

Article

## Assessment of Gardening Wastes as a Co-Substrate for Diapers Degradation by the Fungus *Pleurotus ostreatus*

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**Abstract:** Waste with high biomass content generated in cities in developing countries is sent to landfills or open dumps. This research aims to degrade biomass content in urban waste through cultivation, at pilot scale, of the edible mushroom *Pleurotus ostreatus*. First, the number of diapers used by one baby per week was measured with a survey in day care facilities. Then, cellulose content of diapers was assessed. Finally, cultivation of *P. ostreatus* was carried out using as substrate a mixture of diapers with gardening waste, a co-substrate readily available at urban settings. The factors assessed were strain of *P. ostreatus* (grey BPR-81, white BPR-5), conditioning of the substrate (diapers with and without plastic) and co-substrate (wheat straw, grass, and withered leaves). Results show that diapers are a

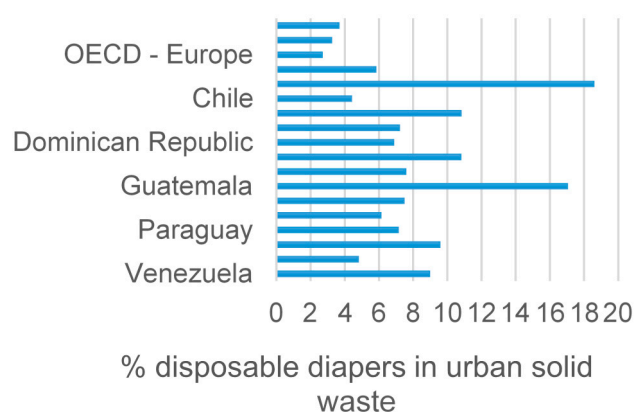
valuable source of biomass, as generation of diapers with urine is 15.3 kg/child/month and they contain 50.2% by weight of cellulose. The highest reductions in dry weight and volume (>64%) of substrates was achieved with the substrate diaper without plastic and co-substrate wheat straw. Although diapers with plastic and grass and leaves showed lower degradation, they achieved efficiencies that make them suitable as a co-substrate (>40%), considering that their biomass is currently confined in landfills.

**Keywords:** biomass; biodegradation; cellulose; biological efficiency

## 1. Introduction

World's population has grown exponentially, in 1950 it was 2.6 billion and by 2015 reached 7.27 billion [1]. This is causing a huge stress on natural resources and increasing waste and emissions of pollutants to the atmosphere, water and soil. Biomass, a natural resource, is used to provide food for humans, feed for animals and fuels. Allocation of crops produced globally varies according to the region; in India 92% of crops become food for humans, 4% feed for animals and 4% have other use whereas in Brazil 39% are food, 14% are feed and 27% have other use [2]. In 2000, only 3% of world's crops were used for production of biofuel, since then volume of produced biofuel has increased more than 450% [2].

Although biomass is a renewable resource, production capacity of this commodity is limited. Sustainable use of this resource is relevant; organic waste such as agricultural byproducts, pruning and gardening waste are important sources of biomass. Agricultural waste can be treated with technologies, such as incineration, gasification, and pyrolysis to produce energy or fuels. Pruning and gardening waste can be composted or sent to final disposition sites. However, in some developing countries this type of organic waste is simply let to rot or burned [3]. Urban centers contribute as well to the generation of waste with high biomass content. One of the relevant sources of biomass in urban waste is diapers, as shown in Figure 1 [4,5]. It can be observed that the percentage of disposable diapers in urban waste is considerably higher in Latin American countries than in North America and the European countries included in the Organization for Economic Co-operation and Development.



**Figure 1.** Percentage of diapers in urban solid waste in some countries in North America, Europe and Latin America [4,5].

In most developing countries disposable diapers are collected un-sorted with general waste and are disposed in sanitary landfills or open dumps. In developed countries, such as the United States of America, Spain, New Zealand, Canada, and the United Kingdom, disposable diapers are collected by private companies and incinerated or composted [6–9].

Developing countries, such as Mexico, need to valorize the biomass contained in urban solid waste with technologies that can be implemented within their economic, cultural and technologic capabilities. Biological treatments that valorize the cellulose content of used diapers, such as composting, and use as substrate in edible mushroom production have been investigated at laboratory or pilot scale [4,10,11].

The production of edible fungus to degrade agricultural waste is a sustainable alternative for biomass recycling. In 1978, Chang *et al.* [12] was a pioneer in the production of *Pleurotus ostreatus* with waste of wheat straw and oil palm, since then a variety of organic wastes have been investigated as substrate for cultivation of this fungus (Table 1). Valorization of diapers as substrate in cultivation of *P. ostreatus* of used baby diapers and wheat straw was carried out by Espinosa-Valdemar [11]. However, straw is not easily obtained in urban centers, which are the higher producers of diapers. In this work, grass and withered leaves (urban gardening wastes) are tested as a co-substrate for the degradation at pilot scale of disposable baby diapers by *P. ostreatus*.

**Table 1.** Substrates used for the cultivation of *P. ostreatus* with agricultural wastes.

Substrate	Country	Reference
Rice straw and banana leaves	Brazil	[13]
Wood dust, wheat straw and leaves	Pakistan	[14]
Pine wood dust and barley straw	Mexico	[15]
Coffee pulp and wheat straw	Mexico	[16]
Populus chips, eucalyptus chips and wheat straw	Chile	[17]
Coffee pulp	Spain	[18]
Corn stubble, sorghum stubble and Bermuda grass	Mexico	[19]
Roselle plant, rice straw, banana leaves and stalks	Mexico	[20]
Wheat, millet, soy and cotton straw	Turkey	[21]
Olive leaves	Israel	[22]
Beet pulp, wood chips, palm, bran wheat, bran rice and soy	Iran	[23]
Wheat straw, baby diapers and grape pomace	Mexico	[11]
Wood dust, yucca skin, cotton waste and banana leaves	Nigeria	[24]
Garlic waste and corn waste	Argentina	[25]
Banana leaves, potato skin and sugarcane bagasse	Colombia	[26]
Wheat straw	Argentina	[27]
Goldenberries skin, pea skin, corncob and oat	Cuba—Ecuador	[28]
White bond paper and printed bond paper	Portugal	[29]

## 2. Experimental Section

This research evaluated the effect of three factors on biodegradation of baby diapers, containing urine, during cultivation of the edible fungus *Pleurotus ostreatus*. The factors assessed were: strain of *P. ostreatus*, conditioning of the substrate and co-substrate. Experimentation was carried out in the following stages: determination of baby diapers composition and number of diapers with urine produced by

one baby in a month, preparation of substrates, cultivation of *P. ostreatus* and evaluation of the biodegradation of substrates. Experimentation was carried out in the Laboratory of Sustainable Technologies at the Autonomous Metropolitan University Campus Azcapotzalco.

### 2.1. Determination of Baby Diapers Composition

Composition of baby diapers was determined to establish the potential to valorize this waste as a biomass source. Ten different brands of baby diapers for children of 10 kg were bought in supermarkets and pharmacies. Three diapers from each brand were weighted (with a balance OHAUS model Scout) and separated into their components (*i.e.*, superabsorbent polymer, non-woven fabric, adhesives, elastic), then the mass of individual components was measured (with a balance OHAUS model TS 120S). Finally, average and standard deviation were calculated for mass and composition of diapers.

### 2.2. Determination of Generation of Diaper Waste with Urine per Child

The study was conducted in 10 baby daycare facilities located in the northern region of Mexico City. A survey was applied to parents and generation of diapers was measured during 4 days. Children up to 24 months old were included in the study. The survey asked parents for the number of disposable diapers used per children per day and the number of diapers only with urine produced per child per day. A total of 600 surveys were handed in daycare facilities.

During 4 days, diapers with urine were separated in a plastic bag in baby rooms in daycare facilities. Every day diapers with urine were collected, and the total weight was measured with a balance ADAN<sup>®</sup> model QBW-30K. The average weight of diapers with urine was calculated selecting 10 diapers in each baby room randomly. Diapers with feces were excluded since this research is in an early stage and pathogens contained in feces were a sanitary concern.

### 2.3. Biodegradation of Diapers by the Edible Fungus *Pleurotus Ostreatus*

Biodegradation was carried out according to the method proposed by Espinosa-Valdemar *et al.* [11]. *P. ostreatus* was cultivated in bags of 5 kg, called experimental units, filled with the mixture substrate-co-substrate in a ratio of 65%–35%. The factors evaluated were strain of *P. ostreatus* (grey BPR-81 and white BPR-5), conditioning of the substrate (diapers with plastic and diapers without plastic) and co-substrate (wheat straw, grass and withered leaves). For each strain of *P. ostreatus*, three blanks were set: wheat straw, grass and withered leaves. The experiment was carried out in triplicates. Table 2 shows the treatment combinations for each strain of *P. ostreatus*.

#### 2.3.1. Preparation of the Substrates

Bright yellow wheat straw was bought clean in 12 kg bales, grass clippings from the University lawn mowing were used, and withered leaves were also collected in the University. Diapers with urine were obtained from baby daycare facilities and milled with a brush chipper Vermeer<sup>®</sup> model BC1000 XL. Plastic components were manually removed from diapers, before milling, for experimental units without plastic.

The mixtures of diapers, wheat straw, grass and withered leaves, prepared according to Table 2, were homogenized manually, and their humidity was adjusted at 60%. Then, substrates were placed in thermo-resistant paper bags and sterilized for 1 h at 121 °C in a vertical autoclave AESA® model CV-300.

**Table 2.** Treatment combinations for each strain of *P. ostreatus* (grey BPR-81 and white BPR-5) for evaluation of biodegradation of diapers.

Treatment	Conditioning of Substrate	Co-Substrate
1	Diapers with plastic	Wheat straw
2	Diapers with plastic	Grass
3	Diapers with plastic	Withered leaves
4	Diapers without plastic	Wheat straw
5	Diapers without plastic	Grass
6	Diapers without plastic	Withered leaves
7	Wheat straw	---
8	Grass	---
9	Withered leaves	---

### 2.3.2. Cultivation of *Pleurotus Ostreatus*

Cultivation of *P. ostreatus* occurs in three stages: sowing, spawning and harvesting. Sowing was carried out in a vertical laminar flow cabinet with inoculation of 500 g of seeds per experimental unit. Weight of experimental units was registered after sowing. Then, experimental units were placed in a dark room to allow the inoculum to invade the substrate and spawn. Degradation of the substrate begins in this phase. When the substrate was fully invaded by the inoculum experimental units were transferred to a light room for fructification of the fungus *P. ostreatus*. In this phase, thermo-resistant paper bags were almost fully removed to allow CO<sub>2</sub> interchange (Figure 2). Experimental units were watered with a drop-by-drop irrigation system. Fungus were harvested when they were fully developed, recording their weights.



**Figure 2.** Fruiting bodies of *Pleurotus ostreatus* on the experimental units during the luminous phase.

## 2.4. Evaluation of the Degradation Process

The degradation process was evaluated by a reduction of dry weight and volume of substrate after cultivation of *P. ostreatus*. The humidity originally contained in substrates (wheat straw, grass, and withered leaves) was determined with the weight loss of substrates after drying them in a laboratory stove (Riossa, model H-62) at 60 °C for 24 h. The initial dry weight of substrates ( $w_s$ ), prepared according to Table 2, was calculated by subtracting the weight due to humidity. After harvesting *P. ostreatus*, substrates were dried (60 °C, 24 h, stove Riossa, model H-62) and their weight was recorded. The balance ADAN model QBW-30 K was used to measure weight of substrates. Reduction of volume of substrates was estimated using a container of known volume. These parameters were statistically analyzed with an analysis of variance test.

Additionally, the biological efficiency (BE) for each treatment combination was calculated. BE, a term commonly used in the edible mushroom industry, reflects the conversion of substrate into mushroom. This was calculated by the following equation [16]:

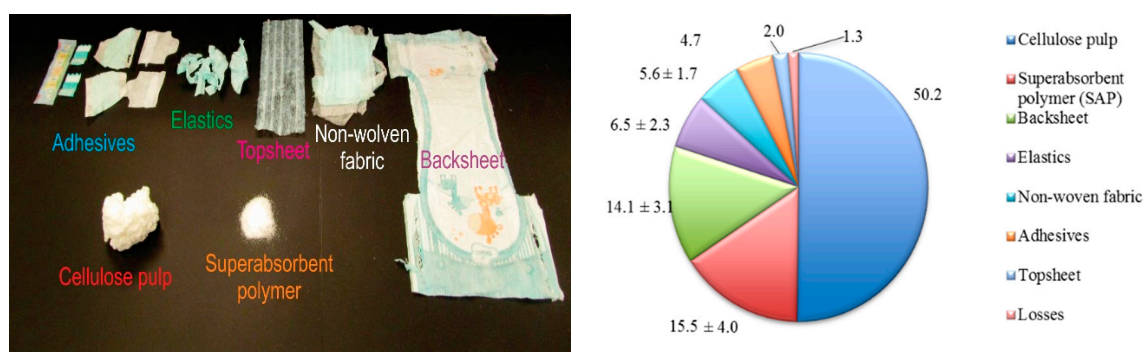
$$\%BE = \frac{w_m}{w_s} \times 100 \quad (1)$$

where %BE is the percentage of biological efficiency,  $w_m$  is the fresh weight of the harvested mushrooms and  $w_s$  is the initial dry weight of the substrate and co-substrate. The fresh weight of mushrooms was obtained weighting these immediately after the harvest and the determination of the initial dry weight of substrate ( $w_s$ ) was explained in the paragraph above.

## 3. Results

### 3.1. Determination of Baby Diapers Composition

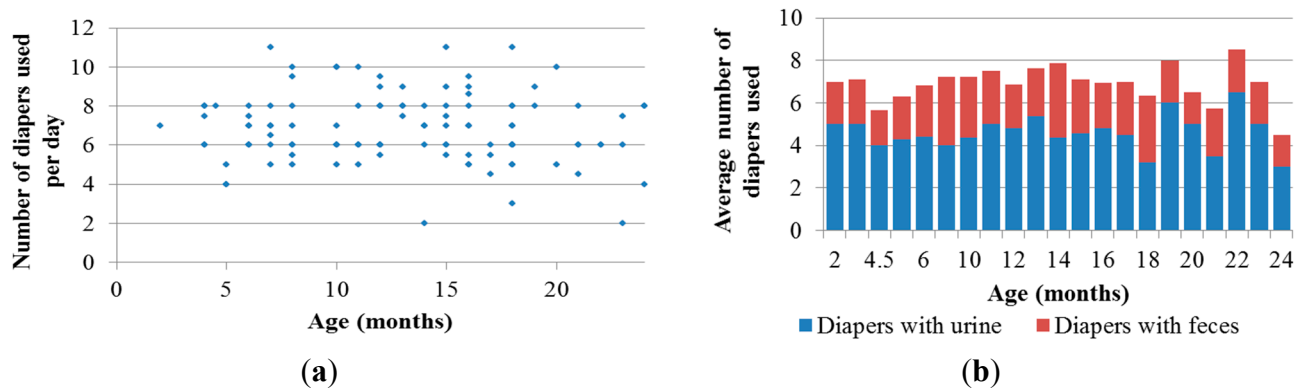
The average mass and standard deviation of the ten brands of baby diapers studied was  $29.1 \pm 3.4$  g. Figure 3 presents the average composition of baby diapers in weight percent (% w); with cellulose representing 50.2% w, superabsorbent polymer 15.5% w and plastic components (*i.e.*, backsheet, elastics, non-woven fabric, adhesives and topsheet) 33.0% w. Although, the composition was calculated with diapers locally bought, the content of cellulose pulp presents a relatively small standard deviation, 7.5% (Figure 2). Additionally, due to globalization many diaper brands are sold in other regions of the world.



**Figure 3.** Average composition of baby diapers (in weight percent) available in the Mexican market.

### 3.2. Determination of Generation of Diaper Waste with Urine per Child

Of the 600 surveys delivered in baby daycare facilities, only 122 were returned and 116 were correctly answered. Figure 4 presents the number of diapers used per day according to the age of children and the average number of diapers with urine. On average a child produces 6.9 diapers per day, 4.6 of these contain only urine.



**Figure 4.** Number of diapers used per day according to the age of children (a) and the average number of diapers with urine (b).

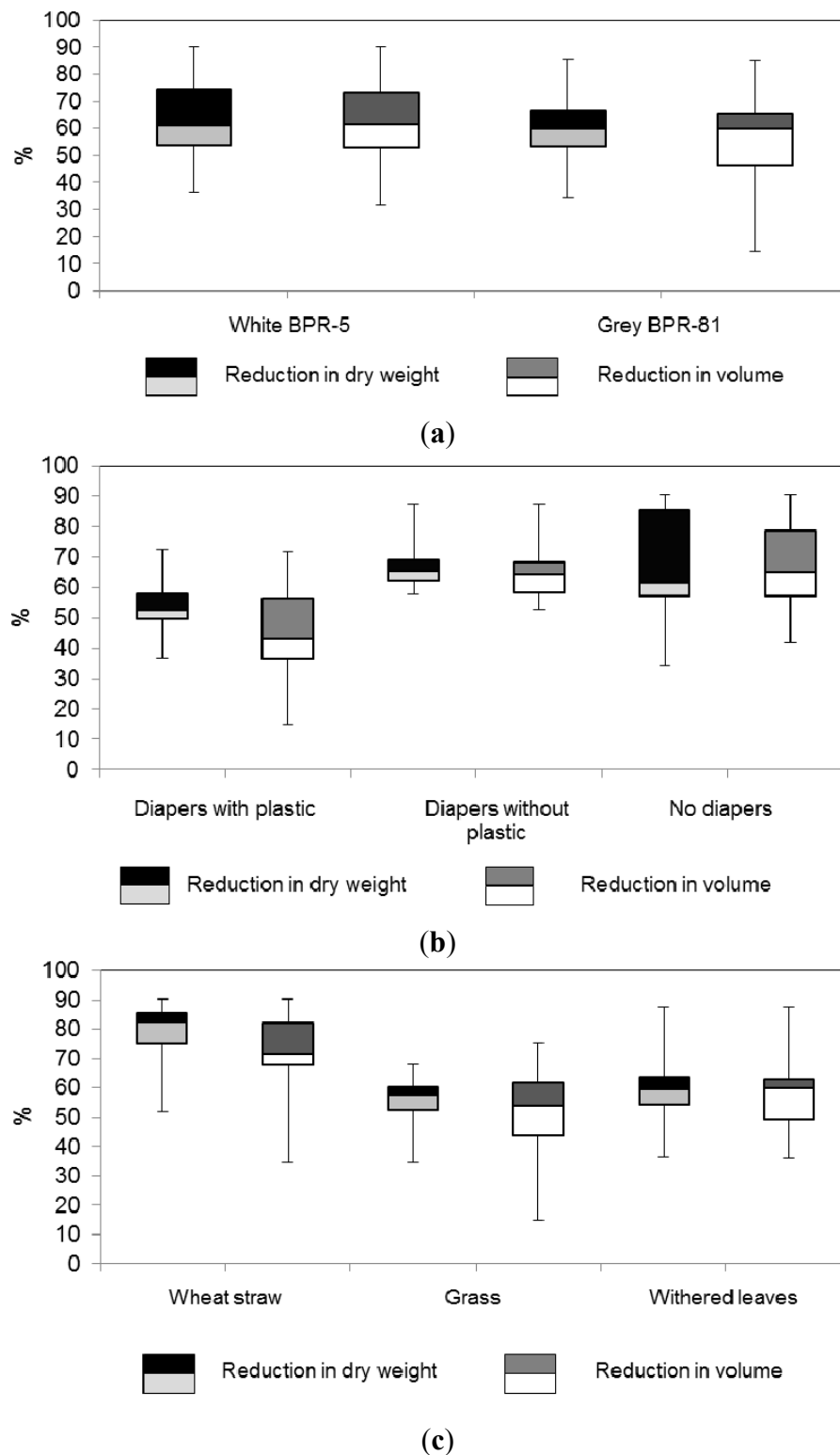
In total 236 kg of diapers with urine were collected, the average weight of diapers for children from 45 days to 12 months of age was  $89.5 \pm 20.07$  g, and for children from 12 to 24 months of age was  $119.0 \pm 24.6$  g. Average weight of diapers with urine, including children of all ages, was 101.7 g. Considering this, a child produces on average 15.3 kg of diapers with urine per month. In comparison, Colon *et al.* [4] estimated with data from United States of America and Spain a generation of 37.98 kg/child/week of diapers with urine and feces.

### 3.3. Evaluation of the Degradation Process

Since experimental data were not normally distributed (Shapiro-Wilk test,  $p < 0.05$ ), the non-parametric Kruskal-Wallis test was used to analyze results. Figure 5 shows box plots of percentage of weight reduction and volume reduction according to *P. ostreatus* strain (Figure 5a), conditioning of substrates (Figure 5b) and co-substrates (Figure 5c).

For the response reduction of weight, the factors conditioning of substrate and co-substrate were found to have a statistically significant difference (Kruskal-Wallis,  $p < 0.05$ ). The Fisher's Least Significant Difference (LSD) test found three different groups for the medians for the factor substrate, diapers with plastic (median 56.7%), experimental units without diapers (median 66.0%) and diapers without plastic (median 72.3%). For the factor co-substrate the Fisher's LSD test found two groups for the medians of reduction of weight; the first group for grass (median 57.3%) and withered leaves (median 58.8%) and the second group for wheat straw (median 79.2%).





**Figure 5.** Box plots of percentage weight and volume reduction according to (a) *P. ostreatus* strain; (b) conditioning of substrate and (c) co-substrates.

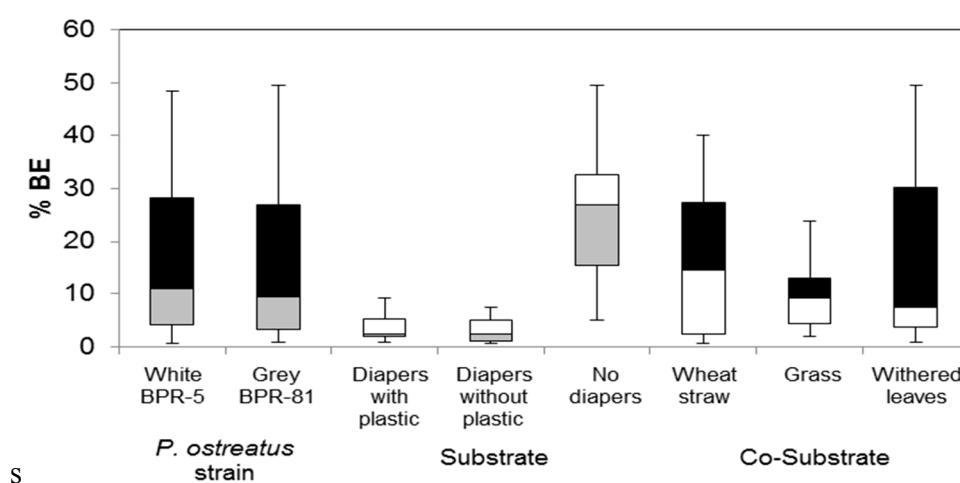
For the response reduction of volume, the factors strain of *P. ostreatus*, conditioning of substrate and co-substrate were found to have a statistically significant difference (Kruskal-Wallis,  $p < 0.05$ ). The Fisher's LSD test found two groups for the factor strain of *P. ostreatus*, white (median 57.6%) and grey (median 64.0%); two groups for the factor substrate, the first group for diapers with plastic (median



47.8%) and the second group for experimental units without diapers (median 65.2%) and diapers without plastic (median 69.4%); and two groups for co-substrate, the first group for grass (median 53.5%) and withered leaves (median 58.1%) and the second group for wheat straw (median 70.8%).

The Kruskal-Wallis test found a statistically significant difference ( $p < 0.05$ ) in the medians of biological efficiency for the factors substrate and co-substrate. Figure 6 shows box plots of percentage of biological efficiency for results grouped by the factors strain of *P. ostreatus*, conditioning of substrate and co-substrate.

The Fisher's LSD test found two groups for the factor substrate, the first group for diapers without plastic (median 2.6%) and diapers with plastic (median 3.8%) and the second group for experimental units without diapers (median 24.4%); and two groups for co-substrate, the first group for grass (median 2.9%) and wheat straw (median 7.4%) and the second group for withered leaves (median 15.3%).



**Figure 6.** Box plot of percentage biological efficiency according to *P. ostreatus* strain, conditioning of substrate and co-substrate.

#### 4. Discussion

This research investigated production of *P. ostreatus* as a biological treatment for used baby diapers, which currently are wasted in landfills. The focus was on finding alternative co-substrates easily available in urban areas. Results showed that content of cellulose in baby diapers was on average 50.2% by weight while plastic components make 33.0%. On average a child produces 4.6 diapers with urine per day, which in a month is 15.3 kg of diapers per day. This makes disposable diapers attractive for biomass recycling in cities.

Degradation of diapers was evaluated using as indicators reduction of weight and volume of substrates. Results showed that conditioning of substrate and co-substrate has an impact on the degradation of this waste. The use of diapers without plastic and wheat straw resulted in higher reduction of dry weight (65.36% without plastic and 82.37% wheat straw) and volume (64.13% without plastic and 71.7% wheat straw) of the substrate. However, considering that for diapers with plastic the median of weight and volume substrate reduction is 13.5% and 21.3% lower than with diapers with plastic, and that separation of plastic materials from diapers can be complex it should be considered to treat the diaper as a whole.

During biological treatment for diapers as a whole, plastic components will not be degraded. However, depending of the type of plastic and environmental conditions (humidity, temperature, oxygen concentration, and UV radiation), it is possible that plastics present some evidence of degradation such as discoloration and loss of mechanical properties. Santa Cruz-Navarro *et al.* [30] reported a reduction of elongation at break, an indicator of loss of mechanical properties, of 33%–70% for different types of plastic films embodied in substrates used in cultivation of *P. ostreatus*.

Although the co-substrate wheat straw showed a higher median reduction of weight and volume than other co-substrates, it is not highly available in cities. In the other hand, withered leaves could be easily obtained in cities. Although the grass is also extensively accessible in cities, this substrate makes difficult CO<sub>2</sub> exchange due to its tendency to stick together.

Biological efficiency, a parameter that indicates the mass of the substrate that is converted to mushroom, was low for all substrates. Low BE were caused by variations in the environmental conditions during the cultivation process [31,32]. Also, the median of BE was lower for experimental units with diapers (2.6% for diapers without plastic and 3.8% for diapers with plastic) than without diapers (24.4%). In comparison, Espinosa *et al.* [11] obtained a BE of 14% with a substrate of diapers without plastic and enriched with grape pomace, and Fernandes *et al.* [29] got a BE of 10.3% for white clean paper and 14.9% for printed paper. The focus of these works was on treating wastes with high cellulose content, not in increasing the yield of edible mushrooms. Nevertheless, the process could be optimized to increase BE.

## 5. Conclusions

The high values achieved for reduction of weight and volume of substrates indicates that biological treatment is an attractive treatment alternative for urban organic waste with high content of cellulose such as wheat straw, withered leaves and diapers. The substrate withered leaves has the advantages of being widely available in urban centers and presents similar values of biological efficiency than wheat straw, which is commonly used in commercial cultivation of *P. ostreatus*. In addition, biomass is recycled, producing high-quality protein for human consumption that is currently confined in landfills or burned.

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## Author Contributions

Rosa María Espinosa-Valdemar, Alethia Vázquez-Morillas and Sara Ojeda-Benítez conceived and designed the experiments; Xochitl Quecholac Piña performed the experiments; Xochitl Quecholac Piña, Maribel Velasco-Perez and Perla X. Sotelo Navarro analyzed the data; all authors contributed to the writing of the paper.

## Conflicts of Interest

The authors declare no conflict of interest.

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