Sustainable and Eco-Friendly Conversions of Olive Mill Wastewater-Based Media by *Pleurotus pulmonarius* Cultures

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Abstract: *Pleurotus pulmonarius* mushroom was cultivated on liquid cultures with olive mill wastewaters (OMWs) of initial phenolic compound concentrations of 0 (control), 1, 2 and 3 g/L and glucose at initial concentrations of 40 and 60 g/L. The ability of the fungus to grow on media containing toxic compounds enriched with glucose was assessed through biomass production, glucose consumption, polysaccharide (IPS) accumulation and total cellular lipids biosynthesis, while the total phenolic compounds (TPC) and antioxidant component monitoring were also assessed during a 43-day fermentation. An analysis of the total simple sugar composition of IPS and the total fatty acid composition of lipids was performed. The phenolic reduction and media decolorization were also monitored. Results showed that the addition of glucose in OMW-based media had a positive effect on biomass, IPS and lipid production and increased the unsaturated fatty acids and TPC concentration. The highest recorded values were the following: biomass 32.76 g/L, IPS 4.38 g/L (14.70%, w/w in dry weight), lipids 2.85 g/L (11.69%, w/w in dry weight). The mycelial lipids were unsaturated and dominated by linoleic acid, whereas IPS were composed mainly of glucose. Significant phenolic compound reduction (87–95%) and color removal (70–85%) occurred. Results strongly suggest the potentiality of *P. pulmonarius* utilization in the OMW treatment.

Keywords: oyster mushroom; phenolic compounds; glucose; lipids; biomass; polysaccharides; antioxidants

1. Introduction

Olive mill wastewater (OMW), a consequence of olive oil production, possesses a dark brown to black color and has high toxicity due to its high organic load, comprising mostly of (poly)phenolic compounds (mainly of toxic phenolic and aromatic compounds) and sugars [1]. The composition of OMW presents a large diversity depending on the variety of olives and their maturity, the growing region and the technology used for oil extraction [2]. The uncontrolled disposal of OMW poses a serious environmental problem for the olive-oil producing countries, such as Greece. Furthermore, the seasonal operation and the wide geographic distribution of traditional three-phase olive mills create additional difficulties for their effective management [3]. Moreover, the large volume of OMW produced (i.e., up to 30 × 10^6 m^3/ year), in combination with the short discarding time, increases the importance of this treatment from both an environmental and financial point of view [4]. When disposed of in the environment, OMW creates serious environmental problems. For
instance, its discarding into watercourses could lead to the deterioration of natural water bodies, which is a major threat to aquatic life. Environmental pollution of ground and surface waters, soil contamination, environmental degradation, as well as effects of toxicity and growth inhibition on species from different trophic levels have also been reported [5].

White-rot Basidiomycetes are among the most potent organisms to biodegrade and detoxify a wide range of wastes and pollutants such as OMW. Therefore, mushroom cultivation could serve as a biotechnological means to convert OMW-based substrates into useful biomass and metabolic compounds. Since many of the organic compounds present in OMW are identical or very similar to lignin biodegradation products, ligninolytic fungi have been exploited for the biodegradation of OMW through phenolic reduction, COD reduction and/or decolorization [1,4]. There are notable examples of Pleurotus strains that have been extensively used for the bioremediation of olive mill wastes, resulting in the removal of phenols and the decolorization of the effluent [5–7] and the generation of value-added products, e.g., edible mushrooms [8]. The conversion of toxic by-products into valuable products, such as mushroom carposomes or mycelium, is a non-sophisticated biotechnological application, yet a profitable and innovative approach to the biological treatments of OMWs based on research involving a great number of mushroom species; P. ostreatus, P. pulmonarius, Pleurotus eryngii, Pleurotus florida, Lentinula edodes, Agaricus bisporus, Agrocybe cylindracea, Ganoderma resinaceum, Ganoderma applanatum, Ganoderma carnosum, etc. have been successfully used for the detoxification of OMW [5,6,9–11].

The capability of mushrooms to grow in the toxic environment of OMWs to produce particular fungal masses and valuable metabolites, such as polysaccharides, lipids, and antioxidant substances, has been highlighted by many researchers [5,7,9,10,12–15]. The quantity and synthesis of the produced metabolites are affected by the substrate formulation as well as the mushroom strain [9,12]. Among these metabolites, intra- and extra-cellular polysaccharides have been well produced in significant quantities by many mushroom species.

Mycelial mass containing proteins and lipids, principally composed of polyunsaturated fatty acids [16–18] and particularly polysaccharides and ganoderic acids [19], could be considered, possibly after treatment, as a foodstuff with high nutritional and medicinal value [20]. Edible mushrooms are characterized by anti-tumor, cardiovascular, anti-inflammatory and anti-allergenic properties due to the variety of secondary metabolites in their fruiting bodies, including polysaccharides, phenolic compounds, and antioxidants [21]. Specifically, fungal biomass and polysaccharide production have already drawn much attention in numerous studies because of their various biological and pharmacological activities, in contrast to mushroom lipids, which, despite their relatively small quantity in basidiocarp or mycelium, contain poly-unsaturated fatty acids (e.g., α-linoleic acid) essential for human nutrition [22,23].

Liquid agro-industrial waste streams such as concentrated glycerol water, molasses, expired glucose syrups and other low-cost carbon-rich substances (e.g., commercial glucose) that also constitute sources of environmental pollution and have already been biologically treated by higher fungi viz. Morchella spp., Tuber aestivum, Lentinula edodes [24], ref. [25] could be diluted with OMWs and be used as potential substrates for mycelial mass and valuable metabolite production. Glucose constitutes one of the most suitable carbon sources for the culture of edible fungi, even in high concentrations, whereas peptone and yeast extract have been commonly used as they enhance mycelial growth in a variety of mushrooms [16,26–29]. The polysaccharide production is also enhanced by glucose and organic nitrogen sources (e.g., yeast extract, peptone, corn steep liquor), as has been reported in many studies [19,28,29].

In this study, the optimization of the OMW degradation and decolorization process by P. pulmonarius was examined through the addition of low-cost commercial glucose (at 40 and 60% g/L) in media in which OMWs were added in several ratios to yield initial phenolic compound concentrations of 0 (no OMWs added), 1, 2 and 3 g/L (viz. phenolic compound concentrations routinely found in various types of OMWs). The principal scope
of the work was to investigate the potential of the biological treatment of wastes rich in polyphenols by cultivating \textit{P. pulmonarius} in liquid cultures with OMWs, used for the first time for this purpose, along with its ability to grow in highly glucose-concentrated substrates. Therefore, in the current investigation, the mushroom strain was evaluated for its ability to convert glucose-enriched OMW-based media into useful metabolic compounds (valuable polysaccharides, antioxidants, and lipids) using a completely eco-friendly and green process.

2. Materials and Methods

2.1. Fungal Species, Substrates and Culture Conditions

\textit{Pleurotus pulmonarius} strain AMRL 177 of the Laboratory of Edible Fungi of Institute of Technology of Agricultural Products/Hellenic Agricultural Organization–Dimitra was used. The strain was maintained on potato dextrose agar (PDA, Merck, Darmstadt, Germany) at 4 ± 1 °C and regularly sub-cultured.

The culture media consisted of OMW (obtained from an olive oil three-phase decanter manufacture located in the Holy City of Missolonghi industrial zone, Western Greece) enriched with carbon sources (commercial glucose, 95% \( w/w \) purity; Hellenic Industry of Sugar S.A., Thessaloniki, Greece), nitrogen sources (yeast extract, Fluka, Steinheim, Germany) and minerals [26]. The OMW solid particles were removed through filtration and subsequent centrifugation at 15,000 rpm, 4 ± 0.5 °C for 30 min. Characteristics of OMW used in the experiments of this study are shown in Table 1. The supernatant was diluted at various rates with deionized water in order to achieve concentrations of total phenolic compounds of 1 g/L (10%, \( v/v \)), 2 g/L (20%, \( v/v \)) and 3 g/L (30%, \( v/v \)) and enriched with two initial concentrations of glucose (40 and 60 g/L). The maximum concentration of 3 g/L was chosen after preliminary experiments where above this value, the fungus presented very small growth. Precisely, when OMWs were added into the medium in order to yield an initial phenolic compound concentration of \( c. 4.0 \) g/L and the initial glucose concentration was adjusted to 40 g/L, after 37 days of cultivation the total biomass concentration achieved was \( 2.0 \) g/L. With even higher OMWs quantity added (initial phenolic compounds concentration = 5.0 g/L), almost no growth at all (viz. at 37 days after inoculation, biomass achieved was \( 0.8 \) g/L) was reported. Based on these preliminary experiments hence, eight different liquid media with glucose as main carbon source and liquid waste oil (OMW) were prepared (Table 2). The addition of glucose in the OMWs resulted in the generation of liquid media with initial concentrations of glucose and phenolic compounds resembling to those of a typical two-and-a-half-phase centrifugation process system (initial glucose concentration adjusted to \( c. 40 \) g/L) or a typical traditional press extraction system (initial glucose concentration adjusted to \( c. 60 \) g/L) [4,30]. The cultures were performed in 100-cc flasks filled with 30 mL of medium, sterilized (at 121 ± 1 °C for 20 min) and inoculated with 0.9 mm diameter mycelial disks cut from the edge of 7-day-old \textit{P. pulmonarius} colonies. The initial pH for all substrates before and after sterilization was 6.4 ± 0.1. The flasks were incubated at 26 ± 1 °C for 43 days. Sampling was carried out every 6 or 7 days (starting seven days after inoculation).

Table 1. Characteristics of olive mill wastewater (OMW) used in the present study.

| Characteristics of OMW                      |          |
|-------------------------------------------|----------|
| Total sugars                              | 30.0 g/L |
| Phenolic compounds                        | 11.5 g/L |
| Free amino nitrogen                       | 70.5 mg/L|
| Proteins                                  | 0.5 g/L  |
| pH                                        | 4.95     |
| EC                                        | 13.86 mS/cm|
Table 2. Composition of the substrates used in liquid state fermentation experiments (before inoculation).

| OMW Concentration (g/L) | Glucose Concentration (g/L) | Culture Media |
|-------------------------|-----------------------------|--------------|
| 0                       | 40                          | S0-G40        |
|                         | 60                          | S0-G60        |
| 1                       | 40                          | S1-G40        |
|                         | 60                          | S1-G60        |
| 2                       | 40                          | S2-G40        |
|                         | 60                          | S2-G60        |
| 3                       | 40                          | S3-G40        |
|                         | 60                          | S3-G60        |

2.2. Analytical Methods

Mycelia were collected by filtration under vacuum (using No.2 Whatman filters, Kent, England) and washed twice with distilled water. Dry biomass (X, g/L) was gravimetrically determined after drying the mycelia at 60 ± 5 °C (in drying chamber, Elvem, Greece) until constant weight, weighing in a 4-digit balance (Kern AGB balance, Germany). Determination of pH was performed by a Crison (Barcelona, Spain) GLP 21 pH-meter. Electrical conductivity (EC, S/cm) was estimated using a Hanna HI-8733 (Padova, Italy) conductivity meter. Glucose consumed (Glc, g/L) in the media was estimated by the 3.5-dinitro-2-hydroxybenzoic acid method [31].

Determination of phenolic compounds’ reduction in broth samples was estimated using the Folin–Ciocalteu (FC) assay by measuring the absorbance at 765 nm [32] with some modifications. Briefly, 0.2 mL of each sample was diluted in 10.8 mL H₂O and mixed with 8 mL of saturated (75 g/L) Na₂CO₃ (Alfa Aesar, Kandel, Germany) and 1 mL of FC (Merck, Darmstadt, Germany) reagent. The mixtures were vortexed and allowed to react in the dark for 2 h. Absorbance was then read at 750 nm using gallic acid (Sigma, Steinheim, Germany) as standard. Samples were measured in three replicates. Absorbance measurement of the inoculated and non-inoculated OMW samples at 395 nm was used for the estimation of decolorization [7].

Intracellular polysaccharides (IPS, g/L) determination was conducted after acid hydrolysis, as described by Diamantopoulou et al. [16,32]. For this purpose, IPS from the dried mycelia (100 mg) was extracted by using 20 mL of 2.5 M HCl (Merck, Darmstadt, Germany) at 100 ± 1 °C for 20 min. The whole mixture was neutralized to pH = 7 using 2.5 M NaOH (Merck, Darmstadt, Germany). The final volume was adjusted to 50 mL and the mixtures were filtered. 0.5 mL of each filtrate was added in 0.5 mL DNS, the whole was then vortexed and incubated at 100 ± 1 °C for 5 min. After cooling the tubes to room temperature in a water bath, the IPS content was determined by measuring the absorbance at 540 nm. All samples were analyzed in triplicate. The composition of individual saccharides of the produced IPS was performed by HPLC analysis. Thus, filtered aliquots of the neutralized samples with NaOH were analyzed by a Waters Association 600E (Milford, Midland, TX, USA) apparatus at a 30.0 cm × 7.8 mm Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA). The mobile phase used was H₂SO₄ at 0.005 M with a flow rate 0.8 mL/min and the column temperature was 65 ± 0.5 °C. Individual simple sugars and sugar-alcohols were detected by a RI detector (differential refractometer 410-Waters).

Methanolic extracts were prepared as follows: 50 mg of fresh colonized substrate were extracted with 1 mL of methanol (Merck, Darmstadt, Germany) using an ultrasonic bath (SKYMEN, JP-060S, China) (15 min, room temperature) followed by vortex and centrifugation (3500 rpm, 15 min, ambient temperature; Micro 22R, Hettich, Kirchlengern, Germany). The same extraction was repeated three times and the supernatants were stored at 4 ± 0.5 °C and used for further analysis. Total phenolic content (TPC) was determined using the FC method [33] and was expressed as mg of gallic acid equivalents per g of dry biomass. Furthermore, the scavenging ability of DPPH• free radicals was used to
estimate antioxidant activity (AC) according to Bondet et al. [34], Molyneux et al. [35] and Musa et al. [36]. AC was expressed as mg of Trolox equivalents per g of dry biomass.

Total cellular lipid was extracted from the following dry biomass by chloroform/methanol (Merck, Darmstadt, Germany): 2/1 (v/v) mixture and determined gravimetrically [37]. Derivatization of fatty acids to methyl esters was performed in a two-stage reaction (to avoid trans-isomerization) using sodium methoxide and methanol/hydrochloride according to the AFNOR method, as described in Sarantou et al. [37]. Fatty acid methyl esters were identified by reference to authentic standards. For this purpose, methyl esters were suspended in hexane and analyzed by GC in a Varian CP-3800 chromatograph equipped with flame ionization detector (Agilent Technologies; Santa Clara, California, USA), in which an Agilent J&W Scientific DB23 capillary column (model n.123–2332, 30.0 m × 0.32 mm, film thickness 0.25 µm) (Agilent Technologies; Santa Clara, California, USA) was used. Helium gas (Air Liquid, Paris, France) was used as the carrier gas with a column flow rate of 2.0 mL/min.

The set-up conditions were as follows: initial oven temperature was set at 150 ± 1 °C, held for 18 min, subsequently rammed to 185 ± 1 °C at a rate of 5 °C/min and held for 2 min. Thereafter, the oven temperature rose to 210 ± 1 °C at a flow rate of 5 °C/min, held for 2 min and then increased to 240 ± 1 °C at 10 °C/min. The injector and flame ionization detector temperatures were set at 260 ± 1 °C and 270 ± 1 °C, respectively. Individual fatty acid methyl-esters were identified by comparison of their retention times with external standard (Supelco 37 Component fatty acid methyl-esters Mix, CRM47885) retention times. The amounts of individual fatty acid methyl-esters identified were expressed in % of the total fatty acid’s areas chromatograms identified.

3. Results and Discussion
3.1. Biomass Production, Sugars Consumption

In previous studies, glucose has been used as a common carbon source for the maximum production of mycelial mass for Pleurotus spp. achieving values ranging from 13.9 to 22.50 g/L [16,28,32,38]. In the present study (Table 3), the media containing OMWs showed biomass production of over 20 g/L, although OMWs are highly toxic due to their high concentration of polyphenols [39]. Particularly, P. pulmonarius produced the maximum mycelial mass (32.76 g/L) at the highest concentration of phenolics, possibly due to its higher nutrient content (macro and trace elements) [2,40] (Figure 1, Table 3) at the end of the fermentation period. Additionally, a biomass production rate of 32.76 g/L, was achieved in the culture with 3 g/L phenolics and 60 g/L glucose. However, pH seemed to affect the biomass production as well, particularly in the control cultures (no OMW added) that showed a significant decrease of values below three after the 21 day, whereas in OMW-added substrates pH values were maintained almost constant (~5.5). A pH reduction has been observed in previous works with the cultivation of strains of the genus P. ostreatus when a pH increase was recorded in the P. sajor-caju [41]. Moreover, an increase in pH was shown by Ganoderma australe and G. carnosum, with a parallel decrease in EC [42]. During this study, a general decrease in the pH values throughout the culture was observed in all media without affecting the biomass production. Therefore, the maximum production of P. pulmonarius dry mycelial mass recorded in the current research (up to c. 33 g/L) compares favorably with the highest values of biomass reported in the literature.
Table 3. Total results of biomass, IPS and lipids production, phenolic removal and decolorization of *P. pulmonarius* in batch-liquid state fermentation cultures, in synthetic media containing commercial glucose and OMWs with various phenolics concentrations. Representation of dry biomass (X, g/L), consumed glucose (Glc, g/L), IPS (in g/L and w/w) and total cellular lipids (L, in g/L and w/w) at the following different fermentation points of each trial: (a) when the maximum biomass ($X_{\text{max}}$, g/L) was produced; (b) when the maximum IPS amount ($Y_{\text{IPS}/X_{\text{max}}}$, g/L) was synthesized; (c) when the maximum IPS in dry weight ($Y_{\text{IPS}/X_{\text{max}}}$, %, w/w) value was achieved and (d) when the maximum total lipid in dry weight ($Y_{\text{L}/X_{\text{max}}}$, %, w/w) value was recorded. Culture conditions: growth on 100-cc flasks, initial pH = 5.6 ± 0.3, incubation temperature $T = 26 \pm 2^\circ C$. Each point is the mean value of three independent measurements.

| Culture Media * | Time (Day) | X (g/L) | Glccons (g/L) | $Y_{X/S}$ (%, w/w) | IPS (g/L) | $Y_{IPS/X}$ (%, w/w) | L (g/L) | $Y_{L/X}$ (%, w/w) |
|-----------------|------------|---------|---------------|-------------------|-----------|---------------------|---------|-------------------|
| **S0-G40**      | a, d       | 43      | 14.53 ± 0.04  | 18.83 ± 0.25      | 0.77 ± 0.02 | 2.08 ± 0.12         | 14.34 ± 0.75 | 0.94 ± 0.08      | 6.47 ± 0.10 |
|                 | b, c       | 37      | 14.44 ± 0.05  | 17.86 ± 0.29      | 0.80 ± 0.01 | 3.25 ± 0.20         | 22.51 ± 0.85 | 0.51 ± 0.06      | 3.53 ± 0.12 |
| **S0-G60**      | a, b, c    | 43      | 19.62 ± 0.56  | 30.09 ± 0.32      | 0.65 ± 0.02 | 4.01 ± 0.05         | 20.41 ± 0.12 | 1.63 ± 0.05      | 8.31 ± 0.09  |
|                 |            | 37      | 18.29 ± 0.26  | 29.14 ± 0.35      | 0.63 ± 0.01 | 3.84 ± 0.04         | 20.99 ± 0.16 | 0.80 ± 0.06      | 4.37 ± 0.09  |
| **S1-G40**      | a, d       | 43      | 22.66 ± 0.12  | 29.22 ± 0.31      | 0.78 ± 0.01 | 2.83 ± 0.22         | 12.79 ± 0.52 | 1.38 ± 0.04      | 6.09 ± 0.06  |
|                 | b, c       | 37      | 19.51 ± 0.56  | 24.84 ± 0.35      | 0.79 ± 0.02 | 3.92 ± 0.21         | 20.08 ± 0.63 | 0.93 ± 0.04      | 4.77 ± 0.11  |
| **S1-G60**      | a, b, d    | 43      | 24.39 ± 0.58  | 31.35 ± 0.58      | 0.78 ± 0.01 | 3.44 ± 0.12         | 14.09 ± 0.28 | 2.85 ± 0.07      | 11.69 ± 0.14 |
|                 | c          | 29      | 21.73 ± 0.06  | 27.41 ± 0.62      | 0.79 ± 0.01 | 4.03 ± 0.18         | 18.55 ± 0.95 | 1.16 ± 0.11      | 5.34 ± 0.03  |
| **S2-G40**      | a, d       | 43      | 25.11 ± 0.71  | 32.53 ± 0.21      | 0.77 ± 0.01 | 3.10 ± 0.22         | 12.35 ± 0.64 | 2.30 ± 0.11      | 9.16 ± 0.12  |
|                 | b, c       | 37      | 24.86 ± 0.62  | 31.59 ± 0.23      | 0.79 ± 0.01 | 4.32 ± 0.14         | 17.39 ± 1.01 | 1.94 ± 0.09      | 7.80 ± 0.23  |
| **S2-G60**      | a, b, d    | 43      | 29.82 ± 0.76  | 41.65 ± 0.14      | 0.72 ± 0.01 | 4.38 ± 0.09         | 14.70 ± 0.05 | 2.08 ± 0.13      | 6.98 ± 0.21  |
|                 | c          | 37      | 25.14 ± 0.52  | 33.71 ± 0.12      | 0.74 ± 0.01 | 4.06 ± 0.07         | 16.15 ± 0.87 | 1.24 ± 0.12      | 4.93 ± 0.35  |
| **S3-G40**      | a, b, d    | 43      | 30.61 ± 0.73  | 35.43 ± 0.32      | 0.88 ± 0.03 | 4.30 ± 0.12         | 14.04 ± 0.14 | 2.19 ± 0.12      | 7.15 ± 0.05  |
|                 | c          | 37      | 26.57 ± 0.73  | 34.69 ± 0.11      | 0.76 ± 0.02 | 3.98 ± 0.09         | 14.98 ± 0.12 | 1.87 ± 0.12      | 6.96 ± 0.02  |
| **S3-G60**      | a, b, d    | 43      | 32.76 ± 0.53  | 42.07 ± 0.24      | 0.78 ± 0.01 | 3.63 ± 0.05         | 11.01 ± 0.09 | 2.00 ± 0.09      | 6.11 ± 0.21  |
|                 | c          | 37      | 28.86 ± 0.45  | 37.63 ± 0.21      | 0.77 ± 0.01 | 3.50 ± 0.02         | 12.13 ± 0.04 | 1.70 ± 0.08      | 5.89 ± 0.32  |

* As at Table 2.
In most studies, the usual way of cultivating edible fungi in liquid cultures in order to receive mass and various metabolites (enzymes, polysaccharides, lipids) is by using simple carbon sources with a predominant glucose source [9,28,43,44], while as a nitrogen source, yeast extract is usually preferred [29,43–46]. Specifically, Diamantopoulou et al. [16] during the cultivation of *P. ostreatus* and *P. pulmonarius* for 16 days in glucose recorded biomass production of 16.9 and 16.2 g/L, respectively (under agitated conditions) and of 4.2 and 8.8 g/L, respectively (under static conditions), which was much lower than the biomass achieved in all the cultures of the present study. Foundoulakis et al. [47] during the cultivation of *P. ostreatus* in heat-treated, undiluted OMW and in sterile, diluted 50% v/v OMW recorded biomass values of 5.7 and 5 g/L, respectively, at the end of the fermentation. In static batch cultures with 25% OMW v/v, *Abortiporus biennis*, *Hapalopilus croceus*, *Pleurotus djamor*, and *P. pulmonarius* provided yields of 2–2.3 g/L [12]. The increased production of biomass by higher fungi because of OMWs addition has been shown previously in the cultures of *G. resinaceum* in medium with 0.8 g/L phenolic compounds [9], of *G. applanatum* in 20% OMWs [14] and of *G. carnosum* in 25% OMWs [12]. Besides, in higher medicinal and pharmaceutical fungi of the species *Lentinula edodes* that presented similar physiological behavior [13], it has been revealed that the addition of OMWs containing toxic phenolic compounds not only did not suppress but, in contrast, stimulated fungal growth. The same was noticed for yeasts of the species *Saccharomyces cerevisiae* [48] and *Yarrowia lipolytica* [49], suggesting again that besides the toxic compounds (phenolic substances), other micronutrients are present in the OMWs, promoting microbial growth. Additionally, in carbon-rich media, many higher fungi have shown good performance in biomass production as stated by Sarris et al. [25] for *L. edodes* in molasses and *A. bisporus* in wheat cereal hydrolysates, whereas other researchers such as Fang and Zhong [19], Diamantopoulou et al. [32] and Ding et al. [50], confirmed an increase in biomass production for *Pleurotus spp.*, *Ganoderma* spp., *Flammulina velutipes*, *Volvariella volvacea* and *Coprinus comatus* in cultures with glucose as a main carbon source up to concentration of 50 or 60 g/L. Therefore, the maximum production of *P. pulmonarius* dry mycelial mass recorded in the current study (up to c. 33 g/L) compares favorably with the highest values of biomass reported in the literature.

Concerning the glucose consumption profile presented in Figure 2a,b it is shown that higher consumption occurred in the media containing OMWs than in the control, which is consistent with the biomass production increase in the same media. Notably, glucose consumption in the substrates of 40 g/L initial glucose concentration was as high as 73.05,
81.32 and 96.74% for 1, 2 and 3 g/L phenolics, respectively. In the case of media with a 60 g/L initial glucose concentration, glucose consumption was lower (up to 70.12% of the initial amount) at the highest phenolic concentration. It seems, therefore, that although in the increased amount of phenolics more glucose was consumed (and the highest biomass was produced), more time was needed for the fungi to totally consume the excess glucose (medium S3G60).

The maximum theoretical dry cell biomass yield on sugars consumed $Y_{X/S}$ (g/g) is 0.80 g/g [51]. In this study, $P. pulmonarius$ achieved values higher than 0.60 g/g, which indicates very good exploitation of glucose for mycelial biomass production (Table 3), whereas in one case, a conversion yield of 0.88 g/g was recorded—presumably yeast extract furnished at a concentration of 2.5 g/L served also as a carbon source. It was also observed that with the increase in the concentration of glucose to 60 g/L in the media, $Y_{X/S}$ decreased, which is in line with the corresponding investigations by Diamantopoulou et al. [26] with $V. volvacea$, where the increase in glucose concentration resulted in a decrease in $Y_{X/S}$. 

![Figure 2](image-url)
However, in the cultivation of *G. resinaceum* in OMW media with 0.8 g/L phenolic compounds and 40 g/L glucose, 14.5 g/L of biomass was produced and glucose was almost totally consumed with $Y_{X/G}$ at only 0.37 [9]. The production of biomass depends to a certain extent on the conditions of growth of the macro-fungi, meaning the pH of the medium, their ambient temperature, aeration, and stirring [52]. Presumably, in the media with increasing initial concentrations of glucose, yield $Y_{X/S}$ values show a slight reduction (Table 3), which would indicate some substrate inhibition exerted by the fungus at the relatively elevated (viz. 60 g/L) initial glucose concentrations in the medium.

### 3.2. Removal of Phenolic Compounds—Decolorization

During the cultivation of *P. pulmonarius*, satisfying reduction of the phenolic compounds of OMW with concurrent decolorization was observed. The kinetics of total phenol degradation and colour removal on OMW media with initial phenolics of 1, 2 and 3 g/L are shown in Figure 3, presenting a similarity with relevant biomass kinetics. A significant reduction of phenolic compounds has been observed since day 13, where it exceeded 50% of the initial concentration. After the 17 day, in substrates with 1 and 2 g/L initial phenolic compounds, the dephenolization process of the media was almost stable, with phenolics being reduced by ~76 and ~79%, respectively. The substrate with 3 g/L of initial phenolic compounds resulted in a similar reduction range after the 21 day. Moreover, the different glucose amounts did not appear to significantly affect the dephenolization process. Results demonstrate that at the end of the fermentation period, the phenolic content reduction was very high (87–95%, *w/w*), as was the media decolorization, with values ranging from 70 to 85%. The strains’ ability to reduce the concentration of phenolic compounds as well as to achieve decolorization of the OMW is based on the fact that fungi can produce ligninolytic enzymes that degrade the phenolic compounds. In particular, the ability of detoxification and decolorization of *P. citrinopileatus* LGAM 28684, in batch cultures with a concentration of OMW (25%, *v/v*) was studied by Zerva et al. [53] and a reduction of phenolic compounds by 86.4% and decolorization by 79.1%, after fermentation of 22 days, was achieved. Yesalida et al. [41] recorded a 60% decrease in phenolics by *P. sajor-caju* and 35–45% by *P. ostreatus*. Moreover, phenolic compound reduction achieved by *Pleurotus* and *Ganoderma* spp. was significantly high (74–81 and 64–67%, respectively) in crops with an OMW of 25% *v/v* after 20 days of fermentation, while decolorization of 60–65% and 40–46%, respectively was observed after 30 days of fermentation [42]. Finally, an exceptionally high rate of phenolic reduction (94.5%) and decolorization (76.5%) was recorded recently in static culture trials performed by a newly isolated *G. resinaceum* mushroom, cultivated on various OMW-based media enriched with glucose [9]. Therefore, great detoxification of OMW was achieved by *P. pulmonarius* in the present study.

![Figure 3](image_url)
The production of IPS at 5 sampling points was also recorded and the maximum values are presented in Table 3. In general, the addition of OMW had a positive effect on the IPS increase (in absolute values, g/L), and the largest amount of IPS was achieved in substrates with a glucose concentration of 60 g/L. The maximum production of IPS was ~4.3 g/L (media S2-G40, S2-G60 and S3-G40), while in the OMW-free media it was the lowest (2.08 g/L). In substrates with 40 g/L glucose, the maximum value was presented on the 37 day, whereas in substrates with 60 g/L glucose, there was a continuous increase up to the 43 day. Higher relative values of IPS (%, w/w in dry weight) were observed in the substrates with a low amount of phenolic compounds and glucose concentration, with the maximum value recorded being 22.51% w/w (control medium). However, Diamantopoulou et al. [32] recorded higher IPS production values (10.9 g/L; 48.4%, w/w) during the cultivation of strain *P. pulmonarius* in agitated and static bottles, with main carbon source of 30 g/L glucose. Very high concentrations of polysaccharides have also been reported for the mycelium of *P. ostreatus* (46.6–81.8%, w/w) [54,55] and *P. cystidiosus* (50.7%, w/w) [54]. Data from liquid cultivations of *V. volvacea* by Diamantopoulou et al. [26] showed an increase in IPS concentration (g/L and %, w/w) with an increase in glucose concentration, and the same was detected with the IPS production of *G. lucidum* [19]. It seems, therefore, that the genus and species significantly affect the ability of fungi for IPS synthesis and that media composition can be used for biomass and IPS stimulation. Additionally, there was a decrease in the IPS amount (%, w/w) on dry biomass after 37 day of fermentations (Figure 4), which is in accordance with many previous studies, such as that by Diamantopoulou et al. [9,16,26,27,32], where, in liquid cultures of *V. volvacea, F. velutipes, Morchella esculenta* and *G. resinaeum*, an IPS (%, w/w) decrease was observed with time after the 12 day of cultivation and that by Diamantopoulou et al. [9], where in *G. resinaceum* grown in media with 0.5 and 0.8 g/L of phenolic compounds, IPS_{max} was achieved at the 17 day of culture, followed by reduction.
IPS produced by higher fungi include simple sugars (e.g., fructose), disaccharides (e.g., trehalose) and polyalcohol-type compounds (e.g., mannitol), but principally polysaccharides, such as glycogen, pullulan, β-glycans, etc. [19,56–60]. Fungal-derived polysaccharides can present potential immuno-modulating, anti-tumor, and hypoglycemic activities [20]. The quantity of total intra-cellular sugars in dry mycelial mass reported in the literature is strain-, substrate-, and mode-of-culture-dependent, since in most cases the biosynthesis of IPS is a secondary anabolic activity performed under nitrogen-limited (and thus sugar-excess) conditions [61]. In fact, nitrogen limitation resulting in obligatory cleavage of AMP, in order for nitrogen requirements of the cells to be secured, leads to inhibition of the 6-phosphoro-fructokinase (6-PFK), given that this enzyme (as the mitochondrial NAD⁺-isocitrate dehydrogenase) is allosterically activated by the cellular AMP [61–63]. The above-mentioned situation can result in the intra-cellular accumulation of polysaccharides.

Figure 4. Production rate of intracellular polysaccharides (IPS) (A) in absolute (g/L) (B) in relative (IPS %, w/w) values during the cultivation of P. pulmonarius in liquid cultures with OMWs containing: (a) 0 g/L phenolic compounds and initial concentration glucose 40 and 60 g/L (S0-G40, S0-G60), (b) 1 g/L phenolic compounds and initial concentration glucose 40 and 60 g/L (S1-G40, S1-G60), (c) 2 g/L phenolic compounds and initial concentration glucose 40 and 60 g/L (S2-G40, S2-G60), (d) 3 g/L phenolic compounds and initial concentration glucose 40 and 60 g/L (S3-G40, S3-G60). Each point is the mean value of at least three independent measurements, SD < 5%.
(principally based on 6-phospho-glucose) [63], with various types of sugars, medium pH values, and agitation rates favoring the above-mentioned bioprocess [64].

The carbohydrate composition of fungal IPS depends on the growth medium and it can be changed by manipulating submerged culture factors [26]. So, in this study, the impact of the increasing concentrations of phenolic compounds at different glucose concentrations in the growing medium on the total sugar composition of IPS was investigated (Table 4).

In all cases, glucose was the most abundant individual sugar identified and quantified in the produced polysaccharides (75–89%, \( w/w \)). Mannitol was found in a significantly smaller quantity than glucose (11–25%, \( w/w \)). Interestingly, in the media with phenolic compound glucose concentration decreased with time, whereas mannitol increased. Similarly, glucose was the most abundant carbohydrate in the mycelium of \( P. \) pulmonarius [32,65] and also of the other following mushrooms: \( G. \) applanatum [32,66], \( M. \) spp. [24], \( A. \) aegerita and \( F. \) velutipes [32]. Likewise, according to Diamantopoulou et al. [32], the amount of mannitol followed that of glucose in the cultures of \( P. \) pulmonarius with glucose as the main carbon source and glucose percentage decreased with fermentation time as mannitol increased.

**Table 4.** Carbohydrate composition of total IPS produced by \( P. \) pulmonarius cultivated in static cultures in glucose (S0-G40, S0-G60) and OMWs (S1-G40, S2-G40, S3-G40 and S1-G60, S2-G60 and S3-G60; see Table 2) substrates.

| Culture Media | Day | Glucose (%) | Mannitol (%) |
|---------------|-----|-------------|--------------|
| S0-G40        | 17  | 83.24       | 16.76        |
|               | 29  | 88.14       | 11.86        |
|               | 43  | 89.20       | 10.80        |
| S0-G60        | 17  | 84.15       | 15.85        |
|               | 29  | 87.32       | 12.68        |
|               | 43  | 88.96       | 11.04        |
| S1-G40        | 17  | 85.28       | 14.72        |
|               | 29  | 82.21       | 17.79        |
|               | 43  | 78.31       | 21.69        |
| S1-G60        | 17  | 89.36       | 10.64        |
|               | 29  | 84.85       | 15.15        |
|               | 43  | 78.45       | 21.55        |
| S2-G40        | 17  | 89.24       | 10.76        |
|               | 29  | 85.56       | 14.44        |
|               | 43  | 79.23       | 20.77        |
| S2-G60        | 17  | 88.49       | 11.51        |
|               | 29  | 86.35       | 13.65        |
|               | 43  | 80.75       | 19.25        |
| S3-G40        | 17  | 88.28       | 11.72        |
|               | 29  | 85.21       | 14.79        |
|               | 43  | 76.52       | 23.48        |
| S3-G60        | 17  | 87.21       | 12.79        |
|               | 29  | 82.65       | 17.35        |
|               | 43  | 75.32       | 24.68        |

### 3.4. Fungal Phenolic Compounds–Antioxidant Components Monitoring

The production of TPC (Figure 5) and AC (Figure 6) differed considerably depending on the growth medium and species. In the eight media, TPC and AC did not have a specific pattern and fluctuated during the fermentation in all culture media. Higher values for TPC are observed on the 37 day of growth. Specifically, significantly higher TPC (mg GAE/g dw) was observed in the medium with the addition of OMW (12.45–7.92 mg GAE/g dw), in contrast to the production of TPC in the medium without OMW (6.6 mg GAE/g dw), which was considerably lower. The wide range of values and inconsistency between the results are
attributed to the different species, origins, the environmental conditions, the maturity stage and the extraction method. Sulistiany et al. [67] determined a TPC\textsubscript{max} value in the mycelial of Pleurotus spp. methanol extracts (2.02 mg GAE/g), while Puttaraju et al. [68] recorded in a water extract TPC\textsubscript{max} values of 13.3 mg/g for P. djamor. In addition, Reis et al. [69] reported the TPC\textsubscript{max} of P. ostreatus (5.19 mg GAE/g) and P. eryngii (9.11 mg GAE/g), which is relatively close to the values of the present study (5.26–12.45 mg/g).

Figure 5. Phenolic compounds (TPC, mg GAE/g dw) produced during the cultivation of P. pulmonarius in liquid cultures with OMWs containing: (a) 0 g/L phenolic compounds and initial concentration glucose 40 and 60 g/L (S0-G40, S0-G60), (b) 1 g/L phenolic compounds and initial concentration glucose 40 and 60 g/L (S1-G40, S1-G60), (c) 2 g/L phenolic compounds and initial concentration glucose 40 and 60 g/L (S2-G40, S2-G60), (d) 3 g/L phenolic compounds and initial concentration glucose 40 and 60 g/L (S3-G40, S3-G60). Each point is the mean value of at least three independent measurements, SD < 5%.

Figure 6. Antioxidant components (AC) produced using DPPH\textsuperscript{•} (mg Trolox/g dw) during the cultivation of P. pulmonarius in liquid cultures with OMWs containing: (a) 0 g/L phenolic compounds and initial concentration glucose 40 and 60 g/L (S0-G40, S0-G60), (b) 1 g/L phenolic compounds and initial concentration glucose 40 and 60 g/L (S1-G40, S1-G60), (c) 2 g/L phenolic compounds and initial concentration glucose 40 and 60 g/L (S2-G40, S2-G60), (d) 3 g/L phenolic compounds and initial concentration glucose 40 and 60 g/L (S3-G40, S3-G60). Each point is the mean value of at least three independent measurements, SD < 5%.
The radical scavenging activity indicates significant differences among the different culture media compositions. The antioxidant activity of methanol extracts was measured as their scavenging capacity to reduce the DPPH• radical, a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule [70]. Our results showed that in the presence of OMW, the production of AC was lower. The AC\textsubscript{max} values for the substrates with OMW were 0.45 Trolox mg/g. Hence, in control substrates with 40 and 60 g/L glucose, the AC\textsubscript{max} values were 0.59 and 0.74 Trolox mg/g, respectively, on the 43 day of growth. In another study, the anti-radical activity of Pleurotus spp. was determined to be 2.76 Trolox mg/mL [67]. Reis et al. [69] reported that fruiting body extracts of P. ostreatus gave much higher DPPH• scavenging activity than its mycelial extracts, with antioxidant activity of 6.54 ± 0.16 and 58.13 ± 3.02 Trolox mg/mL, respectively. Methanolic extracts of P. sapidus, P. ostreatus, and P. eryngii mycelia showed higher values (53.9, 44.1, and 75% at 10 mg/mL) [71], which were significantly lower than the AC\textsubscript{max} achieved in the media of this study. However, the free radical scavenging activity of P. djamor was reported to be much higher, 4.8 and 1.9 mg BHA/g in water and methanol extracts, respectively [68]. Diamantopoulou et al. [9] observed higher values (718 µg GAE/g dw) of antioxidant activity on the 10 day of mycelial growth of G. resinaceum in the media without the addition of OMW and on substrate with 16% v/v OMW antioxidant activity reached a value of 404.0 ± 17.1 µg GAE/g dw on the last day of growth. These results suggest that P. pulmonarius and many other mushrooms may be used as a potential source of natural antioxidants for food supplements, as well as in the development of nutraceuticals.

3.5. Total Lipid Content and Fatty Acid (FA) Composition

The lipid content generally shows marked variations among species belonging to the phylum Basidiomycota [16,72]. The FA composition of the lipids produced by P. pulmonarius is demonstrated in Table 5.

Table 5. FA composition of total lipids synthesized by P. pulmonarius in batch-liquid state fermentation cultures, in synthetic media containing 40 and 60 g/L glucose and OMWs with phenolics concentration adjusted to 1, 2 and 3 g/L for 43 days at 26 ± 1 °C. Each point is the mean value of two independent measurements.

| Fatty Acids (%) | Culture Media * | S0-G40 | S0-G60 | S1-G40 | S1-G60 | S2-G40 | S2-G60 | S3-G40 | S3-G60 |
|----------------|----------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Undecanoic acid (C11:0) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Lauric acid (C12:0) | 2.27 | 0.00 | 0.00 | 2.72 | 0.18 | 0.00 | 0.20 | 0.18 |
| Myristic acid (C14:0) | 3.19 | 1.93 | 0.43 | 0.38 | 0.31 | 0.62 | 0.32 | 0.35 |
| Pentadecenoic acid (C15:1) | 7.32 | 9.17 | 0.00 | 0.00 | 0.39 | 0.00 | 0.35 | 0.00 |
| Palmitic acid (C16:0) | 23.10 | 22.30 | 16.50 | 7.78 | 14.70 | 18.23 | 14.00 | 14.80 |
| Palmitoleic acid (C16:1) | 1.42 | 1.15 | 0.00 | 0.00 | 0.45 | 0.00 | 0.45 | 0.48 |
| Heptadecanoic acid (C17:0) | 0.92 | 0.00 | 0.00 | 0.26 | 0.25 | 0.00 | 0.23 | 0.25 |
| Searic acid (C18:0) | 10.20 | 9.85 | 4.30 | 4.25 | 5.05 | 4.90 | 4.98 | 4.83 |
| Oleic acid (C18:1) | 11.80 | 16.90 | 27.30 | 29.80 | 28.60 | 27.50 | 28.84 | 29.60 |
| Octadecenoic Acid (C18:1) | 0.00 | 0.00 | 0.00 | 0.00 | 0.25 | 0.00 | 0.22 | 0.37 |
| Linoleic acid (C18:2) | 27.42 | 28.55 | 47.43 | 46.10 | 41.75 | 46.86 | 41.08 | 41.95 |
| α-Linolenic acid (C18:3) | 0.00 | 0.00 | 0.00 | 0.00 | 0.23 | 0.20 | 0.26 | 0.26 |
| Arachidic acid (C20:0) | 0.00 | 0.00 | 0.40 | 0.30 | 0.50 | 0.00 | 0.71 | 0.69 |
| Eicosenoic acid (C20:1) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Behenic acid (C22:0) | 0.00 | 0.00 | 0.41 | 0.43 | 0.69 | 0.00 | 0.90 | 0.51 |
| Saturated | 39.68 | 34.08 | 22.08 | 16.12 | 21.68 | 23.75 | 21.34 | 21.61 |
| Monounsaturated | 20.54 | 27.22 | 27.30 | 29.80 | 29.69 | 27.50 | 29.86 | 30.45 |
| Polyunsaturated | 27.42 | 28.55 | 47.43 | 46.33 | 41.95 | 47.12 | 41.34 | 42.23 |
| U.I. | 0.91 | 0.97 | 1.39 | 1.30 | 1.28 | 1.40 | 1.26 | 1.30 |
| Other acids | 7.26 | 3.65 | 0.88 | 1.80 | 1.89 | 0.81 | 2.94 | 1.85 |
| Not identified peaks (35 min–38.5 min) | 5.09 | 6.50 | 2.34 | 5.95 | 4.80 | 0.81 | 4.52 | 3.87 |

* As at Table 2.

The total lipid content in the fungal structures of the strain, ranging from 0.94–2.85 g/L, was in line with values reported in previous studies of higher Basidiomycetes, including...
As regards the total lipid in dry weight (% w/w), the concentration of total lipophilic compounds from mycelia grown in the eight media on the 43 day of cultivation in static cultures was between 6.09% and 11.69% w/w. Several studies have suggested that the cell wall composition of mycelium can be similar to that of a mushroom fruiting body. Smiderle et al. [65] reported total lipids of 0.8% w/w in P. pulmonarius mycelium synthesized when glucose was used as the main carbon source. Kavishree et al. [73] reported fat content of 0.5% w/w in the mycelia produced by many higher fungi in liquid cultures in static conditions, such as Morchella elata, Morchella esculenta, Auricularia auricula, F. velutipes and L. edodes [16]. This high value is significant as a result, since the mushrooms used in the current investigation as well as other edible and pharmaceutical fungi such as P. ostreatus, P. pulmonarius, P. sajor-caju, Stropharia aeruginosa, Phellinus spp., etc. are not considered as oleaginous microorganisms (meaning that their present values are 20% w/w) [17,65,74]. Fungal lipids are the basic construction unit of membranes and cell walls and, in some cases, extracellular products [61,75]. In general, the total fat content of the fungi varies from 0.6 to 18.4% (w/w) on a dry basis [54,72,75,76]. According to Papanikolaou and Aggelis [61], fungal lipids on non-oleaginous microorganisms can theoretically reach up to 20% w/w. However, in the case of oleaginous organisms, the percentage of fat exceeds this value and can reach values of up to 80% w/w [77]. On the other hand, in several investigations total lipids were found as 14.2–16.3% w/w in the mycelia of mushrooms Polyporus hirsutus and Calvatia caelata [78], 25% w/w in G. frondosa [79] and 22% w/w in G. tsugae [80]. In our study, despite the low lipid accumulation, cellular lipid concentration significantly decreased between the 17 and 37 day and increased between the 37 and 43 day as the fermentation proceeded (Figure 7), without this reduction being related to the absence of glucose in the medium and being in accordance with kinetic patterns observed for several yeast or fungal strains (e.g. Y. lipolytica, Zygosaccharomyces rouxii, Candida oleophila, Pichia membranifaciens, Thamnidium elegans, Mucor sp., etc.) growing on sugars (e.g. glucose) or similarly metabolized carbon sources (e.g., glycerol). In the case of V. volvacea and other ascomycetes and Basidiomycetes grown on glucose [16,27,32,81–83], it has been observed that a similar decrease in the quantity of lipids in dry weight when glucose was found in excess in the medium. Having contributed to biomass production, lipid synthesis was increased again.

![Figure 7](image-url)
According to our results, the analysis of the obtained profiles showed that linoleic (C18:2 ω-6), oleic (C18:1 ω-9), palmitic (C16:0) and stearic (C18:0) acids were the predominant in the studied species. The unsaturated FAs dominated over the saturated ones, where C18:2 was the most abundant FA. In the presence of OMWs, the major unsaturated FAs, linoleic and oleic, increased their concentrations from ~27 to 47% and from 12 to 28%, respectively. That was almost twofold compared to the control media. The Unsaturation Index (U.I.) of FAs was increased with the presence of OMW, with the maximum value of 1.4 being presented in the S2-G60 medium. Our findings agreed with the results reported for other Basidiomycetes [17, 26, 27, 88]. Pedneault et al. [18] demonstrated that linoleic, palmitic and oleic acids were the main FAs found in both polar and non-polar extracts of P. ostreatus and Pleurotus cornucopiae, accounting for 83.1–95.9% of total FAs. Beyond these, it is interesting that rare carbon number FA, such as myristic (C14:0) and arachidic (C20:0), were observed, even though in negligible proportions. However, according to Solomko et al. [89], oleic acid (C18:1) was the main FA in the lipids of fruit bodies and mycelium of P. ostreatus (56% of total lipids), whereas in our culture, oleic acid (C18:1) was found in significantly lower values (16.9–29.8% w/w). On the other hand, palmitic acid (C16:0) values (~23%, w/w) were higher in control media (S0-G40, S0-G60) and with the addition of OMW, they were decreased to under 18.23% w/w (in S2-G60) or even lower (7.78%, w/w in S1-G60). Moreover, traces of α-linolenic acid were found in the media.
with OMW. Thus, according to our results, media that contained OMW were found to have higher unsaturated FA concentrations, 71.64–76.13%. In general, the FA composition of eukaryotic microorganisms, including fungi, can often be modified in response to environmental conditions such as pH, media composition, temperature, and aeration [90].

4. Conclusions

*P*. *pulmonarius* mushroom was able to grow in substrates containing OMWs at high concentrations of phenolic compounds (3 g/L) and glucose (60 g/L). It achieved a significant degree of detoxification of the OMWs (>90% phenolic content reduction and >85% decolorization). In the presence of OMW in the culture substrates, the fungus presented great biomass formation, IPS synthesis, and lipid accumulation. Also, the concentration of phenolic compounds in the biomass was increased, adding value to its antioxidant activity. Hence, biological treatment of waste oil products with simultaneous production of high-nutritional-value products can be achieved.

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