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Olive mill waste water treatment by immobilized cells of *Aspergillus niger* and its enrichment with soluble phosphate

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Abstract

Olive mill waste water (OMW), supplemented or not with ammonium sulphate and rock phosphate (RP), was applied as a medium in a shake-flask repeated-batch fermentation with *Aspergillus niger* immobilized on polyurethane sponge. Compared to other treatments, the results showed higher growth of the immobilized mycelium and significant reduction of the total phenols when the waste material was enriched with RP and ammonium sulphate (N). The immobilized fungus solubilized the RP with a maximum level of soluble P of 0·58 g/litre reached during the fourth batch cycle of the OMW+RP treatment. Depending on the medium composition, three types of treated OMW were produced which could be further used for various purposes.

Introduction

In Mediterranean countries, olive production represents 98% of the overall worldwide production [1]. Large quantities of olive mill waste water (OMW) are produced during the extraction of oil from the olive fruit by the traditional mill and press process. Without any practical use, this effluent poses a serious environmental problem. The phytotoxic and antimicrobial effects have been attributed to the phenolic part of the waste material [2,3]. During the last two decades many processes have been described and applied in order to reduce the toxicity and to utilize OMW as food or raw material in various biotechnological processes [4]. Another interesting approach is to use OMW as a fertilizer since it contains up to 11 kg of K_2O, 2 kg of P_2O_5 and 0·5 kg of MgO per m^3 in the mineral fraction [1]. However, the phenolic and toxic nature of the waste prevents its wide disposal on agricultural land. For these reasons, pretreatment of OMW by fungal microorganisms is believed to affect positively the composition of OMW [5,6]. In this paper we report preliminary results on the treatment of OMW by immobilized cells of *A. niger* in order to produce a material with a low level of toxicity. Rock phosphate was added to the fermentation medium to prove its possible solubilization and to formulate a suitable process for OMW-based fertilizer production.

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Materials and methods

Microorganism

An acid-producing filamentous fungus, *Aspergillus niger* NB2, used throughout this study, was maintained on potato-dextrose agar slants.

Culture media

The olive mill waste water (pH, 5·3; total sugars, 27·6 g/litre; total carbon, 28·5 g/litre; total phenol content, 6·0 g/litre; soluble P, 0·198 g/litre) was obtained from a local factory (Viterbo, Italy), which used centrifugation for the mechanical extraction of olive oil. The medium for cell immobilization contained (g/litre of distilled water): glucose, 100·0; NH_4NO_3, 1·5; KH_2PO_4, 1·0; $MgSO_4.7H_2O$, 0·2; $ZnSO_4.7H_2O$, 0·007. The medium

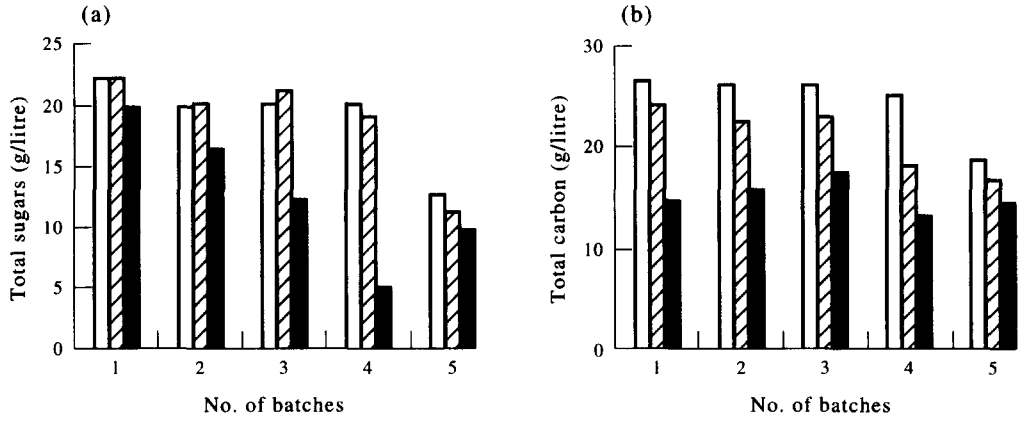

Fig. 1. Residual total sugar (a) and total carbon (b) during repeated-batch fermentation with immobilized *A. niger* on OMW-based media. Open symbol, OMW+N; shaded symbol, OMW+RP; filled symbol, OMW+N+RP.

for OMW treatment contained (g/litre OMW): $(NH_4)_2SO_4$, 2·5; $MgSO_4.7H_2O$, 0·2; rock phosphate (Morocco fluorapatite, 12·8% P; 1 mm mesh), 3·0 g/litre. Ammonium sulphate (N) and rock phosphate (RP) were added when necessary.

Immobilization procedure and fermentation conditions

Polyurethane sponge cubes (0·3 cm^3) used for the immobilization of *A. niger* had an average pore size of 0·6–0·8 mm and were obtained from the local market. Prewashed carrier (2·0 g/litre) was submerged in 100 ml of growth (glucose-based) medium in 250 ml Erlenmeyer flasks and, after sterilization at 115°C/30 min, incubated with $3 \cdot 10^6$ spores/ml. The latter were germinated for 48 h, then washed with sterile distilled water and transferred to 100 ml of OMW-based medium in 250 ml Erlenmeyer flasks. The immobilization procedure and fermentation were carried out (in triplicate) at 30°C in shaken culture at 200 rpm. The OMW-based medium was changed every 48 h.

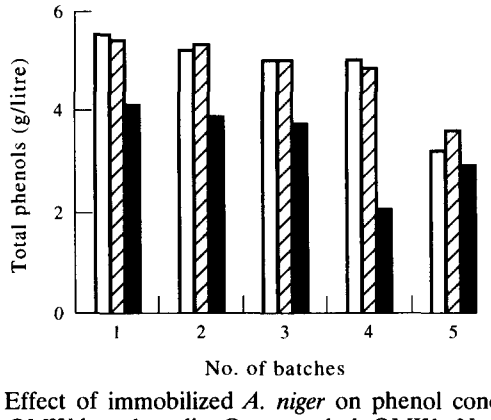

Fig. 2. Effect of immobilized *A. niger* on phenol concentration on OMW-based media. Open symbol, OMW+N; shaded symbol, OMW+RP; filled symbol, OMW+N+RP.

Analytical methods

The dry weight of immobilized cells was determined by subtraction of an average predetermined dry weight of sponge cubes from the weight of cubes plus mycelium after drying overnight at 90°C. Total sugar content was measured following the method of Dubois *et al.* [7]. The amount of total phenols was determined by the method of Swain and Hillis [8]. Organic carbon was determined according to Kalembasa and Jenkinson [9]. Total soluble P was measured by the method of Lachica *et al.* [10]. Medium pH was measured with a glass electrode. Results quoted are the average of three replicates. Standard errors were smaller than the dimensions of the symbols.

Results and discussion

In a preliminary study carried out with freely suspended mycelium of *A. niger* it was not possible to perform a fermentation process under the conditions of surface and submerged cultivation due to the absence of sufficient development of mycelium in the first case and abundant fungal growth in the second case. In this work three types of OMW-based medium were studied: OMW+N, OMW+N+RP and OMW+RP, utilizing the same filamentous fungus but in an immobilized state.

Generally, OMW inhibits the growth of microorganisms and the waste liquid must be diluted prior to microbial treatment [11], but this strain of *A. niger* was not affected by the toxic substances in the OMW. Microscopic examinations showed that filamentous growth favoured cell immobilization in such an open porous carrier, allowing strong biomass retention. As reported earlier, *A. niger* grew well inside the sponge particles although immobilization appeared to induce a thickening of the fungal culture [12]. However, different immobilized biomass concentrations were obtained at the end of the five-cycle repeated-batch

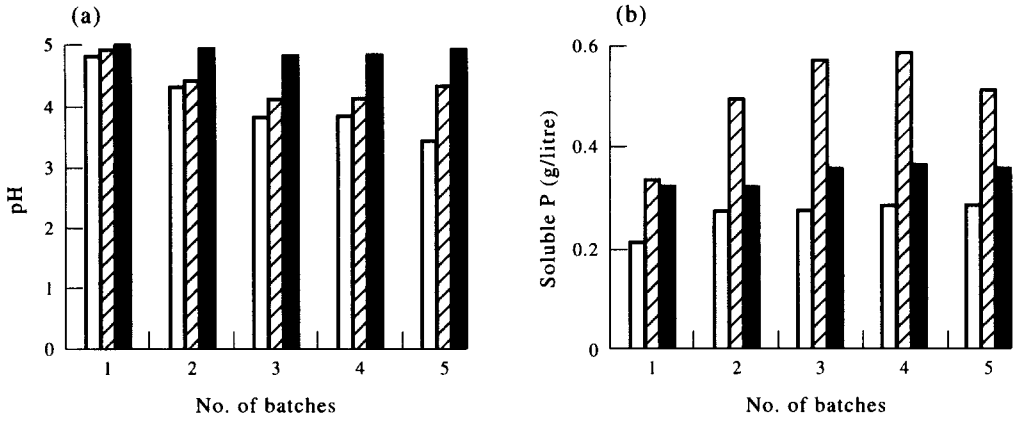

Fig. 3. pH values (a) and soluble phosphate accumulation (b) in repeated-batch process by immobilized *A. niger* on OMW-based media. Open symbol, OMW+N; shaded symbol, OMW+RP; filled symbol, OMW+N+RP.

fermentation process depending on the medium composition. The highest amount of fungal biomass of 5·32 g/g carrier was produced on the medium supplemented with both N and RP compared with 4·60 g/g carrier and 4·15 g/g carrier on OMW+RP and OMW+N treatments, respectively. Not surprisingly, the concentration of total sugars and the total carbon content followed the biomass growth with a maximal reduction to 5·00 g/litre and 13·2 g/litre in the OMW+N+RP, respectively (Fig. 1). The major benefit of the fermentation, however, concerned the effect of the immobilized fungal culture on the phenolic part of the OMW-based medium. *A. niger* was able to reduce the total phenols presented in the medium at an initial concentration of 6·0 g/litre (Fig. 2). Although such a reduction was registered in all studied treatments, the lowest value of total phenols of 2·0 g/litre was obtained at the fourth batch cycle of the OMW+N+RP treatment. On the other hand, it was evident that the presence of additional nitrogen source positively affected the degradation of OMW phenols. Recently, it was reported that ammonium sulphate applied to an OMW medium influenced positively fungal growth and chemical oxygen demand (COD) removal [13]. The extent of decrease of total phenol content in the medium appeared to be related to fungal growth.

The presence of RP influenced the behaviour of the immobilized fungus (Fig. 3). The buffering capacity of RP was recently found to affect the acid-producing activity of immobilized *Penicillium variabile* [14]. It is also known that 0·1% RP added to the fermentation medium increases the pH by 0·5 units [15], accounting for the higher pH values recorded in treatments with RP. Nevertheless, the immobilized *A. niger* was able to overcome the neutralizing effect of the RP and, as a consequence, to increase the amount of soluble P in the medium solution. The highest total concentration of soluble P (OMW-derived plus RP-solubilized fraction) occurred after the first two batches in all RP treatments. A similar trend in the solubilizing activity of the immobilized *A. niger* was observed in glucose-based medium supplemented with RP [16]. The additional presence of ammonium sulphate in the fermentation medium in combination with large available amounts of P stimulated growth and decreased acid formation by the immobilized culture. This effect, in turn, led to a reduction of the solubilizing activity of *A. niger* observed during OMW+N+RP treatment.

The successful application of immobilized *A. niger* in OMW treatment resulted in the production of three types of the waste liquid with different characteristics. Further work should be done to assess their possible effect on plant growth and to scale up the process. The OMW+N treatment could also be applied as a preliminary step to facilitate the anaerobic digestion of the waste material.

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