Biotechnological treatment of oil mill waste-water to produce high value-added mycelial products.

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Abstract. Several trials with *Pleurotus pulmonarius* mushroom were carried out in liquid state cultures, in synthetic media containing commercial glycose and olive mill wastewater (OMW) with phenolics concentration adjusted to 10% and 30% v/v. Results demonstrated that at the end of fermentation period the phenolic content reduction was very high (93-95%), as well as substrate decoloration, with values ranging from 70% to 85% (medium containing 10 and 30% v/v phenolic compounds respectively), indicating the great capability of *Pleurotus* strains for OMW detoxification. Moreover, the highest phenolic concentration (30% v/v) affected positively biomass and intra-cellular polysaccharide (IP) production, reaching 30.61 g/L and 4.30 g/L, respectively. On the other hand, although lower values of biomass and IP in absolute values were synthesized in the medium with 10% v/v phenolic compounds (22.66 and 3.92 g/L respectively), the amount of IP in relative values was as high as 20 w/w. The maximum anti-oxidant activity (expressed as total phenolic compounds) of 0.4 g/L was also determined in *P. pulmonarius* biomass produced in the medium with 10% v/v phenolics. In conclusion, the examined mushroom species could be used with great success as a mean of biological treatment of OMWs with simultaneous production of high nutritional value products (biomass, polysaccharides, phenolic compounds).

Keywords: Liquid fermentation, agro-industrial wastes, *Pleurotus pulmonarius*, polysaccharides, detoxification

1. Introduction

Olive mill wastewater (OMW) is a dark coloured emulsified effluent consisted of olive tissues, branches and processing waters with high organic load (mainly of toxic phenolic and aromatic compounds) (Aytar et al., 2013). Although OMW composition presents a large diversity depending on the variety of olives and their maturity, the region of origin and the technology used for oil extraction (Roig et al., 2006), its uncontrolled disposal consists a serious environmental problem to the countries involved to the olive oil production, like Greece (Kapellakis et al., 2008). Furthermore, the seasonal operation and the wide geographic distribution of three-phase olive mills create additional difficulties for its effective management (Ouzounidou et al., 2010). White-rot basidiomycetes and particularly those of genus *Pleurotus* are among the most potent organisms to biodegrade and detoxify a wide range of wastes and pollutants such as OMW (Tsioulpas et al., 2002). Additionally, mushroom cultivation could serve as a biotechnological mean to convert OMW-base media into useful mycelial mass and metabolic compounds (e.g. intra-cellular polysaccharides – IP) of high economic value (Aggelis et al., 2003).

2. Materials and Methods

Pleurotus pulmonarius strain AMRL 177 is maintained at the Laboratory of Edible Fungi (LEF, ITAP) collection. OMWs were obtained from an olive oil three-phase decanter manufacture located in Missolonghi, Western Greece and subjected to several analyses before use. The cultivation medium consisted of 40 g/L commercial glucose, 1 g/L yeast extract and several mineral elements and OMW was added by adjusting the phenolic compounds' concentration at 1 g/L (10% v/v) and 3 g/L (30% v/v). Cultivation took place in flasks (100 mL) containing 30 mL of the medium that were inoculated with 7 mm agar-plugs with *P. pulmonarius* mycelium and incubated at 26±1 ºC for 43 days under static conditions. Initial pH of cultures was 6.2±0.2 and it was monitored during cultivation period. Biomass concentration was determined gravimetrically in dry weight and expressed as g/L. The residual glucose level in the culture medium was estimated by 3,5-dinitro-2-hydroxybenzoic acid method (Miller, 1959). IP determination was conducted after acid hydrolysis, according to Diamantopoulou et al. (2014), using D-glucose as standard. Determination of total phenolic compounds in the mycelial samples was estimated using the Folin-Ciocalteau assay by measuring the absorbance at 765nm (Singleton and Rossi, 1965). Color removal was performed according to Sayadi and Ellouz (1992) protocol.

3. Results and Discussion

Table 1. Total results of maximum biomass production, endopolysaccharides, phenolic removal and decolorization of *P. pulmonarius* in batch- liquid state fermentation cultures, in synthetic media containing commercial glycose and OMW with phenolics concentration adjusted to 1 (10% v/v) and 3 (30% v/v) g/L for 43 days at 26 (±1) ^{0}C.

Concentration of phenolic compounds/parameters	1 g/L (10% v/v)	3 g/L (30% v/v)
Biomass Production (g/L)	22.14	30.61
IP (g/L)	3.92	4.30
IP (% w/w)	20.08	14.04
Phenolic removal (%)	95.12	93.08
Decolorization (%)	71.40	85.56

The results of *P. pulmonarius* cultivated in liquid cultures with glucose as carbon source and the addition of waste oil (OMW) (at two different concentrations) in static conditions are presented at **Table 1**. The maximum values of biomass (30.61 g/L) and IP production (4.3 g/L) were observed on day 43 in the medium with the highest concentration of phenolics (3 g/L - 30% v/v). On the other hand, in the medium with 1 g/L (10% v/v) phenolic compounds, the max values of biomass (22.61 g/L) and IP production (3.92 g/L) were observed on day 37. It is worth mentioning that *P. pulmonarius* in both media that contained OMWs after a time of adaption presented very high biomass production, although OMWs are highly toxic due to the high concentration of polyphenols (Mulinacci et al., 2001). Diamantopoulou et al. (2012) during the cultivation of *Pleurotus ostreatus* and *P. pulmonarius* for 16 days in media with main carbon source glucose, they recorded lower biomass production values; under agitated conditions 16.9 g/L and 16.2 g/L respectively and under static conditions 4.2 g/L and 8.8 g/L respectively. Fountoulakis et al. (2002) recorded even lower values of ~ 5 g/L biomass during the cultivation of *P. ostreatus* in heat-treated undiluted OMWs and sterile diluted 50% (v/v) OMWs at the end of the fermentation period.

Significant amount of IP in absolute values was synthesized during the fermentation process (**Table 1**), more in the medium with the highest phenolic compounds (4.30 /g/L). As regards the maximum relative (% w/w) values of IP, 20% and 14% w/w were produced at OMW with 1 and 3 g/L phenolic compounds respectively (day 37), the increased amount of which showed a significant negative effect. There was also a decrease in the IP amount on dry biomass towards the end of cultivation period, in cultures with both phenolic compounds concentrations. Diamantopoulou et al. (2012) in liquid cultures of *P. pulmonarius* mushroom with glucose as main carbon source recorded particularly high IP values (10.9 g/L and 48.4% w/w), but under agitation. Very high concentrations of polysaccharides in carposomes have also been reported in solid-state fermentations of lignocellulosic substrates e.g. 61-64% w/w in *P. ostreatus* and *Pleurotus cystidiosus* (Yang and Zhang, 2009), 46.6-81.8% w/w in *P. ostreatus* (Bano and Rajarathaman, 1998; Chang and Miles, 2004) and 50.7% w/w *P. cystidiosus* (Chang and Miles, 2004).

Both substrates showed early (since day 13) significant consumption of phenolic compounds exceeding 40% of the initial concentration (**Figure 1**). Results demonstrated that towards the end of fermentation period, the phenolic content reduction was very high (93-95%), as well as substrate decolorization, with values ranging from 70% to 85% that were among greatest in bibliography. Koutrotsios et al. (2014) reported adequate performance of *P. pulmonarius* on OMW (25% v/v) achieved ~50% decolorization and 76% reduction of total phenolic content after 30 days. Zerva et al. (2017), studied the ability for detoxification and decolorization of *Pleurotus citrinopileatus* LGAM 28684, in liquid batch culture with 25% v/v phenolic compounds and achieved a maximum reduction by 86.4% and maximum decolorization by 79.1% after fermentation of 22 days. Also, Ntougias et al. (2012) studied various strains of genus *Pleurotus* in liquid batch cultures with 25% v/v phenolic compounds, the concentration of which has decreased significantly; 70.3%, 62.5% and 73.9% by *P. pulmonarius*, *P. ostreatus* and *P. eryngii* after 20 days of fermentation. Also, decolorization rates were 44.0% and 64.8% respectively, after 30 days of fermentation In cultures with *Ganoderma resinaceum*, significant phenolic reduction (94.5%) and decolorization (76.5%) occurred, 14.6 g/L of biomass was produced, whereas the presence of OMWs enhanced the synthesis of IP (maximum absolute values 4.0–5.2 g/L corresponding to 35–42% w/w) (Diamantopoulou et al., 2021). Koutrotsios et al. (2016) reported 65% decolorization and 47% total phenolic reduction when *Hericium erinaceus* mushroom cultivated in OMW liquid cultures.

Concluding, *P. pulmonarius* AMRL 177 showed high detoxification ability by significantly reducing the phenolic load and the intensity of the color in OMWs, along with great mycelial mass and polysaccharide production. The potential use of edible fungi as alternative method of biological treatment of waste olive mill oil products is therefore established.

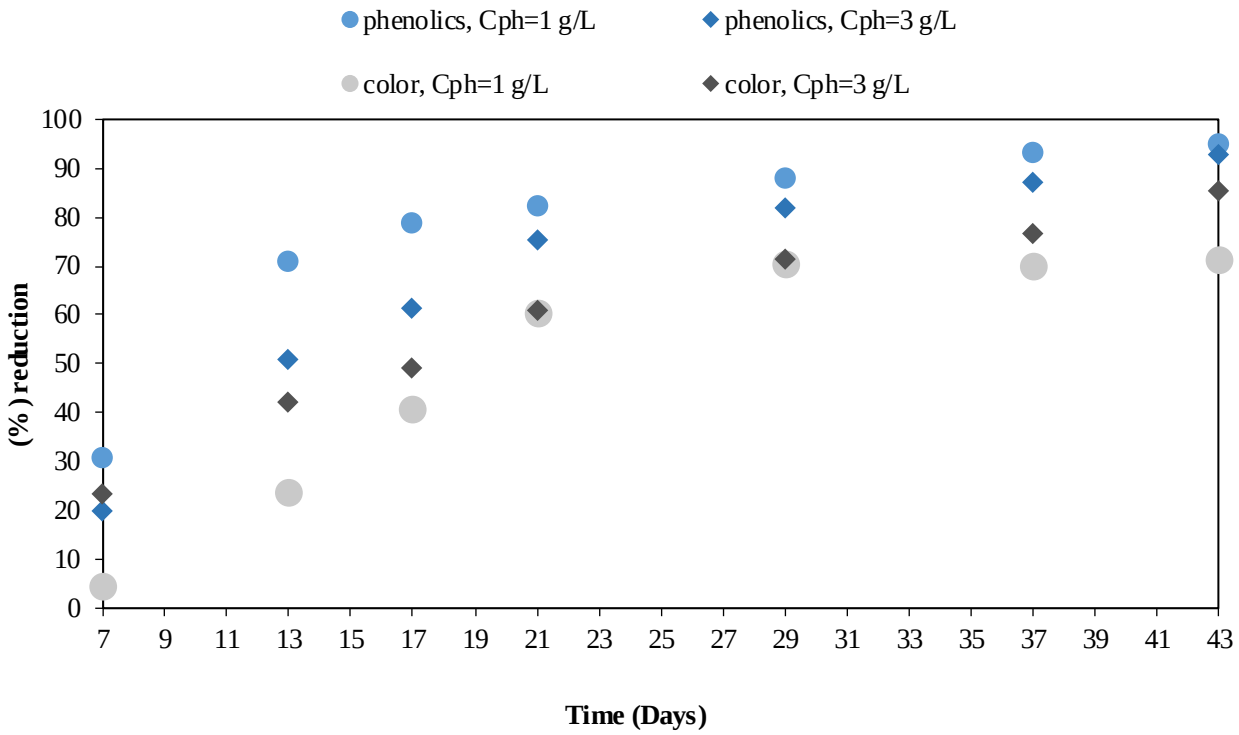

Figure 1. Kinetics of phenol reduction (g/L) and % decolorization of the medium during cultivation of *Pleurotus pulmonarius* in liquid cultures with olive mill wastewaters containing 1 (10% v/v) and 3 g/l (30% v/v) phenolic compounds (C_{ph}). Each point is the mean value of at least three independent measurements, SD < 5%.

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