Biodegradation of Bioplastic Carrier Bag Wastes by the White Rot-Fungus *Coriolus versicolor*

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Abstract

Increasing petroleum cost, legislative pressure and environmental concerns have induced to usage renewable resources in many industrial areas. Recently, replacement of non-biodegradable petroleum-based plastics by bioplastics is major interest. As a result, many countries around the world have begun to manufacture bioplastic products, including carrier bags. This study investigated the biodegradation of bioplastic carrier bag wastes by the white rot fungus *Coriolus versicolor* in suspended solid state fermentation. The total reducing sugar levels were determined by the dinitrosalicylic acid (DNS) method. Types of monomers of biodegradation bioplastic carrier bag wastes were determined by High-Performance Liquid Chromatography (HPLC). DNS sugar analyses results showed that total reducing sugar was very low compared with controls. HPLC analysis results showed that any type of sugar was not detected. All results showed that white rot fungus *C. versicolor* was not grown on the bioplastic carrier bag wastes.

Key words: Biodegradation, bioplastic carrier bag, *Coriolus versicolor*, white-rot fungi.

1. Introduction

Plastic materials are currently considered very important materials due to their exceptional properties and performance over other materials such as metal and wood [1, 2]. However, plastics are non-biodegradable and therefore can remain as waste in the environment for a very long time; it may pose risks to human health as well as the environment. Plastics also have caused extensive environmental problems associated with their disposal. Although recycling is an environmentally attractive solution, only a small percentage of plastics are actually recyclable, and most end up in municipal landfills [3].

The ever-growing environmental problems and concerns, rising petroleum costs and legislations have prompted research on an alternate material that can replace with plastics. As a result, 'bioplastics' have developed and entered to industry. However, the bioplastics that are currently being used for manufacturing industrial and day-to-day products, including carrier bags [4].

'Bioplastics' are made from renewable resources such as corn, sugars, potatoes, etc., [5, 6] or they are produced by a range of microorganisms [7]. Polyhydroxyalkanoate (PHA) is a type of bioplastic [8] and synthesized by numerous microorganisms as a carbon and energy source under conditions of limiting nutrients in the presence of excess carbon source [9].

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One of the most important properties of PHAs is their complete microbial biodegradability to carbon dioxide, water and energy without any toxic byproducts [10, 11]. PHA degradation has been recorded in nature and attributed mainly to bacteria [12, 13].Although fungi, the other potent group of the degrading microbiota, may play an important role in PHA breakdown [11].

One the other hand, white rot fungi are able to degrade a wide variety of aromatic compounds, through the production of lignin peroxidases (LiP), manganese peroxidases (MnP), and cellulases. In addition, some white rot fungi can produce amylases together with other ligninolytic enzymes that support the degradation of a wide range of aromatic compounds [14, 15, 16].

The main objective of the present study was to investigate the biodegradation of bioplastic carrier bag wastes by the white rot-fungus *Coriolus versicolor*. In this study, biodegradation of bioplastic carrier bag wastes also compared with petroleum based plastic carrier bag waste.

2. Materials and Method

2.1. Sample preparation

Commercial bioplastic carrier bag (BCB) that professed to be a PHA bioplastic was purchased from an electronically supermarket located in Mersin, Turkey. Bioplastic carrier bag was cleaned up after using for a while and divided into small pieces about equal size for further use (Figure 1).

Figure 1: Pieces of bioplastic carrier bag wastes

For the purpose of comparing with bioplastic carrier bag wastes, petroleum based carrier bag purchased from a supermarket located in Mersin was used for a while, cleaned up and divided into small pieces about equal size for further use.

2.2. Fungal inoculum and media preparation

C. versicolor was incubated in petri dishes which contain potato dextrose agar (Merck) at static conditions for 7 days. At the end of the incubation, *C. versicolor* was inoculated into flasks containing Yeast Malt Broth (Difco) shaken at 150 rpm, and incubated at 30 ^{0}C. The content of the flask was filtered, washed twice with sterilized 0,009% NaCl (Sigma- Aldrich) solution and washed twice with sterilized distilled water. Washed *C. versicolor* biomass was homogenized at 13500 rpm for 30 seconds in sterile conditions for use as inoculum in suspended-solid state experiments.

For further studies, carbon-deficient basal medium [17] was prepared in pH 5.0 phosphate buffer and contain, as micronutrients 0.3 g/L $MgSO_4.7H_2O$; 0.3 g/L $CaCl_2.2H_2O$; 0.005 g/L $FeSO_4.7H_2O$; 0.0016 g/L $MnSO_4.2H_2O$; 0.0014 g/L $ZnSO_4.7H_2O$.

2.3. Fungal biomass calculation

Homogenized *C. versicolor* was then oven dried at 80 ^{0}C for 24 h, equilibrated in a desiccator to room temperature and measured gravimetrically. It was reported that 1 L of mycelium suspension of *C. versicolor* was at 7,1 ± 1.50 g dry-weight mass.

2.4. Fermentation experiment preparation

In this research, suspended solid state fermentation (SuSF) conditions were prepared to bioplastic carrier bag wastes by *C. versicolor* to determine the potential for biodegradation.

Suspended solid state fermentation experiment was performed by adding 2 g bioplastic carrier bag wastes pieces and 50 mL carbon-deficient basal medium to 250 mL flasks. For the purpose of comparing with bioplastic carrier bag wastes, another flask was added with 2 g petroleum based plastic wastes pieces. The flasks were sterilized at 121 ^{0}C for 20 min. and each flask was inoculated with homogenized 4 mL *C. versicolor*.

Control experiments were performed in 250 mL flasks. First flask was added with 2 g bioplastic carrier bag wastes pieces and 50 mL carbon deficient basal medium. Second and third flasks had 50 mL carbon deficient basal medium added. For the purpose of providing the same condition with SuSF experiments, all control flasks were sterilized at 121 ^{0}C for 20 min. After sterilization, the second flask was inoculated with 4 mL *C. versicolor* mycelium suspension.

All essays were performed in triplicate. The experimental and control flasks were incubated at 24 ^{0}C during 35 days. The content of all experiments are shown in Table 1.

Table 1. Content of Experiment Flasks

Flask Name	Content of material	Amount of Carbon	Inoculated *C.*

	type and amount (g)	deficient Medium (mL)	*versicolor* (7,1 ± 1.50 g dry mass/L)
Experiment flask 1 (E1)	Bioplastic carrier bag wastes pieces- 2 g	50	4
Experiment flask 2 (E2)	Petroleum based plastic wastes pieces-2 g	50	4
Control flask 1 (C1)	Bioplastic carrier bag wastes pieces-2 g	50	-
Control flask 2 (C2)	-	50	4
Control flask 3 (C3)	-	50	-

2.5. Chemical analysis

All flasks of supernatant were harvested and transferred into centrifuge bottles (15 mL) at the 35th day. After centrifuging at 15000 rpm for 20 min, the supernatant was filtered and used for determine to reducing sugar content. The reducing sugar content was determined by the dinitrosalicylic acid (DNS) method [18], using a spectrophotometer (Hach Lange/DR 3900) at 575 nm. The absorbance readings were then converted into equivalent sugar concentration (g/L) using a standard glucose solution curve (Figure 2).

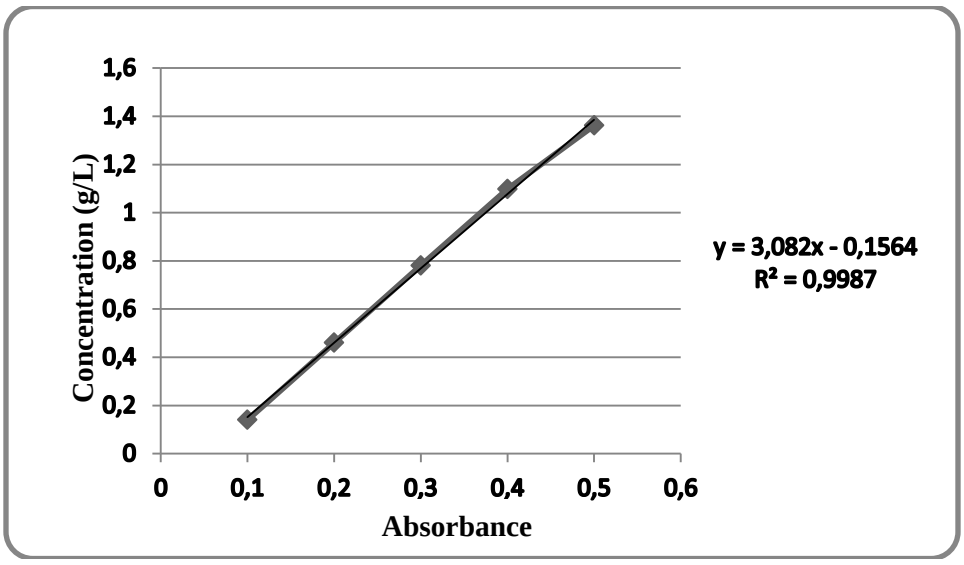

Figure 2: Standard Glucose Solution Curve

2.6. Analytical analysis

All experiment flasks of supernatant were also used for High Performance Liquid Chromatography (HPLC). Control flasks of supernatant were also used for HPLC analysis.

The type of sugar were determined by HPLC (Agilent 1200), using a carbohydrate column (Zorbax- carbohydrate; 4.6 x 250 mm 5µ,) and a refractive index detector, the column temperature was 30 ^{0}C and injection volume was 10 µL. 75 Acetonitrile/ 25 deionized water was used as the mobile phase at a flow rate of 1.4 mL/min. Quantification and identification of peaks were performed using solutions of glucose, arabinose and fructose stock standards.

3. Results

3.1. Fungal Growth

After *C. versicolor* was inoculated into flasks containing Yeast Malt Broth, a dense growth of mycelia was observed in the broth at the end of the ten days (Figure 3).

Figure 3. Growth of *C. versicolor* in Yeast Malt Broth

During the incubation period, *C. versicolor* was not grown in any flask, even containing bioplastic carrier bag wastes pieces (Figure 4).

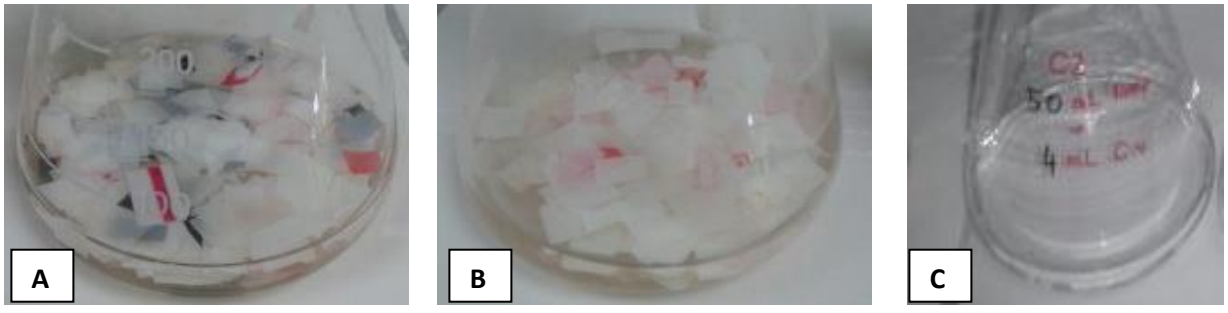

Figure 4. (A) *C. versicolor* in E1 flask; (B) in E2 flask; (C) in C2 flask at the end of the incubation (35th day)

3.2. Chemical analysis

DNS results showed that all experiment and control flasks was contain range of 0, 52-0, 58 g/L total reducing sugar. DNS method of experiments results are shown in Table 2.

Table 2. DNS Results of Experiment and Control Flasks

Flask Name	Total reducing sugar (g/L)
E1	0,58
E2	0,54
C1	0,52
C2	0,52
C3	0,52

3.3. Analytical Analysis

It's expected that any type of sugar was not detected by the HPLC analysis on the control flasks or experiment flasks (Figure 5).

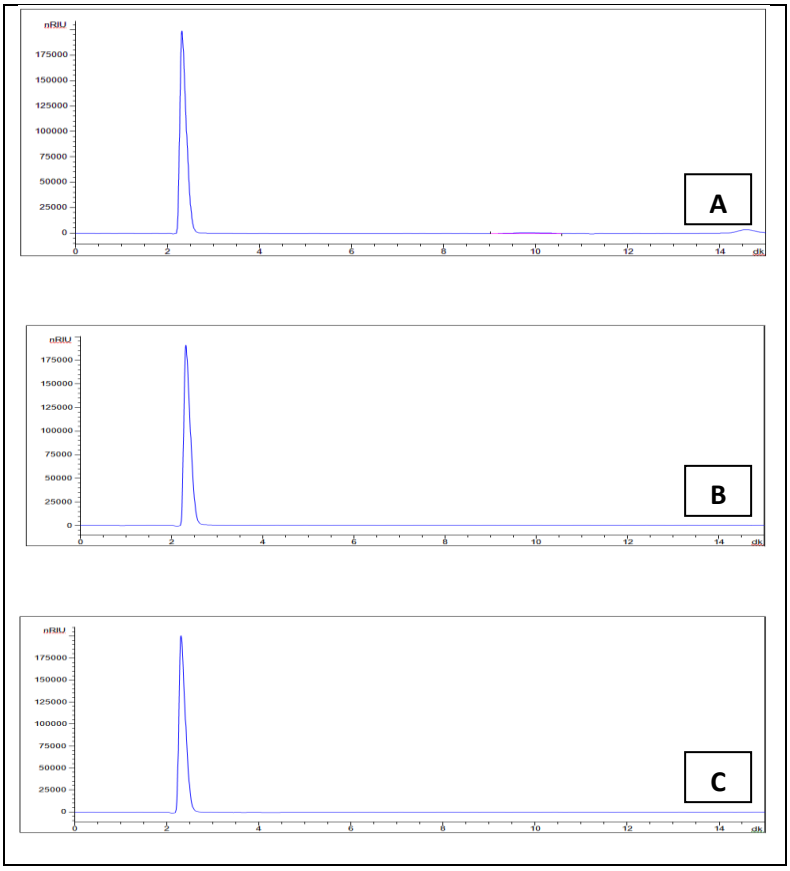

Figure 5. Results of HPLC Analysis of (A) E1 flask (B) E2 flask (C) C1 and C2 flask

4. Discussion

Whereas *C. versicolor* was grown in Yeast malt broth, *C. versicolor* was not grow on neither bioplastic carrier bag wastes pieces or petroleum-based plastic wastes pieces. Our preliminary work of bioplastic biodegradation by *C. versicolor* results showed that *C. versicolor* was grown on bioplastic [19]. For the full growth of microorganisms, the macro elements like carbon are required [20]. Therefore, fungal growth, HPLC and DNS results supported that bioplastic carrier bag used in this study may not contain macro elements like carbon.

Conclusions

In the future, bioplastics can replace to common plastics both in the Turkey and in the world. Some industries have been used to 'bioplastic' name for their packaging materials without following any legislation or standard in Turkey. If every called of 'bioplastics' material were to be released into the environment, they could have some of the same effects on the environment that petrochemical based plastics currently have. Therefore, for the sustainability new guide should develop for bioplastic usage and labeling as soon as possible.

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