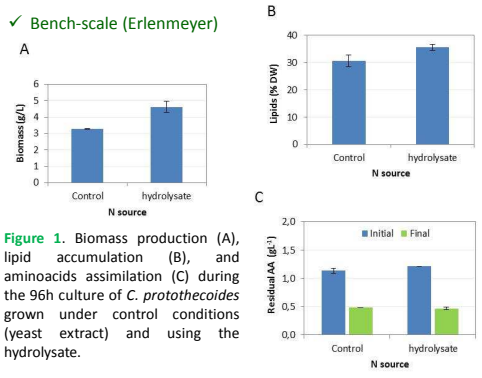
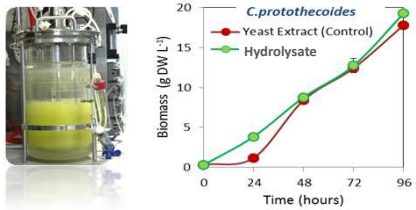


Figure 1. Biomass production (A), lipid accumulation (B), and aminoacids assimilation (C) during the 96h culture of *C. protothecoides* grown under control conditions (yeast extract) and using the hydrolysate.

Results from flask experiments (Figure 1) show that:

- ❖ Biomass production is 40% higher when the hydrolysate is used
- ❖ Lipids accumulate up to 35,5% with the hydrolysate, an increase of 16% over control
- ❖ About 60% of supplied aminoacids are consumed in both cultures

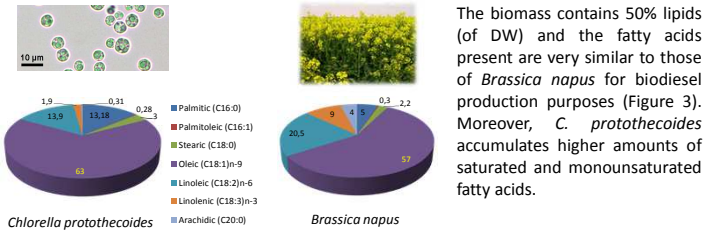
✓ Scale-up to 3.5L



Both nitrogen sources were similarly assimilated by the microalgae in a process where the biomass productivity reached up to 4,5 $gDWL^{-1}d^{-1}$ (Figure 2). This production process resulted in a productivity 3-times higher than in flask cultures.

Figure 2. Growth pattern of *C. protothecoides* in 5L-reactor using yeast extract or the hydrolysate as nitrogen sources.

✓ Fatty acid profile of the biomass:

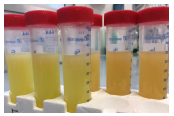


The biomass contains 50% lipids (of DW) and the fatty acids present are very similar to those of *Brassica napus* for biodiesel production purposes (Figure 3). Moreover, *C. protothecoides* accumulates higher amounts of saturated and monounsaturated fatty acids.

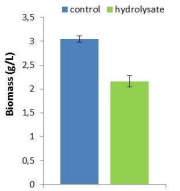
Figure 3. Main fatty acids present in both *C. protothecoides* and *Brassica napus* (rapeseed).

FUTURE PROSPECTS

In order to further improve the sustainability of this biodiesel production process and in an attempt to go towards a circular economy, the deffated *C. protothecoides* residue was hydrolysed and used as sole nutrient source (carbon and nitrogen source) for its own growth.



Preliminary results show that *C. protothecoides* is able to grow although not to the level of control culture. This is due to the lower glucose amount in the residue, which after being supplemented will surely allow similar growth patterns.



CONCLUSIONS

- ➢ The EPH produced a nitrogen-rich "raw material" suitable enough to fulfill the requirements of *Chlorella protothecoides* growth
- ➢ The hydrolysate is an effective alternative for yeast extract yielding similar biomass productivity
- ➢ The fatty acid profile of the biomass is highly appropriate for biodiesel production purposes
- ➢ *C. protothecoides* deffated biomass after lipids extraction can be re-used as nutrient source

REFERENCES

[1] Valorization of organic residues for the production of added value chemicals: A contribution to the bio-based economy. Biochem Eng J (2015)