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Use of *Yarrowia lipolytica* strains for the treatment of olive mill wastewater

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Abstract

The principal aim of this research was to evaluate the ability of different *Yarrowia lipolytica* strains, having different origin, to grow in olive mill wastewater (OMW) and reduce its COD level. All the strains were able to grow in undiluted OMW; the comparison between the data obtained in a semi-synthetic medium and in OMW suggests that lipases with different specificity can be produced in relation to the medium composition.

Under the adopted conditions, the reduction of the OMW COD values varied from 1.47% and 41.22% of the initial value. Some strains determined a significant reduction of polyphenol content, while other ones caused its apparent increase. Moreover, some *Y. lipolytica* strains, isolated from chilled foods, produced the highest citric acid concentrations. These results evidenced that some *Y. lipolytica* strains are good candidates for the reduction of the pollution potential of OMW and for the production of enzymes and metabolites such as lipase and citric acid.

Keywords: *Yarrowia lipolytica*; Olive mill wastewater; Pollution reduction; COD; Polyphenols; Lipases; Citric acid

1. Introduction

Olive mill wastewater (OMW) treatment and disposal are becoming a critical environmental problem for the Mediterranean countries that accounts for about 95% of the world olive production (Al-Malah et al., 2000). In fact, the Mediterranean regions have an annual OMW production of about 3×10^7 m^3 (D'Annibale et al., 1998) of which about 2×10^6 m^3 is produced in Italy (Scioli and Vallaro, 1997). Generally, one tonne of olives yields one/two tonnes of OMW, according to the oil extraction process used. In fact, the continuous process uses about 2 l of water for kg of olives while the discontinuous one requires much less. Although the composition is dependant on the process used, the olive mill wastewater is a stable emulsion constituted by "vegetation waters" of the olives, water from the processing, olive pulp and oil. The organic fraction of OMW includes sugar, tannins, polyphenols, polyalcohols, pectins and lipids. Most of the problems associated with OMW pollution can be attributed to the phenolic fraction. In fact, phenolic compounds are responsible for several biological effects, including antibiosis and phytotoxicity. Ragazzi and Veronese (1989) reported that the antimicrobial activity is principally due to phenolic compounds such as tyrosol and hydrotyrosol. Another negative property of OMW is its extremely high organic content. Generally OMW has BOD values ranging between 12,000 and 63,000 mg/l and COD values between 80,000 and 200,000 mg/l (Al-Malah et al., 2000). These concentrations are around 200–400 times higher than a typical municipal sewage (Cossu et al., 1993). As microorganisms present in the environment consume these materials, oxygen will be depleted from the water with adverse effects on the aquatic life (if this wastewater is discharged to water supplies). Although some Authors have proposed the

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use of OMW as fertiliser, the high content of mineral salts as well as the presence of organic compounds, such as fatty acids and polyphenols, are factors detrimental to soil fertility (Alianiello et al., 1998; Paredes et al., 1999).

Several physico-chemical processes, including simple evaporation, reverse osmosis, and ultra-filtration have been proposed to reduce the polluting effects of OMW (Mameri et al., 2000; Al-Malah et al., 2000). Also the traditional anaerobic system of waste treatment has been experimented for OMW (Andreoni et al., 1993). However this system did not produce acceptable results, due to the inhibition both of enzymatic activity and aerobic bacteria by the phenolic fraction of OMW (Scioli and Vallaro, 1997). Anaerobic treatment of OMW was recently proposed by Erguder et al. (2000) in batch reactors. This anaerobic treatment resulted in the production of about 57.1 l of methane from 1 l of OMW. However, the anaerobic culture required an adaptation period of 15–25 days with consequent increase of storage costs. Several Authors used OMW as growth substrate for suitable microorganisms in order to reduce COD, BOD as well as to produce enzymes and biomass. Because toxicity has been directly linked to the phenolic fraction, higher fungi such as *Lentinus edodes*, *Pleorotus ostreatus* as well as yeast such as *Geothricum* spp. (Ehaliotis et al., 1999; D'Annibale et al., 1998; Kissi et al., 2001; Assas et al., 2002; Fadil et al., 2003) have been proposed to detoxify and de-colorize the waste. However their use on large scale is difficult compared to that of bacteria and yeast. Yeast species such as *Candida tropicalis* and *Yarrowia lipolytica* as well as bacteria belonging to the species *Azotobacter vinelandii*, *Pseudomonas* spp., *Sphingomonas* spp., *Ralstonia* spp. resulted suitable for the aerobic biodegradation and detoxification of OMW (Ehaliotis et al., 1999; Di Gioia et al., 2001, 2002). In particular, Scioli and Vallaro (1997) have indicated *Y. lipolytica* as a good candidate for the wastewater purification and reduction of pollution due to its ability: (i) to grow in OMW; (ii) to reduce COD and BOD and (iii) and produce biomass. However these authors considered only one *Y. lipolytica* strain, belonging to an international collection.

The principal aims of the work were to evaluate the ability of several *Y. lipolytica* strains, having different origin, to grow in OMW and metabolise its lipidic fraction. Verified the suitability of the different strains for the required functions, the ability to reduce the COD values as well as the potential of these strains to produce citric acid, and metabolise polyphenols were studied.

2. Methods

OMW used. The OMW used in this study originated form and olive oil production plant located in the southern Italy, which uses a continuous process for the extraction of olive oil.

Microorganisms used. Sixty two strains of *Y. lipolytica*, belonging to the culture collection of Dipartimento di Protezione e Valorizzazione Agroalimentare of Bologna University, were employed. The strains used in this research originated from previous examinations of chilled foods (Y labelled strains), light butter (RO labelled strains), superficial water of lagoon of the Po river delta (PO labelled strains) and irradiated poultry meat (the other strains) (Guerzoni et al., 1993; Sinigaglia et al., 1994).

Microbial counts. The cell loads of the different strains, inoculated at levels of 3 log CFU/ml, in undiluted OMW were determined by plate counting on Sabouraud Dextrose Agar (Difco, Detroit, USA), after an incubation of 72 h at 25 °C. The Petri dishes, after inoculation, were incubated at 25 °C for 48 h.

Lipase activity. To determine the extracellular lipase activity the 62 *Y. lipolytica* strains were cultured in Sabouraud broth or in OMW for 72 h at 25 °C under continuous stirring at 170 rpm. After cell elimination, by centrifugation at 12,000*g* for 10 min using an Avanti J-25 centrifuge (Beckman Coulter International S.A., Nyon Switzerland), the lipase activity was assessed by titrating fatty acids, liberated from olive oil by 7 ml of supernatant, with alkali according to the method of Fu et al. (1995). One unit of lipase activity was defined as the amount that released 1 mmol of fatty acid under the above conditions.

Polyphenols determination. Twenty strains of *Y. lipolytica* were precultured in 25 ml of Sabouraud broth for 48 h at 25 °C. The cells were harvested by centrifugation at 12,000*g* for 10 min and inoculated in 20 ml of OMW. After incubation at 25 °C of 72 h the total polyphenol content was determined according to the method of García García et al. (2000) based on the use of the Folin–Ciocalteau's phenol reagent.

Citric acid production. Twenty strains of *Y. lipolytica* were inoculated at levels of about 4 log CFU/ml in a synthetic medium containing 20 g/l of glucose, KH_2PO_4 1 g/l, $FeSO_4 \cdot 7H_2O$ 0.1 g/l, $MgSO_4 \cdot 7H_2O$ 0.25 g/l, thiamine HCl 0.25 mg/l. After incubation at 25 °C for 72 h under continuous stirring at 170 rpm, analysis of citric acid concentrations were performed by an HPLC (Model 1050, Hewlett-Packard, Germany) equipped with a Bio-Rad Aminex (Bio-Rad Laboratories, Hertfordshire, UK) HPX-87H column (300×7.8 mm), connected with a refractive index detector. Samples from fermentation broths were analysed after filtration through a 0.45 mm membrane filter (Paul et al., 1999).

COD determination. The chemical oxygen demand (COD) was determined according to the AOAC analytical methods (AOAC, 1990).