

STUDIES ON THE BIOMASS PRODUCTION OF  
PLEUROTUS OSTREATUS USING PEAT  
EXTRACT AS SUBSTRATE

CENTRE FOR NEWFOUNDLAND STUDIES

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WIREKO MANU-TAWIAH









**STUDIES ON THE  
BIOMASS PRODUCTION OF  
Pleurotus ostreatus USING  
PEAT EXTRACT AS SUBSTRATE**

By



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B.Sc. Hons (Food Science)**

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## ABSTRACT

Pleurotus ostreatus mushroom mycelium was grown in submerged culture with acid extract from peat as a basic substrate. The proximate chemical composition of the peat extract was determined. The total reducing sugars constituted about 50% of the total carbohydrate (TCH) concentration. The total nitrogen content was low at about 0.6 g/L of the peat extract. The total ash was about 4.5%, of which 1.2% was  $P_2O_5$  and 0.87%  $K_2O$ .

Different concentrations of water-diluted peat extract were tested in shake flask experiments in an attempt to overcome the effect of growth inhibitors apparently present in non-diluted peat extracts. The best results were obtained with a ratio of one part peat extract diluted with one part water. Several operating variables were studied to optimize the growth of the mycelial biomass of P. ostreatus. The best results produced approximately 5 g/L of dry biomass with a yield of 60% and an efficiency of 33%. The results were obtained in 192 hours at an inoculum ratio of 5.0% (v/v), an incubation temperature of  $28 \pm 1^\circ C$ , an initial pH of  $5.0 \pm 0.1$  and an agitation speed of 150 rpm. The addition of 1.5% glucose to the diluted peat extract medium increased the growth to approximately 14 g/L of dry biomass with a yield of 70% and an efficiency of 45%, indicating that the diluted peat extract was deficient in carbon.

The nutritional requirements of the P. ostreatus mycelium grown in peat extract were studied to enhance the growth of the mycelial biomass. The fungus grew better on hexose sugars (glucose and mannose) than on pentose sugars (xylose and arabinose) and required

additional nitrogen, phosphorus and potassium for growth. Growth was also better on organic sources of nitrogen than on ammonium salts of inorganic acids. The addition of 5.0 g/L yeast extract and 4.0 g/L  $\text{KH}_2\text{PO}_4$  to the peat extract media produced approximately 7 g/L dry biomass with a yield of 73% and an efficiency of 46%.

Agitation and aeration were optimized and the dissolved oxygen concentration was monitored in a 2 Liter fermenter to study the growth of the P. ostreatus mycelium biomass in the peat extract. The best results produced approximately 9 g/L of biomass with a yield of 72% and a productivity of 70 mg/L/hour. These results were obtained in 96 hours at an agitation speed of 200 rpm, an aeration rate of 1.0 vvm, an inoculum ratio of 5.0% (v/v), an incubation temperature of  $28 \pm 1^\circ\text{C}$  and a pH of  $5.0 \pm 0.1$ . Higher agitation speeds and aeration rates produced lower growth and a dispersed mycelial growth form. The morphology of the P. ostreatus growth form was also affected by the pH and the media composition.

The chemical composition of the P. ostreatus mycelium biomass was analyzed and compared with that of the fruiting body produced on solid peat to study the nutritional quality of the product. The mycelium contained approximately 40% crude protein, 8% ash, which contained K, P, Ca and Mg as major minerals, 36% TCH, 6% fiber and 3% total fat. The protein contained 17 amino acids, including all the essential amino acids, and was high in threonine, lysine, tryptophane, arginine, leucine and phenylalanine, but was deficient in methionine when com-

pared with egg protein. Three essential fatty acids (palmitic, oleic and linoleic acids) were identified in the biomass.

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Nana, Esie ne Kagya Eni Aseda.

W. M. T.

## ABBREVIATIONS

AR = aeration rate

AS = agitation speed

CP = crude protein content

D.O. = dissolved oxygen

DR = dilution ratio

E = efficiency

FpH = final pH

FT = fermentation time

IpH = initial pH

ITCH = initial total carbohydrate

PE:W = peat extract:water ratio

RTCH = residual total carbohydrate conc.

T = incubation temperature

TCH = total carbohydrate

vvm<sub>a</sub> = volume of air per volume of medium per minute

X = dry biomass

Y = yield

YE = yeast extract

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## CHAPTER 1.

### INTRODUCTION

#### 1.1. Background

The expanding world population brings with it an enormous increase in the demand for food, particularly protein. It is estimated that more than two-thirds of the world's population live in less developed countries, where there are limited resources and food production. It has also been shown that some of the protein sources consumed by man and domestic animals in most less-developed countries are of low quality (Bano and Rajarathnam, 1982). With the world's population estimated to double by the year 2000, the supply of protein per person is likely to decrease (Steinkraus, 1980) since conventional measures such as the cultivation of additional land, widespread use of fertilizers, and species improvement may not be able to meet the higher protein demand.

Science and technology are therefore faced with new tasks to search for further sources of protein. This search has led to the refinement and genetic improvement of vegetable proteins, "krill" fishing in the Antarctic and single cell protein (SCP) production (Dimmling and Seipenbush, 1978; Robinson and Davidson, 1959).



## 1.2. Single cell protein (SCP)

SCP is cultured microbial biomass from certain types of microorganisms that can produce valuable proteins. These microorganisms include algae, bacteria, yeasts and fungi (Reed, 1982). They have contributed traditionally to the supply of food through their use of fermentation processes, such as those for the alcoholic drinks, cheese, vinegar, bread, meso, tempe and soy sauce, and in the form of mushrooms. In some of these foods, both microbes and substrates are consumed by humans. The incidental consumption of microbes by humans in fermented foods and by domestic animals in feeds is, therefore, quite old (Reed, 1982). In almost all of the above fermented foods, microbial protein contributes greatly to the nutritional value of the diet.

### 1.2.1. Nutritional value of SCP

SCP compares well with high quality protein sources in terms of crude protein supply and the overall pattern of nutrients (Miller, 1968), and is suitable for use in foodstuffs for animal husbandry and aquaculture and, in a refined form, possibly as a protein supplement in the human diet (Dimmeling and Seipenbush, 1978; Litchfield, 1977). Table 1.1 shows the gross chemical composition of various classes of microorganisms. In general, microbial proteins are rich in lysine and relatively poor in sulfur-containing amino acids (Reed, 1982).

Table 1.1. Chemical composition of the microbial biomass for classes of microorganisms (% of dry weight)<sup>1</sup>

Chemical composition	Filamentous fungi	Algae	Yeast	Bacteria
Nitrogen (N)	5.0 - 8.0	7.5 - 10.0	7.5 - 9.0	11.5 - 13.3
Protein (N $\times$ 6.25)	31.0 - 50.0	47.0 - 63.0	47.0 - 56.0	72.0 - 83.0
Nucleic acids	9.2	3.0 - 8.0	6.0 - 12.0	8.0 - 16.0
Ash	9.0 - 14.0	8.0 - 10.0	5.0 - 9.5	3.0 - 7.0
Lipids	2.0 - 8.0	7.0 - 20.0	2.0 - 6.0	1.5 - 3.0

<sup>1</sup> Source: Reed (1982).

The high nucleic acid content of microbial biomass is, however, a problem when it comes to using microbial biomass as food for humans since man has lost the ability to synthesize enzymes that can break down purine compounds, the accumulation of which leads to metabolic disturbances, specifically gout.

The estimated safe intake of nucleic acids for a healthy adult person is about 2 g per day (Reed, 1982). This limits the possible usefulness and application of microbial biomass for human consumption (Waslien and Steinkraus, 1980).

### 1.3. Edible fungi

Edible fungi are among the few microorganisms used as food by man (Bunker, 1964; Waslien and Steinkraus, 1980). They have advantages over other microorganisms when the choice of SCP is for foodstuffs. They are clearly nontoxic and have high food values (Crisan and Sand, 1978; Eddy, 1958), are delicious, and are more acceptable to the consumer because they are used in existing foodstuffs. Their naturally occurring texture and flavor make them particularly suitable for human foodstuff (Solomons, 1975). They have, therefore, been used in the past and are used at present for or in foods in many parts of the world (Litchfield, 1963). The ancient Romans and Egyptians respected the delicious taste and flavor of mushrooms (Litchfield, 1967a). The Greeks regarded mushrooms as providing strength for warriors in battle, while the Chinese treasured them as health food (Change and Miles, 1982). Today, mushrooms are eaten by almost all people of the world (Bano et al., 1963; Kurtzman, 1979a).

### 1.3.1. Mushrooms as a source of protein

Mushrooms and other edible fungi represent one of the world's greatest untapped resources of nutritious and palatable foods (Bano *et al.*, 1963; Chang, 1980) and may, therefore, be considered as a source of food both for human beings and for livestock (Reusser *et al.*, 1958a), and as a possible means of directly increasing food production (Gray, 1970; Kurtzman, 1979a). They are thought to be an attractive source of protein (Bano *et al.*, 1963; Robinson and Davidson, 1959; Solomons, 1975; Sugihara and Humfeld, 1954). Their protein is easily digested and of higher quality than that of vegetables (Chan, 1981; Chang, 1980; Tseng and Luong, 1984) and in some cases equal to muscle protein (Bano *et al.*, 1963). Mushroom protein contains all the essential amino acids (Chan, 1981; Crisan and Sands, 1978) in high concentrations when compared with egg protein (Hayes, 1976) and it is especially rich in lysine and leucine. The efficiency of protein production is higher for mushrooms and other fungi than for any conventional protein source (Tseng and Luong, 1984). In addition to proteins, mushrooms are a good source of the B vitamins (Crisan and Sands, 1978), fat and phosphorus, low in calories (Chang, 1980) and are used as flavoring agents in foods (Litchfield, 1967c; Tseng and Luong, 1984).

Mushrooms, like other fast-growing organisms, contain higher amounts of nucleic acid than do conventional foods (Table 1.2). However, on the basis of 2 g of nucleic acid per day as the estimated safe intake for a healthy adult person, it is quite safe for one to consume as much as 300 g of fresh mushrooms daily (Chang, 1980). Since this is

Table 1.2. Nucleic acid contents of mushrooms compared with those of other foods<sup>1</sup>

Sample	Nucleic acid (% of dry weight)
<u>Agaricus bisporus</u>	7.4
<u>Pleurotus cystidiosus</u>	6.2
<u>Pleurotus florida</u>	6.0
<u>Volvariella volvacea</u> (fruiting bodies)	8.8
<u>Volvariella volvacea</u> (mycelium)	5.4
Microorganisms	3.0 - 25
Cereal	1.1 - 4.0
Meat and fish	2.2 - 5.7

<sup>1</sup>Source: Chang (1980).

generally beyond the human daily consumption, the content of nucleic acid in edible mushrooms should not limit their use as a daily vegetable.

### 1.3.2. Rate of mushroom consumption

In most industrialized Western countries, the consumption of edible mushrooms has been increasing rapidly during the last few decades (Hayes and Nair, 1975). Tseng and Luong (1984) have reported that in the last 20 years, consumption of mushrooms has increased at an annual compound rate of 13% in West Germany, 11% in Canada, 9.5% in the U.S.A., and 6% in the United Kingdom and France.

The total world production of edible fungi was estimated at about 1,135,000 tons in 1981 (Zadrazil and Grabbe, 1983) which was far below the total world demand for edible mushrooms (Tseng and Luong, 1984).

### 1.4. Cultivated mushrooms

There are more than 2,000 species of edible fungi in the world, but so far only about 25 of them are widely accepted for human consumption (Bessey, 1961; Chang, 1980). The five most important cultivated mushrooms are: the white mushroom/button mushroom (Agaricus bisporus), the black forest mushroom/shiitake (Lentinus edodes), the winter mushroom (Flammulina velutipes), the straw mushroom (Volvariella volvacea) and the oyster mushroom (Pleurotus spp.) (Chang and Miles, 1982; Tseng and Luong, 1984). Agaricus is cultivated in seventy-four countries but is commonly consumed mainly in Europe and North America. Lentinus and Flammulina are commonly grown in China and Japan. China is also the main producer of Volvariella, but it

Pleurotus is gaining popularity in Europe and Asia (Tseng and Loung, 1984).

### 1.5. Methods of mushroom cultivation

Although edible mushrooms are still collected in the wild in most parts of the world, their scientific cultivation began as far back as the 17th century in France (Lambert, 1938; Litchfield, 1967a). Today, cultivation of mushrooms is worldwide (Riviere, 1977; Smith, 1969). Cultivation techniques are based on liquid or solid substrates (Zadrazil and Grabbe, 1983).

#### 1.5.1. Solid culture of mushrooms

Mushrooms possess extensive enzyme complexes which enable them to flourish successfully on a wide variety of inexpensive substrates, such as lignin, cellulose, hemicellulose, pectin and other industrial wastes which are not suitable even for animal feed (Bano et al., 1983). Traditionally, mushroom fruiting bodies are produced on solid culture. This process utilizes waste materials such as manure, cereal straw, corn cobs, wood bark, sawdust, cotton seeds and other plant wastes as substrates (Tseng and Luong, 1984). Three major stages are involved in the solid culture production of mushrooms; the mycelium (vegetative) growth, the formation of primordia and the development of primordia into fruiting bodies. In some cases the process requires composting of the substrates and the use of casing soil to initiate primordia. The solid culture process is thus lengthy, complex, and requires a lot of space and manpower. This makes the production costs and the cost of fresh mushrooms very high.

### 1.5.2. Submerged culture of the mushroom mycelium

Because the demand for mushrooms is greater than the supply, work was undertaken to see if they could be grown in submerged culture. This process is considered to offer the promise of a large scale, low-cost production of mushroom mycelium for foods and feeds (Hashida et al., 1967). The development of submerged culture methods for producing mushroom mycelium came from the experience gained during World War II in penicillin and other fungal antibiotic production processes (Litchfield, 1967a). The first commercially orientated research on submerged culture production of mushrooms was carried out by Humfeld (1948). He reported that a good commercial product can be grown on any medium that contains a suitable sugar and other essential nutrients, that does not contain an ingredient inhibitory or toxic to the growth of the microorganisms, and that does not impart a characteristic flavor of its own during its use as a medium. Since then, there has been an attempt by many workers to intensify the process of biomass production from higher fungi. Humfeld and Sugihara (1952) demonstrated that Agaricus campestris could be grown in the mycelial form in submerged culture and that nutrient requirements for the growth of this organism were quite simple. In subsequent studies, Sugihara and Humfeld (1954) reported the submerged culture growth of several mushroom species. Eddy (1958) studied the growth of 20 species of mushrooms in synthetic culture. In a series of patents, Szuecs (1958), Humfeld (1954) and Cirillo et al. (1960) disclosed methods for producing mushroom mycelium in submerged culture. Szuecs (1958) claimed that the mycelia of several mushrooms, including Pleurotus ostreatus,



Morchella esculenta and A. campestris, could be grown in submerged culture. The growth requirements of some wood-rotting fungi (Jennison et al., 1955) and some mushrooms (Whitaker, 1951) grown in submerged culture, have also been studied. Reviews of the work done on the submerged culture of mushroom mycelium have been presented by Litchfield (1967a, 1968) and Worgan (1968). The cultivation of basidiomycetes in fermentors is mostly used in physiological studies in order to determine single parameters for biomass production or to produce special enzymes, or it is used as a screening program for antibiotics (Zadrazil and Grabbe, 1983). The potential of mushroom as a source of fungal protein, a source of spawn, or as a flavoring agent makes the production of fungal mycelium in submerged culture a most attractive prospect (Hadar and Cohen-Arazi, 1986).

#### 1.5.2.1. Nutritive value of the mushroom mycelium grown by submerged culture

Mushroom mycelium grown in submerged culture has been reported to be capable of producing materials with good nutritive value (Block et al., 1958; Reusser et al., 1958b), and has potential use as a food, food additive or food supplement (Cirillo et al. 1960; Janardhanan et al., 1970; Litchfield, 1968). Litchfield (1963) has stated that a pleasantly flavored protein supplement made from mushroom could be of particular interest to the developing countries of the world where chronic protein shortages exist.

### 1.5.2.2. Advantages of the submerged culture process

The submerged culture process has the advantage of producing large amounts of mushroom mycelium in a comparatively short period of time and in all areas of the world throughout the year (Litchfield and Overbeck, 1965). This is because the growth rates of fungi on liquid media are often higher (Taguchi *et al.*, 1968) than on solid media. Also, factors such as pH, nutrient concentration and aeration can be controlled and, hence, a product of uniform quality can be produced (Szuëcs, 1958). Submerged culture processes can also make use of industrial and food processing wastes as substrates (Litchfield, 1977) and, hence, reduce biological oxygen demand (BOD) in the environment. The process is relatively simple and requires relatively little labor and capital investment.

### 1.5.2.3. Acceptability of the product

Despite its advantages, submerged culture production of mushroom mycelium has not attracted much commercial interest (Kurtzman, 1979a). Commercial production of morel mushroom mycelium by submerged culture based on the process developed by Szuëcs (1958) was reported by Kils (1963). The process was, however, not a complete success and production had to be discontinued (LéDuy, 1974). Block (1960) reviewed the developments that had been made in the production of mushroom mycelium in submerged culture and noted that several times during the preceding decade commercial production had seemed imminent but that it had not yet materialized. The failure of the submerged production of mushrooms to attract commercial interest could be attributed to the lack of acceptability of the product. It has been reported

that the submerged culture process does not produce fruiting bodies (Block *et al.*, 1953; Eddy, 1958; Zadrazil and Grabbe, 1983), and that only a few strains of cultivated mushrooms retain their characteristic mushroom flavor during submerged cultivation (Block, 1960; Lambert, 1938; Litchfield, 1967c).

#### 1.5.2.4. Mushroom mycelium as a flavoring agent

Mushrooms are mostly used as a flavoring agent in soups, sauces and gravy mixes rather than as food staples (Chang, 1980; Eddy, 1958). Therefore, if the mycelium produced by submerged culture was to have the characteristic mushroom flavor, there should be no problem of consumer acceptance. If the characteristic flavor was present, the product could help to alleviate the current shortage of protein (Litchfield, 1963, 1967c).

Ginterova (1973a) has suggested that the lack of fruiting body formation and flavor in submerged culture was due to dedikaryotization during agitation. One of the phases of the life cycle of basidiomycetes is the mating of two compatible monokaryotic cells to produce dikaryotic cells from which fruiting bodies are formed. However, Kurtzman (1978) was of the view that the loss of flavor was not due to dedikaryotization but to contamination by other microorganisms. Dijkstra (1976) and Litchfield (1967c) have reported that the flavor of the mushroom mycelium was dependent on the substrate and the strain.

#### 1.5.2.5. Enhancement of the mushroom mycelium flavor

Szuecs (1949) developed a method of enhancing the mushroom flavor of some edible mushroom mycelium grown in submerged culture.

The method included autolysis, heating and cooking, chemical treatment, aging, agitation and aeration of cultures after the growth has ceased and all the carbohydrates have been consumed, and supplementing the medium with complex nutrients. Litchfield (1967c), however, has reported that almost all of these methods have been tried and found to produce little or no flavor enhancement, although Heineman (1963) claimed to have obtained better flavor when he supplemented the medium with skimmed milk than with corn steep liquor.

#### 1.6. The oyster mushrooms, Pleurotus species

##### 1.6.1. Biology of the Pleurotus mushroom

The genus Pleurotus belongs to the class Basidiomycetes and to the family Polyporaceae (Fig. 1.1). The genus is cosmopolitan (Eger, 1978; Zadrazil and Grabbe, 1983), and there are 39 known species (Singer, 1975). Most of these species have common morphological characteristics and, hence, it is difficult to identify them by morphology (Eger, 1978). It is, therefore, the view of some workers that the taxonomic difficulty in the Pleurotus spp. can be approached from the viewpoint of sexuality rather than pure morphology (Eger, 1978; Eger et al., 1979). The color of the mycelium is white, but that of the sporophore varies (Eger, 1978). The latter may be as white as the mycelium or it may be creamy, brown, gray or pink.

##### 1.6.2. Physiology of the Pleurotus mushroom

Pleurotus species have relatively simple growth requirements. It is reported that for both mycelial growth and fruiting body development, lignin-cellulose materials are sufficient (Block, 1965), and

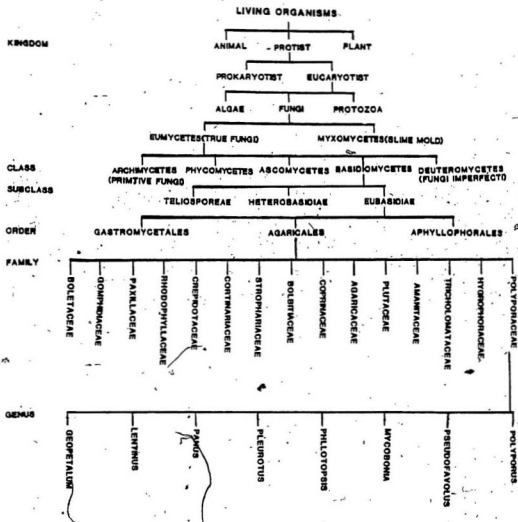


Fig. 1.1. Classification of the genus Pleurotus according to Bessey (1961) and Singer (1975)

that substrates containing high amounts of protein depress yield (Hayes and Wright, 1979). Platt et al. (1984) have studied the activities of Pleurotus involved in lignocellulose degradation. Cultivation of Pleurotus species does not require composting (Jandaik and Kapoor, 1974; Worgan, 1968) nor, in some cases, pasteurization or sterilization of the substrate (Tan, 1981, Rangaswami et al., 1975). Zadrazil (1975, 1980) has reported that carbon dioxide is required for the growth of some species of Pleurotus, and that supplementing the substrate with some organic and/or inorganic sources of nitrogen could increase yield and total nitrogen content of Pleurotus. The possibilities of nitrogen fixation by Pleurotus have been comprehensively reviewed by Kurtzman (1979b). Pleurotus species can be cultivated at a wide range of temperatures (21 to 33°C) and relative humidity (67 to 72%) (Bano et al., 1983).

#### 1.6.3. Nutritional value of the Pleurotus mushroom

Pleurotus species are exotic mushrooms and are treasured for their taste and flavor, are non-toxic and have a high content of crude protein in which all the essential amino acids, except methionine and phenylalanine, are present in high concentrations (Bano et al., 1983). Although the average protein content of Pleurotus mushrooms is low compared to that of A. bisporus, the nutritional value of Pleurotus is not inferior to that of Agaricus because the yield of the former mushroom is almost two times higher than that of the latter, which makes the protein output, in the form of edible fruiting bodies, higher in the Pleurotus mushroom (Bano and Rajarathnan, 1982).

#### 1.6.4. Cultivation of the Pleurotus mushroom

Pleurotus species are relatively new cultivated mushrooms (Declaire, 1978). In recent years, they have gained prominence as a type of edible mushroom in eastern countries (Bano and Rajarathnan, 1982). They are grown in Southeast Asia, Taiwan, Japan (Kurtzman, 1979a; Tan, 1981), Germany (Ng et al., 1977) and Hungary (Heltay, 1979), and are also becoming very popular in India (Samajpati and Bandyopadhyay, 1982). Pleurotus spp. formed about 2.7 percent of the total world production of cultivated edible mushrooms in 1979 (Chang and Miles, 1982).

##### 1.6.4.1. Substrates for cultivating the Pleurotus mushroom

Artificial cultivation of the Pleurotus mushroom began in the 20th century (Zadrazil, 1978). In 1958, Block et al. successfully cultivated Pleurotus species on sawdust, but mass production of Pleurotus substratum on straw basis was first carried out by Bano and Srivastava (1962). In addition, Zadrazil and Kurtzman (1982) studied aspects of the industrial production of Pleurotus species. Recently, many Pleurotus species have been successfully cultivated on a wide range of different substrates, such as cotton wastes (Chang et al., 1981; Cho et al., 1981; Nout and Keya, 1983; Tan, 1981), paddy straws (Khanna and Garcha, 1982; Jandaik and Kapoor, 1974; Samajpati and Bandyopadhyay, 1982), banana stems (Jandaik and Kapoor, 1974), coffee pulp (Guzman and Martinez, 1986), oil-palm pericarp (Ng et al., 1977) and paper (Steinkraus and Cullen, 1978). Comprehensive literature on the cultivation of Pleurotus species has been summarized by Zadrazil (1978), Kurtzman and Zadrazil (1982), and Zadrazil and Kurtzman (1982).

### 1.6.5. Pleurotus ostreatus (Jacq. ex Fr.) Kummer

#### 1.6.5.1. Commercial importance of P. ostreatus

P. ostreatus is the second most important edible mushroom in Europe (Lelly, 1982). It has been used both for the commercial production of fruiting bodies (Zadrazil, 1978) and as a model in the studies of lignin biodegradation (Platt et al., 1984). It is the most widely cultivated of all the Pleurotus species (Hayes and Wright, 1979). The first attempt to cultivate P. ostreatus in Europe was in 1969 (Kostadinov et al., 1972). Some special medicinal properties have also been claimed for P. ostreatus (Yoshioka et al., 1972).

#### 1.6.5.2. Morphology of P. ostreatus

The pileus of P. ostreatus is stemmed at the side, shelled, spatula or tongue-shaped, and is depressed. The lamellae are whitish or grey and decurrent; the stipe is short, eccentric or lateral. Two strains of P. ostreatus have been isolated (Eger et al., 1976): one from Florida and the other from Germany. The first is well known to growers throughout the world as the "Florida type". It is identical with P. sapidus Kalchbr. The German type differs from the American isolate in that the fruiting process is sensitive to temperatures above 15°C and will, therefore, not fruit in the range of 15 - 25°C, in which the Pleurotus from Florida is usually grown commercially. There is, however, little or no difference in appearance between the two isolates (Eger et al., 1976).

P. ostreatus has gymnocarpous fruiting bodies, which means that during sporophore development the lamellae are covered by a velum



which is produced soon after the first lamellae are generated. The spores are liberated in great quantities, and may elicit allergic responses in many persons who inhale them repeatedly. Eger (1976) has reported the isolation of sporeless strains of P. ostreatus.

#### 1.6.5.3. Genetics of the P. ostreatus mycelium

Pure cultures of the Pleurotus mycelium can be obtained by spore germination or by tissue culture (Ginterova and Janotkova, 1975). P. ostreatus has typical monokaryotic and dikaryotic cells (Elliott, 1982). The basic life cycle of the higher fungi is from spore to mycelium to fruiting body and, following a cell division, back to spore again (Fig. 1.2). The monokaryon produces asexual spores called oidia (Elliott, 1982). Therefore, reproduction is either sexual or asexual. P. ostreatus shows tetrapolar incompatibility (Anderson *et al.*, 1973; Eugenio and Anderson, 1968), i.e., the basidium or fruiting body bears four types of single spore isolates with different incompatibility (two different A and two different B) factors essential for the production of fruiting bodies. Such mycelia interact to form a dikaryon, bearing clamp connections and revealing conjugated nuclear division that carries different A and different B incompatibility factors (Räper, 1966). The bifactorial heterothallism of P. ostreatus was first described by Vandendries (1932) and has since been confirmed by a number of authors including Eugenio and Anderson (1968). Toyomasu *et al.* (1986) have studied interspecific protoplast fusion between P. ostreatus and P. salmoneostramineus.

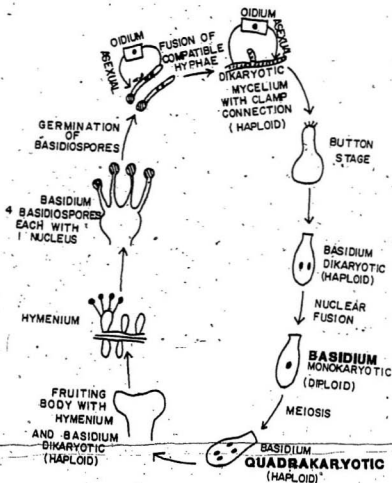


Fig. 1.2. Schematic representation of the life cycle of *P. ostreatus*

#### 1.6.5.4. Growth conditions for the P. ostreatus mycelium

P. ostreatus is characterized by the rapidity of its mycelial growth (Zadrazil, 1975). The reported optimal temperature range for the growth of the P. ostreatus mycelium is 20 - 30°C (Zadrazil and Grabbe, 1983). P. ostreatus requires oxygen (Zadrazil, 1978), and a carbon dioxide concentration in the air of up to 28% (Zadrazil, 1975) for mycelial growth. It is also reported that a very acidic condition (pH 4 and below) inhibits mycelial growth, and that rising pH values of 4 to 6 affect mycelial growth favorably (Zadrazil, 1978). Light is not required for mycelial growth, but is necessary for the formation of fruiting body primordia from the mycelium (Cho et al., 1981; Worgan, 1968; Zadrazil and Grabbe, 1983). However, some workers (Block et al., 1959; Jandaik and Kapoor, 1974) have reported normal growth of P. ostreatus fruiting bodies in the dark. Other factors such as the sources of carbon and energy, nitrogen, and the carbon/nitrogen ratio are important for the growth of P. ostreatus mycelium (Zadrazil and Grabbe, 1983).

#### 1.6.5.5. Some studies on the submerged growth of the P. ostreatus mycelium

Pleurotus species grow well and rapidly in submerged culture (Zadrazil, 1978). The method has, therefore, been recently used for physiological studies and for the industrial production of the P. ostreatus mycelium (Jennison et al., 1955; Worgan, 1968). Volt (1968) studied the submerged growth of strains of P. ostreatus by comparing growth in media with 44 carbon and energy sources and 31 nitrogen sources. In addition, he examined the effects of vitamins and growth

hormones on the growth of P. ostreatus in submerged culture. He reported that the fungus grew well in synthetic media with ammonium citrate as the nitrogen source. Zadrazil (1975), using sulfite waste liquor as the sole organic source, reported that low concentrations of the sulfite liquor are a good medium for the P. ostreatus mycelium production. The ability of P. ostreatus to fix nitrogen by submerged culture was investigated by Ginterova (1973b). Labenel et al. (1977), who studied the factors influencing cultural conditions of some mushrooms including P. ostreatus in submerged culture, reported that inoculum size did not affect the yield and the protein content to a great extent, and that the favorable temperature range for growth of the P. ostreatus mycelium in submerged culture was 20 - 25°C. Dijkstra (1976) studied the production of flavor compounds by the P. ostreatus mycelium in submerged culture. He reported that P. ostreatus, produced in submerged culture, had few or none of the components which contributed to flavor development. Eddy (1958) investigated the production of mushroom mycelia by submerged cultivation, and reported that satisfactory harvests of mycelium including that of P. ostreatus could be obtained. However, flavor, if present, was only weak and appeared to be chemically and physiologically dissimilar from the usually accepted mushroom flavor. Sugihara and Humfeld (1954) found that Pleurotus mycelium did not produce pleasant flavor when grown in submerged culture. Shannon and Stevenson (1975) used brewery wastes as substrates for the production of mycelium of some mushrooms, including P. ostreatus. Sugimori et al. (1971) have reported the production of fruiting bodies of P. ostreatus on the liquid surface of

submerged culture. It has been reported that submerged culture could be used to produce *P. ostreatus* liquid spawn for solid culture of fruiting bodies (Kostadinov et al., 1972).

## 1.7. Peat

### 1.7.1. Accumulation of peat

Peat is an accumulated plant residue at various stages of microbial decomposition (Chang, 1985), due to a gradual decrease in oxygen supply from the surface of the accumulated wet plant matter to a level below the surface where oxygen from the air and/or the plant residues is virtually absent (Fuchsman, 1980). The permanently wet and partially decomposed remains of the bog plants accumulates very slowly. On the average, it takes 3,000 to 4,000 years to accumulate a meter of peat (Fuchsman, 1980).

### 1.7.2. Classification of peat

Peat is classified according to its geological, botanical and physiochemical characteristics (Fuchsman, 1980). Geologically, there are low-moor, transitional and high-moor peats. In low-moor peats, the bog water system is continuous with the mineral and ground water system. The bog water system for high-moor peats is significantly above the ground water system for mineral soils. Thus low-moor peats are frequently covered with water and are, therefore, wetter and have higher contents of dissolved inorganic material than high moor peats (Fuchsman, 1980). Botanically, peats are named according to the plants that grow most abundantly in the bog, since they are likely to be the same as the plants whose decomposed residues form the peat.

immediately beneath the surface. High-moor plants are mostly mosses (Sphagnum), since they can thrive in mineral-poor water. Low-moor plants are those which require higher levels of solutes and are mostly woody plants. Physiochemical classification of peats is based on the degree of decomposition or humification. Low-moor peats are commonly more decomposed than high-moor peats (Fuchsman, 1980). Some workers (Morita et al., 1980; Morita and Montgomery, 1980) have suggested that analyses of sugars derived from hot acid hydrolysis of peats may be useful taxonomic aids.

The main varieties of peat can also be distinguished by their acidity and their ash content. Low-moor peats have higher ash contents but are less acidic than high-moor peats (Fuchsman, 1980).

In Newfoundland, peatlands are comprised largely of a complex array of unforested bog and fen types (Pollett, 1972). The bog vegetation layer is a combination of Sphagnum mosses, particularly S. fuscum and S. magellanicum, along with sheep laurel (Kalmia angustifolia). The fen type is richer in nutrients, less acidic, has higher bulk density and is generally more humified than the bog type (Pollett and Wells, 1977).

#### 1.7.3. Composition of peat

Peat is composed, mainly of water (80 - 90%) with only about 10 - 20% of its mass as partially decomposed residue of dead plants and microorganisms. Chemically, peats are largely organic material with very little inorganic matter. The composition of the organic residue of

peat is shown in Table 1.3. The bitumens include waxes, resins and other substances soluble in organic solvents; the humic substances are soluble in aqueous alkaline media; the carbohydrate-related materials such as cellulose and hemicellulose, and some proteinlike substances can be dissolved by treatment with acid; lignins are soluble in strong bases and are characteristically phenolic in their reactions. There are also small quantities of simple sugars, free amino acids and other water soluble components present. The cellulose and hemicellulose content of peat is decreased by decomposition (Puustjarvi and Robertson, 1977). Thus high-moor peats have a high polysaccharide content compared to low-moor peats. The high polysaccharide content of high-moor peats makes them particularly suitable for processes such as SCP production, which require large amounts of potentially metabolizable carbohydrates (Fuchsman, 1980).

Humic acids are mainly gelatinous colloids which, in high concentrations, feel soapy to the touch. They are formed as a result of the decomposition in lignin and other substances (Puustjarvi and Robertson, 1977).

Peat is normally deficient in most plant nutrients. It is reported that the nitrogen content of peat increases with decomposition (Puustjarvi and Robertson, 1977). Sphagnum peat moss, therefore, has a low content of nitrogen.

Approximately 40% of the total nitrogen in peat is present as amino acid-N, and/or as amino sugar-N. Therefore, a considerable chemical action is required to convert the organic nitrogen into soluble form (Puustjarvi and Robertson, 1977).

Table 1.3. Chemical composition of high-moor Sphagnum peats and low-moor sedge peats (% dry weight of peat)<sup>1</sup>

Components	<u>Sphagnum</u>	Sedge
pH	3.0 - 5.0	4.0 - 7.5
Moisture <sup>2</sup>	80.0 - 90.0	80.0 - 90.0
Bitumen	3.1 - 9.1	3.2 - 3.9
Hemicellulose	9.0 - 21.0	6.0 - 10.0
Cellulose	10.3 - 23.7	7.8 - 8.1
Lignin and humic substances	26.3 - 64.3	56.1 - 62.2
Protein (%N x 6.25)	5.6 - 6.9	10.0 - 13.8
Total reducing sugars (as glucose)	20.0 - 41.9	16.3 - 20.0
Total ash	1.5 - 3.0	7.7 - 14.5

<sup>1</sup>Source: Fuchsman (1980).

<sup>2</sup>% wet weight of peat.



The inorganic fraction of peat consists of calcium, iron, magnesium, sulfur and phosphorus, as well as many micro-elements (Quierzy et al., 1979; Smith et al., 1958).

#### 1.7.4. Distribution of the world peat reserves

Peat covers more than 1% of the world's land area (Rohrer, 1981). The total exploitable peat reserves in the world are about 230 million hectares (LeDuy, 1979). It is estimated that about 90% of the world's peat resources are located within three countries (Table 1.4). Peat is one of Canada's most abundant, and yet, least exploited resources (Chornet et al., 1980). About 2 million hectares of Canada's peat reserves are located in Newfoundland (Pollett, 1972).

#### 1.7.5. Uses of peat

As an organic material, peat can be used for a variety of purposes including agricultural, metallurgical, energy and medical uses (Lishtvan, 1981). So far, the largest industrial application of peat is as a fuel (Rohrer, 1981; Chornet et al., 1980), especially in Finland, Ireland and the Soviet Union (Fuchsman, 1980). The low ash content and sulfur level, and the rapid burning characteristics of dehydrated peat product make it a potential substitute for oil in many utility and industrial boilers (Rohrer, 1981). In Finland and Germany, high-quality formed coke for foundry or blast furnaces is made from peat for the metallurgical industry (Fuchsman, 1980). In Britain, experiments have shown that peat is a potential source of gaseous fuel (Morita, 1980). In Canada, studies have been conducted for the possible use of peat as a source of energy (Gallo and Sheppard, 1981; Chornet et al., 1981).

Table 1.4. World peat resources<sup>1</sup>

Country	Extent of Resources	
	(million hectares)	% of total world resources
CANADA	129.50	56.18
U.S.S.R.	71.50 <sup>a</sup>	31.01
FINLAND	10.00	4.33
U.S.A.	7.50	3.24
NORWAY	3.00	1.29
U.K.	1.58	0.68
SWEDEN	1.50	0.64
POLAND	1.50	0.64
WEST GERMANY	1.13	0.48
IRELAND	1.00	0.42
INDONESIA	0.70	0.29
EAST GERMANY	0.49	0.20
CUBA	0.20	0.08
JAPAN	0.20	0.08
IRELAND	0.17 <sup>a</sup>	0.06
NEW ZEALAND	0.17	0.06
HUNGARY	0.10	0.03
NETHERLANDS	0.10	0.03
DENMARK	0.06	0.02
FRANCE	0.06	0.02
ITALY	0.06	0.02
CZECHOSLOVAKIA	0.03	0.01
AUSTRIA	0.02	0.01
OTHERS	0.40	0.18

<sup>1</sup>Source: LeDuy (1979).<sup>a</sup>These are exploitable reserves only.

Peat is also used for horticultural and agricultural purposes in North America and Europe (Fuchsman, 1980; Sinclair, 1981). In Newfoundland, about 2% of the total peat reserves have been used for afforestation trials and agriculture (Pollet, 1972). Peat soils in Europe are used extensively for forestry and pasturage, while in Canada they are mainly used for vegetable production.

Peat is also a potential source of organic and industrial materials such as phenols, nitrogen bases and some aromatics, which are useful for the production of plastics, plant protectives and pharmaceuticals (LeDuy, 1981). Peat tar, which is a useful organic intermediate, contains asphaltenes, wax and paraffin, neutral oils, and hydrocarbons. The pyrolysis process used in the fuel industry to dewater crude peat produces such by-products as furfurals, acetones and methanol (Rohrer, 1981). Peat wax may be used in industrial lubricants and protective coating applications, and in the Soviet Union production has been increased recently from 200 to 800 tons (Fuchsman, 1981). Peat humates can be used as drilling mud additives and also to improve flow properties of Portland cement mixtures (Rohrer, 1981).

#### 1.7.5.1. Peat as a fermentation substrate

Carbohydrates comprise a large fraction of the organic matter of peat (Morita and Montgomery, 1980). In addition, peat contains high proportions of nitrogenous compounds of both organic and inorganic nature (McLoughlin and Kuster, 1972a). The extractable and soluble components of peat can, therefore, serve as a medium for the production of SCP and in other fermentation processes (Forsberg *et al.*, 1986; Fuchsman, 1980; LeDuy, 1981; Mulligan and Cooper, 1985), and have,

therefore, been used for a long time as culture media for bakers yeast and fodder yeast (Fuchsman, 1980). In the Soviet Union, the second largest industrial application of peat is as a raw material for production of high-protein yeast (Carter, 1981).

Recently, extensive studies have been carried out on the utilization of peat extracts as fermentation substrates for the production of SCP (LeDuy, 1981; Martin, 1983b; Martin and White, 1985; Quierzy et al., 1979), solvents (LeDuy and Laroche, 1983) and polysaccharides (Mulligan and Cooper, 1985; LeDuy and Boa, 1983). A summary of the various fermentation processes utilizing peat extract has been published by LeDuy (1979). Martin (1986) has reviewed the fundamental process aspects for the production of mushroom mycelium in submerged culture with peat extract as the main nutrient source.

#### 1.7.5.2. Methods of preparing peat extract

Different extraction procedures have been used to treat peats and their components so that their nutrients are rendered available to microorganisms, for the synthesis of protein. These methods included Soxhlet extraction with organic solvents, cold and hot water extraction, dilute acid and dilute alkaline hydrolysis carried out at different temperatures (McLoughlin and Kuster, 1972a; Fuchsman, 1980), and enzymatic hydrolysis (Fuchsman, 1980).

Hydrolysis is the principal technique employed in the treatment of peat carbohydrates for further use. The process converts the cellulose to glucose, and also extracts other carbohydrates in addition to several organic substances, some of which may be utilized as nutrients by

microorganisms. Factors such as temperature, retention time, type of catalytic agent, peat-catalyst concentration ratio (McLoughlin and Kuster, 1972a,c; Chang, 1985; Quierzy et al., 1979; Boa and LeDuy, 1982) and the peat particle size (Morita and Levesque, 1980) have been reported to affect the amount of the utilizable organic fraction in peat extract. Also, bitumen and other water-insoluble components of peat affect the release of nutrients from the peat into solution during acid hydrolysis (Chang, 1985).

The hydrolysis process produces a liquid fraction called peat extract which is rich in both organic and inorganic substances (Fuchsman, 1980). In general, peat extract produced by dilute acid hydrolysis of peat in an autoclave constitutes the most appropriate culture medium (McLoughlin and Kuster, 1972a). Quierzy et al. (1979) have reported that acid-catalyzed hydrolysis of peat results in a comparatively high total carbohydrate concentration in the extract due to higher decomposition of peat cellulose (a "difficult-hydrolysable carbohydrate" in peat).

Steam explosion has recently been used to extract nutrients from peat for microbial production of solvents (Forsberg et al., 1986). Water expressed during the drying of fuel-grade peat resembles peat extract obtained from acid hydrolysis (Quierzy et al., 1979) and has been used as substrate for microbial production of extracellular polysaccharides (Mulligan and Cooper, 1985).

### 1.7.5.3. Composition of peat extract

Peat extract contains mostly monosaccharides and non-volatile organic acids such as ~~car~~aronic acids, hydroxycarboxylic acids and  $C_2$ - $C_5$  dicarboxylic acids as the main carbon substrates (LeDuy and Boa, 1983). Peat extracts are also rich in some organic substances such as humic acids and bitumen (Chang, 1985, Fuchsman, 1983), and minerals (LeDuy, 1981). It is, however, too deficient in nitrogen and phosphorus for the optimal growth of microorganisms (Boa and LeDuy, 1982; LeDuy, 1979; Mulligan and Cooper, 1985).

The concentrations of the various substances in peat extract depend on the extraction procedure. Water and alcohol extracts of peat have low monosaccharide composition (Fuchsman, 1980). Acid-catalyzed hydrolysis of peat results in comparatively higher total carbohydrates and other organic nutrients than other methods of extraction (Quierzy *et al.*, 1979; McLoughlin and Kuster, 1972a).

The monosaccharides in acid peat extract are mostly hexose (Morita and Montgomery, 1980). LeDuy (1981) has reported that the major sugar in the  $H_2SO_4$  extract is glucose while xylose is the major sugar in the HCl extract. Chang (1985) has also reported that HCl extract from peat contained slightly higher concentrations of reducing sugars and total nitrogen than that produced by  $H_2SO_4$  solution.

### 1.7.5.4. Effect of some components of peat extract on the growth of microorganisms

The hydrolysis process also yields, in addition to carbohydrates, other substances, both nutrients and non-nutrients (Fuchsman, 1981).

Peat hydrolysate might, therefore, have inhibitory, promoting or no effect on microbial growth and product synthesis (McLoughlin and Kuster, 1972b, c). McLoughlin and Kuster (1972a) have reported that alkaline extract with NaOH in an autoclave and solvent extract with benzene-ethanol in a Soxhlet apparatus inhibited growth of Candida utilis due to high ionic concentrations. Chang (1985) also found that even though HCl extract of peat contains more reducing sugars and total nitrogen than  $H_2SO_4$  extract the latter is a better growth medium. The HCl extract of peat contains high concentrations of salt (sodium chloride) which have an inhibitory effect on the growth of microorganisms (Dady and Chang, 1983). McLoughlin and Kuster (1972b) also reported that the best yeast growth in acid catalyzed peat extract, using different acids, was in the following decreasing order:  $H_2SO_4$ , HCl,  $HClO_4$ , humic (water extraction), and  $HNO_3$ .

The reports on the effects of humic substances and bitumen in peat extract on the growth of microorganisms are mixed, depending on the concentration of humic acids and on the organism in question. Fuchsman (1983) has stated that humic substances have inhibitory effects on the growth of microorganisms. Similar observations have been made by Chang (1985), who obtained an increased yield of C. utilis when bitumen and humic substances were removed from the peat extract, and by Martin (1983b), who obtained good growth of mushroom mycelium after diluting the peat extract with water. However, Dady and Chang (1983) reported that increasing humic acid concentrations of up to 0.5% in the peat extract increased the biomass of C. tropicalis significantly. McLoughlin and Kuster (1972c), who studied the effect of

humic substances on the metabolism of microorganisms, reported that humic substances had no effect on the microbial metabolism. Dady and Chang (1983) have reported that some microorganisms can utilize humic acids as sources of carbon and nitrogen. Some vitamins and hormones present in peat extracts are thought to favor growth of microorganisms (Chang, 1985).

#### 1.8. Objectives of this work

The main objectives of this work could be divided into five parts as follows:

1. To extract and study some of the organic components present in peat and use them in the production of a valuable source of protein;
2. To study the operational variables and kinetic parameters in the submerged growth of the P. ostreatus mycelium in peat extract;
3. To obtain scientific information on fungal metabolism and the feasibility of peat and peat extract as fermentation media, using P. ostreatus as a model;
4. To compare the nutritional values of the P. ostreatus mycelium and fruiting bodies produced with peat as the main fermentation substrate; and
5. To provide preliminary information on the potential nutrient source for the production of exotic mushrooms as a food and as a flavoring agent, utilizing a Canadian raw material.

#### 1.9. Scientific information to be obtained from this work

This work will provide scientific information on:

1. The usefulness of the peat from some peat reserves of Newfoundland as an inexpensive substrate for producing food-stuffs;
2. Optimal growth conditions, kinetic parameters of growth, and the physiology, including pellet formation and nutrient requirement for the growth of the P. ostreatus mycelium produced by fermentation with peat extract as substrate; and



3. The composition, including protein content and essential amino acid composition, of the P. ostreatus mycelium and fruiting body produced with peat as substrate.

#### 1.10. Investigation program

In these studies,  $H_2SO_4$  extract from peat was used. The proximate composition, the amino acid composition and the composition of some macro and micro elements of the raw peat, and of the acid peat extract, were determined. The composition of the raw peat and the peat extract provided information on the minimum essential composition of the peat extract as a growth medium.

Due to a lack of information in the literature regarding the optimal growth conditions of the P. ostreatus mycelium cultivated in submerged culture, the studies were designed to optimize growth conditions in terms of temperature, initial pH, dilution ratio (substrate concentration), agitation speed, aeration rate, inoculum size and nutrient supplements of the culture medium, in shake flasks, and then in batch fermentors. Some studies were also conducted on the morphology of the P. ostreatus grown in the peat extract and in synthetic media.

The protein, amino acids, fat, carbohydrate, fiber, mineral and fatty acid compositions of the biomass were determined to find the overall pattern of nutrients present in the P. ostreatus biomass produced by submerged culture using peat extract as substrate.

## CHAPTER 2

## MATERIALS AND METHODS

## 2.1. Materials

2.1.1. Sphagnum peat moss

A high-moor Sphagnum peat moss was obtained from Sundew peat bog, near the city of St. John's, Newfoundland, Canada. Samples were taken from the upper layer to a depth of 20 cm. The peat was of a relatively low degree of decomposition and the humification value, in the von Post Scale, corresponded to H2, (Martin and Bailey, 1983). The particle size ranged from coarse to > 60 mesh.

## 2.1.2. Culture

The culture of P. ostreatus No. 152, was obtained from the Dept. of Plant Science, University of Western Ontario, London, Canada. It was maintained at 4°C on slopes of peat extract-YM agar (Martin, 1983a) for growth in a peat extract medium or on slants of potato-dextrose agar for growth in a synthetic medium (Srivastava and Bano, 1970), and was transferred every two months.

## 2.1.3. Chemicals

All the chemicals used in this work were of reagent or laboratory grade.

Those listed below were purchased from Fisher Scientific Company Ltd: sulfuric acid, hydrochloric acid, boric acid, sodium hydroxide, ammonium hydroxide, ammonium sulfate, ammonium phosphate dibasic, ammonium citrate heptahydrate, potassium nitrate, potassium phosphate dibasic, potassium phosphate monobasic, potassium citrate monohydrate, magnesium sulfate heptahydrate, manganese sulfate monohydrate, zinc sulfate heptahydrate, ferrous sulfate heptahydrate, sodium molybdate dihydrate, D-glucose anhydrous, ethyl ether (anhydrous), pyridine, ethanol (95%) methylene blue (water soluble), sodium borohydride, acetic acid (glacial), acetic anhydride, aluminum potassium sulfate (alum), hydrogen peroxide (30%), and urea.

The following were obtained from Difco Laboratories, Detroit, Michigan, U.S.A.: yeast extract, peptone and YM agar.

Anthrone and D-xylose were purchased from J. T. Baker Chemical Co. Phillipsburg, NJ, U.S.A.

Lithium citrate was purchased from Beckman Industries, Inc.

Nitrogen gas was obtained from Liquid Air Canada.

The following chemicals were purchased from Sigma Chemical Company, St. Louis, MO, U.S.A.: thiamine, cyanocobalamin, pyridoxine, riboflavin, biotin, niacin and p-aminobenzoic acid, D(+) mannose, D-galactose, D-arabinose, and calcium chloride.

Panthothenic acid was purchased from Eastman Organic Chemicals, Rochester 3, NY, U.S.A.

Folic acid was obtained from Mann Research Laboratories, NY, U.S.A.

## 2.2. Methods

### 2.2.1. Preparation of peat extract

The peat, as received (with a moisture content of approximately 80%), was air-dried (to a moisture content of 20%), and the peat extract was prepared according to the method of Martin and Bailey (1985). It involved mixing peat with 1.5%  $H_2SO_4$  in a ratio of 20 g of dry peat to 100 g of acid solution, and autoclaving it at 15 psi ( $121 \pm 1^\circ C$ ) for 2 hours. The extract was separated by pressing the autoclaved product in a Carver Laboratory Press (Model C, F.S. Carver Inc., WI, U.S.A.) followed by filtration through Whatman no. 1 filter paper. The peat extract was stored in plastic containers at  $4^\circ C$  until required for use.

### 2.2.2. Preparation of the synthetic medium

The basal medium was prepared, according to the method of Srivastava and Bano (1970), by dissolving the following chemicals in a liter of deionized water:  $KH_2PO_4$ , 1 g;  $MgSO_4 \cdot 7H_2O$ , 0.5 g;  $MnSO_4 \cdot H_2O$ , 3 mg;  $ZnSO_4 \cdot 7H_2O$ , 3 mg;  $FeSO_4 \cdot 7H_2O$ , 3 mg;  $Na_2MoO_4$ , 3 mg. To the solution was added a mixture of vitamins containing thiamine, niacin, riboflavin, panthothenic acid, p-aminobenzoic acid, each at the rate of 100 g/L, and cyanocobalamin, biotin, pyridoxine, and folic acid, each at the rate of 50 g/L.

### 2.2.3. Optimization of growth conditions in shake flasks

The growth and biomass production of the *P. ostreatus* mycelium in the peat extract and in the synthetic medium was studied in shake flasks. The pH of the growth medium was adjusted to the required

values by the addition of either lactic acid (to the synthetic medium) or 10 M NaOH (to the peat extract medium) before sterilization at  $121 \pm 1^\circ\text{C}$  for 30 minutes.

#### 2.2.3.1. Inoculum preparation

The inocula for the shake flask experiments were prepared from fresh culture slants (Martin, 1983a): peat extract-YM agar slants for the peat extract medium; potato-dextrose agar slants for the synthetic medium. The entire growth of one slope was transferred aseptically to a sterile Waring blender jar containing 50 mL sterile water and blended for 30 seconds. The suspension was used to inoculate 50 mL sterile growth media in 250 mL shake flasks, which were inoculated in a Gyrotory water bath shaker (model G-76, New Brunswick Scientific Co. Inc., Edison, NJ, U.S.A.).

#### 2.2.3.2. Preliminary studies

Preliminary experiments were conducted to determine the appropriate peat extract concentrations for the media, and the best inoculum ratio. Peat extract/water dilution ratios of 1:0, 1:0.5, 1:1, 1:2 and 1:3, plus 5 g/L yeast extract, and inoculum ratios of 2.5, 5.0, 7.5 and 10.0% (v/v), were used at  $28^\circ\text{C}$  with an agitation speed of 150 rpm, and 192 hours of fermentation time. The yeast extract was used to supplement the nitrogen content of the peat extract.

#### 2.2.3.3. Studies on growth conditions

Using peat extract diluted to a 1:1 ratio with water, an inoculum ratio of 5.0% (v/v) (which were the best conditions obtained from the

preliminary studies) and 5 g/L yeast extract, the effects of temperature (19, 22, 25, 28 and 31°C), initial pH (4, 5, 6 and 7), agitation speed (100, 150 and 200 rpm) and fermentation time (48, 96, 144, 192, 240 and 288 hours) were investigated in 60 separate experiments.

#### 2.2.4. Determination of the nutritional requirements of P. ostreatus grown in shake flasks

The effect of different sources of carbon and nitrogen, and different concentrations of phosphorus, potassium, magnesium and manganese on the growth of the P. ostreatus mycelium in the synthetic medium and in the peat extract medium were studied. The following optimal growth conditions were employed: temperature of 28°C, an initial pH of 5, an inoculum ratio of 5.0% (v/v), an agitation speed of 150 rpm and a fermentation time of 192 hours.

##### 2.2.4.1. Addition of different sources of carbon and nitrogen

The effect of different carbon sources (glucose, galactose, mannose, arabinose and xylose) on the growth and the biomass production of P. ostreatus in a synthetic medium was studied to determine which of the above major monosaccharides in the peat extract were best utilized by the fungus. The amount of sugar added was calculated so that it provided approximately 6.0 g/L of carbon in the medium. The results of this study were verified by determining the concentrations of these sugars in the peat extract medium before and after fermentation.

The effect of different sources of nitrogen (ammonium citrate, ammonium phosphate, ammonium sulfate, ammonium nitrate, potassium nitrate and urea) was also studied to determine the possibility of sup-

plementing the low nitrogen content in the peat extract with nitrogen from other sources, since yeast extract is an expensive source of nitrogen. The different sources of nitrogen were calculated to provide 0.5 g/L of nitrogen to the synthetic medium. The effect of different concentrations of ammonium citrate (which was found to be the best nitrogen source in the synthetic medium) on the growth of the *P. ostreatus* mycelium, was also studied.

#### 2.2.4.2. Addition of phosphorus and potassium

Experiments were conducted to study the effects of phosphorus and potassium on the growth and the biomass production of *P. ostreatus* in the synthetic medium. Both phosphorus and potassium were supplied in the form of  $\text{KH}_2\text{PO}_4$ . The other nutrients in the medium were kept at uniform concentrations in all the treatments.

Phosphorus was tested at four different concentrations (0, 0.11, 0.22 and 0.33 g/L). A complete absence of phosphorus (0 g/L) was achieved by not adding any  $\text{KH}_2\text{PO}_4$  to the medium, but substituting for it sufficient potassium citrate to maintain the level of potassium. The second treatment (0.11 g/L) was also supplemented with sufficient potassium citrate to maintain the level of potassium. A higher concentration of phosphorus (0.33 g/L) was added by supplementing the medium with  $(\text{NH}_4)_2\text{HPO}_4$ .

Potassium was tested at four different concentrations (0, 0.14, 0.28, and 0.42 g/L). A complete absence of potassium (0 g/L) was achieved by withholding  $\text{KH}_2\text{PO}_4$  and substituting for it sufficient  $(\text{NH}_4)_2\text{HPO}_4$  to maintain the level of phosphorus. The second treatment

(0.14 g/L) was also supplemented with sufficient  $(\text{NH}_4)_2\text{HPO}_4$  to maintain the level of phosphorus. A higher concentration (0.42 g/L) of potassium was achieved by supplementing the medium with potassium citrate.

## 2.2.5. Nutrient supplementation of the peat extract

The peat extract was tested without additional nutrients (non-supplemented), and with the addition of different concentrations of carbon, nitrogen, phosphorus, potassium, magnesium and manganese alone, and in combinations. The growth conditions were the same as those in section 2.2.4.

### 2.2.5.1. Supplementation of the peat extract with carbon and nitrogen

Glucose, which was one of the best carbon sources for growth of the *P. ostreatus* mycelium in the synthetic medium, was added to peat extract diluted to a 1:1 ratio with water at six different concentrations (0, 10, 15, 20, 25 and 30 g/L). The experiment was conducted to determine whether the diluted peat extract was deficient in carbon.

The effect of different sources of nitrogen on growth was also studied. The organic sources were yeast extract, peptone, and urea, and the inorganic nitrogen sources were ammonia solution, ammonium phosphate dibasic, ammonium citrate, ammonium nitrate and potassium nitrate. The concentrations of the different sources of nitrogen were calculated to provide 0.5 g/L of nitrogen to the peat extract medium. The ammonia solution was used to provide nitrogen and also to adjust the pH of the peat extract medium. Different concentrations of yeast extract (1.0, 3.0, 5.0, 7.5 and 10.0 g/L) and  $(\text{NH}_4)_2\text{HPO}_4$  (0, 2.5, 5.0



and 7.5 g/L) (which were the best nitrogen sources) were added to the peat extract medium to study their effects on growth and on crude protein production.

#### 2.2.5.2. Supplementation of the peat extract with phosphorus and potassium

Potassium phosphate monobasic ( $\text{KH}_2\text{PO}_4$ ) was used as a source of both phosphorus and potassium. Different concentrations of  $\text{KH}_2\text{PO}_4$  (0, 1.3, 2.6, 3.9, 5.2, 6.5 and 7.8 g/L) were added to the peat extract medium, and their effect on growth and on crude protein production of the P. ostreatus mycelium was studied.

#### 2.2.5.3. Addition of yeast extract and $\text{KH}_2\text{PO}_4$ to the peat extract

Different concentrations of yeast extract (0, 1.0, 3.0 and 5.0 g/L) were combined with different concentrations of  $\text{KH}_2\text{PO}_4$  (0, 1.3, 2.6 and 3.9 g/L) and used to supplement the peat extract. The experiment was conducted to study the combined effect of the two sources of nutrients on the growth of the P. ostreatus mycelium in the peat extract. This was because when the two sources of nutrients were added individually to the peat extract, they produced a very good growth.

#### 2.2.5.4. Supplementation of the peat extract with magnesium and manganese

The peat extract was supplemented with magnesium (0.08 g/L) and manganese (0.02 g/L) individually and in combination. Magnesium

sulfate heptahydrate was used to supply magnesium, and manganese sulfate monohydrate was used as the source of manganese.

#### 2.2.6. Batch fermentation

Batch fermentation experiments were conducted to optimize the mechanical agitation speed and aeration rate, and also to study the kinetics of the growth of the *P. ostreatus* mycelium in the fermenter. The experiments were conducted in a 2-Liter Bioflo fermenter (model F-2000, New Brunswick Scientific Co.), operated under total control of pH, aeration, agitation and temperature. The fermenter was equipped with a turbine-type impeller (with four blades that mixed the medium, and a built-in mechanical pump that delivered filtered air to the culture through a hollow agitator shaft (Appendix A1.)). The pH of the growth medium was kept constant by using an automatic pH controller (model pH-40) and a pump module (New Brunswick Scientific Co.). The dissolved oxygen concentrations at various times of fermentation were measured using a D.O. Analyzer (model DO-40, New Brunswick Scientific Co.) (Appendix A2.).

The fermenter system was operated under sterile conditions, at a controlled temperature of  $28 \pm 1^\circ\text{C}$  and at a controlled pH of  $5.0 \pm 0.1$ . The pH was automatically adjusted by the addition of 0.5 M NaOH or 1.0 M  $\text{H}_2\text{SO}_4$  solution. Microscopic observations of culture samples, together with streak plate technique, were used to check the sterile conditions during the experiments.

#### 2.2.6.1. Inoculum

The inocula for the batch fermentation experiments were propagated in 250 mL shake flasks with 50 mL of growth medium. The growth medium consisted of peat extract diluted to a 1:1 ratio with water, 3 g/L yeast extract, 2.6 g/L  $\text{KH}_2\text{PO}_4$  and 0.1 g/L  $\text{MnSO}_4\text{H}_2\text{O}$ . The propagation conditions were as follows: incubation temperature of  $28.0 \pm 1.0^\circ\text{C}$ , an initial pH of  $5.0 \pm 0.1$ , an inoculum ratio of 5.0% (v/v) and an agitation speed of 150 rpm. A fermentation time of 168 hours was used in order to obtain mycelium which were in the accelerated phase of growth.

The growth of 1 shake flask was blended in a sterile Waring blender for 30 seconds and used to inoculate 1 Liter of sterile growth media at a ratio of 5.0% (v/v).

#### 2.2.6.2. Growth media

The growth media for the batch fermentation experiments consisted of peat extract diluted to a 1:1 ratio with water, yeast extract (3 g/L),  $\text{KH}_2\text{PO}_4$  (2.6 g/L) and  $\text{MnSO}_4\text{H}_2\text{O}$  (0.1 g/L). This was the best combination of nutrient supplements as determined by the shake flask experiments. The media were sterilized at  $121 \pm 1^\circ\text{C}$  for 30 minutes.

#### 2.2.6.3. Optimization of agitation speed and aeration rate

Three levels of mechanical agitation speed (150, 200 and 250 rpm) and three levels of aeration rates (0.5, 1.0 and 1.5 vvm) were tested by growing the culture in the fermenter for 192 hours. All the batch cultivation experiments were performed separately under the same operational conditions.

#### 2.2.6.4. Determination of the growth kinetic parameters

The optimal conditions of temperature (28°C), initial pH (5.0), inoculum ratio [5.0% (v/v)], mechanical agitation speed (200 rpm) and aeration rate (1.0 vvm) were employed to study the kinetics of the growth of the P. ostreatus mycelium. The cultivation progress was followed over a period of 192 hours for three separate fermentations by determining the growth in terms of the biomass concentration, the TCH concentration and the dissolved oxygen concentration every 24 hours for each fermentation.

#### 2.2.7. Solid culture cultivation

The method of Manu-Tawiah and Martin (1986) was employed to produce fruiting bodies. The proximate chemical composition of the mycelium produced by submerged culture was then compared with that of the fruiting bodies.

### 2.3. Analytical methods

#### 2.3.1. Determination of the pH of the raw peat

The pH of the raw peat was determined according to the method of the A.O.A.C. (2.17a, 1980). The method involved weighing about 3.0 g air-dried peat into a 100 mL beaker and adding 50 mL of H<sub>2</sub>O. The peat was allowed to soak for 30 minutes with occasional stirring and the pH was read with a pH meter (model 5652-00, Cole-Parmer Instrument Co.).

### 2.3.2. Determination of total solids

The total solids content of the peat extract was determined according to the method outlined by Reusser *et al.* (1958a). The pH of the sample was adjusted to 7.0 with 10 M NaOH solution. The sample was transferred to a previously dried and weighed glass dish and then evaporated to dryness in a vacuum oven at 70°C to constant weight.

### 2.3.3. Determination of dissolved solids

About 50 mL of peat extract was filtered through a previously dried and weighed Whatman no. 541 filter paper using a Buchner funnel. The filter paper was dried in a vacuum oven at 70°C to a constant weight. The dissolved solids content of the peat extract was calculated as the difference between the total solids content and the total weight of the dry residue on the filter paper per unit volume of the peat extract.

### 2.3.4. Determination of total reducing sugars

The sample was purified according to the method described by Morita and Montgomery (1980). It involved neutralizing 50 mL of the peat extract with a saturated solution of aqueous barium hydroxide. The mixture was centrifuged at 2000 x g for 30 minutes and the supernatant was concentrated to 15 mL. The neutralized hydrolysate was purified through ion-exchanged chromatography using 3 columns (Rexyn 101 cation exchanger, Rexyn 201 anion exchanger, and Rexyn 101 cation exchanger, respectively) and deionized water for elution. The effluent from the columns was made up to 1000 mL with deionized water.

The total reducing sugars content of the solution was estimated using the Nelson and Somogyi colorimetric method (Hodge and Hofreiter, 1962). The method involved the addition of an equal volume of low-alkalinity copper reagent to 1 mL of peat solution containing not more than 0.6 mg of D-glucose or its equivalent, and to 1 mL of each standard sugar solution (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mg/mL of D-glucose). The sample and standard sugar solutions were heated for 10 minutes in a vigorously boiling water bath and then cooled. Two mL of arsenomolybdate reagent was added and mixed thoroughly to allow dissolution of all the cuprous oxide. The solution was diluted to 25 mL with deionized water and allowed to stand for about 20 minutes. The absorbances were read at 500 nm in a Beckman UV/Vis Spectrophotometer (model Du-8). The sugar content was computed from a curve established with the standard sugar solutions.

#### 2.3.5. Monosaccharide analysis

The concentrations of glucose, galactose, rhamnose, mannose, arabinose and xylose in the peat extract medium before and after fermentation were determined using a modified form of the method described by Morita and Montgomery (1980). The peat extract was purified as under section 2.3.4. The effluent from the columns was subsequently concentrated to 15 mL and evaporated to dryness, followed by conversion of the monosaccharides to alditols by treating, for 1 hour at room temperature, with 2 - 3 drops of 0.2 M  $\text{NaBH}_4$ . Excess  $\text{NaBH}_4$  was destroyed by the addition of 2 - 4 drops of acetic acid, and the solution was evaporated to dryness. The residues were taken up in 0.3

mL of water and lyophilized. The sample was kept in a desiccator overnight and acetylated for 15 minutes at 100°C with 50  $\mu$ L of pyridine and 50  $\mu$ L of acetic anhydride. A 4  $\mu$ L aliquot was injected for gas chromatography.

Gas chromatography of the acetylated sugars was run on a Perkin Elmer gas chromatograph (model 8310) fitted with dual glass columns (2 mm i.d. x 180 cm) packed with 3% Silar 10CP on 100-200 mesh Chromosorb WHP. Dual flame ionization detectors were used. The operating conditions were: a column temperature of 200°C, and a carrier gas ( $N_2$ ) flow rate of 56 mL/minute.

#### 2.3.6. Determination of the biomass

For the determination of the dry biomass weight, the total biomass in the culture medium after fermentation was filtered through oven-dried (at 105°C to constant weight) Whatman no. 1 filter paper. The filter paper with the mycelium was washed twice with distilled water to remove the fermentation broth and oven-dried at 60 - 65°C to a constant weight. The initial inoculum dry weight was subtracted from the total biomass dry weight to obtain the dry weight of the mycelium produced. The biomass yield was calculated as grams of the mycelium dry weight produced per gram of the total carbohydrate consumed, and the efficiency was calculated as grams of the mycelium dry weight produced per gram of the total carbohydrate supplied.

#### 2.3.7. Determination of the total carbohydrate (TCH) concentration

The TCH concentration of the growth medium before and after fermentations were determined by the Anthrone reagent method (Morris,

1948; Neish, 1952). The samples were diluted to the appropriate range (ca. 20 - 200 mg/L of equivalent glucose) and they were read after 10 minutes against distilled water at 540 nm, using a Beckman UV/VS Spectrophotometer (model DU-8). The results were expressed as equivalent glucose concentrations. The TCH concentrations in the dry mycelium and in the dry fruiting bodies were determined based on the same reagent procedure. About 0.25 g of ground sample was extracted with ether for two hours (i.e., until essentially fat-free). The extracted sample was transferred to an evaporating dish and dried at 60°C to constant weight. The sample was heated for three hours with 100 mL of water and 10 mL of concentrated hydrochloric acid in a 250-mL flask provided with a reflux condenser. The sample was cooled and filtered through Whatman no. 1 filter papers. Ten mL of this solution was pipetted to a volumetric flask to make 100 mL of solution. Two mL of this solution and the standard glucose solutions were pipetted separately into test tubes and allowed to react with 4 mL of anthrone-sulfuric acid reagent. Mixing was done thoroughly with the aid of a Fisher Scientific Vortex Genie.

#### 2.3.8. Determination of moisture

The moisture contents of the raw peat, the fresh mycelium and fresh fruiting bodies were determined according to the method of the A.O.A.C. (7.003; 1980). It involved drying a quantity of sample containing approximately 1 g of dry matter to constant weight at 95 - 100°C under vacuum.



### 2.3.9. Determination of the total nitrogen and the crude protein

The total nitrogen contents of the raw peat, the peat extract, the mycelium and fruiting bodies of *P. ostreatus* were determined by a modified micro-Kjeldahl method (A.O.A.C. 47.021; 1980). The method involved placing 1 g of the ground dried sample in a Kjeldahl flask and adding 2 special Kjel tabs (S.3.5), 20 mL of concentrated sulfuric acid and 10 mL of 30% hydrogen peroxide into the flask. The sample was digested, using the Kjeltec Digestion System 6 (1007 digester, Tector Inc., Boulder, CO, U.S.A.), for 3 hours until the solution had become colorless or clear. The digestion was continued for another 30 minutes, and the solution was allowed to cool. About 40 mL of water was added, carefully and a little at a time, to the flask. The flask was cooled under running water and was transferred to an Ammonia Distillation Unit (Kjeltec system 1002). About 50 mL of 40% NaOH solution was carefully added to the flask without agitation, and the solution was distilled for 30 - 40 minutes into a receiving flask containing 50 mL of 4% boric acid. A few drops of a methyl red indicator was added to the distillate and the latter was titrated with a standard 0.1 M hydrochloric acid solution.

A blank determination was carried out exactly as above, but without any of the samples.

The crude protein content in the sample was calculated from the percent nitrogen content using the conversion factor ( $N \times 6.25$ ).

### 2.3.10. Determination of fiber

The crude fiber contents of the dry mycelium and the dry fruiting bodies were determined according to the method of the A.O.A.C. (7.061; 1980). One gram of dried sample was extracted with ether for 2 hours (until fat-free), and then transferred to a 250 mL beaker. About 1 g of asbestos and 100 mL of boiling 1.25%  $\text{H}_2\text{SO}_4$  were added to the beaker and the mixture was digested for exactly 30 minutes on a preadjusted hotplate. The beaker was rotated periodically during digestion to keep solids from adhering to the sides. About 0.5 g of alum ( $\text{K}_2\text{SO}_4 \cdot \text{Al}_2(\text{SO}_4)_3 \cdot 24\text{H}_2\text{O}$ ) was added and the solution was then heated to boiling again. The alum was added to assist filtration. The beaker was removed from the heater and the contents filtered through a Buchner funnel using Whatman no. 541 filter paper. The beaker was then rinsed with 20 - 30 mL of boiling water and the water was used to wash the sample through the funnel without breaking the suction or raising the filter. The washing was repeated with three 25 mL portions of water and suctioned dry. The mat formed from the asbestos, and the residue were removed and transferred to the original beaker. About 100 mL of boiling 1.25% NaOH solution were added and the mixture was boiled for exactly 30 minutes. The beaker was removed from the heater and the contents transferred as above. The residue was washed with 25 mL of boiling 1.25%  $\text{H}_2\text{SO}_4$ , three 25 mL portions of water and 25 mL of 95% ethanol. The mat and residue were carefully transferred to a porcelain crucible which had been ignited at  $600^\circ\text{C}$  for 2 hours. The mat and the residue were dried for 2 hours at  $130^\circ\text{C}$ , cooled in a desiccator and weighed. They were then ignited for 30

minutes at 600°C, cooled in a desiccator and reweighed. A blank determination was carried out by treating the same quantity of asbestos with the acid and the base, as was done in the determination of fiber.

### 2.3.11. Determination of total lipids

The total lipids contents of the raw peat, the peat extract, the mycelium and fruiting bodies were determined using a modified form of the procedure used by Folch *et al.* (1957). The method involved homogenizing 1 g of the powdered sample with 19 mL of chloroform:methanol (2:1 v/v) mixture using a polytron at setting 20 for 2 minutes. The homogenate was allowed to equilibrate and the final volume adjusted to 20 mL with the chloroform/methanol mixture. The homogenate was filtered through glass wool into a 50 mL glass-stoppered graduated cylinder and the glass wool washed with 7.5 initial volumes of the chloroform/methanol mixture. The crude extract was mixed thoroughly with 0.2 volumes of 0.9% NaCl solution. The mixture was shaken vigorously and the phases allowed to separate upon standing. The volume of the chloroform layer was recorded and the alcoholic layer was removed by siphoning. The inside of the cylinder was washed with methanol and the final volume was made up to 20 mL by the addition of the chloroform/methanol mixture. The mixture was allowed to separate into two phases by standing and the upper layer removed as before. The chloroform (lower) layer, which contained the lipids, was transferred to a flask and evaporated to dryness in a vacuum at 50°C to remove the solvent. The lipid residue was redissolved in chloroform and the non-soluble portion was separated by filtration. The

filtrate was finally evaporated to dryness in a vacuum at 50°C and the flask was weighed for total lipids.

### 2.3.12. Determination of ash

The ash contents of the raw peat, the peat extract, the mycelium and fruiting bodies were determined according to the method of the A.O.A.C. (14.006; 1980). The method involved weighing 2 - 3 g of dried sample into a previously ignited, cooled and weighed porcelain crucible. The sample was ignited in a muffle furnace at about 550°C for 16 hours (until only grey ash remained). The crucible and the contents were allowed to cool in a desiccator to room temperature. The ash was moistened with distilled water to dissolve the soluble salts, dried slowly on a hotplate, and ignited again at 550°C to constant weight.

### 2.3.13. Determination of P, Fe, Ca, Mg, Mn, Na, K, Zn, Co, Ni and Cu

The concentrations of these minerals in the raw peat, peat extract, the mycelium and the fruiting bodies of P. ostreatus were determined according to the method of the Water Quality Branch, Environmental Canada (Anon., 1974). The method involved destroying the organic matter of the sample by placing about 1 g of the dried sample in a porcelain crucible and igniting it for 6 hours in a muffle furnace at 500°C, and then allowing it to cool. The ash was wetted with about 10 drops of water, and about 3 - 4 mL of nitric acid were added. The crucible was returned to the furnace and the sample was ignited again for another 4 - 5 hours at 500°C. The crucible was cooled and the

residue was then taken up in 100 mL of 0.2% nitric acid for flame atomic absorption analysis with a Varian AA - 5 atomic absorption spectrophotometer to measure the cations listed above.

#### 2.3.14. Determination of amino acids

The dried samples were hydrolyzed with 6 N HCl under vacuum for 24 hours at 110°C. The sample was reconstituted with 0.6 M lithium citrate buffer and analyzed with a Beckman 121 MB amino acid analyzer using a single column method. The concentrations of tryptophane and cystine were determined according to the methods of Penke et al. (1974), and Blackburn (1968), respectively.

#### 2.3.15. Determination of fatty acids

The modified form of the procedure by Folch et al. (1957) as described in section 2.3.11, was used to extract and purify the total lipids from the sample. The extract was stored at -29°C in chloroform to which 0.5% hydroquinone had been added. The fatty acids composition was determined using a modified form of the procedure by Thompson (1969): It involved taking about 0.5 - 2.0 mL aliquots of the lipid extract and evaporating them to dryness under a stream of nitrogen gas. The residues were transmethylated by adding 2 mL of 6% H<sub>2</sub>SO<sub>4</sub> in 99.9 mol% methanol and a little hydroquinone, and placing the mixture in an oven at 55 - 60°C overnight. After that, 1 mL deionized water was added and the sample was extracted 3 times with 1.0 mL portions of high-quality hexane. The hexane extract was washed 2 times with 1.5 mL portions of deionized water and the hexane was then evaporated. The lipid was dissolved in about 15 - 40 L CS<sub>2</sub> and about

4  $\mu$ L was analyzed with a Perkin Elmer gas chromatograph (model 8310) with a flame ionization detector and a Perkin Elmer graphic printer (model GP-100). The column was packed with SP 2330 (Supelco) and operated isothermally at 170°C and with a sensitivity of  $3 \times 10^{-10}$  amp/mv.

Fatty acid esters were identified qualitatively by comparing their retention times to those of standard fatty acid esters.

#### 2.4. Statistical analysis

All the data for the shake flask experiments represent mean values of three flasks. All the data for the batch cultivation experiments in the fermenter represent mean values of three fermentations. The data for proximate analyses of samples represent mean values of at least three determinations of three replicate samples.

Comparison between means was made using the T and F statistics (Bender et al., 1982).

Percentage values were subjected to angular transformations to find the mean values  $\pm$  standard deviations (Snedecor and Cochran, 1980).

Relationships between parameters were obtained by regression analyses.

## CHAPTER 3

## RESULTS AND DISCUSSION

3.1. Chemical composition of the Sphagnum peat moss and the acid ( $H_2SO_4$ ) peat extract

The chemical compositions of the raw peat and the peat extract were studied from the point of view of the ingredient requirements for the fermentation medium (carbon source, nitrogen, phosphate and oligo-elements). The results of the chemical analyses of the raw peat and the peat extract are summarized in Tables 3.1, 3.2, 3.3 and 3.4.

## 3.1.1. Composition of the raw peat

## 3.1.1.1. Proximate composition

Table 3.1 shows the proximate composition of the raw peat. It was acidic. Its pH was within the range for the maritime bog peats (pH 4.2 - 4.8) reported by Smith et al. (1958), and the range (pH 3 - 5) for Sphagnum peat reported by Fuchsman (1980). However, the raw peat was less acidic than some Newfoundland samples analyzed by Pollett (1972). The lower acidity of the raw peat used in this work (when compared to the average values for peat bogs in Newfoundland) could be attributed to the higher precipitation and greater salt concentrations experienced by coastal bogs (Pollett and Wells, 1977). The initial moisture content of the raw peat was less than 90%, which is the

Table 3.1. Chemical composition of the raw peat and the peat extract

	Raw peat (% of dry weight) <sup>1</sup>	Peat extract (g/L) <sup>1</sup>
pH <sup>2</sup>	4.85 ± 0.11	0.99 ± 0.15
Moisture <sup>3</sup>	80.50 ± 1.30	nd <sup>5</sup>
Total solids <sup>4</sup>	19.50 ± 0.50	62.26 ± 1.59
Dissolved solids	nd <sup>5</sup>	49.41 ± 0.77
TCH	nd <sup>5</sup>	32.75 ± 1.23
Total reducing sugars	nd <sup>5</sup>	16.47 ± 0.51
Total fat	2.52 ± 0.17	0.99 ± 0.03
Total nitrogen	0.80 ± 0.08	0.60 ± 0.01
Ash	2.60 ± 0.58	4.51 ± 0.01

<sup>1</sup>These are the mean values of three determinations of three replicate samples ± standard deviations.

<sup>2</sup>No units.

<sup>3</sup>% of wet weight.

<sup>4</sup>Determined by difference.

<sup>5</sup>nd = not determined.



Table 3.2. Amino acid composition of the raw peat and the peat extract

Amino acid	Raw peat (mg/g of dry weight) <sup>1</sup>	Peat extract (mg/L) <sup>1</sup>
Ala	1.82 ± 0.12	2.65 ± 0.18
Arg	0.58 ± 0.02	1.22 ± 0.05
Asp	1.93 ± 0.07	4.37 ± 0.18
Cys	0.73 ± 0.01	4.64 ± 0.18
Glu	1.93 ± 0.15	2.45 ± 0.21
Gly	1.91 ± 0.12	2.95 ± 0.12
His	0.62 ± 0.07	0.79 ± 0.14
Ile	0.62 ± 0.07	0.84 ± 0.08
Leu	1.31 ± 0.11	1.71 ± 0.13
Lys	0.86 ± 0.13	1.22 ± 0.08
Met	trace	0.42 ± 0.03
Phe	0.86 ± 0.04	0.78 ± 0.03
Pro	1.06 ± 0.00	2.24 ± 0.12
Ser	1.54 ± 0.21	1.93 ± 0.24
Thr	1.54 ± 0.02	2.52 ± 0.11
Trp	trace	0.00
Tyr	0.74 ± 0.01	0.88 ± 0.06
Val	1.24 ± 0.04	1.68 ± 0.15

<sup>1</sup>These are the mean values of three determinations of three replicate samples ± standard deviations.

Table 3.3. Mineral composition of the raw peat and the peat extract

Mineral	Raw peat (mg/g of dry weight) <sup>1</sup>	Peat extract (mg/L) <sup>1</sup>
P (P <sub>2</sub> O <sub>5</sub> )	0.40 ± 0.05	50.00 ± 3.88
Fe	0.20 ± 0.03	140.00 ± 1.50
Ca	0.30 ± 0.03	165.00 ± 1.10
Mg	0.72 ± 0.04	257.00 ± 2.10
Na	0.14 ± 0.01	190.00 ± 6.84
K	0.30 ± 0.01	39.00 ± 0.97
Zn	0.01 ± 0.00	7.20 ± 0.20
Mn	0.01 ± 0.00	6.20 ± 0.10
Co	0.01 ± 0.00	0.05 ± 0.00
Cu	0.01 ± 0.00	0.16 ± 0.02
Ni	0.02 ± 0.00	0.10 ± 0.00

<sup>1</sup>These are the mean values for two determinations of three replicate samples ± standard deviations.

Table 3.4. Major monosaccharide composition of the acid peat extract

Monosaccharide	% of total reducing sugars <sup>1</sup>
Glucose	38.20 $\pm$ 1.31
Galactose	19.07 $\pm$ 1.67
Rhamnose	6.96 $\pm$ 1.62
Mannose	16.46 $\pm$ 1.83
Arabinose	2.48 $\pm$ 1.11
Xylose	12.03 $\pm$ 1.15

<sup>1</sup>These are the mean values of three determinations of three replicate samples  $\pm$  standard deviations.

average moisture content of Sphagnum peat moss under natural conditions (Puustjarvi and Robertson, 1977; Fuchsman, 1980). However, the moisture content corresponded very well with that obtained by Martin and Bailey (1983), who analyzed samples from the same bog. The moisture content of the peat from the Sundew bog was not constant and the peat tended to lose moisture during storage. The total nitrogen content of the raw peat was within the range reported for Newfoundland peat (Pollett, 1972). The nitrogen content of peat increase with decomposition (Puustjarvi and Robertson, 1977), and the depth from which the peat samples are taken (Black et al., 1955). The raw peat sample used in this work was slightly decomposed Sphagnum peat and, therefore, had a low nitrogen content. Generally, the nitrogen content of slightly decomposed Sphagnum high-moor peat is in the range of 0.5 - 1.0% (Fuchsman, 1980).

The total lipids content of the raw peat corresponds very well with the percentage concentration of ether-soluble components in Sphagnum peat. Fuchsman (1980) reported that the content of ether-soluble components in peat moss ranges between 2 - 6% on a dry weight basis. Lipids in peat are composed of triglycerides of fatty acids, waxes, steroids, terpenes, fat-soluble vitamins and some pigments. These substances, and any others extractable with non-aqueous organic solvents, are called bitumens (Fuchsman, 1980). Black et al. (1955) obtained crude fat contents between 3.9 to 5.2% for Scotch Sphagnum peat, and reported that crude fat contents increased with depth. Peat consists chiefly of organic matter with a small percentage of ash. The ash content of the peat sample used in this work was low compared with

the value obtained by Quierzy et al. (1979) for Sphagnum peat moss from Quebec, but was within the range of 1.5 - 3.0% reported by Fuchsman (1980).

#### 3.1.1.2. Amino acid composition

The raw peat had a low concentration of amino-acids (Table 3.2, page 58), which confirms its low concentration of nitrogen. About 40% of the total nitrogen in peat is present as amino acid-N, which is preserved as amino-sugar-N (Puustjarvi and Robertson, 1977). Thus, a considerable chemical action is required to convert the organic nitrogen into a soluble form. The presence of glucosamine in the peat was detected.

#### 3.1.1.3. Mineral composition

The results of the analysis of the mineral composition of the raw peat are shown in Table 3.3 (page 59). The results indicate that the raw peat had low concentrations of P, Fe, Mg, K, Zn and Mn, which are nutrients for both plants and microorganisms. Apart from the phosphate, the contents of the other mineral nutrients in the raw peat were below the range reported by Pollett and Wells (1977), and also lower than the values obtained by Quierzy et al. (1979). The results show that the concentration of Ca was lower than Mg in the raw peat. However, Pollett and Wells (1977) and Quierzy et al. (1979) obtained a higher concentration of Ca than Mg in their Sphagnum peat samples.

### 3.1.2. Composition of the peat extract

#### 3.1.2.1. Proximate composition

The proximate composition of the peat extract is shown in Table 3.1 (page 57). The concentration of the total solids in the peat extract was low compared to that reported by LeDuy (1981). It was observed that 80% of the total solids in the peat extract were present as dissolved solids. This was higher than the value reported by LeDuy (1981), which was about 70% of the total solids. The difference in the concentration of total solids and dissolved solids between the two samples of  $H_2SO_4$  peat extract can be attributed to the type of raw peat used, their degree of decomposition, and the temperature and retention time during hydrolysis. The method of separating the extract from the residue can also affect the total solid concentration in the peat extract. The peat extract contained about 3% (w/v) carbohydrate and this corresponds reasonably well with that reported by Martin (1986), and LeDuy and Laroche (1983), using the same conditions for the extraction. The TCH concentration in the peat extract sample was about 66% of the dissolved solids concentration. This percentage was within the range of 66 - 72% reported by Fuchsman (1980). The total reducing sugars accounted for about 50% of the TCH in the peat extract.

Acid peat extracts normally have low total lipid concentrations because fatty substances do not dissolve easily in aqueous solutions. The concentration of total lipids in the peat extract sample compares favorably with that reported by Fuchsman (1980). The total nitrogen concentration in the peat extract sample was high compared with that reported by Martin (1986) and about the same as that reported by

LeDuy (1981), and McLoughlin and Kuster (1972a). It has been reported that the total nitrogen concentration in acid extracts prepared from Sphagnum peat ranges from a little below 1.0 to 1.6 g/L (Fuchsman, 1980).

#### 3.1.2.2. Amino acid composition

Amino acids in peat extracts constitute a minor nitrogen source for fermentation. Table 3.2 (page 58) shows the amino acid composition of the peat extract samples. The amino acids present were in low concentrations when compared with those reported by Fuchsman (1980). The results indicated that cysteic acid, aspartic acid, glycine, glutamic acid, threonine and alanine were the major amino acids in the peat extract. The high concentration of cysteic acid in peat extract has also been reported by Black *et al.* (1955). Tyrosine has been reported to be prominent in  $H_2SO_4$  peat extract, but the results are contrary to this. The comparatively low concentrations of valine, lysine, arginine and proline can be attributed to the fact that these amino acids go into solution only under more severe conditions. The absence of tryptophane in the peat extract is due to its destruction during acid hydrolysis of the raw peat. Black *et al.* (1955) found tryptophane in peat extract produced by alkaline hydrolysis.

#### 3.1.2.3. Mineral composition

The mineral composition of the peat extract is shown in Table 3.3 (page 59). The results indicate that the major minerals in the peat extract were Mg, Na, Ca and Fe, and that the extract contained low amounts of P (as  $P_2O_5$ ), K, Zn, Mn and other micro-elements.

Similar observations were also reported by LeDuy (1981), except that in his study the concentration of Ca was higher than that of Mg. Low concentrations of phosphorus are a characteristic of acid peat extract (McLoughlin and Kuster, 1972c). Peat extracts are, therefore, normally supplemented with external sources of phosphorus when used as a substrate for the growth of microorganisms.

#### 3.1.2.4. Composition of the major monosaccharides

The composition of the major monosaccharides in the peat extract sample is summarized in Table 3.4 (page 60). The results indicated that the hexoses represented more than 50% of the total sugars, and that glucose was the most abundant sugar, followed by galactose and mannose. Similar observations have also been reported by Fuchsman (1980), Morita and Levesque (1980), Morita and Montgomery (1980), and Black *et al.* (1955). Among the pentoses, xylose was the most abundant, with low concentrations of rhamnose and arabinose. Generally, the amount of hexoses are decreased with an increasing degree of decomposition in the raw peat (Morita and Montgomery, 1980).

#### 3.1.2.5. General discussion: free sugar content in the acid peat extract

The amount of free sugars released during acid hydrolysis of peat is affected greatly by temperature and the time of hydrolysis. Longer exposure times at the higher temperatures will result in a net decrease in the free sugar concentration (LeDuy and Laroche, 1983; Forsberg *et al.*, 1986), presumably as a result of increased destruction of free sugars. Normally, temperatures of 150°C (Quierzy *et al.*, 1979) or



higher have been employed. However, if the liquid extract is intended to be used as a nutrient source for biological processes, milder conditions ( $121^{\circ}\text{C}$ ) would be preferred, although the time required for the hydrolysis would be lengthened, and the process yield would be lower.

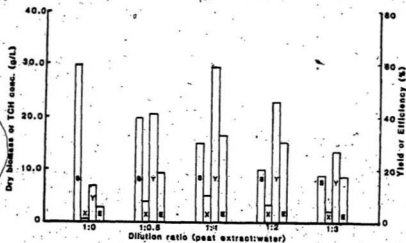
### 3.2. Shake flask experiments

#### 3.2.1. Optimal operating conditions

Preliminary experiments were conducted to determine the appropriate substrate concentration (peat extract:water ratio) for the media composition, and the best inoculum ratio (v/v). Afterwards, the effect of temperature (5 levels), initial pH (4 levels) and agitation speed (3 levels) were investigated in 60 different sets of operating conditions. With the best set of temperature, pH, and agitation speed, new experiments were conducted to confirm the previously-found best values of substrate concentration and inoculum ratio. Finally, different fermentation times were tested.

##### 3.2.1.1. Substrate concentration

When peat extracts obtained from the hydrolysis process were inoculated with *P. ostreatus*, practically no growth was observed. The fungus was, therefore, tested on various dilutions of peat extract with water. The results of the growth and the TCH concentrations at different dilutions are presented in Fig. 3.1. It was observed that a maximal growth, in terms of the dry biomass concentrations, yield and efficiency, was obtained at a 1:1 dilution ratio. Higher dilution ratios hampered the growth, probably because of lack of nutrients. For example, the highest dilution ratio of 1:3 corresponded to a TCH con-



S - TCH concentration.  
 X - dry biomass concentration.  
 Y - % yield.  
 E - % efficiency.

Fig. 3.1. Effect of the substrate concentrations (dilution ratios) on the growth of the *P. ostreatus* mycelium in the peat extract in the shake flask experiments

centration of approximately 7.5 g/L, as compared to about 15.0 g/L at the optimal dilution ratio of 1:1. The lower growth obtained at lower dilution ratios (higher peat extract concentrations) could be due to the higher concentrations of inhibitory substances, since the TCH concentration was the highest in the non-diluted peat extract (30 g/L), and yet only traces of growth were observed.

### 3.2.1.2. Effect of increasing carbon concentrations in the peat extract on growth

Table 3.5 shows that there was a progressive increase in the values of the growth parameters when glucose was added to the peat extract. It is apparent, therefore, that carbon was limiting in the diluted peat extract. Maximum growth parameters (a dry biomass concentration of 13.35 g/L, a yield of 69.40% and an efficiency of 44.52%) were obtained when 15 g/L glucose was added to the peat extract, and the statistical analysis indicated that significant differences ( $P < 0.05$ ) occurred when glucose was added to the diluted peat extract, up to a concentration of 15 g/L. A linear relationship between reducing sugars and biomass concentrations has been reported for Candida utilis in an acid-depituminized peat extract (Chang, 1985).

Further addition of glucose above 15 g/L (1.5%), however, had a less favorable effect. It has been reported that, for fungi, there are optimal sugar concentrations which, through osmotic effects or specific enzyme effects, are the most favorable for the consumption of sugars (Foster, 1949). However, Solomons (1975) was of the view that the decrease in the growth rate at high substrate concentrations was almost certainly due to oxygen limitation, since the fermenter vessel appeared

Table 3.5. Effect of increasing carbon (glucose) concentrations on the growth of the *P. ostreatus* mycelium in the peat extract<sup>1</sup>

Glucose (g/L)	Dry biomass <sup>2</sup> conc. (g/L)	Yield <sup>2</sup> (%)	Efficiency <sup>2</sup> (%)
0	4.98 ± 0.05 <sup>a</sup>	60.04 ± 1.14 <sup>a</sup>	33.02 ± 0.20 <sup>a</sup>
10	11.47 ± 0.66 <sup>b</sup>	69.85 ± 1.91 <sup>b</sup>	45.68 ± 0.88 <sup>b</sup>
15	13.35 ± 0.60	69.40 ± 1.11 <sup>b</sup>	44.52 ± 1.07 <sup>b</sup>
20	11.76 ± 0.84 <sup>b</sup>	60.06 ± 1.53 <sup>b</sup>	32.79 ± 1.36 <sup>a</sup>
25	9.54 ± 0.51	42.13 ± 1.84	24.38 ± 0.85
30	4.84 ± 0.09 <sup>a</sup>	23.05 ± 1.88	10.88 ± 2.26

<sup>1</sup>Peat extract diluted to a 1:1 ratio with water, plus 5.0 g/L yeast extract, an incubation temperature of 28°C, an initial pH of 5.0, an agitation speed of 150 rpm and a fermentation time of 192 hours were used.

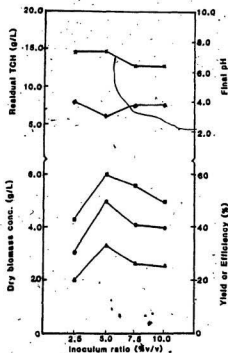
<sup>2</sup>These are the mean values of at least three replicate samples ± standard deviations. The % yield and the % efficiency were subjected to angular transformation to find the mean values ± standard deviations. Values in the same column with the same superscript are not significantly different at the 5% level.

to have a limit to the dry weight of mycelium it can support. Other investigators have also observed the detrimental effects of higher sugar concentrations on the growth of mushroom mycelium in submerged culture. Volt (1966) observed that the growth of *P. ostreatus* in a synthetic medium with various concentrations of glucose levelled off at 5.0% and showed little variation between 5.0% and 7.0%. Moustafa (1960) obtained the best growth of *A. campestris* in malt sprout extract medium supplemented with 2.3% glucose, and observed a long lag period and low yields when the concentration of the glucose supplements was raised to 5.0%. Falanghe *et al.* (1964) have reported that the mycelial yield of *Tricholoma nudum* greatly decreased with the addition of more than 1.0% of glucose to soybean whey.

### 3.2.1.3. Inoculum ratio

The highest values of the biomass dry weight, yield and efficiency were obtained with 5% (v/v) inoculum ratio (Fig. 3.2). Lower values were obtained at higher and lower inoculum concentrations. The values for the growth parameters at 7.5% and 10.0% (v/v) were not significantly different ( $P > 0.05$ ).

The relationship between the inoculum ratio and the final pH of the medium indicate that, generally, there was an increase in the pH values of the medium. However, the increases in the final pH values were lower at higher inoculum concentrations. The residual TCH concentration in the medium was the lowest at 5.0% (v/v) inoculum ratio. This means that when the inoculum ratio was optimal, the fungus utilized more of the TCH in the medium than at any other inoculum ratio tested. There was no significant difference ( $P > 0.05$ ) between the residual



- (●) - Dry biomass concentration.
- (■) - % yield.
- (▲) - % efficiency.
- (◆) - Residual TCH concentration.
- (×) - Final pH values.

Fig. 3.2. Effect of the inoculum ratio on the growth of the *P. ostreatus* mycelium in the peat extract in the shake flask experiments

TCH concentrations obtained at the 2.5%, 7.5% and 10.0% (v/v) inoculum ratios.

#### 3.2.1.4. General discussion: the effect of inoculum size on the biomass production

It was observed that the size of the inoculum affected the growth and the biomass production of the P. ostreatus mycelium in the peat extract. A similar observation was made by Martin (1983a), who obtained an optimal inoculum ratio of 7 - 10% (v/v) for Morchella esculenta grown in peat extract. Labuaneh et al. (1977) obtained an increase in yield of the P. ostreatus mycelium when the inoculum size was increased. However, in this study the highest inoculum ratio tested produced the best growth. The effect of inoculum size on the biomass production of Morchella crassipes (Kosaric and Miyata, 1981), and Collybia velutipes (Hashida et al., 1967) have also been reported. However, Yoshida et al. (1965) did not observe any significant effect of inoculum size on the biomass production of Lentinus edodes.

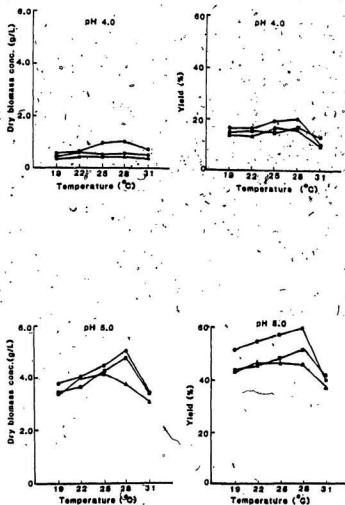
The lower growth values obtained in this work for inoculum concentrations higher than 5.0% (v/v), and the correspondingly higher residual TCH concentrations, indicate that the effect of higher inoculum ratios was not due to the depletion of nutrients as a result of higher initial cell concentrations in the medium. Carryover of inocula nutrients and other metabolites from one medium to another could occur during inoculum transfer (Jennison et al., 1955). Some of the metabolites have inhibitory effects, at higher concentrations, on the growth of microorganisms (Martin and White, 1985). The lower growth at the higher inoculum ratios could therefore be due to increased concentrations of

inhibitory metabolites produced during the propagation stages and transported with the inoculum. The lower growth values obtained at 2.5% (v/v) inoculum ratio could, however, be due to the low initial cell concentration. It has been reported that low cell concentrations increased the lag time for the growth of microorganisms in a liquid medium, since the time for the lag phase of growth varies inversely with the inoculum size (Bailey and Ollis, 1977).

### 3.2.1.5. Temperature

The optimum temperature range for the mycelial growth of P. ostreatus has been reported to be between 20 - 30°C (Zadrazil and Grabbe, 1983). Temperatures ranging from 19°C to 31°C, at 3 degree intervals, were studied in this work, and the results are presented in Fig. 3.3. It was observed that there was an increase in the growth parameters with increasingly higher temperatures of 19 to 28°C. A further increase in temperature to 31°C produced a significant reduction in the growth. The influence of the initial pH values and agitation speed on the effect of temperature on the growth was also observed. For example, at all levels of pH, the optimal temperature for the growth at 200 rpm was between 22 and 25°C, while the growth was significantly less at higher temperatures. There was no significant difference between the corresponding values of the growth parameters at these two temperatures at 200 rpm. It was also observed that at pH 7.0 the differences between the growth parameters at 25°C and those at 28°C were not significantly different ( $P > 0.05$ ) when the agitation speed was 100 or 150 rpm.





- (■) - 100 rpm.  
 (●) - 150 rpm.  
 (▲) - 200 rpm.

Fig. 3.3.. Effect of temperature on the growth of the *P. ostreatus* mycelium in the peat extract in the shake flask experiments

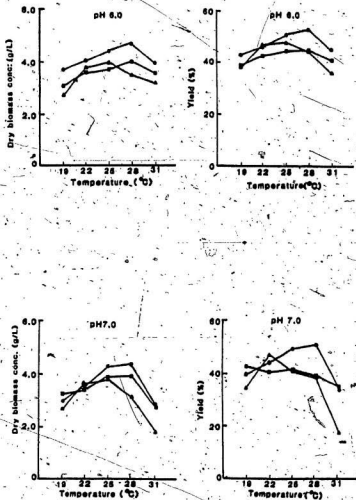


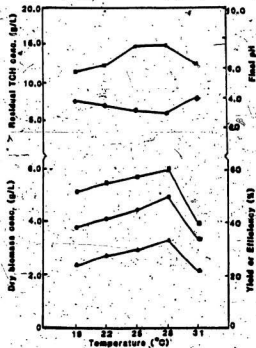
Fig. 3.3. (continued)

Fig. 3.4 shows more clearly the observation that the optimal temperature for the growth of the *P. ostreatus* mycelium in the peat extract was  $28 \pm 1^{\circ}\text{C}$  and that a further increase of the temperature to  $31^{\circ}\text{C}$  adversely affected the growth of the fungus. Bukhalo and Solomko (1978) and Labanelah et al. (1977) have reported an optimal temperature of  $25^{\circ}\text{C}$  for the growth of the *P. ostreatus* mycelium in submerged culture. However, Hadar and Cohen-Arazi (1986) used  $28^{\circ}\text{C}$  for growing *P. ostreatus* in submerged culture.

There was a slight increase in the carbohydrate utilization rate (the decrease in the residual TCH concentration) from  $19^{\circ}\text{C}$  to  $28^{\circ}\text{C}$ , where the maximum growth was obtained. The lowest carbon utilization rate (highest residual TCH concentration) was obtained at  $31^{\circ}\text{C}$ , where the growth was lowest. There was a general increase in the pH values of the medium at each of the various temperatures. The highest final pH values were obtained at  $25^{\circ}\text{C}$  and  $28^{\circ}\text{C}$  and the lowest final pH value at  $31^{\circ}\text{C}$ . Litchfield (1977) reported that mushroom mycelium appears to be less tolerant of temperatures above  $30^{\circ}\text{C}$ .

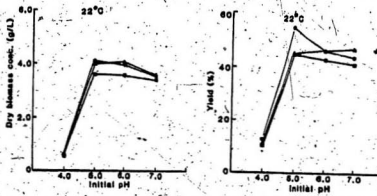
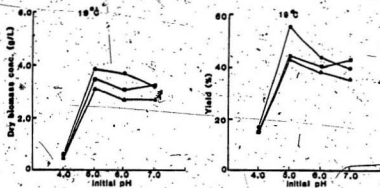
### 3.2.1.6. Initial pH

Studies were conducted on the effect of initial pH values ranging from 4.0 to 7.0 on the growth of *P. ostreatus*. The effect of initial pH at various temperatures and agitation speeds are presented in Fig. 3.5. It was observed that the growth at initial pH 4.0 was very poor, but there was a great increase in the growth when the initial pH was increased to 5.0, followed by slight decreases in the growth at both initial pH values higher than 5.0. It was also observed that the effect of the initial pH was sometimes influenced by the temperature and



- (●) - Dry biomass concentration.
- (■) - % yield.
- (▲) - % efficiency.
- (◆) - Residual TCH concentration.
- (I) - Final pH values.

Fig. 3.4. Effect of temperature on the growth of the *P. ostreatus* mycelium in the peat-extract in the shake flask experiments, at an initial pH of 5.0 and an agitation speed of 150 rpm



- (●) - 100 rpm.  
 (○) - 150 rpm.  
 (▲) - 200 rpm.

Fig. 3.5. Effect of initial pH on the growth of the *P. ostreatus* mycelium in the peat extract in the shake flask experiments

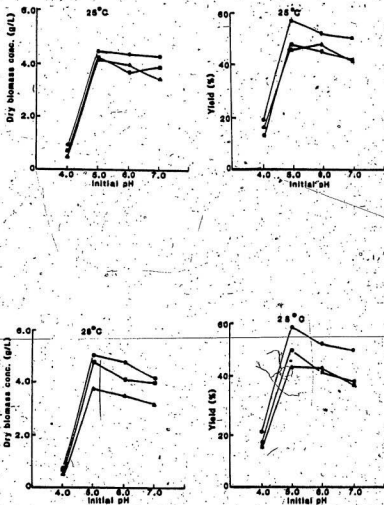


Fig. 3.5. (continued)

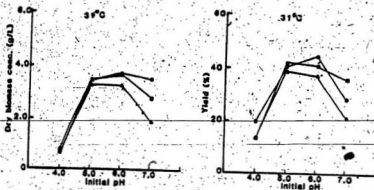
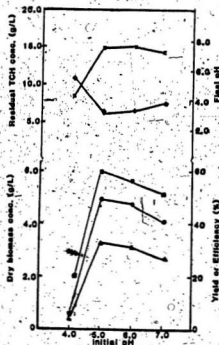


Fig. 3.5 (continued)

agitation speed. For example, at temperatures of 19, 25 and 28°C, the maximum growth parameters were obtained at an initial pH of 5.0, but when the temperature was 22 or 31°C there was no significant difference ( $P > 0.05$ ) between the corresponding growth parameters at pH 5.0 and those at pH 6.0. It was also observed that at an agitation speed of 100 rpm, the growth at initial pH 7.0 was either not significantly different ( $P > 0.05$ ) from or better than the growth at pH 6.0 at all temperatures tested.

Fig. 3.6 shows more clearly the observation that the optimal initial pH for the growth of P. ostreatus was 5.0. There was no significant difference ( $P > 0.05$ ) between the values of the dry biomass concentration and efficiency obtained at pH 5.0 and those obtained at pH 6.0. However, a lower yield value was obtained at pH 6.0, which indicates that the optimal initial pH had been exceeded. Sugimori et al. (1971), and Hadar and Cohen-Arazi (1986) also obtained an optimal initial pH of 5.0 for the growth of P. ostreatus mycelium in submerged culture. It has been reported that an acid pH inhibits the growth of Pleurotus species and that increasing the pH from 4 to 6 affects the mycelial growth favorably (Zadrazil, 1978). Block et al. (1959) observed that the P. ostreatus mycelium grew best at pH values between 5 and 6.2, but the growth was restricted by pH values of 4.2 and 6.7. Hashimoto and Takahasi (1974) have also observed poor growth of P. ostreatus mycelium at pH 3.0. Volt (1966) reported that P. ostreatus growth was best at pH 6.5. The optimal pH values for the growth of mushroom mycelium depends on the species or the strain and the culture medium





- (●) - Dry biomass concentration.
- (■) - % yield.
- (▲) - % efficiency.
- (◆) - Residual TCH concentration.
- (X) - Final pH values.

Fig. 3.6. Effect of the initial pH on the growth of the *P. ostreatus* mycelium in the peat extract in the shake flask experiments at 28°C and 150 rpm

for growth under particular fermentation conditions (Kosaric and Miyata, 1981; Zadrazil, 1978).

The pH values of the media were changed during the mycelial growth. Fig. 3.6 shows that there was a rise in the pH values with increasing mycelial growth. There was a comparatively big change in the pH value at the optimum initial pH of 5.0 (i.e., from 5.0 to 7.4). The final pH value obtained when the initial pH was 6.0 was about the same as that obtained with an initial pH of 5.0, while there was only a slight increase in the pH value at an initial pH of 7.0 (from 7.0 to 7.2). Thus, within the optimal limits, the final pH value varied only slightly. This is in agreement with the observation made by Zadrazil (1978). The lowest residual TCH concentration (highest TCH utilization) was obtained at the optimal pH value of 5.0. Higher residual TCH concentrations were obtained both at lower and at higher initial pH values.

#### 3.2.1.7. Agitation speed

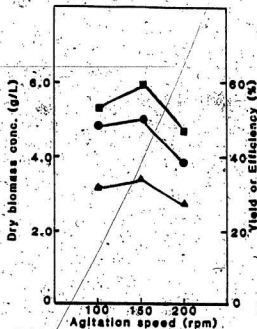
The effect of agitation speed on the growth of the P. ostreatus mycelium in shake flasks can be seen in Fig. 3.3 (page 74) and Fig. 3.5 (page 78). It is apparent that the maximum growth was observed at 150 rpm, with decreased growth both at lower and at higher agitation speeds. Agitation enhances mixing and makes nutrients and oxygen more available. Therefore, one would have expected that growth would be higher at 200 rpm than at 150 rpm. However, due to the nature of the growth of filamentous fungi, the detrimental effect of the shearing force on the growing hyphae overcame the beneficial effect of mixing,

and hence affected the physiology and growth of the fungi (Martin, 1983b).

It was observed that the temperature and the initial pH values did not have much influence on the effect of the agitation speed on the growth of the P. ostreatus mycelium. An agitation speed of 150 rpm remained optimal at most levels of temperature and initial pH studied. The only noticeable influence was that at 22°C: there was no significant difference between the corresponding biomass concentrations at 150 and 200 rpm at all levels of initial pH tested. The presentation of the experimental data in Fig. 3.7 shows clearly that 150 rpm was the optimal agitation speed for the growth of the P. ostreatus mycelium in shake flask cultures.

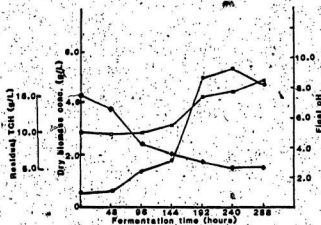
#### 3.2.1.8. Fermentation time

The effect of fermentation time on submerged growth of the P. ostreatus mycelium in the peat extract is shown in Fig. 3.8. There was no appreciable growth before 48 hours of fermentation (Fig. 3.8a). In general, there was a growth adaptation period which lasted for 144 hours. The accelerated growth phase occurred between 114 hours and 192 hours of fermentation followed by a decline in the growth rate. The highest dry biomass concentration (5.15 g/L) was obtained at 240 hours of fermentation. This value, however, was not significantly different ( $P > 0.05$ ) from that (4.98 g/L) obtained at 192 hours. There was also a decrease in the yield value at 240 hours (Fig. 3.8b) which indicated that the accelerated phase of growth has passed. The growth began to decrease after 240 hours of fermentation. The TCH concentration in the medium decreased sharply during the first 96 hours of



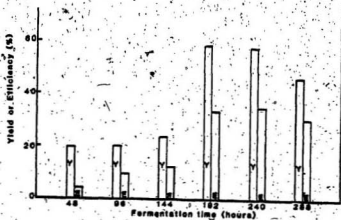
- (●) - Dry biomass concentration.  
 (■) - % yield.  
 (▲) - % efficiency.

Fig. 3.7. Effect of agitation speed on the growth of the *P. ostreatus* mycelium in the peat extract in the shake flask experiments at an incubation temperature of 28.0°C and an initial pH of 5.0.



- (●) - Dry biomass concentration.
- (●) - Residual TCH concentration.
- (x) - Final pH values.

Fig. 3.8a. Effect of fermentation time on the growth of the *P. ostreatus* mycelium in the peat extract in the shake flask experiments at an incubation temperature of 28.0°C, an initial pH of 5.0 and an agitation speed of 150 rpm



(Y) - Yield.  
(E) - Efficiency.

Fig. 3.8b. Effect of fermentation time on the growth of the *P. ostreatus* mycelium in the peat extract in the shake flask experiments at an incubation temperature of 28.0°C, an initial pH of 5.0 and an agitation speed of 150 rpm

fermentation and then the decrease became gradual until 240 hours. The residual TCH concentration became constant after 240 hours.

Pellet growth was observed after 144 hours of fermentation. The turbidity of the peat extract medium changed during growth; the peat extract cleared after 144 hours of fermentation. Fig. 3.8a also shows that the pH values of the media were fairly constant during the first 144 hours of fermentation. The pH then rose sharply from a little below 5.8 to about 8.0, between 144 hours and 192 hours of fermentation. This corresponded with the period of rapid growth. The pH continued to rise, but gradually, after 192 hours of fermentation.

### 3.2.2. Nutritional requirements of the P. ostreatus mycelium

Studies conducted using non-supplemented peat extracts to grow the P. ostreatus mycelium produced very poor results, i.e., the growth of the mycelium was very poor. This could be due to the lack of some nutrients in the peat extract. Studies on the growth in a synthetic medium and in a nutrient-supplemented peat extract were carried out to determine the nutritional requirements of P. ostreatus grown in submerged culture and also to maximize the growth of the P. ostreatus mycelium.

#### 3.2.2.1. Carbon sources

The major monosaccharides, which were present in the peat extract were glucose, mannose, xylose, galactose and arabinose (Table 3.4, page 80). These sugars were tested in a synthetic medium for their abilities to support the growth of the P. ostreatus mycelium. Table 3.6 shows that P. ostreatus grew best and produced maximal dry biomass

Table 3.6. Effect of different carbon sources (sugars) on the growth of the *P. ostreatus* mycelium in the synthetic medium<sup>1</sup>

Carbon source (15 g/L)	Dry biomass <sup>2</sup> conc. (g/L)	Yield <sup>2</sup> (%)	Efficiency <sup>2</sup> (%)
D - Glucose	7.22 ± 0.94 <sup>a</sup>	52.29 ± 1.80 <sup>a</sup>	48.11 ± 1.28
D (+) Galactose	4.11 ± 0.10	31.79 ± 1.13	27.40 ± 0.80
D (+) Mannose	7.34 ± 0.64 <sup>a</sup>	53.21 ± 1.13 <sup>a</sup>	49.05 ± 1.25
L (+) Arabinose	1.34 ± 0.06	28.28 ± 0.40	8.94 ± 0.38
D - Xylose	trace	nd <sup>3</sup>	nd <sup>3</sup>

<sup>1</sup>The synthetic medium consisted of the basal medium, plus 3.5 g/L ammonium citrate. An incubation temperature of 28°C, an initial pH of 5.0 and a fermentation time of 192 hours were used.

<sup>2</sup>These are the mean values of at least three replicate samples + standard deviations. The % yield and the % efficiency were subjected to angular transformation to find the mean values + standard deviations. The values in the same column with the same superscript are not significantly different at the 5% level.

<sup>3</sup>nd = not determined.



concentration, yield and efficiency, when D (+) mannose and D - glucose were used as carbon sources. The values for the growth parameters showed no significant difference ( $P > 0.05$ ) between D (+) mannose and D - glucose. Growth was very poor when L (+) arabinose or D - xylose was used as a carbon source. This could mean that the pentoses were not as well metabolized as the hexoses.

Analyses of the peat extract medium after fermentation indicated that the fungus utilized more of the glucose, mannose and xylose (their concentrations were lower than before fermentation) than other sugars in the peat extract (Table 3.7).

#### 3.2.2.2. General discussion: utilization of carbon sources

Carbohydrates are usually the best sources of carbon and energy for microbial biomass production. The different growth parameters obtained with different sugars in the synthetic medium and the greater utilization of glucose and mannose in the peat extract, indicate that the response of P. ostreatus to various carbon sources may be specific, so that one substrate might have been utilized readily while another of closely similar chemical structure might have been poorly utilized (Hawker, 1968). Bukhalo and Solomko (1978) have reported that P. ostreatus readily assimilated glucose, sucrose and mannitol; less readily, raffinose, maltose, xylose, starch and cellulose, and poorly, lactose. Hashimoto and Takahashi (1974), also obtained poor growth of P. ostreatus when xylose or arabinose was used as the only carbon source. The poor growth of P. ostreatus on xylose or arabinose was, however, unexpected as it has been reported that these two sugars are usually satisfactory sources of carbon for wood-destroying filamentous fungi

Table 3.7. The composition of the major monosaccharides in the peat extract medium after fermentation

Monosaccharide	Composition (% of total reducing sugars) <sup>2</sup>
Glucose	6.45 $\pm$ 0.95
Galactose	38.18 $\pm$ 1.78
Rhamnose	28.37 $\pm$ 1.37
Mannose	10.61 $\pm$ 1.44
Arabinose	6.51 $\pm$ 1.25
Xylose	8.42 $\pm$ 1.64

<sup>1</sup>Peat extract diluted to a 1:1 ratio with water, plus 5.0 g/L yeast extract, an incubation temperature of 28°C, an initial pH of 5.0 and a fermentation time of 192 hours were used.

<sup>2</sup>These are the mean values of three determinations of three replicate samples  $\pm$  standard deviations.

(Hawker, 1968). Mannose has been reported to be a good source of carbon for mushroom mycelial growth by several investigators (Hashimoto and Takahashi, 1974; Yoshida et al., 1965; Reusser et al., 1958a; Srivastava and Bano, 1970). However, glucose is more available and less expensive than mannose.

Analyses of the peat extract medium after fermentation indicated that more glucose was consumed than mannose, even though they were equally utilized when tested singly in the synthetic medium. Studies on the utilization of mixtures have shown that glucose has general repressive and inhibitory effects on the utilization of other carbon sources (Griffin, 1981).

The 15 g/L concentration of glucose and 3.5 g/L concentration of ammonium citrate in the synthetic medium provided about the same TCH (15.0 g/L) and nitrogen (0.5 g/L), respectively, as did the peat extract diluted to a 1:1 ratio with water, and supplemented with 5.0 g/L yeast extract, yet a higher dry biomass concentration was obtained in the synthetic medium (7.22 g/L), than in the peat extract (4.98 g/L). An explanation for the difference in the growth could be that, in addition to the probable presence of inhibitory substances in the peat extract, the TCH in the peat extract represented a mixture of different carbohydrates some of which could not be utilized efficiently. The fungus did not have the capacity to produce small quantities of several enzymes to utilize the different carbohydrates in the peat extract, but was capable of producing a large quantity of the enzyme that utilized the glucose in the synthetic medium (Hawker, 1968). It was observed that at 15 g/L glucose concentration, the fungus was able to utilize

about 99% of the available TCH in the synthetic medium, while it could only utilize about 57.0% of the TCH concentration in the peat extract medium (the final TCH concentration in the fermented peat broth was about 6.5 g/L). Another explanation for the lower growth obtained in the peat extract could be that the complex nature of the medium might have caused some of the utilizable carbohydrates to be diverted into certain metabolic pathways that did not synthesize microbial carbon (Foster, 1949). Failure to completely utilize all the carbohydrate in a complex medium seems to be a characteristic of the mushroom mycelium metabolism. For example, reducing sugars utilization by P. ostreatus in brewery wastes ranged from 52.7 to 74.7% (Shannon and Stevenson, 1975). Litchfield and Overbeck (1965) obtained carbohydrate utilization values ranging from 50 to 70% with Morchella cultures grown in food producing wastes. Reusser et al. (1958a, b) obtained a value of 82.8% for M. hybrida in waste sulfite liquor medium, and 90% for T. nudum in a sugar beet molasses medium. In peat extract, Martin (1983a) reported that M. esculenta only utilized about 50% of the available carbohydrate, while Quierzy et al. (1979) observed that in a batch cultivation of C. utilis, only 55 - 60% of the initial TCH was consumed by the organism. This suggested that some carbohydrates in complex media might not be utilizable.

### 3.2.2.3. Nitrogen sources

Growth of the P. ostreatus mycelium in a non-nitrogen supplemented peat extract produced very little growth. Therefore, different organic and inorganic sources of nitrogen were individually used to supplement the peat extract. Studies were also conducted using a

synthetic medium with different ammonium salts as sources of nitrogen. The addition of nitrogen, in most cases, produced higher mycelium concentrations than a non-supplemented peat extract (Table 3.8), which indicated that the peat extract was too deficient in nitrogen for a good growth of the *P. ostreatus* mycelium. However, the effect of the nitrogen supplementation on the growth of the *P. ostreatus* mycelium depended on the nitrogen source. Maximal growth values were obtained in the peat extract supplemented with yeast extract as the nitrogen source, and in the synthetic medium with ammonium citrate as the nitrogen source (Table 3.9). Ammonium citrate and ammonium nitrate did not produce any significant increase in the growth in the peat extract as compared with the non-supplemented peat extract, while ammonia solution and potassium nitrate produced about 27.0 and 25.0% increase in the growth, respectively. Ammonium phosphate produced a dry biomass concentration and yield values higher than those produced by urea in the peat extract, but the reverse was true in the synthetic medium.

The organic nitrogen sources and ammonium citrate increased the pH of the medium, while the ammonium salts of inorganic acids and potassium nitrate decreased the pH during the growth of *P. ostreatus* (Tables 3.8 and 3.9).

#### 3.2.2.4. General discussion: utilization of nitrogen sources

It was observed that the growth was comparatively poor when the peat extract was supplemented with some ammonium salts of inorganic acids. This is in agreement with the observation that, generally, organic nitrogen sources are better than inorganic nitrogenous salts for

Table 3.8. Effect of different sources of nitrogen on the growth of the *P. ostreatus* mycelium in the peat extract<sup>1</sup>

Nitrogen source <sup>2</sup> (0.5 g/L N)	Dry biomass <sup>3</sup> conc. (g/L)	Yield <sup>3</sup> (%)	Final pH of medium
None	1.06 ± 0.02 <sup>a</sup>	35.35 ± 1.92 <sup>a</sup>	4.44
Ammonia solution (29.8%) <sup>4</sup>	1.35 ± 0.02 <sup>b</sup>	35.05 ± 1.07 <sup>a</sup>	4.43
Ammonium citrate	1.01 ± 0.02 <sup>a</sup>	29.65 ± 1.76 <sup>b</sup>	5.17
Ammonium phosphate (dibasic)	3.40 ± 0.04	37.30 ± 1.14 <sup>a</sup>	4.95
Ammonium sulfate	1.20 ± 0.03	28.20 ± 1.92 <sup>bc</sup>	4.31
Ammonium nitrate	0.91 ± 0.07 <sup>a</sup>	23.20 ± 1.48	4.35
Potassium nitrate	1.32 ± 0.02 <sup>b</sup>	14.10 ± 0.85	4.68
Yeast extract	4.98 ± 0.05	60.02 ± 1.14	6.47
Peptone	2.89 ± 0.07	31.00 ± 1.90 <sup>b</sup>	7.39
Urea	2.46 ± 0.02	26.20 ± 1.14 <sup>c</sup>	5.98

<sup>1</sup> Peat extract diluted to a 1:1 ratio with water, an incubation temperature of 28°C, an agitation speed of 150 rpm and a fermentation time of 192 hours were used.

<sup>2</sup> Amount of nutrient added was calculated so that approximately the same quantity of nitrogen was added from each source.

<sup>3</sup> These are the mean values of at least three replicate samples ± standard deviations. The % yield was subjected to angular transformation to find the mean values ± standard deviations. The values in the same column with the same superscript are not significantly different at the 5% level.

<sup>4</sup> Quantity required to bring the pH of the peat extract to 5.

Table 3.8. Effect of different sources of nitrogen on the growth of the *P. ostreatus* mycelium in the peat extract

Nitrogen source <sup>2</sup> (0.5 g/L N)	Dry biomass <sup>3</sup> conc. (g/L)	Yield <sup>3</sup> (%)	Final pH of medium
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Ammonium citrate	1.01 ± 0.02 <sup>a</sup>	29.55 ± 1.76 <sup>b</sup>	5.17
Ammonium phosphate (dibasic)	3.40 ± 0.04	37.30 ± 1.14 <sup>a</sup>	4.95
Ammonium sulfate	1.20 ± 0.03	28.20 ± 1.92 <sup>bc</sup>	4.31
Ammonium nitrate	0.91 ± 0.07 <sup>a</sup>	23.20 ± 1.48	4.35
Potassium nitrate	1.32 ± 0.02 <sup>b</sup>	14.10 ± 0.85	4.68
Yeast extract	4.98 ± 0.05	60.02 ± 1.14	6.47
Peptone	2.89 ± 0.07	31.00 ± 1.90 <sup>b</sup>	7.39
Urea	2.46 ± 0.02	26.20 ± 1.14 <sup>c</sup>	5.98

<sup>1</sup> Peat extract diluted to a 1:1 ratio with water, an incubation temperature of 28°C, an agitation speed of 150 rpm and a fermentation time of 192 hours were used.

<sup>2</sup> Amount of nutrient added was calculated so that approximately the same quantity of nitrogen was added from each source.

<sup>3</sup> These are the mean values of at least three replicate samples ± standard deviations. The % yield was subjected to angular transformation to find the mean values ± standard deviations. The values in the same column with the same superscript are not significantly different at the 5% level.

<sup>4</sup> Quantity required to bring the pH of the peat extract to 5.

fungus growth, especially in unbuffered medium (Litchfield, 1967a; Bukhalo and Solomko, 1978). For example, Hashimoto and Takahashi (1974), and Hackaylo et al. (1954) reported that organic sources of nitrogen supported good growth of P. ostreatus while ammonium salts and nitrates produced less growth. Yoshida et al. (1965) observed that P. japonicus grew better in a medium with yeast extract and/or peptone than in one with ammonium sulfate or ammonium tartrate as the nitrogen source. Yeast extract has been found to be a good source of nutrients for the growth of microbial biomass. Sugimori et al. (1971), comparing different organic nitrogen sources, found that yeast extract produced the highest mycelial concentration of P. ostreatus. Bukhalo and Solomko (1978) obtained the highest biomass growth of P. ostreatus when yeast extract was used to supplement some complex media. The problem with yeast extract is that it is too expensive for an industrial production of microbial biomass.

### 3.2.2.5. General discussion: ability of the mushroom mycelium to utilize ammonium salts as nitrogen sources in submerged culture

Fungi differ in their ability to utilize various ammonium salts as nitrogen sources for their growth (Hawker, 1968). In this work P. ostreatus grew very well in the synthetic medium with ammonium citrate as a nitrogen source, but Brock (1951) found that ammonium citrate was toxic to M. esculenta. The apparent differences in the utilization of the various ammonium compounds may be due to the increase of the hydrogen ion concentration in the media. Hawker (1968) has reported



that when a fungus was grown with an ammonium salt of an inorganic acid as a source of nitrogen, the medium became more acidic. The acidity resulted from a rapid utilization of the ammonium ion. Srivastava and Bano (1970) have reported that when ammonium salts of inorganic acids were used as sole sources of nitrogen for the growth of P. flabellatus, the final pH of the medium dropped to 3.0. A similar observation was made in this work (Tables 3.8 and 3.9, pages 95 and 98). Therefore, the low pH values developed in the medium could be responsible for the poor growth observed in the peat extract medium when some ammonium salts were used as supplements (Hashimoto and Takahashi, 1974).

The good growth obtained when ammonium phosphate was used, however, was due to the strong buffering capacity of the phosphate ions. Thus, they were able to maintain the pH values of the medium over a range suitable for growth of the mushroom mycelium (Litchfield, 1967a). However, this strong buffering capacity was lost at higher concentrations of the ammonium salt, and acid reactions began to increase in the medium (Reusser et al., 1958b; Shannon and Stevenson, 1975). It was, therefore, observed that the growth of P. ostreatus decreased when the concentration of the ammonium phosphate increased from 0.25 to 0.50 g/L (Table 3.10).

Ammonium citrate did not enhance the growth of the P. ostreatus mycelium in the peat extract, although it was the best nitrogen source for the fungus in the synthetic medium. It could be that the ability to utilize a particular nitrogen source might depend, among other factors,

Table 3.10. Effect of different concentrations of ammonium phosphate on the growth and the crude protein content of the *P. ostreatus* mycelium in the peat extract

Ammonium phosphate conc. (g/L)	Dry biomass <sup>2</sup> conc. (g/L)	Yield <sup>2</sup> (%)	Efficiency <sup>2</sup> (%)	Crude protein (% of dry mycelium)
0	1.06 ± 0.08 <sup>a</sup>	25.35 ± 1.92	7.55 ± 0.65	9.81
2.5	3.40 ± 0.04	37.30 ± 1.14	22.65 ± 1.21	13.75
5.0	1.10 ± 0.05 <sup>a</sup>	32.90 ± 1.50	18.25 ± 1.29	22.19
7.5	trace	nd <sup>3</sup>	nd <sup>3</sup>	nd <sup>3</sup>

<sup>1</sup>Peat extract diluted to a 1:1 ratio with deionized water, an incubation temperature of 28°C, an initial pH of 5.0, an agitation speed of 150 rpm and a fermentation time of 192 hours were used.

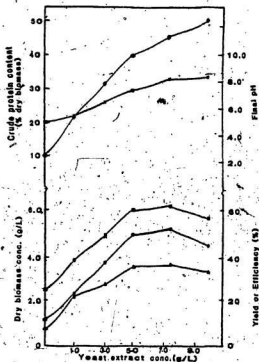
<sup>2</sup>These are the mean values of at least three replicate samples ± standard deviations. The % yield and the % efficiency were subjected to angular transformation to find the mean values ± standard deviation. The values of the same column with the same superscript are not significantly different at the 5% level.

<sup>3</sup>nd = not determined.

on the nature of the carbon source used in the medium (Hawker, 1968). *P. ostreatus* grew poorly, in both the synthetic medium and the peat extract, when potassium nitrate or ammonium nitrate was used as the nitrogen source. A similar observation was made by Sriyastava and Bano (1970) with *P. flabellatus*, and by Hashida *et al.* (1967) with *Collybia velutipes*. Hackaylo *et al.* (1954) have reported that basidiomycetes utilize nitrate nitrogen very slowly or not at all.

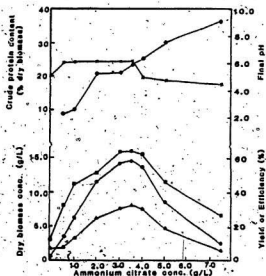
#### 3.2.2.6. Effect of increasing concentrations of nitrogen on the mycelial growth and the crude protein concentration

It was observed that increased concentrations of nitrogen had a positive influence on the growth and the crude protein concentration of the *P. ostreatus* mycelium. There was a smooth increase in the growth and in the crude protein concentration when the concentration of yeast extract in the peat extract medium (Fig. 3.9), or when the concentration of ammonium citrate in the synthetic medium (Fig. 3.10), was increased. Maximal growth values were obtained at 5.0 g/L yeast extract and at 3.5 g/L ammonium citrate in the peat extract medium and in the synthetic medium, respectively. Higher concentrations produced a less favorable effect on growth. There was no significant difference ( $P > 0.05$ ) between the corresponding values of the growth parameters at 5.0 and 7.5 g/L yeast extract in the peat extract or at 3.0 g/L and 4.0 g/L ammonium citrate in the synthetic medium. However, higher concentrations of the yeast extract or ammonium citrate produced an increase in the crude protein content in the mycelium. The addition of



- (●) - Dry biomass concentration.
- (■) - % yield.
- (▲) - % efficiency.
- (◆) - Crude protein content.
- (x) - Final pH.

Fig. 3.9. Effect of different concentrations of yeast extract on the growth and the crude protein content of the *P. ostreatus* mycelium grown in the peat extract in the shake flask experiments at a temperature of 28°C, an initial pH of 5.0, an agitation speed of 150 rpm and a fermentation time of 192 hours



- (●) - Dry biomass concentration.
- (■) - % yield.
- (▲) - % efficiency.
- (◆) - Crude protein.
- (x) - Final pH.

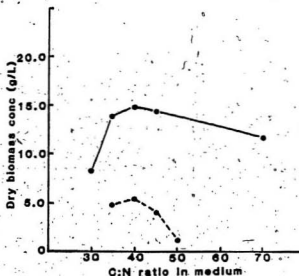
Fig. 3.10. Effect of different concentrations of ammonium citrate on the growth and the crude protein content of the *P. ostreatus* mycelium grown in the synthetic medium in the shake flask experiments at an incubation temperature of 28.0°C, an initial pH of 5.0, an agitation speed of 150 rpm and a fermentation time of 192 hours.

ammonium phosphate to the peat extract followed a similar pattern. Table 3.10 (page 99) shows that concentrations of ammonium phosphate higher than 2.5 g/L had a detrimental effect on the growth, but it produced an increase in the protein content of the mycelium. This corresponds with the observations made by other investigators, that the mycelial protein concentration of a variety of mushrooms could be increased markedly by increasing the amount of nitrogen in the medium (Humfeld and Sugihara, 1952; Reusser *et al.*, 1958b; Falanghe, 1962; Litchfield, 1963, 1967b; Srivastava and Bano, 1970).

Increased concentrations of yeast extract or ammonium phosphate also produced increased pH values in the media during the growth of the fungal mycelium in the peat extract. The pH of the synthetic medium also increased during the mycelial growth when the concentration of the ammonium citrate was increased to 3.5 g/L. However, higher concentrations produced a decrease in the pH.

### 3.2.2.7. General discussion: effect of C:N ratio on the mushroom mycelial growth

Several factors may influence the growth of the mushroom mycelium in submerged culture. Among these factors are the adequate amount and the kinds of carbon and/or nitrogen sources, and a favorable quantitative balance (C:N ratio) of these nutrients. Moustafa (1960) and Falanghe *et al.* (1964) have reported that an optimal C:N ratio was needed for the effective reduction of the lag period, a maximum sugar consumption, and a good yield. Fig. 3.11 shows the dry biomass concentration of the *P. ostreatus* mycelium as a function of the C:N



(—) Synthetic medium.  
 (---) Peat extract.

Fig. 3.11. Relationships between the dry biomass concentrations of the *P. ostreatus* mycelium and the C:N ratio in the shake flask experiments at a temperature of 28°C, an initial pH of 5.0, an agitation speed of 150 rpm and a fermentation time of 192 hours.

ratios in the synthetic medium and in the peat extract. The optimal C:N ratio for P. ostreatus was about 40:1 in both media. This compares very well with the optimal C:N ratio for the growth of P. flabellatus (Srivastava and Bano, 1970). The optimal C:N values for mushroom biomass production vary widely depending upon the species, the growth medium, and the operating conditions such as temperature, pH, agitation and aeration. Reusser et al. (1958a) reported that the highest yields of A. campestris NRRL 2335 mycelium grown in a glucose ammonium tartrate synthetic medium were obtained in the C:N range of 20:1 to 25:1. However, with another strain of this organism (NRRL 2334), Moustafa (1960) observed the best growth in a malt sprout extract glucose medium in the C:N range of 8:1 to 12:1. Shannon and Stevenson (1975) obtained the best growth for Calvatia gigantea mycelium grown in brewery waste-ammonium sulfate medium, in the C:N range of 30:1 to 40:1.

Approximately the same optimal C:N ratio found for the growth of the P. ostreatus mycelium in the synthetic medium and in the peat extract could mean that the C:N ratio which affords the maximum growth of mushroom mycelium in submerged culture is independent of the carbon and the nitrogen sources. Similar observations have been made by other investigators. For example, Morchella hortensis grown in a glucose ammonium phosphate synthetic medium alone, and in a medium supplemented with corn steep liquors, produced the highest growth at a C:N range of 5:1 to 10:1 (Litchfield, 1963). Litchfield and Overbeck (1965) obtained optimal yields of three species of morel mushrooms at C:N ratio of 8:1 in pumpkin waste and in cheese whey media.



### 3.2.2.8. Effect of phosphorus and potassium

Omission of phosphorus or potassium from the synthetic medium greatly reduced the dry biomass concentration, yield and efficiency of *P. ostreatus* mycelial growth (Tables 3.11 and 3.12). The best growths were obtained with minimum phosphorus and potassium concentrations of 0.22 g/L and 0.28 g/L, respectively. Higher concentrations did not significantly ( $P > 0.05$ ) improve the growth of the *P. ostreatus* mycelium in the synthetic medium.

Fig. 3.12 shows that the addition of potassium phosphate greatly enhanced the growth of the *P. ostreatus* mycelium in the peat extract. There was a rapid increase in the growth when 1.3 g/L of potassium phosphate was added to the medium. The growth continued to increase with further additions of potassium phosphate, but at a slower rate until maximal growth values were obtained at 6.5 g/L. A higher concentration had an unfavorable effect on the growth.

The pH of the medium rose slightly during the growth and then became almost constant. Sugimori *et al.* (1971) also observed that concentrations of potassium phosphate higher than 3.0 g/L produced lower yields. When potassium phosphate was added to the medium, the crude protein concentration of the mycelium did not increase significantly over that of the dry mycelium produced with non-supplemented peat extract. Thus, little or no improvement was made in the protein content of the mycelium by varying the amount of potassium phosphate. Similar observations were made by Falanghe (1962), and Srivastava and Bano (1970).

Table 3.11. Effect of different concentrations of phosphorus on the growth of the *P. ostreatus* mycelium in the synthetic medium<sup>1</sup>

Phosphorus conc. (g/L)	Dry biomass <sup>2</sup> conc. (g/L)	Yield <sup>2</sup> (%)	Efficiency <sup>2</sup> (%)
0	0.35 ± 0.30	2.81 ± 0.44	0.78 ± 0.06
0.11	12.44 ± 0.54	61.26 ± 2.74 <sup>a</sup>	27.64 ± 1.21
0.22	14.68 ± 0.69 <sup>a</sup>	64.17 ± 1.62 <sup>ab</sup>	32.62 ± 1.76
0.33	15.27 ± 1.25 <sup>a</sup>	66.20 ± 2.24 <sup>b</sup>	35.04 ± 1.03

<sup>1</sup>The synthetic medium consisted of the basal medium, plus 3.0 g/L ammonium citrate and 45 g/L glucose. An incubation temperature of 28°C, an initial pH of 5.0, an agitation speed of 150 rpm and a fermentation time of 192 hours were used.

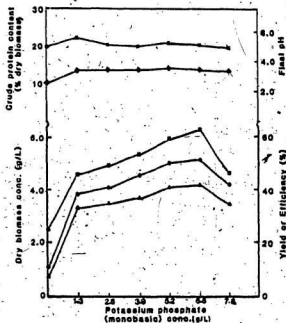
<sup>2</sup>These are the mean values of at least three replicate samples ± standard deviations. The % yield and the % efficiency were subjected to angular transformation to find the mean values ± standard deviations. The values in the same column with the same superscript are not significantly different at the 5% level.

Table 3.12. Effect of different concentrations of potassium on the growth of the *P. ostreatus* mycelium in the synthetic medium

Phosphorus conc. (g/L)	Dry biomass <sup>2</sup> conc. (g/L)	Yield <sup>2</sup> (%)	Efficiency <sup>2</sup> (%)
0	0.43 ± 0.00	3.04 ± 0.37	0.96 ± 0.01
0.14	12.68 ± 0.89	57.95 ± 1.84 <sup>a</sup>	28.18 ± 1.98
0.28	14.68 ± 0.69 <sup>a</sup>	64.17 ± 1.62 <sup>ab</sup>	32.62 ± 1.76
0.42	15.49 ± 1.19 <sup>a</sup>	5.77 ± 2.09 <sup>b</sup>	34.41 ± 2.66

<sup>1</sup>The synthetic medium consisted of the basal medium, plus 3.0 g/L ammonium citrate and 45 g/L glucose. An incubation temperature of 28°C, an initial pH of 5.0, an agitation speed of 150 rpm and a fermentation time of 192 hours were used.

<sup>2</sup>These are the mean values of at least three replicate samples ± standard deviations. The % yield and the % efficiency were subjected to angular transformation to find the mean values ± standard deviations. The values in the same column with the same superscript are not significantly different at the 5% level.



- (●) - Dry biomass concentration.
- (■) - % yield.
- (▲) - % efficiency.
- (◆) - Crude protein.
- (×) - Final pH.

Fig. 3.12. Effect of  $\text{KH}_2\text{PO}_4$  on the growth and the crude protein content of the *P. ostreatus* mycelium grown in the peat extract in the shake flask experiments at a temperature of  $28^\circ\text{C}$ , an initial pH of 5.0, an agitation speed of 150 rpm and a fermentation time of 192 hours

### 3.2.2.9. General discussion: effect of phosphorus and potassium on the growth of the mushroom mycelium

The results obtained with potassium phosphate, added to both the synthetic medium and the peat extract, indicate that the P. ostreatus mycelium had a significant requirement for the phosphorus and potassium. It was observed that the numerical value for the dry biomass concentration obtained in the peat extract medium supplemented with 5.2 g/L potassium phosphate was not significantly different ( $P > 0.05$ ) from that supplemented with 5.0 g/L yeast extract. The latter provided, in addition to nitrogen, trace nutrients to the medium. The good growth observed for the P. ostreatus mycelium when potassium phosphate was added to the medium was in agreement with the observations made by other investigators, that phosphorus enhances the growth of mushroom mycelium. For example, Srivastava and Bano (1970) observed that the omission of phosphate from the medium hampered the growth of P. flabellatus in a glucose-ammonium citrate medium. Kosaric and Miyata (1981) also observed an enhanced growth of M. esculenta when potassium phosphate was added to a cheese whey medium. Hawker (1968) and Sinskey (1978) have reported that fungal cells have large quantities of phosphorus and, therefore, it should be included in the medium for their growth.

The good growth observed by supplementing the peat extract with phosphate also confirmed the observations made by several investigators, that peat extract supplemented with phosphate was a very good medium for microbial biomass production (Quierzy et al., 1979; McLoughlin and Kuster, 1972b). Most likely, the addition of phosphate

maintained the pH in a range suitable for the growth and compensated for any phosphorus deficiencies in the peat extract. Phosphorus is used in the cells for the production of energy-rich phosphorus compounds required for synthesizing complex organic substances (Hawker, 1968).

#### 3.2.2.10. The combined effect of potassium phosphate and yeast extract

The results of the nutrient supplementation of the peat extract showed that potassium phosphate or yeast extract could be used to enhance the production of the *P. ostreatus* mycelium in a peat extract medium. Consequently, it appeared worthwhile to investigate the effect of combining the two sources of nutrients on the growth and biomass production of the *P. ostreatus* mycelium in peat extract. Various combinations of concentrations of potassium phosphate and yeast extract were used, and the results are summarized in Table 3.13. It was apparent that the addition of both nutrient sources to the peat extract enhanced the growth.

The highest dry biomass concentration of 6.8 g/L was obtained at the maximum supplement concentrations tested, i.e., when 5.0 g/L yeast extract and 3.9 g/L potassium phosphate were added to the peat extract. This combination produced an increase in the biomass concentration of about 37% over that produced by adding 5.0 g/L yeast extract alone and an increase of 51% over that produced by adding 3.9 g/L potassium phosphate alone. However, it was observed that there were no significant differences in the growth when other combinations

Table 3.13. The combined effect of yeast extract and  $\text{KH}_2\text{PO}_4$  on the growth of the *P. ostreatus* mycelium in the peat extract

Yeast extract conc. (g/L)	$\text{KH}_2\text{PO}_4$ conc. (g/L)	Dry biomass <sup>2</sup> conc. (g/L)	Yield <sup>2</sup> (%)	Efficiency <sup>2</sup> (%)
0	0	1.08 ± 0.08	35.35 ± 1.92	7.55 ± 0.15
	1.3	3.80 ± 0.03 <sup>a</sup>	45.30 ± 1.84 <sup>a</sup>	25.35 ± 1.92 <sup>a</sup>
	2.6	4.11 ± 0.11 <sup>b</sup>	48.37 ± 2.20 <sup>ab</sup>	27.37 ± 0.74 <sup>a</sup>
	3.9	4.51 ± 0.11 <sup>bc</sup>	52.99 ± 1.49 <sup>bc</sup>	30.03 ± 1.92 <sup>b</sup>
1.0	0	2.24 ± 0.05 <sup>cd</sup>	30.35 ± 0.21	19.50 ± 1.23 <sup>bc</sup>
	1.3	4.76 ± 0.27 <sup>b</sup>	53.80 ± 2.60 <sup>c</sup>	31.45 ± 1.48 <sup>bc</sup>
	2.6	5.25 ± 0.02 <sup>b</sup>	62.45 ± 1.35 <sup>dc</sup>	34.75 ± 1.21 <sup>ce</sup>
	3.9	5.51 ± 0.15 <sup>b</sup>	65.70 ± 1.70 <sup>ef</sup>	36.70 ± 0.71 <sup>c</sup>
3.0	0	3.84 ± 0.06 <sup>a</sup>	49.73 ± 1.15 <sup>b</sup>	26.26 ± 0.08 <sup>a</sup>
	1.3	6.07 ± 0.19 <sup>a</sup>	68.90 ± 2.12 <sup>f</sup>	40.40 ± 0.99 <sup>d</sup>
	2.6	6.48 ± 0.16 <sup>f</sup>	71.05 ± 2.47 <sup>gh</sup>	41.04 ± 1.58 <sup>d</sup>
	3.9	6.48 ± 0.18 <sup>f</sup>	71.25 ± 1.80 <sup>gh</sup>	41.65 ± 1.25 <sup>d</sup>
5.0	0	4.98 ± 0.05 <sup>d</sup>	60.02 ± 1.14 <sup>d</sup>	33.20 ± 1.14 <sup>e</sup>
	1.3	6.18 ± 0.06 <sup>e</sup>	70.25 ± 1.19 <sup>g</sup>	41.20 ± 1.20 <sup>d</sup>
	2.6	6.50 ± 0.16 <sup>f</sup>	71.25 ± 1.14 <sup>gh</sup>	43.20 ± 1.20 <sup>df</sup>
	3.9	6.80 ± 0.18 <sup>f</sup>	73.05 ± 2.47 <sup>h</sup>	45.05 ± 1.46 <sup>f</sup>

<sup>1</sup>Peat extract diluted to a 1:1 ratio with water, an incubation temperature of 28°C, an initial pH of 5.0, an agitation speed of 150 rpm and a fermentation time of 192 hours were used.

<sup>2</sup>These are the mean values of at least three replicate samples ± standard deviations. The % yield and the % efficiency were subjected to angular transformation to find the mean values ± standard deviations. The values in the same column with the same superscript are not significantly different at the 5% level.

of nutrients were used. For example, there was no significant difference ( $P > 0.05$ ) in the growth parameters at 3.0/2.6 g/L and those at 5.0/3.9 g/L for yeast extract/potassium phosphate combinations.

### 3.2.2.11. Addition of magnesium and manganese

It has been reported that some minerals, when added in small concentrations to culture media, enhance the growth and the flavor development of the mushroom mycelium (Humfeld and Sugihara, 1952). Therefore, the effect of magnesium and manganese on the growth of the P. ostreatus mycelium was studied in this work. Table 3.14 shows that the addition of magnesium to the peat extract had an inhibitory effect on the growth of P. ostreatus. The concentration of magnesium in a synthetic medium needed for the maximal growth of A. campestris was 20 mg/L (Humfeld and Sugihara, 1952). The magnesium concentration of the peat extract diluted to a 1:1 ratio with water was about 130 mg/L. Thus, further increases of the magnesium concentration might have exceeded the optimum required for the growth of the P. ostreatus mycelium. Varying the concentration of manganese sulfate added to the medium produced a significant difference ( $P > 0.05$ ) in the growth in the range 0.10 and 0.15 g/L, but there was no significant difference ( $P < 0.05$ ) in the growth at concentrations below or above this range and the growth in the non-manganese supplemented peat extract (Table 3.15). Kosaric and Miyata (1981) observed that addition of either magnesium or manganese did not increase the biomass production of M. crassipes 13227 in a cheese whey medium.



Table 3.14. Effect of magnesium and manganese on the growth of the *P. ostreatus* mycelium in the peat extract<sup>1</sup>

MgSO <sub>4</sub> (0.80 g/L)	MnSO <sub>4</sub> (0.20 g/L)	Dry biomass <sup>2</sup> conc. (g/L)	Yield <sup>2</sup> (%)	Efficiency <sup>2</sup> (%)
0	0	6.48 ± 0.16 <sup>a</sup>	71.05 ± 2.47 <sup>a</sup>	41.04 ± 1.58 <sup>a</sup>
+	0	5.25 ± 0.02	60.87 ± 1.30	34.43 ± 1.43
0	+	6.66 ± 0.12 <sup>a</sup>	72.01 ± 1.45 <sup>a</sup>	41.83 ± 0.78 <sup>a</sup>
+	+	5.51 ± 0.11	63.40 ± 1.57	38.05 ± 1.21

<sup>1</sup>Peat extract diluted to a 1:1 ratio with water, plus 3.0 g/L yeast extract and 2.6 g/L KH<sub>2</sub>PO<sub>4</sub>, an incubation temperature of 28°C, an initial pH of 5.0, an agitation speed of 150 rpm and a fermentation time of 192 hours were used.

<sup>2</sup>These are the mean values of at least three replicate samples ± standard deviations. The % yield and the % efficiency were subjected to angular transformation to find the mean values ± standard deviations. The values in the same column with the same superscript are not significantly different at the 5% level.

Table 3.15. Effect of different concentrations of manganese on the growth of the *P. ostreatus* mycelium in the peat extract<sup>1</sup>

MnSO <sub>4</sub> conc. (g/L)	Dry biomass <sup>2</sup> conc. (g/L)	Yield <sup>2</sup> (%)	Efficiency <sup>2</sup> (%)
0	6.48 ± 0.16 <sup>a</sup>	71.05 ± 2.47 <sup>a</sup>	41.04 ± 1.58 <sup>a</sup>
0.05	6.60 ± 0.16 <sup>ab</sup>	71.80 ± 1.42 <sup>a</sup>	41.50 ± 1.45 <sup>a</sup>
0.10	6.80 ± 0.14 <sup>b</sup>	72.50 ± 1.24 <sup>a</sup>	43.14 ± 0.85 <sup>b</sup>
0.15	6.83 ± 0.11 <sup>b</sup>	72.35 ± 1.45 <sup>a</sup>	43.48 <sup>b</sup> ± 1.47
0.20	6.66 ± 0.12 <sup>ab</sup>	72.01 ± 1.50 <sup>a</sup>	41.83 ± 0.78 <sup>ab</sup>

<sup>1</sup>Peat extract diluted to a 1:1 ratio with water, plus 3.0 g/L yeast extract and 2.6 g/L KH<sub>2</sub>PO<sub>4</sub>, an incubation temperature of 28°C, an initial pH of 5.0, an agitation speed of 150 rpm and a fermentation time of 192 hours were used.

<sup>2</sup>These are the mean values of at least three replicate samples ± standard deviations. The % yield and the % efficiency were subjected to angular transformation to find the mean values ± standard deviations. The values in the same column with the same superscripts are not significantly different at the 5% level.

### 3.3. Batch fermenter experiments

#### 3.3.1. Agitation and aeration

The effect of agitation speeds (150 to 250 rpm at 50 rpm-intervals) and aeration rates (0.5 to 1.5 vvm at 0.5 vvm intervals) on the growth of the P. ostreatus mycellum in a batch fermenter was tested. The results are presented in Fig. 3.13. Maximum growth was obtained at 200 rpm. There was less growth at higher and lower agitation speeds, at all the aeration rates tested. Fig. 3.13 also shows that the best growth was obtained at 1.0 vvm at all the agitation speeds tested. Thus, the optimal agitation speed and aeration rate for the growth of P. ostreatus (within the ranges of the values tested) were 200 rpm and 1.0 vvm, respectively. The influence of the aeration on the effect of the agitation speed on the growth, and vice versa, were also observed. For example, the dry biomass concentration was slightly higher at 150 rpm than at 200 rpm when the aeration rate was 1.5 vvm, while there was no significant difference ( $P > 0.05$ ) between corresponding values of the dry biomass concentration at 200 rpm and 250 rpm for the same aeration rate. However, a higher yield value was obtained at 200 rpm, indicating that the growth was, in general, better at 200 rpm. It was also observed that at all levels of agitation speed tested, the growth was better at 0.5 vvm than at 1.5 vvm. The growth parameter values at 200 rpm and 0.5 vvm were not significantly different ( $P > 0.05$ ) from those at 150 rpm and 1.0 vvm or those at 250 rpm and 1.0 vvm.

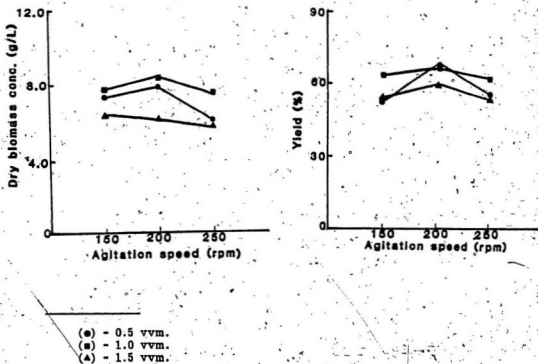


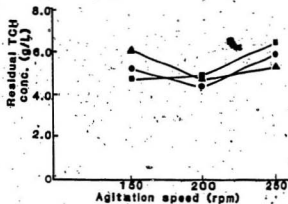
Fig. 3.13. Effect of mechanical agitation and aeration on the growth of the *P. ostreatus* mycelium in a batch fermentor at a temperature of 28°C, a pH of 5.0 and a fermentation time of 192 hours.

### 3.3.1.1. Effect of agitation and aeration on the substrate (TCH) consumption

The relationship between the residual TCH concentration in the peat extract medium and the agitation speed at various aeration rates is shown in Fig. 3.14. It can be seen that the consumption rate of carbohydrates follows a similar pattern as the growth parameters. That is, more carbohydrates were consumed at 200 rpm and 1.0 vvm, the optimal values for the growth of the fungus, than at other agitation speeds and aeration rates tested. It was also observed that at 1.5 vvm, more carbohydrates were consumed at 150 rpm than at any other agitation speed tested. This corresponds with the observation that the highest dry biomass concentration was obtained, at 1.50 vvm, when the agitation speed was 150 rpm. Also, at 250 rpm the amount of carbohydrates consumed was greater at 0.5 vvm than at 1.0 vvm, even though growth was better at the latter aeration rate. This means that the process at 1.0 vvm was more productive, as it produced a higher yield.

### 3.3.1.2. General discussion: effect of agitation and aeration on the growth of the mushroom mycelium

The dissolved oxygen concentration depended on the medium agitation, and higher levels of agitation increased the oxygen transfer capability of the fermenting system. The higher the agitation speed and/or aeration rate, the higher the concentration of dissolved oxygen in the medium (Yoshida *et al.*, 1965; Martin and Bailey, 1985), which is



- (Δ) - 0.5 vvm.  
 (●) - 1.0 vvm.  
 (■) - 1.5 vvm.

Fig. 3.14. Effect of agitation and aeration on the substrate (TCH) utilization during the batch cultivation of the *P. ostreatus* mycelium in the peat extract at an incubation temperature of 28°C, a pH of 5.0 and a fermentation time of 192 hours

essential for the production of biomass by aerobic microorganisms. However, it was observed that the growth was reduced at 250 rpm and/or 1.5 vvm.

The detrimental effect of higher agitation speeds on the growth of the mushroom mycelium in submerged culture could be due to high local stress or impeller shock on the growing hyphae (Taguchi *et al.*, 1968). Growth of filamentous fungi involves an increase in the hyphal length (Righelato, 1975), which appears to occur at the tips of the hyphae (Katz and Rosenberger, 1971). Duckworth and Harris (1949), and Finn (1967) have reported that increased agitation speeds distorted and fragmented the hyphae, especially at the growing tip. This affects the physiology and hence the growth of the fungi (Finn, 1967). Martin (1983b) obtained a decrease in the growth of the *A. campestris* mycelium when the agitation speed was increased to about 300 rpm.

The detrimental effect of excessive aeration rates on the growth of fungi (Foster, 1949) was observed in this work when a reduction in the growth of the *P. ostreatus* mycelium was obtained at 1.5 vvm at all agitation speeds tested. Carbon dioxide has been reported to stimulate growth of several fungi (Hartman *et al.*, 1972), including *Pleurotus* species (Zadrazil, 1978). Nitrogen has a sweeping effect in removing carbon dioxide from a medium (Finn, 1967). Therefore, increasing the aeration rate to 1.5 vvm might have caused the concentration of carbon dioxide, which is required for the growth of the *P. ostreatus* mycelium to be reduced. Another explanation for the detrimental effect of excessive aeration could be that when air was blown into a medium the latter

was stirred (Finn, 1967). Thus, higher aeration rates increased the overall agitation of the medium and, thus, caused a detrimental shearing stress effect on the culture. The growth was, therefore, higher at 150 rpm than at 200 rpm when the aeration rate was 1.5 vvm. The growth was also better at 0.5 vvm than at 1.5 vvm at all agitation speeds tested. Other investigators have also observed the detrimental effect of higher aeration rates on the growth of the mushroom mycelium. Litchfield (1967a) pointed out that aeration rates in the range commonly used in other aerobic fermentations could be detrimental to mushroom mycelium growth. Litchfield and Overbeck (1965) obtained higher yields of the morel mushroom mycelium at 0.5 vvm than at 0.7 vvm. Kosaric and Miyata (1981) and Kosaric *et al.* (1973) have also reported that a very low aeration rate was required for the growth of the morel mushroom mycelium. In their studies, an aeration rate of 0.25 vvm was used.

#### 3.4. Morphology of the *P. ostreatus* mycelium growth forms

Filamentous and pellet growth forms of *P. ostreatus* were both observed. The morphology of the growth form was affected by the agitation, the aeration, the pH of the medium and the nutrients in the medium. In both the shake flask and the batch fermenter experiments, with agitation speeds of 150 and 200 rpm and aeration rates of 0.5 and 1.0 vvm, the growth took place in the form of pellets (Fig. 3.15). The size of the pellet depended on the agitation speed and/or the aeration rate. The pellet size decreased with increasing agitation speeds. In the shake flask experiments, the average diameter of the pellet was 4.5, 4.0 and 2.5 mm at 100, 150 and 200 rpm, respectively. In the fermenters, the growth was in the form of dispersed mycelia at 250 rpm.



Fig. 3.15. Pellet growth of the *P. ostreatus* mycelium in the peat extract in the shake flask experiments at an incubation temperature of 29°C, an initial pH of 5.0, an agitation speed of 150 rpm and a fermentation time of 192 hours



and 1.5 vvm. Some of the dispersed mycelia were collected on the walls of the fermenter vessel and on the impeller and shaft (Fig. 3.16). A similar observation was made by LeDuy (1974), and Metz and Kossen (1977).

The addition of yeast extract, potassium phosphate and manganese to the peat extract increased the frequency of pellet formation and their size in the shake flask experiments (Table 3.16). The increase in the pellet size was about 10 times over that produced in non-supplemented peat extract and about 2 times over that produced in the peat extract supplemented only with yeast extract. The effect of nutrients on fungal pellet size has also been observed by Morton (1961).

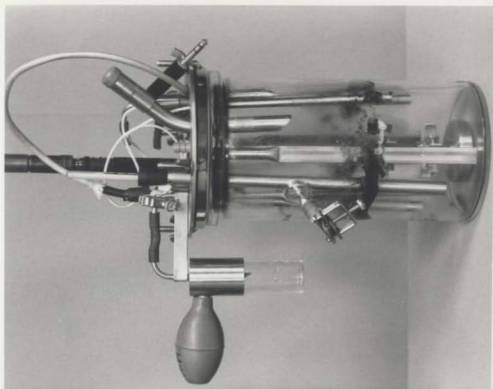
The growth of pellets was not observed when the initial pH was 4.0. In the shake flask experiments, the growth was in the filamentous form at all temperatures and all agitation speeds tested when the initial pH was 4.0. This was in agreement with the observations made by Righelato *et al.* (1968), and Bent and Morton (1963), that growth of fungal mycelium occurred only in the filamentous form at the minimum pH values for growth.

Pellets produced in the synthetic medium were smaller, smoother and more compact than those produced in the peat extract (complex media) (Fig. 3.17). This was in disagreement with the observation by Bukhalo and Solomko (1978). They observed that pellets of P. ostreatus grown in complex media were smoother and more compact than those produced in a synthetic medium.

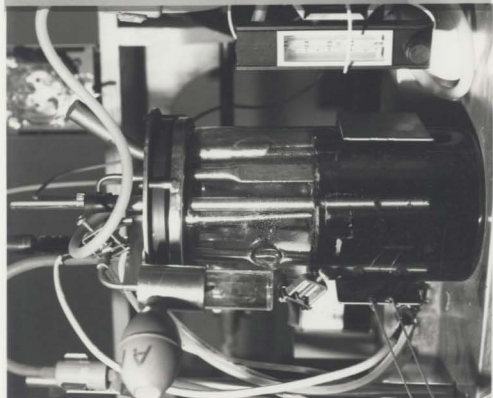
[A], pellets (3.0 mm in diameter) after 144 hours of fermentation at an aeration rate of 0.5 vvm, an agitation speed of 200 rpm and an incubation temperature of 28°C.

[B], dispersed growth on the sides of the fermenter and on the impellers, after 144 hours of fermentation at an aeration rate of 0.5 vvm, an agitation speed of 250 rpm and an incubation temperature of 28°C.

Fig. 3.16. The growth of the P. ostreatus mycelium in the peat extract in a 2 - liter fermenter



B



A

Table 3.16.. Effect of nutrients on the *P. ostreatus* pellet size in the shake flask experiments.

Medium	Pellet size (mm diameter)
Peat extract <sup>1</sup>	0.5 - 1.5
Peat extract + 5.0 g/L yeast extract	3.0 - 5.0
Peat extract + 3.0 g/L yeast extract, 2.6 g/L $\text{KH}_2\text{PO}_4$ and 0.1 g/L $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$	7.0 - 10.0

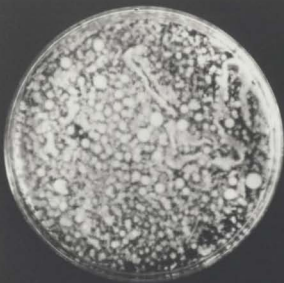
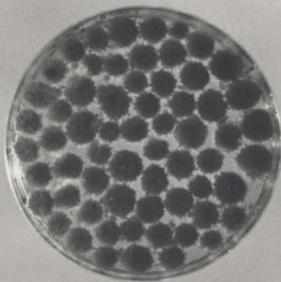
<sup>1</sup>Peat extract diluted to a 1:1 ratio with water; an incubation temperature of 28°C, an initial pH of 5.0, agitation rate of 150 rpm and a fermentation time of 192 hours were used.



[A], synthetic medium.

[B], peat extract.

Figure 3.17. Morphology of the *P. ostreatus* pellets in different media in the shake flask experiments at an incubation temperature of 28°C, an initial pH of 5.0, an agitation speed of 150 rpm and a fermentation time of 192 hours

**A****B**



### 3.4.1. Pellet formation

The formation of pellets is shown in Fig. 3.18. It can be seen that the formation of pellets was due to the agglomeration or aggregation of the hyphae (Foster, 1949). Thus, small portions of the fungal hyphae (A) grew and aggregated into groups of mycelium (B) and then the initial type of pellet was formed (C). A young pellet (D) of about 2.5 mm in diameter showed many growing tips of hyphae around its peripheral surface. Similar stages of pellet formation of morel mushroom mycelium in sulfite waste liquor have been reported by LeDuy (1974). Martin and Bailey (1985) also observed pellet growth of A. campestris by agglomeration.

#### 3.4.1.1. General discussion: effect of agitation and aeration on the growth form

It was observed that pellet growth occurred in the shake flask experiments at all agitation speeds tested, but the size of the pellet decreased with increasing agitation speed. In the fermenter, pellet growth was observed at agitation speeds of 150 and 200 rpm when the aeration rate was 0.5 and 1.0 vvm. When the aeration rate was 1.5 vvm, only dispersed mycelia were observed at either 150 or 200 rpm. The growth was in the form of dispersed mycelia at 250 rpm at all aeration rates tested. The size of the pellets formed in the fermenter was smaller than that of the pellets formed in the shake flasks. Thus, the size of the pellets depended on the type of agitation and aeration of the medium (Block et al., 1953). Yoshida et al. (1965) obtained a decrease in the pellet size with increasing agitation speeds and aeration

[A], portions of fungal hyphae.

[B], aggregation of hyphae into groups of mycellum

[C], initial pellet formed.

[D], young pellet (2.5 mm in diameter) showing growing tips of hyphae around its peripheral surface.

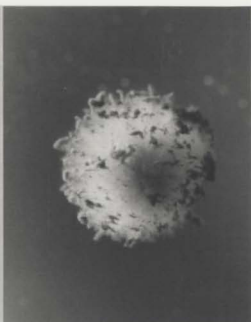
Scale applies to all photographs.

Figure 3.18. The formation of the *P. ostreatus* pellets in the peat extract

B



D



A



C



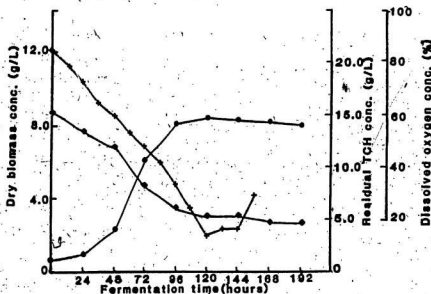
rates. Kosaric and Miyata (1981), and LeDuy (1974) observed a decrease in the pellet size and, in some cases, obtained dispersed mycelial growth at higher aeration rates. Litchfield and Overbeck (1965) reported that mycelia produced at 0.25 vvm were in the pellet form, those produced at 0.5 vvm were in the filamentous form, while those produced at 0.7 vvm were filamentous and slimy. Taguchi *et al.* (1968) have reported that the physical effects of agitation on the mycelial pellets in mechanically agitated and baffled fermenters included a decrease in the diameter of the pellets due to the chipping off of pellicles from the surface of the pellets, and also due to the direct rupture of the spherical shape of the pellets by the tip of the impeller blades.

### 3.5. Kinetics of the growth of the *P. ostreatus* mycelium in the peat extract medium

The optimal conditions of temperature, initial pH, inoculum ratio, substrate (dilution ratio) and nutrient concentrations, agitation speed and aeration rate were employed to study the growth characteristics of the *P. ostreatus* mycelium in a fermenter. The progress of batch cultivations over a fermentation time of 192 hours is shown in Fig. 3.19, for the dry biomass concentration, the residual TCH concentration and the dissolved oxygen concentration.

#### 3.5.1. Lag phase

There was no appreciable growth within the first 24 hours of fermentation. This period could therefore be described as the lag phase. During this period, presumably, the cells adjusted themselves to the new medium by inducing the production of enzymes required for



- (●) - Dry biomass concentration.  
 (●) - Residual TCH concentration.  
 (x) - Dissolved oxygen concentration.

Fig. 3.19. Progress of batch cultivation of the *P. ostreatus* mycelium in peat extract, at incubation temperature of 28°C, pH of 5.0, agitation speed of 200 rpm and aeration rate of 1.0 vvm

metabolic synthesis and by increasing the protein synthesis machinery (Righelato, 1975). However, there was a rapid consumption of carbohydrates and a rapid utilization of the dissolved oxygen. This could be due to the fact that the rates of enzyme activities such as oxygen consumption, carbon dioxide production and the evolution of heat were the same on a per unit of dry weight basis during the lag phase as during the logarithmic phase (Luedeking, 1972). The lag phase was shorter in the fermenter than in the shake flasks, as there was no appreciable growth in the shake flask within the first 96 hours of fermentation (Fig. 3.8a, page 87). Moustafa (1960) reported that a 96 - 120 hour lag period existed before spontaneous growth of A. campestris took place in a synthetic medium. Kosaric and Miyata (1981) observed a lag period of 48 hours during the growth of M. crassipes in cheese whey. Bukhalo and Solomko (1978) observed that the culture of P. ostreatus IMVF-1300 grew practically without a lag phase in a fermenter. In this work, the shorter lag phase in the fermenter could be due to better controlled growth conditions. The less controlled growth conditions in the shake flask might have affected the culture, since cells in the lag phase are said to be "physiologically young" (Luedeking, 1972), and are more sensitive to environmental changes.

### 3.5.2. Accelerated growth of the cell mass

It was observed that there was a rapid growth of the cell mass after 24 hours of fermentation (Fig. 3.19). This period, after the lag phase, could be described as a steady state when all the numerous linked reactions which comprise the metabolism of the cell had built up

to a maximum rate (Luedeking, 1972). Therefore, with an excess of all the substrate necessary for growth (and in the absence of inhibitors), there was a rapid increase in the cell mass. It was observed that the growth was faster, with a higher concentration of the dry biomass, in the fermenter than in the shake flask. The maximum biomass concentration in the fermenter reached 8.70 g/L (as compared to 6.8 g/L in shake flask) within 96 hours (as compared to 192 hours in the shake flask). The amount of cell mass produced by a unit amount of cells in a unit time (the specific growth rate) is constant during this period of rapid growth and is dependent on the cell concentration (Pirt, 1966; Righelato, 1975). It is represented by the equation:

$$\frac{dx}{dt} = \mu X \quad [a]$$

Upon integration of the equation [a]:

$$\ln \frac{X}{X_0} = \mu t \quad [b]$$

where X is the cell concentration,

$\mu$  is the specific growth rate, and

t is any time after the lag phase.

Thus a logarithmic relationship between cell concentration and time could be established during the accelerated phase of growth. A graph of the logarithms of the values of the dry biomass concentration of the microorganisms versus time should produce a straight line for the accelerated phase of growth, if the growth is exponential. The slope of this line is considered as the value of the specific growth rate.

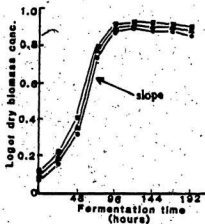
Several workers have shown that filamentous fungi grew exponentially in submerged culture (Pirt, 1966; Pirt and Callow, 1960; Borrow *et al.*, 1964; Trinci, 1969). However, Swanson and Stock (1966), and Mandels (1965) are of the view that the filamentous fungal growth in submerged culture is not exponential. The biggest slope obtained in this work was between 48 and 72 hours of fermentation (Fig. 3.20). However, there was not enough data to conclude that the growth during this period followed an exponential pattern.

The carbohydrate consumption and oxygen utilization in the batch fermenter corresponded well with the growth of the fungus. The carbohydrate and the oxygen were utilized as the biomass concentration increased towards its maximum value. The carbohydrate utilization continued after the maximum dry biomass concentration was obtained. The sugars might have been further assimilated by the cell population, and used for cell maintenance or for the accumulation of cellular components (Kosaric and Miyata, 1981).

#### 3.5.2.1. Decline in the growth rate

The accelerated growth rate continued for only 72 hours after onset, and was followed by a decline in the growth rate which was associated with a constant value of the residual TCH concentration and a gradual increase in the D.O. concentration. It is probable that the most common cause of the declining growth rate was a decrease in the concentration of one of the required nutrients to a growth limiting concentration (Righelato, 1975). The declining growth phase associated with the constant value of the residual TCH concentration in the





(●) - Batch 1.

(■) - Batch 2.

(▲) - Batch 3.

Figure 3.20. Growth rates of the *P. ostreatus* mycelium in the peat extract in three batch cultivations at an incubation temperature of 28°C, a pH of 5.0, an agitation speed of 200 rpm and an aeration rate of 1.0 vvm

medium, after 96 hours of fermentation, suggested that these residual carbohydrates represented the sugars that were non-assimilable for the fungus (Quierzy et al., 1979). Analysis of the fermented broth showed that only 0.2 to 0.4 g/L of total reducing sugars remained after 96 hours of fermentation (as compared with about 5.0 g/L residual TCH). Thus, the declining growth after 96 hours could be caused by a growth-limiting concentration of the assimilable sugars for the P. ostreatus mycelium. Righelato (1975) has reported that oxygen (due to its low solubility in aqueous media) and carbon sources often become growth-limiting in fungal cultures. Therefore, the growth rate became a function of the substrate concentrations (Monod, 1949), and is represented by the equation:

$$\mu = \mu_{\max} \left( \frac{S}{k_s + S} \right) \quad [c]$$

where  $\mu_{\max}$  is the maximum specific growth rate,

$k_s$  is the half saturation constant for the uptake of the growth limiting substrate, and

S is the concentration of the growth limiting substrate.

Another cause of the declining growth rate could be the growth of P. ostreatus mycelium in the pellet form. Pellet growth was observed after 96 hours of fermentation, which corresponds with the time the D.O. concentration began to rise. Pirt (1966) has reported that when pellets exceed a certain diameter, substrates, including oxygen, will not diffuse inwards fast enough to maintain an exponential growth of the whole pellet mass. Thus, the restriction of the diffusion of oxygen into

the pellets might have caused the D.O. concentration to rise after the onset of the pellet growth, and a decline in the growth rate due to autolysis at the center of the pellet (Phillip, 1966). Another explanation for the rise in the D.O. concentrations is that pellet forms reduce the viscosity of the medium and enhance the oxygen transfer to the fluid (Righelato, 1975).

Because of the resistance to the diffusion of the substrate into the centers of the pellets, it has been reported that the growth proceeds only at the periphery, since only that zone would have sufficient substrate to maintain the exponential growth (Pirt, 1966). Hence, the growth of the pellets fit a cube root pattern better than it fit the logarithmic plot (Marshall and Alexander, 1960; Pirt, 1966), and can be represented by the following equation:

$$X^{1/3} = X_0^{1/3} + kt \quad [d]$$

where k is a constant.

Due to weak diffusion of the oxygen to the mycelial cells in the centers of the pellets, these cells undergo lysis, which decreases the yield and the protein content of the biomass (Bukhalo and Solomko, 1978). However, one of the advantages of the growth of P. ostreatus in the pellet form was that the recovery of the biomass only involved a filtration operation.

### 3.5.2.2. Biomass yield (Y)

As it was defined elsewhere in this work, the yield is given by the equation:

$$Y = \frac{X}{S_0 - S_f} \quad [1]$$

where  $S_0$  is the initial substrate concentration (g/L), and

$S_f$  is the final substrate concentration (g/L).

The substrate was assumed to be the TCH (Solomons, 1975), because it was the carbon and energy source employed in this work.

The value obtained, when expressed as a percentage, (Table 3.17) compares very well with that reported by Shannon and Stevenson (1975) for the P. ostreatus mycelium grown in press liquor (brewery waste). They obtained a yield value of 69.1%. Eddy also reported a P. ostreatus yield of about 50 to 62% when it was cultivated in a medium with complex carbon and nitrogen sources. However, Sugihara and Humfeld (1952) reported a value of 100.0 to 125.0% for the same organism when it was cultivated in a synthetic medium. Crueger and Crueger (1984), and Sinskey (1978) have stated that yield values are not constant and are dependent on biological parameters such as the biomass concentration and the specific growth rate, and on chemical parameters such as the D.O. concentration, the C:N ratio, and the phosphorus content of the medium.

The yield value obtained in this work and those cited above are quite high, when the maximum theoretical yield expected from glucose of approximately 45 to 50% (Sinskey, 1978) is considered. However, in a complex medium, there could be many other sources of carbon besides the carbohydrates present, such as amino acids, lipids, organic acids and higher alcohols (Solomons, 1975; Hawker, 1968). LeDuy (1981) has reported that about 80% of the amino acids, 50% of the non-volatile

Table 3.17. Estimated biomass yield and productivity values for the *P. ostreatus* mycelium grown in the peat extract<sup>1</sup> in batch cultivations in a controlled fermenter

Parameter	Mean <sup>2</sup>
Yield (%)	71.89 ± 1.83
Productivity (P, mg/L hour)	70.16 ± 1.25

<sup>1</sup>Peat extract diluted to a 1:1 ratio with water, plus 3.0 g/L yeast extract, 2.6 g/L  $\text{KH}_2\text{PO}_4$  and 0.1 g/L  $\text{MnSO}_4\cdot\text{H}_2\text{O}$ , an incubation temperature of 28°C, a pH<sup>2</sup> of 5.0, an agitation speed of 200 rpm and an aeration rate of 1.0 vvm were used.

<sup>2</sup>These are the mean values of three replicate samples ± standard deviation.

organic acids and 50% of the hydroxymethylfurfural in peat extract are utilized as carbon sources by microorganisms for the production of biomass. Consequently, high yield values have been reported by other investigators for some mushroom mycelia cultivated in complex media. For example, yield values of 74.7% for A. campestris grown in beet molasses (Reusser et al., 1958a), 86.1% for Boletus indecisus grown in vinasse (Falanghe, 1962), and 64.0% for T. nudum grown in sulfite waste liquor (Reusser, et al., 1958a) have been reported. The most accurate yield value can, therefore, be obtained if it is calculated as the amount of cell mass produced per gram of unit weight of carbon in the substrate consumed (Solomons, 1975; Sinskey, 1978; Shannon and Stevenson, 1975). Since the carbon source for the SCP production is almost always the most expensive component supplied, Solomons (1975) has suggested that high yield factors could be of great importance in batch cultures.

### 3.5.2.3. Productivity (P)

Productivity is the dry biomass concentration, in grams per liter of medium, produced per unit time, and it is represented by the equation:

$$P = \frac{X_f - X_0}{t} \quad [1]$$

where  $X_f$  is the final biomass concentration (g/L),  
 $X_0$  is the initial biomass concentration (g/L), and  
 $t$  is the cultivation time (hours) from the lag phase to the beginning of the stationary phase.

The productivity, or the amount of biomass produced per unit volume per hour, was approximately 70 mg/L/hour (Table 3.17). Unfortunately, there is no data in the literature available on the productivity of mushroom mycelium grown by submerged culture. However, Sinskey (1978) has reported that a high productivity is more essential than high growth rates, especially in continuous culture processes. Paredes-Lopez *et al.* (1976), and Chang (1985) have observed that increased substrate concentration produced higher productivity values.

### 3.5.3. General discussion: growth kinetic parameters of the mushroom mycelium produced by submerged culture

Many papers on the submerged growth of mushrooms (fungi) refer to the biomass concentrations obtained after a certain number of hours of batch growth, rather than to the growth rate and the doubling time. In this work, the maximum growth of *P. ostreatus* was obtained in 192 hours in shake flask experiments and in 96 hours in an agitated and aerated fermenter. Hadar and Cohen-Arazi (1986), using the same operational variables (an incubation temperature of 28°C, a pH of 5.0, an agitation speed of 200 rpm and an aeration rate of 1.0 vvm) obtained a maximum biomass concentration of *P. ostreatus* in 72 hours in a fermenter, and in 144 to 168 hours in a shake flask, in a glucose medium. Bukhalo and Solomko (1978) obtained the maximum growth of the *P. ostreatus* mycelium in 96 hours in a fermenter and 120 to 168 hours in a shake flask. However, the *P. ostreatus* growth rate of 8.7 g/L dry biomass in 96 hours in this work was superior to that of both

A. campestris (5.0 g/L in 192 hours in peat extract) (Martin and Bailey, 1985) and M. crassipes (22 g/L in 360 hours in cheese whey) (Kosaric and Miyata, 1981).

### 3.6. Composition of the biomass

#### 3.6.1. Proximate composition

The concentrations of the main components of the mycelium biomass are presented in Table 3.18, together with the corresponding values for the fruiting bodies grown in solid peat. It can be seen that there is a high similarity between the composition of the mycelium produced in the peat extract and that of the fruiting bodies. The only notable difference was the higher total lipids content and lower moisture and ash contents of the mycelium. The moisture content of the fruiting bodies compares very favorably with those reported for the P. ostreatus mushroom by other investigators (Bano et al., 1983; Khanna and Garcha, 1984). When the values of the TCH (N-free) and the crude fiber are combined, the value obtained compares very well with that reported for the TCH content for P. ostreatus by Cho et al. (1981), but lower than those reported by Bano and Rajarathnam (1982), and Khanna and Garcha (1984). The total lipids content of the mycelium was within the range of values reported by Bukhalo and Solomko (1978), but higher than that obtained by Hadar and Cohen-Arazi (1986). The ash content of the fruiting bodies was similar to the value obtained by Bano et al. (1983) on a dry weight basis, but lower than that reported by Khanna and Garcha (1984).



Table 3.18. Proximate composition of the *P. ostreatus* biomass (% of dry weight)<sup>1</sup>

	Mycelium		Fruiting bodies
	Peat extract <sup>3</sup>	Synthetic medium <sup>4</sup>	
Moisture <sup>2</sup>	80.5 ± 1.8 <sup>a</sup>	78.8 ± 1.5 <sup>a</sup>	85.1 ± 1.3
TCH	35.2 ± 1.5 <sup>a</sup>	36.5 ± 1.1 <sup>a</sup>	35.6 ± 1.3 <sup>a</sup>
Crude fiber	5.9 ± 0.5 <sup>ab</sup>	5.0 ± 0.3 <sup>a</sup>	6.5 ± 0.5 <sup>b</sup>
Crude protein	40.1 ± 1.8 <sup>a</sup>	25.7 ± 1.8 <sup>a</sup>	36.0 ± 2.7 <sup>a</sup>
Total lipids	3.7 ± 0.4 <sup>a</sup>	3.0 ± 0.3 <sup>a</sup>	2.2 ± 0.2
Ash	7.9 ± 0.6	4.2 ± 0.7	10.1 ± 1.1

<sup>1</sup>These are the mean values of three determinations of three replicate samples ± standard deviations. The values of the same row with the same superscript are not significantly different at the 5% level.

<sup>2</sup>Moisture (% wet weight).

<sup>3</sup>Peat extract diluted to a 1:1 ratio with H<sub>2</sub>O, plus 3.0 g/L yeast extract, 2.6 g/L KH<sub>2</sub>PO<sub>4</sub> and 0.1 g/L MnSO<sub>4</sub> was used.

<sup>4</sup>The synthetic medium consisted of the basal medium, plus 45 g/L glucose and 3.5 g/L ammonium citrate.

### 3.6.1.1. Protein content.

The crude protein content of the mycelium produced in the synthetic medium was low, but the protein content of the mycelium grown in the peat extract was not significantly different ( $P > 0.05$ ) from that of the fruiting bodies. The crude protein content of the mycelium produced in the synthetic medium being lower than that of the mycelium produced in the complex medium is a common phenomenon among fungi. For example, Reusser *et al.* (1958a) reported that *M. hybrida* had only 10.5% of protein in a glucose medium, but 34.8% and 37.5% of protein in molasses and waste sulfite liquor media, respectively. Kosaric *et al.* (1973) obtained 48.0% crude protein in model mushroom mycelium in Mg-waste sulfite liquor, in comparison with 19.7% protein when grown in a glucose-based synthetic medium.

The high protein content of the *P. ostreatus* mycelium grown in the peat extract is a most significant characteristic. This value was higher than those reported for the *P. ostreatus* mycelium grown on molasses (Hadar and Cohen-Arazi, 1986), and on brewery wastes (Bukhalo and Solomko, 1978; Shannon and Stevenson, 1975). The crude protein content of the fruiting bodies grown on solid peat was also higher than those reported for *P. ostreatus* produced on other substrates. For example, Khanna and Garcha (1984) reported a crude protein content of 27.38% for *P. ostreatus* cultivated on straw. Thus, peat extract has a beneficial effect on the protein synthesis of *P. ostreatus*. Martin and Bailey (1985) reported that the protein content of *A. campestris* grown on peat extract was higher than those reported for the same organism grown on the other substrates. A similar obser-

vation was made by LeDuy (1981), who reported that C. utilis grown in peat extract contained a higher protein content than the commercial yeast.

### 3.6.2. Amino acid composition

The amino acid profile of the mycelium and the fruiting bodies of P. ostreatus produced with peat as the basic substrate showed the presence of 17 amino acids including all the essential amino acids (Table 3.19). It was observed that the concentrations of the amino acids in the mycelium were, generally, higher than those in the fruiting bodies. Histidine and glutamic acid were, however, higher in the fruiting bodies than in the mycelium. It was also observed that the mycelium produced in the peat extract medium had higher concentrations of amino acids than in the mycelium produced in the synthetic medium.

The essential amino acid concentrations in the fruiting bodies were lower than those reported for the P. ostreatus mushroom grown on other substrates (Khanna and Garcha, 1984). However, the essential amino acid concentrations in the mycelium grown in the peat extract compared very favorably with those reported for the P. ostreatus mycelium grown in beer wort (Bukhalo and Solomko, 1978); and in molasses (Hadar and Cohen-Arazi, 1986). Comparing with other mushroom mycelia grown in various substrates, it was observed that the essential amino acid concentrations of the P. ostreatus mycelium grown in the peat extract were higher than those reported for the morel mushroom mycelium grown in waste sulfite liquor (Kosaric et al., 1973; LeDuy et al., 1974), for A. campestris grown in peat extract (Martin and Bailey, 1985), and for T. nudum grown in glucose medium (Reusser

Table 3.19. The amino acid composition of the *P. ostreatus* biomass (g/100g protein)

Amino acid	Mycelium		Fruiting body
	Peat extract <sup>2</sup>	Synthetic medium <sup>3</sup>	
Ala	5.9 ± 0.2	3.7 ± 0.4	5.0 ± 0.3
Arg	4.3 ± 0.4	2.4 ± 0.2	3.9 ± 0.4
Asp	6.2 ± 0.5 <sup>a</sup>	3.8 ± 0.5	6.5 ± 0.6 <sup>a</sup>
Glu	6.7 ± 0.9	8.4 ± 1.1 <sup>a</sup>	9.1 ± 1.0
Gly	3.7 ± 0.4	2.1 ± 0.2	2.9 ± 0.3
His	1.1 ± 0.4 <sup>a</sup>	0.8 ± 0.1 <sup>a</sup>	1.9 ± 0.1
Ile	3.5 ± 0.2	3.0 ± 0.1	2.0 ± 0.1
Leu	6.1 ± 0.3	4.7 ± 0.3 <sup>a</sup>	4.2 ± 0.4 <sup>a</sup>
Lys	5.7 ± 0.4	4.1 ± 0.2 <sup>a</sup>	3.9 ± 0.3 <sup>a</sup>
Met	1.0 ± 0.1 <sup>a</sup>	0.6 ± 0.1	1.1 ± 0.1 <sup>a</sup>
Phe	3.4 ± 0.3	2.5 ± 0.2 <sup>a</sup>	12.4 ± 0.3 <sup>a</sup>
Pro	2.8 ± 0.3	2.1 ± 0.3	3.3 ± 0.1
Ser	3.8 ± 0.5 <sup>a</sup>	2.1 ± 0.3	3.6 ± 0.5 <sup>a</sup>
Thr	4.9 ± 0.3	3.7 ± 0.2 <sup>a</sup>	3.2 ± 0.4 <sup>a</sup>
Trp	1.2 ± 0.1 <sup>a</sup>	1.0 ± 0.0 <sup>a</sup>	1.0 ± 0.1 <sup>a</sup>
Tyr	2.5 ± 0.3 <sup>a</sup>	1.9 ± 0.4 <sup>a</sup>	2.3 ± 0.1 <sup>a</sup>
Val	3.9 ± 0.2	2.2 ± 0.1 <sup>a</sup>	2.4 ± 0.2 <sup>a</sup>
Total	66.7	49.1	58.7

<sup>1</sup> These are the mean values of three determinations of three replicate samples ± standard deviations. The values of the same row with the same superscript are not significantly different at the 5% level.

<sup>2</sup> Peat extract diluted to a 1:1 ratio with water, plus 3.0 g/L yeast extract, 2.6 g/L  $\text{KH}_2\text{PO}_4$  and 0.1 g/L  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  was used.

<sup>3</sup> The synthetic medium consisted of the basal medium, plus 45 g/L glucose and 35 g/L ammonium citrate.

et al., 1958b). The essential amino acid composition of the mycelium grown in the peat extract was compared with that of hen's eggs and with the FAO/WHO (1973) reference amino acid pattern (Table 3.20). It was observed that the mycelium was primarily deficient in methionine but had high concentrations of lysine and leucine, which are deficient in cereals.

### 3.6.3. Mineral composition

Table 3.21 shows the mineral composition of the mycelial biomass and the fruiting bodies of the P. ostreatus mushroom. It can be seen that the concentrations of the minerals were higher in the mycelium than in the fruiting bodies. The higher concentration of the minerals in the mycelium than in the fruiting bodies could be due to the better absorption capability of the fungus when metals are in solution. Humfeld and Sugihara (1952) reported that apparently all the phosphorus in the medium were absorbed by A. campestris grown in a synthetic medium. Conditions of absorption of metals appeared to be better in mushrooms (fungi) than for other organisms (Bano and Rajarathnam, 1982).

The major minerals in both the mycelium and the fruiting bodies grown with peat as the basic substrate were P, Mg, Ca, Fe and Mn in order of decreasing concentrations. The concentrations of P and Ca in the fruiting bodies were comparable to the FAO values for P. ostreatus. The concentrations of the minerals in the mycelium and in the fruiting bodies were also comparable to those reported for other Pleurotus species and to those of other mushrooms (Bano and Rajarathnam, 1982).

Table 3.20. Comparison of the essential amino acid concentrations in the *P. ostreatus* mycellum produced in the peat extract with those in hen's eggs

Essential amino acid	Mycellum (A)	Egg <sup>1</sup> (B)	Ratio <sup>2</sup> (A/B)	FAO/WHO <sup>3</sup>
Arg	4.3	6.1	0.71	-
His	1.1	2.4	0.46	-
Ile	3.5	6.3	0.56	4.0
Leu	6.1	8.9	0.67	7.0
Lys	5.7	7.0	0.81	5.5
Met	1.0	3.4	0.29	-
Phe	3.4	5.7	0.60	-
Thr	4.9	5.1	0.96	4.0
Trp	1.2	1.5	0.80	1.0
Val	3.9	7.3	0.53	5.0

<sup>1</sup>Data from Khanna and Garcha (1984).

<sup>2</sup>Calculation based on the method of Kurtzman (1975).

<sup>3</sup>FAO/WHO reference protein pattern (1973).

Table 3.21. The mineral composition of the *P. ostreatus* mushroom  
(mg/100g sample)

Mineral	Mycelium	Fruiting Body
Ca	215 $\pm$ 15	527 $\pm$ 5
Mg	231 $\pm$ 20	283 $\pm$ 15
Mn	147 $\pm$ 13	35 $\pm$ 2
Fe	197 $\pm$ 25	23 $\pm$ 4
Cu	12 $\pm$ 3	trace
Zn	15 $\pm$ 2	trace
P	1320 $\pm$ 20	1050 $\pm$ 43

<sup>1</sup>These are the mean values of two determinations of three replicate samples  $\pm$  standard deviation.

#### 3.6.4. Fatty acids

Table 3.22 shows the composition of the fatty acids in the mycellium and the fruiting bodies of P. ostreatus produced with peat as the basic substrate. It was observed that the fatty acid concentrations in both the mycellium and the fruiting bodies, with the exception of oleic and linoleic acids, were not significantly different. Two saturated fatty acids (myristic and palmitic) and three unsaturated fatty acids (Palmitoleic, oleic and linoleic) were identified in the samples tested.

The essential fatty acids (palmitic, oleic and linoleic) were present in large amounts. The most abundant fatty acid was linoleic acid, which was also reported as the major fatty acid in P. ostreatus grown in molasses (Hadar and Cohen-Arazi, 1986), and in M. crassipes grown in cheese whey (Kosaric and Miyata, 1981). Linolenic acid was not identified in the samples tested, and was not also reported by Hadar and Cohen-Arazi (1986), but was found in morel mushroom mycellium (LeDuy et al., 1974).



Table 3.22. The fatty acid composition of the *P. ostreatus* mushroom (% of total methyl esters)<sup>1</sup>

Fatty acid	Mycelium		Fruiting body
	Peat extract <sup>2</sup>	Synthetic medium <sup>3</sup>	
Myristic (14:0)	1.5 ± 0.3 <sup>a</sup>	1.5 ± 0.4 <sup>a</sup>	1.3 ± 0.3 <sup>a</sup>
Palmitic (16:0)	17.0 ± 1.1 <sup>a</sup>	18.3 ± 1.9 <sup>a</sup>	16.6 ± 1.3 <sup>a</sup>
Palmitoleic (16:1)	1.0 ± 0.3 <sup>a</sup>	1.5 ± 0.4 <sup>a</sup>	1.1 ± 0.2 <sup>a</sup>
Oleic (18:1)	18.6 ± 1.3 <sup>a</sup>	18.4 ± 1.6 <sup>a</sup>	15.2 ± 1.2
Linoleic (18:2)	44.9 ± 1.4 <sup>a</sup>	43.2 ± 1.5 <sup>a</sup>	36.3 ± 1.5

<sup>1</sup>These are the mean values of three determinations of three replicate samples ± standard deviation. The values in the same row with the same superscript are not significantly different at the 5% level.

<sup>2</sup>Peat extract diluted to a 1:1 ratio with water, plus 3.0 g/L yeast extract, 2.6 g/L  $\text{KH}_2\text{PO}_4$  and 0.1 g/L  $\text{MnSO}_4\cdot\text{H}_2\text{O}$  was used.

<sup>3</sup>The synthetic medium consisted of the basal medium, plus 45 g/L glucose and 3.5 g/L ammonium citrate.

## CHAPTER 4

## CONCLUSIONS AND SUGGESTIONS

## 4.1. Conclusions

This work has shown that P. ostreatus can be cultured in acid peat extract media supplemented with only nitrogen, phosphorus and potassium and that a higher yield can be obtained from this species than for other mushroom species grown in other complex media. The use of peat extract as an inexpensive source of carbon and energy, and the simple nutritional requirements of P. ostreatus make the use of peat-based media for the large scale production of the P. ostreatus biomass an attractive possibility.

This work has also established an optimal range of values for the most important operational variables involved in the submerged growth of the P. ostreatus mushroom mycelium in a peat-based culture medium. The optimal operational variables were as follows: incubation temperature of  $28 \pm 1^{\circ}\text{C}$ , pH of  $5.0 \pm 0.1$ , agitation speed of 200 rpm (150 rpm in the shake flask), aeration rate of 1.0 vvm, inoculum ratio of 5.0% (v/v), and fermentation time of 96 hours (192 hours in the shake flask).

It has also been shown in this work that P. ostreatus can grow in both the filamentous and the pellet forms, and that the formation of pellets was due to agglomeration or aggregation of the hyphae. The

size of the pellet has also been shown to decrease with increasing agitation speed and aeration rate, with lower initial pH values and with low nutrient concentrations in the medium.

The P. ostreatus mycelium, grown on peat-based medium, which was supplemented with external sources of nitrogen, phosphorus and potassium, was characterized by a high protein content, a favorable amino acid composition, high concentrations of essential fatty acids and minerals that permit its consideration, as a potential ingredient of food. The results of this work are in accordance with conclusions of other investigators in that the cultured mushroom mycelium obtained under conditions of submerged cultivation has a higher yield and food significance than the mushroom fruiting bodies.

#### 4.2. Suggestions

It is suggested that substances in the peat extract that may have inhibitory effects on the microbial growth should be identified and then removed from the peat extract, in order to enhance the growth and the biomass production of P. ostreatus. This treatment is indicated because P. ostreatus grown in the non-diluted peat extract whose TCH concentration was about 30 g/L produced only traces of growth. However, when glucose was added to a peat extract diluted into a 1:1 ratio with water, in order to give approximately the same TCH concentration as in the non-diluted peat extract, approximately 14 g/L dry biomass concentration was obtained. Unfortunately, supplementing diluted peat extract with glucose will make the fermentation process more expensive on a large scale production.

The high lysine and leucine contents in the P. ostreatus mycelium grown in the peat extract make it a possible supplement for cereal foods. Animal nutrition trials should be conducted, and the level of nucleic acids determined before consideration is given for its use in human foods. It has been reported that fungi grown at high growth rates normally have higher protein contents as well as increased levels of nucleic acids. Studies could also be conducted to produce a product with acceptable flavor for use as a food ingredient.

It is further suggested that scale-up studies should be done, including checks on the stability of the organism in continuous culture in terms of yield and product specification. The performance of the organism in continuous culture should also be compared with that in a laboratory batch fermenter.

Analysis of the growth media after fermentation should be carried out to identify the by-products, since P. ostreatus has been reported to produce certain polysaccharides that have anti-tumor properties.

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**APPENDIX A**

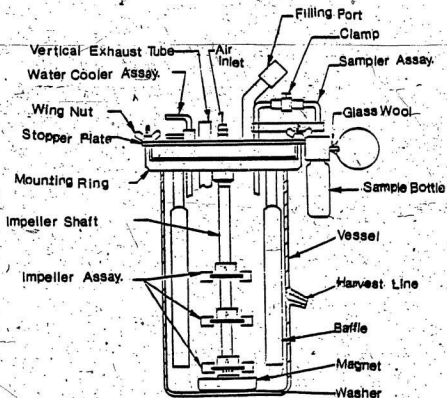
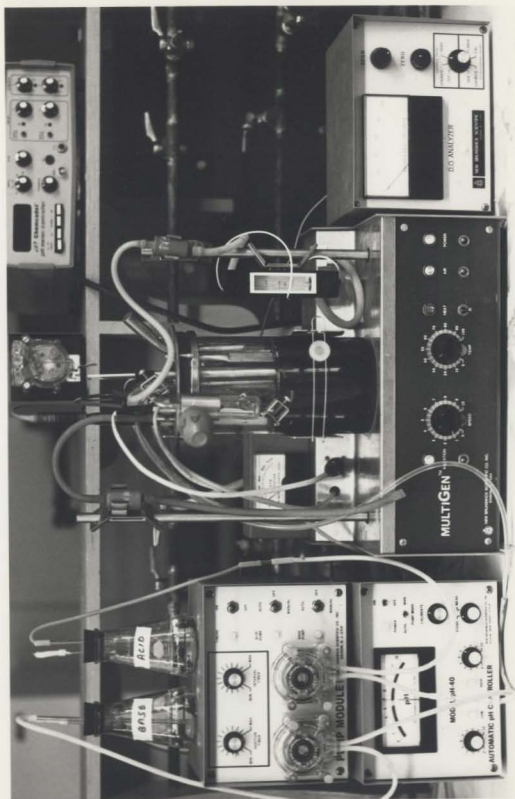


Figure A.1. Multigen fermenter vessel assembly M 15052 - 2770 -  
2 Liter

Figure A.2: Arrangement of the pH controller, fermenter and D.O. analyzer



## APPENDIX B



## Appendix B.1. Shake flask experiments

Table B.1.1. Effect of substrate concentration (dilution ratio and different concentrations of yeast extract) in the peat extract on the growth of the *P. ostreatus* mycelium

YE (g/L)	DR (PE:W)	ITCH (g/L)	X <sup>a</sup> (g/L)	Y <sup>a</sup> (%)	E <sup>a</sup> (%)
0	1:3	7.5	0.40 ± 0.01	17.77 ± 2.73	7.92 ± 0.03
	1:2	10.0	0.63 ± 0.07	18.65 ± 2.16	6.34 ± 0.74
	1:1	15.0	1.06 ± 0.08	35.35 ± 0.92	7.55 ± 0.15
	1:0.5	20.0	0.80 ± 0.09	20.88 ± 4.33	3.98 ± 0.47
	1:0	30.0	0.33 ± 0.01	7.97 ± 0.33	1.11 ± 0.03
1	1:3	7.5	1.11 ± 0.03	30.81 ± 0.90	22.13 ± 0.65
	1:2	10.0	1.92 ± 0.02	31.35 ± 0.37	19.22 ± 0.23
	1:1	15.0	3.13 ± 0.02	37.28 ± 1.16	20.88 ± 1.12
	1:0.5	20.0	2.77 ± 0.20	35.73 ± 1.17	13.85 ± 1.04
	1:0	30.0	0.66 ± 0.03	6.57 ± 0.59	2.21 ± 0.11
3	1:3	7.5	1.67 ± 0.14	54.79 ± 5.95	33.38 ± 2.86
	1:2	10.0	2.59 ± 0.55	38.15 ± 0.78	25.93 ± 0.55
	1:1	15.0	3.84 ± 0.06	49.73 ± 0.15	26.26 ± 0.80
	1:0.5	20.0	3.23 ± 0.08	40.32 ± 0.97	16.23 ± 0.38
	1:0	30.0	1.43 ± 0.07	12.74 ± 1.20	4.75 ± 0.33
5	1:3	7.5	1.94 ± 0.11	28.05 ± 2.83	18.80 ± 1.52
	1:2	10.0	3.00 ± 0.14	42.99 ± 2.20	30.09 ± 1.09
	1:1	15.0	4.98 ± 0.05	59.81 ± 1.14	33.20 ± 0.20
	1:0.5	20.0	3.74 ± 0.20	41.38 ± 2.57	18.84 ± 1.18
	1:0	30.0	1.65 ± 0.62	13.77 ± 0.52	5.51 ± 0.15
7.5	1:3	7.5	2.24 ± 0.20	64.35 ± 1.21	43.85 ± 1.14
	1:2	10.0	3.58 ± 1.10	42.65 ± 1.20	34.95 ± 0.64
	1:1	15.0	5.17 ± 0.51	60.87 ± 1.03	34.43 ± 1.43
	1:0.5	20.0	3.85 ± 0.12	41.85 ± 1.48	23.55 ± 1.20
	1:0	30.0	1.78 ± 0.37	20.46 ± 1.54	6.15 ± 0.89

<sup>1</sup> An incubation temperature of 28°C, a pH of 5.0, an inoculum ratio of 5.0% (v/v) and a fermentation time of 192 hours were used.

<sup>a</sup> These are the mean values of three replicate samples ± standard deviations.

Table B.1.2. Effect of the inoculum ratio on the growth of *P. ostreatus* mycelium in the peat extract in the shake flask experiments

IR (% v/v)	X <sup>a</sup> (g/L)	Y <sup>a</sup> (%)	E <sup>a</sup> (%)
2.5	3.11 ± 0.16	43.15 ± 1.34	20.00 ± 0.99
5.0	4.98 ± 0.05	59.80 ± 0.14	33.20 ± 2.00
7.5	4.03 ± 0.14	53.80 ± 2.12	25.95 ± 0.92
10.0	3.95 ± 0.03	49.10 ± 1.90	25.50 ± 0.28

<sup>1</sup> Peat extract diluted to a 1:1 ratio with water, plus 5.0 g/L yeast extract, an agitation speed of 150 rpm and a fermentation time of 192 hours were used.

<sup>a</sup> These are the mean values of three replicate samples ± standard deviations.

Table B.1.3. Effect of temperature, initial pH and agitation speed on the growth of the *P. ostreatus* mycelium in the peat extract

T (°C)	RPM	IpH	X <sup>a</sup> (g/L)	Y <sup>a</sup> (%)	E <sup>a</sup> (%)	FpH
19	100	4.0	0.50 ± 0.01	15.20 ± 0.28	3.20 ± 0.00	3.96
		5.0	3.45 ± 0.04	44.25 ± 1.21	23.00 ± 1.28	5.68
		6.0	3.00 ± 0.12	39.95 ± 1.60	20.10 ± 0.85	6.51
		7.0	3.29 ± 0.21	42.75 ± 1.91	22.20 ± 1.40	6.99
	150	4.0	0.55 ± 0.04	15.80 ± 0.11	3.80 ± 0.25	4.03
		5.0	3.85 ± 0.10	51.55 ± 1.40	23.83 ± 1.00	6.38
		6.0	3.68 ± 0.15	43.28 ± 2.50	22.30 ± 1.27	6.95
		7.0	3.00 ± 0.28	39.50 ± 3.65	23.25 ± 3.50	6.75
	200	4.0	0.46 ± 0.12	14.05 ± 1.04	3.20 ± 0.85	4.09
		5.0	3.38 ± 0.10	44.20 ± 2.40	24.10 ± 1.00	5.85
		6.0	2.66 ± 0.09	38.10 ± 2.90	18.95 ± 0.84	6.74
		7.0	2.68 ± 0.38	34.65 ± 3.04	19.10 ± 1.83	7.02
22	100	4.0	0.54 ± 0.16	12.85 ± 2.18	3.55 ± 1.06	3.95
		5.0	3.67 ± 0.05	44.85 ± 0.78	24.45 ± 1.35	4.53
		6.0	3.58 ± 0.10	42.65 ± 1.20	23.95 ± 0.84	5.19
		7.0	3.41 ± 0.05	40.85 ± 1.07	22.85 ± 1.35	5.70
	150	4.0	0.59 ± 0.03	13.30 ± 0.99	4.00 ± 0.14	3.99
		5.0	4.10 ± 0.20	54.70 ± 1.30	27.50 ± 1.14	5.88
		6.0	4.00 ± 0.13	46.25 ± 1.63	26.50 ± 1.85	6.12
		7.0	3.53 ± 0.14	43.90 ± 1.30	23.70 ± 0.99	7.10
	200	4.0	0.42 ± 0.04	10.20 ± 1.30	2.65 ± 1.21	3.70
		5.0	4.05 ± 0.03	45.50 ± 1.42	25.35 ± 1.21	6.78
		6.0	4.12 ± 0.10	46.50 ± 1.13	25.85 ± 1.64	7.05
		7.0	3.61 ± 0.05	47.45 ± 3.46	22.85 ± 1.35	7.95
25	100	4.0	0.53 ± 0.03	13.40 ± 1.57	3.45 ± 1.21	4.07
		5.0	4.29 ± 0.10	48.25 ± 1.35	28.50 ± 1.00	7.46
		6.0	3.72 ± 0.21	44.45 ± 1.35	25.75 ± 1.50	6.95
		7.0	3.91 ± 0.27	41.95 ± 2.60	26.40 ± 1.84	5.69

Table B.1.3. (continued)

$T$ (°C)	RPM	IpH	$X^a$ (g/L)	$Y^a$ (%)	$E^a$ (%)	FpH
28	150	4.0	$0.96 \pm 0.03$	$19.30 \pm 0.70$	$6.40 \pm 1.14$	3.95
		5.0	$4.45 \pm 0.03$	$56.90 \pm 1.71$	$29.75 \pm 1.21$	7.67
		6.0	$4.38 \pm 0.04$	$52.15 \pm 1.06$	$29.20 \pm 1.20$	7.55
		7.0	$4.35 \pm 0.05$	$50.50 \pm 1.14$	$28.90 \pm 1.28$	7.99
	200	4.0	$0.75 \pm 0.06$	$16.80 \pm 1.14$	$4.85 \pm 1.07$	3.93
		5.0	$4.20 \pm 0.14$	$46.25 \pm 3.45$	$28.85 \pm 1.92$	7.47
		6.0	$4.02 \pm 0.23$	$48.25 \pm 1.74$	$20.70 \pm 1.56$	7.73
		7.0	$3.54 \pm 0.11$	$41.80 \pm 3.25$	$20.30 \pm 1.71$	7.69
	100	4.0	$0.58 \pm 0.05$	$17.10 \pm 1.27$	$3.90 \pm 0.28$	4.09
		5.0	$4.76 \pm 0.22$	$51.90 \pm 1.37$	$31.05 \pm 1.48$	7.70
		6.0	$4.08 \pm 0.23$	$43.30 \pm 1.57$	$27.30 \pm 1.40$	7.00
		7.0	$3.93 \pm 0.22$	$39.45 \pm 1.77$	$26.05 \pm 1.48$	6.93
31	150	4.0	$0.61 \pm 0.11$	$20.00 \pm 1.26$	$4.00 \pm 0.71$	3.87
		5.0	$4.98 \pm 0.05$	$59.80 \pm 1.14$	$33.20 \pm 1.20$	7.96
		6.0	$4.75 \pm 0.22$	$53.55 \pm 3.46$	$31.45 \pm 1.48$	7.52
		7.0	$4.09 \pm 0.09$	$51.45 \pm 1.63$	$27.25 \pm 1.64$	8.33
	200	4.0	$0.56 \pm 0.01$	$15.65 \pm 2.19$	$3.85 \pm 1.07$	3.99
		5.0	$3.71 \pm 0.18$	$45.35 \pm 1.75$	$25.75 \pm 1.20$	7.95
		6.0	$3.45 \pm 0.55$	$44.55 \pm 4.46$	$24.35 \pm 3.89$	8.18
		7.0	$3.13 \pm 0.27$	$38.80 \pm 2.83$	$21.50 \pm 1.56$	8.24
	100	4.0	$0.50 \pm 0.05$	$11.06 \pm 0.60$	$3.00 \pm 0.56$	3.99
		5.0	$3.36 \pm 0.04$	$39.70 \pm 2.69$	$21.50 \pm 1.28$	7.35
		6.0	$3.57 \pm 0.08$	$40.65 \pm 2.19$	$22.90 \pm 1.42$	7.71
		7.0	$2.70 \pm 0.21$	$35.50 \pm 1.41$	$17.35 \pm 1.34$	7.54
	150	4.0	$0.71 \pm 0.09$	$9.60 \pm 0.57$	$4.50 \pm 0.57$	3.97
		5.0	$3.38 \pm 0.23$	$39.70 \pm 1.59$	$21.50 \pm 1.40$	7.85
		6.0	$4.02 \pm 0.09$	$44.45 \pm 1.64$	$25.60 \pm 1.57$	5.99
		7.0	$2.52 \pm 0.16$	$27.75 \pm 2.62$	$16.05 \pm 1.06$	7.70

Table B.1.3. (continued)

T <sub>o</sub> (°C)	RPM	IpH	X <sup>a</sup> (g/L)	Y <sup>a</sup> (%)	E <sup>a</sup> (%)	FpH
200	4.0		0.50 ± 0.10	8.50 ± 1.05	3.35 ± 0.95	3.99
	5.0		3.19 ± 0.18	38.85 ± 1.48	23.55 ± 1.20	7.25
	6.0		3.13 ± 0.12	36.60 ± 3.11	20.30 ± 1.85	8.17
	7.0		1.78 ± 0.37	20.60 ± 3.54	12.35 ± 2.48	7.18

<sup>1</sup> Peat extract diluted to a 1:1 ratio with water; plus 5.0 g/L yeast extract, an inoculum ratio of 5.0% (v/v) and a fermentation time of 192 hours were used.

<sup>a</sup> These are the mean values of three replicate samples ± standard deviations.

Table B.1.4. Effect of fermentation time on the growth of the *P. ostreatus* mycelium in the peat extract in the shake flask experiments

FT (hr)	X <sup>a</sup> (g/L)	Y <sup>a</sup> (%)	E <sup>a</sup> (%)	RTCH (g/L)
48	0.60 ± 0.05	19.25 ± 1.63	4.00 ± 0.28	13.10
96	1.34 ± 0.09	20.15 ± 1.20	8.90 ± 0.51	8.25
144	1.82 ± 0.05	23.30 ± 0.10	12.10 ± 0.28	7.20
192	4.98 ± 0.05	59.80 ± 0.14	33.20 ± 0.20	6.40
240	5.33 ± 0.22	58.10 ± 1.40	35.55 ± 1.48	5.15
288	4.66 ± 0.16	46.15 ± 0.92	31.05 ± 1.06	5.20

<sup>1</sup> Peat extract diluted to a 1:1 ratio with water, plus 5.0 g/L yeast extract, an inoculum ratio of 5% (v/v), an incubation temperature of 28.0°C, an initial pH of 5.0 and agitation speed of 150 rpm were used.

<sup>a</sup> These are the mean values of three replicate samples ± standard deviations.

Table B.1.5. Effect of different concentrations of yeast extract on the growth and the crude protein content of the P. ostreatus mycelium grown in the peat extract in the shake flask experiments

YE (g/L)	X <sup>a</sup> (g/L)	Y <sup>a</sup> (%)	E <sup>a</sup> (%), (% dry wt.)	CP
0.0	1.06 ± 0.08	35.35 ± 0.92	7.55 ± 0.15	10.03
1.0	3.13 ± 0.02	37.28 ± 0.19	20.88 ± 0.12	21.50
3.0	3.84 ± 0.06	49.73 ± 0.15	26.28 ± 0.08	30.08
5.0	4.98 ± 0.05	59.80 ± 0.14	33.20 ± 0.20	40.10
7.5	5.17 ± 0.51	60.87 ± 0.30	34.43 ± 0.43	45.06
10.0	4.75 ± 0.22	53.55 ± 3.46	31.45 ± 1.48	51.08

<sup>1</sup> Peat extract diluted to a 1:1 ratio with water, an inoculum ratio of 5% (v/v), an incubation temperature of 28.0°C, an initial pH of 5.0, a fermentation time of 192 hours and an agitation speed of 150 rpm were used.

<sup>a</sup> These are the mean values of three replicate samples ± standard deviations.

## Appendix B.2. Batch fermentations in a 2-Liter fermenter

Table B.2.1. Effect of mechanical agitation speed and aeration rate on the growth of the *P. ostreatus* mycelium in the peat extract

AS (rpm)	AR vvm	Time (h)	X <sup>a</sup> (g/L)	Y <sup>a</sup> (%)	E <sup>a</sup> (%)
150	0.5	24	1.35 ± 0.25	35.46 ± 1.33	7.05 ± 1.40
		48	1.44 ± 0.04	34.42 ± 1.69	8.01 ± 1.23
		72	4.40 ± 0.11	52.68 ± 1.11	23.20 ± 1.71
		96	7.22 ± 0.23	62.71 ± 1.66	36.10 ± 1.38
		120	7.30 ± 0.13	59.31 ± 1.66	36.50 ± 1.38
		144	6.98 ± 0.30	58.11 ± 1.83	33.49 ± 1.25
		168	6.95 ± 0.40	55.16 ± 1.88	33.47 ± 1.33
		192	6.76 ± 0.11	52.29 ± 1.03	33.35 ± 1.95
	1.0	24	1.57 ± 0.27	34.41 ± 1.59	7.85 ± 0.58
		48	1.84 ± 0.20	40.95 ± 2.25	9.20 ± 1.18
		72	4.48 ± 0.25	50.49 ± 2.49	24.49 ± 1.46
		96	7.77 ± 0.15	69.03 ± 1.68	38.45 ± 1.08
		120	7.70 ± 0.24	68.28 ± 1.23	38.08 ± 1.11
		144	7.77 ± 0.19	68.25 ± 1.65	38.41 ± 1.27
		168	7.69 ± 0.17	65.87 ± 1.65	38.41 ± 1.16
		192	7.46 ± 0.19	63.20 ± 2.55	37.53 ± 1.80
1.5	1.5	24	1.52 ± 0.16	30.02 ± 0.74	7.95 ± 0.40
		48	1.74 ± 0.18	34.03 ± 1.98	8.70 ± 1.11
		72	3.96 ± 0.20	48.85 ± 1.01	20.85 ± 1.51
		96	6.34 ± 0.20	58.43 ± 1.83	32.58 ± 1.40
		120	6.44 ± 0.15	57.27 ± 0.56	33.03 ± 1.33
		144	6.37 ± 0.20	56.72 ± 1.40	32.69 ± 1.23
		168	6.32 ± 0.10	54.94 ± 1.21	32.81 ± 1.08
		192	6.30 ± 0.25	53.86 ± 1.59	32.68 ± 1.33



Table B.2.1. (continued)

AS rpm	AR vvm	Time (h)	X <sup>a</sup> (g/L)	Y <sup>a</sup> (%)	E <sup>a</sup> (%)
200	0.5	24	1.32 ± 0.10	24.62 ± 1.23	6.86 ± 0.96
		48	2.34 ± 0.14	35.04 ± 1.50	11.18 ± 1.04
		72	3.41 ± 0.26	48.02 ± 1.20	17.67 ± 1.50
		96	7.55 ± 0.35	69.90 ± 2.64	37.75 ± 1.15
		120	7.77 ± 0.23	69.94 ± 2.03	38.65 ± 1.20
		144	7.72 ± 0.18	68.55 ± 2.11	38.60 ± 1.85
		168	7.80 ± 0.38	69.27 ± 3.18	38.88 ± 1.88
		192	7.77 ± 0.29	68.05 ± 1.99	38.45 ± 1.50
	1.0	24	1.08 ± 0.42	25.91 ± 1.93	7.98 ± 1.01
		48	2.47 ± 0.24	46.23 ± 1.61	13.07 ± 1.59
		72	6.23 ± 0.11	55.60 ± 2.60	31.57 ± 1.07
		96	8.77 ± 0.21	73.99 ± 1.69	40.09 ± 1.39
		120	9.00 ± 0.11	71.03 ± 1.92	40.66 ± 1.34
		144	8.95 ± 0.10	70.64 ± 1.48	40.81 ± 1.78
		168	8.66 ± 0.15	68.66 ± 1.36	40.04 ± 1.90
		192	8.40 ± 0.17	66.97 ± 1.52	38.99 ± 1.15
200	1.5	24	1.38 ± 0.11	24.07 ± 1.02	6.44 ± 1.24
		48	1.20 ± 0.09	25.00 ± 1.00	6.69 ± 1.34
		72	3.81 ± 0.21	49.48 ± 1.48	20.42 ± 1.59
		96	6.02 ± 0.15	61.40 ± 1.34	30.59 ± 1.92
		120	6.04 ± 0.13	60.40 ± 1.65	30.61 ± 1.63
		144	6.04 ± 0.15	59.22 ± 1.05	30.63 ± 1.21
		168	6.00 ± 0.18	58.82 ± 1.12	30.02 ± 1.05
		192	6.02 ± 0.20	59.02 ± 2.50	30.07 ± 1.25

Table B.2.1. (continued)

AS rpm	AR vvm	Time (h)	X <sup>a</sup> (g/L)	Y <sup>a</sup> (%)	E <sup>a</sup> (%)
250	0.5	24	0.64 ± 0.08	22.2 ± 1.22	4.50 ± 1.05
		48	1.57 ± 0.30	32.22 ± 1.54	7.85 ± 1.11
		72	2.53 ± 0.13	37.76 ± 1.69	12.85 ± 1.71
		96	5.72 ± 0.32	55.53 ± 1.98	28.90 ± 1.21
		120	5.99 ± 0.26	55.98 ± 1.32	30.01 ± 1.08
		144	6.04 ± 0.43	55.41 ± 1.16	30.12 ± 1.28
		168	6.02 ± 0.21	55.22 ± 1.34	30.09 ± 1.09
		192	6.01 ± 0.16	55.14 ± 1.86	29.98 ± 1.14
	1.0	24	1.40 ± 0.14	35.68 ± 1.14	7.89 ± 0.53
		48	1.99 ± 0.34	36.54 ± 1.20	10.70 ± 1.40
		72	5.23 ± 0.32	50.10 ± 2.76	27.15 ± 1.57
		96	7.24 ± 0.26	68.05 ± 1.60	36.11 ± 1.17
		120	7.52 ± 0.16	68.34 ± 1.47	37.88 ± 1.39
		144	7.30 ± 0.26	66.61 ± 1.26	36.60 ± 1.16
		168	6.88 ± 0.18	64.84 ± 1.86	35.05 ± 1.15
		192	6.90 ± 0.10	61.88 ± 1.19	34.10 ± 1.61
	1.5	24	0.46 ± 0.15	15.33 ± 2.32	2.37 ± 1.66
		48	0.43 ± 0.08	14.95 ± 1.65	2.19 ± 1.21
		72	1.01 ± 0.24	24.43 ± 2.11	5.08 ± 1.19
		96	3.04 ± 0.11	35.76 ± 1.87	15.31 ± 1.38
		120	5.57 ± 0.21	55.70 ± 2.39	27.55 ± 1.57
		144	5.61 ± 0.43	52.94 ± 1.99	28.80 ± 1.72
		168	5.65 ± 0.36	53.30 ± 3.44	28.84 ± 1.32
		192	5.65 ± 0.29	53.30 ± 1.62	28.86 ± 1.34

<sup>1</sup>Peat extract diluted to a 1:1 ratio with water, plus 3.0 g/L yeast extract, 2.6 g/L  $\text{KH}_2\text{PO}_4$  and 0.1 g/L  $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$ , an incubation temperature of 28°C and a pH of 5.0 were used.

<sup>a</sup>These are the mean values of three replicates ± standard deviations.

**APPENDIX C**

[A], growth in raw peat 25 days after spawning, at room temperature and 70% relative humidity: (1) side view, (2) aerial view.

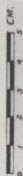
[B], growth in hydrolyzed peat, 22 days after spawning, at room temperature and 70% relative humidity: (1) side view, (2) top view.

Fig. C.1. Fruiting bodies of the P. ostreatus mushroom grown on the solid peat

BI



B2



AI



A2







