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BIOTECHNOLOGICAL PRODUCING OF NATURAL FERTILIZERS THROUGH MICROBIAL COMPOSTING OF FRUIT WASTES

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Abstract

In Romania, to protect the natural environment and save its biodiversity there is an urgent need to solve a lot of serious damages caused by the pollution effects induced through discharging of fruit wastes into natural aquatic ecosystems (streams, rivers, lakes), in huge amounts, after their fermentation and alcohol extraction made in private horticultural farms. The efficient valorising of such organic compounds, represented by many derived wastes from fruit processing, through biotechnological means was establish to be the main aim of certain research experiments whose results are presented in this paper. There were carried out laboratory works to test the optimal needs of bacterial and fungal pure cultures to grow inside different marc made of apple, cherry and plum wastes (chemical composition, temperature, pH, oxygen/carbon dioxide concentration). In this respect, there were used pure bacterial cultures of Bacillus genus as well as the fungal ones belonging to species of Pleurotus for microbial transformation of different fruit wastes. The biotechnology of microbial composting was applied by using a laboratory-scale bioreactor of 15 L working volume. The submerged fermentations of different fruit wastes were set up for the following parameters: constant temperature, 23°C; agitation speed, 80-100 rev. min^{-1}; pH level, 5.7–6.0 units; dissolved oxygen tension within the range of 30-70%. After a period of submerged fermentation lasting between 140- 230 h, there was produced fermented composts containing the microbial biomass that was developed through biochemical transforming of marc into natural fertilizers. By using pure cultures of bacterial and fungal species, in strict hygienic conditions and permanent control of microbial processes induced for fruit waste composting it was ensured the optimum performance of tested biotechnological proceedings to increase the biochemical transformations of marc as compounds with a high content in proteins and other organic compounds that are very useful as natural fertilizers of agricultural crops.

Key words: *bacteria, composting, fertilizers, fruit wastes, mushrooms, submerged fermentation*

INTRODUCTION

It is well known that, annually, huge amounts of wastes and residues are produced in orchards during agro-industrial activities of fruit processing. The reuse and recycling of all fruit wastes could allow self-sustainable processes through the natural fertilizers that are produced by microbial conversion of cellulose into protein-rich biomass with beneficial effect on soil as well as efficient environmental protection tool (Bae *et al.*, 2000; Verstraeate and Top, 1992; Chahal, 1994; Moser, 1994).
The present work reports on the preliminary results carried out to study the bioconversion of cellulose wastes from fruit processing agro-industry into protein biomass by using pure cultures of bacteria and edible mushrooms (Carlile and Watkinson, 1996; Wainwright, 1992). The main aim of this study was to establish the optimal submerged culture conditions for controlled microbial co-fermentation in order to improve the bioconversion of cellulose from fruit wastes like apple and plum wastes into organic biomass with high protein content to be used as natural fertilizers in horticulture.

MATERIALS AND METHODS

Microbial strains and culture media

During the experiments there were tested two microbial strains of cellulolytic bacterial species, namely *Bacillus subtilis* as well as mushroom species *Pleurotus ostreatus*. These

two microbial species were used as pure cultures from the culture collection of microorganisms belonging to the University of Pitesti (Petre and Petre, 2008).
The stock cultures of bacterial species *Bacillus subtilis* were kept in viable shape on 0.5 LB agar plate (1% tryptone, 0.5% yeast extract, 0.5% NaCl and agar-agar 1.5%) and the mushroom species of *Pleurotus ostreatus* were maintained on malt-extract agar (MEA) slants. The culture medium composition for microbial conversion and protein synthesis was made of apple wastes 50%, previously treated by mixing with wheat bran 10%, barley bran 5%, d-glucose 5% and hydrated with pure water 30%. This was the first variant of culture substrate for mushroom growing (substrate 1). The second variant composition of culture substrate was prepared from plum wastes 50% improved by adding barley bran 10%, wheat bran 5%, d-glucose 5% and tap water 30% (substrate 2). The two variants of culture substrates were used in experiments for growing both monocultures and co-cultures of *B. subtilis* and *P. ostreatus*. In this respect, the optimal temperatures during the growth of bacterial and mycelial co-cultures were registered between 23–25 °C corresponding to initial pH levels of 4.5–6.0. The agitation speed was tested in the range of 30-90 rpm (Beguin and Aubert, 1994).

Methods used in experiments

The microbial strains of *B. subtilis* and *P. ostreatus* were used in pairs as well as separately to compare the efficiency of their biological potential in bioconversion of fruit wastes into protein biomass (Petre and Petre, 2013a).
These strains were tested both in monocultures and co-cultures for growing on two variants of culture substrates made of apple and plum wastes mixed with cereal wastes. The medium composition, pH levels, incubation temperature, agitation rate, inoculum age as well as inoculum volume during the submerged co-fermentation were registered as significant physical and chemical factors that could influence the bioconversion of fruit wastes used as growth substrates into protein biomass as well as microbial biomass formation (Ropars *et al*., 1992; Chahal, 1994; Lamar *et al.*, 1992).

The experiments concerning the testing of microbial strains with the best potential of bioconversion of cellulosic wastes from fruit processing were carried out by using a laboratory scale bioreactor (Figure 1).

Figure 1. General view of the laboratory-scale bioreactor

The design of this bioreactor incorporates a device to keep the constant temperature, inoculum reservoir, sterile air supply in aerobic processes, culture vessel as well as an automation panel for bioprocess monitoring and management (Petre and Petre, 2013b).

RESULTS AND DISCUSSION

Bioconversion of apple and plum wastes requires a suitable environment for growth of pure bacterial and fungal cultures, in order to increase efficiency of submerged fermentation made by mono- and co-cultures of *B. subtilis* and *P. ostreatus* (Petre *et al*., 2012; Smith, 1998; Stamets, 1993; Leahy and Colwell, 1990). The content of reducing sugars was determined by Kubicek technique and the total nitrogen content was analyzed by Kjeldahl method (Chahal and Hachey, 1990; Glazebrook *et al*., 1992). The experimental data determined as total reducing sugars contents (Kubicek *et al.*, 1981) were correlated by complementary investigations with those values of dry weight loss measurements of fruit wastes bioconversion, for both mono- and co-cultures of *B. subtilis* and *P. ostreatus*.
All registered data regarding the evolution of total reducing sugars as well as total nitrogen contents during bioconversion of apple and

plum wastes into protein biomass by using monocultures and co-cultures of *B. subtilis* and *P. ostreatus* are presented in Tables 1 and 2.

The evolution of dry weight loss of the same fruit wastes used as substrates for microbial cultures is shown in Table 3.

Table 1. Total reducing sugars evolution during bioconversion of apple and plum wastes into protein biomass by using monocultures and co-cultures of *Bacillus subtilis* and *Pleurotus ostreatus*

Culture time (h)	Total reducing sugars (mg g^{-1})					
	Bacillus subtilis (Monoculture)		*Pleurotus ostreatus* (Monoculture)		*B. subtilis – P. ostreatus* (Co-cultures)	
	Substrate 1	Substrate 2	Substrate 1	Substrate 2	Substrate 1	Substrate 2
72	2.10	2.80	4.50	6.90	9.30	12.80
144	4.10	4.90	5.80	8.10	11.10	15.50
216	5.70	6.80	7.90	10.40	14.90	18.30
288	7.80	8.10	10.70	12.80	18.30	21.80
360	9.50	10.90	14.10	15.50	21.90	25.30
432	10.70	12.50	16.30	18.20	24.50	27.50
504	11.45	15.30	19.70	21.50	26.30	30.10
576	12.50	17.70	21.80	23.30	28.80	32.50
648	14.80	19.30	23.50	25.80	30.10	33.90
720	15.10	20.50	25.10	28.30	30.50	35.10

All data are representative of three replicated determinations.

Table 2. Total nitrogen content evolution during bioconversion of apple and plum wastes into protein biomass by using monocultures and co-cultures of *Bacillus subtilis* and *Pleurotus ostreatus*

Culture time (h)	Total nitrogen content of fungal protein biomass (g % dry weight)					
	Bacillus subtilis (Monoculture)		*Pleurotus ostreatus* (Monoculture)		*B. subtilis – P. ostreatus* (Co-cultures)	
	Substrate 1	Substrate 2	Substrate 1	Substrate 2	Substrate 1	Substrate 2
72	3.50	3.90	4.50	5.10	7.90	9.50
144	4.10	4.75	5.80	6.40	9.30	12.10
216	5.70	6.55	7.70	8.50	14.10	15.80
288	7.80	7.90	9.80	10.10	15.80	18.10
360	9.50	9.80	12.10	12.50	18.30	21.90
432	10.70	11.10	14.00	14.40	21.50	23.30
504	11.45	12.70	16.70	17.30	23.60	25.70
576	12.10	13.50	18.50	20.10	25.90	27.10
648	12.80	14.30	20.80	21.80	27.20	28.90
720	12.90	14.50	21.30	23.20	28.10	30.30

All data are representative of three replicated determinations.

Table 3. The evolution of dry weight loss of apple and plum wastes used as substrates for *Bacillus subtilis* and *Pleurotus ostreatus* growing as monocultures and co-cultures

Culture time (h)	Dry weight loss (g %)					
	Bacillus subtilis (Monoculture)		*Pleurotus ostreatus* (Monoculture)		*B. subtilis – P. ostreatus* (Co-cultures)	
	Substrate 1	Substrate 2	Substrate 1	Substrate 2	Substrate 1	Substrate 2
72	2.50	4.30	5.10	6.40	7.30	10.40
144	3.10	5.50	5.90	7.00	8.50	12.80
216	3.90	6.70	6.70	8.50	9.70	14.10
288	4.80	7.90	7.90	9.40	10.80	15.30
360	5.50	8.80	8.80	10.50	12.50	16.50
432	6.40	9.50	10.90	12.80	14.80	18.30
504	7.30	10.90	12.70	14.30	16.30	20.10
576	8.50	12.10	13.50	15.90	17.70	21.80
648	9.30	13.70	14.90	17.40	18.50	23.70
720	10.10	14.30	15.80	18.50	20.80	25.50

All data are representative of three replicated determinations.

Finally, after 720 h of submerged fermentation the protein biomass obtained through bioconversion of apple and plum wastes by using the co-cultures of *B. subtilis* and *P. ostreatus* was collected from the culture vessel of laboratory scale bioreactor as it is shown in Figure 2 and Figure 3.

Figure 2. Protein biomass obtained through the bioconversion of apple wastes by using the co-cultures of *B. subtilis* and *P. ostreatus*

Figure 3. Protein biomass obtained through the bioconversion of plum wastes by using the co-cultures of *B. subtilis* and *P. ostreatus*

The amounts of protein biomass obtained through bioconversion of apple and plum wastes by using co-cultures of *B. subtilis* and *P. ostreatus* contain between 28.1 and 30.3 g % dry weight after 720 h of submerged fermentation in the culture vessel of the laboratory-scale bioreactor.

CONCLUSIONS

The microbial strains of *B. subtilis* and *P. ostreatus* were used in pairs as well as separately to compare the efficiency of their biological potential in bioconversion of fruit wastes into protein biomass.

These strains were tested both in monocultures and co-cultures for growing on two variants of culture substrates made of apple and plum wastes mixed with cereal wastes.

The experiments concerning the testing of microbial strains with the best potential of bioconversion of cellulosic wastes from fruit processing were carried out by using a laboratory scale bioreactor as controlled biotechnological system.

The optimal temperatures for both bacteria and mycelia cultures to produce microbial biomass through controlled submerged fermentation as mono- and co-cultures, were registered between 23–25 °C, corresponding to initial pH levels of 4.5–6.0 and the agitation speed was tested in the range of 30-90 rpm.

The registered results revealed an increasing of reducing sugars correlated with an increasing of protein content analysed as total nitrogen for the microbial biomass of co-cultures, in comparison with the control samples represented by the monocultures of the same bacterial and fungal species used in experiments

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