Effects of Nutrient Sources and Environmental Factors on the Cultivation and Yield of Oyster Mushroom (Pleurotus ostreatus)

V.I. Ibekebe¹, P.I. Azubuike¹, E.U. Ezeji² and E.C. Chinakwe¹
¹Department of Industrial Microbiology, Federal University of Technology, P.M.B. 1526, Owerri, Nigeria
²Department of Biotechnology, Federal University of Technology, P.M.B. 1526, Owerri, Nigeria

Abstract: Oyster mushroom (Pleurotus ostreatus) was cultivated on different carbohydrate substrates (millet, corn, rice and rye). Millet gave the highest mycelia yield while rye gave the lowest. Further growth on different concentrations of millet extract showed that 1.00 mg mL⁻¹ concentration of millet extract gave an optimum mycelia growth of 4.0 mg mL⁻¹. Cultivation of the mushroom on different nitrogen sources namely, lima beans extract, soya beans (Glycine max) and vigma species (brown beans) showed that soya beans (Glycine max) gave the highest mycelia yield while lima beans gave the lowest yield. Optimum mycelia yield was also achieved at pH 6.5. This study shows that given the right substrate and optimal environmental conditions, oyster mushroom can be mass-produced to meet the nutritional requirements of the Nigerian populace.

Key words: Edible mushroom, Pleurotus ostreatus, mycelia growth, substrates

Introduction
Mushroom belongs to the genus known as fungi. They are classified based on edibility and shape of the fruit body. Pleurotus ostreatus (oyster fungus) is an edible mushroom having excellent flavour and taste (Shah et al., 2004). It belongs to class Basidiomycetes, subclass Holobasidiomycetidae, order Agarics. It grows wild in the forests of hilly areas and is cultivated in temperate and subtropical regions of the world. Mushroom has been widely cultivated since the 1700’s and presently more than 30 unknown species are cultivated as foods. Total world production of edible mushrooms in 1994 was 4.9 million tonnes (Chang, 1999). Mushroom cultivation has various advantages: it is easy to propagate due to its numerous spores, has high nutritional quality, utilizes a large variety of agricultural wastes such as cotton wastes, wood wastes, palm wastes and rice bran. Pharmacologically mushroom functions in the stimulation of blood lymphocytes, muscle relaxation and disease resistance. Mushroom has also been successfully used in the bioremediation of polluted environment (Stamat, 1993). Mushroom can be grown on different carbon sources. Fasidi and Kadiri (1993) reported the successful growth of mushrooms on lignocellulose wastes such as begusse, banana, plantain leaves, cereal straw, cassava peals, coconut core, cotton waste (k p o l a) and paper wastes, which provided the essential nutrients required for its growth. Other factors that affect mushroom growth include moisture content, temperature, pH and light intensity (Stamat, 1993; Kadiri and Kehinde, 1999).

This study examines the factors that enhance the optimum growth and cultivation of the oyster mushroom.

Materials and Methods
Sources of oyster mushroom: The cultivated mushroom was collected from an Agricultural Multinational Farm, Zartech at Ibadan, Nigeria. The fruit bodies of the mushroom were cut with a sterile knife and taken to the laboratory in an aseptic plastic transparent Ziploc bag.

Growth substrates and reagents: The cereal grains (millet/sorghum, maize rice, rye) and nitrogen sources (lima beans, soya beans and vigma beans species) were purchased from Ekeonunwa market, Owerri, Nigeria. All reagents and chemicals used were of analytical grades.

Isolation of spores: The mushroom spores were isolated by removing the stem from the mushroom and placing it on a dark coloured paper with the gills down onto the paper. It was then covered with a glass and allowed for 26 h after which a visible white outline of the spore print of the mushroom was obtained. Pure culture spores were obtained by culturing on a Malt extract Agar and Gentamicin sulphate.

Cultivation of mushroom: The isolated mushroom spores were seeded on a prepared millet grain in a Mason Quart jar. The grain jar was stored in a low area and shaken intermittently until grain colonization was completely ensured. The colonized grain was mixed with sawdust contained in a sterile Ziploc transparent bag. Small holes were punctured for adequate aeration and sufficient drainage. The mycelia-colonized sawdust block was placed in a bucket filled with ice and allowed to soak for 2-3 days after which it was drained and the mushroom block
Ibekwe et al.: Yield of Oyster Mushroom

Effect of different nitrogen sources on mycelia growth: Nitrogen sources used were lima beans extract, soya beans (glycine max) and vigna species (brown beans) at 0.5 mg mL\(^{-1}\) concentration each in malt extract broth medium. After 3 days of incubation the different mycelia growth were obtained and used to assay each nitrogen source.

Effect of initial pH of substrate medium on mycelia growth: The effects of different initial pH on mycelia growth of Pleurotus ostreatus was investigated in a Malt Extract Broth medium. The pH ranges were 5.0, 5.5, 6.0, 6.5, 7.0, 8.5 and 9.0. They were obtained using strong and mild acetic acid solution and strong and mild dilutions of sodium hydroxide solution. After 3 days of incubation at room temperature 28±2°C, the effect of initial pH of substrate on mycelia growth was carried out to determine the optimum initial pH of substrate for mycelia growth.

Results
The effect of different carbohydrate sources on the mycelia growth is shown in Fig. 1. The highest mycelia growth (9.71 mg mL\(^{-1}\)) was achieved using millet extract while rye gave the least mycelia growth (9.47 mg mL\(^{-1}\)). Growth of the mushroom in different concentrations of millet extract shows that 1.00 mg mL\(^{-1}\) gave the highest mycelia yield (Fig. 2). Fig. 3 shows the mycelia yield in different nitrogen sources. Maximum yield was achieved using soya beans extract (glycine max). Fig. 4 shows the effects of pH on the mycelia growth. Optimum mycelia yield was observed at pH 6.4 and in the absence of light. There were no differences in mushroom yield at pH lower than 5.3, while a decrease in mycelia yield was obtained between pH 6.4 and 7.5.

Discussion
In this study, the mycelia of oyster mushroom (Pleurotus ostreatus) were isolated through its spores from a freshly harvested fruit body on a Malt Extract Agar medium and gentamicin sulphate as a bacteria inhibitory agent. The study suggested that the rate of production of oyster mushroom from its mycelia stage was significantly influenced by the carbohydrate source, different concentrations of carbohydrate, nitrogen sources, availability of light, initial pH of substrate, incubation period and the control of contamination during spawn generation. This agrees with the earlier report of Chang and Miles (1989) that mushroom production was influenced by pH of substrate medium, type of substrate medium, light availability, temperature and degree of aeration.
Among the different carbohydrate sources (millet, rice, maize and rye) studied, millet proved the best carbohydrate source for the production of mushroom.

Fig. 1: Growth Yield of Oyster Fungi on Different Carbon Sources

![Graph showing growth yield of oyster fungi on different carbon sources](image1)

Fig. 2: Mycelia growth of Oyster Fungi on different concentrations of millet extract

![Graph showing mycelia growth on different concentrations of millet extract](image2)

set for fruiting. Fruiting is carried out at temperatures between 70 and 80°C and carried out between 80 and 90% sunlight and fresh air.

Effects of different carbohydrates sources: The effects of different carbohydrate sources were investigated in a solution containing starch. The different sources used were millet, corn, rice and rye at 0.5 mg mL\(^{-1}\) concentrations each in a Malt Extract broth medium. After 3 days of incubation the different dry weights were obtained and used to assay against each carbohydrate source.

Effects of different concentrations of millet extract: The effects of different concentrations of millet extract on mycelia growth were investigated in a MEB medium. The concentrations were 0.025, 0.050, 0.075, 0.100, 0.125, 0.150, 0.175, 0.200, 0.225 and 0.250 mg mL\(^{-1}\). After 3 days incubation at 28±2°C, various dry weights were obtained using a digital balance.
Ibekwe et al.: Yield of Oyster Mushroom

Fig. 3: Growth of oyster fungi on different nitrogen sources

Fig. 4: Effect of different initial pH of substrate on mycelia growth

mycelia. This is in agreement with the work of Cangy (1994). It should be noted that millet contain more protein than others and its small size provides a large surface area for the mycelia hyphae.

The study showed that maximum mycelia growth of *P. ostreatus* was obtained at 1.00 mg mL$^{-1}$ concentration of millet extract while other concentrations such as 0.025 mg mL$^{-1}$, 0.050 mg mL$^{-1}$, 0.075 mg mL$^{-1}$, 0.150 mg mL$^{-1}$, 0.175 mg mL$^{-1}$, 0.200 mg mL$^{-1}$, 0.225 mg mL$^{-1}$, and 0.250 mg mL$^{-1}$ concentration gave unstable trends of increase and decrease in mycelia production as this could be due to the direction of light.

The production of mycelia in mushroom cultivation is very sensitive to light availability. In this study, the absence of light was found to yield the best, while the alternation of light and darkness gave good yield but continuous light gave little growth yield. This result is similar to that of Zandrazil (1982) who reported that most mushroom mycelia growth gave better yield in the absence of light while fruit bodies gave better yields during light and darkness alternation.

Optimum mycelia production was recorded at pH 6.4 while pH range less than 5 showed no significant growth. This report is in line with the findings of Quinio et al. (1990). Decrease in mycelia growth at lower pH could be due to the toxicity of very acidic pH to the hyphae. However Bilgrami and Verma (1992) reported that mushroom mycelia are more tolerant to acidic media than basic media. Zandrazil (1978) reported that the growth of *Pleurotus ostreatus* and *P. eryngi* mycelia were positively affected by pH range of 4-6.

References