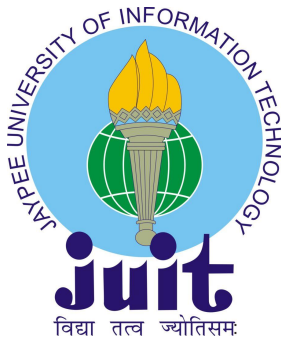


**DESIGNING, FABRICATION, AND PHYSICOMECHANICAL
CHARACTERIZATION OF PINE NEEDLE AND SAWDUST
REINFORCED WHEAT STRAW-PLEUROTUS OSTREATUS
BIOCOMPOSITE MATERIAL.**

THESIS SUBMITTED TO
DEPARTMENT OF BIOTECHNOLOGY AND BIOINFORMATICS,
JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY
SOLAN,[HP].



In the partial fulfilment of the requirement for the degree of
Bachelors of Technology in Biotechnology

By

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Under the Supervision of

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SELF-DECLARATION

I hereby declare that the work reported in the B.Tech. dissertation thesis entitled "Designing, Fabrication and Physicomechanical characterization of pine needle and sawdust reinforced wheat straw-*Pleurotus ostreatus* biocomposite material. " submitted at Jaypee University of Information Technology, Wagnaghat, Himachal Pradesh, India, is an authentic record of my work carried out under the supervision of Dr. Anil Kant (Associate Professor) at the Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Wagnaghat, Himachal Pradesh- 173234, India. I have not submitted this work elsewhere for any other degree or diploma.

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CERTIFICATE

This is to certify that the work reported in the B.Tech. dissertation thesis entitled "Designing, Fabrication and Physicomechanical Characterization of pine needle and Sawdust reinforced Wheat straw-*Pleurotus ostreatus* Biocomposite Material." submitted by Himanshu Kumar(191802) at Jaypee University of Information Technology, Wagnaghat, Himachal Pradesh, India, is a bonafide record of his original work and has not been submitted elsewhere for any other degree or diploma programme.

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ACKNOWLEDGEMENT

Without the direction and assistance of numerous people who in one way or another contributed with their significant time, aid, and support in the preparation and execution of this study, this thesis would not have been feasible. It is a joy to express to them all my sincere appreciation in this humble acknowledgment.

First and foremost, I would like to take this opportunity to thank my supervisor, Associate Professor Dr. Anil Kant. I want to thank him for all of his hard work, perseverance, confidence, and availability over the years. By giving me the freedom to do my work in my own way and by emphasising the value of critical thinking and logical reasoning in research, he has motivated me to work independently as a researcher.

I want to express my gratitude to Dr. Sudhir Syal (HOD), Department of BT & BI, JUIT. Waknaghat (HP), He is incredibly understanding and helpful.

I also want to convey my sincere gratitude and devotion to Dr. Jitendraa Vashisht, an associate professor at JUIT, and Ms. Suhani Bhagta. Dr. Saurabh Bansal, an associate professor at JUIT, for his ongoing support and collaboration throughout the process.

Alka Sharma, Megha Sharma, and Nidhi Tyagi, all of them are in their final year of study for a master's degree in biotechnology, have been a tremendous help to me in obtaining and completing my major project at the aforementioned firm. My friends and the department personnel who assisted me in finishing this assignment are greatly appreciated.

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Abstract

Completely degradable biocomposite is a material that is composed of two or more fully biodegradable distinct materials which are brought together to form a new material with improved properties over all the constituents. The objective of the study was to develop a proof of concept of reinforcing mycelium-based composite with pine needles and sawdust thereby improving its mechanical properties. In this study *Pleurotus ostreatus* was used for the formation of biocomposite as its mycelial network is dense and strong enough to bind, and firmly holds the substrate together. *P. ostreatus* were inoculated in a mixture containing varying proportions of Substrate- Wheat straw[WS], Filler material-sawdust[SD] and reinforcement-Pine needle[PN], and allowed to grow at 25°C in complete darkness for a period of 15 days, resulted in formation of a three-dimensional mycelium network that effectively binds all three components. To eliminate chances of further growth in the final composite, all living mycelia is terminated by heat killing which also reduces moisture content drastically, effectively increasing shelf life of this mycelium based biocomposite. Environmental and technological application of this sustainable material can be achieved by utilising this biocomposite as a cushion-providing internal packaging material, alternative cattle feed, fuel blocks for domestic usage and organic fertilisers in crop fields.

Keywords- Mycelium based biocomposite, *Pleurotus ostreatus*, Pine needle, Wheat straw.

Chapter 01- Introduction

Waste management and disposal is a time, capital, and energy-consuming process, Which requires a well-managed workforce to perform different standardised operations for different industrial, municipal, and domestic waste categories. Recycling of some selected items requires additional unit operations and protocols which is highly energy and time consuming for most of the materials. One of such materials is a polymer of class- polystyrene called expanded polystyrene or [EPS]. Recycling polystyrene in its expanded polystyrene [EPS] configuration is a very tedious process because of some properties of [EPS] mentioned below.

- (1). Extremely low density i.e.-(density range of 11 to 32 kg/m³).
- (2). Brittle nature due to low elasticity.
- (3). Porous nature of the material.

Due to all mentioned reasons approximately 2.3 million tons of expanded polystyrene end up in landfills every year world wide and occupy 30 percent of landfill space globally [03].

By many accounts, it takes around 500 years for [EPS] to decompose and even then it can leach chemicals into soil and water bodies. Global waste management cost for expanded polystyrene in 2020 to 2021 is \$400 billion[02]. And only 1% of total EPS which is considered waste is ever recycled and repurposed[02],[03].

Not only from the viewpoint of waste management and recycling but expanded polystyrene is an environmental hazard as it also generates toxic gases during its production, According to a report EPS producers and manufacturers were the 5th largest producer of toxic gases in 1986.

And till date, the global annual production of EPS is at million metric tons[02],[03].

On the other hand, there are different types of waste like agricultural waste. In the region of Haryana and Punjab main crops are cereal grains and pulses, for example- chickpeas (gram), pigeon peas (arhar) moong beans, lentil (masoor) peas, and various kinds of beans. wheat, rice, sugarcane, cotton, barley, corn, millet, and paddy[08]. stalks of most of these plants are rich in lignocellulose, cellulose, and hemicellulose. After harvesting the crops, stalks are of no use to farmers which leads to open-field burning of crop residue in regions of Haryana and Punjab. In recent decades this practice of burning crop residue has become a major consult. This malpractice had led to depletion in the air quality index, heat waves, smog haze, and many

health issues[07],[08]. The effect of burning crop residue is not only evident in Haryana and Punjab but also reports of heat waves are a common occurrence during harvesting season in neighboring regions of these States every year[07]. In the absence of an effective agriculture waste disposal management system, for farmers burning down the harvested crop residue is the easiest, most viable, and cost-effective method to get rid of agriculture waste[08].

In some regions of Himachal Pradesh, a species of pine tree native to the Himalayas is called *pinus roxulrghii*. Commonly known as “Chir Pine ” or “ Long leaves Indian pine ”. Almost every year during the summer season the floor of the mountains gets covered by dried pine needles which fall from these trees. This creates a very critical fire hazard situation because these pine needles are very flammable due to the high content of lignocellulose. Every year reports of wildfire is a common occurrence during the summer season in Himachal Pradesh causing loss to local flora and vegetation.

The basic aim of this project is to solve 3 problems listed below-

1. reduce the use of styrofoam or expanded polystyrene by developing an alternative material with similar properties
2. eliminate the practice of burning down the harvested crop residue by creating useful products using agro waste as raw material.
3. reduce the occurrence of the forest fire caused by dried and fallen pine needles by collecting and using it as raw materials for bio-friendly applications.

This will be achieved by Designing, Fabrication, and Physicomechanical characterization of pine needle and sawdust reinforced wheat straw-*Pleurotus ostreatus* biocomposite material.

With objectives listed bellow-

01. Growth of *Pleurotus ostreatus* on wheat straw supplemented with wheat straw extract.
02. Growth of *Pleurotus ostreatus* on wheat straw medium reinforced with pine needles and sawdust in different proportions.
03. Characterization of bio-composite material for mechanical and physical properties.

CHAPTER 02- Review of the literature.

2.1 Problems caused by styrofoam.

Polystyrene is essentially a hard, brittle plastic (similar to throwaway plastic cups), and Styrofoam is created by injecting a "blowing agent" into it to make it 30 times lighter than when it was first created. Once it changes from Polystyrene to Polystyrene, the term doesn't change because the chemical makeup stays the same. In order to create polystyrene, specific gases are pumped into the plastic, creating microscopic holes that, as the material cools, turn into gas and air-filled pockets[09]. Over 90 distinct dangerous compounds, including dioxins and styrene vapours, are released when Styrofoam is burned. These vapours can be controlled if burned in highly specialised facilities, but most commonly, incineration facilities lack the significant financial resources necessary to maintain their facility at these tightly regulated levels. Living close to these plants puts residents at a higher risk for health issues[09]. Polystyrene prevents water from penetrating the soil, which permits water to absorb waste to the point that it resembles soup. When it rains heavily, this soup leaks through the Styrofoam barrier and ends up in our soil and groundwater or, more likely, on the landfill liner[09]. The environment's mercury compounds may build in Styrofoam waste. In comparison to virgin styrofoam and beach sand, the amount of mercury discovered in beach debris was an order of magnitude greater. The greatest level of mercury found in styrofoam waste exceeds the limits for soil and bottom sediment. Polystyrene waste should thus be considered a possible mercury transporter throughout ecosystems. The bioaccumulation and biomagnification of mercury, as well as the entry of plastic trash into the food chain by several aquatic and likely also terrestrial creatures, make these findings appear to be particularly significant[10]. Mercury binds to Styrofoam waste in water, and it may also do so through contact with the Earth. The intricate interaction of biotic elements like the existence of biofilm and abiotic ones like solar radiation and mercury-related changes play a significant effect. As a result, seasonal and regional variations in mercury amounts in styrofoam waste occur[10]. While geographical variation appears to mirror the degree of pollution in the environment, high concentrations are prevalent in the summer. The debris gathered near the open Baltic shore, where mercury contamination in the air and water is lower than in the Gulf of Gdansk and Godap Lake,

contained the lowest mercury values. The debris found along the cliffs of the Gulf of Gdansk had the greatest mercury concentrations because, given the form of the shoreline, it had likely been there for a very long time[10].

2.2 Problems caused by the Combustion of agricultural residues.

Agricultural residues are distinguished by their high volatile matter concentration. It has been discovered that devolatilization begins at extremely low temperatures, and the volatiles that are emitted mostly include flammable gases including CO, H₂, and C_xH_y. Therefore, the design and operation of combustion systems for agricultural wastes must take volatile release and combustion into account. This is crucial when deciding on the fuel feeding system and how the combustion air will be distributed[11]. High concentrations of the unburned pollutants as well as ash may be predicted in the flue gas due to the high contents of volatile matter and the low particle densities of the agricultural wastes as well as the necessity to operate at temperatures below the melting points of the ash. However, by using an appropriate furnace design and staged combustion, it is feasible to regulate these pollutants[11]. Agricultural residues have relatively low levels of N and S, hence modest SO₂ and N₂O emissions are to be anticipated. However, it's possible that some waste burning will result in more Nitrogen compound emissions[11]. Due to the region's use of a rice-wheat cropping pattern, Punjab produces close to fifteen million tonnes of rice straw annually. Out of this, it is estimated that around 7-8 million tonnes of rice residue are often set afire in open areas. There are several explanations for this. It is commonly believed that farmers find it to be the simplest and most cost-effective method of elimination of rice stubble[12]. Farmers have no choice but to burn the wheat crop since there isn't enough time to seed it once the rice crop is harvested. The burning of soil organic matter is one of the recognised challenges to the sustainability of the rice-wheat farming system. The straw that is gathered from the fields has tremendous economic worth as fuel, animal feed, and a starting point for manufacturing. While rice straw is fed to animals in southern India, wheat straw is favoured in the north. The rice-wheat cropping method produces residue that may be used for a variety of purposes, but doing so requires that the residue be taken out of the field and separated from the grain[12]. Burning significantly decreases the amount of straw that is available to animals, which is already scarce. However, with combined picking, the majority of the waste is left in the soil for

burning, which has a negative impact on the rice-wheat cropping system's overall sustainability. When using a harvester, the majority of the leftover is left in the soil for burning, which has a negative impact on the rice-wheat farming system's overall environmental sustainability[13]. Today, many farms use zero tillage following stubble burning. About ten per cent of the total wheat-sown area in the years 2005–2006 was planted using zero-till equipment. It appears that less than 1% of farmers integrate the rice straw since doing so requires more digging than doing so after combustion[14]. In India, both wheat and rice residue loads make up 36 and 41 per cent, correspondingly, of the entire amount of stubble, whilst Punjab contributes 11 and 36%, to be specific, of the total amount of burned rice and wheat residue[15]. It is estimated that 347 million metric tonnes of agricultural leftovers were generated in India, of which over 200 million metric tonnes were made up of both wheat and rice crop wastes[16]. In Punjab, 18.8 million tonnes of paddy stubble were produced in 2004–2005, out of which 15 million tonnes were burned on open fields. 80% of all the rice picked with a combination harvester, according to the study, is burned in areas that are left open[17].

2.3 Problems caused by forest fire in Himachal Pradesh due to pine needles.

Pine trees are abundant in the Himalayan woods, and pine needles are a significant source of fuel for forest fires. The pinus needles can be used in a variety of ways to alleviate these issues. It is also possible to enhance the bulk density of unstructured biomass, which is normally between 40 and 200 kg/m³, to densities between 600 and 1200 kg/m³[62]. In the Himalayas and around the world, forest fires continue to decimate huge tracts of forest, harming ecosystem services, eradicating biodiversity, and endangering sources of livelihood. As a result of burning biomass and the resulting degradation of soils, forest fires ultimately contribute to global warming. These effects are anticipated to intensify as a result of rising fire incidents, which are in part made worse by climate change. Despite these difficulties, the region's capacity for building, mechanised tools, and adequate policies are all lacking[18]. Each year, forest fires continue to decimate substantial portions of the planet's forests[19]. Approximately 27.9 million hectares of woodlands were burnt by forest fires in 2016, according to statistics from the University of Maryland in the USA[20]. There are both natural

and man-made causes of forest fires, including lightning, rock slides, and volcanic eruptions [21]. Forest fires have historically played a significant role in promoting ecological succession by acting as an ecological filter, selecting species and characteristics, and establishing ecosystem communities[22]. excessively many flames Burning outside its traditional range can alter the environment and its ecology permanently[23]. Forest fires are occurring more frequently as a result of rising temperatures, less precipitation, and dryness in particular. Such a fast decline in forest to fires can worsen the scope and severity of socio-ecological effects, such as a lack of fuelwood, eviction of residents, and damaged ecosystem services[24]. In addition to having an effect on people, the natural world, and biodiversity, forest fires also contribute to global warming. Studies examining the effects of forest fires (both man-made and natural) on the ecosystem and ecosystem revealed significant carbon emissions[25]. Adopting climate-resistant adaptive routes entails recognising vulnerabilities to the effects of climate change, evaluating the potential for risk reduction, and designing and putting into practice measures that are in line with the objectives of sustainable development. These interventions have the potential to offer a combination of incremental and transformative responses that account for a variety of variables[26].

2.4 *Pleurotus ostreatus*.

The binomial name is *Pleurotus ostreatus*.

Taxonomic Description[32]

Kingdom : Fungi

Phylum : Basidiomycota

Class : Agaricomycetes

Order : Agaricales

Family : Pleurotaceae

Genus : *Pleurotus*

Species : *P. ostreatus*.

Considering A, B, and A, B₂; A, B₂ and A₂ B₁ are incompatibility factors. FIGURE 2.1 Diagrammatic represents the nature of inheritance in a typical *Pleurotus* species[33].

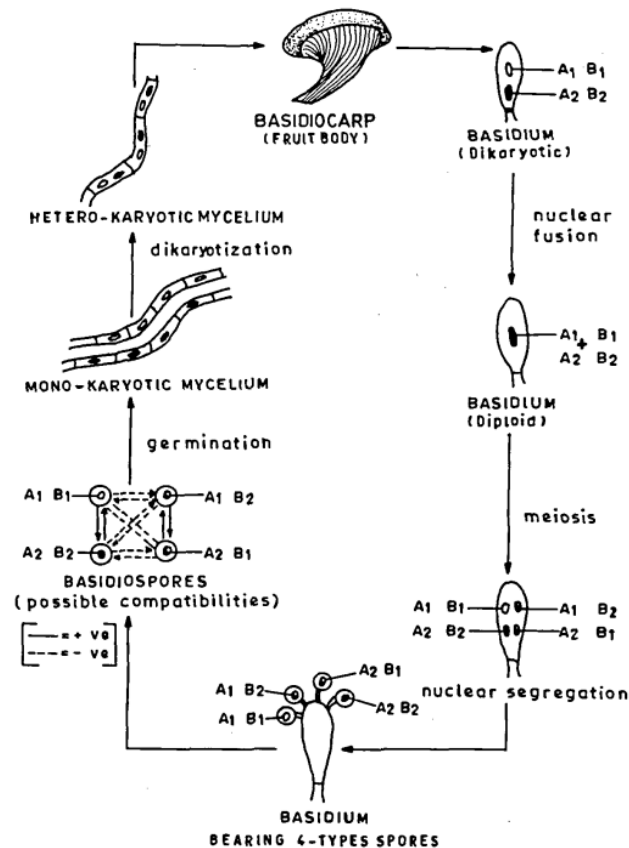


FIGURE 2.1- Diagrammatic representation of the nature of inheritance in a typical *Pleurotus* species. A, B, and A, B₂; A, B₂ and A₂ B₁ are incompatibility factors.

About 40 distinct species of the "oyster mushroom" genus *Pleurotus* exist. *Pleurotus ostreatus* (*P. ostreatus*), one of several species in this genus, for example, *P. eryngii*, *P. tuberegium*, *P. ulmarium*, *P. pulmonarius*, *P. citrinopileatus*, and *P. geesteranus* are all members of the *Pleurotus* genus. *P. ostreatus*, *P. sajorcaju*, *P. florida*, *P. flabellatus*, *P. cystidiosus*, *P. sapidus* [32]. is widely consumed across the world because of its flavour, high nutritional content, and therapeutic characteristics[27]. *P.ostreatus* is a brand-new edible fungus with significant nutritional and biological value. This is because it has a variety of bioactive components that enable it to perform a wide range of therapeutic functions[27]. The first cultivation of *Pleurotus* was documented by Kaufer, who also carried out the first cultivation in Germany as a means of storing food through the First World War[28]. Due to its high mineral content, therapeutic benefits, short lifespan, repeatability in recovering the value of specific agricultural and commercial wastes, and cheap resource and technological requirements, many different kinds of *Pleurotus* are now grown commercially[29]. The substrate used to cultivate *Pleurotus* mushrooms is beneficial to plant development as a fertiliser and soil conditioner[30]. It has been demonstrated that the mycelium and mushroom culture broth have positive biological benefits[31]. There are roughly 40 species in the genus *Pleurotus*, which is also known as the "oyster mushroom" which is frequently produced artificially and grows in tropical as well as subtropical regions. Essential characteristics of morphological differentiation in the genus *Pleurotus* are represented in Figure 2.2 [33].

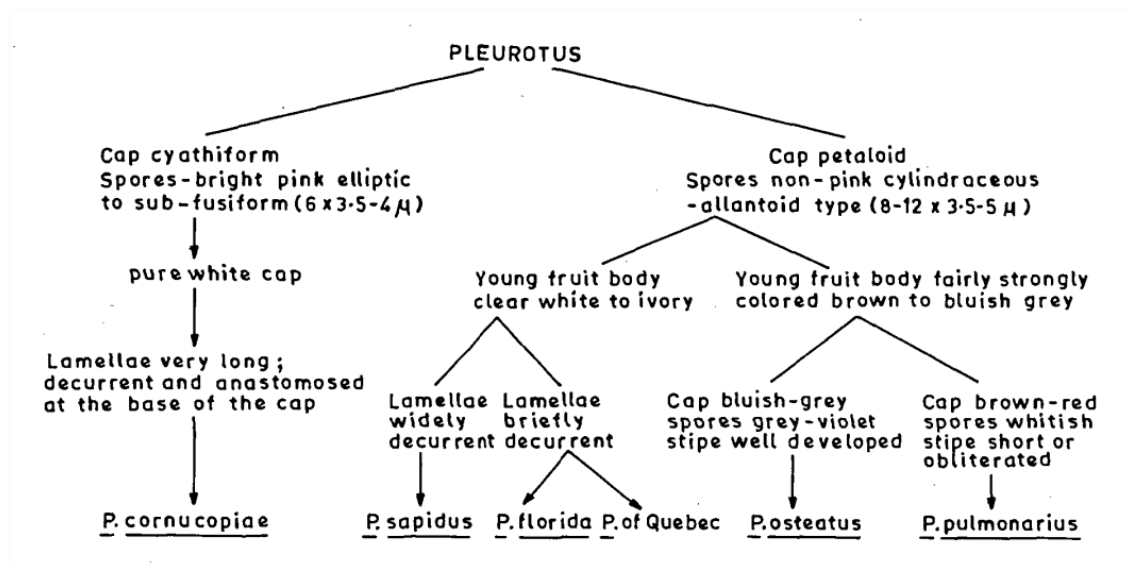


Figure 2.2 - Essential characteristics of morphological differentiation in the genus *Pleurotus*.

2.4.1 Mycological characteristics.

The scientific and colloquial names both allude to the fruiting body's form. Although the Latin term *ostreatus* and the English common name oyster allude to the form of the cap that resembles the bivalve of the same name, *Pleurotus* (sideways) signifies the sideways development of the stem with regard to the cap. The broad, fan-shaped or oyster-shaped cap of *P. ostreatus* measures 5 to 25 cm; natural examples range in colour from white to charcoal grey or brown to dark brown; the outermost layer is smooth when young[32]. White, hard, and varying in thickness owing to the stipe arrangement is the flesh mushroom. Gills: If present, the mushroom's gills appear in colour from white to cream, and descend on the stem. Stipe: The stipe has a lateral connection to wood and is off-centre. Spore print: The mushroom's spore print is best seen on a dark surface and ranges in colour from white to lilac-grey[34]. There are about 150 aromatic compounds found in various mushroom species. According to Mau and Hwang, carbonyl compounds and octavalent carbonate alcohols, such as 1-octanol, 3-octanol, 3-octanon, 1-caprynyl-3-ol, 1-octynol-3-ol, and 1-caprynyl-3-on, are primarily responsible for the scent of the majority of edible mushrooms. The fructifications in the sp. of *P. ostreatus* are dominated by the chemical 1-octynol-3-ol[35]. The presence of amino acids, nucleotides, as well as other elements like nitrogen, sulphur, phosphorus, potassium, iron, and zinc, as well as the autoxidation of unsaturated fatty acids, all affect the scent of the mushroom[36].

2.4.2 Nutritional values.

P. ostreatus's macronutrients (g/100g) of nutrients in dried mushrooms is measured and it indicates that this mushroom consist of 17-42% proteins, Carbohydrates 37–48% Lipids 0.55–10%, Fibers 24-31%, Minerals 4-10% and Moisture 85-87%[37]. It is quickly coming to light that mushrooms are an intriguing source of new proteins. The isolation of many proteins with distinctive properties includes lignocellulolytic enzymes, lectins, protease inhibitors, and hydrophobins. Numerous medical and biotechnological issues, like microbial drug resistance, poor agricultural yields, and the need for renewable energy sources, can be resolved by them. Conversely, the mass synthesis and commercial use of some fungal proteins demonstrate its technological promise and establish higher fungi as a viable, though little-known, source of

novel proteins[38]. According to several *P. ostreatus* mushroom investigations, the amount of protein level varies between 17 to 42 g per 100 g of dried fruit bodies[39]. Because some of the *Pleurotus* sp. mushroom species comprise complete proteins with a balanced distribution of essential and non-essential amino acids, including ornithine, an intermediate in the biosynthesis of arginine, and GABA, which acts as a neurotransmitter, the proteins from these species are of superior quality[40]. Despite having a low-fat content, *Pleurotus* mushrooms do contain certain necessary fatty acids[41]. Glycogen and other indigestible types of carbohydrates, such as dietary fibres, cellulose, chitin, - and -glucans, and other hemicelluloses including mannans, Xylans, and galactans, are represented by the polysaccharides that make up the majority of the carbohydrates in *P. ostreatus*[42],[41]. In most cases, between 50 and 60 percent of the dry matter in mushrooms is made up of carbohydrates. In addition to monosaccharides, their derivatives, and oligosaccharides (usually referred to as sugars), the carbohydrate also includes reserve and building polysaccharides (glucans)[27]. The group B vitamins, such as thiamine, riboflavin, pyridoxine, nicotinamid, pantotene acid, nicotinic acid, folic acid, and cobalamin, are particularly rich. Other vitamins include ergosterol, biotin, phytochromon, and tocopherols[43].

2.5 Biocomposite.

The word "biocomposite" is currently used to describe a dizzying array of materials made entirely or partially from renewable biomass resources[44]. The necessity to protect our environment has made the idea of bio-based products increasingly important. Biofibers including hemp, sisal, coir, and oil palm are currently being used in a variety of sectors. The automobile industry, particularly for interior applications, is the key sector where the use of these composite materials is expanding[44]. Composite materials made of green resources might lead the material revolution of this century. Sustainability, 'cradle-to-grave' design, industrial ecology, ecological efficiency, and environmentally friendly chemistry are not simply recently popularised buzzwords[44]. The development of biodegradable polymers has been pursued due to the extensive usage of petroleum-based resources. This is based on agricultural and plant products with renewable biofuels that can operate in marketplaces where petroleum-based goods are now king[44]. The creation of entirely biodegradable materials to replace petroleum-based goods is not a cost-effective alternative. Combining petroleum with

biobased resources to create a product with broad uses would be a more practical approach[44]. In order to render the biocomposite revolution a reality, scientists are exploring the different options for mixing biofibres like sisal, hemp, flax, jute, banana, wood, and other plants with matrices of polymers from non-renewable and sustainable sources[45]. Starch and cellulose are the most well-known renewable materials that may be used to create biodegradable polymers[46]. The creation of biodegradable polymers from vegetable oils such as soybean oil, sesame oil, peanut oil, walnut oil, and sunflower oil is another topic that has drawn attention from all around the world. Green composites made of natural fibres and bioplastics based on soy protein have the potential to be used in stiff packaging, housing, and transportation applications[47]. The market for fibre-reinforced composites is currently a multi-billion dollar industry[48]. In order to address the issue of expensive trash disposal, researchers at the BioComposites Centre at the University of Wales, Bangor are producing high-quality packaging for commodities[48]. The main benefits of using environmentally friendly composites were that they are sustainable, ecologically benign, and entirely biodegradable, making them genuinely "green" in every sense. They may be simply discarded or composted after the end of their intended use without causing environmental damage[49]. In various applications, including as mass-produced consumer goods with brief lifespans or goods designed for single-use or short-term usage before disposal, green composites are being employed successfully. Additionally, interior applications with a long useful life are possible for green composites. Bismarck has emphasised the use of biofibres as reinforcement in green composite materials[50]. Hybrid biocomposites have been created as a result of combining many fibre types into a single matrix. The behaviour of hybrid composites is a weighted average of the constituent components, where the inherent benefits and drawbacks are more favourably balanced. Additionally, the benefits of one type of fibre might be combined with the shortcomings of another by utilising a hybrid composite that combines two or more forms of fibre. As a result, good material design might be used to strike a compromise between cost and performance[51]. The amount of fibres in the composite, their length, their orientation, how much they are intertwined, how they are bonded to the matrix, and how they are arranged all have a major role in how the composite behaves. Individual fibre failure strain affects the hybrid composite's strength as well[52].

2.5.1 Mycelium biocomposite.

The vegetative portion of fungi, known as mycelium, has the ability to cement particle substrate and, when produced in a mould, can take on any shape. Expanded Polystyrene (EPS), which is utilised as insulation in the building sector, is one of several hydrocarbon-based materials that Mycelium Biocomposites (MBs) are quickly replacing as environmentally friendly alternatives[53]. Due to the extra variables involved in working with living organisms, new design techniques will also need to be taken into account while constructing MB. A price that may be suitable for large-scale manufacturing low cost products like advanced packaging items and composite sandwich frameworks with specially made porous internal strengthening elements is offered by MB, which gives the designer an additional level of freedom to create shapes and inside geometries previously only seen when using 3D printing[53]. In Europe, the building industry is responsible for almost half of all resources that are taken, a third of all energy used, and about a third of all garbage produced. In order to solve the significant "cradle to grave" environmental challenges with present practices, a green material revolution is unquestionably necessary[54]. Rapid advancements in MB technology over the past 10 years have demonstrated their potential as an environmentally friendly replacement for many materials used in production today, like building insulation or packaging, and we are now witnessing the rise of their commercial application[55]. Many investigations have shown that this material has a wide range of insulating and mechanical qualities that are equivalent to traditional foams made of plastic or even soft woods; heat pressing changed the performance of mycelium composites from foam-like functionality to cork and wood-like efficiency[56]. It is nonetheless possible if given a growth scaffold-like those used in three-dimensional printed tissue manufacturing since mycelium structures are generated rather than manually put together[57]. When high-performing natural insulators like hemp or straw are used as a growth substrate, both of which exhibit low thermal conductivity (0.039–0.08W/mK) and density (57–99 kg/m³), this is also evident with MBs. When compared to conventional EPS insulation, which has a thermal conductivity of 0.03-0.04 W/mK, these figures place them inside the competitive range[58]. The potential for MBs as acoustic insulation is far greater than that of thermal insulation. The power of reflected noise and sound accumulation is decreased by acoustic absorbers, which transform the kinetic movement of molecules of air moving in acoustic waves into heat energy[59]. At 1000 Hz,

which is the dominating frequency from road noise, Pelletier tested MB boards utilising a variety of organic substrates and observed a level of sound absorption of over 70–75%. The maximum absorption was found using a growing substrate made up of 50–50% switchgrass–sorghum, according to comparisons of audio spectra[60]. Mycelium doesn't naturally exhibit any observable fire-retardant properties. However, MBs can get these by adding substrates or fillers that are high in lignin and naturally occurring phenolic polymers like silica (SiO_2). These have shown significantly better thermal degradation, fire reactivity, and safety characteristics[61]. These mycelium composites outperformed the synthetic insulating materials under consideration in terms of the mean peak rate of heat release and the projected time to flashover[61]. Studies on MBs have revealed that they normally exhibit a preference for water-quick absorption, gaining between 40 and 580 weight per cent when exposed to fluids for 48 to 192 hours[62],[56]. When compared to EPS (XPS, AU\$491/m³), volume-specific expenses for MBs were 6 to 12 times less expensive (AU\$40/m³ and AU\$81/m³, respectively). This further supports their competitiveness in terms of manufacturing costs. Agricultural waste is also significantly less expensive in relation to the wholesale cost of polyurethane (8.2-10.4 \$US/kg) and polystyrene (2.1-2.3 \$US/kg)[61].

Chapter 03-

Materials and Methods.

3.1 Materials, Instrumentation and equipment, and Chemicals.

3.1.1 Materials.

	MATERIALS	MANUFACTURER	USAGE
1	Reagent bottle.	Borosil.	Culture maintenance.
2	petri plates.	Tarsons.	Culture maintenance.
3	Inoculation loop.	E-outstanding	Inoculation.
4	Burner.	Pasco.	Inoculation.
5	Measuring cylinders.	Polylab.	Liquid measurement.
6	Surgical blades.	Ruettgers Surgical Pvt. L.t.d.	Inoculation.
7	Plywood.	Greenply.	Mould making.
8	Wooden blocks.	Greenply.	Moulds making.
9	Nails.	Balaji Industries.	Moulds making.
10	adhesive tape.	Cello.	Mould making.
11	Polymer beaker.	Polylab.	Container.
12	Glass rods.	Borosil	[WSE] preparation.
13	Autoclavable bags.	Hi-Media.	incubation.
14	Aluminium foil.	Hindalco Industries Ltd.	Mould covering.
15	latex gloves.	Perfect Surgicare Industries.Pvt.Ltd.	Aseptic handling.

3.1.2 Instrumentation and equipment.

0	INSTRUMENTATION AND EQUIPMENT.	MANUFACTURER	USAGE
1	Laminar air flow cabinet.	S.M. Industries ltd.	Aseptic handling.
2	Compound microscopes.	Olympus.	Microscopy.
3	Hot plate with a stirrer.	Micro scientific works Pvt.Ltd.	Substrate preparation.
4	Incubator.	Orbotech.	Incubation.
5	Autoclave.	Hicon.	Sterilisation.
6	weighing balance.	Citizen.	Weight measurement.
7	dovetail chisel.	Taparia.	Mould making.
8	Angle grinder.	Bosch.	Mould making.
9	Handheld electric planer.	Bosch.	Mould making.
10	Wood cutting cross blades.	Taparia.	Mould making.
11	Mortise chisel.	Taparia.	Mould making.
12	Water baths.	Mac flow.	Substrate preparation.
13	Bench vise.	Stanley.	Mould making.
14	Helico cube mould.	Helico.	Standardised mould.
15	Bajaj gx3 grinder.	Bajaj.	Substrate size reduction.
16	Thermometer.	Labworld.	Temperature monitoring of substrate.
17	311 DS Labnet oven.	Labnet.	Drying and heat killing.

3.1.3 Chemicals.

	CHEMICALS	MANUFACTURER	USAGE
1	Potato dextrose agar [PDA].	Hi-Media.	Culture maintenance.
2	potato dextrose broth [PDB].	Hi-Media.	Culture maintenance.
3	Lacto phenol cotton blue.	Lab Chemie pvt.Ltd.	Staining for microscopy.
4	Distilled water.	Milipore.	Multiple general usages.
5	Calcium sulphate.	Fisher scientific.	Substrate pH maintenance.
6	Calcium carbonate.	Fisher scientific.	Substrate pH maintenance.

BIOLOGICAL MATERIAL.

0	BIOLOGICAL MATERIAL.	Source	USAGE
1	Wheat straw[WS]	Local source.	Substrate.
2	Pine needle[PN]	Local source.	Reinforcement.
3	sawdust[SD]	Local source.	Filler material.
4	<i>Pleurotus ostreatus</i>	ICAR-DMR.Chambaghat,Solan H.P. India.	Binding agent.

3.2 Culture Maintenance.

The culture is maintained on Potato Dextrose Agar plates having composition of 200 g potato infusion, 20 g dextrose and 20 g agar in 1000 ml water. as well as in potato dextrose broth. Potato Dextrose Broth is the same formula as Potato Dextrose Agar, but agar has been omitted. First step for Subculture of *P.ostreatus* is media preparation which is performed by weighing 3.3 grams of potato dextrose agar and 2.4 grams of potato dextrose broth then transferring both separately in two reagent bottles containing 100 ml distilled water. The media is sterilised in an autoclave at a temperature of 121 °C for a duration of 20 minutes. Then the autoclaved media is allowed to cool down at ambient room temperature for 15 minutes In LAF shown in Figure 03.01, 100 ml PDA is then poured into 4 petri plates (25 microliters each) and allowed to solidify without covering with a lid at room temperature inside LAF, whereas PDB was allowed to cool inside same reagent bottle, And then inoculated with *p.ostreatus*.



Figure 03.01.a- (250ml autoclaved PDB)x2.**03.01.b-**inoculated with 1/8th section PDA.

03.01.c-Submerged mycelium culture of *Pleurotus ostreatus* after 18 days of incubation.

3.3 Moulds

For growing a continuous three-dimensional mycelium network, substrates should be of continuous nature and also have uniform packing density. Moulds are necessary for containing the substrate and providing a uniform packing of the substrate with other components present in the substrate.

3.3.1 Standardised mould

The HELICO cube mould shown in Figure 03.02 for this study was provided by the Department of Civil Engineering, Jaypee university of information technology, Solan. Each cubicle mould has 4 side plates of thickness 7mm and a base plate of thickness 4mm and are made up of steel. After inoculation the substrate along with reinforcement and filler material is filled in these moulds and allowed to incubate for a defined period. Specification of these moulds are-

Size 70.6x70.6x70.6 mm.

Cat no. HC 42.10.1 IS 10080.

Model No. L 1258650. Sr.no 3288.

Wall thickness- 7mm.

Base plate thickness- 4mm.



Figure 03.02.a-HELICO cube mould. **03.02.b**-top view, - front view

3.3.2 Wooden mould preparation

Two types of wooden moulds shown in Figure 3.3 were crafted in the workshop lab of the department of civil engineering at Jaypee University of information technology.

Dimensions of the moulds are-

1. **Inner volume - 30cm x 23cm.**
Wall thickness- 5.2 cm. (on each side)
Base thickness- 0.75 cm.

2. **Inner volume - 17cm x 12cm.**
Wall thickness- 1.2 cm. (on each side)
Base thickness- 0.75 cm.

Both moulds are laminated with polyethene adhesive tape to provide a smooth surface finish which will be helpful during the demolding process.

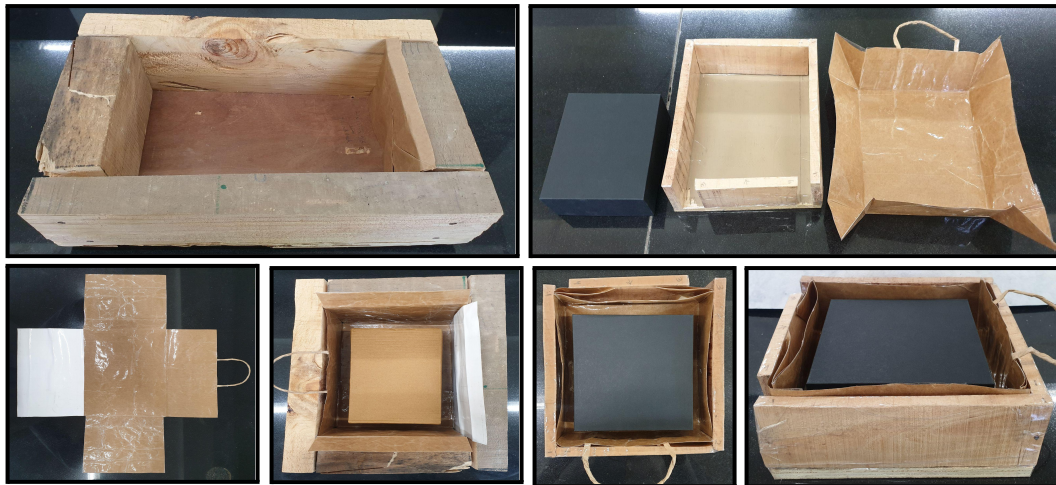


Figure 3.3.a-large primary mould, **3.3.b**-large laminated lining, **3.3.c**-complete large mould with laminated lining and negative mould. **3.3.d**-negative mould, small primary mould, small laminated lining. **3.3.e**- complete large mould with laminated lining and negative mould top view. **3.3.f**-complete large mould with laminated lining and negative mould front-top view.

3.4.1 Measurement of water retention capacity of wheat straw and estimation of the compressed density of wheat straw under no weight.

20 grams of wheat straw is taken in a container and kept in the oven for dehydration at 60 degree celsius for 24 hours then oven-dried wheat straw was weighed and it measured 16.54 grams, 10 grams of wheat straw was separated and compressed in a polymer beaker into a volume of 200 cc. 30 ml distilled water is measured in a measuring cylinder and then poured into the beaker containing the compressed and dried wheat straw then water is allowed to get soaked by the dry wheat straw for 10 minutes at room temperature and then excess water is drained out of the container. The volume of drained water out of the container after soaking was measured to be 15 ml.

The flowchart is shown below in (figure 3.4).

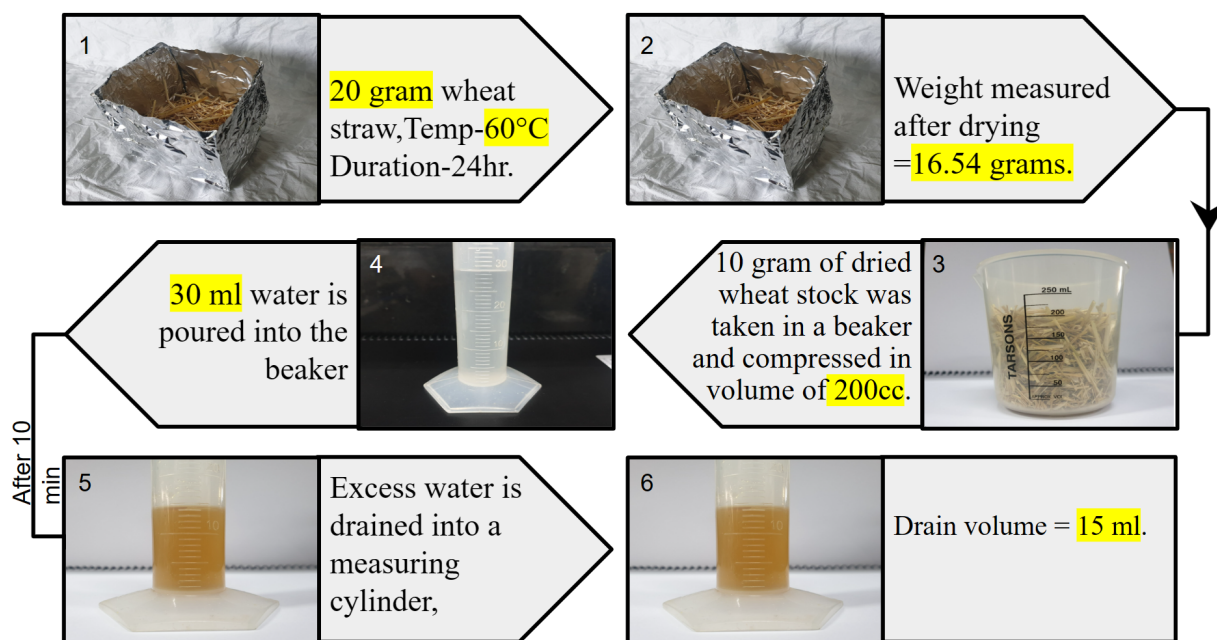


Figure 3.4 -Flowchart representing process of measurement of water retention capacity of wheat straw.

3.4.2 Wheat straw extract[WSE] preparation.

100 grams of wheat straw is weighed and transferred into a beaker. 700 ml of distilled water is added to the beaker containing wheat straw then the beaker is kept over the MH 2lt hot plate at temperature= 80°C and for the duration=1 hour[05] .Total of two batches of the wheat straw extract[WSE] were prepared. Calculated for both batches, the net volume of distilled water used was 1400 ml and the total mass of wheat straw was 200 grams and the total [WSE] formed was estimated to be 1400 ml. However, the total recovery of the final product [WSE] was measured at only 1000 ml. It was assumed that during the process of heating net volume loss due to evaporation=400ml.

3.4.3 Substrate preparation.

Selected substrate for this experiment was wheat straw[WS]. The average size of sourced wheat straw was more than 4 cm which was not fit for the desired results[05]. Size reduction was required to bring down the particle size of [WS] to a smaller size and for that an attempt was made to reduce [WS] size by grinding it in the Bajaj GX3 mixer grinder, but the results were not satisfying enough then an alternate approach was taken for reducing the size by firstly drying the [WS] making it more firm and brittle which eventually makes it better suited for grinding operations. To remove moisture wheat straw is dried in a 311DS Labnet oven for the duration of 24 hours at°C [05]. Then [WS] average size is reduced to 2.6cm ±1.1cm* by grinding it in a Bajaj GX3 mixer grinder, at this stage the processed [WS] is ready for mixing [WSE] and inoculation.

*Average straw size is determined by measuring 10 random straws and measuring their size then calculating its mean.

3.4.4 Biocomposite fabrication by the growth of *p.ostreatus* on wheat straw supplementary with the wheat straw extract.

For the fabrication of biocomposite, A clean polymer container is taken. Then 40 grams of substrate[WS] is weighed on a weighing balance and added to the container, then 60 ml freshly prepared [WSE] is measured in a measuring cylinder and added to the container along with [WS]. All the calculations for required quantities of [WSE] in (ml) per gram of [WS] and estimation of the volume of final biocomposite are performed by the data generated in the the previous experiment named [Measurement of water retention capacity of wheat straw and estimation of the compressed density of wheat straw under no weight.] 60 ml of [WSE] is thoroughly mixed with 40 grams of [WS] by hand for 10 minutes. The container containing this mixture along with Two HELICO cube moulds and a wooden mould is placed under ultraviolet radiation inside the laminar airflow cabinet for 30 minutes for sterilisation.

Two HELICO cube moulds and a wooden mould are completely and tightly filled with the substrate enriched with its own extract. During the process of filling the substrate into the mould, a lining of polyethylene is applied to prevent the biocomposite from getting adhered to the wall of the mould. Then finally the substrate filled in the moulds is aseptically inoculated with 7 days old submerged cultured mycelia of *p.ostreatus*. The mould is then covered with transparent polyethylene film which is then perforated for gaseous exchange.

All the moulds which are filled with the substrate[WS],[WSE] and inoculated with mycelia of *p.ostreatus* was kept for incubation inside a Scigenic Biotech ORBITEK incubator for 20 days at the temperature of 25 degrees celsius and for photoperiod of 24 hours darkness.

3.4.5 Demolding and heat Killing.

After incubation of 15 days the freshly formed biocomposite was now ready for removal from the mould. On the 16th day the mould was taken out of the incubator and kept inside the laminar airflow cabinet under the ultraviolet radiation for 5 minutes[05][06].with all personal safety and precaution like wearing gloves, a face mask , and handling the material under laminar air flow[LAF] cabinet (to avoid backward contamination). Firstly the outer layer of polyethylene was removed from the mould and then the block of freshly formed biocomposite is raised in upward direction by lifting the inner lining out of the moulds, Demonstrated in (figure 3.5). After removal of freshly formed biocomposite from the wooden mould and cube moulds it is now transferred to an autoclavable polymer bag of appropriate size and sealed.

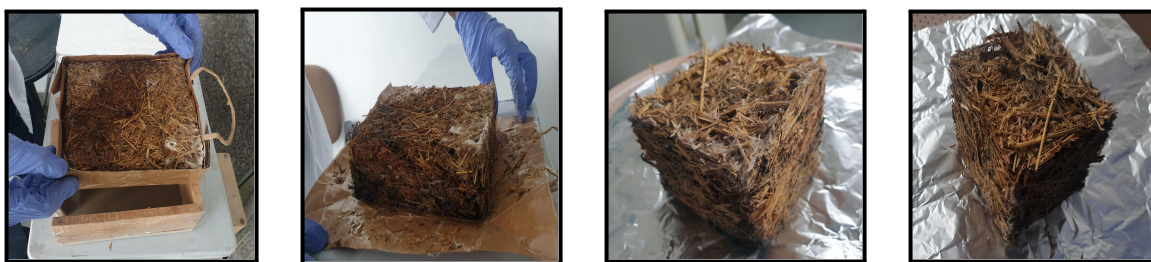


Figure 3.5.a-Growth of *Pleurotus ostreatus* after 15 days on wheat straw supplemented with wheat straw extract in small wooden mould,

3.5.b- demoulded biocomposite from small wooden mould.

3.5.c-demoulded biocomposite from HELICO *cube mould 01*,

3.5.d- demoulded biocomposite from HELICO *cube mould 02*.

Now the bags containing biocomposite are taken out of [LAF] and placed into a convection oven at a temperature of 90 degrees celsius for the duration of 6 to 7 hours[06].During this operation for the first 2 hours the bag remains sealed and then the bag is opened to release pressure caused by the formation of water vapour. After 6 hours, the samples are taken out of the oven and weighed and then they are put back inside the oven without changing the temperature for 20 minutes[06]. After 20 minutes samples are taken out and weighed again, this process is repeated n number of times until weight of sample stabilises [05].

3.5- Growth of *Pleurotus* on wheat straw medium reinforced with pine needles (*p.roxulrghii*) and sawdust in different proportions.

Rationale of this objective is to measure the effect of each individual component of the mixture over the variance and relation with physical and mechanical properties of the finally formed biocomposite with varying proportions of [WS],[PN] and [SD]. The basic components of this process are represented by figure 3.6. For this experiment different proportions of [WS],[PN] and [SD] will be mixed for forming a total of 11 batches, the main issue with dividing quantities of [WS],[PN] and [SD] was there are different moisture content levels, If the mixture components are weighed without drying and mixed together, then due to moisture reduction during drying process the resultant proportion will not remain same as initial state. To solve this problem all the components should be added into the mixture in such a way that their dry masses are divided in desired proportion upon drying at the final stage of formulation. And to achieve this, dry mass is calculated for each individual component that is [WS],[PN] and [SD] in the following experiment.

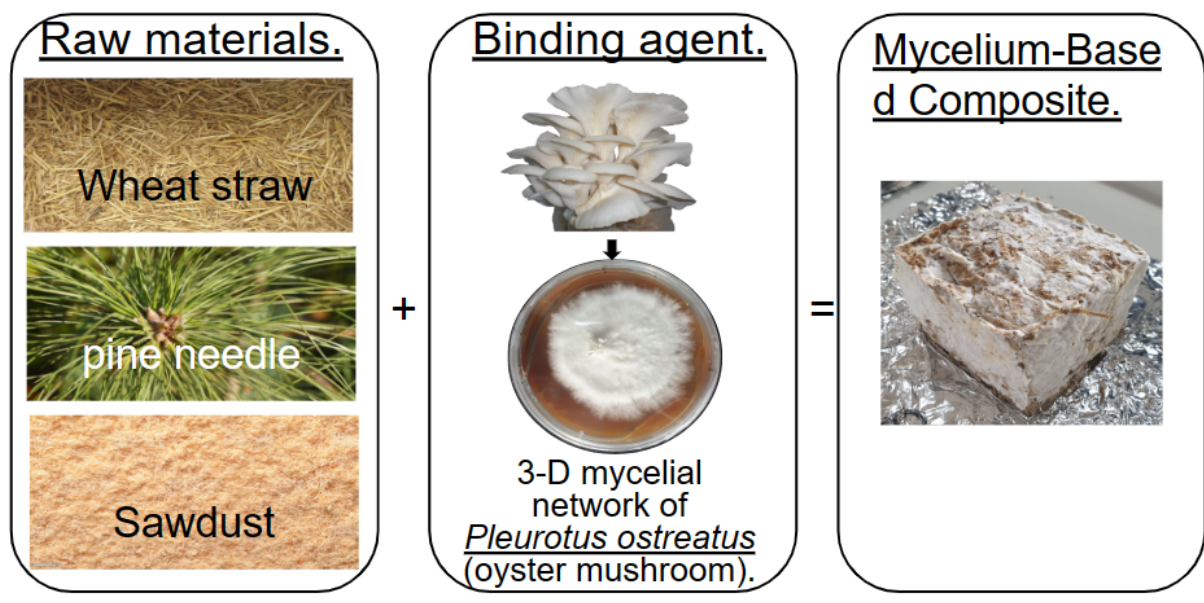


Figure 3.6 - Basic illustration of the process of formulation of mycelium based biocomposite.

3.5.1 Measurement of the dry weight of wheat straw, pine needle, and sawdust by ISO 11465: 1993.

After measurement of the dry weight of wheat straw, pine needle, and sawdust a table is prepared for distributing all the components of the mixture in varying percentages, for example, if we compare batch 03 which is composed of 0%[WS], 100%[PN] and 0%[SD] with batch 07 which contains 33%[WS], 33%[PN] and 33%[SD] according to their dry mass for each, which translate to 0 grams[WS], 53.27 grams[PN] and 0 grams[SD] in batch 03 and 8.96 grams[WS], 17.53 grams[PN] and 10.12 grams[SD] in batch 07, Which eventually add up to 53.27 grams and 36.61 grams for batch 03 and 07 respectively. For the pH regulation, two basic compounds(CaSO₄ calcium sulphate and CaCO₃ calcium carbonate) are also added into each mixture batch in quantities of 0.5 grams and 0.125 grams respectively[04]. Which makes the final mixture 03 and 07 weigh at 53.895 and 37.325 grams respectively.

3.5.2 Table preparation and batch distribution.

Batch	SD%	PN%	WS%	Dry Wt. SD(grams)	Dry Wt. PN(grams)	Dry Wt. WS(grams)	damp wt. SD(grams)	damp wt. PN(grams)	damp wt. WS(grams)	CaSO ₄ (grams)	CaCO ₃ (grams)	Net dry weight	Net weight
1	100%	0%	0%	25	0	0	26.89	0	0	0.5	0.125	25.625	27.515
2	0%	0%	100%	0	0	25	0	0	30.39	0.5	0.125	25.625	31.015
3	0%	100%	0%	0	25	0	0	53.27	0	0.5	0.125	25.625	53.895
4	0%	50%	50%	0	12.5	12.5	0	26.63	15.19	0.5	0.125	25.625	42.445
5	50%	50%	0%	12.5	12.5	0	13.44	26.63	0	0.5	0.125	25.625	40.695
6	50%	0%	50%	12.5	0	12.5	13.44	0	15.19	0.5	0.125	25.625	29.255
7	33.30%	33.30%	33.30%	8.33	8.33	8.33	8.96	17.53	10.12	0.5	0.125	25.615	37.235
8	25%	10%	65%	6.25	2.5	16.25	6.72	5.32	19.75	0.5	0.125	25.625	32.415
9	50%	10%	40%	12.5	2.5	10	13.44	5.32	12.15	0.5	0.125	25.625	31.535
10	25%	20%	55%	6.25	5	13.5	6.72	10.65	16.49	0.5	0.125	25.375	34.485
11	50%	20%	30%	12.5	5	7.5	13.44	10.65	9.11	0.5	0.125	25.625	33.825

Table-01-Table for wheat straw medium reinforced with pine needles and sawdust in different proportions supplemented with gypsum(CaSO₄·2H₂O.) and chalk powder(CaCO₃).

SD%, WS%, PN% vs. Batch

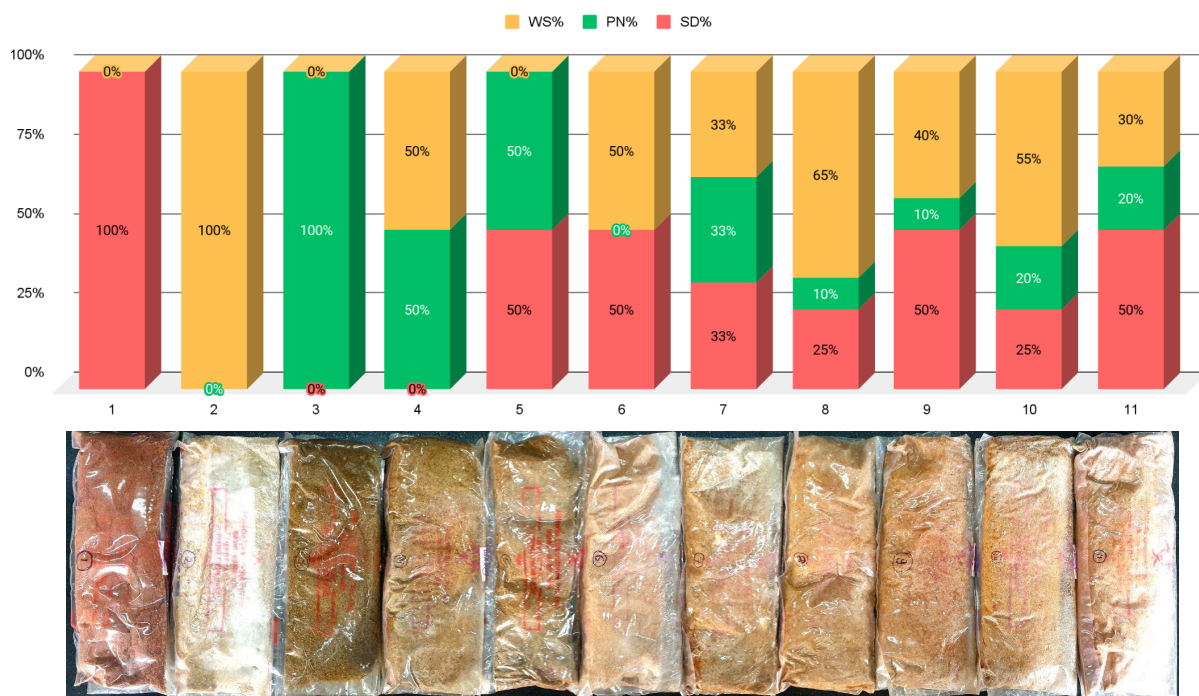


Figure 3.7- Graphical representation for wheat straw medium reinforced with pine needles and sawdust in different proportions with corresponding bar graph.

3.5.3 Substrate preparation [wheat straw].

The selected substrate for this experiment was wheat straw[WS]. The average size of sourced wheat straw was more than 4 cm which was not fit for the desired results[05]. Size reduction was required to bring down the particle size of [ws] to a smaller size. An attempt was made to reduce [WS] size by grinding it in the Bajaj GX3 mixer grinder, but the results were not satisfying enough. Therefore, An alternate approach was taken for reducing the size by firstly drying the [WS] making it more firm and brittle which eventually makes it better suited for grinding operations. To remove moisture wheat straw is dried in a 311DS Labnet oven for the duration of 24 hours at 55 degrees Celsius [05]. Then [WS] average size is reduced to 2.6cm \pm 1.1cm* by grinding it in a Bajaj GX3 mixer grinder. To soften hard substrates for easy mycelial growth, soak them in hot water for an hour at 65-70°C or for 60-120 minutes at 80°C, draining and cooling, It should be noted that this technique is not recommended for large-scale

commercial cultivation[07]. At this stage, the processed [WS] is ready for mixing with other components.

*Average straw size is determined by measuring 10 random straws and measuring their size then calculating its mean.

3.5.4 Reinforcement preparation [pine needles].

The reinforcement material that was the pine needle [PN] selected for the experiment was too large, with an average size of over 12 cm, which was unsuitable for the desired results. To achieve the desired particle size, the [PN] needed to be reduced in size. Initially, an attempt was made to grind the [PN] in a Bajaj GX3 mixer grinder, but the results were not satisfactory. An alternative approach was taken, which involved drying the [PN] to make it more firm and brittle, thereby making it easier to grind. The pine needle[PN] was dried in a 311DS Labnet oven for 24 hours at 55 degrees Celsius to remove moisture and then ground in a Bajaj GX3 mixer grinder, resulting in an average size of $2.6\text{cm} \pm 1.1\text{cm}^*$. To facilitate mycelial growth, the [PN] was softened by soaking it in hot water for an hour at 65-70°C or for 60-120 minutes at 80°C. It is important to note that this technique is not recommended for large-scale commercial cultivation. At this stage, the processed WS is ready to be mixed with other components.

*Average pine needle size is determined by measuring 10 random pine needles and measuring their size then calculating its mean.

3.5.5 Biocomposite fabrication by the growth of *p.ostreatus* on wheat straw media reinforced by pine needles with sawdust as filler material.

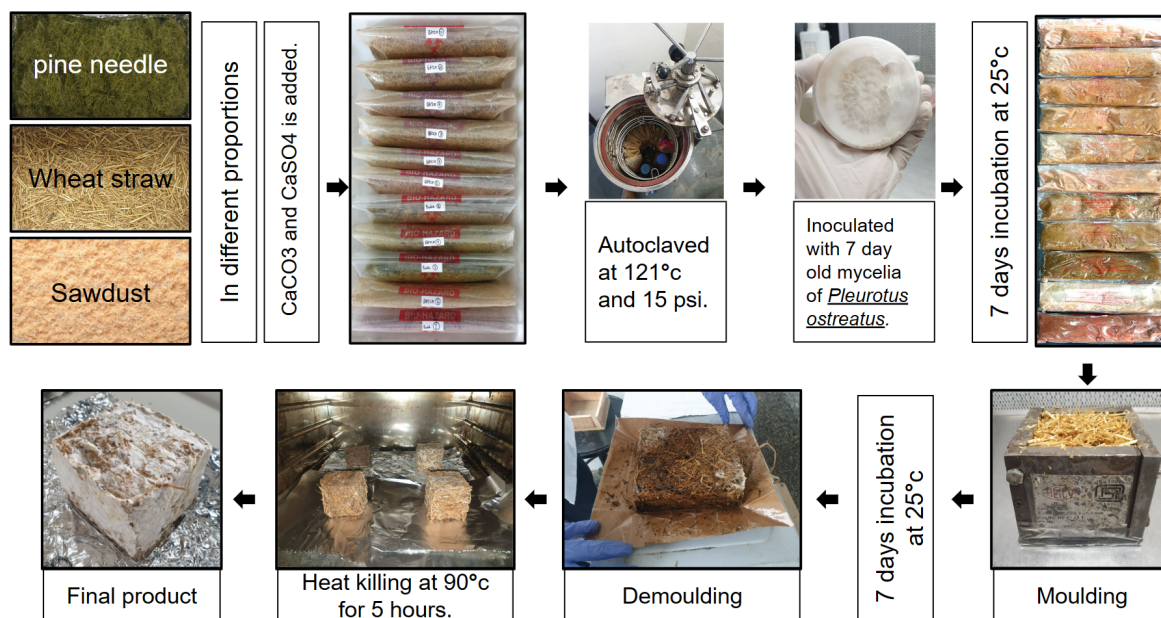


Figure 3.8-flowchart representing the complete process of biocomposite fabrication by the growth of *p.ostreatus* on wheat straw media reinforced by pine needles with sawdust as filler material.

Different quantities of pine needle wheat straw and sawdust are weighted according to the table shown above and kept in autoclavable bags separately without mixing. 11 bags of each material is prepared to add to 33 total bags, Components like -[WS] and [PN] was prepared according to 3.5.3, 3.5.4, and 3.5.5 respectively. Then 3 consecutive bags are mixed together to form batches, Total of 11 batches are prepared and in each batch required amount of basic salts ie- sodium sulphate and sodium carbonate are added according to the distribution table mentioned above. All 11 batches are sterilised in an autoclave at temperatures of 121 degrees Celsius and pressure of 15 psi for 20 minutes. Sterilised bags were taken out of the autoclave and allowed to cool at ambient room temperature for 2 hours. Then all 11 bags are kept under UV radiation inside LAF for 30 minutes. Then inoculation is performed by aseptic transfer of 7 days old mycelium cultures of *p.ostreatus* on a PDA Petri plate to each bag very carefully.

Bags are sealed airtight with the help of adhesive tape. And allowed to incubate at 25 degrees Celsius for 7 days in complete darkness in the inside Scigenic Biotech ORBITEK incubator[05]. After completion of incubation duration of 7 days, bags are taken out of the incubator and kept in LAF. Note - ultraviolet lamps are turned off during the entire process of mould filling. All 11 bags are wiped with ethanol to prevent contamination. Then moulds are also wiped with ethanol for the same reason. Then all the constituents of each bag are packed in separate moulds inside LAF. Eleven HELICO cube moulds are completely and tightly filled with the constituents of 11 respective batches. During the process of filling the substrate into the mould a lining of polyethylene is applied to prevent the biocomposite from getting adhered to the wall of the mould. Then each mould is then transferred inside individual polymer bags and sealed airtight. All the moulds which are filled with 7 days incubated substrate[WS], reinforcement[PN] and filler material[SD], and inoculated with mycelia of *p.ostreatus* was kept for incubation inside Scigenic Biotech ORBITEK incubator for next 7 days at the temperature of 25 degrees celsius and for photoperiod of 24 hours darkness[05]. All the above steps are diagrammatically shown in the form of flowchart in figure 3.8.

3.5.6 Demolding and heat Killing.

After incubation of 7+7 days, the freshly formed biocomposite was now ready for removal from the mould. On the 15th day, the mould was taken out of the incubator and kept inside the laminar airflow cabinet under ultraviolet radiation for 5 minutes[05][06].with all personal safety and precaution like wearing gloves, a face mask , and handling the material under laminar air flow[LAF] cabinet (to avoid backward contamination). Firstly the outer layer of polyethylene is removed from the mould and then the block of freshly formed biocomposite is raised in the upward direction by lifting the inner lining out of the moulds. After the removal of freshly formed biocomposite from the wooden mould and cube moulds it is now transferred to an autoclavable polymer bag of appropriate size and sealed.

Now the bags containing biocomposite are taken out of [LAF] and placed into a convection oven at a temperature of 90 degrees Celsius for a duration of 6 to 7 hours[06]. During this operation for the first 2 hours, the bag remains sealed and then the bag is opened to release pressure caused by the formation of water vapour. After 6 hours, the samples are taken out of

the oven and weighed and then they are put back inside the oven without changing the temperature for 20 minutes[06]. After 20 minutes, samples are taken out and weighed again, this process is repeated “n” number of times until the weight of the sample stabilises [05]. The formulation of biocomposite was completed at this stage and it was packed in aluminium foil and kept inside an airtight plastic bag and then in a cardboard box.

3.6 Composite characterization.

3.6.1 Moisture content (ISO 16979:2003).

Moisture content is an important parameter for materials like biocomposite, It is a major factor and affects the shelf life drastically. Estimation of moisture content is very essential for any composite as it affects the final weight of the product and economy of transportation and shipment of final product is highly dependent over its weight.

The above standards do apply to new building materials, in a satisfactory state of conservation, without sampling restrictions.

To measure the moist weight of the final biocomposite was measured just after the demolding stage which is indicated by “ W_w ”. And dry weight is measured after the drying and heat-killing process until weight stabilises and is denoted by” W_d ” . To calculate the moisture content the following formula is used and the graph is plotted for each of the batches on the x-axis and moisture content on the y-axis.

$$M = \frac{(W_w - W_d) \times 100}{W_d}$$

Where,

M = Moisture content (%).

W_w = Wet weight (g).

W_d = dry weight (g). ³

3.6.2 Dry density (ISO 9427:2003)

ISO 9427 was prepared by the Technical Committee ISO/TC 89, Wood-based panels. This Standard specifies a method for determining the density of wood-based panels. Dry density of the biocomposite is calculated by the following formula and a table was generated.

$$\text{Dry density} = \frac{\text{Oven-dry mass of composite block}}{\text{Volume of composite block.}}$$

Batch	Length (cm)	Width (cm)	Height (cm)	Volume (cm ³)	Mass (g)	Density (g/cm ³)
1	N/A	N/A	N/A	-	-	-
2	6	6.5	6.8	265.2	19.05	0.07183257919
3	7.6	7.6	5.9	340.784	19.33	0.05672214658
4	7.6	7.6	6.1	352.336	21.17	0.06008469189
5	6.3	6.4	6.3	254.016	14.52	0.05716175359
6	6	5.9	6.2	219.48	21.63	0.09855112083
7	6.2	6.4	6	238.08	20.91	0.08782762097
8	6.2	6.3	4	156.24	20.1	0.1286482335
9	6.6	6.6	4	174.24	20.4	0.1170798898
10	7.6	7.6	4	231.04	21.5	0.09305747922
11	7.6	7.6	4	231.04	21.1	0.09132617729

Table 2- Dry density of biocomposites.

CHAPTER 04-RESULTS AND DISCUSSION

4.1 Water retention capacity of [WS].

The water retention capacity of wheat straw was calculated with the following equation:

$$\text{Water retention capacity (ml/g)} = \frac{\text{volume of H}_2\text{O added} - \text{volume of H}_2\text{O drained}}{\text{Weight of wheat straw}}$$

$$\text{Water retention capacity (ml/g)} = \frac{(30 \text{ ml} - 15 \text{ ml})}{10 \text{ grams}} = \frac{15 \text{ ml}}{10 \text{ grams}} = 1.5 \text{ ml / gram.}$$

4.2 Growth of *P.ostreatus* on [WS] supplemented with [WSE].

Significant growth was not observed when *p.ostreatus* was grown on wheat straw supplemented with [WSE], This substrate was also very susceptible to bacterial and fungal contamination. Maximum growth of *p.ostreatus* was observed around the edges of the moulds and contaminations was observed at the centre position of the upper surface directly below the aeration holes, suggesting the source of contamination was ambient air which entered through a perforation in the laminating sheet carrying air-born microbes. After demolding and heat killing the final biocomposite blocks were able to stand their own weight without collapsing and showing any sign of cracking.



Figure 04.1.a- demoulded and heat killed biocomposite from small wooden mould.

04.1.b- demoulded and heat kill biocomposite from HELICO *cube mould 01*,

04.1.c- demoulded and heat killed biocomposite from HELICO *cube mould 02*.

4.3 The dry weight of [WS],[SD], and [PN].

4.3.1 Measuring the dry weight of wheat straw[WS].

Dry weight of wheat straw was calculated using the following equation :

Freshly dried wheat straw weighs = 12 grams.

Oven dried at 75°C. for 4 hours.

Dry weight = 9.87 grams.

Dry mass of 100 grams of wheat straw (g) =	$\frac{\text{Oven dried weight of sample (g) X 100}}{\text{Weight of sample before drying process (g).}}$
--------------------------------------------	-----------------------------------------------------------------------------------------------------------

$$\begin{aligned}
 \text{Dry mass of 100 grams of wheat straw} &= \frac{9.87 \text{ grams X 100}}{12.00 \text{ grams.}} \\
 &= 82.25 \text{ grams}
 \end{aligned}$$

4.3.2 Measuring the dry weight of sawdust[SD].

Freshly dried sawdust weighs = 10 grams.

Oven dried at 75°C. for 4 hours.

Dry weight = 9.295 grams.

Dry mass of 100 grams of fresh sawdust (g) =	$\frac{\text{Oven dried weight of sample (g) X 100}}{\text{Weight of sample before drying process (g).}}$
----------------------------------------------	-----------------------------------------------------------------------------------------------------------

$$\begin{aligned}
 \text{Dry mass of 100 grams of fresh sawdust} &= \frac{9.295 \text{ grams X 100}}{10 \text{ grams.}} \\
 &= 92.95 \text{ grams}
 \end{aligned}$$

4.3.3 Measuring the dry weight of pine needles [PN].

$\text{Dry mass of 100 grams of fresh pine needle (g)} = \frac{\text{Oven dried weight of sample (g)} \times 100}{\text{Weight of sample before drying process (g).}}$

$$\begin{aligned} \text{Dry mass of 100 grams of fresh pine needle} &= \frac{8.05 \text{ grams} \times 100}{17.15 \text{ grams.}} \\ &= 46.93 \text{ grams} \end{aligned}$$

It was observed that the dry weight of [WS],[SD] and [PN] are not the same. Calculation indicates [PN] is lightest with (46.93) grams per 100 grams of freshly picked green [PN], Similarly, [WS] was found to have dry weight of (82.25) grams per 100 grams of sun dried [WS]. Whereas, Dry weight of [SD] was measured (92.95) grams per 100 grams of ambient moist [SD], Giving order of dry weight of mixture compositions are ([SD]>[WS]>[PN]). Percentage weight loss due to evaporation caused during drying by the heating process was also not the same for obvious reasons.

It was measured that upon heating [PN] lost maximum weight (53.07%), [WS] lost some weight (17.75%), and [SD] lost negligible weight (07.05%), Resulting order of weight loss percentage ([PN]>[WS]>[SD]), which is inverse of dry weight order.

Since, water is not the only compound which evaporated on exposure to heat, But other volatile compounds must have been evaporated during the process as olfactory detection of different characteristic aroma and fragrance were registered, Suggesting the above assertion.

4.4 Growth of *P.ostreatus* on [WS] substrate and [SD] as filler material reinforced with [PN] in different proportions.

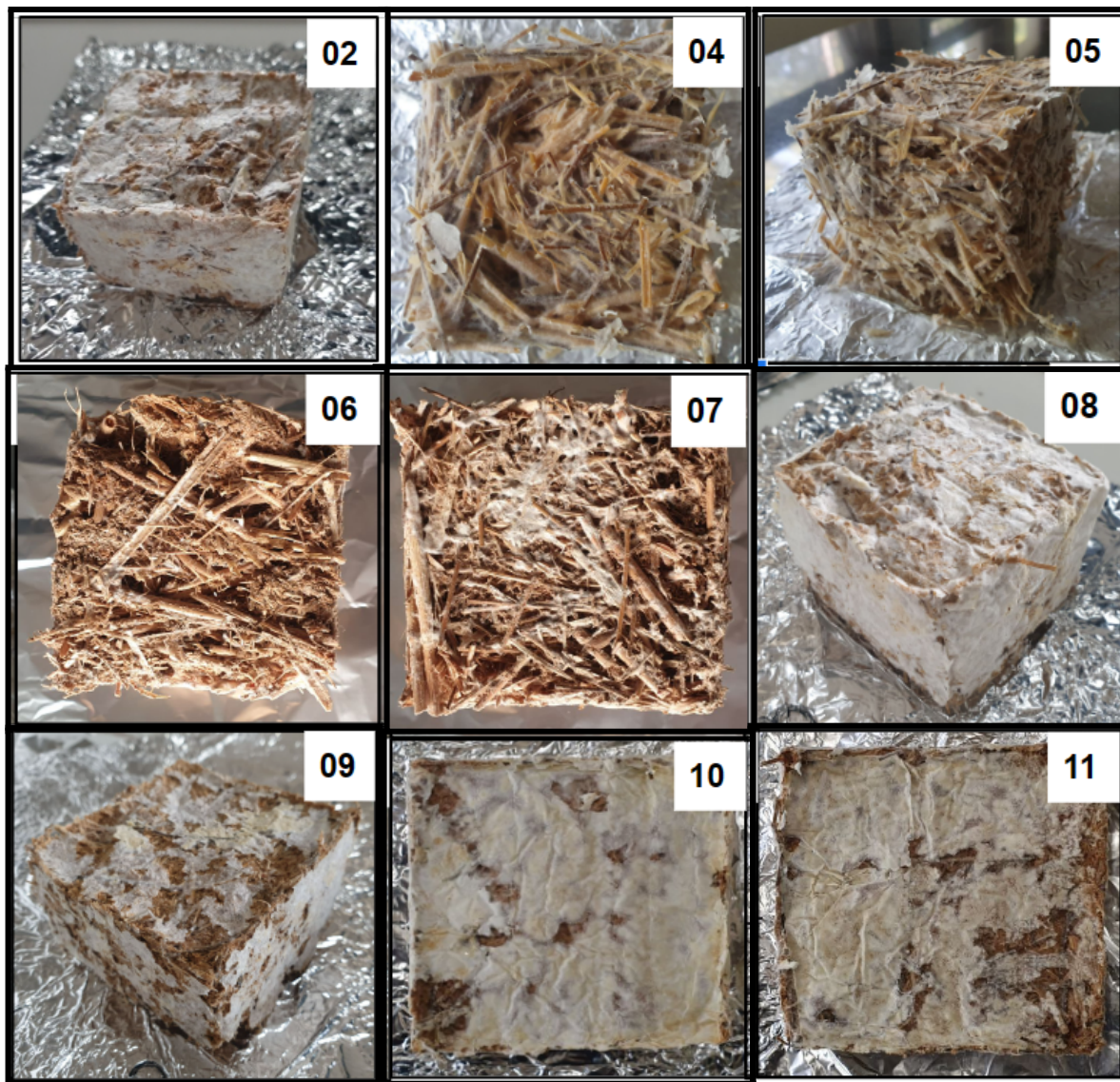


Figure 4.2-(batch 02,04,05,06,07,08,09,10,11) Blocks of heat killed and dried biocomposite showing growth of *Pleurotus ostreatus* after 7+7 days on wheat straw medium reinforced with pine needles and sawdust in different proportion.

The mycelium of *p.ostreatus* grows very quickly on the substrate.

After inoculating all the batches of [WS], [SD] and [PN] mixture, (from batches 01-11), mycelia of *p.ostreatus* covers the entire upper surface of the substrate.

Upon visual inspection, the estimation of growth is assumed on a scale of (01 to 10), by which maximum coverage by a white filamentous sheet-like appearance over the substrate surface is rated (10) and minimum or no growth is rated (00).

Conformation of growth of the desired mycelium of selected fungal strain is achieved via microscopy.

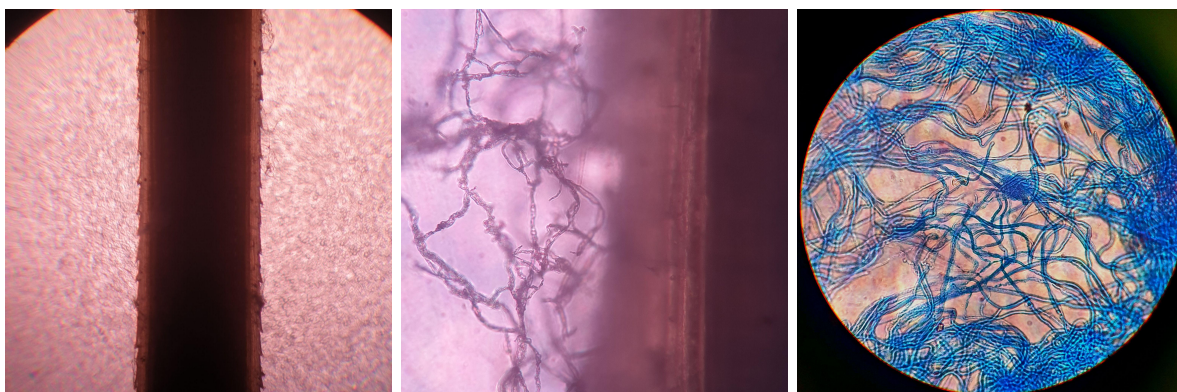


Figure 4.3.a- Transverse plane view of pine needle at 10X magnification under the the microscope .

4.3.b-mycelium of *p.ostreatus* attached over the outer surface of pine needle at 40X magnification under a microscope,

4.3.c- mycelium of *p.ostreatus* sampled from the biocomposite surface before heat killing and dyed with lactophenol cotton blue dye, captured at 40X magnification under the microscope.

Growth of *p.ostreatus* mycelium is also visually inspected on different substrate [WS], Filler material [SD] and reinforcement [PN] composition and it is observed that mycelia of *p.ostreatus* shows better growth on the mixture that contain more amount of substrate [WS], and with increasing percentage of reinforcement [PN], mycelium growth was observed decreasing. Filler material [SD] does not show any substantial effect on the growth of mycelium of *p.ostreatus*. The following graph plotted between growth (y-axis) and different batches (x-axis) indicated the growth of mycelium of *p.ostreatus* on different mixtures of [WS],[SD] and [PN].

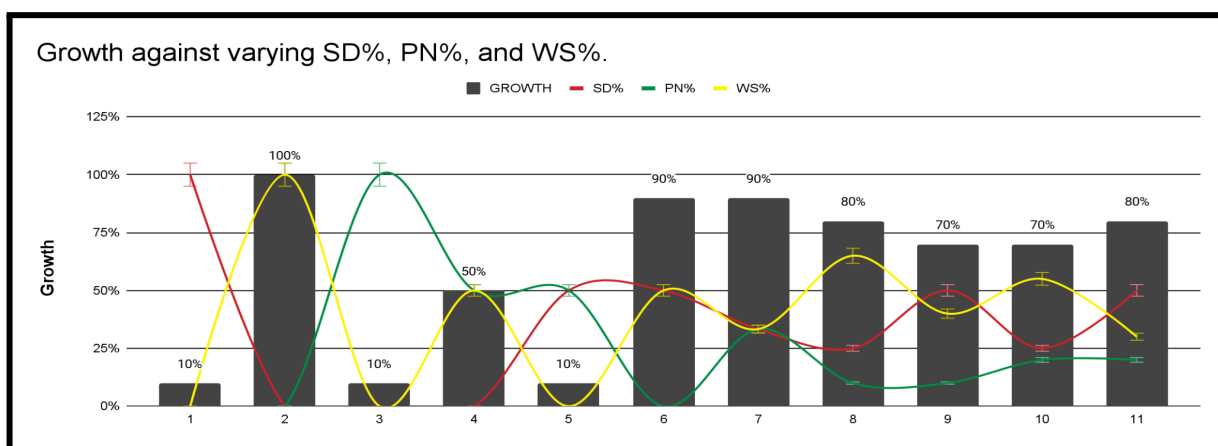


Figure 4.4-Graph of Growth of *Pleurotus ostreatus* after 7+7 days on wheat straw medium reinforced with pine needles and sawdust in different proportions.

From the above graph, it can be interpreted that mycelia of *p.ostreatus* shows better growth on the mixture that contain more amount of substrate [WS], Batch 02 (100%[WS]) shows maximum growth, and with an increasing percentage of reinforcement [PN], mycelium growth was observed decreasing, Batch 03 (100%[PN]) shows minimum growth. Filler material [SD] was unable to support growth in the absence of other components, Batch 01 (100%[SD]) shows minimal growth, but does not show any substantial effect on the growth of mycelium of *p.ostreatus* upon its addition.

4.5 moisture content of moist and freshly de-moulded biocomposite of *P.ostreatus* on [WS] substrate and [SD] as filler material reinforced with [PN] in different proportions.

Moisture content of batch 03 was calculated 90.80%, Which was highest among all 11 batches, And lowest moisture content was calculated for batch 01 which was only 7.37, However the resultant biocomposite from batch 01 and 03 were not able to withstand their own weight and collapsed, and considered unsuccessful. So, Among the successful batches (i.e.-except batch 01 and 03) batch 06 was having least moisture content =14.16%.

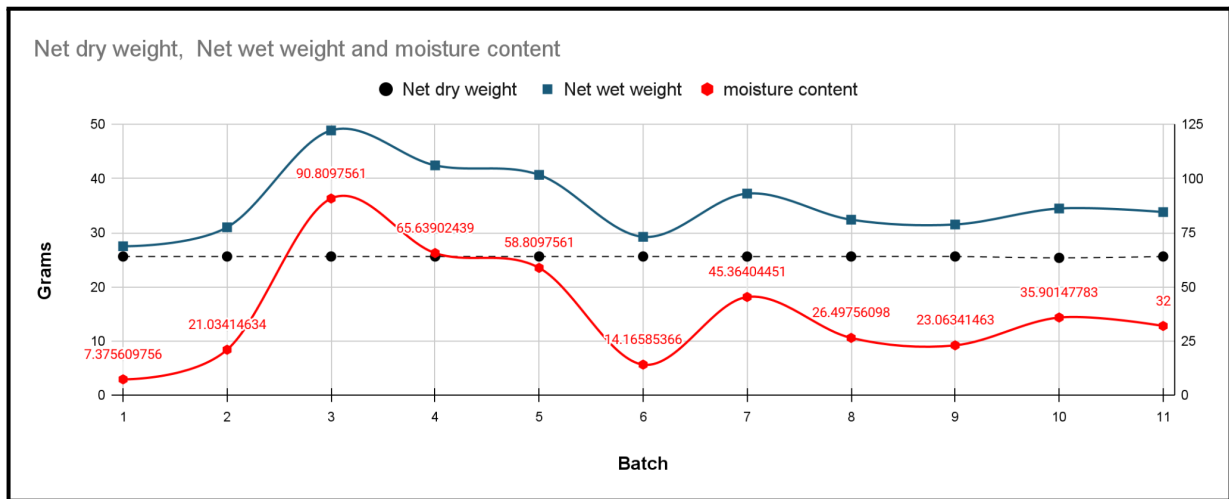


Figure 4.5- Net dry weight, Net wet weight and moisture content against batches.

4.6 Dry density of heat-killed and dried biocomposite of *P.ostreatus* on [WS] substrate and [SD] as filler material reinforced with [PN] in different proportions.

The maximum dry density of final heat-killed and dried biocomposite is observed in batch 08 mixture = 0.128 grams/cm³ which was composed of 65% WS, 25% SD and 10% PN. And least dry density was observed in batch 03 = 0.056 grams/cm³ which contains 100%PN only. However the resultant biocomposite from batch 01 and 03 were not able to withstand their own weight and collapsed, and were considered unsuccessful. So, Among the successful batches (i.e.-except batch 01 and 03) batch 05 was having dry density = 0.0571 grams/cm³.

A trend can be observed between dry density and percentage of [SD] in the mixture composition which indicates increasing dry density with increasing [SD]%, Scope of discussion emerges for the dependency of dry density over [SD]% only, And the possible reason for the above-stated dependency can be described as [SD] is relatively smaller in size as compared to [PN] and [WS], And occupied the voids in the 3D matrix of substrate and reinforcement, Also [SD] is denser than [WS] and [PN] resulting in a tight arrangement and high dry density of final biocomposite.

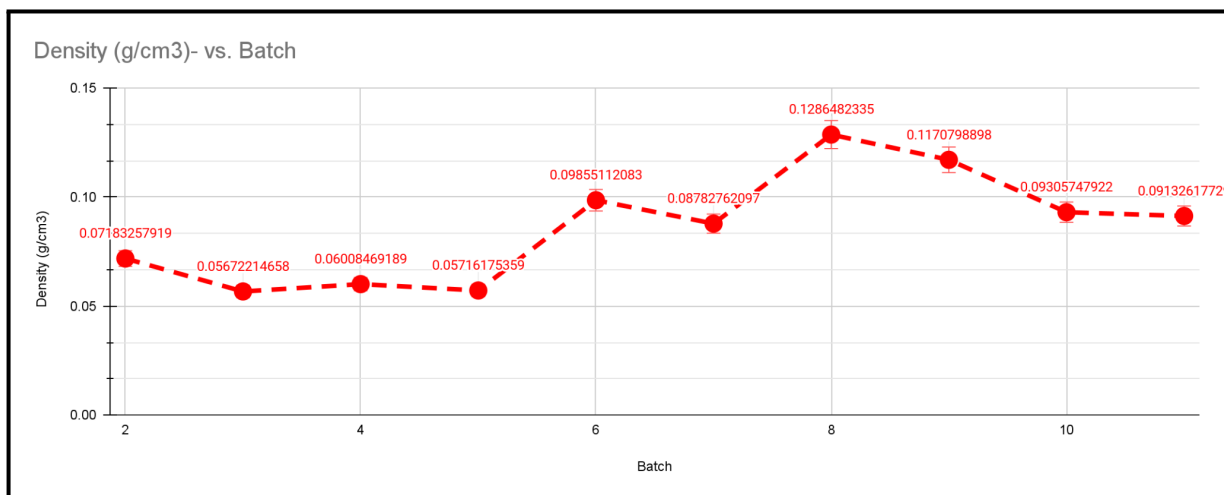


Figure 4.6- Dry density vs batch

CONCLUSION-

Thus it can be concluded that the mycelium based biocomposite fabricated using wheat straw as a substrate, pine needles as reinforcement and sawdust as filler material shows promising possibilities for various applications. In addition, utilisation agro wastes helps in reducing the mountain range of wastes, converting them into a green biocomposite which can be used as a cushion-providing internal packaging material, alternative cattle feed, Fuel blocks for domestic usage and organic fertilisers in crop fields. This represents one of the world's untapped resources of green composite in the future and because mycelium based composite fabrication needs only a small space, land can be conserved. Thus the use of wastes can provide more food, more jobs, better family income, and improved living standard, curb global warming and clear up the crop residues on road sides and forest margins.

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