Microbial composting of fruit tree wastes through controlled submerged fermentation

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Abstract

The ecological valorising of organic compounds represented by many derived wastes from fruit processing through the controlled microbial composting was established as the main aim of research experiments 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 140-230 h, the fermented composts, containing the microbial biomass developed through biochemical transforming of marc into natural fertilisers, were produced.

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Introduction

An efficient method to convert cellulose materials, in order to produce unconventional high-calorie foods or feeds, is the direct conversion through the controlled metabolism of cellulolytic microorganisms. Theoretically, any microorganism that can grow in the shape of pure culture on cellulosic substrates, using them as carbon and energy sources, should be considered a potential organism for *single-cell protein* or *protein rich feed* producing (Chahal and Hachey, 1990).

In the last decades, many researches were done to biodegrade and convert different cellulosic wastes into useful products by microbial composting with bacterial and fungal species through their specific metabolism (Verstraete and Top, 1992; Beguin and Aubert, 1994; Moser, 1994; Carlile and Watkinson, 1996).

In this respect, one of the most efficient biotechnological method to convert the cellulosic wastes resulted alcoholic distillation of fermented fruit tree wastes, such as juice and pulps of apples, pears and plums, is their controlled submerged fermentation through the enzymatic activity of bacterial cells and edible mushroom mycelia by using automatic biotechnological devices to get nutritive microbial biomass through controlled composting of the fruit tree wastes to be used as natural bio-fertilisers of horticultural crops (Petre and Petre, 2013).

The main purpose of this work consists in testing the completely new biotechnology for controlled composting of different liquid wastes of tree fruits through the submerged cultivation of bacterial cells and edible mushroom species in order to get high nutritive organic fertilisers in a very short time of producing cycle. This original biotechnology has already been registered at Romanian Office of Patents and Trade Marks.

Materials and methods

Microorganisms and culture media

According to the main aim of this work, there were tested two cellulolytic microorganisms, namely the bacterial species *Bacillus subtilis* and the fungal one, respectively, *Pleurotus ostreatus* (Jacquin ex Fries) Kummer from the cultures collection of University of Pitesti.

B. subtilis was isolated from the apple wastes resulted from alcoholic distilleries and was maintained in culture medium containing (g/L): peptone 5.0; meat extract 3.0 and agar 2.0. The medium was autoclaved at 121°C for 15 min. The cultures were kept at 4°C and renewed every 45 days. The seed culture medium was composed by 100 g/L of sucrose (1%) and NaCl (0.5%).

The edible mushroom *P. ostreatus* was used as pure culture in all carried out experiments. The stock cultures were maintained on malt-extract agar (MEA) slants. Slants were incubated at 25°C for 5-7 d and then stored at 4°C. The mushroom cultures used as inoculum for the

cultivation inside the culture vessel of laboratory-scale bioreactor were grown in 250-mL flasks containing 100 mL of MEA medium (20% malt extract, 2% yeast extract, 20% agar-agar) at 23°C on rotary shaker incubators at 110 rev min^{-1} for 5-7 d (Ropars *et al*., 1992; Wainwright, 1992; Smith, 1998).

The natural substrates for the controlled microbial composting were prepared from different sorts of organic fruit wastes such as juices and pulps, resulted from the industrial processing through alcohol distillation of fermented apples, pears and plums. These substrates were made by apple and plum wastes resulted from alcohol distillation mixed with other needed natural ingredients, such as, barley and wheat bran, in small amounts (1.5-3% w/w), in order to improve the enzymatic activity of bacterial and mushroom mycelia and convert the cellulose content of these fruit wastes into protein biomass.

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, 2013).

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).

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 and Petre, 2012; Smith, 1998; Stamets, 1993; Leahy and Colwell, 1990).

All the cultivation substrates were steam sterilised at 123°C for 30 min at 1.2 atm inside the culture vessel of the bioreactor and after cooling, the substrates from the glass vessel were inoculated aseptically with the seed cultures grown in the rotary shaker incubator as it was previously described.

The steam sterilisation of the substrates for bacterial and fungal growing is compulsory because all these liquid wastes are contaminated with competitive and opportunistic microbial species, many of them being phytopatogenic. In this way, even the energy expenses were registered between 5-10% from the final price of the bio-fertilisers, the effectiveness cost justifies this physical treatment to eliminate the competitors on the same substrates made of fruit wastes to be converted in a nutritive biomass. After inoculation into the bioreactor vessel, the submerged fermentation was set up at 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%. The mushroom growing cycle lasted from 7 to 10 days, depending on cultivated species, and there were registered thousands of mushroom pellets with variable dimensions that were developed inside the culture vessel of the laboratory-scale bioreactor as it is presented in Figure 1.

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

The content of reducing sugars was determined by Kubicek technique and the total nitrogen content was analysed by Kjeldahl method (Glazebrook *et al*., 1992; Chahal and Hachey, 1990). The experimental data determined as total reducing sugars contents (Kubicek *et al.*, 1993) 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*.

Results

During the whole bioconversion of apple and plum wastes as juices and pulps from alcohol distillation 48 h samples were collected and then chemically analysed to assess the progress 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 progress of dry weight loss of the same fruit wastes used as substrates for microbial cultures is shown in Table 3.

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 Figures 2 and 3.

Figure 1. The laboratory-scale bioreactor for submerged cultivation of microbial cells.

Table 1. Total reducing sugars during bioconversion of apple and plum wastes.

Time (h)	Total reducing sugars (mg/g)*					
	B. subtilis (monoculture)		*P. 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	14.90	19.50	23.10	25.30	30.50	33.10

*All data are representative as means of three repeated determinations.

Table 2. Total nitrogen content during bioconversion of apple and plum wastes.

Time (h)	Total nitrogen content of fungal protein biomass (g % dry weight)*					
	B. subtilis (monoculture)		*P. 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	12.70	14.10	20.30	21.50	27.10	28.30

*All data are representative as means of three repeated determinations.

Table 3. Dry weight loss of apple and plum wastes used for microbial composting.

Time (h)	Dry weight loss (g %)*					
	B. subtilis (monoculture)		*P. 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	14.10	14.90	17.40	18.50	23.70
720	9.10	14.30	14.80	17.30	18.30	23.50

*All data are representative as means of three repeated determinations.

Figure 2. Biocompost obtained through the bioconversion of apple wastes by using the co-cultures of *B. subtilis* and *P. ostrea*tus.

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

Discussion

Analysing the registered data, it was revealed the fact that by applying this biotechnology, the fruit wastes can be recycled as useful raw materials for mushroom growing in order to get significant mushroom biomass production in contrast with actual composting techniques that are not suitable to control and monitor the microbiological and chemical qualities of the final products of fruit waste bioconversion process as reported by Chahal (1994) and Smith (1998).

Thus, it can be noticed that 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 agitate-on speed was tested in the range of 30-90 rpm.

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

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.

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 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 the significant level 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.

Due to its nutritive protein content, the final biomass resulted from the biotechnological process of bioconversion of apple and plum wastes by using monocultures and co-cultures of *B. subtilis* and *P. ostreatus* is appropriate to be used as natural bio-fertilisers of horticultural crops, being produced by controlled composting of organic wastes.

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