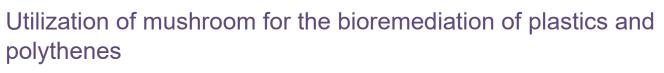


Review



Odufa Patience Ikhimalo ¹, & Anthony Moses Ugbenyen ^{2,*}

Abstract

Plastics found important usage across all industries and are mostly produced from fossil sources, however, the additive used in the plastic production process makes these plastics non-hydrolyzable and deters the formation of biofilms, which are important for microbial colonization and subsequent degradation. The use of living organisms, specifically fungi, in the degradation of waste is known as mycoremediation. This has been applied to different waste categories, including lignocellulose, petrochemical, and wastewater with successes recorded. Plastic waste is ubiquitous and is a challenge to waste management due to its durability and recalcitrant nature; the remediation process of plastic waste produces by-products that could be destructive to humans and the environment. Mushrooms which have been consumed since time immemorial for their medicinal and pharmacology properties have been widely used in the mycoremediation process due to their rapid growth, biomass production, and extracellular enzymes. The enzyme system of mushrooms and those found in spent mushroom compost have degradational prowess which has shown the ability to digest plastic polymers. Mushrooms such as Pleurotus ostreatus, Agaricus bisporus, Auricularia auricular, and Pestalotiopsis microspore amongst several others have prospects in the mycoremediation of plastics and polythenes. Fourier Transform Infrared (FTIR) spectrophotometry confirms biodegradation breakage of chemical bonds in the plastic by revealing bands for oxidative products like esters, aldehydes, and carboxylic for mushroom-treated polythene films, nylons, and polythenes. Mycoremediation of plastic waste is purported to be sustainable in the large-scale degradation of plastic waste and should be exploited.

Introduction

Our world is highly polluted and this is getting worse each day as the entrant level of contaminant supersedes the rate at which they are removed; Environmental contaminants from natural and anthropogenic activities pollute the environment making it unsafe for all living things, emerging contaminants such as pharmaceutical waste, personal care products, antibiotics and pesticides are also area of concern nowadays (D'Surney & Smith, 2005; Chopra & Sharma, 2019; Ali et al., 2022; Hazra et al., 2022). These pollutants negatively affect air, soil, and water which are major ecosystems for living organisms.

Researchers have raised these alarms about the exponential increase in environmental pollutants (Fuller et al., <u>2022</u>). Nations have set some minimal level of release of such harmful substances by industries; world leaders have set some benchmarks and milestones to dealing with these issues such as is contained in the millennia development goals and the sustainable development goals.

Plastic waste at a time was emerging but has now become ubiquitous and, on the increase, due to its indispensable nature (Caruso, <u>2015</u>; Gomiero et al., <u>2019</u>).

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¹Department of Biological Sciences, Plant Biology and Biotechnology Unit, Edo State University, Uzairue, Edo State, Nigeria.

²Department of Biochemistry, Edo State University, Uzairue, Edo State, Nigeria.

*Correspondence: ugbenyen.anthony@edouniversity. edu.ng

Keywords

Bioremediation; Fungi; Mushroom; Plastic; Polyethenes

Citation

Ikhimalo, O. P., & Ugbenyen, A. M. (2023). Utilization of mushroom for the bioremediation of plastics and polyethenes. Syncytia, 1(1), 16-26. DOI: 10.52679/syncytia.2023.0bt5 hn Several solutions for eradicating plastic wastes have been proffered such as thermal degradation and pyrolysis, however, these processes are costly and may cause environmental problems through the emission of harmful substances (Qureshi et al., 2020) while other solutions such as recycling and energy recovery cause more concern (Sharuddin et al., 2016). The urgency is upon us to find sustainable means of cleaning up our ecosystems of waste generated from plastics. Some mushrooms have been implicated in the biodegradation of such waste generated from plastics e.g., *Pleurotus ostreatus, Agaricus bisporus,* and *Auricularia auricular* (da Luz et al., 2013; Brunner et al., 2018; Liu et al., 2019).

The main objective of this current review is to look at the biological treatment of plastic and polyethene waste using mushrooms.

Plastics and Polyethynes

Plastics are polymers that can be shaped into various valuable products; they are of excessive molecular mass and are composed of a chain of polymeric molecules, unusual bonds, and halogen substitutions (Pathak & Navneet, 2017). Plastics have become indispensable in everyday life (Siracusa, 2019), they are used in virtually most industries, especially for packaging. The euphoria that heralded its advent has dwindled due to the ecological and economic threat they have constituted; An eyesore of plastic waste is seen in virtually all ecosystems with the marine ecosystem being the worst hit (Beaumont et al., 2017; Pathak & Navneet, 2017). The breakdown of plastics as a result of oxygen and UV reactions forms microplastics. Microplastic and plastic waste are now ubiquitous and a growing concern (Caruso, 2015; Gomiero et al., 2019). Microplastics have been isolated from air, water, and land and there are rising trepidations on their debilitating effects, which include amongst others; pseudo satiation in Pisces causing starvation and leaching of plastic-based chemicals into marine organisms and the environment (Critchell & Hoogenboom, 2018)

Nowadays, alternatives to plastics are highly sought out for pari-pasu a means of healing our plastic-infested ecosystems. It is worthy of note that several research are underway and some concluded in a bid to solve this problem. Several solutions have been proffered, how be it these are expensive and may not be environmentally friendly (Kumar et al., <u>2011</u>).

There are several types of plastic with applications across varying industries (Table 1). Based on the source of biomass, plastics are categorized as natural or synthetic. Natural plastics include poly- β -hydroxybutyrate (PHB), polyhydroxyvalyrate (PHV), cellulose acetate, nitrocellulose, polyhydroxyhexanoate (PHH), polylactic acids (PLAs), and polyamide 11 (PA 11). Synthetic plastics are produced from petrochemicals and include polyethylene also known as polythene (PE), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polystyrene (PS), polyurethane (PUR), polypropylene (PP), and polycarbonate (PC). Conventional plastics with sources from fossils are recalcitrant to biodegradation; it is no news that synthetic plastics form the crux of environmental and ecological concern. In addition, several additives in plastic production render them non-hydrolyzable and hinder the formation of biofilms crucial to the colonization of microbes (Cregut et al., <u>2013</u>).

Polyethylene is a polymeric chain of the ethylene molecules. It is the most common of all synthetic plastics and has applications in a variety of packaging solutions such as plastic bags, water, and milk bottles, and packaging films. Polyethylene is of three types, viz, low-density polyethylene (LDPE), Medium-density polyethylene (MDPE), and high-density polyethylene (HDPE). HDPE is more recalcitrant to biological degradation than the other types of polyethenes. HDPE is recalcitrant to biological degradation; thus, HDPE may linger more in the environment. Thermal degradation, pyrolysis, photo-degradation, and bioremediation are used to reduce plastic infestation with bioremediation being the most preferred.

	Synthetic Plastics	Industrial Applications
1	Polyurethane (PU)	Automotive, sponges, life jackets, clothing
2	Polyvinyl chloride (PVC)	Automobiles, curtains, raincoats, bottles, agriculture, shoes, soles, garden hoses, electricity pipes
3	Polypropylene (PP)	Bottle cups, food, packaging, straws, car seats, batteries, bumpers, syringes
4	Polycarbonate (PC)	Lens in glasses, safety visors, roofs, baby bottles
5	Polystyrene (PS)	Food packaging materials, disposable cups, electronic devices, laboratory wares
6	Polyethylene (PE)	Plastic bags, food packaging films, water and milk bottles, agriculture pipes, motor oil bottles
7	Nylon	Shoes, clothing, rainwear
8	Polyethylene terephthalate (PET)	Carbonated soft drink bottles, meat packaging, food packaging, clothing, textile fibers
9	Polytetrafluoroethylene (PTFE)	Chemicals, electronics, kitchen utensils

Table 1. Synthetic plastics and their industrial uses.

Bioremediation

Bioremediation is the use of living organisms to degrade harmful pollutants into harmless substances. It is employed to clean up polluted sites and environments either in situ or ex-situ. Heavy metals, wastewater, organic compounds, lignocellulose biomass, and xenobiotic compounds are examples of substances that have been bio-remediated with complete mineralization of contaminants as the ultimate aim (Kulshreshtha et al., 2014). Several literatures exist on the application of some organisms such as plants, bacteria, and fungi as bioremediators. The bioremediators are biological agents used to perform bioremediation processes. For a season and even now, the use of plants in bioremediation (known as phytoremediation) has gained wide attention in academia. However, the time required, the effect of season, weather and climate, toxicity level of plants, and accumulation of contaminants in edible parts of the plants are factors that affect phytoremediation; moreover, the process depends on root depth which is also a drawback of this system. The heavy recalcitrant are not bio-remediated by Plants but are accumulated at the root zone, thus rendering phytoremediation unsuitable to heavier bio-recalcitrant substances (Ouvrard, 2013).

Recalcitrant pollutants such as persistent organic pollutants (POP), polycyclic aromatic hydrocarbons (PAH), and xenobiotic and halogenated compounds have become crux issues in bioremediation due to the non-bioavailability of building blocks but there are positive results in the use of bacteria and fungi in achieving mineralization of these recalcitrant; the use of fungi is considered as the most sustainable and efficient bioremediation.

Mycoremediation

The use of fungi in bioremediation is known as mycoremediation; this process is cheap and has wide application in bio-remediating quite an array of toxins and organic compounds including the recalcitrant. The extracellular enzymes produced by fungi give them a natural ability to break down cellulose and lignin. The success of mycoremediation may be attributed to the presence of the structure mycelium in fungi; this structure like plant roots infiltrates the substrate and exudes extracellular enzymes for the breakdown of substrate and nutrient uptake (Ali, <u>2017</u>). Notwithstanding, effective mycoremediation depends greatly on the selection of specific fungal species for target pollutants amidst other factors (Rhodes, <u>2014</u>). Several researchers have dealt extensively with the subject matter of bioremediation and mycoremediation (Kulshreshtha et al., <u>2014</u>; Rhodes, <u>2014</u>; Dorr, <u>2017</u>).

Mycoremediation studies utilize fungal species from different genres such as *Aspergillus, Saccharomyces,* and a few mushrooms. Mushrooms have gained popularity due to their short growth period, high biomass formation, and the variety of extracellular enzymes produced.

Mushrooms and their role in bioremediation

Mushrooms are found in the fungi family Basidiomycetes and Ascomycetes and are estimated to be over 140,000 species, they are a macro fungus with distinctive fruiting bodies that are epigenous or hypogenous, sufficiently visible to the naked eye and can be hand-picked (Kortei et al., <u>2018</u>). Mushrooms are non-photosynthetic, obtaining nutrition by saprophytic, parasitic, and symbiotic means. They are mostly found in the soil and have long been known to play a role in the breakdown of forest litter (Girma & Tasisa, <u>2018</u>).

The use of Mushrooms for food has a long history, there are about 2000 edible species from 140,000 mushroom varieties (Julian et al., <u>2019</u>), however, some are non-edible and others are poisonous. Species of the genus *Agaricus, Pleurotus, Lentinus, Ganoderma,* and *Huitlacoche* are the main edible mushrooms. Worldwide, mushroom cultivation is a profitable agribusiness having an annual global value of 45 billion dollars, with about 35 commercially cultivated species around the world (Valverde et al., <u>2015</u>; Kortei et al., <u>2018</u>; Julian et al., <u>2019</u>). Mushrooms are rich in proteins, vitamins, and minerals and could serve as a substitute for meat. Some secondary metabolites isolated from mushrooms include quinones, benzoic acid derivatives, terpenes, steroids, and quinolones (Kakon et al., <u>2012</u>; Feeney et al., <u>2014</u>; Valverde et al., <u>2015</u>)

The fruiting body of mushrooms has a high medicinal value and is used as a nutraceutical and dietary supplement (Singh, <u>2017</u>). Mushrooms have applications in several industries such as the culinary, pharmaceutical (anti-oxidant, hypoglycaemic, hypocholesterolemic, antihypertensive, antiviral, anticancerous properties), and waste management and bioremediation with biotransformation of lignocellulose biomass to protein being the most prominent (Julian et al., <u>2019</u>); more recent is their application in the bioremediation of plastics and plastic wastes.

An array of enzymes such as cellulase, hemicellulase, amylase, pectinase, protease, laccase, and glucosidase are produced by mushrooms. These enzymes can degrade cellulose, lignin, dyes, pesticides, heavy metals, petroleum hydrocarbons, and polychlorinated biphenyls. Enzymes of the ligninolytic system were also isolated from Spent Mushroom Compost (SMC) (Nakajima, <u>2018</u>). SMC is the soil and

substrate waste of mushroom cultivation; it's been reported to be rich in nutrients and enzymes and has been applied as a soil conditioner (Wiafe-Kwagyan et al., <u>2018</u>). Mushroom fruiting bodies, SMC, and enzymes are major components of mushrooms significant in bioremediation.

Lignocellulose biomass may be the predecessor of the use of mushrooms in bioremediation studies. Lignin, cellulose, and hemicellulose with varying levels of ash, minerals pectin, proteins, and salts are the constituents of lignocellulose biomass (Cortes-Tolalpa et al., 2017; Tsegaye et al., 2019), they are generated mostly from agricultural waste such as wood residues and vegetable feedstock. The inoculation of several species of mushroom for the production of fruiting bodies has been achieved by the use of biomass from agricultural wastes such as wheat straw, rice straw, sawdust, winery and vineyard waste for the cultivation of mushroom species including *P. ostreatus*, *Ganoderma lucidum* and *A. bisporus* (Petre et al., 2016; Julian et al., 2019) has greatly reduced the arduous task of disposing these wastes.

Wild mushrooms were investigated for their heavy metal content; it was observed that *A. augusta* and *B. subvelutipes* accumulated high levels of cadmium, copper, iron, manganese, lead, and zinc. This implies that mushrooms could be investigated for remediation of heavy metal pollution and could also serve as bio-indicators of these metals because they accumulate high concentrations of metals in their fruiting bodies. Investigations by Wang et al. (2017) and Falandysz (2015; 2017) revealed the bioaccumulation of mercury by *Coprinus comatus* and species of *Lactarius*. Njoki et al., (2017) specified a reduction of heavy metals such as manganese, copper, and zinc by *Pleurotus pulmonarius* in hydrocarbon-polluted soil. *C. comatus* served as a bioindicator for mercury-polluted soil and recently, the bioremediation capacity of mushroom species was improved by augmenting with bacteria inoculum.

The advent of the oil and gas industry has exceedingly benefited the economy of several nations, in terms of fuel generation moreover by-products of this industry have found relevance in the food, pharmaceutical, and agricultural industries. Nevertheless, the environment and humans are affected by pollution from this industry such as oil spills, emissions, and effluents; it is also a source of chemicals contributing to global warming, ozone layer depletion, and acid rain (Sharma, 2017). The mushroom *P. pulmonarius* has been featured in several studies such as Adenipekun et al. (2013), Mohammadi-Sichani et al. (2017), and Njoki et al. (2017) for the bioremediation of petroleum-contaminated site. Njoki et al. (2017) inoculated pure cultures of *P. pulmonarius* in soil contaminated with petrol, diesel, spent petrol engine oil, and spent diesel engine oil in a ratio of 1:1:1:1 at a concentration of 2.5%, 5%, 10%, and 20% respectively. Results from this study unveil a percentage loss after a 62-day incubation of the mushroom of the total petroleum hydrocarbon which decreases with concentration increase of petroleum added.

Emerging contaminants are organic or synthetic substances and their transformed products that are not currently monitored which have the potential to cause health and ecological issues; Pharmaceutical wastes, flame retardants, hormones, personal care products, antibiotics, and pesticides are examples of emerging contaminants (Rosenfeld & Feng, <u>2011</u>; Dey et al., <u>2019</u>). It is amazing that at a time in the past, plastic and polythenes wastes didn't attract the level of attention being given.

Paracetamol and 17 α -ethynyl estradiol (EE2) were remediated by *A. bisporus* and *Lentinula edodes* (de Jesus Menk et al., 2019); while wastewater containing the

drugs Acetaminophen and sulfonamide were remediated by *P. eryngii* (Chang et al., <u>2018</u>). Remarkably, *Pleurotus ostreatus* has also found application in the waste management of diapers (Espinosa-Valdemar et al., <u>2011</u>). Yang et al. (<u>2017</u>) maintained that SMC addition in sludge affected a high degradation of the flame retardant tetrabromobisphenol-A.

Aflatoxin contamination of agricultural produce is of great concern and has been implicated as a carcinogenic and hepatoxic substance (Kowalska et al., <u>2017</u>). A bioreactor with high bioconversion would be most appropriate due to the challenge of disposing of contaminated bioreactor biomass after bioremediation (Branà et al., <u>2017</u>).

Pesticides and herbicides have found widespread use in the world however, leaching of these substances contaminates ground water, causes eutrophication, and decreases diversity in vegetable; in addition, wind drifts from these causes' respiratory issues, skin, and eye irritation in persons beyond the target area and may induce genetic disorders (Bernardes et al., <u>2015;</u> Özkara et al., <u>2016</u>).

Pesticides such as DDT (1, 1-trichloro-2, 2-bis (4-chlorophenyl) ethane) were degraded and mineralised by *Pleurotus ostreatus* in DDT contaminated soil (Purnomo et al., 2010), there was amelioration of the toxic effect of the carbamate pesticide Carbofuran in rats by the administration of *Ganoderma lucidum* and *Auricularia polytricha* (Hossen et al., 2018). The use of mulch aids in the reduction of weed infestation on soils, tSaoir & Mansfield (1998) used SMC as a mulch while Matute et al. (2012) and Kadian et al. (2008) utilized SMC to degrade the herbicides Metsulfuron methyl and atrazine respectively.

Role of mushroom in the bioremediation of plastic and polythenes

The bioavailability of polymers is limited to molecular weights less than 1000, plastics have molecular weights ranging from 10,000-500,000, thus this inhibits degradation by biological organisms. Despite this, some microorganisms have passed this hurdle and can digest plastics. Microorganisms such as bacteria and fungi can create biofilm on plastics that have been weathered by the environment. A fungus Aspergillus tubingensis isolated from a waste disposal site was confirmed to be able to degrade plastic polyurethane (PUR) (Brunner et al., 2018). The study used scanning electron microscopy to determine inoculation of the fungal mycelia on the PUR and Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy bands to confirm biodegradation breakage of chemical bonds in the plastic. The benefits of using mushrooms which are one of the most potent decomposers include their rapid growth, biomass production, and extracellular enzymes produced (Adenipekun et al., 2013). Oxidation of plastic material has been purported as the major mechanism of bioremediation of plastic materials by mushrooms (Chonde et al., 2012; Nwogu et al., 2012); the following mushrooms have been reported for the biodegradation of plastics and polythenes.

Pleurotus ostreatus

da Luz et al., (2013) reported that *P. ostreatus* were inoculated in media containing strips of oxo-biodegradable plastics (D2W). The result showed the formation of cracks and holes on the surface of the plastic after 45 days of incubation. Other changes observed were wrinkles, discoloration, and biofilm formation; significant in this study was the formation of mushrooms by *P. ostreatus* on the plastic strips.

Oxo-biodegradable plastics are formed from polymers synthesized chemically with the integration of pro-oxidants which hastens their physical and biological degradation (da Luz et al., 2013; 2014). Pretreatment through treating with enzymes and physico-chemical treatment such as hydrolysis and pyrolysis of oxo-biodegradable plastics are required before biodegradation. In this study, oxidation was achieved by the activity of laccase without prior treatment with enzymes, heat, or water.

This mushroom was used to biodegrade green polyurethane by da Luz et al. (2015). In this study, the green polyethylene was exposed to sunlight to induce photodegradation before being inoculated with fragments of *Pleurotus ostreatus*. Results revealed a reduction in the chemical composition of the plastic although no cracks, pits, and formation of new functional groups in the GP structure were observed. Upon inoculation and incubation with the mushroom, there was a massive decrease in the mechanical properties of the plastic and the formation of new chemical bonds with bands typical of hydrogen-oxygen bonds. In the same study, there were changes in the physical nature of the plastic treated with the mushroom alone without prior treatment with the abiotic stress of sunlight. It can be deduced that temperature is an important factor for bioremediation in the biodegradation of PU by *Aspergillus tubingensis* which shows that the rate of hyphae growth was higher at 37°C than at lower temperatures (Khan, <u>2017</u>).

Agaricus bisporus

Brunner et al. (2018) reported that the mushroom Agaricus bisporus was among the fungi with PU degradability others are the liter fungi *Cladosporium cladosporioides*, *Xepiculopsis graminea*, and *Penicillium griseofulvum* and plant pathogen *Leptosphaeria* which were isolated from plastic debris were also able to biodegrade polyethylene. Nonplastic isolates showing PU biodegradability were *Agaricus bisporus* and *Marasmius oreades*. The authors argued that the growth of fungi on plastic debris (both in the macro, micro, and nano forms) does not connote biodegradability potentials, citing the ever-increasing plastic debris which is yet to be biodegraded in ecosystems that one would assume is populated by "plastic-eating" microbes as an example. PAH removal ability by the application of Spent *A. bisporus* substrate was investigated in three different methods on soil polluted with PAH and Pb (Liu et al., 2019). Spent *A. bisporus* substrate application is significant in achieving maximum PAH removal.

Auricularia auricular

Spent mushroom substrate obtained from *Auricularia auricular* and *Sarcomyxa edulis* in combination with the coccoid bacterium *Paracoccus* sp. LXC and humic acid (HA) were used to remediate agricultural soil contaminated with aged polycyclic aromatic hydrocarbons (PAHs). It also revealed an increase in acquired nutrients and organic matter for soil treated with *Paracoccus* sp. LXC combined with HA and SMS from *A. auricular* (Liu et al., <u>2019</u>)

Pestalotiopsis microspore

Polyurethane (PUR) generated by the condensation of a polyisocyanate and a polyol was reported to be degraded by *Pestalotiopsis microspore*; serine hydrolase produced by these fungi was used to degrade PUR which served as the only carbon source (Russel et al., <u>2011</u>).

Pycnoporus sanguineus

Landfills are composed of composites of both natural and synthetic polymer waste. A choice of recycling plastics in municipal waste is faced with the huddle of separating the plastic from other wastes present in the composites, this is not a cost-friendly approach; moreover, the recycled product may be weaker than the original plastics. A consideration of bioremediating composites of natural and synthetic polymers by mushrooms was achieved in an investigation by Catto (2014). Flake form of post-consumer waste from bottle caps of polypropylene and ethylene-vinyl acetate mixed with wood flour of *Eucalyptus grandis* and *Pinus elliottii* showed a reduction of sample mass compared to *Trametes villosa* (TV) for Plastic-wood composite of *Eucalyptus grandis*.

Phanerochaete chrysosporium

Ali et al. (2014) reported a strain identified as *Phanerochaete chyrosporium* isolated from sewage-buried films of polyvinyl chloride blended with cellulose indicating the utilization of PVC as a nutrient source. Sturm test and FTIR showed mineralization of the polymer through carbon dioxide production and peak changes respectively. Nylon 6 polymer serving as the only nitrogen source was degraded by *P. chyrosporium* after 75 days (Chonde et al., 2012).

Pleurotus tuberegium and pulmonarius

The bioremediation of HDPE polyethylene films by 4 mushrooms: *Lentinus squarrosulus*, *Pleurotus tuberegium*, *P. pulmonarius*, and *Rigidoporus lignosus* which were inoculated in a medium with powdered polyethylene films as the only carbon source (Nwogu et al., <u>2011</u>). A weight loss of 13.26% and 9.67% were observed for *P. tuberegium* and *P. pulmonarius*, respectively.

Conclusion

The recalcitrant nature of plastics has rendered them unsusceptible to biological attack, however, some mushrooms have breached this recalcitrant barrier posed by these plastics by producing an array of enzymes in the lignolytic enzyme system having oxidative properties and rendering these mushrooms to be ferocious decomposers. Although expensive, there is a possibility of isolating these enzymes for use at industrial scale degradation of plastic polymers into smaller fragments which could be further channeled into the production of other products.

Declarations

Author Contribution: Odufa Patience Ikhimalo & Anthony Moses Ugbenyen conceived of the presented idea, and wrote and revised the manuscript.

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