OLIVE MILL WASTEWATERS BIOREMEDIATION TOWARDS DETOXIFICATION

Paixão S.M., Ribeiro B., Sàágua M.C., Baeta-Hall L., Correia A., Duarte J.C.

LNEG – Laboratório Nacional de Energia e Geologia, I.P.
Estrada do Paço do Lumiar 22, 1649-038 Lisboa, Portugal. E-mail: jose.duarte@ineti.pt

ABSTRACT

Olive oil production is a traditional agricultural industry in Mediterranean countries and Portugal is one of the ten major producers. This industry generates an effluent, olive mill wastewater (OMW), which does not undergo any treatment and, usually, is stored in evaporation lagoons or spread on the land. Disposal of olive oil mill wastewaters is a serious environmental problem due to its high organic loading, presence of polyphenols and tannins, high content in suspended solids and acidity, which contributes to its ecotoxicity. In this work it was intended to study the biodegradation of OMW by microrganisms naturally present in these wastewaters. Thus, an aerobic biological treatment system: a packed-bed batch reactor was applied to a OMW from a mill on northern of Portugal, exploring its autochthon microbial population as inoculum. The biodegradation ability of OMW by microrganisms naturally present in these wastewaters was assessed, by following the evolution of the process and monitoring several of its physico-chemical parameters. Furthermore, an ecotoxicological evaluation, using chronic toxicity tests (*Pseudomonas putida* growth inhibition test and *Vibrio fischeri* growth inhibition test), was performed to follow the detoxification capacity of the system as well as its potential to be used in the treatment of this type of agroindustrial effluent.

Keywords: OMW; detoxification; packed-bed batch reactor; ecotoxicity tests.

1. INTRODUCTION

Olive oil production is a traditional agricultural industry in Mediterranean countries and Portugal is one of the ten major producers. This industry generates an effluent, olive mill wastewater (OMW), which does not undergo any treatment and, usually, is stored in evaporation lagoons or spread on the land.

Disposal of olive oil mill wastewaters is a serious environmental problem due to its high organic loading, presence of polyphenols and tannins, high content in suspended solids and acidity, which contributes to its ecotoxicity.

In this work it was intended to study the biodegradation of OMW by microrganisms naturally present in these wastewaters. Thus, an aerobic biological treatment system: a packed-bed batch reactor was applied to an OMW from a mill on northern of Portugal, exploring its autochthon microbial population as inoculum. The biodegradation ability of OMW by microrganisms naturally present in these wastewaters was assessed, by following the evolution of the process and monitoring several of its physico-chemical parameters. Furthermore, an ecotoxicological evaluation, using chronic toxicity tests (*Pseudomonas putida* growth inhibition test and *Vibrio fisheri* growth inhibition test), was performed to follow the detoxification capacity of the system as well as its potential to be used in the treatment of this type of agroindustrial effluent.

2. MATERIALS AND METHODS

Effluent sampling and characterization - Sampling of olive mill wastewaters was carried out from a lagoon of a Portuguese mill farm (Alfândega da Fé, Trás-os-Montes, Portugal). The characterization of

OMW, untreated and treated samples, generally was conducted according to the physico-chemical methods described in Standard Methods for the Examination of Water and Wastewater [1].

Biological Packed-Bed Batch Reactor - A 60 litter's vessel was filled with a packaging of plastic material consisting of a cubic geometry (Biological Carrier Media from Rauschert). The non-inoculated reactor was filled with OMW and the effluent was re-circulated daily for homogeneity. The vessel was kept closed and at the average room temperature (≈ 23ºC).

Microbial Characterization - The OMW microbial population was characterised at the beginning of the assay and during the treatment process (0 days, 60 days and 140 days). Microbial colonies were counted as colony forming units (CFU/ ml sample) in a complete medium (total bacteria and fungi) using the spread plate method. The phenol-degrading microorganisms were screening in mineral agar medium with selective phenolic substrates.

Ecotoxicological Evaluation - The ecotoxicological evaluation of OMW samples, collected during the treatment process (0 days, 60 days and 140 days), was carried out using two miniaturized chronic bacterial toxicity tests: The *Pseudomonas putida* growth inhibition test [3] and the *Vibrio fischeri* growth inhibition test [2].

3. RESULTS AND DISCUSSION

The biodegradation ability of OMW by the microrganisms naturally present in these wastewaters was assessed monitoring several physico-chemical parameters (pH, COD, TSS, phenol content, total phosphorus and nitrates) during a biological treatment process carried out in a packed-bed batch reactor. The system studied in this work led to a reasonable removal of COD, TSS and phenol content from OMW (see Fig.1). There was a removal of 80% COD, 71% TSS and 61% phenol content in 140 days.

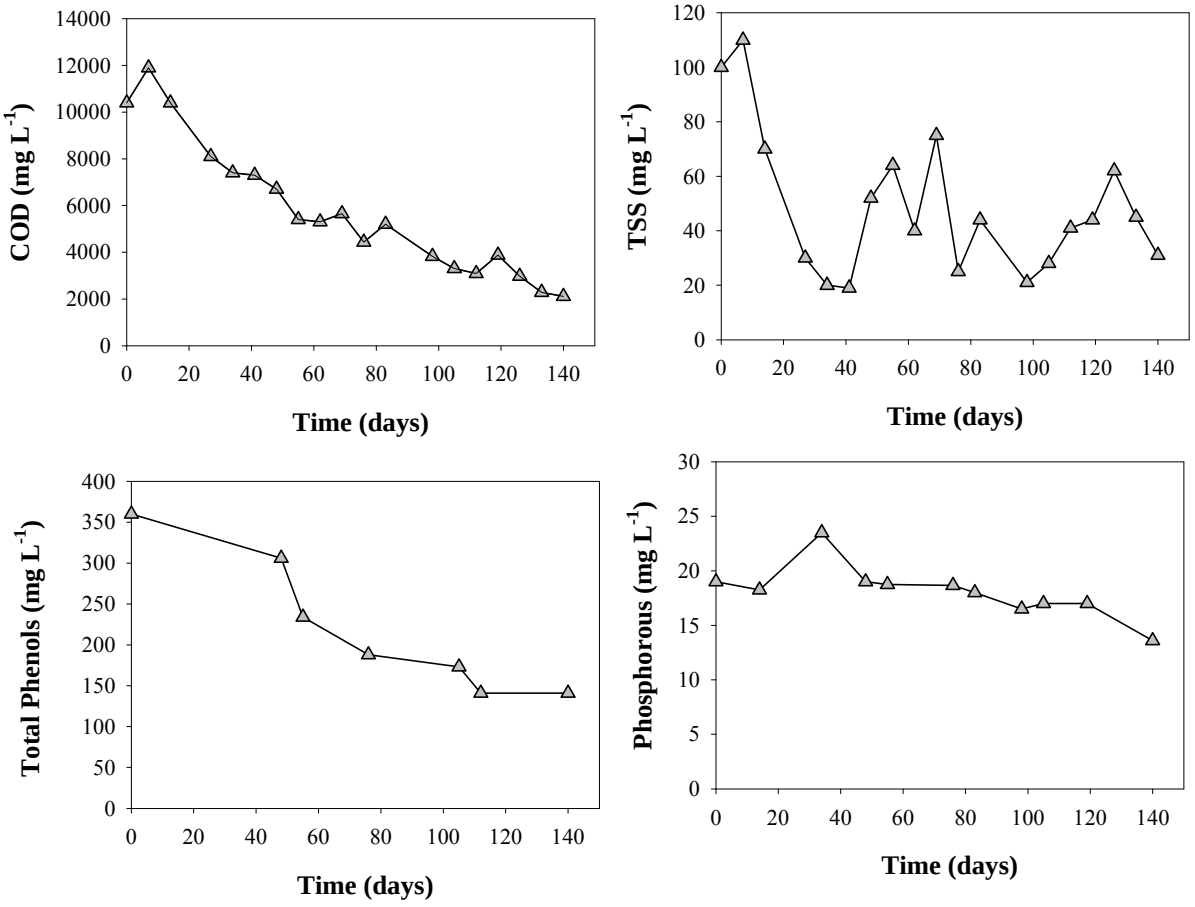

Fig 1. *Monitorization of the physico-chemical characteristics of OMW during the biological treatment in the aerobic packed-bed reactor, at 22 ± 2 ºC.*

The results of the microbial characterization are presented in Table 1. These results demonstrated that this active microbial community present is able to grow under the established conditions. The high number of colonies observed in caffeic and siryngic plates (10^8 - 10^9) during the later stage of the treatment ($T_{140\ d}$) demonstrated a significant polyphenols degrading potential by the microbial population. This high number of active degraders present at the end of the treatment highlights for their potential to extent the treatment process, enhancing the efficiency of process.

Table 1. *Microbial characterization (CFU/ml) of OMW samples collected during the biological treatment process (0, 60 and 140 days of treatment).*

OMW Samples	Microorganisms (CFU/ml)			
	Heterotrophic bacteria	**Yeast/Molds**	**Caffeic acid degrading colonies**	**Siryngic acid degrading colonies**
$T_{0\ d}$	$1.6x10^8$	$2.5x10^8$	$3.9x10^6$	$4.0x10^6$
$T_{60\ d}$	$2.8x10^9$	$2.6x10^8$	$4.8x10^6$	$1.2x10^6$
$T_{140\ d}$	$>1.0x10^{11}$	$2.0x10^6$	$9.2x10^8$	$1.5x10^9$

The ecotoxicity results (Table 2) showed a significant decrease in the chronic toxicity of the treated OMW to both bacteria, *V. fischeri* (62.8%) and *P. putida* (64.3%), after 140 d of treatment, however the OMW sample-$T_{140\ d}$ is still toxic to be discharged directly in the environment (IC_{50}-6h = 23.8% for *V. fischeri* and IC_{50}-16h = 32.4% for *P. putida*).

Previous works already shown that *V. fischeri* in a bioluminescent bioassay was the most sensitive microorganism in testing OMW acute toxicity [4], but this study using *V. fischeri* in a growth inhibition assay for the determination of the OMW chronic toxicity, also demonstrated its higher sensitivity. *V. fischeri* presented lower values of IC_{50}s than *P. putida* for OMW samples.

Table 2. Ecotoxicity results, IC_{50}-16h (%) and IC_{50}-6h (%), obtained in *P. putida* and *V. fischeri* growth inhibition tests, respectively, for OMW samples collected during the treatment process (0 days, 60 days and 140 days).

OMW Samples	***P. putida* Growth Inh. test**		***V. fisheri* Growth Inh. Test**	
	IC_{50}-16h (%)	CI (95%)	IC_{50}-6h (%)	CI (95%)
$T_{0\ d}$	11.57	10.60-14.85	8.91	5.16-10.98
$T_{60\ d}$	13.48	12.06-15.07	8.67	7.96-9.17
$T_{140\ d}$	32.43	28.07-39.23	23.98	16.38-32.88

Inh. – Inhibition, IC_{50} – Median Inhibitory Concentration, CI – Confidence Interval

4. CONCLUSIONS

The system studied in this work led to a reasonable removal of COD, TSS and phenol content from OMW. There was a removal of 80% COD, 71% TSS and 61% phenol content in 140 d.

The presence of the autochthon microbial population was detected in the olive oil wastewater samples since the beginning but the treatment induced a great increase in the active microbial community, with a significant increase of polyphenol degrader population (10^8-10^9 CFU/ml), permitting that an effective degradation and mineralization of the complex organic matter took place.

Together with the COD, TSS and phenol removal, a significant decrease (> 60%) in the chronic

toxicity of the treated OMW, after 140 d of treatment, was also observed to both bacteria tested (*P. putida* and *V. fischeri*), highlighting the detoxification potential of the system studied.

So, the high reduction of the organic loading and its associated toxicity make this simple and inexpensive biological aerobic reactor system, based on the stimulation of the OMW'autochthon microbial population potential, a promising technique to be used as an aerobic pretreatment stage to feed a complementary enhanced anaerobic digestion system of OMW, after detoxification, on an integrated aerobic-anaerobic system.

Acknowledgments

This study was partially supported by the European LIFE Programme (LIFE04/ENV/ IT/000409).

5. REFERENCES

[1] APHA (1998) *Standard Methods for the Examination of Water and Wastewater*. 20th edition. American Public Health Association, American Water Works Association, Water Environment Federation, Washington DC, USA.

[2] DIN 38412-L37 (1999) "Determination of the inhibitory effect of water on the growth of bacteria (*Photobacterium phosphoreum* cell multiplication test)". DIN Deutches Institut fur Normung e.V. Berlin, 12 p.

[3] ISO 10712 (1995) " Water Quality – *Pseudomonas putida* growth inhibition test (*Pseudomonas* cell multiplication inhibition". International Organization for Standardization. Geneve, Switzerland, 11 p.

[4] Paixão S.M., E. Mendonça, A. Picado, A.M. Anselmo (1999) "Acute toxicity evaluation of olive oil mill wastewaters - A comparative study to three aquatic organisms", Environmental Toxicology 14, 263-269.