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Olive mill wastewater remediation by means of *Pleurotus ostreatus*

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Abstract

Results of a research program on raw olive-mill wastewater (OMW) bioremediation are presented. Bioremediation experiments have been carried out both in an airlift bioreactor and in aerated flasks, using *Pleurotus ostreatus*. The process was investigated under controlled non-sterile operating conditions, representative of industrial operation. Growth of *P. ostreatus* as well as polyphenols conversion were assessed. OMW bioconversion was characterized in terms of total organic carbon, polyphenols concentration, phenol oxidase activity, extent of decolourization and pH as a function of time. Results demonstrate that: *P. ostreatus* effectively grows on raw OMW; polyphenols abatement is controlled by the availability of nutrients and can be as large as 95%; bioconversion of non-sterilized OMW does not result into appreciable decolourization of the liquid medium. The use of an internal loop airlift bioreactor as a candidate for the full-scale implementation of an OMW aerobic bioremediation process is demonstrated.

Keywords: Olive mill wastewater; Airlift; Polyphenols; Laccase; Bioremedation; *Pleurotus ostreatus*

1. Introduction

Remediation of olive mill wastewater (OMW) is a critical issue associated with olive-oil manufacturing, a widespread activity in the Mediterranean area. Environmental impact of OMW is related to its large organic content and to the phytotoxic and antibacterial action of polyphenols that are abundant in it [1,2]. OMW discharge into sewerage systems, treatment in wastewater systems and spreading on land are discouraged because of its dark colour, due to chromophoric lignin-related compounds [3]. Remediation by means of physical and chemical methods (evaporation and incineration, ultrafiltration, reverse osmosis, anaerobic digestion, addition of chemicals, etc.) is generally technically or economically unfeasible [4], unless specific local factors come into play to improve its feasibility [5]. Preliminary assessment of OMW remediation by aerobic biological treatments demonstrated the potential of these processes.

The potential of ligninolytic fungi as biocatalysts for aerobic OMW remediation has been recently demonstrated. Basidiomycete fungi belonging to the "white-rot" group prove to be active in the reduction of the phytotoxic and antibacterial activity of OMW. Among the others, *Pleurotus ostreatus* (strain Florida) expresses phenol oxidases and peroxidase activities [6–9]. OMW remediation by means of ligninolytic fungi has been mostly addressed with an emphasis on the identification of the ligninolytic enzymes responsible for polyphenols conversion and on the effects of operating conditions on enzyme secretion. Previously published studies most typically address remediation of sterilized OMW in the presence of additional nutrients [7,8,10,11]. Exceptions are the studies carried out by Tsioulpas et al. [9], Fountoulakis et al. [12] and Aggelis et al. [13] who examined bioremediation of sterilized OMW without supplementing nutrients. Preliminary techno-economic assessment indicates that sterilization of OMW prior to remediation would severely affect its feasibility. Bioremediation of raw non-sterile OMW by sequential aerobic (using *Phanerochaete chrysosporium*) and anaerobic biotreatments followed by ultrafiltration was recently investigated by Dhouib et al. [14]. In the present paper the effectiveness of the aerobic stage of bioremediation of raw non-sterile OMW by free cell culture of *P.*

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Nomenclature

C_{Ph}	polyphenols concentration (g/L)
$\dot{C}_{Ph}$	polyphenols conversion rate (g/(L day))
k	first order kinetic constant (L/(g_{DM} day))
MW	molecular weight (g/mol)
OCR	oxygen consumption rate (g_{O_2}/(h g_{DM}))
r_i	reaction rate of component i (g_i(g_{DM} day))
t	time (day)
TOC	total organic carbon concentration (g/L)
X	biomass concentration (g/L)
Y	yield coefficient (g/g)

Greek letters

Φ	conversion coefficient (g/g)
ω_C^X	biomass carbon content (g/g)
ω_C^{Ph}	polyphenols carbon content (g/g)

Subscripts

F	value at the end of the run
C	carbon
Ph	polyphenols
0	value at the beginning of the run

ostreatus is addressed, with an emphasis on the characterization of rates and yields of polyphenols conversion and of *P. ostreatus* growth under controlled process conditions. The potential of internal loop airlift (ILA) bioreactors as efficient and reliable candidates for full scale implementation of the process is also assessed.

2. Materials and methods

2.1. Materials

Olive mill wastewater derived from the continuous solid-liquid centrifugation production technology and was collected from an olive-oil factory located in southern Italy. OMW was delivered to the laboratory within 1 week after the production, then stored frozen at −20 °C. OMW was centrifuged (45 min at 10,000 × g) to remove residual oil and solids, prior to experiments. The main properties of the tested OMW are: TOC = 31 g/L, pH 5.2, COD = 50 g/L, polyphenols concentration 3.6 g/L, dry solid content (at 105 °C) 10.5 g/L.

2.2. Microorganism and inoculum preparation

P. ostreatus (strain Florida) was maintained, through periodic subculture every 3 weeks, at 4 °C on agar–potato dextrose (Difco Laboratories, Detroit, MI) plates containing 24 g/L potato dextrose (Difco Laboratories, Detroit, MI), 15 g/L agar (Sigma), 5 g/L yeast extract (Difco), and 5% (v/v) of OMW. Mycelium growth was carried out in liquid medium in agitated flasks at 28 °C in the dark for 4 days. Each culture flask contained 500 mL of sterilized broth (20 min at 120 °C), consisting of 24 g/L potato dextrose and 5 g/L yeast extract, and five 10 mm plugs collected from the subculture plates. The 4-day culture was then filtered to remove the broth. The mycelium was re-suspended in bi-distilled water and homogenized by means of a mixer for few seconds. Pre-set volumes of a homogeneous suspension of the mycelium were inoculated into reactors.

2.3. Apparatus

Erlenmeyer flasks (400 mL) immersed in a thermostatic bath were continuously oxygenated by sparging water-saturated air through an immersed nozzle. The continuous pneumatic agitation of the suspension provided effective mixing.

A 5 L internal-loop airlift bioreactor was also adopted. Main characteristics of the airlift are reported in Olivieri et al. [15]. The reactor was equipped with: a heating apparatus and on-line diagnostics. The design/operational variables of the airlift were optimized with reference to bed hydrodynamics as discussed in Olivieri et al. [15]. Metered water-saturated air was sparged into the draft tube by means of a perforated pipe distributor. The heating apparatus consisted of an external heater/cooler connected to an internal heat exchanger.

2.4. Operating conditions and procedure

Table 1 reports the operating conditions of a representative set of experimental runs. All experiments were carried out using raw (non-sterilized) OMW, batchwise with respect to both liquid and solid phases. No pH control was accomplished.

Raw OMW was diluted with bi-distilled water to adjust the initial polyphenols concentration to pre-set values comprised between 0.11 and 1.4 g/L. The culture pH did not change as a consequence of dilution, being approximately 5. The air stream fed to reactors was water-saturated, in order to limit water stripping from the reacting medium. Operating temperature was set at 28 °C.

Columns A13 and A14 of Table 1 report operating conditions adopted in the internal loop airlift (ILA) bioreactor. Air was sparged into the draft tube at a volumetric rate of 200 nL/h (superficial gas velocity 0.5 cm/s). Correspondingly, the liquid circulation time in the airlift was 2.5 s and the gas–liquid mass transfer coefficient about 15 h^{-1} [15].

2.4.1. Procedure

OMW charge was prepared by diluting raw OMW at pre-set polyphenols concentrations with distilled water. Initial *P. ostreatus* concentration (X_0) ranged between 0.1 and 0.2 g_{DM}/L. Total volume of the culture was 0.3 L in experiments carried out in flasks and 5 L in the airlift. Culture sampling was carried out typically every 12 h for 9 days. Samples were centrifuged and phases were analyzed off-line.

P. ostreatus growth and oxygen consumption rate were determined by monitoring a set of flasks operated under the same initial conditions. Respiration rate and the dry biomass content were estimated at the end of each run.

Data regarding biomass growth, polyphenols conversion and total organic carbon (TOC) (see next section) estimated during