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INVESTIGATION OF FUNGAL BIODEGRADATION OF STARCH BASED BIOPLASTIC SPOON WASTES

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Keywords

Bioplastic,
Biopolymer,
Coriolus versicolor,
Starch,
White rot fungus.

Abstract

Recently, environmental and economic concerns have risen due to excessive and unconscious consumption of traditional plastics. These concerns have led to the development of environmentally friendly plastics produced from renewable raw materials called "bioplastic". Recent studies have focused mainly on physical, mechanical characteristics and reduction in the cost. It was expected that bioplastics can be completely biodegraded in nature because of being called biodegradable. On the other hand, there are few studies on the biodegradability of bioplastics in the literature. In this study, one of the most consumed type starch-based bioplastics spoon wastes biodegradability was investigated with the fungus. For this purpose, the white rot fungus *Coriolus versicolor* and the starch-based bioplastic spoon wastes were incubated for 93 days under suspended solid fermentation conditions. Results of reduced sugar analyses showed that the reducing sugar was increased because of fungal attack on starch-based bioplastics and decreased by fungi because of using of these sugars for growth. The results of HPLC as the glucose content indicated that, starch in the structure of bioplastics were biodegraded to glucose. Weight loss analysis showed that starch-based bioplastic spoon waste was 20% biodegraded by *C. versicolor* in 93 days under suspended solid fermentation conditions.

NİŞASTA ESASLI BİYOPLASTİK KAŞIK ATIKLARININ FUNGAL BİYOLOJİK PARÇALANABİLİRLİĞİNİN ARAŞTIRILMASI

Anahtar Kelimeler

Beyaz çürükçül fungus,
Biyoplastik,
Biyopolimer,
Coriolus versicolor,
Nişasta.

Öz

Son zamanlarda, petrol esaslı plastiklerin aşırı ve bilinçsiz tüketimi nedeni ile çevresel ve ekonomik endişeler ortaya çıkmıştır. Bu endişeler, "biyoplastik" adı verilen ve yenilenebilir ham maddelerden üretilen çevre dostu plastiklerin geliştirilmesine imkân tanımıştır. Son yıllarda yapılan çalışmalar, biyoplastiğin mekanik ve fiziksel özelliklerinin geliştirilmesine ve maliyetinin azaltılmasına odaklanmış durumdadır. Biyolojik parçalanabilir olarak adlandırılması nedeni ile biyoplastiklerin doğada tamamen parçalanabilmesi beklenmektedir. Ancak literatürde, biyoplastiklerin biyolojik parçalanabilirliğine dair az sayıda çalışma mevcuttur. Bu çalışmada, bakterilerden daha dayanıklı olduğu bilinen funguslarla, en çok tüketilen biyoplastik türlerinden biri olan nişasta esaslı biyoplastik atığının parçalanabilirliği araştırılmıştır. Bu amaçla, beyaz çürükçül fungus türü olan *Coriolus versicolor* ile nişasta esaslı biyoplastik kaşık atığı, yarı katı fermantasyon koşullarında 93 gün boyunca inkübe edilmiştir. Toplam redükte şeker analizi sonuçları, toplam redükte şekerin fungusların nişasta esaslı biyoplastiği parçalaması ile arttığını, parçalama sonucunda oluşan şekerlerin fungus tarafından kullanılması ile de azaldığını göstermiştir. HPLC analiz sonuçları, *C. versicolor*'un nişasta esaslı biyoplastik kaşık atığının yapısındaki nişastayı, glikoza parçaladığını göstermiştir. Kütle kaybı analizleri ise *C. versicolor*'un yarı katı fermantasyon koşulları altında 93 günde nişasta esaslı biyoplastik kaşık atığının %20'sini parçalayabildiğini göstermiştir.

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1. Introduction

According to the Association of Plastics Manufacturers in Europe, 335 million tons of plastics are produced in 2016 (Wang et. al., 2018). They have been used in a various industrial area because of their cheap, durable and light features. In the worldwide, the total volume of plastics produced has surpassed to steel. Therefore, without a doubt, humanity have lived the Age of Plastics (Stevens, 2002; Iskandar, 2011)

Mostly using oil, coal, and natural gas as raw materials for plastic's manufacturing (Di Gregorio, 2009). They are flexible materials and used wide range of applications, from simple packaging to complex engineering (Philp et. al., 2013a). Due to plastics are used in a wide range of applications economic and environmental problems has raised. On the other hand, in recent years, for reduce to the dependence and consumption of petrochemical resources and decrease to the environmental pollution, there has been a great interest to utilize renewable biomass in the manufacture (Gonzalez-Gutierrez et. al., 2009). One of the most innovative manufactured materials is "bioplastic" considered as renewable biomass originated plastic.

2. Scientific Literature Research

Generally, 'Bioplastics' are made from renewable resources such as corn, sugars, potatoes, etc., (Sarasa et. al., 2008; Karana et. al., 2012) including proteins, lipids and polysaccharides (Averous, 2004; Siracusa et. al., 2008) or they are produced by a range of microorganisms (Luengo et. al., 2003) under certain conditions. Bioplastics are novel materials of the twenty-first century and would be of great importance to the materials world (Mohanty et. al., 2002). Many countries around the world have already begun to integrate bioplastic into their markets, especially in the packaging industry (Stevens, 2002). They generally use for short service life applications such as spoon, tableware, fork and knife or edible films in food industry, personal-care disposals, bags and bone plates and screws in medical fields, agricultural goods such as mulch films, and golf tees (www.european-bioplastics.org). They have also been used even automotive parts and building/construction (Song et al., 2009) where durability is important.

Global bioplastics production capacity has been increasing from around 2.05 million tons in 2017 to approximately 2.44 million tons in 2022 (https://www.european-bioplastics.org/market/). It showed that the bioplastic production and consumption will grow bigger in the future.

Recent studies have focused mainly on physical, mechanical characteristics and reduction in the cost. Recently, to improve the plastic behavior bioplastics, commercial starch-based plastics are chemically modified or blended with synthetic polymers (Sagnelli et. al., 2016). The result is an increasingly diverse range of bioplastics that make it difficult to define any unifying characteristics (Philp et. al., 2013b). It was expected that bioplastics can be completely biodegraded in nature because of being bio-origin. On the other hand, there are few studies in the literature on the biodegradability of bioplastics. Therefore, these materials need to be evaluated carefully for sustainability.

Coriolus versicolor (also known as *Trametes versicolor*), is taxonomically a member of the phylum Basidiomycota, order Polyporales, and family Polyporaceae (Hsu et. al., 2013). It is a unique microorganism that has a widespread host and act as great carbon recycler in the natural forest ecosystems (Karim et. al., 2017). Also, *C. versicolor* is a member of white rot fungi and they are able to degrade a wide variety of aromatic compounds, through the production of lignin peroxidases (LiP), manganese peroxidases (MnP), and cellulases. Some white rot fungi can produce amylases together with other ligninolytic enzymes that support the degradation of a wide range of aromatic compounds (Hatakka, 1994; Andersson et. al., 2000; Levin et. al., 2005; Singh et. al., 2013). Therefore, it is thought that White rot fungus *C. versicolor* can be a good candidate for biodegradation of starch-based bioplastics.

In this study, fungal biodegradability of starch-based bioplastic wastes by the *Coriolus versicolor* was investigated under suspended solid fermentation conditions.

3. Materials and Methods

3.1. Starch- Based Bioplastic Wastes

The starch-based bioplastic spoons (Conserve, Made in China) (Figure 1A) were purchased from www.amazon.com. Starch-based bioplastic spoons wastes (BSW) were cleaned up after using for a while

and divided into small pieces about equal size for further use (Figure 1B).

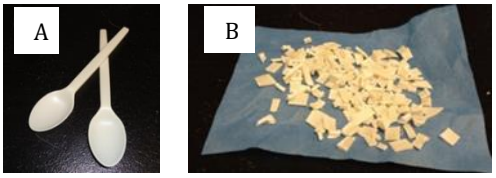

Figure 1. A) Starch based bioplastic spoons; **B)** Granulated starch based bioplastic spoon wastes

3.2. Fungal Strain and Media Preparation

White rot fungus *Coriolus versicolor* (ATCC #200801) was obtained from Environmental Biotechnology Laboratory, Environmental Engineering Department, Mersin University, Turkey. Fungal spores maintained on potato dextrose agar (PDA, Merck) at 4 ^{0}C and re-cultured every 15 days.

For further use, carbon deficient stock mineral medium (SMM) 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 $MnSO_4.2H2O$; 0.0014 g/L $ZnSO_4.7H_2O$ (Kirk et al., 1978).

Iodine solution was prepared also for further use. For this purpose, 0.5 g potassium iodide and 1 g iodine were added into volumetric flask and dissolved in 100 mL distilled water.

3.3. Fungus ability of degrade to starch

It is known that starch is composed of macromolecular component, α- amylose and β-amylose (Be Miller and Whistler, 2009; Bertolini, 2010). β- amylose reacts with iodine forming a deep blue color (Patnaik, 2010). For the ability of *C. versicolor* degrade to starch, fungus was inoculated to containing 1% soluble starch solution and agar-agar petri dishes. These petri dishes incubated at 30 ^{0}C for 10-15 days at static conditions (Jo et. al., 2010) for growth. After a dense growth of mycelia was formed in petri dishes, iodine solution was added to petri dishes for observing deep blue color.

3.4. Biodegradation Experiments

3.4.1. Preparation of fungal inoculum

C. versicolor spores were harvested from petri dishes and inoculated into 250 mL Erlenmeyer flasks containing 100 mL Yeast Malt Broth (YMB, Difco). Inoculated flasks were incubated aerobically by shaking at 30 ^{0}C and 150 rpm for 7 d. Then, *C. versicolor* was filtered from YMB and washed with 0.9% NaCl solution for removal YMB and then washed sterilized distilled water for removal NaCl solution. After removal from YMB and NaCl solution, fungal biomass was homogenized at 13500 rpm for 30 seconds for use as inoculum for biodegradation studies.

For dry cell weight determination, homogenized *C. versicolor* biomass was oven dried at 60 ^{0}C for 24 h, equilibrated in a desiccator to room temperature and measured gravimetrically (Liao, 1990). It was reported that 1 mL of mycelium suspension of *C. versicolor* was 11.60 ± 1.50 mg dry-weight mass.

3.4.2. Fermentation experiments

Suspended solid state fermentation (SuSF) experiments (Sample No 1) were performed by adding 0.4 g granulated starch-based BSW and 25 mL carbon deficient SMM into 250 mL erlenmayer flasks. Then, flask was sterilized at 121 ^{0}C for 20 min. and inoculated with homogenized 1 mL *C. versicolor* mycelium of the suspension.

For control experiments (Sample No 2, 3, 4), 250 mL erlenmayer flasks were added with 25 mL carbon deficient SMM. One of the flasks (Sample No 2) was added with just 0.4 g granulated starch-based BSW and then all flasks were sterilized at 121 ^{0}C for 20 min for the purpose of providing the same condition with SuSF sample (Sample No 1). After sterilization, one of the flasks (Sample No 3) was inoculated with 1 mL *C. versicolor* mycelium suspension.

All experiments were performed in triplicate. The experimental samples were incubated at 30 ^{0}C for 93 days. The overall experimental contents are shown in Table 1.

Table 1. Content of experimental samples

Sample No	Amount of starch-based BSW (g)	Inoculated fungus (11,60± 1,50 mg dry mass/mL)	SMM (mL)
1	0.4	1.0	25. 0
2	0.4	NI	25.0
3	NA	1.0	25. 0
4	NA	NI	25. 0

NI: Not Inoculated; NA: Not Added

3.4.3. Total reducing sugar analysis

Brown rot fungus *C. versicolor* can produce α-amylase (Huang and Zhang, 2011) and when starch degrades by this enzyme, sugars are formed (Sarikaya et al., 2000). Therefore, in this study total reducing sugar analysis was performed. For this purpose, supernatant of all samples were harvested and transferred into centrifuge bottles (15 mL) daily. Following the harvesting, sterilized carbon deficient SMM were added to the amount of collecting supernatant to

flasks at sterile conditions for the purpose of ensuring SuSF conditions. After centrifuging at 15000 rpm for 20 min, the supernatant was filtered and used for determine to total reducing sugar content. The total reducing sugar content was determined by the dinitrosalicylic acid (DNS) method (Miller, 1959), using a spectrophotometer (Hach/DR 2010) at 575 nm. The absorbance readings were then converted into equivalent sugar concentration (g/L) using a standard glucose solution curve.

3.4.4. Analytical methods

At the end of the incubation, supernatant of all samples were collected, centrifuged as mentioned before and filtered with syringe filter (0.2 µm) for High Performance Liquid Chromatography (HPLC). HPLC (Shimadzu) were used for determination types of sugars, using a Coregel-87H3 (Transgenomic) colon and a refractive index detector (Shimadzu RID-10A), the operating conditions were 70 atm and column temperature 85 0C. Acetonitrile/ deionized water (1/4, v: v) was used as the mobile phase at a flow rate of 0.5 mL/min. Quantification and identification of peaks were performed using solutions of glucose, galactose, fructose, and mannose standards.

3.4.5. Weight loss of starch-based bioplastic spoon wastes

At the end of the incubation, Sample No 1 (Inoculated with 1 mL *C. versicolor*, 0.4 g BSW, 25 mL SMM) and 2 (Not Inoculated with *C. versicolor*, 0.4 g BSW, 25 mL SMM) were filtered and starch-based BSW were separated from the fungal biomass to determine the loss of biodegradable starch-based BSW mass. Then, Starch-based BSW was dried at 60 ^{0}C for 24 h and equilibrated to room temperature in a desiccator before weighing. At the end of the 24 hours, starch-based BSW was measured gravimetrically. Rate of biodegradation was calculated by the following equation 1 (Ismail et al., 2016).

$$WL\ (\%) = [(W_0\text{-}W) / W_0] \times 100 \quad (1)$$

In equation 1, W_0 and W, is the initial and final weight of bioplastic samples, respectively. Also WL refers to Weight Loss.

4. Results and Discussions

4.1. Fungus Ability of Degrade to Starch

After adding iodine solution, colorless and deep blue zones were observed in the petri dishes (Figure 2).

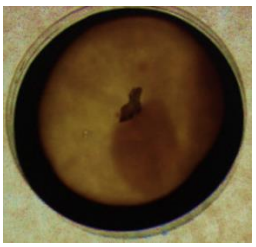

Figure 2. After adding iodine solution to *C. versicolor* solid culture medium containing 1% soluble starch

The blue zone indicated the presence of starch and the colorless zone indicated the absence of starch.

A large colorless zone was observed *C. versicolor* which grown in petri dishes. Therefore, this colorless zone showed that *C. versicolor* was produced the enzymes or enzyme groups for degrade of starch as a carbon source. So, it was concluded that *C. versicolor* was the capable of degrade to the starch**.**

4.2. Fungal Growth

After 10 days of the inoculation, Sample No 1 (Inoculated with 1 mL *C. versicolor*, 0.4 g BSW, 25 mL SMM) flask's surface was covered by C. versicolor biomass (Figure 3A). It is indicated that starch-based BSW was used as a carbon source by the fungus. On the other hand, no fungal growth was observed in Sample No 3 (Inoculated with 1 mL *C. versicolor*, 25 mL SMM) as expected (Figure 3B). It was also supported that SMM were not contain any carbon source.

Figure 3. *C. versicolor* growth in **A)** Sample No 1; **B)** Sample No 3

4.3. Total Reducing Sugar Analysis Results

As expected, Sample No 2 (Not Inoculated with *C. versicolor*, 0.4 g BSW, 25 mL SMM), 3 (Inoculated with 1 mL *C. versicolor*, Not added BSW, 25 mL SMM) and 4 (just 25 mL SMM) of total reducing sugar concentration were constant and zero during the incubation time. For the Sample No 1 (Inoculated with 1 mL *C. versicolor*, 0.4 g BSW, 25 mL SMM), total reducing sugar analysis results showed that the highest peak of total reducing sugar concentration was determined at 14th day (1.22 g/L), 51th day (1.33 g/L) and 56th day (1.42 g/L), respectively. Total reducing sugar of Sample No 1 (Inoculated with 1 mL *C. versicolor*, 0.4 g BSW, 25 mL SMM) time-dependent changes results are shown in Figure 4.

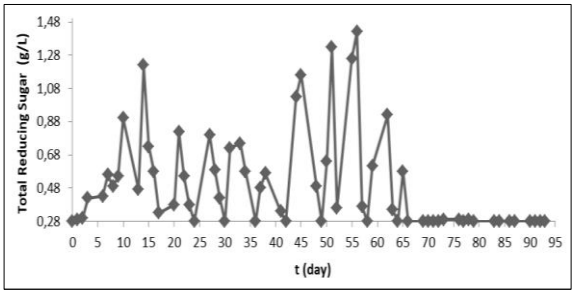

Figure 4. Time dependent changes in total reducing sugar analyses results of Sample No 1

On the other hand, Sample No 1 (Inoculated with 1 mL *C. versicolor*, 0.4 g BSW, 25 mL SMM) contained 16 g BSW /L SMM at the beginning of the incubation period. But the highest concentration of total reducing sugar was 1.42 g/L (at 56th day) Therefore, it is thought that all starch-based BSW was not fully biodegraded during the incubation period.

Also, it was determined that total reducing sugar concentration was increased and decreased until 65th day. It was thought that total reducing sugar concentration was increased due to fungal biodegradation of starch into BSW. Then, total reducing sugar concentration was decreased due to the usage of sugars for fungal growth. Between 65 and 93th day, total reducing sugar concentration was constant and lower than before. In these weeks, fungus may be stressed due to decreasing oxygen level and carbon source concentration.

4.4. The Results of HPLC as The Glucose Content

For Sample No 2 (Not Inoculated with *C. versicolor*, 0.4 g BSW, 25 mL SMM), 3 (Inoculated with 1 mL *C. versicolor*, Not added BSW, 25 mL SMM) and 4 (just 25 mL SMM) (control samples), any type of sugar was not be detected by the HPLC. It showed that any physical or chemical events such as sterilization and incubation temperature were not affected to the structure of starch-based BSW.

On the other hand, glucose was determined at the end of the incubation (at 93th day) on Sample No 1 (Inoculated with 1 mL *C. versicolor*, 0.4 g BSW, 25 mL SMM) (Figure 5). Starch is a natural polymer, the monomeric unit of which is glucose (Mohameed, 2006). The results of HPLC as the glucose content showed that starch into BSW biodegraded to the monomeric units of glucose by *C. versicolor.* The other hexoses of starch (fructose, mannose and galactose) weren't determined in the Sample No 1. These types of hexoses could be formed in another day of the incubation and used by the *C. versicolor*.

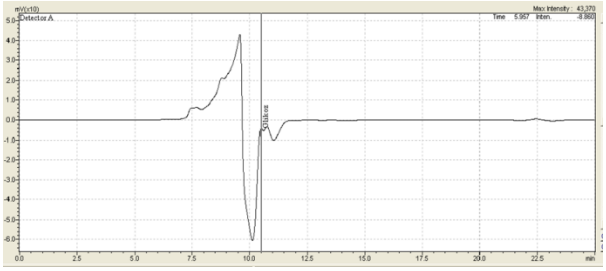

Figure 5. Glucose determined by HPLC for Sample No 1

4.5. Results of Weight Loss

Weight loss calculations results showed that the control Sample No 2 (Not Inoculated with *C. versicolor*, 0.4 g BSW, 25 mL SMM) had no weight losses as expected. For Sample No 1(Inoculated with 1 mL *C. versicolor*, 0.4 g BSW, 25 mL SMM), weight loses of BSW are shown in Table 2. The results showed that the total WL of starch-based BSW was about 20% for Sample No 1 and just this part of starch-based BSW was biodegraded by *C. versicolor* in 93 days.

Table 2. Weight Loss of starch-based BSW for Sample No 1

Sample No	W_0 (g)	W (g)	Total loss of BSW mass (g)
1	0.4000	0.3197	0.0803

On the other hand, biodegradability depended on the starch proportion (Guohua et al., 2006). But the starch content of BSW was not specified on the product. Therefore, weight loses of starch-based BWS was indicated that starch proportion into BSW could be lower such as 20%.

5. Conclusion

According to our results, it was found that the starch based bioplastic spoon wastes was partially biodegraded by *C. versicolor* under suspended solid fermentation conditions. It was also concluded that *C. versicolor* biodegraded to starch into bioplastic spoon waste to glucose. Although it is called biodegradable and it is served in this way, it was not completely biodegraded in 3 months in controlled conditions. Furthermore, natural conditions could be more difficult than laboratory conditions. Therefore, it was thought that biodegradability of this bioplastic could be more difficult and longer in nature. Also, biodegradability studies should perform with different bioplastic commercial products and different microorganisms. Consequently, to be called biodegradable, some tests and standards must be improved or reformed to the production and servicing.

Conflict of Interest

There is no conflict of interest.

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