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Polyphenol removal from olive mill waste waters by selected mould strains

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RESUMEN

Eliminación de los polifenoles del alpechín por cepas de mohos seleccionados.

Cepas de moho fueron aisladas del alpechín (OMW), caracterizadas y analizadas para determinar sus actividades en la hidrólisis de polifenoles mediante su recultivo en el mismo alpechín. Las cepas seleccionadas se usaron posteriormente para la eliminación de polifenoles en ensayos de cultivos puros en matraces. Antes de la inoculación, el alpechín fue diluido para obtener tres concentraciones iniciales de polifenoles (2,2g/L; 4,5g/L; 14,5g/L). Las características químicas, incluyendo polifenoles y la demanda química de oxígeno (DQO), fueron analizadas en estos ensayos. Los resultados muestran que los polifenoles eliminados por algunas cepas fueron del 95.14% y la DQO disminuyó un 43%. El alpechín es normalmente oscuro por la oxidación de los polifenoles. Las cepas microbianas al hidrolizar los polifenoles decoloraron completamente estos efluentes y un líquido claro fue obtenido tras 12-15 días de tratamiento bajo agitación aerobia en laboratorio. Los polifenoles pueden tener algún efecto inhibidor que podría causar problemas en el tratamiento biológico de estos efluentes. Su eliminación facilitaría el tratamiento del residuo.

PALABRAS-CLAVE: Alpechín - Depuración aerobia - Eliminación - Moho - Polifenol.

SUMMARY

Polyphenol removal from olive mill waste waters by selected mould strains.

Mould strains were isolated from Olive Mill Waste (OMW) waters, characterized and screened for their activities on polyphenols hydrolysis by reculturing on the olive mill waste waters itself. The selected strains were then used for polyphenols removal in pure culture assays in flasks. Prior to the inoculation, OMW water was diluted to prepare three initial concentrations of polyphenols (2.2 g/L; 4.5g/L; 14.5g/L). Chemical characteristics including polyphenols and Chemical Oxygen Demand (COD) were followed up in these assays. Results showed that the polyphenols removal by some strains was estimated to around 95.14% and the COD was also decreased by 43%. Olive mill waste waters are normally colored in dark because of the polyphenols oxidation. The microbial strains by hydrolysing the polyphenols had decolored completely these effluents and a clear liquid was obtained afier 12-15 days of treatment under aerobic shaking in laboratory. Polyphenols may have some inhibitory effect which would cause problems to the biological treatment of these effluents. Their removal would facilitate the treatment of the waste.

KEY-WORDS: Aerobic treatment - Mould - Olive mill waste water - Polyphenol - Removal.

1. INTRODUCTION

The olive mill waste (OMW) water is a dark liquid generated by the olive oil processing. Two kinds of olive mill waste waters may exist due to the oil processing procedures. The oil extraction can be achieved by centrifugation (new process) or by pressing (traditional procedure). These two kinds of procedures for extracting oil may generate certainly OMW waters with different chemical composition and hence different polluting powers. The centrifugation may generate more OMW waters than the traditional procedure because of the high amounts, of water used in the process.

These effluents are very resistant to biological and physico-chemical treatments. OMW waters may contain high amounts of polyphenols (Mouncif *et al.*, 1993) which would render the effluent hard to treat biologically by inhibiting the microorganisms responsible of the organic matter decomposition. Mouncif, *et al.* (1995) had tentatively succeded in culturing some yeast strains on olive mill waste waters for polyphenols removal. They obtained a reduction percentage for the polyphenols of 50% and a COD decrease from 54.14 to 21.56 g/kg.

Several works were carried out on OMW treatment by the use of microbial inoculation. Borja-Padilla *et al.* (1991) and Maestro-Durán *et al.* (1991) combined an aerobic pretreatment (polyphenol removal) with an anaerobic treatment. Hamdi *et al.* (1992) used *Aspergillus niger* in an integrated biological system to remove the polyphenols. Borja-Padilla *et al.* (1992a,b) used a two steps system for OMW treatment consisting of an aerobic treatment with *Geotrichum candidum* followed by an anaerobic digestion process. Mouncif *et al.* (1995) could reach a relatively high reduction of polyphenols in OMW waters by the use of yeast strains.

In the present work, the removal process of polyphenols and the COD reduction were highly improved by the use of moulds isolated from OMW waters.

2. MATERIAL AND METHODS

2.1. Microbial strains isolation

Appropriate dilutions (10^{-1} to 10^{-2}) of OMW samples were pour plated on Potato-Dextrose Agar

(PDA) (Biokar, France). The plates were incubated at 28°C for 5 days. The appeared cultures on the medium were purified by re-isolating two times on the same medium and pure cultures streaked on agar slants were collected for the treatment assays.

2.2. Screening tests

The isolated strains of moulds from OMW waters were screened for growth and polyphenols utilization on a medium prepared from OMW water solidified by agar at 15g/L as described by Mouncif *et al.* (1995). All the isolates were spot inoculated on the medium and incubated for one week at 30°C. Growth and clearing zones around the cultures were used to select the active strains in polyphenols removal. The selected isolates were used in the pure culture assays in flasks.

2.3. Cultures in flasks

The OMW water was centrifuged at 2000 g for 15 min and diluted to 1/5 in distilled water. This solution was supplied with agar (15g/l) and heated to boiling. The prepared medium was dispensed in 250 mL erlen-meyer flasks. A volume of 40 mL/flask was used to make a thick layer in the flask and autoclaved at 120°C for 20 min. The flasks were allowed to cool to room temperature (24°C) and inoculated with the selected mould strains and incubated at 30°C until a heavy culture developped in the flasks. OMW water was then added to the flasks so that the cultures were flowded and the flasks were placed on a shaker (type IKA, Germany). OMW water was diluted in such a way to obtain 3 concentrations of polyphenols (2.2, 4.5 and 14.5g/L). Polyphenols and COD were determined in the initial cultures to know the initial concentration, and after 6, 9, 12 and 15 days.

2.4. Polyphenols determinations

Total polyphenols were determined by the method described by Maestro-Durán *et al.* (1991). A calibration curve was realized in the same conditons using tannic acid as standard at the concentrations: 0, 1, 2, 2.4, 3.6, 4.8, 6 and 7.2 ppm. The absorbance (OD) was measured in a spectrophotometer at 720 nm against a blank without tannic acid.

2.5. COD

The COD was determined according to the standards for the examination of water and waste waters (APHA, 1989).

3. RESULTS AND DISCUSSION

3.1. Strains selection

Growth of moulds strains on OMW waters are reported in table I. Data indicated that several strains could grow on the OMW water solidified with agar. Only few isolates were inhibited and could not grow on the medium. The strains were selected on the basis of their decoloring power and growth. All the strains that showed a heavy growth and wide decoloring zones were qualified as the most efficient strains to be used in the polyphenol removal assays. 4 isolates were selected for their high activities in decoloring the OMW waters.

More selection was also made by the culture conditons including pH and temperature. Variations in growth on OMW water as well as its decoloration among the different strains were observed. The most important growth assessed by the diameter of cultures in the plates was observed between pH 3.2 and 6. One can figure out that the strains grow easily in a wide range of pH as it was indicated. The optimal temperature for growth of most strains was around 30°C. The glucose concentration was not a factor that would have an effect on growth since all the strains could have the same growth under different concentrations in glucose.

These results revealed the growth conditions that would have an effect on the yield of polyphenols removal in the treatment assays. However the activity of the strain due to enzyme production is not

Table I
Growth of the mould strains on OMW waters solidified by agar

	1:10*	1:5	1:2	OMW**
m1	+	+	+	-
m2	+++	+	+	-
m3	+	+	+	-
m4	+++	++	+	-
m5	+	+	-	-
m6	+++	+++	+	-
m7	+	+	-	-
m8	+++	++	+	-
m9	+	+	-	-
m10	+	-	-	-
m11	+	+	+	-
m12	+	+	+	-

Legends: +: growth; -: no growth; *: OMW diluted to 1:10
**: non diluted OMW.

usually correlated to growth. In our case the strains could grow under these normal conditions and produce enzymes which were evaluated by the decoloring zones around the cultures.

3.2. Cultures in flasks

Polyphenols and COD removal

In the trial made with OMW waters containing 2.2g/L of polyphenols and having a COD of 15.5 gO_2/L, all the strains could grow well and reduce the polyphenols to a low level so the removal percentage reached 87.8% for the strain m4 and 86.84 % for the strain m6 (Figure1). The most important reduction had been reached with the strain m4. Only slight variations were observed among the different strains. The reduction time was long so the most important reduction percentage was obtained after 12-15 days at ambient temperature (22-24°C).

OMW waters with the characteristics mentionned above was also analysed for the COD reduction and results are reported in figure 2. The strain m8 was the most efficient and showed the highest reduction percentage (35.81%). Even the COD reduction was low compared to the polyphenols decrease by the same strain, the efficiency of the process is normally aimed to remove polyphenols first and then the COD can be taken in a second phase for finishing the treatment.

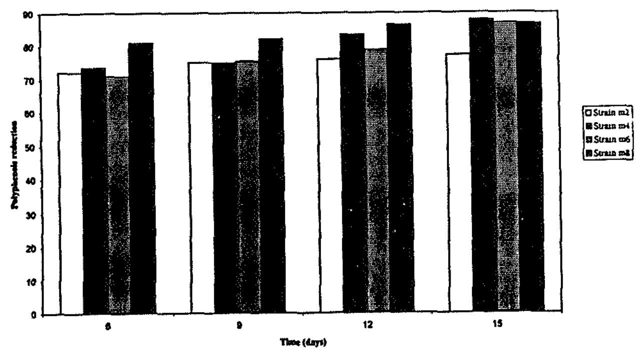

Figure 1
Polyphenols reduction (as %) by the mould strains in OMW water containing 2.2 g/l polyphenols (in %).

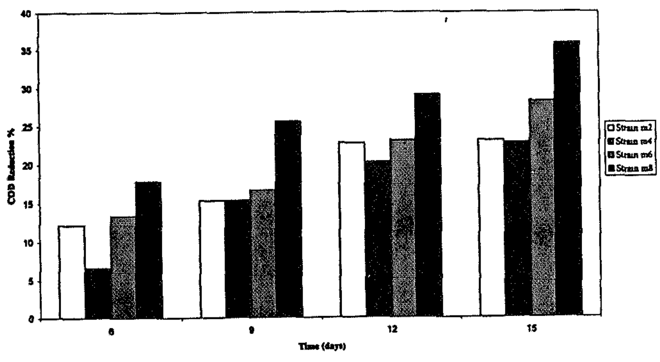

Figure 2
COD reduction (as %) by the mould isolates in OMW water containing 15.5 g O_2/L (in%)

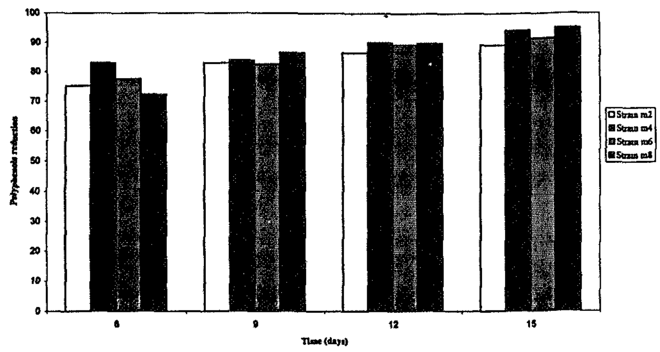

Figure 3
Polyphenols reduction (as %) by the mould strains in OMW water containing 4.5 g/l polyphenols (in %).

Almost the same pattern in polyphenols reduction was observed in the assays made with a higher concentration of polyphenols (4.5g/L) and a higher COD (34.5g O_2/L (Figure 3). The removal percentage ranged from 95.14 (m8) and 89.12 (m2). Only slight variations were observed for the different strains in the polyphenols reduction and the high removal percentage obtained in these assays may give the evidence that the strains could have activities in OMW waters at these concentrations of polyphenols. The low time of reduction 12-15 days should be improved. Further studies are needed to define the kinetics conditions for lowering the time of the process.

For the COD, the reduction percentage was higher than the assays made with lower dilution of the OMW waters. The COD reduction ranged from 39.70% to 45.96% (Figure 4). No important variation between the strains were observed after 15 days but between the 6th day and the 15th day the difference was clear.

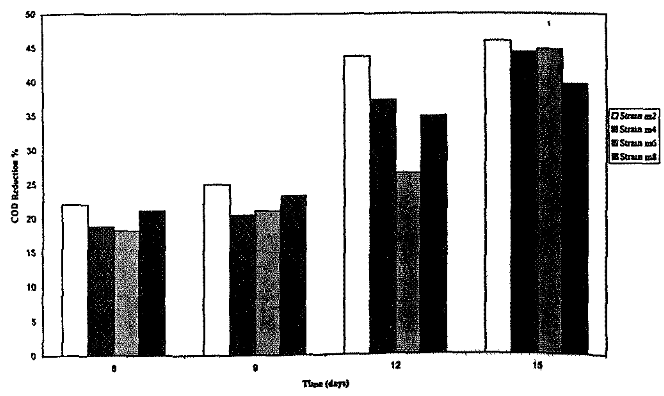

Figure 4
COD reduction (as %) by the mould isolates in OMW water containing 34.5 g O_2/L (in %).

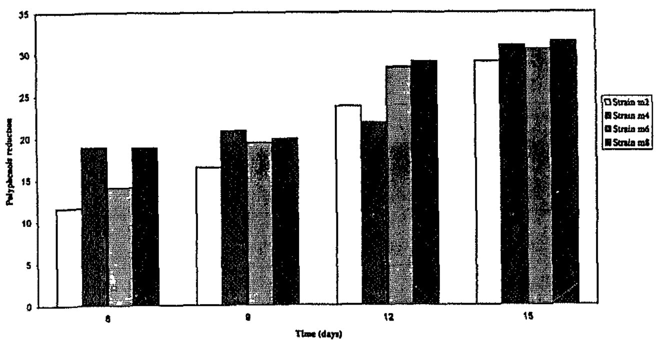

Figure 5
Polyphenols reduction (as %) by the mould strains in OMW water containing 14.5 g/l polyphenols.

In the assays made with non diluted OMW waters, the polyphenols reduction was lowered to around 30%. Polyphenols concentration may have an effect on the reaction and this can be observed for these assays (figure 5). OMW waters from the traditional units are heavily contaminated with organic matter and polyphenols. Our objective was to study the possibility of reducing the dilution of OMW waters so the water volume is also lowered but there was an effect of the non diluted OMW water on the microbial strain and also on the reaction.

The COD reduction in these assays was lower than that in the other assays (Figure 6). This phenomenon would be related to the inhibitory activities of high concentrations of polyphenols in the crude OMW waters. This phenomenon was also studied to state the effect of crude OMW waters on the microorganisms. The COD reduction is not as important as the removal of the inhibitory substances represented by the polyphenols in OMW waters. First research works should concentrate on the reduction of polyphenols and then the usual treatment can be applied.

Results showed a decrease pattern in the polyphenol concentration in the assays made with

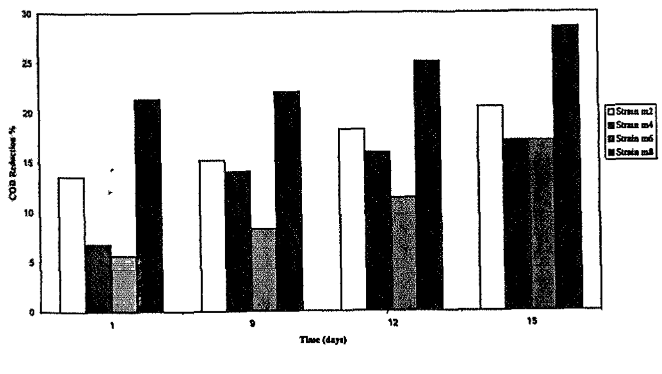

Figure 6
COD reduction (as %) by the mould isolates in OMW water containing 85.5 g O_2/L (in%).

diluted OMW waters (2.2 to 4.5 % polyphenols). This would tell about the inhibitory effect of polyphenols. The conversion process is related to the growth of the microbial strains inoculated in the OMW waters. Polyphenols biodegradation reached a removal percentage of 95.14% by strain m8 in OMW waters containing 4.5% polyphenols. This percentage is the highest one in the field of OMW waters treatment. The process can be improved by a post chemical or biological treatment for more purification. Our results showed higher reduction of the polyphenols than those reported by Martínez-Nieto *et al.* (1992) who reported a total biodegradation of polyphenols by *A. terreus* ranging from 42.08% to 68.90% respectively for non diluted OMW water and a 20% dilution. Maestro-Durán *et al.* (1991) reported a similar reduction of the polyphenols by an aerobic treatment from 300 ppm to 103 ppm which may represent a 67% reduction. In our case the OMW waters were diluted to 1/5 which may represent a higher concentration in polyphenols and the activity of the strain was higher (95.14%). The polyphenols reduction would be a suitable procedure for reducing the inhibiting action of these effluents and consequently the resistance to the usual biological treatments. Hamdi *et al.* (1992) reached a percentage reduction of 58.7% for the COD in OMW waters using an aerobic treatment system by *A. niger*.

The COD reduction may suggest a possible uptake of the organic matter by the microorganisms (oxidation). This uptake may result in biomass formation by the inoculated strains. This phenomenon was observed in most assays and there is a correlation between the COD reduction and the biomass production. The COD reduction would not constitute a major problem in the treatment of OMW waters but the removal of the highly inhibiting compounds such as polyphenols is the only problem that would stop any biological reaction in these effluents and consequently would lead to a high resistance in the usual systems for waste waters treatments. Organic matter hydrolysis by these strains was assessed by the COD slow-down and the most interesting property of these strains was the removal of polyphenols.

Other strains were also used such as *G. candidum* (Borja Padilla *et al.*, 1992a) and *A. terreus* (Martínez-Nieto *et al.*, 1992) to remove polyphenols from OMW waters and to reduce the COD in a pretreatment system for the biomethanization process. In the same way, Hamdi *et al.* (1992) used *A. niger* in an aerobic treatment system. Rannalli (1991) had combined various yeast strains in the aerobic treatment of OMW waters. None of these works reached the removal yield reported in the present work.

Donosa-Arce (1979) described some valorization processes of OMW waters and stated that the production of single cell proteins by yeasts on OMW waters was inhibited and the obtained product was colored.

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