KINETICS OF A PACKED-BED BATCH REACTOR FOR THE TREATMENT OF OLIVE OIL WASTEWATERS FROM A PORTUGUESE MILL

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SUMMARY: 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. This effluent does not undergo any treatment and is usually stored in evaporation lagoons or spread on the land. This can have a negative impact in the environment since this effluent has a high level of organic matter leading to a high chemical oxygen demand. In addition it has also a high content of polyphenols that contributes to the ecotoxicity of this effluent.Different techniques for the treatment of these wastewaters have been studied. In this work a 60 litre 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 effluent from an olive mill farm (from Alfândega da Fé, Trás-os-Montes) and the effluent was re-circulated daily for homogeneity. COD, colour, nitrogen, solids and phosphorous were measured to follow the evolution of the system. Microbial composition and polyphenols were also evaluated. As an indicator of the microbial activity in the reactor, lipase activities were measured. Ecotoxicity tests were carried out to follow the detoxification capacity of the system as well as its potential for using in the treatment of this type of agroindustrial effluent.

1. INTRODUCTION

Olive oil production is a traditional agricultural industry in Mediterranean countries that accounts for about 95% of the world production and Portugal is one of the ten major producers. (Capasso, 1995)

The olive oil extraction process leads to the production of two by-products: a solid residue called bagasse and the mixture of the vegetation waters and the waters resulting from the production process that is called olive mill wastewater (OMW).

There are different techniques to extract the olive oil. The oldest extraction technique consists on a pressing system. However, nowadays, continuous processes of two and three phases have been more and more used. The two phase's system produces a lower volume of olive mill wastewater but bagasse with a higher percentage of humidity.

Olive mill wastewater shows a high phytotoxicity and antibacterial potential mainly due to its high chemical oxygen demand (COD) and high content of phenolic compounds. The disposal of these wastewaters in water courses causes acidification, a high oxygen demand, colour alteration

and a resulting bad smell. For these reasons these effluents must be treated before discharging.

The maximum accepted values for the discharge of effluents in water courses in Portugal are shown in table 1.

Table 1 – Maximum values permitted for the disposal of effluents in water courses in Portugal. [law 236/98 Anex XVIII]

	Concentration (mg/L)
Phenols	0.5
COD	150
BOD_5	40
Fat	15
Nitrogen	15
Total solids	60
Phosphorus	10

An alternative for the disposal of OMW in water courses would be its spreading in agricultural soils. It is allowed in Italy and in Portugal with a special permission. (Casa, 2003)

To be able to dispose OMW in soils in Portugal it is needed to have permission from the Environmental Department and it has to follow several rules: the existence of a storage vessel or lagoon to store the total amount of wastewater produced by year, a pre-treatment is mandatory including correction of pH, the disposal of OMW must be done between Mars and November depending on the weather conditions of the year, OMW can only be disposed in arboreal land, the volume of OMW disposed can not be over 80 m^3/ha/year, the olive mill must do a report with the volume of OMW disposed and the period of its disposal and the disposal of these wastewaters may be prohibited in certain places such as natural parks, land less than 35 m away from water courses, etc. (law 236/98 Anex XVIII)

Usually, in Portugal, OMW are kept in evaporation lagoons. This is a rather easy technique for the discharge of OMW, however, it has several disadvantages such as the bad smell, the possible contamination of water courses, the demand of large areas and the long period of time required for the evaporation of the water.

Another possible way to treat these effluents is incineration. This technique leads to the total destruction of OMW but it is costly, it causes atmospheric pollution and it requires specialized manpower.

It is also possible to treat OMW by anaerobic digestion. This has the advantage of producing biogas and not producing much sludge. However, due to the high content of COD and phenolic compounds in the OMW, it is sometimes needed to dilute the effluent prior to its treatment by anaerobic digestion or it has to be pre-treated. (Marques, 2001)

Alternatively, OMW can also be treated by aerobic digestion. This can lead to different reductions of phenolic compounds and COD depending on the performance of the strain used. Also it can lead to the production of several products. However, this technique can be hard to use due to the need for aeration.

In the last decades a big effort has been made to find an efficient way to solve the problem of the OMW disposal. Many techniques have been developped, however, it has been difficult to find an compromise between the efficiency of the technique and the costs associated. In this work it was intended to study the biodegradation of OMW by microrganisms naturally present in these wastewaters.

2. MATERIALS AND METHODS

2.1 Wastewater

The OMW was collected on February from a lagoon of an olive mill lagoon at Alfândega da Fé (Province of Trás-os-Montes) and its characteristics are shown in table 2.

Table 2 - Physical and chemical characteristics of the OMW used.

Parameter	mg/L
pH	4.69
COD	10400
Phosphorous	19
Colour*	7650
Nitrogen**	50
TSS	102
SSV (g/L)	102

* Colour is measured in units of platinum-cobalt

**from nitrates

Abbreviations: COD – chemical oxygen demand; TSS – total suspended solids; VSS – volatile suspended solids

2.2. Analysis

Analysis of chemical oxygen demand (COD), total suspended solids (TSS), volatile suspended solids (VSS) and nitrogen were carried out following the Standard Methods for the Examination of Water and Wastewater (1995). Phosphorous determination was performed using Phosver 3 (Ascorbic Acid) Method HACH. Colour was measured using the platinum-cobalt method, where the unit of colour is that produced by 1 mg platinum/L in the form of choroplatinate ion.

Lipase activity in the OMW was determined using p-nitrophenyl palmitate (pNPP) as the substrate. A 30 mg of pNPP dissolved in 10 ml propanol-2-ol was emulsified in 90 ml 50 mM phosphate buffer pH 8.0, containing 207 mg of sodium deoxycholate and 100 mg of gum arabic. A 0.3 ml of OMW was mixed with 2.7 ml of the pNPP containing emulsion and the absorbance was measured spectrophotometrically at 410 nm. One unit (U) was defined as the amount of enzyme that liberated 1 μmol p-nitrophenol per min.

Phenol content was determined using the method of Singleton & Rossi as follows: Folin-Ciocalteu reagent at 1:10 ($5cm^3$) and sodium carbonate (7.5%-w/v, $8cm^3$) were added to diluted (1:50) crude OMW sample ($2cm^3$). This solution was kept for 2 hours and determination was made at 765 nm.

2.3. Equipment

A 60 litter's vessel was filled with a packaging of plastic material consisting of a cubic geometry (Biological Carrier Media from Rauschert) and pebbles. 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).

2.4. Microbial characterisation

The microbiological population was characterised at three different times (T0 days, T60 days and T140 days). Microbial colonies were counted as colony forming units (CFU) using the spread plate method (Standard Methods for the Examination of Water and Wastewater, 1998).

Bacterial and fungal isolation was carried out on Tryptic Soy Agar (TSA) and Rose Bengal Chloramphenicol Agar Base (CRB), respectively. The phenol-degrading microorganisms were screening in mineral agar medium (Pettigrew et al., 1990) with caffeic or siryngic acids, as test substrate, at final concentration of 200mg l-1.

The incubation was carried out at 28ºC during 3 days.

2.5. Ecotoxicological evaluation

The ecotoxicological evaluation of OMW samples, collected during the treatment process (0 days, 60 days and 140 days), was carried out using a chronic bacterial toxicity test. A miniaturized growth inhibition test in 96-well microplates (NUNC^{TM}, Denmark) using a culture of a bacterium, *Pseudomonas putida* (MIGULA DSM 50026*)*, was performed according to ISO 10712 (1995), but adapted to the microtitration plates. The results are expressed in IC_{50}, the concentration responsible for the growth inhibition in 50% of the tested population. IC_{50}-16h values were estimated from the sigmoidal concentration - inhibition curves fitted by the maximum likelihood - logit method using the ToxCalc V5.0.23F (Tidepool Scientific Software, McKinleyville, CA, USA).

3. RESULTS AND DISCUSSION

The reactor was operated during 140 days and the effluent was re-circulated daily for homogeneity and aeration. The dissolved oxygen, pH and the temperature was measured during the working period of the reactor.

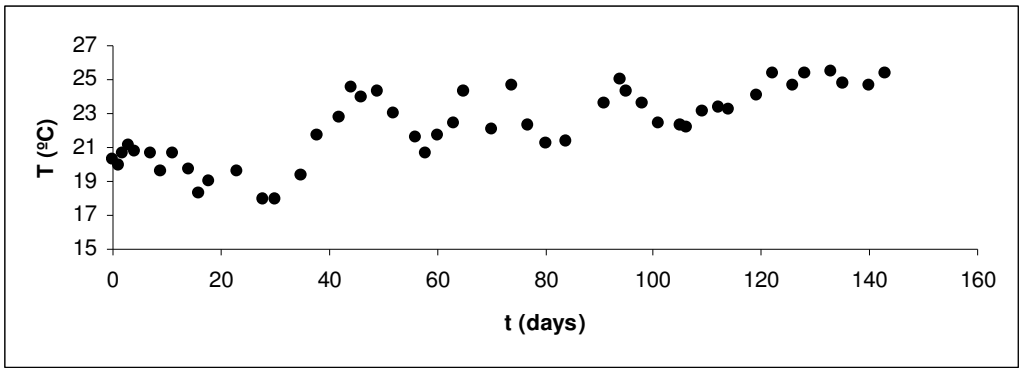

Figure 1 – Variation of the temperature, in ºC, during the working period, in days.

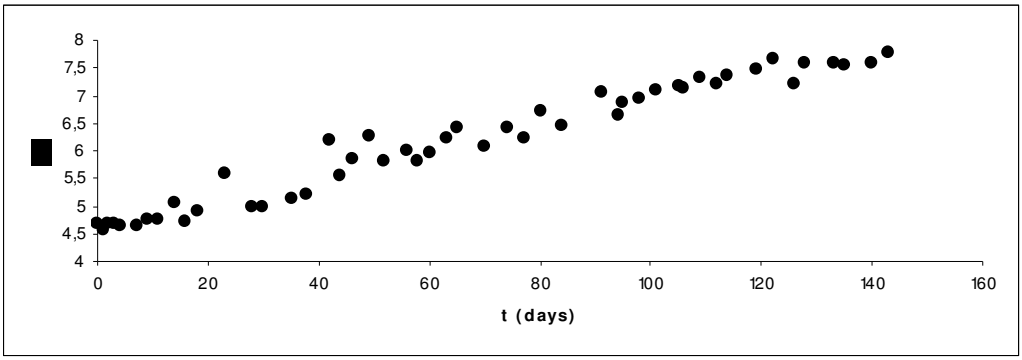

Figure 2 – Variation of the pH during the working period, in days.

As we can see by Figure 1 the temperature varied from 17.9 ºC to 25.5 ºC. Also, by figure 2, we can see that the pH increased from 4.7 to 7.7.

The daily measure of the dissolved oxygen showed that it was very close to zero during the period of time studied.

Parameters such as COD, phenol content, phosphorous, nitrogen, colour, total and volatile suspended solids were also followed. There was a removal of COD of about 80%. However, after 140 days of operation, the COD was still higher than the minimum value allowed for discharge (150 mg/L).

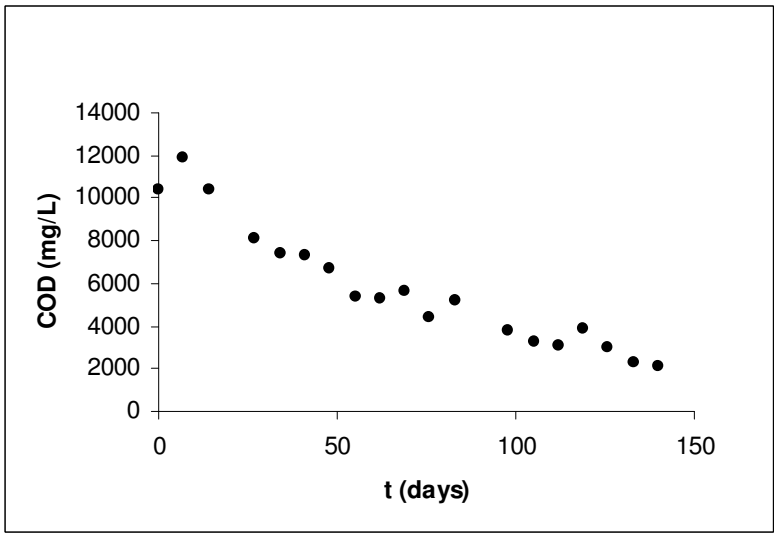

Figure 3 – Decrease of COD, in mg/L, during the period of time studied.

As can be seen by Figure 4, the content of phenols also decreased during the period of time studied. There was a removal of phenol content of about 61%. However, after 140 days of operation, the phenol content was also still higher than the minimum value allowed for discharge (0.5 mg/L). Colour remained constant during the process.

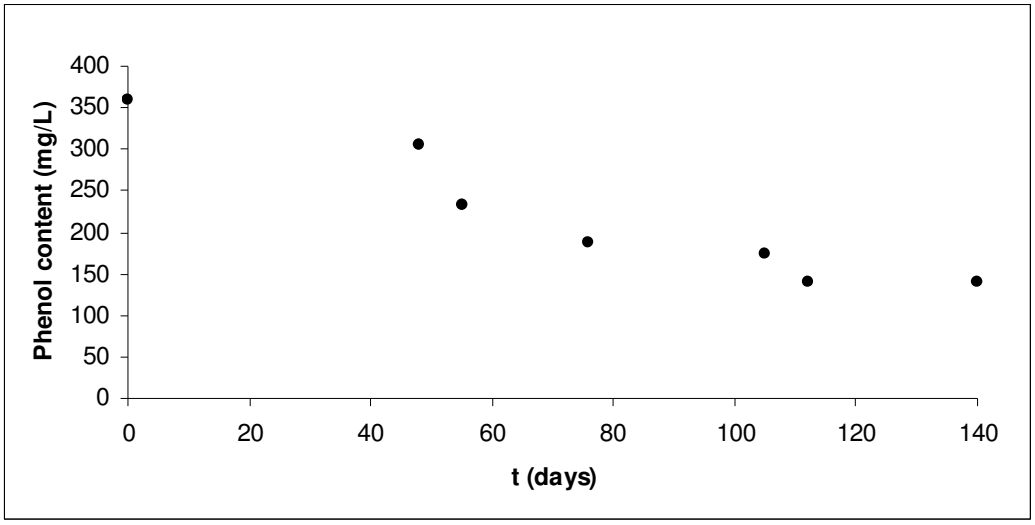

Figure 4 – Decrease of phenol content, in mg/L, during the period of time studied

As it can be seen by Figure 3 and Figure 4, the removal rate of COD (62.0 $mgL^{-1}day^{-1}$) was higher than the removal rate of phenolic compounds (1.7 $mgL^{-1}day^{-1}$).

Nitrate concentration was measured along the process. However no large differences were observed among the samples and compared with the initial nitrate concentration (see table 2). As ammonia was not measured most of the used nitrogen for microbial growth (see table 3) should be of organic origin.

As it can be seen from Figure 4, phosphorous did not show a clear trend during the period of time studied. The concentration of phosphorous was almost constant in the beginning of the experiment and to decrease near the end. There is a possibility that some of the phosphorus may be coming out from the sandy rock layer at the bottom of the reactor compensating for some of the loss of phosphorous due to the microbial growth .

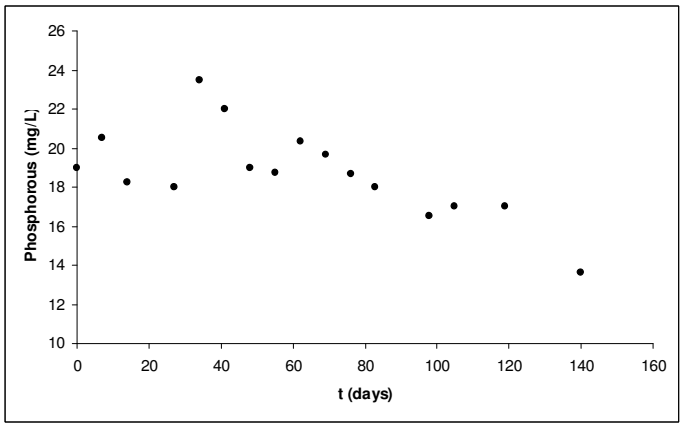

Figure 4 – Concentration of phosphorous, in mg/L, during the period of time studied

The lipase activity in the OMW was also determined and, for the beginning of the experiment, it was 5.7 U/mL. The results showed that there was not a significative variation in the lipase activity for the period of time studied.

There was a decrease of the total suspended solids during the period of time studied The results of the microbial characterisation are presented in Table 3.

Table 3 – Microbial characterisation (CFU/ml) at 28ºC of OMW at different times.

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

Considering the results obtained for the CFU, the different samples (T_0, T_{60} and T_{140d}) hold an active microbial community and an increase in the cell number in bacterial population was observed, ranging from 10^8 CFU/mL OMW (T_0) to 10^{11} CFU/mL OMW (T_{140}) (heterotrophic bacteria) and from 10^6 CFU/mL OMW (T_0) to $10^8/10^9$ CFU/mL OMW (T_{140}) for caffeic and siryngic acids degrading colonies, respectively. However, a decrease (two orders of magnitude) was obtained for yeast/fungi population, along the process what is reasonable due to the anoxic conditions.

The results demonstrated that an active microbial community is present and is able to grow under the established conditions. The high numbers of colonies observed in caffeic and siryngic plates (10^8 /10^9) appeared mainly during the later stages of the treatment and demonstrate a significative presence of polyphenols degrading potential of the microbial population.

The results of the ecotoxicological evaluation of the OMW samples during the treatment process (0 days, 60 days and 140 days), using the P. putida growth inhibition test, are presented in Table 4.

Table 4 – Ecotoxicity results, EC_{50}-16h (%), obtained in *P. putida* growth inhibition tests for OMW samples collected during the treatment process (0 days, 60 days and 140 days).

OMW Sample	IC50-16h (%)	CI (95%)	% Toxicity Reduction
$T_{0\ d}$	11.57	10.60-14.85	---
$T_{60\ d}$	13.48	12.06-15.07	---
$T_{140\ d}$	32.43	28.07-39.23	64.35

Abbreviations: IC – Inhibitory concentration, CI – Confidence interval

These results showed a significant decrease (64.35%) of the chronic toxicity of the treated OMW to the bacteria *P. putida*, after 140 days of treatment, however the OMW sample-$T_{140days}$ is still toxic to be discharged directly in the environment.

4. CONCLUSIONS

The system studied in this work led to a reasonable removal of COD and phenol content from OMW. There was a COD removal of 80% and phenol content removal of 60% in 140 days.

The presence of an active microbial community was detected in the olive oil wastewater samples since the beginning but the treatment induced a great increase of the microbial population and an effective degradation and mineralization of the complex organic matter took place.

Together with the COD and phenol removal, a significant decrease in the chronic toxicity of the treated OMW to *P. putida*, after 140 days of treatment, was also observed, highlighing the detoxification potential of the system studied.

This is a rather simple technique, which may be of relatively low cost and with a rather important degradation potential of the organic load. However, it takes a long time for the degradation to take place and it is still needed a storage vessel to keep the OMW before its disposal. It may be of some advantage when a full treatment plant is available nearby or the Ecotoxicity decrease makes it more amenable for land disposable. Alternatively a complementary treatment may be devised for allowing disposal or recycle of the water.

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