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The absorption of polyphenols from olive oil mill wastewaters by sawdust and biodegradation by the fungus *Phanerochaetae chrysosporium*

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RESUMEN

La biodegradación del alpechín por sawdust y por *Phanerochaetae chrysosporium.*

Este trabajo describe la decoloración y la disminución de la demanda química de oxígeno del alpechín (OMW) por *Phanerochaetae chrysosporium,* crecido en cultivos estáticos, agitados e inmovilizados. Cuando *P. chrysosporium* fue cultivado en agitación, no se observa ninguna decoloración de OMW crudo, la decoloración ocurre solamente después de eliminar los polifenoles mediante adsorción en el serrín (Disminución del 39% del contenido en polifenoles). La utilización de la lignina peroxidasa generada en el medio da lugar a la mayor decoloración de alpechín y a las eficiencias de eliminación de DQO más altas. Las pruebas de la decoloración realizadas en las muestras de OMW que fueron pretratadas por la adsorción de madera del serrín, y usaron cultivos inmovilizadas demostraron resultados mejores. Por tanto, la eficiencia de eliminación de DQO alcanzó un 70%. La reducción de los polifenoles alcanzó los niveles más altos siempre, i.e. 62%. Se observó una decoloración significativa del efluente.

PALABRAS-CLAVE: Alpechín – Biodegradación –- Decoloración - Polifenoles – Serrín.

SUMMARY

The biodegradation of Olive Oil Mill Wastewaters by Sawdust and by a *Phanerochaetae chrysosporium.*

This paper discusses decolorization and chemical oxygen demand (COD) abatement in olive mill wastewaters (OMW) by *Phanerochaetae chrysosporium* grown in static, stirred and immobilized cultures. When *P. Chrysosporium* is used in cultures, no decolorization of crude OMW is observed. Decolorization occurs only after the removal of polyphenols by adsorption in sawdust, which allows a 39% polyphenol removal. The use of a High lignin peroxides (Lip) producing medium, yields the highest OMW decolorization and COD removal efficiencies. The use of *P. Chrysosporium* immobilized on polyurethane foam leads to significant abatements of OMW polluting characteristics. And COD abatement reached 70%. The reduction of polyphenols reached its highest level at 62%. A significant effluent decolorization is apparent.

KEY-WORDS: *Biodegradation – Decolorization – Olive mill wastewaters – Polyphenols – Sawdust.*

1. INTRODUCTION

Pollution by olive mill wastewaters (OMW) is becoming a crucial problem in the Mediterranean area, particularly for the main producers of olive oil, such as Spain, Italy, Greece, Tunisia and Morocco which produce more than 3×10^7 m^3 of OMW per year.

Industrial olive oil production processes in Morocco consist of (a) continuous solid-liquid centrifugal systems (industrial scale) reaching 190 units with a global capacity of 405.000 tonnes and (b) a traditional press process with 15.991 units having a global extraction capacity of more than 50% of the total national production.

In Morocco large volumes of OMW are produced annually. These effluents are currently discarded in natural water streams particularly in Fès, causing severe damage to water treatment plants and to the natural environment. Oxygen depletion was observed at 100 km downstream the city of Fès.

OMW are highly polluting. Biological oxygen demand (DBO) and chemical oxygen demand (COD) can be as high as 100 and 200 g l^{-1} respectively. The phytotoxic, antibacterial and color effects of the OMW have been attributed to its phenolic compounds, which inhibit biological treatment.

P. Chrysosporium is one of the organisms whose ligninolytic enzyme system has been studied extensively. Because of its high ligninolytic activity, rapid growth, and ability to produce asexual spores, several biotechnological applications were tested using this particular species (Sayadi and Ellouz, 1992; 1993).

A recent work has shown that the *P. Chrysosporium* species is able to degradate a wide variety of phenolic compounds (Sayadi and Ellouz, 1992; 1993). Besides *P. Chrysosporium* produces two types of extra-cellular peroxidases involved in lignin biodegradation:

Lignin peroxidase (Lip) catalyzes the oxidation of various aromatic compounds and Manganese peroxidase (Mnp) which catalyzes the oxidation of Mn (II) to Mn (III), which in turn can oxidize several phenolic substrates.

Several studies have reported that the use of immobilized white rot basidiomycetous, prove to be a promising approach, since it opened the way to the possibility of mycelia biomass recycling in effluent treatment applications (Sayadi et al., 1996).

This research work aims to develop an integrated process to treat OMW. The integrated process comprizes the following treatment steps:

- Coagulation–decantation which allows for the reduction of COD and BOD_5 .
- Absorption of OMW in sawdust which improves OMW biodegradability with *P. Chrysosporium* species in static and stirred chemostat cultures and with polyurethane immobilized mycelia of *P. Chrysosporium.* Sawdust, a relatively abundant and inexpensive material is currently being investigated as an absorbent to remove polyphenols from olive oil mill wastewaters.

2. MATERIALS AND METHODS

OMW samples were obtained from Al Idrissi olive oil mill, located at Dokkarat in Fès, which uses a continuous solid-liquid centrifugal processing system. All OMW samples were screened at 0.16 mm in order to eliminate coarse solids.

2.1. Physico-Chemical Treatments

2.1.1. Flocculation- decantation

Classic Jar tests were carried out. Several coagulants such as ferrous sulphate ($FeSO_4.7H_2O$), ferric chloride ($FeCl_3.6H_2O$) and aluminium sulphate $Al_2(SO_4)_3$ were tested at concentrations ranging from 20 and 80 mg l^{-1}. pH, COD, BOD_5 and color were measured in supernatants.

2.1.2. Absorption in wood sawdust

Absorption was carried out using sawdust, on decantation supernatant samples, in 500 ml stirred flasks. Sawdust concentrations of 5 to 25 g l^{-1} were used. The measured variables were pH, COD, BOD_5, phenol concentration and color.

2.1.3. Source of organisms

P. Chrysosporium fungi were received from MUCL (Collection Mycothec of the Catholic University of Louvain, Belgium) and were received in 10 ml closed containers in slanted agar.

2.1.4. Culture media

Two culture media were prepared: Manganese peroxidase (Mnp) producing medium (medium A) and Lignin peroxidase (LiP) producing medium (medium B) at pH 6.5. The incubation temperature was 37°C (Hernández et al., 1990). Reticulated polyurethane foam cubes (volume of 1.5 cm × 1.5 cm × 1.5 cm, an internal void fraction of 0.97 µm and a density of 22 kg·m^{-3}), were used for *P. Chrysosporium* biomass immobilization. Cultures were inoculated at 20% and incubated at 37°C for up to 5 days until the foam surface appeared to be uniformly colonized.

2.1.5. Aerobic treatments of olive mill wastewater

Aerobic treatments of OMW were performed in one litter flasks using 250 ml of effluent incubated at 37°C, for 12 days. The cultures were purged daily for 10 min with sterilized oxygen at a rate of 0.40 l min^{-1}.

All measurements were made in two replicates and the results presented are the average of the two measurements.

2.1.6. Analytical techniques

Polyphenol determinations: Total polyphenols were determined by a spectrophotometric method (Maestro et al., 1991). Calibration was carried out under the same conditions using cafeic acid as standard at concentrations of 0, 1.2, 2.4, 3.6, 4.8, 6 and 7.2 mg l^{-1}. Absorbance was measured at 720 nm against a blank sample.

Decolorization assay: Two cultures were harvested and the mycelia washed filtered on Whatman Glass Microfiber Filters GF/D. 30 fold diluted supernatants were used for absorbance measurements at 395 nm (UV-Visible Spectrophotometer).

COD determination: COD was carried out according to the method described in the standards for the examination of water and waste waters handbook (APHA, 1989).

BOD determination: BOD_5 was measured using the direct manometric method using a Hach (model 2173 B) BOD meter.

2.1.7. Statistical analysis

Experiments were conducted following 2^{nd} order factorial plan (Espensen, 1981). The effects of four factors were considered i.e., initial polyphenol concentration [polyphenols]i, initial inoculum concentration [inoc]i, incubation temperature [Ti] and initial pH (pHi). Two response-variables were obtained: average maximum polyphenol biodegradation rate (Max.Avr.Rate), and final pH (pH_f).

Optimal polyphenols biodegradation and inhibition conditions were obtained (This research part was accepted for publication in Statistics Probabilities Letters (Mebirouk et al., 2007).

3. RESULTS AND DISCUSSION

3.1. Olive Mill Wastewater composition

A typical composition of used raw OMW samples is given in Table 1. Two parameters directly

Table 1
OMW Typical physico-chemical composition.

Parameter	Value
pH	5.5
Conductivity (ms cm^{-1})	14
MES (g l^{-1})	9.0
COD (g l^{-1})	196
BOD5 (g l^{-1})	96
COT (g l^{-1})	40.5
Kjeldhal nitrogen (g l^{-1})	0.9
Polyphenols (g l^{-1})	17.5
Oleuropeine (g l^{-1})	2.8
Color (Cob-Pb)	180
Na+ (g l^{-1})	0.9
Cl^- (g l^{-1})	1.1

related to OMW chemical composition are, pH and polyphenols compounds. The pH varies from 4.5 and 5.5. Polyphenolic compounds deserve special attention because of their influence on three important properties of the effluent: its antibacterial effect, its phytotoxic effect and its blackish color (Sáinz et al., 1986). The phenolic compounds with low molecular weight seem to be responsible for the first two effects and those with high molecular weight are responsible for the black color.

3.2. Flocculation-Decantation

Flocculation-decantation allowed a significant reduction in the organic components of OMW. The BOD_5, COD, TOC, and suspended matter (MES) removal rates achieved with 80 mg l^{-1} aluminium sulphate were 60%, 36%, 30% and 33% respectively. However, no decolorization was observed. This leads to the conclusion that the color is mainly due to soluble organic compounds.

The pH of OMW was neutralized after coagulation-flocculation. The coagulated-flocculted OMW is used therefore for the action of sawdust.

3.3. Absorption on Sawdust

Sawdust absorption is a pre-treatment step before biodegradation-decolorization. Figure 1 shows pH evolution and figure 2 shows polyphenol, COD and BOD_5 reduction ratios in OMW samples respectively.

3.3.1. pH evolution in OMW in contact with sawdust

Figure 1 shows the pH increase during sawdust absorption, due to the release of basic compounds from sawdust (The surface of sawdust contains important oxygen sites mainly in basic functional form: phenolic hydroxyl groups). This contributes to OMW acidic pH neutralization and this contribution increases with sawdust concentration. Polyphenols are absorbed in the sawdust by dispersive interactions between the basic carbons and basics polyphenol aromatics in the sawdust (Dutta et al., 2001).

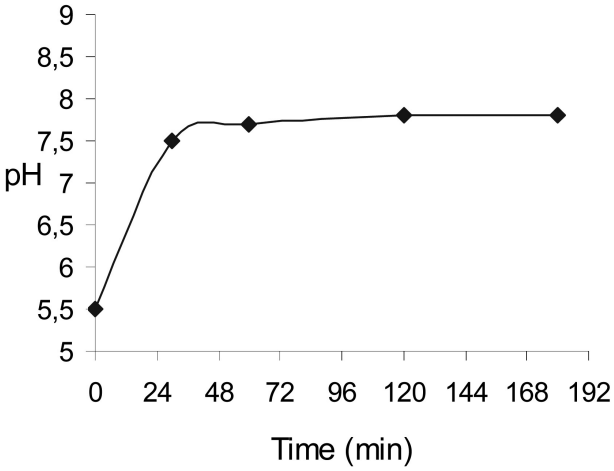

Figure 1
OMW pH evolution in contact with sawdust.

3.3.2. Polyphenol, COD, BOD5 evolution in OMW in contact with sawdust

Sawdust absorption allows for polyphenol, COD and BOD_5 reduction ratios of about 39%, 53% and 44% respectively (Figure 2). This leads to the conclusion that the soluble organic matter contained in OMW is partially absorbed in sawdust.

A COD decrease is due to a solid organic matter physical retention into sawdust porosity and soluble COD absorption in sawdust.

Absorption in sawdust produced an improvement in OMW decolorization. This is due to the removal of High Molecular (HM) polyphenolic fractions. Previous physico-chemical research, reported that the HM fraction inhibited OMW decolorization and also showed that these fractions completely inhibit LiP activity (Sayadi et al., 1996).

3.4. Biological Treatment

We specify that OMW samples submitted to biological treatments are those resulting from physico-chemical sawdust pre-treatment.

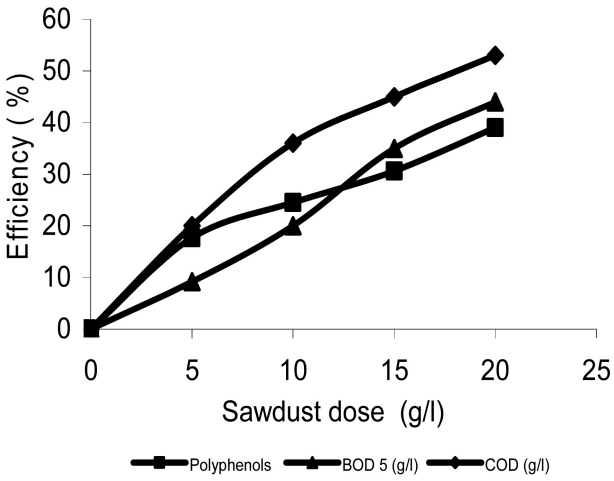

Figure 2
OMW polyphenol content evolution by sawdust absorption.

Correspondence factorial analysis was used to determine optimized growth conditions for *P. Chrysosporium*. Thus, the best polyphenol biodegradation kinetics was obtained in the following conditions: pH range, varying from 5.5 to 7.5 with an optimum of 6.5 and Ti range, varying of 32°C with 42° with an optimum of 37°C. The final pH is 7.1.

3.4.1. *OMW decolorization by* P. Chrysosporium *grown in stirred cultures*

The results from stirred cultures show that, raw OMW polyphenol abatement ratios and discoloration are about (0%), however, in OMW samples pre-treated, polyphenol abatement ratios reached in 3, 6 day treatments, 75% and 65% respectively in (A) and (B) mediums. Likewise , the OMW discoloration reached approximately 65% and 45% respectively in the two mediums (figure 3).

The COD abatement rates were about 22% in raw OMW, whereas in pre-treated OMW samples, rates reached, 75% and 50% respectively in (A) and (B) mediums for the same residence time (figure 4).

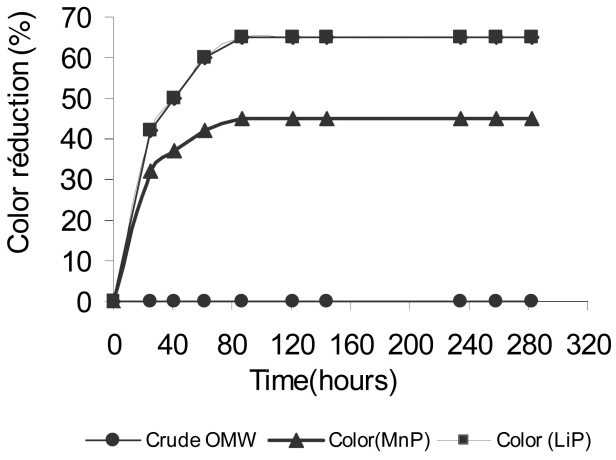

Figure 3
Decoloration by *P. Chrysosporium* in stirred cultures.

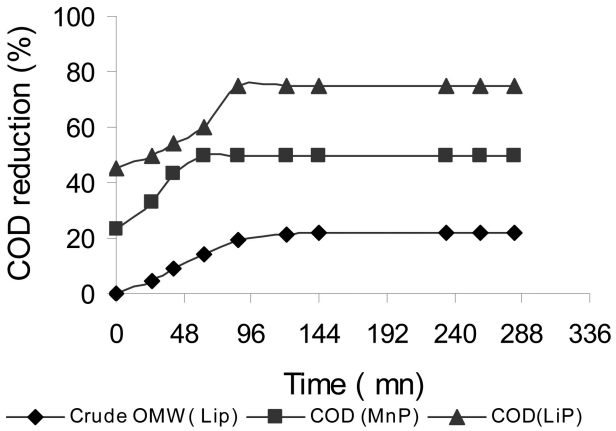

Figure 4
COD evolution of crude and pre-treated OMW in stirred cultures.

The biological treatments showed that the COD part is reduced during the first two days; this means that the *P. Chrysosporium* specie, first oxidizes easily biodegradable organic matter. The COD kinetics degradation was then stabilized further than 3, 6 days; this means that the medium lacks substrate.

The results obtained, show best abatements obtained in a lignin peroxidase (Lip) producing medium compared to lower abatement in a manganese peroxidase (MnP) producing medium.

3.4.2. *OMW decolorization by* P. Chrysosporium *grown in static cultures*

The figures 5(a,b), show the phenolics compound degradation evolution and discoloration in medium (A) static cultures for 10 days respectively . The phenolic compound reduction ratio was about 20% in raw OMW samples. Whereas, in pre-treated samples, this rate reached approximately 86%. The discoloration reached approximately 75%.

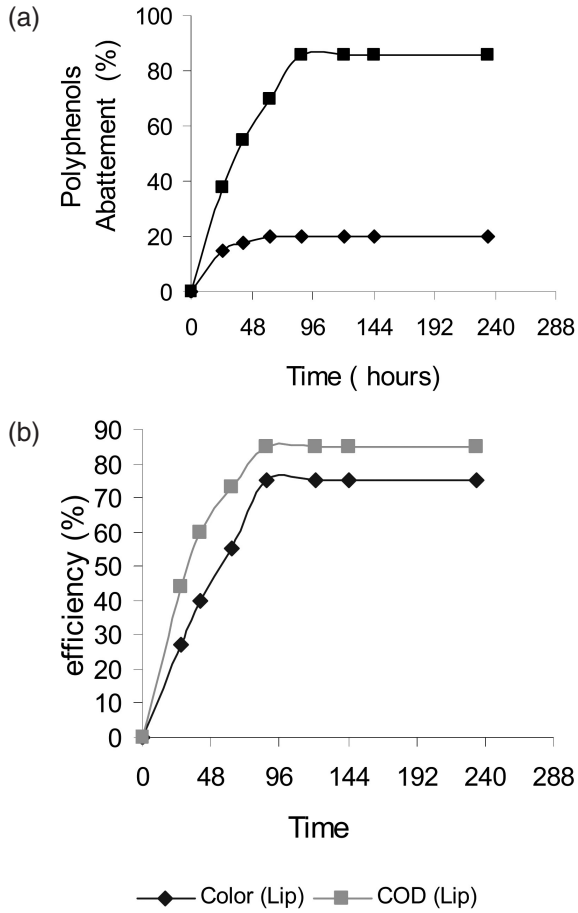

Figure 5 (a, b)
OMW treatment efficiency by *P. Chrysosporium* grown in static cultures.

Previous physicochemical pre-treatment tests showed high molecular weight retention polyphenols in sawdust (Dutta et al., 2001). In a biological system these fractions inhibited lignin

peroxidase action and consequently deteriorated the growth of the microorganism.

The results obtained, showed that biological static treatments are more efficient than biological stirred treatments. This difference is explained (a) by the negative stirred effect on biological biodegradation and (b) by large amounts of organic matter which inhibit fungus species growth.

Indeed several studies reported that the lignin degrading enzymes produced by *P. Chrysosporium* species are detached easily from the fungus cells and partially lose their active capacity in stirred cultures(Kirk and Farrell 1987; Sayadi et al., 1996).

3.4.3. OMW biological treatment efficiencies in immobilized cultures

The figure shows that OMW discoloration appeared after 24 hours of treatment and reached 70% in 62 hours. These results lead to the conclusion that polyurethane, cultures immobilized *P. Chrysosporium,* give more efficiency compared to those in stirred cultures (figure 6).

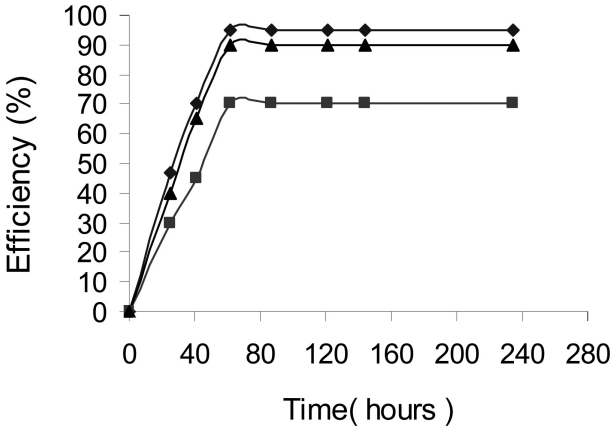

Figure 6
Polyphenols evolution of crude and pre-treated OMW in immobilized cultures.

4. CONCLUSION

Results show that the use of a combined sawdust absorption pre-treatment prior to aerobic biodegradation with *P. Chrysosporium*, produces a significant organic matter reduction from olive mill wastewaters.

Sawdust has proven to be a promising material for organic matter removal. In fact, polyphenol reduction is about 39%.

Table 2, synthesizes, batch OMW biological treatment efficiencies according to various cultures types (stirred, static or immobilized). The efficiencies are reported after the same residence time of about 3.5 days in stirred and static cultures. In immobilized cultures, the average residence time is about 2.5 days.

The biological treatments showed that, fungus biological biodegradation potential is better as it is cultivated on the lignin producing medium.

The liquid static cultures concept seems more appropriate to OMW phenolic degradation compounds, suggesting that the production of lignino-degrading enzymes is better in immobilized cultures than in suspension cultures.

The present developed treatments, combining sawdust absorption and biological treatment with immobilized *P. Chrysosporium,* leads to total polyphenols, color, and COD reduction efficiencies of 95 %, 75% and 90% respectively. Polyphenol abatement rates reached 2, 65 g/l/d for an average residence time of 2,6 days in an immobilized reactor culture compared to 1,53 g/l/d in reactor suspension cultures for an initial polyphenol concentration of 8000 mg/l. This justifies the higher efficiency of the immobilized cells.

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Table 2
OMW biological batch treatments efficiencies %.

	Stirred cultures				Static cultures				Immobilized cultures
	Raw OMW		Pre-treated OMW		Raw OMW		Pre-treated OMW		Pre-treated OMW
	Lip	MnP	Lip	Mn	Lip	MnP	Lip	MnP	Lip
Polyphenols	0%	0%	75%	60%	20%	–	86%	–	95%
Color	0%	0%	65%	45%			75%	–	75%
COD	22%	–	75	50%			85	–	90%

–: No data.
Lip: Lignin peroxidase producing medium.
MnP: Manganese peroxidase producing medium.

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