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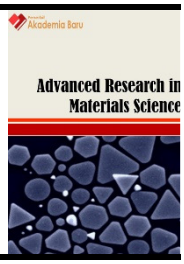

Development of mushroom-based film from waste and its role in mycoremediation

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ABSTRACT

Recently, extensive attention has been paid on the management of environmental pollution caused by hazardous pollutants such as heavy metals and various xenobiotic compounds. The objective of this study is to develop biodegradable film from mushroom waste of Pleurotus sajor-caju and to investigate the ability of the biodegradable film in biosorption of pollutant through mycoremediation. The strength of the film was determined using the Universal Tensile Machine by comparing the biodegradable film with a control film and synthetic plastic. In the soil burial degradation test, the intensity of degradation was tested for all three types of film and the biodegradable film degraded at a rapid rate which is 100% within 9 days compared to control film (68%) whilst the synthetic plastic degradation rate is the lowest (5%). The batch biosorption test was conducted according to optimum parameters including temperature, pH, contact time, speed and biomass weight. The present data demonstrates the suitability of film from Pleurotus sajor-caju waste as an efficient biosorbent for the removal of toxic heavy metal.

1. Introduction

Biological approaches based on industrial and environmental biotechnology is focusing on the development of "clean technologies" which emphasizes on the maximum production, reduced waste generation, treatment and conversion of waste in some useful form. These clean technologies focus on the use of biological methods for the remediation of waste. One such biological method is mycoremediation which is based on the use of fungi and mushroom for the removal of waste from the environment. Mushrooms and other fungi which consist of chitin enable it to be used for the degradation of waste pollutants and therefore, can be applied for a wide variety of pollutants. This had attracted research attention in the field of mushroom cultivation and waste remediation. Many

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reports have published to emphasize the role of mushroom in bioremediation of wastes by the process of biodegradation, biosorption and bioconversion [1,2].

Mycoremediation is a process of using fungi to return an environment contaminated by pollutants to a less contaminated state. Fungi are unique organisms due to their morphological, physiological and genetic features, also they are ubiquitous, able to colonize all matrices (soil, air, water) in natural environments, in which they play key roles in maintaining the ecosystems equilibrium. As a result of adaptation to their environment, fungi developed unique bioremediation properties [3]. Mycoremediation plays a pivotal role in breaking down numerous toxic substances like petroleum hydrocarbons, polychlorinated biphenyls, heavy metals (by biosorption), phenolic derivatives, persistent pesticides etc. Mushroom uses different methods to decontaminate polluted spots and stimulate the environment. These methods include biodegradation, biosorption and bioconversion [4].

Biosorption is considered as an alternative to the remediation of industrial effluents as well as the recovery of metals present in effluent. Biosorption techniques are now becoming very popular for the removal of pollutants. Biosorption is a process based on the sorption of metallic ions, pollutants or xenobiotics from effluent by live or dried biomass which often exhibits a marked tolerance towards metals and other adverse conditions [5]. Biosorbents can be prepared from mushroom mycelium and spent mushroom compost. In the case of biosorption, dead biomass of mushroom offers certain advantages over living cells. Dead mushroom biomass can be obtained from industries as a waste of fermentation processes. The uptake of xenobiotic by living cells depends on fungal species and contact time. Biosorption is an effective method due to the high uptake capacity and very cost effective source of the raw material. In the mushroom industry, a massive amount of the biowaste accumulated during mushroom production and harvesting, mainly consist of stalks and mushrooms of irregular dimensions and shapes. [6] claim that the amount of biowaste obtained can be up to approximately 50,000 metric tons of waste material per year. Waste accumulated during mushroom production and harvest consist mainly stalks and mushroom of irregular dimensions and shapes. Waste disposal create environmental problems for producers due to the large volume and volatile degradation products.

This study will examine the viability of using *Pleurotus sajor-caju* waste as a biosorption tool for the removal of toxic heavy metals. The development of this film able to provide a potential strategy for cost-effective and eco-friendly remediation of contaminated soils or water as well as a solution for accumulation of mushroom waste.

2. Methodology

2.1. Preparation of powder form of waste mushroom

Waste mushrooms such as the root, irregular shape and rotten mushrooms were collected from *Rumah Cendawan*, Politeknik Nilai. The waste mushrooms were then ground until the powder form was obtained.

2.2. Preparation of control film and mushroom-based biodegradable film

Firstly, 3ml sodium hydroxide (NaOH), 3ml hydrochloric acid (HCl), and 5ml acetic acid ($C_2H_4O_2$) solutions were prepared. Next, 10g starch powder and 5ml glycerin were weighed and measured respectively. After that, hot plate was warmed up to 215 ◦C. Subsequently, 60ml of water was added to the starch and stirred well. Then HCl, glycerin, acetic acid and NaOH was added. The mixture was heated slowly, until it started to become pasty. When it stopped boiling, the mixture was taken off

the heat. It was poured into a mould and spread. Three replicates were done. Approximately, 3 days was taken for it to dry. These steps were repeated to make biodegradable film from mushroom waste by adding 3g mushroom powder.

2.3. Soil burial degradation test

The biodegradable films were cut into 2.5cm x 2.5cm and weighed to measure the weight before the test. Then, the films were buried in 5cm depth. Water was sprinkled 2 days once. In 3 days of time interval, the specimens from the soil were taken and washed with distilled water. Later, the specimens were dried in oven at 60 ◦C and finally was weighed to measure after burial weight. This test was done for 3 weeks with 3 days of time interval.

2.4. Tensile strength properties

The tensile strength was tested using Universal Testing Machine where the biodegradable film was stretched with 5Kn static load cell. The films were cut into dumbbell shape with 15mm x 15mm gauge length.

2.5. Preparation of ammonium sulphate solutions

Stock solution of ammonium was prepared by dissolving 2.2g of ammonium sulphate in 1 L deionized water. Initial stock solution having concentration 1000 mg/L was subsequently diluted with appropriate amount of deionized water to get desired standard solutions. The resultant concentration of lead and ammonium ions in the biosorption experiment was determined using UV Spectrometer.

2.6. Batch biosorption experiment

The batch biosorption studies was conducted to investigate the effect of different factors on biosorption of metals onto the biosorbent and also to determine the metal uptake capacity of the biosorbent. Experiment was conducted at room temperature (25°C) on orbital shaker. The film was control by the thickness,weight and dimension. Then the samples was placed in a beaker, filtered and was left for about 30 minutes. The residual metal concentrations was analyzed by UV Spectrometer. The efficiency of the biosorption (E) was calculated using the following equation

$$E = (Ci – Cf) / Ci \times 100 \quad (1)$$

where, Ci is the initial and Cf is the final concentration of metal ions (mg / L) in the test samples.

3. Result

Starch was used as the base element to prepare the control film and starch-mushroom blend film in this study. Starch-mushroom blend films look more transparent with slight yellowish in color compared to the control film.

3.1. Biodegradability test

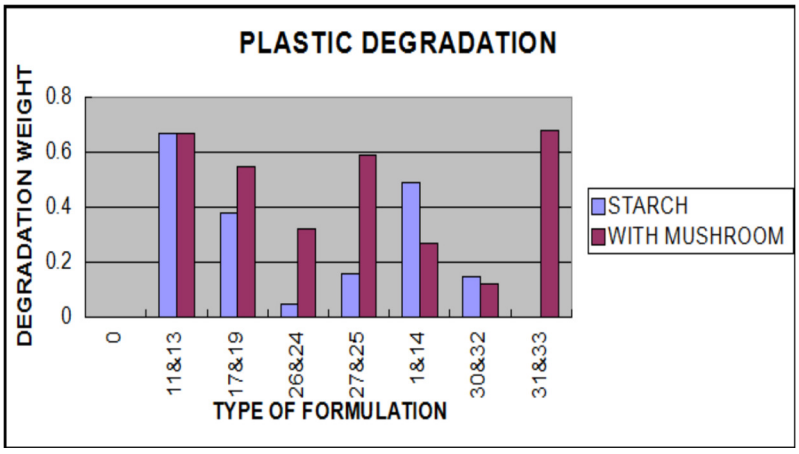

Fig. 1. Biodegradability Test (Soil Burial Method)

Biodegradability test for selected formulation of both starch-mushroom blend films shows Table 1 shows the density of films before and after soil burial test. The film started to degrade in 3 days and become darker in color. At day 9, degradation occurred rapidly and the films lost the actual shape. On day 12 the starch and mushroom-based film completely degraded except for the plastic.

Table 1
Rate of degradation

	Day	%	Days	%	Days	%	Days	%
	0		3		6		9	
Plastic	0.148	100	0.145	98	0.141	95	0.140	95
Mushroom-based film	0.662	100	0.541	82	0.428	65	0.000	0
Control	0.916	100	0.799	87	0.687	75	0.286	31

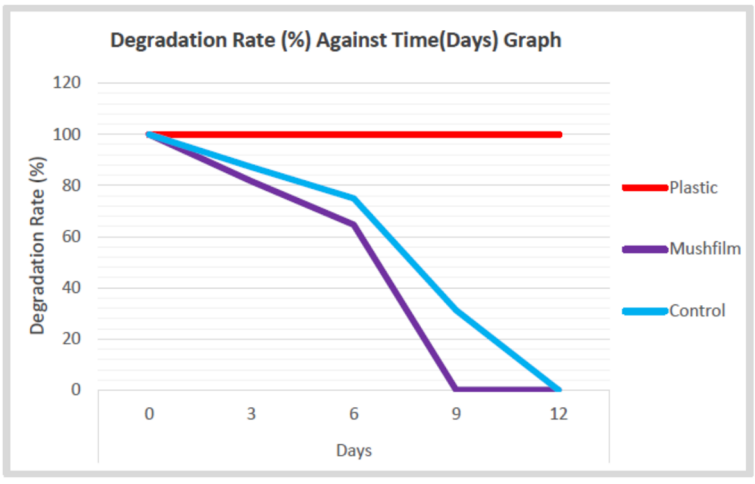

Fig. 2. Degradability Rate of Control Film and Starch-mushroom Films in the Soil Burial Test

Plastic does not show any degradation rate as its content does not have a proper biodegradable ability. The degradation development in soil of the biodegradable mushroom-based film during the experimental period was shown in Fig. 2. From the figure, it can be observe that the biodegradable

rate increase with the present of mushroom content. The films had shown a fast degradation within 9 days. The films were shown to start degradation after 3 days and achieved 100% degradation within 12 days. Dependent T-test shows that there is a significant difference between the mushroom-based film and the rate of the degradation.

3.2. Tensile strength test

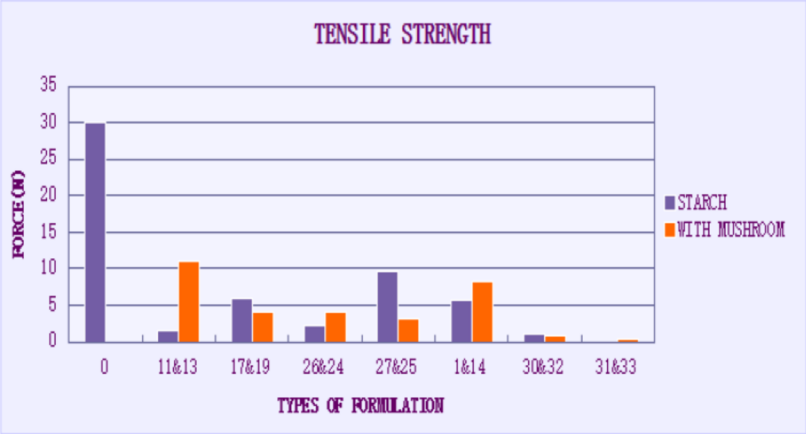

Fig. 3. Strength of starch and mushroom film

Based on the result, Fig. 3 shows the tensile strength of the selected formulation starch and mushroom based film. Formulation 13 (film that contain mushroom) are more rigid and flexible compared to sample 11 (no mushroom added). Sample 13 needs 11N break the film apart but sample 11 needs 2N only.

3.3. Batch Biosorption Testing

Table 2
Average absorbance and standard deviation for ammonium sulphate

TIME	BIOMASS		MUSHFILM		CONTROL		PLASTIC		BLANK	
	ABS	S.D	ABS	S.D	ABS	S.D	ABS	S.D	ABS	S.D
1	0.085	±0.00212	0.071	±0.01061	0.094	±0.03959	0.075	±0.01626	0.077	±0.00494
2	0.093	±0.00636	0.066	±0.01131	0.166	±0.04525	0.082	±0.01626	0.074	±0.01272
3	0.091	±0.00282	0.059	±0.01131	0.142	±0.01979	0.079	±0.01555	0.074	±0.00707
4	0.108	±0.02828	0.059	±0.00494	0.145	±0.01838	0.081	±0.01202	0.07	±0.00071
5	0.199	±0.01838	0.054	±0.01555	0.159	±0.00565	0.076	±0.01202	0.068	±0.00636
6	0.125	±0.00071	0.053	±0.02333	0.184	±0.01202	0.075	±0.00071	0.075	±0.00707
7	0.126	±0.01555	0.041	±0.03818	0.189	±0.00989	0.075	±0.00919	0.067	±0.00636
8	0.13	±0.00424	0.026	±0.04808	0.212	±0.00071	0.078	±0.02051	0.066	±0.00071
9	0.135	±0.00212	0.015	±0.03747	0.229	±0.00424	0.083	±0.01484	0.076	±0.00353
PH	5.03		5.04		5.03		5.02		5.05	
INITIAL READING	0.082		0.092		0.094		0.087		0.088	

Based on the results obtained from the measurement of absorbance of ammonium sulphate in Table 2 there is a significant drop in the absorbance reading of mushroom-based film in ammonium sulphate solution. The reading was measured for biomass, mushroom-based film, control, plastic and

blank of the solution. All these samples were measure for ammonium sulphate at average pH of 5.0 and at 25°C.

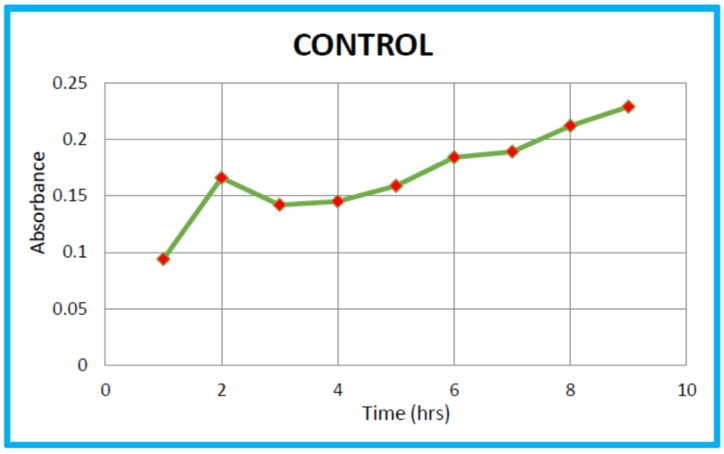

Fig. 4. Ammonium sulphate absorbance reading of control

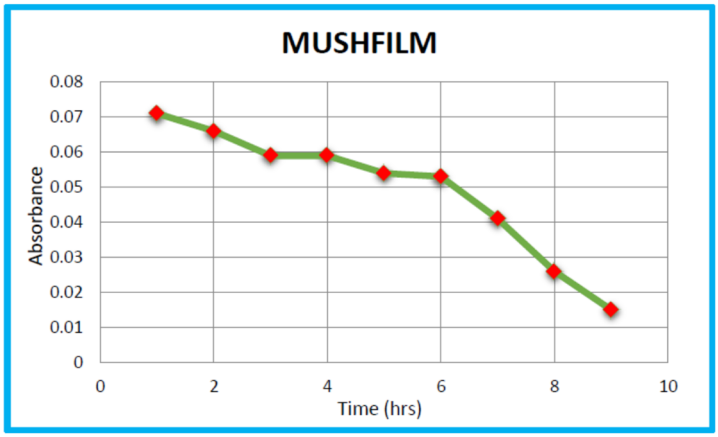

Fig. 5. Ammonium sulphate absorbance reading of mushroom-based film

4. Discussion

4.1. Biodegradability test (soil burial method)

The degradation of both starch-mushroom blend films and control films begins to at the Day 3 and totally that all degraded within 12 days. The presence or incursions of microorganism from the soil may cause the weight loss and degradation of the mushroom-based film. Carbohydrate polymers attacked by microorganism that have the capability to hydrolyze these polymers with its specific enzymes and change the polymers into digestible units where starch structure is weakened and starch strength is reduced [7].

4.2. Tensile Strength

The bar graph indicates the effects of Force (N) against different types of films' formulations. Based on the result, Formulation 13 (mushroom-based film) is more rigid and flexible compared to sample of Formulation 11 which does not contain any of mushroom powder. By comparing the result of Formulation 13 and Formulation 11 (control film), sample of Formulation 13 can withstand 11 N

whilst sample of Formulation 11 can only withstand 2 N of force. Therefore, mushroom-based film is more rigid and flexible compared to control film. This might happened because of the characteristics of mushroom itself, which contain chitosan in the mycelium of mushrooms which can give the strength towards the films. According to [6], chitosan in fungi are as fibrillary polymer of the cell wall. Thus, the unique properties of it make it useful for various properties of industrial applications like adhesive and paper strengthening agent. Moreover, [6] claimed that the chitosan have good film forming properties, which are valuable for wound dressing, artificial skin or packaging.

4.3. Biosorption

The initial absorbance and the absorbance reading for every one hour interval of total 9 hours were taken. The samples were left on orbital shaker during the period of this test to ensure aeration and a complete reaction could take place. The results show that the mushroom-based film in ammonium sulphate absorbs the metal ion significantly as the absorbance reading was decreasing. Furthermore, the reading for control and biomass increased gradually which shows that there is leaching of chemicals from the film and biomass itself.

5. Conclusion

In conclusion, the film produced from mushroom waste has the potential to be a new alternative for mycoremediation. However, there are still rooms needed for the growth and expansion of this biodegradable film. In further research, other pollutants will be tested and mushroom-based film will be applied on-site at polluted area.

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