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SEARCHING FOR OLIVE MILL WASTE WATER (OMWW) SOLUTIONS: IS THERE A POTENTIAL ON MICROALGAE TREATMENT?

SILVA, Olinda [1], VASCONCELOS, Vitor [2], AIRES, Alfredo [3], FERNANDES, Conceição [1*]

(1) Mountain Research Centre (CIMO), ESA-Polytechnic Institute of Bragança, Portugal. * conceicao.fernandes@ipb.pt
(2) Interdisciplinary Centre for Marine and Environmental Research (CIIMAR/CIMAR), Faculty of Sciences-Porto University, Portugal.
(3) Centre for Research and Technology for Agro-Environment and Biological Sciences (CITAB), University of Trás-os-Montes e Alto Douro, Portugal.

Introduction

Olive Mill Waste Water (OMWW) is the principal industry extraction residue of olive oil and still one of the most serious environmental problems [1]. The main purpose of this study was to evaluate the ability growth of *Chlorella vulgaris* under different OMWW concentrations and assess its phenolic content variations to get OMWW toxicity reduction.

Material and Methods

OMWW was collected in a continuous 3-phases olive mills. **Growth screening:** *C. vulgaris* (CBSC 15-2075) was plated on agar solid with 5%, 10%, 20%, 30% and 40% OMWW dilutions. Incubation was in a controlled chamber: 22±1 ºC, light of 4500 lux with16:8h light:dark. Algae growth was assessed by comparison with control (Walne Modified medium) after 4, 7 and 12 days. **Batch cultures:** 500 mL flasks with 20% and 40% diluted OMWW were inoculated with pre-adapted cultures (5% OMWW) and incubated in same controlled chamber, with continuous light and aeration, for 20 days. *C. vulgaris* growth was assessed by cell counting. Total phenols was measured by Folin-Ciocalteau reagent and gallic acid as standard (765 nm). **Germination Assay:** 20% diluted OMWW treated samples were subjected to toxicity test with lettuce (*Lactuca sativa*) seeds. Petri dishes were incubated in a growing chamber at 26º C, for 7days.

Results and Discussion

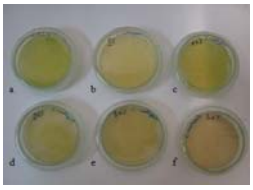

Fig 1. Comparison of *C. vulgaris* growth after 12d incubation, for higher inoculum concentration: control (a), OMWW dilutions (b=5%, c=10%, d=20%, e=30%, f=40%).

Generally, *C. vulgaris* showed ability to grow in tested dilutions, being dependent of time and inoculum concentration. Fast-grow were observed at 10 %, 20% and 30%. Lowest density of cells was observed at 5%, suggesting low nutrients amount.

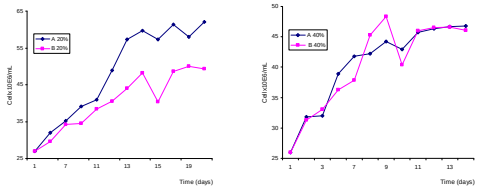

Fig 2. Growth of *C. vulgaris* in batch cultures with 20% and 40% of diluted OMWW (A and B = essays duplicates).

Cultures have been started with high concentration (26 × 10-6 cells ml^{-1}) and cells increased slowly during fermentation (Fig 2.). At 40% OMWW dilution, cultures showed a short exponential grow phase, with slight higher specific growth rates (μ), comparing cultures with 20% dilution. However, a growth index of 130% and 83% was achieved in cultures with 20% dilution, after 20 days.

As no other sources were added to batch cultures and grow, as well as a decrease in phenol were detected, these mean that *C. vulgaris* can used the present phenolic compounds as carbon source.

Results showed that maximum phenol removal were achieved by microalgae grown at 20% dilution (62 and 57%). Others biological processes have been showed a greatly variation on reduction of OMWW phenolic compounds [2-7].

Tab 1. Biological parameters and total phenolic loss obtained in *C. vulgaris* batch cultures at 20 and 40% of diluted OMWW (A and B = essays duplicates).

	20%		40%	
Parameters	A	B	A	B
Total days	20	20	14	14
Exponential days	14	14	9	9
[1] Specific Growth Rate (μ day $^{-1}$)	0,059	0,042	0,061	0,066
[2] Growth Index (GI)	130 %	83 %	80 %	77 %
[3] Phenolic Loss Index (PLI μ g/cell)	57 %	62 %	45 %	43 %

(1) $\mu = \text{Ln } N_t = \text{Ln } N_0 + \mu\, t$; (2) GI = $(N_t - N_i)/N_i$; (3) PLI = $[(Phn_i / N_i) - (Phn_f / N_t)] / (Phn_i/N_i)$. ($N_t$ = cells concentration at time; N_0 = cells concentration at beginning of exponential grow; t = time; N_t = final cells concentration; N_i = inoculum concentration; Phn_i = initial total phenolics concentration; Phn_f = final total phenolics concentration)

Tab 2. Germination index and mean growth root of lettuce with untreated and treated OMWW 20% dilutions (A and B = batch essays duplicates).

Parameters	Control (water)	Untreated 20% diluted OMWW	Treated 20% diluted OMWW	
			A	B
Germination Index (Gi)	96 %	63 %	67 %	79 %
Root growth (cm/day)	5,16 ± 1,07[a]	4,88 ± 1,11[a]	7,01 ± 1,73[b]	6,58 ± 1,67[b]

Data are expressed as mean of 3 replicates for each treatment ± standard deviation; Means with different letters differ significantly (ANOVA, 5%). GI = percentage of germinated seeds; Root growth = (mean length at day) / (number of days)

Treated OMWW 20% showed highest germination percentage (79 and 67%) and an increase in root growth, than untreated one, in accordance with previous results. In fact, several authors attribute OMWW toxicity to their phenolic compounds which inhibits germination of seeds of different plant species [8].

Conclusions

- For the OMWW dilutions tested, *C. vulgaris* in batch cultures is able to use phenolic compounds as carbon source;
- Biotreatments showed a potential reduction in phenol content, which are responsible for toxicity;
- Also, an increase in germination percentage and in root growth of lettuce were achieved, suggesting phyotoxicity decrease;
- Incorporation of microalgae processes on OMWW can be a viable option for treatment;
- Further researches are in course in order to optimise high OMWW dilutions and phenolic compounds removal.

References

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