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Biodegradation of plastics by *Gleophyllum sepiarium* and *Pleurotus ostreatus* isolated from wood

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ABSTRACT

Gleophyllum sepiarium and *Pleurotus ostreatus* isolated from trees and window woods were tested for their ability to degrade plastic granules in minimal salt medium using weight loss, CO_2 evolution and mycelia extension as indicators. The results revealed that *P. ostreatus* degraded plastic bags and plastic granules by 5% and 2%, respectively; while *G. sepiarium* recorded 3% and 1% degradation, respectively. The results of CO_2 evolution indicated 31.4 dm^3 and 22.4 dm^3 CO_2 evolution in sample inoculated with *P. ostreatus* while 22.4 dm^3 and 17.9 dm^3 were recorded in plastic bag and plastic granules inoculated with *G. sepiarium*, respectively. The results of the study demonstrated the ability of *P. ostreatus* and *G. sepiarium* to partially degrade plastic bags better than plastic granules.

INTRODUCTION

Plastics are synthetic polymers that are either thermoplastic or thermosets[1]. They are highly visible part of solid waste stream constituting environmental nuisance. The disposal of plastic into the environment causes severe land pollution problem because they are not easily degraded by microorganisms. The use of plastic for packaging water in developing countries has greatly increased the plastic littering problem in the environment. Burning of plastic is not a good alternative because it leads to liberation of toxic vapors or gases into the atmosphere[2]. Divers group of microorganisms possessed metabolic capabilities to partially degrade plastic. Of such group of microorganisms are Basidiomycetes[3,4]. Basidiomycetes have received considerable attention from various biological and industrial points of view. The high ability to degrade the lignin-cellulose by Basidiomycetes can also be used in degradation of xenobiotic pollutants such as pentachlorophenol, dioxin and polythene[5]. *P. ostreatus* and *G. sepiarium* used in this study are Basidiomycetes; their biodegradative ability of plastic was explored in this study.

The objectives of this study are to isolate *P. ostreatus* and *G. sepiarium* capable of degrading plastics from decay wood. The study also explored the ability of these organisms to degrade plastics.

MATERIALS AND METHODS

Collection of samples

The plastic bags and plastic cups were collected from refuse dump site in Minna, Nigeria. They were thoroughly washed with distilled water and air-dried. The bags were cut into rectangular shape of known weight (2 g); the plastic cups were chopped into granules and air-dried. The fresh saw dust used was collected from timber market in Minna, Nigeria.

Isolation and identification of *P. ostreatus* and *G. sepiarium*

G. sepiarium was isolated from decay wooden win-

dow, while *P. ostreatus* was isolated from bark of a dead tree. The wooden window/bark of the tree was cut into smaller pieces with the aid of saw blade. The small pieces of the wood/bark were put in a bowl and wet saw dust added to enhance the process of decay for one month. After one month, a small piece of the wood/bark was collected with sterile forceps and dipped into boiling water for about 3 minutes[6]. It was then inoculated into plates of malt extract agar (PDH) and incubated at room temperature for 72 hours. The isolates were identified physically based on colour that changes gradually and microscopically based on clamp connection, nature of hyphae and shape of asexual spores.

Utilization of plastic bags by *G. sepiarium* and *P. ostreatus*

Two grams (2 g) of plastic bag cut into rectangular shape was immersed into 200 ml of mineral salt media (2.78 g NH_4NO_3, 0.98 g KH_2PO_4, 0.001g $ZnSO_4$. $7H_2O$, 0.005 g $MnSO_4$. $4H_2O$, 0.05 g $CaCl_2$. $2H_2O$, 0.001 g $CaCl_2$. $6H_2O$, 0.001 g Thiamine hydrochloride, 1000 ml water, pH 6) and sterilized in an autoclave at 121^0C for 15 minutes. The medium (after cooling) was inoculated with spores of *G. sepiarium* and *P. ostreatus* separately and incubated at room temperature for 6 months to monitor the degree of degradation. The procedure was also used for plastic granules obtained from plastic cups. Percentage of degradation was determined using percentage weight loss and calculated with the formula:

$$\text{Percentage bio deg radation} = \frac{W_o - W_f}{W_o} \times 100$$

W_o W_o = Original weight of plastic before degradation; W_f = Final weight of plastic after 6 months of degradation studies

Measurement of CO_2 evolution as evidence of plastic degradation by *G. sepiarium* and *P. ostreatus*

Two grams (2 g) of plastic bag or plastic cup granules were put in conical flask containing 250 ml mineral salt medium. The mixture was sterilized at 121^0C for 15 minutes. The medium was inoculated with the spores of *G. sepiarium* and *P. ostreatus* separately. $Ca(OH)_2$ solution (0.2 g in 10 ml distilled water) in vials were suspended into the medium to absorb CO_2 released due to biodegradative activities of the organisms. The flasks were incubated at room temperature 28^0C ± 2^0C for 28 days. Control experiment without the organisms was set up as well. At 7 days interval, the vials were removed and replaced with new ones containing the same solution of $Ca(OH)_2$. The contents of the vials were titrated against 1 N HCl using methyl orange as indicator. The volume of CO_2 released and absorbed was calculated using the stoichiometric method and equation below.

$$Ca(OH)_2 + 2CO_2 \rightarrow Ca(HCO_3)_2 \quad \text{Step I}$$

$$Ca(HCO_3)_2 + 2HCl \rightarrow CaCC_2 + 2H_2O + 2CO_2 \quad \text{Step II}$$

1 mole of $Ca(HCO_3)_2$ = 2 moles of CO_2

$$C_B = \frac{(C_A \times V_A \times b)N}{V_b \times a}$$

C_B = Concentration of Ca(OH)2; V_b = Volume of Ca(OH)2; b = number of moles of base in step II equation C_A = Concentration of HCl used; V_A = Volume of HCl used; a = number of moles of acid in step II; N = Normal

Measurement of *G. sepiarium* and *P. ostreatus* mycelia extension while growing on medium containing plastic

Plates of mineral salt agar containing 2 g of plastic granules as a source of carbon and energy were inoculated with the spores of *G. sepiarium* and *P. ostreatus*. The plates were incubated at room temperature 28 ± 2^0C for 28 days during which the mycelia was measured using the method described by Smith[7]

RESULTS AND DISCUSSION

Percentage weight loss of plastic

The percentage weight loss of the plastic inoculated into the mineral salt medium by *G. sepiarium* and *P. ostreatus* is shown in TABLE 1. The percentage biodegradation of plastic bags cut into rectangular shape and plastic granules from plastic cup were 3% and 1%, respectively by *G. sepiarium* and 5% and 2%, respectively by *P. osteratus* at the end of 6 months. The results demonstrated the potential of *G. sepiarium and P. ostreatus* to utilize plastic bags better than plastic granules from cup. The reason might be due to the differences in the content of the chemical constituents of the two plastics. It might also be due to differences in their texture, plastic bags are more flexible than plastic cup.

Co_2 evolution from plastic biodegradation by *G. Sepiarium* and *P. Ostreatus*

The volume of CO_2 evolved during biodegradation of plastic granules by *G. sepiarium* and *P. ostreatus*

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are shown in TABLE 2. The volume of CO_2 evolved from plastic bag and plastic granules are 22.4 dm^3 and 17.9 dm^3 by *G. sepiarium*, respectively while 31.4 dm^3 and 22.4 dm^3 were recorded in sample inoculated with *P. ostreatus*, were recorded in sample inoculated with *P. ostreatus*, respectively. This result demonstrated the potential of the two test organisms to degrade plastic. This might be due to the presence of metabolically active enzymes in the two organisms which enhance their ability in breaking down the plastic polymers. This is in agreement with the findings of Odukuma and Okara[8] who reported that polythenes can be a source of nutrient to microorganisms.

TABLE 1 : Percentage weight loss of plastic by *G. sepiarium* and *P. ostreatus* sample organisms W_O(g) W_f(g) % weight loss.

Sample	Organisms	W_O(g)	W_f(g)	% Weight loss
Plastic bag	*G.sepiarium*	2.0	1.94	3.0
Plastic bag	*P. ostreatus*	2.0	1.90	5.0
Plastic bag	uninoculated	2.0	2.00	0.0
Plastic granules	*G.sepiarium*	2.0	1.98	1.0
Plastic granules	*P. ostreatus*	2.0	1.96	2.0
Plastic granules	uninoculated	2.0	2.00	0.0

TABLE 2 : CO_2 evolution from plastic degradation by *G. sepiarium* and *P. ostreatus*.

Sample	CO_2 (dm^3)
Plastic bag + *G. sepiarium*	22.4
Plastic granules + *G. sepiarium*	17.9
Plastic bag + *P. ostreatus*	31.4
Plastic granules + *P. ostreatus*	22.4

Mycelial extension

The mycelial of the test organisms within the period of nine days intervals is shown in TABLE 3. Both organisms did not show any sign of growth within the first two days after inoculation. This might be due to the fact that the organisms were trying to adapt to the plastic environment within the 2 days period. The mycelial extension of *P. ostreatus* reached their maximum length of 4 cm on the 8th day; while that of *G. sepiarium* reached their maximum length of 4 cm at 8th day. The result is an indication that *P. ostreatus* grows faster on MSM enriched with plastic granules than that of *G. sepiarium*. There was a significant difference between the lengths of mycelia at ($P<0.005$) within the nine days period.

TABLE 3 : Mycelial Extension

Days	Plastic granules + *P.ostreatus*	Plastic granules + *G.sepiarium*
1	$0^a \pm 0.00$	$0^a \pm 0.00$
2	$0^a \pm 0.00$	$0^a \pm 0.00$
3	$0.5^a \pm 0.00$	$0.4^a \pm 0.00$
4	$1.3^{ab} \pm 0.58$	$0.9^{ab} \pm 0.58$
5	$2.0^{ab} \pm 0.58$	$1.4^{ab} \pm 0.58$
6	$2.9^{bc} \pm 0.58$	$2.4^{cd} \pm 0.58$
7	$3.4^{cd} \pm 0.58$	$3.0^{cd} \pm 0.88$
8	$4.0^{d} \pm 0.58$	$3.6^{de} \pm 0.58$
9	$4.0^{d} \pm 0.58$	$4.0^{e} \pm 0.58$

CONCLUSION

The results of this study clearly revealed the potential of *G. sepiarium* and *P. ostreatus* to partially utilize plastic bags and plastic granules as a source of carbon and energy. *P. ostreatus* demonstrated better ability in degrading plastic better than *G. sepiarium*. Therefore, the activities of this organism can be enhanced for biodegradation of plastic products.

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