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Training Manual on Mushroom Cultivation Technology

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Acknowledgments

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ABSTRACT

1. Inadequate regional food supplies, diminishing quality of health, and increasing environmental deterioration are three key underlying problems affecting the future well-being of humankind. The magnitude of these problems is set to increase as the world's population continues to grow. The three facets of Applied Mushroom Biology combined offer partial but meaningful solutions through (1) the generation of relatively cheap source of high quality food protein (*Mushroom Science*), (2) the provision of health-enhancing dietary supplements/ mushroom nutriceuticals (*Mushroom Biotechnology*), and (3) the bioconversion/bioremediation of environmental adulterants and maintenance of balanced ecosystems (*Mushroom Mycorestoration*).

2. Mushrooms are very nutritious products that can be generated from lignocellulosic waste materials; and are in rich in crude fibre and protein. In fact, mushrooms also contain low fat, low calories and good vitamins. In addition, many mushrooms possess multi-functional medicinal properties.

3. Mushroom cultivation technology is friendly to the environment. The production of edible and medicinal mushrooms utilising, for example, paddy straw, cotton wastes, coffee waste, water hyacinth, tree saw dust, sugar cane bagasse, wild grasses and various categories of refuse and lignocellulosic wastes, could readily be adopted in Asian and Pacific communities in sophisticated, but low technology approaches.

4. The spent substrate left after harvesting the mushrooms, which is entangled with innumerable mushroom threads (collectively referred to as mycelia) will have been biochemically modified by the mushroom enzymes into a simpler and more readily digestible form, which is thus more palatable to livestock, when used as a livestock feed supplement. Additionally, it will significantly have been enriched with protein, by virtue of the remains of the protein-rich mycelia, left after harvesting the mushroom fruiting bodies. The residue could also be utilised as organic garden mulch, which is good for the soil.

5. Mushroom mycelia can produce a group of complex extracellular enzymes which can degrade and utilize the lignocellulosic wastes in order to reduce pollution. It has been revealed recently that mushroom mycelia can play a significant role in the restoration of damaged environments. Saprotrophic, endophytic, mycorrhizal, and even parasitic fungi/mushrooms can be used in mycorestoration, which can be performed in four different ways: mycofiltration (using mycelia to filter water), mycoforestry (using mycelia to restore forests), mycoremediation (using mycelia to eliminate toxic waste), and mycopesticides (using mycelia to control insect pests). These methods represent the potential to create a clean ecosystem, where no damage will be left after fungal implementation.

6. The key objectives in each of the participating countries will be, firstly, to develop *Pleurotus* (oyster) mushrooms as additional, highly nutritious vegetable crops. We should start with these because they are easiest to grow; yet they are also high in protein. Secondly**,** efforts will be directed towards cultivating *Lentinula* mushrooms, which require less complicated and costly

set-up and equipment (eg. compared with *Agaricus* mushrooms). Thirdly, efforts will be made to produce selected medicinal mushrooms as dietary supplements, especially mushrooms which are known to have a strong potency in invigorating the body's immuno-response systems, such as *Ganoderma lucidum*.

7. A comprehensive training programme for both researchers and mushroom growers will need to be formulated. The identified team of researchers will be brought together for an intensive training course, which will equip them with more skills on how to train others, and also help prepare them on how to succeed in this promising venture. Mushroom farming is both a science and an art. The science, in its broad form, will come through training workshops/courses. The art will come through practical involvement and experience, and will have to be modified in accordance with the prevailing conditions on site.

8. The potential of mushroom farming in generating new employment opportunities is another positive element emanating from mushroom farming ventures, which can be labour intensive. Phase one of this project will aim at providing initial skills for cottage level mushroom production. Later, large scale development can be expected to lead to the establishment of commercial mushroom farms, and international marketing ventures. It is anticipated that Governments of the countries where Phase One of Project implementation will be based, will grant their political good-will, which will be a positive stimulus for private sector involvement.

9. Due to advances in both basic knowledge and practical technology relevant to mushroom farming (mushroom themselves), mushroom products (mushroom derivatives) and mushroom bioremediation (mushroom mycelia), these principles can be applied globally, but must be implemented according to locally available substrates, labour and climatic conditions.

1. INTRODUCTION

Mushrooms are the fruiting bodies of macrofungi. They include both edible/medicinal and poisonous species. However, originally, the word "mushroom" was used for the edible members of macrofungi and "toadstools" for poisonous ones of the "gill" macrofungi. Scientifically the term "toadstool" has no meaning at all and it has been proposed that the term is dropped altogether in order to avoid confusion and the terms edible, medicinal and poisonous mushrooms are used.

Edible mushrooms once called the "food of the gods" and still treated as a garnish or delicacy can be taken regularly as part of the human diet or be treated as healthy food or as functional food. The extractable products from medicinal mushrooms, designed to supplement the human diet not as regular food, but as the enhancement of health and fitness, can be classified into the category of dietary supplements/mushroom **nutriceuticals** (Chang and Buswell, 1996). Dietary supplements are ingredients extracted from foods, herbs, mushrooms and other plants that are taken without further modification for their presumed health-enhancing benefits.

There is an old Chinese saying which states that “MEDICINES AND FOODS HAVE A COMMON ORIGIN”. Mushrooms constitute a most rapidly growing new food category which the current health-oriented public is increasingly enjoying.

Since mushrooms lack chlorophyll they can not, like green plants, get their energy from the sun through photosynthesis. Instead, during their vegetative growth stage, mushroom mycelia secrete enzymes that break down compounds such as cellulose and lignin present in the substrate. The degraded compounds are then absorbed by the hyphae and the mycelium enlarges-usually laterally, and in some cases growing several meters in diameter with the substrate.

Partially understood environmental factors (temperature and light are known to be critical) stimulate the second or reproductive growth stage. Cells of one mycelial strain fuse with cells of the opposite type to form a mycelium that contains both types of nuclei. The new mycelium continues to grow and eventually develops into a mature fruiting body, the gills of which are lined with spore bearing cells called basidia. Various mechanisms trigger the dispersal of spores, which in turn lodge in a substrate, become hyphae and begin the cycle anew.

Mushroom cultivation has great scope in China, India and in some of other developing countries because of the cheap and easily available raw materials needed for this activity, coupled with faster means of communication and marketing (as a fresh commodity), and better purchasing power of the people. Using China as for example, in 1978, the production of edible mushrooms was only 60,000 tonnes. In 2006, China’s mushroom production was over 14 million tonnes. Now there are more than 30 million people directly or indirectly engaged in mushroom production and businesses, and now China has become a leading mushroom producer and consumer in the world.

It is hoped that the avocation of mushroom farming will become a very important cottage industry activity in the integrated rural development programme, which will lead to the economic betterment of not only small farmers but also of landless labourers and other weak sections of communities. The advantages of mushroom cultivation can be summarized as:

1. Wastes such as cereal straws are largely burnt by the farmers, which causes air pollution. However, these raw materials can actually be used for the cultivation of mushrooms. This kind of bioconversion exercise can greatly **reduce environmental pollution**.
2. Mushroom cultivation can be a labour intensive activity. Therefore, it will serve as means of generating employment, particularly for rural women and youths in order to **raise their social status**. It will also provide additional work for the farmers during winter months when the farming schedule is light.
3. It will provide the people with an additional vegetable of high quality, and enrich the diet with high quality proteins, minerals and vitamins which can be of direct **benefit to the human health and fitness.** The extractable bioactive compounds from medicinal mushrooms would **enhance human’s immune systems and improve their quality of life.**
4. Mushroom cultivation is a cash crop. The harvested fruiting bodies can be sold in local markets for additional family income or exported for an important source of foreign exchange that will definitely **improve the economic standards** of the people.

5. Some warm mushrooms, e.g. *Volvariella volvacea* (Straw mushrooms) and *Pleurotus sajor-caju* (Oyster mushrooms) are relatively fast growing organisms and can be harvested in 3 to 4 weeks after spawning. **It is a short return agricultural business and can be of immediate benefit to the community.**

Mushroom farming is both a science and an art. The science is developed through research the art is perfected through curiosity and practical experience. However, mushroom farming is a business which requires precision. Indeed, it is not as simple as what some people often loosely stipulate. It calls for adherence to precise procedures. If you ignore one critical step, you are inviting trouble, which could lead to a substantially reduced mushroom crop yield. For example, if you fail to adjust the pH of the substrate to a critical level required by the specific mushroom species your are cultivating, or if you do not properly pasteurize the substrate (to free the mushroom spawn of other moulds and various bacteria), your planted mushroom could be out-competed by unwanted, intrusive micro-organisms.

The following ideas need to be emphasized as a conclusion of this introduction. Although scientific research and farming practice have led to the development of some universal or general concepts concerning mushroom cultivation, the diverse biological nature of the process (in which large numbers of mushroom species and natural organic substrates are involved) also means that a wide spectrum of variations in farming methods must be employed. Thus the transfer of mushroom farming from one region or country to another cannot be treated in the same way as the transfer of non-biological industrial technology, such as that of complete complex of factory equipment for textile or chemical fertilizer industries. Since the cultivation of mushrooms deals with living organisms, one should consider, not only the unique attributes of the mushroom itself, and of the various micro-organisms growing with it (including both the harmful and beneficial ones), but also the physical and biochemical natures of the substrates. Therefore, the cultivation methods must be tailored in accordance with the prevailing unique natural resources, heritage, local climate, and socio-economic conditions of each farming community. All these considerations call for a critical mass of well trained mushroom scientists and mushroom growers. Thus the training activities (workshops and courses) need to be supported by UN agents as well as Governments of the various countries concerned.

2. OVERVIEW OF THE MUSHROOM INDUSTRY (CONTENTS FOR A ONE-WEEK MUSHROOM TRAINING WORKSHOP)

2.1 Mushrooms and Mushroom Biology

It has been well known that the 20th century has been an explosive time for the accumulation of knowledge. Modern technology for human civilisation is expanding every day. However, human beings still face and will continue to face three basic problems: shortage of food; pollution of the environment; and diminishing quality of human health, due to the continued increase of the world population. The 20th century began with a world populated by 1.6 billion people and ended with 6 billion inhabitants-- with most of the growth occurring in the developing countries. The growing world population is increasing by about 80 million people per year. At the present, about 800 million people in the world are living in poverty. On the other hand, it has been observed that over 70 % of agricultural and of forest products has not been put to total productivity, and have been wasted in processing. Macrofungi (mushrooms) not only can convert these huge lignocellulosic biomass wastes into human food, but also can produce notable immune enhanced products, which have many health benefits. Another significant aspect of mushroom cultivation is using the biota in creating a pollution-free environment.

2.1.1 Definition of a Mushroom

Mushrooms with other fungi are something special in the living world, being neither plants nor animals. They have been placed in a kingdom of their own called the kingdom of Myceteae. But what are mushrooms? The word mushroom may mean different things to different people and countries. It has emerged that specialised studies and the economic value of mushrooms and their products had reached a point where a clear definition of the term "mushroom" was warranted. In a broad sense "Mushroom is a macrofungus with a distinctive fruiting body, which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand" (Chang and Miles, 1992). Thus, mushrooms need not be basidiomycetes, nor aerial, nor fleshy, nor edible. Mushrooms can be ascomycetes, grow underground, have a non-fleshy texture and need not be edible. This definition is not a perfect one but can be accepted as a workable term to estimate the number of mushrooms on the earth. The most common type of mushrooms is umbrella shaped with a pileus (cap) and a stipe (stem) i.e. *Lentinula edodes*. Other species additionally have a volva (cup) i.e. *Volvariella volvacea* or an annulus (ring) i.e. *Agarius campestris* or with both of them i.e. *Amanita muscaria*. Furthermore, some mushrooms are in the form of pliable cups; others round like golf balls. Some are in the shape of small clubs; some resemble coral; others are yellow or orange jelly-like globs; and some even very much resembles the human ear. In fact, there is a countless variety of forms.

The structure that we call a mushroom is in reality only the fruiting body of the fungus. The vegetative part of the fungus, called the mycelium, comprises a system of branching threads and cord-like strands that branch out through soil, compost, wood log or other lignocellulosic material on which the fungus may be growing. After a period of growth and under favourable conditions, the established (matured) mycelium could produce the fruit structure which we call the mushroom. Accordingly mushrooms can be grouped into four categories: (1) those which are fleshy and edible fall into the edible mushroom category, e.g., *Agaricus bisporus*; (2) mushrooms which are considered to have medicinal applications, are referred to as medicinal mushrooms, e.g., *Ganoderma lucidum*; (3) those which are proven to be, or suspected of being poisonous are named as poisonous mushrooms, e.g., *Amanita phalloides*; and (4) a miscellaneous category which includes a large number of mushrooms whose properties remain less well defined, which may tentatively be grouped together as 'other mushrooms'. Certainly, this approach of classifying of mushrooms is not absolute and not mutually exclusive. Many kinds of mushrooms are not only edible, but also possess tonic and medicinal qualities.

Mushrooms are devoid of leaves, and of chlorophyll-containing tissues. This renders them incapable of photosynthetic food production. Yet, they grow, and they produce new biomass. How? For their survival, for their growth, and for their metabolism, they rely on organic matter synthesized by the green plants around us, including organic products contained in agricultural crop residues. The organic materials, on which mushrooms derive their nutrition, are referred to as **substrates**. Mushrooms are a unique biota which assembles their food by secreting degrading enzymes and decompose the complex food materials present in the biomass where they grow, to generate simpler compounds, which they then absorb, and transform into their own peculiar tissues. These substrate materials are usually by-products from industry, households and agriculture and are usually considered as wastes. **And these wastes, if carelessly disposed of in the surrounding environment by dumping or burning, will lead to environmental pollution and consequently cause health hazards.** However, they are actually resources in the wrong place at a particular time and mushroom cultivation can harness this waste/resource for its own beneficial advantage.

Mushrooms lack true roots. How then are they anchored into the substrates where we find them? This is affected by their tightly interwoven thread-like hyphae, which also colonise the substrates, degrade their biochemical components, and siphon away the hydrolysed organic compounds for their own nutrition.

2.1.2 Mushroom Hunting

Fungi are found just about everywhere. Mushrooms, a special group of macro-fungi, are rather more selective than other fungi in that the size of the fruiting body requires the availability of more nutrients than are required for the production of asexual spores by micro-fungi. In damp places, such as tree-fern ecosystems and tropical rain forests, plentiful moisture leads to abundant mushroom formation. There, mushrooms can be collected at most times of the year. But in drier regions, they occur only after seasonal rains. In these ecosystems there may be a particular flora of mushroom species associated with the seasons of autumn, summer and spring. Relatively few mushrooms are produced during the cold winter months, although there are perennial fruiting bodies that persist during the winter. The formation of mushroom fruiting bodies depends very much on the pattern of rains and, in some years, there may be virtually total lack of mushroom fruiting.

Mushroom hunters, in addition to carrying along with them the basic equipment and field guide references, which will vary depending on personal requirements and regional conditions, should record such items as date, time, location, smell, substrate (host) colour, habitat and anything at all unusual about the specimen. Some important characteristics for identification disappear rapidly as the mushroom matures. These characteristics have to be recorded accurately at the time of collection.

2.1.3 Ecological Classification of Mushrooms

Ecologically, mushrooms can be classified into three groups: the saprophytes, the parasites and the symbiotic (which include mycorrhizal) species (Fig. 1). There are only a few parasitic mushrooms. Most of the cultivated gourmet mushrooms are saprophytic fungi. Some of the edible mushrooms are mycorrhizal species, e.g. Perigold black truffle, *Tuber melanosporum*, and matsutake mushroom, *Tricholoma matsutake*. It is difficult to bring these highly celebrated wild gourmet species into cultivation because they are mycorrhizal. These species have a symbiotic relationship with some vegetation, particularly trees, i.e. there is a relationship of mutual need.

Saprophytes obtain nutrients from dead organic materials; parasites derive food substances from living plants and animals and causing harm to the hosts; and mycorrhiza live in a close physiological association with host plants and animals – thereby forming a special partnership where each partner enjoys some vital benefits from the other.

However, some mushrooms do not fall neatly within these man-made categories and can share two of these categories. For example, some *Ganoderma spp.* including *G. lucidum* are common saprophytes, however they can be pathogenic too; also *Tricholoma matsutake,* while initially appearing to be mycorrhizal on young roots, soon becomes pathogenic and finally exhibits some saprobic ability.

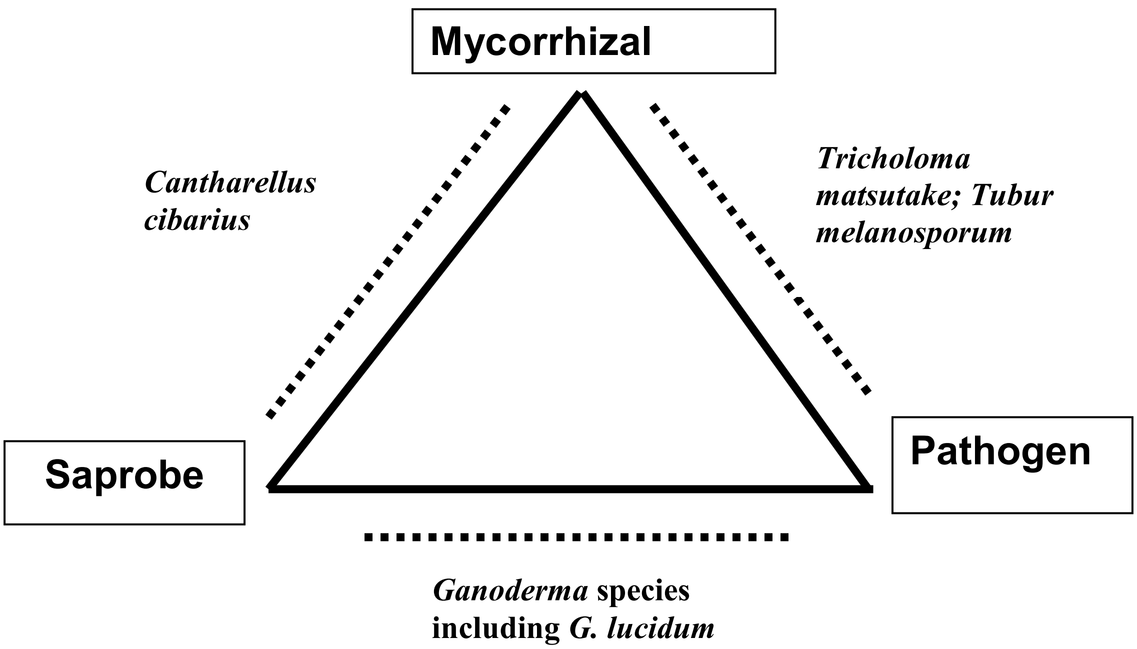

Figure1. Modified triangular model for the ecological classification of mushrooms.
Source: Hall et al., 2003.

2.1.4 Magnitude of Mushroom Species

In 1990, the number of known species of fungi was about 69,000 (Hawksworth, 1991) while it was conservatively estimated that 1.5 million species of fungi actually existed (Table 1). On average 700 species were described as new to science each year from 1920 to 1950. However, the annual total catalogued fungi reached around 1,400 in 1961, 1,500 in 1968 and averaged 1,700 each year for 1986 to 1990.

Fungi are regarded as being the second largest group of organisms in the biosphere after the insects. Known fungal species constitute only about 5% of their species in the world. Thus, the large majority of fungi are still unknown. Out of about 70,000 described species of fungi, it has been suggested that around 14,000-15,000 species produce fruiting bodies of sufficient size and suitable structure to be considered as macrofungi (mushrooms). Of these, about 5,000 of the species are considered to possess varying degrees of edibility, and more than 2,000 species from 31 genera are regarded as prime edible mushrooms. But only 100 of them are experimentally grown, 50 economically cultivated, around 30 commercially cultivated, and only about 6 to have reached an industrial scale of production in many countries. Furthermore, about 1,800 are medicinal ones. The number of poisonous mushrooms is relatively small (approximately 10%), of these some 30 species are considered to be lethal (Miles and Chang, 1997).

Table 1. Comparison of the numbers of known and estimated total species in the world of selected groups of organisms

Group	Known species	Total species	% known species
Vascular plants	220,000	270,000	81
Bryophytes	17,000	25,000	68
Algae	40,000	60,000	67
Fungi	69,000	1,500,000	5
Bacteria	3,000	30,000	10
Viruses	5,000	130,000	4

Source: Hawksworth (1991)

2.1.5 The Concept of Mushroom Biology

The biological science that is concerned with fungi is called mycology. Mushroom biology is the branch of mycology that deals with mushrooms in many disciplines. When knowledge increases and areas of specialisation develop within the discipline, it is convenient to indicate that area of specialisation with a self-explanatory name. In biology, there are such specialisations as neurobiology, bacteriology, plant pathology, pomology, molecular biology, virology, fungal physiology, embryology, endocrinology, phycology, and entomology. These names indicate either a group of organisms (e.g., bacteria, algae, and insects) and /or an approach to the study (e.g., disease, development and physiology).

Although several terms for this important branch of mycology that deals with mushroom have been used, and each of these has its merit, when we get down to the matter of definitions, it seems that there is a place for a new term. The new term is mushroom biology. Mushroom biology is a new discipline concerned with any aspect of the scientific study of mushrooms, such as: taxonomy; physiology; genetics; etc.

2.2 Applied Mushroom Biology

Applied mushroom biology is concerned with all aspects of the application of mushroom biology. It consists of three main components: mushroom science; mushroom biotechnology; and mushroom mycorestoration (Fig. 2). As previously outlined, mushroom biology is concerned with any aspect of the scientific study of mushrooms, therefore it will feature in each of the three components of applied mushroom biology.

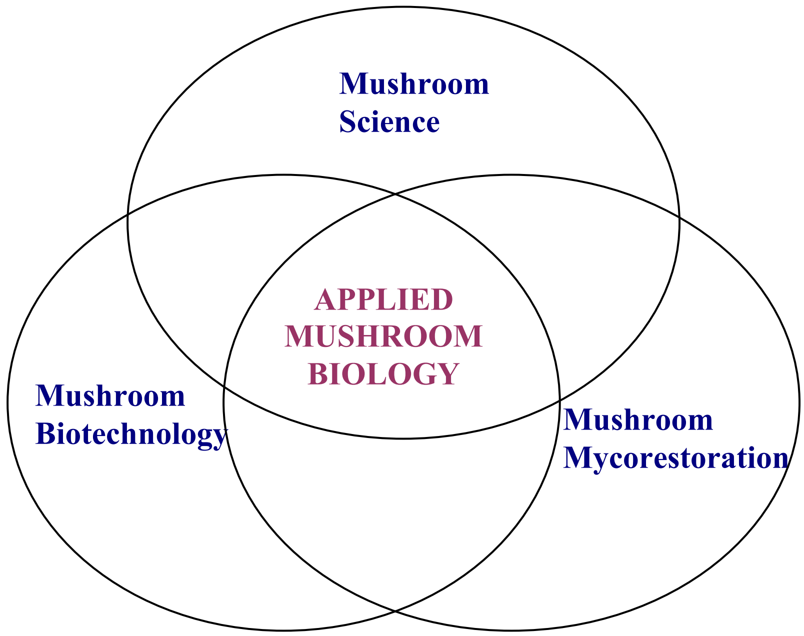

Figure2. The three components of Applied Mushroom Biology: Mushroom Science; Mushroom Biotechnology; and Mushroom Mycorestoration. Source: Chang and Buswell (2008).

Mushroom science deals with mushroom cultivation and production (mushrooms themselves) and encompasses the principles of mushroom biology/microbiology, bioconversion/composting technology and environmental technology (Fig. 3).

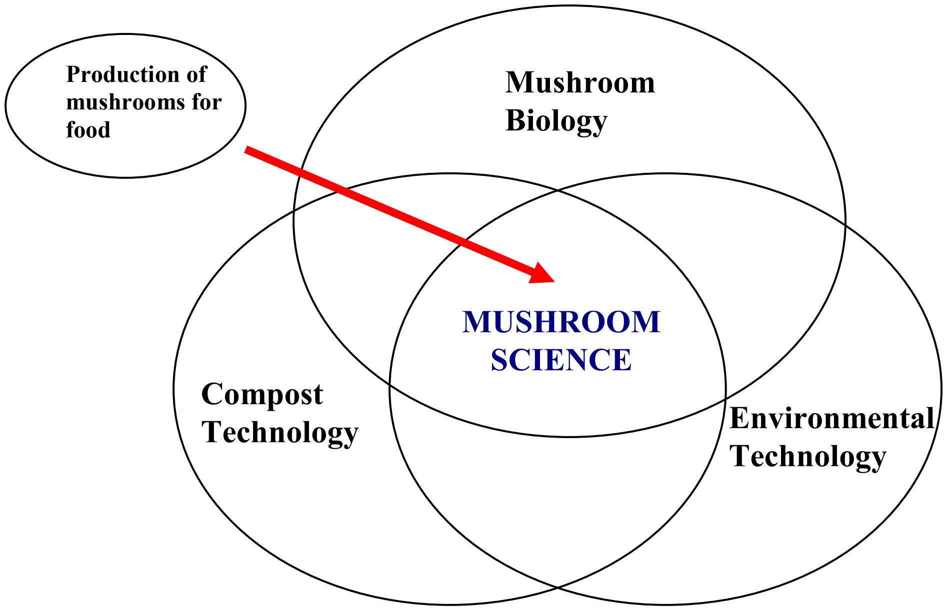

Figure3. Mushroom science: mushroom cultivation and production. Source: Chang and Buswell (2008).

Mushroom biotechnology is concerned with mushroom products (mushroom derivatives) and encompasses the principles of mushroom biology/microbiology, fermentation technology and bioprocess (Fig. 4). Mushroom biotechnology, both as a technology and as the basis for new mushroom products, requires industrial development. It, like many bioscience industries, operates at the cutting edge of science and involves numerous regulatory issues.

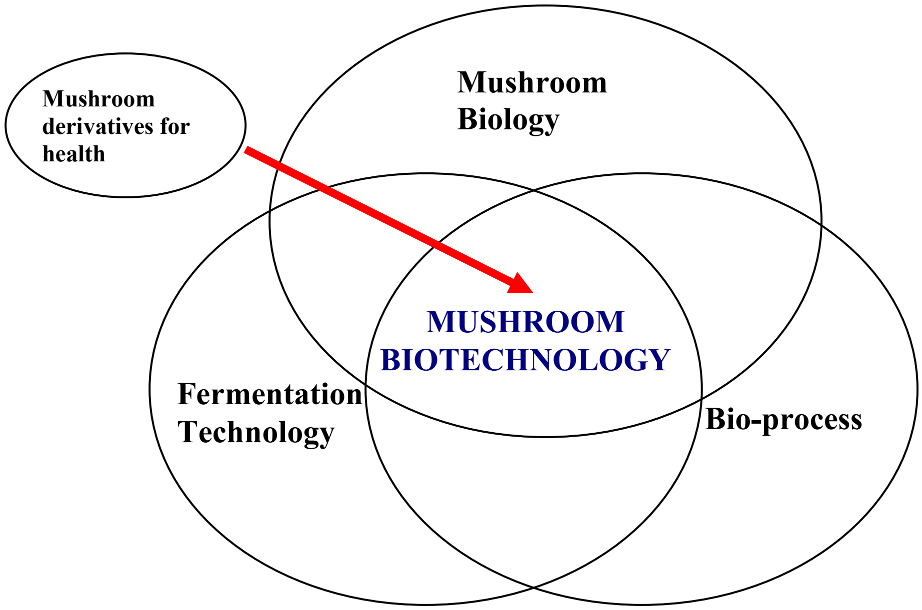

Figure 4.Mushroom biotechnology: mushroom products (mushroom nutriceuticals/dietary supplements) for health enhancement. Source: Chang and Buswell (2008).

The third component of applied mushroom biology has been developed in recent years. This is mushroom bioremediation which is concerned with the beneficial impacts of mushrooms on the environment (from mushroom mycelia) and encompasses principles of mushroom biology/microbiology, ecology and bioremediation technology (Fig. 5).

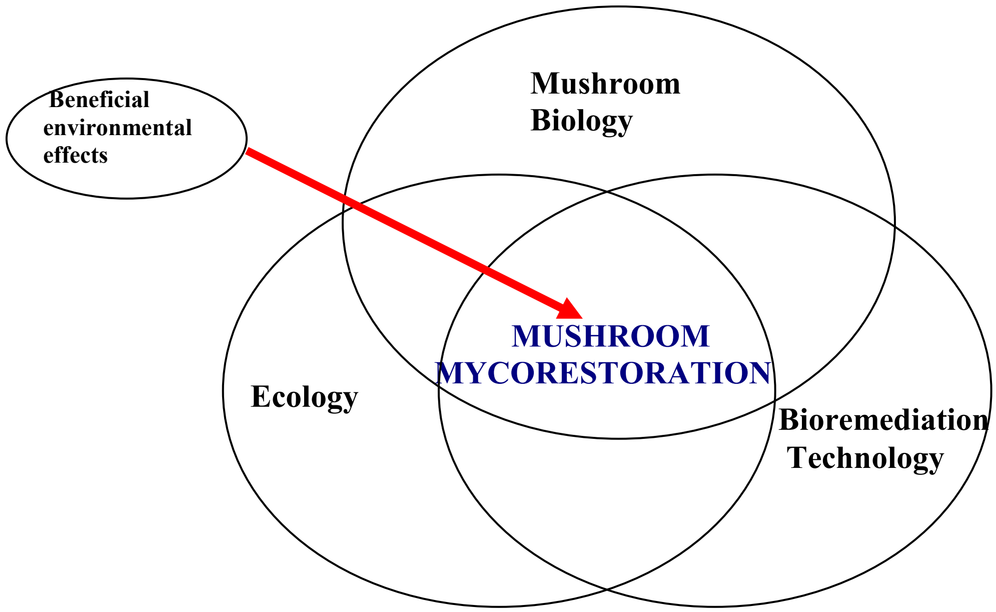

Figure5. Mushroom mycorestoration: the beneficial impacts of mushrooms on the environment. Source: Chang and Buswell (2008).

Therefore, the aims of the discipline of applied mushroom biology are to tackle the three basic problems: shortage of food, diminishing quality of human health and pollution of the environment, which human beings still face, and will continue to face, due to the continued increase of the world population. The 20th century began with a world population of 1.6 billion and ended with 6 billion inhabitants. The world's population is likely to reach 9.2 billion in 2050 from the current 6.7 billion with most of the growth occurring in developing countries. The growing world population is increasing by about 80 million people per year. At present, about 800 million people in the world are living in poverty. On the other hand, it has been observed that over 70% of agricultural and of forest products have not been put to total productivity, and have been discarded as waste. Applied mushroom biology not only can convert these huge lignocellulosic biomass wastes into human food, but also can produce notable nutriceutical products, which have many health benefits. Another significant aspect of applied mushroom biology is using the biota in creating a pollution-free and beneficial environment. The three components of applied mushroom biology are closely associated with three aspects of wellbeing - food, health and pollution.

2.2.1 Mushroom Science: Food Supplies through Mushroom Themselves

The discipline that is concerned with the principles and practice of mushroom cultivation is known as mushroom science. The establishment of principles requires facts, which are arrived at through systematic investigation. The systematic investigation must involve the practical aspects of mushroom cultivation as well as scientific studies. The consistent production of successful mushroom crops necessitates both practical experience and scientific knowledge (see Section 3 on mushroom cultivation technology).

2.2.2 Mushroom Biotechnology: Enhance Human Health through Mushroom Derivatives

It has been pointed out that mushroom biotechnology is concerned with mushroom products and encompasses the principles of fermentation technology, mushroom biology/microbiology and bioprocess. Mushroom products have a generalized or tonic effect, which in some cases may act prophylactically by increasing resistance to disease in humans from the balancing of nutrients in the diet and the enhancing of the immune systems.

(1) Nutritional value of mushrooms

The greatest difficulty in feeding man is to supply a sufficient quantity of the body-building material -- protein. The other three nutritional categories are: the source of energy food—carbohydrates and fats; accessory food factors-- vitamins; and inorganic compounds which are indispensable to good health. Of course, water, too, is essential.

In terms of the amount of crude protein, mushrooms rank below animal meats, but well above most other foods, including milk, which is an animal product (Chang and Miles 1989). Furthermore, mushroom protein contains all the nine essential amino acids required by man.

The moisture content of fresh mushrooms varies within the range 70 - 95% depending upon the harvest time and environmental conditions, whereas it is about 10 - 13% in dried mushrooms.

In addition to their good proteins, mushrooms are a relatively good source of the following individual nutrients: fat, phosphorus, iron, and vitamins including thiamine, riboflavin, ascorbic acid, ergosterine and niacin. They are low in calories, carbohydrates and calcium. Mushrooms also contain a high proportion of unsaturated fat.

In recent years, there has been a trend toward discovering ways of treating mushrooms so as to give them added value. For example, Wermer and Beelman (2002) have reported on growing mushrooms enriched in selenium.

The desirability of a food product does not necessarily bear any correlation to its nutritional value. Instead, its appearance, taste, and aroma, sometimes can stimulate one’s appetite (preference). In addition to nutritional value, mushrooms have some unique colour, taste, aroma, and texture characteristics, which attract their consumption by humans.

(2) Medicinal value of mushrooms

The second major attribute of mushrooms, their medicinal properties, has also been drawn to our attention for study, e.g., hypotensive and rental effects (Tam et. al., 1968; Yip et al., 1987), immunomodulatory and antitumour activities of polysaccharide-protein complex (PSPC) from mycelial cultures (Liu et al., 1995, 1996; Wang et al., 1995, 1996, Ng et al., 1999), immunomodulatory and antitumour activities of lectins from edible mushrooms (Wang et al., 1995, 1996, 1997), isolation and characterization of a Type I Ribosome-Inactivation protein from

V. volvacea (Yao et al., 1998), and medicinal effects of *Ganoderma lucidum* (Chang and Buswell 1999, Chang and Miles, 2004).

(3) Nutriceuticals and dietary supplements

The recent upsurge of interest in traditional remedies for various physiological disorders and the recognition of numerous biological response modifiers in mushrooms have led to the coining of the term "*mushroom nutriceuticals*" (Chang and Buswell, 1996). A mushroom nutriceutical is a refined/partially defined mushroom extractive which is consumed in the form of capsules or tablets as a dietary supplement (not a food) and which has potential therapeutic applications. A regular intake may enhance the immune responses of the human body, thereby increasing resistance to disease and, in some cases, cause regression of a disease state.

For more detailed coverage of the nutritional and medicinal value and for comprehensive lists of mushrooms used in dietary supplements and in medicines, readers are referred to Section 2.5.

2.2.3 Mushroom Bioremediation: Benefit the Environment through Mushroom Mycelia

This component of applied mushroom biology deals mainly with the aspects of benefits to the Earth from the activities of mushroom mycelium. Environmental contamination can be ameliorated by the application of mushroom mycelial technologies. For example, (1) the use of bioconversion processes to transform the polluting substances into valuable foodstuffs, e.g., the proper treatment and reutilization of spent substrates/composts in order to eliminate pollution problems (Beyer, 2005, Noble, 2005). One of the most intriguing opportunities offered by mushroom mycelia in the area of bioconversion is the exploitation of their ability to degrade pollutants, many of which are highly carcinogenic, released into the environment as a consequence of human activity. And (2) the use of fungi/mushroom mycelia as tools for healing soil, what Stamets (2005) called "mycorestoration", which is the use of fungi/mushrooms to repair or restore the weakened or damaged biosystems of environment. The processes of mycorestoration include the selective use of mushrooms for mycofiltration, to filter water; mycoforestry, to enact ecoforestry policy; mycoremediation, to denature toxic wastes; and mycopesticides, to control insect pests. Mycoresoration recognizes the primary role fungi/mushrooms can play in determining the balance of biological populations.

2.3 Mushroom Genetics and Breeding

Studies of the genetics of fungi have played a leading role in the development of the modern era of molecular genetics. The chemical studies of Beadle and Tatum beginning in the 1940's on the biochemical genetics of the ascomycete *Neurospora* brought forth techniques that were subsequently employed with bacteria. Those studies then raised questions concerning the nature of the genetic material which culminated in the elucidation of the structure of DNA by Watson and Crick in 1953. Since that time, fundamental investigations on the molecular level have progressed with ever-increasing rapidity.

The basidiomycetes were not in the fore-front of research during the early days when molecular biologists preferred the rapidity of the cell cycle of the unicellular bacterium *Escherichia coli* to the slower growth of the filamentous basidiomycetes with their complex incompatibility control systems of sexuality. In recent years, however, basidiomycetes have attracted the interest of investigators concerned with problems of differentiation and the control of development because they are eukaryotic organisms and do have some distinct but simple morphological stages which are not present in single cell organisms. However, mushroom (macrofungus) genetics is a young discipline in science as compared to biotechnology, which is correlated with the beginning of human civilisation. It was almost exclusively devoted to fundamental research before attracting serious attention during the early 1770s in relation to mushroom production.

The overall objective of breeding is to improve the quality of extant strains in the most efficient way possible through the target selection, and controlled crossing, and progeny selection. The desired result should be defined with reasonable clarity in terms of measurable traits desired, such as yield, quality of flavour, texture, appearance of mushrooms, disease resistance, and general vigour, all within the context of a chosen set of standard conditions which are employed consistently throughout the breeding programme.

2.3.1 Discovery of Sexuality by Kniep and Bensaude

Although Blakeslee in 1904 had demonstrated sexuality in the Mucorales (class Zygomycetes) through matings of mycelia established from single spores, it was not until 1918-20 that the mechanism of sexuality in the basidiomycetes was discovered. It was demonstrated that the mycelium arising from single spores was made up of hyphae with simple septa, and that the hyphae of the fruiting bodies and of mycelium giving rise to fruiting bodies bore clamp connections. Furthermore, when mycelia which had originated from single spores were confronted with various combinations, only certain combinations gave rise to clamped mycelium, while in other combinations the hyphae had simple septa.

2.3.2. Sexuality in the Edible Mushrooms

Although the process of sexuality is complicated by nutritional and physiological conditions, genetic constitution is the most critical factor determining both the occurrence and the morphology of the fruiting bodies in the edible mushrooms.

Sexuality in fungi consists of three important stages. The first essential stage is plasmogamy which is the fusion of cytoplasm of the two mating individuals. By plasmogamy the nuclei from two strains are brought together in a common cytoplasm. The second essential stage in sexuality is known as karyogamy or nuclear fusion. The third essential stage is meiosis, the nuclear division in which the chromosome number is reduced from the diploid to the haploid number. The product of meiosis is the formation of a tetrad. Through the process of sexuality, genetic recombination and segregation subsequently occurs.

2.3.3 Mating Systems (Patterns of Sexuality) in Fungi

Edible mushrooms contain both self-fertile and self-sterile species. Self-fertilisation (homothallism or homomixis) is probably the commonest mode of sexual reproduction in the fungi as a whole but in the higher fungi (e.g., basidiomycetes) self-fertile species are in a distinct minority of only about 10 %. Self-sterilisation (heterothallism or dimixis) is about 90 %, in which 25 % is bipolar and 65 % is tetrapolar.

(1). Homothallism. Fruiting structure can be produced by a single, monosporous mycelium. Potentially self-fertile fungi are not always necessarily homozygous and a variety of situation and rather imprecise regulating system can result in heterozygosity. Two types of homothallism are found among self-fertile species: (a) primary homothallism, in which a homokaryotic mycelium, established from a single meiotic nucleus, has the potentiality to progress through heterokaryosis to the completion of the sexual cycle; and (b) secondary homothallism, in which a fertile dikaryotic mycelium is established from a basidiospore carrying two meiotic nuclei of different mating types.

(2). Heterothallism. Cross mating between homokaryotic mycelia is required to complete the sexual cycle. Two mating systems of heterothallism are commonly found in edible mushrooms: (a) Bipolar mating system, in which the mating competence is determined by incompatibility factors of a single series, the A factor. Therefore, only two mating types are produced in equal frequency by a single fruiting body. (b) Tetrapolar mating system, in which the mating competence is determined by incompatibility factors of two series, A and B, which assort and segregate independently at meiosis. There are four rather than two mating types produced in equal frequency by a single fruiting body.

2.3.4 Life Cycle

If a section of the gills is cut and examined under the microscope, spores will be observed on their surface. The spores will start to fall as the cap fully expands, indicating maturity of the mushroom. The spores are so minute that they float in the air and are carried by the wind. Eventually, they fall to the ground, usually with rain. If conditions are favourable (optimum temperature and moisture), the spores will germinate to form a mass of mycelium. This is the start of the vegetative phase of the mushroom. Given an unrestricted amount of nutrients and favourable growing conditions, it is capable of unlimited growth. The mycelium developing from the germinating spore is the so-called primary mycelium and is usually uninucleate and haploid. This stage is short-lived because mycelia from different spores tend to ramify and fuse to form the secondary mycelium with two compatible nuclei, which continues to grow vegetatively and is able to form fruiting bodies.

2.3.5 Strain Improvement (Breeding Programme)

The reservoir of edible mushrooms, like other micro-organisms of use for industrial purposes, is not unlimited. It is generally recognised that in order to maintain and breed high-yielding strains, the techniques employed in mushroom breeding should now and then be modified and improved in accordance with new findings and progress in the scientific world as a whole, and in microbiology and genetics in particular.

(1).By selection. The common white mushroom, *Agaricus bisporus*, strains are usually based on selection from multi-spore or single-spore cultures. Tissue culture of selected sporophores has also been used to fix desired variants. In the short term, selection alone appears to have some role to play in mushroom strain improvement, but genetic improvement by selection becomes increasingly difficult. Therefore, strain improvement through hybridisation has become a recourse and also necessity.

(2). By hybridisation. In addition to the conventional method of matings between two genetically compatible strains through which dikaryon mycelia and fruiting bodies are formed, steps towards a broader spectrum of hybridisation can be achieved in strain improvement of edible mushrooms by the following ways.

(A) Use of auxotrophs. Auxotrophs can be obtained naturally or induced by mutagenesis. The contrasting auxotrophs can be paired and the products can be screened for hybridity on minimal medium. Certainly, the feasibility of auxotrophs to be used as a tool for hybridisation depends on how easily auxotrophs can be obtained in the strains of the mushroom.

(B). Use of resistance markers. Mutants resistant to antimetabolites have been suggested recently as alternatives to auxotrophs for use in mushroom breeding programmes. The treated spores or hyphal fragments, which can grow on a medium containing an inhibitory concentration of the anti-metabolite, would be considered to possess the marker. Complementary resistant strains would be grown together and, hybridity can be confirmed by transferring it to a medium containing the two appropriate antimetabolites.

(C). Protoplast fusion. One of the most effective barriers to sexual reproduction is the inability of hyphae from two selected strains to fuse. Several laboratories have reported that protoplasts can be isolated from plant and microbial cells by enzymatic breaking of the cell wall, in the presence of an osmotic stabiliser. Such protoplasts can be effectively induced to fuse in the presence of polyethylene glycol (PEG). After a short period of time protoplasts can regenerate their cell walls and start to propagate as normal cells or hyphae. These cells are heterokaryotic if fusion occurred between cells from genetically different strains. This can serve to increase the frequency of intraspecies crosses in organisms in which natural matings rarely occur. The technique has even a much wider application, and can be used for interspecies and intergeneric crosses in some organisms, which normally cannot be crossed. Although such an approach has been carried out in several laboratories, until now, no clear and economically applicable results have been reported in edible mushrooms. I hope that this does not embrace the truth that "*beautiful hypotheses are destroyed by ugly facts"*. More detailed information on mushroom genetics and breeding can be found in "Mushroom Biology--Concise basics and current developments" by Miles and Chang (1998) pp 65-85.

Due to the nature and the limited time of the training course, the molecular biology of mushrooms was not touched upon in this course, although it has become an important sector of mushroom genetics and breeding. For further reading, readers are referred to Chang et al (1993).

It should be emphasised that sexuality in any species has to be clearly understood in order to conduct rational genetic studies as well as breeding programmes. Mushrooms representing three types with respect to life cycle and genetic controls for mating have been considered. It is hoped that the examples discussed in this course will provide some basic and useful guidelines for procedures as they might be applied generally to any breeding programme for each type of mushroom. Although it seems likely that selection from existing commercial strains of edible mushrooms may continue to enjoy some improvement, by analogy with other crop species, hybridisation seems to offer the best prospects for real progress, especially with regard to multiple gene transfers mediated by protoplast fusion, during the next few decades. Finally, the experience of breeding in a variety of organisms has demonstrated the wisdom of preserving the starting strains used in all breeding programmes for the purpose of maintaining a pool of genetically diverse individuals. Such a practice would allow the possibility of carrying out additional breeding programmes to either improve or restore strains, which are in current use.

2.4 Principle of Mushroom Cultivation and Production

This section provides an overview of mushroom cultivation and mushroom production. Detailed information on mushroom cultivation technology is in Section 3.

2.4.1 Mushroom Cultivation: Both a Science and an Art

The cultivation of mushrooms can be both a relatively primitive farming activity, and a high technology industry. In each case, however, continuous production of successful crops requires both practical experience and scientific knowledge. Mushroom cultivation is both a science and an art. The science is developed through research; the art is perfected through curiosity and practical experience.

Mushroom growth dynamics involve some technological elements, which are in consonance with those exhibited by our common agricultural crop plants. For example, there is a vegetative growth phase, when the mycelia grow profusely; and a reproductive (fruiting) growth phase, when the umbrella-like body that we call mushroom develops. In the agricultural plants, e.g., sunflowers, when the plants switch from the vegetative growth to the reproductive growths, retarded tips for further growth (elongation) is an obvious phenomenon of mature. It is the same principle in mushroom production. After the vegetative (mycelial) phase has reached maturity, what the mushroom farmer needs next is the induction of fruiting. This is the time the mycelial growth tips should be retarded by regulating the environmental factors. These factors generally called “triggers” or “environmental shocks”, such as, switching on the light, providing fresh air, and lowering temperatures, can trigger fruiting (Fig. 6).

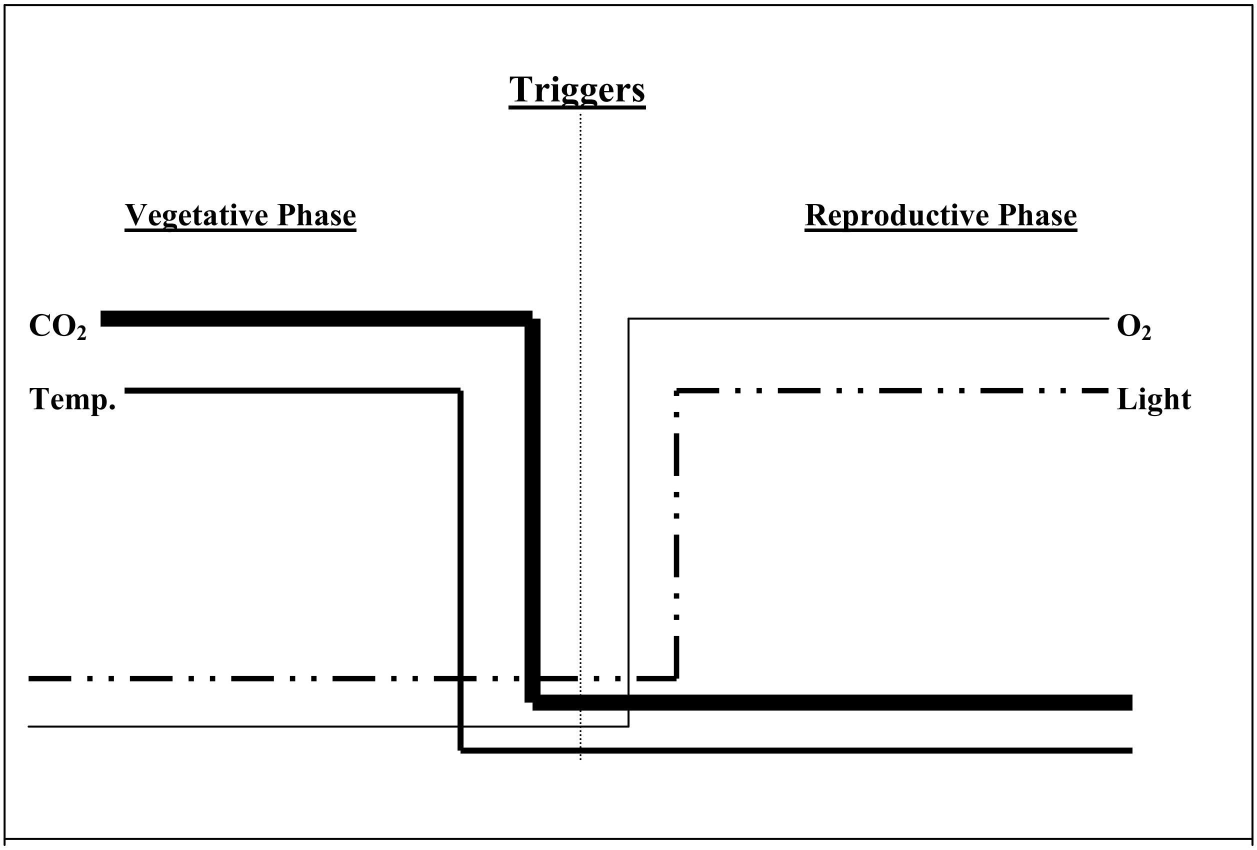

Figure 6.The two major phases of mushroom growth and development - vegetative phase and reproductive phase. The triggers for the transition from the vegetative phase to the reproductive phase are usually regulated by environmental factors.

Although the principles of cultivation are commonly similar for all mushrooms, the practical technologies can be quite different for different species cultivated. The technologies have to be modified and adjusted according to the local climatic conditions, materials available for substrates and varieties of the mushroom used.

2.4.2 World Mushroom Production

Generally, cultivated mushrooms should play a greater role in the endeavour to increase food protein. This is especially true in developing countries, since growth substrates for mushrooms are basically agricultural and industrial discards that are inedible for humans (Chang and Miles 1984). Biological (bioconversion) efficiency, i.e., the yield of fresh weight mushrooms in proportion to the spawning compost in *Agaricus* or to the air-dried substrates in other non-composting mushrooms, can reach 60-100% for *Agaricus* and 15-100% depending on the cultivation conditions for other species.

The following statistics (Table 2) serve to illustrate dramatic increases in the production of farmed mushrooms during the period 1978 to 2006, with particular emphasis on China's contribution to total world production, given its current status as the leading mushroom producer (Chang 1999, 2006b, Delcaire 1978; Sharma 1997):

Table 2.Total world mushroom production and China's contribution since 1978

Year	Total production (x1,000 tonnes)	China's production (x1,000 tonnes)	China's contribution (%)
1978	1,060.0	60.0	5.7
1983	1,453.0	174.5	12.0
1990	3,763.0	1,083.0	28.8
1994	4,909.3	2,640.0	53.8
1997	6,158.4	3,918.0	63.6
2002	12,250.0	8,650.0	70.6
2006	n.a	14,000.0	n.a

Sources: Chang 1999, 2006b, Delcaire 1978; Sharma 1997 and courtesy communications with the China Edible Fungi Association. n.a.: not available

Whereas in 1997, Asia contributed 74.4% of the total world mushroom tonnage, Europe, 16.3% and North America, 7.0%, both Africa and Latin America's shares were less than 1%. This is largely due to lack of know-how, lack of understanding that mushroom can play vital roles towards enhancing human health when used as dietary food supplements, lack of reliable sources of good quality mushroom spawn for supporting the efforts of local mushroom growers, lack of venture capital to support mushroom farming entrepreneurs, and absence of systematic government support towards promoting mushroom farming as a valuable non-traditional new food and cash crop (comparable to coffee, tea, cotton, tobacco, etc.).

2.4.3 Differences in Mushroom Production Patterns

The mushroom industry in UK and in some other Western countries is often overwhelmingly focused on one mushroom species, *Agaricus bisporus*. These industries are nearly 100% dominated by *Agaricus bisporus* (Gaze, 2005). In the US, it accounts for about 98% of its mushroom industry, *Lentinula edodes* for 1% and *Pleurotus* spp for only about 0.5% (Table 3). However, it should be noted that *Agaricus bisporus* is only but one of many edible fungi cultivated globally.

This production pattern is slightly less skewed towards *Agaricus bisporus* in Spain, the third largest mushroom producer in the EU. In 2004, mushroom production in Spain was 110,000 tonnes compared with 26,512 tonnes in 1992, increasing 315%. Production consists of 80% of *Agaricus* mushrooms, 15% of *Pleurotus* mushrooms and 5% of *Lentinula* mushrooms.

Table 3. The US mushroom industry is dominated by *Agaricus Bisporus*

Mushroom Production (t)	02-03	03-04	04-05
Agaricus	379318.8	381479.4	380083.0
	(98.6%)	**(98.4%)**	**(98.2%)**
Lentinula	3390.5	3506.1	4118.4
	(0.9%)	(0.9%)	(1.1%)
Pleurotus	1812.6	2008.2	2453.0
	(0.5%)	(0.5%)	(0.6%)
Sub-total	384521.9	386993.7	386654.4
	(99.9%)	**(99.9%)**	**(99.9%)**
Others	197.3	541.0	253.5
	(0.01%)	(0.01%)	(0.01%)
Grand total	384719.2	387534.7	386907.9
	(100%)	(100%)	(100%)

Source: National Agricultural Statistics Service (NASS) 2005, USA.
Note: share of total US mushroom production is in brackets.

On the other hand, specialty mushrooms in East Asian countries are far more popular than *A. bisporus* as shown in Table 4. *Agaricus* accounted for 12.8% of total mushroom production in China in 2003, 11.6% in S. Korea and 0% in Japan. Furthermore, while the production of the three important mushrooms, *Agaricus, Lentinula* and *Pleurotus* mushrooms together make up nearly 100% of the mushroom industry in U.S and Spain, the production of these three mushrooms account for 72.7% of total mushroom production in S. Korea, 58.2% for China and only 12.3% in Japan (Table 4). This means there are more other culinary-medicinal mushrooms being cultivated and marketed in those three Asian countries particularly in Japan.

Table 4. Asia's mushroom industry is more diverse (2003 data)

Production	China (x1000t)	Japan (t)	S. Korea (t)	Taiwan (t)
Agaricus	1330.4	-	19790	4276
	(12.8%)		**(11.6%)**	**(4.0%)**
Lentinula	2228	35294	41876	36000
	(21.5%)	(10.7%)	(24.6%)	(33.4%)
Pleurotus	2488	5219	61965	4540
	(24.0%)	(1.6%)	(36.5%)	(4.2%)
Sub-total	6046.4	40513	123631	44816
	(58.2%)	**(12.3%)**	**(72.7%)**	**(41.6%)**
Others	4340.5	290333	46369	62984
	(41.8%)	(87.8%)	(27.3%)	(58.4%)
Grand total	10386.9	330846	170000	107800

	(100%)	(100%)	(100%)	(100%)

Source: Chang, 2006; Cui, 2004; Ho and Peng, 2006
Note: shares of total mushroom production are in brackets.

2.4.4 World Mushroom Market

The world market for the mushroom industry in 2001 was valued at over US$40 billion. The mushroom industry can be divided into three main categories: edible mushrooms valued about US$30 billion; medicinal mushroom products were worth about US$9 - 10 billion; and wild mushrooms, US$4-5 billion. International bodies/forums have developed for each of these segments of the mushroom industry that has helped to bring them to the forefront of international attention: 1) International Society of Mushroom Science (ISMS), for edible mushrooms established in 1950 in England; 2) World Society for Mushroom Biology and Mushroom Products (WSMBMP), for mushroom biology and medicinal mushroom products formed in 1993 in Hong Kong; and 3) International Workshops on Edible Mycorrhizal Mushrooms, for some wild mushrooms initiated in 1999 in Sweden. The three international bodies/forums (see next section for further details) have done much to promote each of their respective fields, not least of which is bringing scientists together for useful discussions, encouraging research and the dissemination of valuable research and commercial information.

The outlook for many of the known mushroom species is bright. Production of mushrooms worldwide has been steadily increasing, mainly due to contributions from developing countries, such as China, India, Poland, Hungary and Vietnam. There are also increasing experimentally based evidence to support centuries of observations regarding the nutritional and medicinal benefits of mushrooms. The value of mushrooms has recently been promoted to tremendous levels with medicinal mushrooms trials conducted for HIV/AIDS patients in Africa, generating encouraging results. However, harvests of highly prized edible mycorrhizal mushrooms are continuously decreasing. This has triggered research into devising methods for improved cultivation of wild mushroom. It is hoped that there will be even more research into this area, so that larger quantities of wild mushrooms can be massively harvested through artificial cultivation or semi-cultivation methods. Technological developments in the mushroom industry in general has seen increasing production capacities, innovations in cultivation technologies, improvements to final mushroom goods, capitalising on mushrooms' nutritional and medicinal properties, and utilising mushrooms' natural qualities for environmental benefits.

2.4.5 Development of World Mushroom Industry Movements (Organizations)

Although mushrooms have been collected from the wild and cultivated artificially for human food and for medicine uses for hundreds and thousands of years, it is only recently that the three main segments of the mushroom industry could be identified. These three segments have received international recognition as important inter-related components (Fig. 7), with each deserving its own special patronage and paths of development: (a) cultivated edible mushrooms (mushroom themselves-used directly or indirectly as food); (b) medicinal mushrooms (mushroom derivatives-used as nutriceutical therapy/dietary supplements); and (c) wild mushrooms including edible mycorrhizal, symbiotic and poisonous mushrooms (collected, up to now, only from the wild). The development of three important international bodies/forums has helped to bring each of these three components of the mushroom industry to the forefront of international attention, showcasing their positive contributions to human welfares (Chang, 2006b).

(1). The international movement for edible mushrooms, mainly concerned with mushroom production (mushroom themselves), was initiated during the first International Conference on Mushroom Science held in Peterborough, UK, 3rd to 11th May 1950. Chairman F. C. Atkins with P. J. Bels, E. B. Lambert and R. L. Edwards were on the organising committee. The committee members later formed the International Commission on Mushroom Science which eventually developed into the International Society for Mushroom Science (ISMS) (Personal communication with Peter Flegg 2005).

The 17th International Congress of ISMS will be held in May 2008 in Cape Town, South Africa. Traditionally, the focus of the ISMS has been on the *Agaricus bisporus* mushroom industry. In recent years, the interests of the ISMS have become more diversified but *A. bisporus* is still its main concern. The ISMSC-18 will be held in Beijing, China, in 2012.

(2). The international movement for medicinal mushrooms, mainly concerned with mushroom products (mushroom derivatives), was instituted during the first International Conference on Mushroom Biology and Mushroom Products held in Hong Kong, 23-26 August, 1993. Chairman S. T. Chang with J. A. Buswell, V.E.C. Ooi, K.W.K. Liu and S.W. Chiu were on the organising committee.

The World Society for Mushroom Biology and Mushroom Products (WSMBMP) was launched in January 1994 in response to strong interest expressed at the conference in Hong Kong in the previous year. The object of the WSMBMP is to promote the enhancement and application of knowledge related to the basic and applied aspects of mushroom biology and mushroom products (mushroom derivatives possessing medicinal properties from edible, medicinal and wild mushrooms) through publications, meetings and other means deemed appropriate. The WSMBMP holds a mushroom meeting, International Conference for Mushroom Biology and Mushroom Products (the ICMBMP) every three years. The sixth one is to be held in September 2008 in Bonn, Germany.

The international movement for the medicinal segment of the mushroom industry has been given a further boost with the launch of the International Journal of Medicinal Mushrooms (IJMM) in 1999 by Solomon P. Wasser as Editor-in-Chief with Takashi Mizuno, Shu Ting Chang, and Alexander L. Weis as editors. This then led to the inaugural International Medicinal Mushroom Conference (IMMC) held in Kiev, Ukraine, 12-14 September, 2001. It has been agreed that there is an IMMC after an interval of two years. The 2nd IMMC was held in Pattaya, Thailand, 17-19 July 2003 and the 3rd was held in Port Townsend, Washington, USA, from 12 to 17 October 2005. IMMC 4 will be in Slovenia, 2007 and the IMMC 5 will be in Nantong, China, 2009.

(3). The international movement for wild mushrooms, mainly concerned with edible mycorrhizal mushrooms, was born as a Pre-Congress activity during the 2nd International Conference on Mycorrhizas in Uppsala, Sweden, in 1999. Two years later, the 2nd International Workshop on Edible Mycorrhizal Mushrooms (IW-EMM) was held in Christchurch, New Zealand, 3-6 July

2001. The 3rd IW-EMM was hosted by the University of Victoria, Canada, 16-22, August 2003 and the 4th was held in Murcia, Spain, 29 November to 2 December, 2005. The 5th IW-EMM was held in Yunnan, China, 2007. It should be noted that edible mycorrhizal mushrooms belong to a special group of wild mushrooms which include other symbiotic mushrooms, e.g., termite mushrooms, hallucinogenic, and poisonous mushrooms.

These three international bodies/forums have done much to promote each of their respective fields, not least of which is bringing together scientists in international forums for useful discussions, encouraging research and the dissemination of valuable information. These three segments of the mushroom-based industry are not for competition but for complementation.

2.5 Mushroom Nutritional and Medicinal Properties

Edible mushrooms provide high quality of protein that can be produced with greater biological efficiency than animal protein. They are rich in fibre, minerals and vitamins, and have low crude fat content, with a high proportion of polyunsaturated fatty acids (72 to 85 %) relative to total content of fatty acids. These properties are major contributing factors to the traditional recognition of mushrooms as “healthy” foods.

A large number of mushroom species are not only edible and nutritious but also possess tonic and medicinal qualities. However, some mushrooms are lethally poisonous, and one should eat mushrooms only if one knows their names and their properties with considerable precision.

In the past, the mushroom industry concentrated mainly on the production of fresh, canned and dried mushrooms for food. Thus, the industry had only one leg. In the present era, high-pressure work demands are causing greater stress to the human body, and resulting in the weakening of the human immune system. A variety of proprietary products based on mushroom nutriceuticals and mushroom pharmaceuticals have already been produced and marketed. This trend is expected to increase with wider consumer satisfaction and acceptability. This is the second leg of the industry. These two legs/segments of the mushroom-based industry will not compete but will complement each other.

2.5.1 Nutritional Value of Mushrooms

The greatest difficulty in feeding man is to supply a sufficient quantity of the body-building material -- protein. The other three nutritional categories are: the source of energy food—carbohydrates and fats; accessory food factors-- vitamins; and inorganic compounds which are indispensable to good health. Of course, water, too, is essential.

The moisture content of fresh mushrooms varies within the range of 70 - 95% depending upon the harvest time and environmental conditions, whereas it is about 10 - 13% in dried mushrooms. The protein content of the cultivated species ranges from 1.75 to 5.9 % of their fresh weight. It has been estimated that an average value of 3.5 to 4.0 % would be more representative. This means that the protein content of edible mushrooms in general, is about twice that of onion (1.4 %) and cabbage (1.4%), and four times and 12 times those of oranges (1.0 %) and apples (0.3 %), respectively. In comparison, the protein content of common meats is as follows: pork, 9-16

%; beef, 12-20 %; chicken, 18-20 %; fish, 18 -20 %; and milk, 2.9- 3.3 %. On a dry weight basis, mushrooms normally contain 19 -35 % protein, as compared to 7.3 % in rice, 12.7 % in wheat, 38.1 % in soybean, and 9.4 % in corn. Therefore, in terms of the amount of crude protein, mushrooms rank below animal meats, but well above most other foods, including milk, which is an animal product (Chang and Miles 1989). Furthermore, mushroom protein contains all the nine essential amino acids required by man.

Quantitative data relating to the nutritive value of mushrooms is sparse. In the absence of feeding trials, alternative methods have been used to determine or predict the nutritional value of foods based on their content of essential amino acids (Crimson & Sands 1978).The Essential Amino Acid Index (EA. Index) rates dietary protein in terms of an essential amino acid pattern based on known adult human dietary requirements. The Amino Acid Score (Chemical Score) is the amount of the most limiting amino acid in the food protein expressed as a percentage of the same amino acid present in the reference protein. In an attempt to resolve the difficulties inherent in comparisons between those mushrooms containing small amounts of high quality protein with those containing larger amounts of a protein of lesser nutritional quality, Crisan & Sands (1978) proposed the use of a Nutritional Index calculated as:

$$\text{Nutritional Index} = \frac{(\text{EAA index x percentage protein})}{100}$$

The EAA Indices, the Amino Acid Scores, and Nutritional Indices for various mushrooms and other foods, are reported by Crisan & Sands (1978). EAA Indices and Amino Acid Scores of the most nutritive mushrooms (highest values) rank in potential nutritive value with those of meat and milk, and are significantly higher than those for most legumes and vegetables. The least nutritive mushrooms rank appreciably lower but are still comparable to some of our common vegetables.

In addition to their good proteins, mushrooms are a relatively good source of the following individual nutrients: fat, phosphorus, iron, and vitamins including thiamine, riboflavin, ascorbic acid, ergosterine and niacin. They are low in calories, carbohydrates and calcium. It has also been reported that a total lipid content varying between 0.6 and 3.1 % of the dry weight, is found in the commonly cultivated mushrooms. At least 72 % of the total fatty acids are found to be unsaturated in all the four tested mushrooms (Huang, et al., 1985). It should be noted that unsaturated fatty acids are essential and significant in our diet and to our health.

In recent years, there has been a trend toward discovering ways of treating mushrooms so as to give them added value. For example, Wermer and Beelman (2002) have reported on growing mushrooms enriched in selenium. By adding sodium selenite to compost over a range of 30-300 parts per million, they found that the mushrooms increasingly absorbed selenium according to the amount in the compost, so that it is possible to grow mushrooms containing a desired concentration. Selenium is an essential micronutrient that has generated much recent interest in nutritional and medical research—and, more recently, within the food industry (Beelman and Royse, 2006). Selenium has numerous physiological functions, but is best known as necessary cofactor for the glutathione peroxidase enzyme system. This system is responsible for removing free radicals from the body, thus reducing oxidative damage.

The desirability of a food product does not necessarily bear any correlation to its nutritional value. However, its appearance, taste, and aroma, sometimes can stimulate one's appetite (preference). In addition to nutritional value, mushrooms have some unique colour, taste, aroma, and texture characteristics, which attract their consumption by humans.

2.5.2 Medicinal Properties of Mushrooms

The second major attribute of mushrooms, their medicinal properties, has long been recognised in China, Korea, and Japan. There has been a great upsurge in activities related to the use of mushroom products for medicinal purposes in recent years. In 2001, the figure of US$9-10 billion was cited as representing the value of medicinal mushroom products, including tonics and medicines. The application of modern analytical techniques can be used to establish a scientific basis for the empirical observations that have been made centuries before. According to Chang and Buswell (1996), the term "*mushroom nutriceutical*" is used for a new class of new compounds extractable from either the mycelium, or the fruiting body of the mushroom. Mushroom nutriceuticals may possess both nutritional and medicinal properties.

Due to present day high pressured work demands resulting in great stress to the human body and causing a weakening of the human immune system, there are now many new diseases. These have developed as a consequence of lower natural body resistance. There is some evidence that the beneficial treatment of these diseases can be obtained by consumption of mushrooms as a functional food, or through the use of extracted biologically active compounds as a dietary supplement, in order to enhance immune response of the human body, thereby increasing resistance to disease and, in some cases, causing regression of a diseased state. Differing from most pharmaceuticals, these biologically active compounds extracted from medicinal mushrooms have extraordinarily low toxicity, even at high doses. Long viewed as tonics, now it has been known that they can profoundly improve the quality of human health.

Mushrooms produce several biologically active compounds that are usually associated with the cell wall. Most notably, a group of polysaccharides comprising high molecular weight sugar polymers has been reported to contribute to their immune enhancing and tumour retarding effects. It has been reported that the anti-tumour and anti-cancer effects of the polysaccharides are based on the enhancement of the body's immune systems, including activated macrophages, natural killer cells, cytotoxic T cells, and their secretory products, such as the tumour necrosis factor, reactive nitrogen and oxygen intermediates, and interleukins, rather than direct cytocidal effects (Mizuno et al. 1995; Liu et al. 1996). It should be noted that immune responses are complex reactions involving several types of cells, such as macrophages and lymphocytes. The killing mediated by cytotoxic T lymphocytes and natural killer cells, represents an important mechanism in immune defence against tumours, virus-infected cells, parasites and other foreign invaders. Another group of medicinal compounds found in *Ganoderma* spp. are triterpenoids, steroid-like compounds, which contribute cytotoxic, hepatoprotective, and hypolipidmic influence on platelet aggregation, inhibition of angiotensine-converting enzyme, and inhibition of histamine release (Lindequist 1995). Lectins, another group of mushroom bioactive compounds, are proteins or glycoproteins with specific binding sites for sugars, which are not antibodies or enzymes. These have become useful tools in structural studies of the cell surface, oligosaccharides and/or carbohydrate moieties of glycoproteins. Some lectins have been shown to have anti-tumour and immunomodulatory activities (Wang et al. 1996). Other lectins preferentially agglutinate mammalian cells that have been transformed by oncogenic viruses or by chemical carcinogens, as well as spontaneously transformed cells. These and related findings indicate that studies with lectins may lead to a better understanding of cancer. Moreover, some lectins may be used to inhibit the growth of malignant cells. A novel single-chained ribosome-inactivating protein (RIP) was recently isolated from fruit bodies of the edible mushroom, *V. volvacea* (Yao et al. 1998). The mushroom RIP, designated volvarin, exhibited a potent inhibitory action on protein synthesis in the rabbit reticulocyte lysate system. Like most plant RIPs, volvarin acted as an N-glycosidase that depurinated rRNA from rabbit reticulocyte lysate, releasing a characteristic RNA fragment after treatment with aniline. It also exerted a strong abortifacient effect in mice.

Furthermore, the aqueous extracts of *Pleurotus sajor-caju* (Tam et al. 1986) and *Volvariella volvacea* (Chiu et al. 1996) have been reported to produce a hypotensive effects in normotensive rats. Feeding powdered maitake (*Grifola frondosa)* mushrooms to spontaneous hypertensive rats resulted in a lowering of the blood pressure (Kyoko et al. 1988). It has also been reported that dried powder of another two edible mushrooms, *Auricularia auricula* and *Tremella fuciformis*, after being fed to the rats, has demonstrated to be effective in lowering both the serum total cholesterol and the low density lipoprotein (LDL) cholesterol levels (Cheung 1996). Since the mushrooms did not affect the concentration of serum high density lipoprotein (HDL) cholesterol, the reduction of serum total cholesterol by the mushroom diets is believed to be attributable to the fall in LDL cholesterol. It should be noted that LDL is“bad” cholesterol and HDL is “good” cholesterol.

2.5.3 Mushroom Nutriceuticals

There has been a recent upsurge of interest in mushrooms not only as health vegetables (food) but also as a source of biological active compounds of medicinal value, including use as complementary medicine/dietary supplements for anticancer, antiviral, immunopotentiating, hypocholesterolaemic and hepatoprotective agents. This new class of compounds termed ‘mushroom nutriceuticals’ (Chang and Buswell 1996), are extractable from either the fungal mycelium or fruiting body and represent an important component of the expanding mushroom biotechnology industry.

Of the 14,000-15,000 species of so-called mushrooms in the world, around 400 have known medicinal properties. However, it has been estimated that there are about 1,800 species of mushrooms with the potential of medicinal properties. Both these mushrooms and their root-like structure (called mycelium) produce several medicinal or nutriceutical (general immune enhancing) compounds, central of which are the polysaccharides (high molecular weight strings of sugars), triterpenes, and immunomodulatory proteins. Although virtually all mushrooms and many foods have polysaccharides in their cell walls, certain mushroom species have been found to contain polysaccharides which are particularly effective in retarding the progress of various cancers and other diseases, and in alleviating the side effects of chemotherapy and radiation treatment (through cell-level regenerative effects). There are now many studies in Asia, particularly in China and Japan, documenting life span increases of cancer patients undergoing conventional cancer treatment plus mushroom extract consumption or injection (Mizuno et al., 1995; Liu 1999). At the same time, due to the enhancement of the immune systems, it can help people reduce the possibility of being infected by other diseases.

Between 80% - 85% of all medicinal mushroom products are derived from the fruiting bodies, which have been either commercially farmed or collected from the wild, e.g., Lentinan, a high-molecular weight (1-3)-β-D-glucan, from *Lentinula edodes* and various products from *Ganoderma lucidum*. Only about 15% of all products are based on extracts from mycelia. The notable examples include PSK-trade name (Krestin) of a polysaccharide peptide, and PSP (polysaccharide-bound peptide) extracted from *Coriolus versicolor*. A smaller percent of mushroom products are obtained from culture filtrates, e.g., schizophllan, a high-molecular weight (1-3), (1-6)-β-D glucan, prepared from *Schizophyllum commune* Fr. and PSPC (a protein-bound polysaccharide complex) from *Tricholoma lobayense* Hein. However, due to increased quality control and year round production, mycelial products are the wave of the future.

The market value of medicinal mushrooms and their derivative dietary supplements worldwide was about US$1.2 billion in 1991, and about US$3.6 billion in 1994 (Chang 1996). In 1999, it was estimated to be US$6.0 billion (Wasser et al., 2000). The market value of *Ganoderma*-based nutriceuticals alone in 1995 was estimated at US$1.6 billion (Chang and Buswell 1999). The corresponding monetary values were also generated by another famous mushroom, *Lentinula edodes*. Ninety nine percent of all sales of medicinal mushrooms and their derivatives occurred in Asia and Europe with less than 0.1 percent in North America. The 1999 US market for dietary supplements based mainly on mushrooms was estimated to be US$35 million. However, in recent years, the North American demand is increasing between 20%-40% annually, depending upon species.

3. MUSHROOM CULTIVATION TECHNOLOGY (CONTENTS FOR A MUSHROOM TRAINING COURSE)

3.1 Major Phases of Mushroom Cultivation

Mushroom farming is a complex business, which requires precision. Indeed, it is not as simple as what some people often loosely stipulate. It calls for adherence to precise procedures. The major practical steps/segments of mushroom cultivation are: (a) selection of an acceptable mushroom species; (b) secreting a good quality fruiting culture; (c) development of robust spawn; (d) preparation of selective substrate/compost; (e) care of mycelial (spawn) running; (f) management of fruiting/ mushroom development; and (g) harvesting mushrooms carefully (Chang and Chiu, 1992, Chang 1998). If you ignore one critical step/segment, you are inviting trouble, which could lead to a substantially reduced mushroom crop yield and mushroom marketing value.

3.1.1 Selection of Acceptable Mushroom Species/Strains

Before any decision to cultivate a particular mushroom is made, it is important to determine if that species possess organoleptic qualities acceptable to the indigenous population or to the international market, if suitable substrates for cultivation are plentiful, and if environmental requirements for growth and fruiting can be met without excessively costly systems of mechanical control.

3.1.2 Secreting a Good Quality Fruiting Culture

A "fruiting culture" is defined as a culture with the genetic capacity to form fruiting bodies under suitable growth conditions. The stock culture which is selected should be acceptable in terms of yield, flavour, texture, fruiting time, etc.

(1) Sources of the cultures

(A). Tissue culture. A large healthy mushroom should be chosen either in the later button or egg stage. It should be cleaned with 75% alcohol. The mushroom should be split in half by hand longitudinally and some inside tissue taken from the upper part of the stipe. It should be placed centrally on the surface of the medium with a sterilised needle. The quicker this is done the better. As soon as we transfer the tissue, the test tube should be closed and dated before it is returned to the incubator between 25°C and 34°C depending on the mushroom used. Within two or three days some white, delicate mycelia will be produced from the small piece of the tissue. They grow upwards encircling the inner wall of the test tube. About ten days later the mycelium will grow rapidly and cover the surface of the agar medium. Then it is ready to transfer to spawn substrate to make spawn.

(B). Spore culture. Individual spores properly collected can be transferred singly to a test-tube or petri dish and allowed to develop and germinate into mycelium. Some single-spore isolates from homothallic mushrooms, e.g. *Volvariella volvacea* (primary homothallism) or *Agaricus bisporus* (secondary homothallism) can be used as fruiting culture to make spawn. However if single-spore isolates are from heterothallic mushrooms, e.g. *Lentinula edodes, Pleurotus sajor-caju* and *Ganoderma lucidum*, then they cannot form fruiting cultures and thus cannot make spawn. They have to be mated with a compatible single-spore isolate. After mating they form a dikaryon/fruiting culture. Then they can be used to make spawn.

(C). Pure culture from other laboratories. As an alternative to culturing in the laboratory, as outlined, a test tube culture may be obtained from a research laboratory. The advantage of this is that cultures maintained in reputable culture collections are already tested for their production characteristics and are guaranteed to be pure.

(D). Cultures from another source. Cultures also may be grown from spawn obtained from another source. A piece of the spawn is aseptically transferred to agar slants. However, this is risky because the number of transfers that the spawn culture has undergone is rarely known. If this procedure is followed, it may be advisable to first grow the spawn into fruiting bodies, then make the necessary isolations from the fruiting body.

(2) Culture media

Mushrooms grow on a variety of culture media and on different agar formulas, both natural and synthetic, depending on the organism to be cultivated and the purpose of the cultivation. Synthetic media are often expensive and time-consuming in preparation hence they are not commonly used for routine purposes in mushroom laboratories.

(A). PDA (potato dextrose agar), is the simplest and the most popular medium for growing mycelia of most cultivated mushrooms. It can be purchased commercially as ready mixed powder which can be used directly to make the medium in the laboratory, with a concentration of 20gm/1 litre of distilled water.

(B). Alternatively, it can be prepared in the laboratory with the following ingredients: Potato, diced - 200 gm (1/5kg); Dextrose - 20 gm; Powdered agar or agar bars - 20gm; Water - 1 litre. Procedure: Peeled potatoes are washed, weighed, and cut into cubes. They are boiled in a casserole with at least one litre of water until they become soft (around 15 minutes). The potatoes are removed and water is added to the broth to make exactly 1 litre. The broth is returned to the casserole, and dextrose and the agar added. The solution is heated and stirred occasionally until the agar is melted. The hot solution is then poured into clear flat bottles filling to about 2.5cm from the bottom. When using test tubes for the stock cultures, they are filled with at least 10ml of the liquid agar solution. The bottles or test tubes are plugged with cottonwool. When Petri dishes are available and are used to produce mycelial colonies, the solution can be poured into the dishes to form a layer on the bottom.

(C). A ready-made MEA (malt extract agar) powder is also available commercially. The recommended amount of powder (20gm) is mixed with 1 litre of water, then melted and sterilised. One percent peptone or 0.5% yeast may be added for faster mycelial growth for both PDA and MEA.

3.1.3 Development of Robust Spawn

A medium through which the mycelium of a fruiting culture has grown and which serves as the inoculum of "seed" for the substrate in mushroom cultivation is called the "mushroom spawn". Failure to achieve a satisfactory harvest may often be traced to unsatisfactory spawn used. Consideration must also be given to the nature of the spawn substrate since this influences rapidity of growth in the spawn medium as well as the rate of mycelial growth and filling of the beds following inoculation.

(1) Mushroom Spawn Preparation

(A). Definitions of spawn and spawning**.** The word “spawn” is derived from an old French verb, *espandre*, meaning to spread out or expand, which was derived from the Latin, *expandere*, meaning to spread. Spawn is also defined by Webster’s Dictionary as “the mycelium of fungi, especially of mushrooms grown to be eaten, used for propagation”. In the mushroom industry, spawn is a substrate into which a mushroom mycelium has impregnated and developed, and which will be used as a seed in propagation for mushroom production. In addition the verb, to spawn, is used to mean inoculation of a substrate with mushroom spawn.

The simple definition of spawning is the planting of mushroom spawn in the prepared compost/substrate. Along with advances in spawn making, the methods of spawning have also been continuously developed and improved, making it possible for the mushroom mycelium to grow through the compost more quickly.

(B). Spawn Substrates. A number of materials, mostly agricultural wastes, can be used to prepare mushroom spawn. The type of waste available varies from region to region. Some of these wastes are chopped rice straw, sawdust, water hyacinth leaves, used tea leaves, cotton wastes and lotus seed husks. In most laboratories, cereal grains (wheat, rye or sorghum) are used as mother spawn, and agricultural wastes as the planting spawn substrates. The mother spawn is used to inoculate the final spawn container in which the planting spawn will be produced. The planting spawn is used to inoculate the mushroom cultivation compost/substrate for fruiting/mushroom production.

(C). Preparation of mother spawn. Here using wheat grains as an example, they are soaked in water for 2 hours or over night. Dead seeds or those that float on water should be carefully removed. Then the grains are washed again and boiled in water for at least 10 to 15 minutes until they expand but not quite broken. The grains are drained and allowed to cool. Precipitated chalk (1.5% on wet basis) is added to the grains. The grains are then loosely packed in bottles which are 2/3 full. These are plugged with cotton wool or covered by double-layered aluminium foil. The grains are sterilised in a pressure cooker for about 1 hour at 121°C, alternatively, they are steamed for 3 to 4 hours in a large cast-iron casserole. The bottles are then cooled prior to inoculation.

(D). Preparation of planting spawn. Here we shall use rice straw or water hyacinth leaves as an example for *Pleurotus sajor-caju* planting spawn. The rice straw (or water hyacinth leaves) is chopped into pieces about 2 to 3 cm (1 inch) long, then soaked in water for 4 - 12 hours. The excess water is drained off and the straw pieces mixed with a solution of 1% sucrose, 1.5% chalk and 2% wheat/rice bran in water. The final moisture content is to be adjusted to about 60%. The mixture is then put into glass bottles or plastic bags and sterilised for at least one hour at 121°C before being inoculated with the mother spawn.

(E). Remarks for spawn making. Autoclaved substrate can only be justified for spawn production if it is properly done. Otherwise there will be wastage of energy and money through contamination losses.

(F) What means properly?

Substrates such as saw dust, straw, cotton seed hulls and so on should not be wet (if water stands on the bottom, mycelia will not enter). Water that is held by capillary forces, and swollen water, will not as readily produce vapour pressure, as does standing water. If the container of spawn is tightly sealed, air cannot escape, and steam cannot enter properly. Autoclaving is thus imperfect. After proper sterilisation all moulds inside are killed.

Prevent entry of moulds from outside by: Using very clean cotton stoppers only (which can be used several times, if you observe cleanliness!); leaving at least 3-4 cm free space between lower surface of cotton stopper and the substrate; avoiding the soilage of the container walls around the stopper, and between the stopper and the substrate surface; preventing the cotton plug from getting wet during autoclaving (cover loosely with aluminium-foil, so that the outside of the container is protected all around, at least 3 cm deep); and keeping the outside of the containers clean, to where the aluminium-foil reaches ("collar" of plastic bags).

Inoculate under clean conditions in a room without air movement (close door and windows): clean the table with a disinfectant; wash your hands thoroughly with soap, dry with a clean towel, and disinfect them; transfer the container from the autoclave directly into the clean room. Cover them with a freshly washed and ironed towel if the journey from the autoclave to the inoculation place is long; keep the containers during inoculation in a position that minimises contamination risks; use pure culture spawn only for inoculation (this may be from another spawn batch); and cover the opening after inoculation with aluminium-foil and press around the neck of the container. Place the spawn in the first days under optimal growth conditions. Spawn which is not needed for inoculation can be used for fruit body production under suitable conditions. NOTE: Actually, one test tube of pure culture ("starter") can prepare 1,200 commercial planting spawns through the steps of stock culture, then the mother grain spawn. This process is called the Multiplication of cultures.

(2) Mushroom Spawn Handling

(A). Maintenance of spawn quality**.** Mushroom spawn, whether prepared as a family home project or on industrial scale using modern equipment, should be in excellent condition when delivered to growers. Spawn of most mushrooms can be refrigerated, but it should be warmed to normal room temperature before it is used as an inoculum or as planting spawn. Vigorous growth of the planting spawn is a prerequisite to good growth and yield. If the spawn is not vigorous, the mushroom mycelium will be overgrown by competitor organisms. If it is vigorous it will overcome many of the competitive organisms and produce more mushrooms. When purchasing spawn, ask the spawn maker how long the spawn can be kept before planting. Old spawn is not acceptable because its vigour may have decreased. Buyers or users should know the "expiration" date of the spawn.

(B). Spawn Quantities. The quantity of the spawn used does not directly affect yield. However, the use of more spawn may reduce the effect of competitive organisms present in the planting substrates. The greater the amount of spawn used, the faster it will colonise the substrate. As a result, the growth of competitors is hindered, and yield will be regular and not affected by this competition. 2-4% of spawn is suggested to be inoculated into the spawning substrate. Once the container is opened, spawn should be used in its entirety. Unused and opened bottles or bags of spawn, however, can be kept in the refrigerator for 2 to 6 days as long as they are not contaminated (i.e., no unwanted fungi are growing on the surface) during storage.

(C). Notes on commercial spawn supplies. When growing mushrooms on a small scale, it is not necessary to prepare your own spawn. Commercial suppliers of spawn who provide material to small growers are usually available. Spawn should be ordered ahead of time so that it will be of the right age. Contaminated, old or no-growth spawn should never be sold to growers. Spawn makers should maintain a testing facility where they can test each batch of spawn for production characteristics. Sales personnel should visit growers using their spawn so that they can observe problems at first hand. Problems related to spawn production must be corrected quickly.

3.1.4 Preparation of Selective Substrate/Compost

While a sterile substrate free from all competitive micro-organisms is the ideal medium for cultivating edible mushrooms, systems involving such strict hygiene are generally too costly and impractical to operate on a large scale. Substrates for cultivating edible mushrooms normally require varying degrees of pre-treatment in order to promote growth of the mushroom mycelium to the practical exclusion of other micro-organisms. The substrate must be rich in essential nutrients in forms which are readily available to the mushroom, and be free of toxic substances which inhibit growth of the spawn. Moisture content, pH and good gaseous exchange between the substrate and the surrounding environment are important physical factors to consider.

(1). Mushroom substrate. Mushroom substrate may be simply defined as a kind of lignocellulosic material which supports the growth, development, and fruiting of mushroom mycelium. The process of preparation of substrate is broadly termed "composting". The final product of "composting" is called the "compost" or prepared substrate. The process for preparation of substrates has been the subject of much scientific and practical interest over the past two decades. It should be noted that different types of mushrooms require different types or substrate/compost. *Agaricus bisporus* grows on fermented compost which is traditionally developed from wheat straw mixed with horse manure, and it requires higher nitrogen content. Wheat straw contains about 0.62% nitrogen, and horse manure contains about 1.5 to 1.8% nitrogen. The optimum C:N (carbon:nitrogen) ratio for the mushroom is about 17 to 1 for mycelial running. *Volvariella volvacea*, the paddy straw mushroom, and *Stropharia rugoso-annulata* are grown on almost raw or less composted plant residues, such as rice straw and cotton waste materials. These cellulosic plant materials contain different amounts of nitrogen: rice straw 0.58%, cotton waste 0.65 to 1%, and banana leaves 1.71%. It should be understood that *V. volvacea* is capable of growing on plant material with low nitrogen content. The optimum C:N ratio is about 75 to 80:1, but C:N ratios from 32 to 150:1 are almost as effective. *Lentinula edodes* and *Pleurotus* spp. are fungi that can grow on wood. In addition to being distinguished by its high lignin content, wood can also be distinguished from other plant materials by its very low nitrogen content. Woody tissues contain 0.03 to 1.0% nitrogen as compared to 0.85 to 1.71% in herbaceous residues. The C:N ratio in most woody tissues is in the order of 350 to 500:1. Wood-inhabiting mushrooms are unique in that they can grow in such substrates. This suggests that these mushrooms can metabolise large amounts of carbohydrates, including lignin, in the presence of a very small amount of nitrogen.

(2). Composting. Some early composting investigators had a good appreciation of the biological nature of the composting process. These early researchers had some appreciation of the role of various ecological factors such as temperature, oxygen, moisture, pH and nutrition in determining the general activity and population dynamics that occur during composting. As a working hypothesis, it may be assumed that composting conditions which produce a favourable medium for the development of mushrooms probably do so because they encourage the development of microbial population that is best able to pave the way for the subsequent growth and fructification of mushrooms. Such a hypothesis must take full cognisance of the effect of the staling products of different groups of organisms on the mushroom development as well as the actions of these organisms in producing changes in the manure favourable to the nutrition of mushrooms under competitive conditions.

The substrate left after the mushrooms have been harvested is known as spent compost. This is present in large amounts, and raises the question of what can be done with it. It is certainly not desirable to leave it as a possible source of pollution. It is known that there still remains in the spent compost a considerable amount of lignocellulosic material in addition to the mushroom mycelia and also other products formed by the metabolic activities of the mycelium. Thus, the spent compost should be capable of supporting further biological activities, e.g., the growth of another species of edible mushroom; use as fodder for livestock; as a soil conditioner and fertiliser; and also in bioremediation.

3.1.5 Care of Mycelial (Spawn) Running

Following composting, the substrate is placed in beds where it is generally pasteurized by steam to kill off potential competitive microorganisms. After the compost has cooled, the spawn may be broadcast over the bed surface and then pressed down firmly against the substrate to ensure good contact, or inserted 2 to 2.5 cm deep into the substrate. Spawn running is the phase during which mycelium grows from the spawn and permeates into the substrate. Good mycelial growth is essential for mushroom production.

3.1.6 Management of Fruiting/Mushroom Development

Under suitable environmental conditions, which may differ from those adopted for spawn running, primordial formation occurs and then followed by the production of fruiting bodies. The appearance of mushrooms normally occurs in rhythmic cycles called "flushes".

3.1.7 Harvesting Mushrooms Carefully

Harvesting is carried out at different maturation stages depending upon the species and upon consumer preferences and market value.

3.2 Mushroom Diseases

The mushroom, like any other cultivated crop, is subject to attack by pathogens and pests. The mushroom diseases can be caused by both fungi and bacteria. There are four important fungal diseases of the cultivated mushrooms, particularly referring to *Agaricus bisporus* and these are:

(1) <u>Dry Bubble caused by *Verticillium fungicola*</u>. At vary early stages on infection, brown spots can be seen on mushroom caps. This stage of the disease can be confused with the brown blotch disease caused by a bacterium. However, in the case of dry bubble, if the infected mushrooms continue to be incubated in a humid chamber, greyish white mycelium of the pathogen will develop from the brown spots on the mushroom caps. CONTROL: The environment conditions most conducive to the growth of *Verticillium* - high relative humidity and high relative temperature. Therefore, an effective ecological control would be to reduce the relative humidity

and temperature in the growing rooms. A reduction in relative humidity from 90-95% to 80-85% and in temperature to 14^{o} C can result in a reduction in the incidence of the disease. Control of the disease depends also on farm hygiene consisting of prompt disposal of spent compost and debris and disinfection of affected bed areas. If soil is used as a casing material, it should be pasteurized at 60^{o}C for half an hour with aerated steam.

(2) Wet Bubble caused by *Mycogone permiciosa.* Infection of mushroom fruit bodies results in malformed stalk and cap. The surface of diseased mushrooms is covered by a white felt of mycelium of the pathogen. The undifferentiated tissues of the cap and stalk become necrotic with a wet soft rot dripping a brown liquid. The name "wet bubble" is derived from the foul-smelling, mis-shapen wet mushroom tissues. CONTROL: Disinfection of casing material or steam-air pasteurization of soil, if soil is used as a casing material, can control the disease. It is necessary to protect the developing mushroom fruiting bodies throughout the period of cropping. It is essential that management gives full attention to hygiene such as disinfecting the work area, removing all spent materials, disinfecting all harvesting tools and carrying out after-crop sterilization using steam at 70^{o}C for 12 hours.

(3) Mildew caused by *Cladobotrym sp.* This disease is characterized by a cotton wool type of growth of the pathogen on the mushroom fruiting bodies and spreads over the casing layer to neighbouring mushrooms. The greyish-white mycelium of the pathogen can envelop the mushroom fruiting body completely. Infected mushrooms die with a soft wet rot. CONTROL: Disinfection of the casing material and strict hygiene can control the disease.

(4) Aphanocladium Disease caused by *Aphanocladium album.* Infected mushrooms develop brown spots which, under conditions of high relative humidity, show greyish-white mycelium of the pathogen. The gill tissues of the mushroom fruiting body are commonly infected by the pathogen. CONTROL: Disinfection of the casing material appears to control the disease. Management of the crop environment similar to that of the Dry and Wet Bubble diseases can be effective in controlling the disease.

In addition, there are three important bacterial diseases of the cultivated mushrooms, particularly referring to *Agaricus bisporus*:

(1) Brown Blotch caused by *Pseudomonas tolaasii.* The disease causes brown, slightly sunken blotches on the developing mushroom fruiting body. It affects only the surface layers of the mushroom and, in severe cases, brown streaks develop on the stalk. The blotches on the cap can, sometimes, be yellowish to pale brown in colour. CONTROL: The disease can be controlled by manipulating the crop environment. Since the pathogen can spread by splashing water, the surfaces of mushrooms should be maintained relatively dry. If the surfaces of mushrooms dry within an hour or two after becoming wet, no infection occurs. The drying can take place in an atmosphere of high relative humidity as long as there is no fluctuation in temperature and there is a circulation of air over the mushroom beds. The temperature of the air coming into the growing room should, therefore, be closely controlled.

(2) Mummy Disease caused by *Pseudomonas sp.* In diseased mushrooms the stalk is bent and the cap tilted. Often there is a dense growth of mycelium around the base of the stalk on the surface

of the casing layer. Infected mushrooms remain intact for a relatively long period after which they are invaded by secondary bacteria. Mushrooms often fail to mature and remain in the "button" stage with unopened veil. CONTROL: Strict hygiene is the best means of controlling the disease. Disinfecting the casing layer can help to reduce the infection.

(3) Drippy Gill caused by *Pseudomonas agarici.* The pathogen damages the gill tissues of mature mushroom fruiting bodies. Damage is restricted to the gills. Dark brown, round spots appear on the side and bottom edges of the gills. At the centre of each spot, a creamy grey droplet may show on the gill surface. If the infection is severe, bacterial droplets join to form long streaks of slime which may lead to distortion and collapse of the gills. CONTROL: Disinfection of casing soil can control the disease.

3.3 Post-Harvest Handling

Like all fruits and vegetables, mushrooms are perishable, and after harvest they often change in ways that make them unacceptable for human consumption. The most readily observable of these changes include wilting, ripening, browning, liquefaction, loss of moisture, and loss of texture, aroma and flavour. To ensure that mushrooms are acceptable and nutritious to the consumer at the time of purchase, it is necessary to delay or prevent senescence. Expansion of the pileus by growth of gills and elongation of the stipe post harvest is supported by increased cell wall chitin and protein. Chitin synthase can be activated by proteinases. It has shown that during ageing, there is a major redistribution of dry weight between tissues with gill dry weight increasing during storage while that of the pileus and stipe diminishing. The rate of cap opening depends on the stipe length (the longer the stipe, the greater the expansion), indicating that the stipe is acting as a major nutrient source for the expanding gill tissue.

Recently, the biochemistry and genetic regulation in mushrooms post harvest has been studied by identifying genes with higher transcript levels after harvest. Technologies such as cooling and modified atmosphere packaging can be use to delay the rate of senescence, while preservative technologies such as canning, drying, pickling, and freezing and γ-irradiation arrest biological function to prevent senescence. Depending on the species, the shelf life of mushrooms may vary from one day to two weeks. Fresh mushrooms are best-stored unwashed in brown paper bags in the refrigerator, preferably on the lowest shelf. Bags should be available from your mushroom retailer; otherwise, a paper lunch bag is fine. Generally, it is important that fresh mushrooms are packaged in materials that allow them to breathe, so they do not 'sweat' and become slimy. At the same time, the material should ensure mushrooms do not dry out too much.

3.4 Cultivation of Several Selected Mushrooms

The cultivation of edible mushrooms can be divided into two major stages. The first stage (vegetative) involves the preparation of the fruiting culture, stock culture, mother spawn and planting spawn, while the second stage (reproductive) entails the preparation of the growth substrates for mushroom cultivation. Cultivation conditions for a few selected mushroom species are briefly described in the following sections. Examples of formulas in the following sections are for reference only. They should be modified according to the local available materials and climatic conditions.

3.4.1 *Agaricus bisporus* (Champignon, Button mushroom) as shown in Figure 7

Figure7. *Agaricus* mushrooms grown on horse manure compost.

Agaricus bisporus is variously known as **the white mushroom, button mushroom, champignon,** or simply the **common cultivated mushroom**. In Western countries this mushroom has developed over the past 60 years from beginning as a risky venture to a largely predictable and controllable industrial process, particularly in Great Britain and the Netherlands. In no small measure this remarkable achievement in modern mushroom industrial development may be attributed to contributions resulting from the vigorous research activities conducted at mushroom research laboratories, centres and stations.

The basic principles and practical methods of cultivation of this mushroom have been very well established through repetitive practical experiences during the last 60 years. It is not intended to summarize those in this manual, instead readers are referred to San Antonio (1975), Chang and Hayes (1978), Van Griensven (1988), Quimio et al. 91990) and Kaul and Dhar (2007). However, the compost and composting are reviewed here because they play an important role in the cultivation of this very popular edible mushroom (this discussion is an extension of that in Section 3.1.4 and more specific for this mushroom – further reading: Hayes 1975; Nair 1991; Nair 1994).

Generally, composting refers to the piling up of substrates for a certain period of time and the changes due to the activities of various micro-organisms, which result in the composted substrate being chemically and physically different from the starting material. This is sometimes referred to as a solid state fermentation. Two types of composting are commonly described. One type involves the decomposition of heaps of organic wastes and the subsequent application of the residue to the soil. The aim of this type of composting is to reduce, in a sanitary manner, both the volume and the C:N ratio of the organic waste so that is it suitable for manuring the soil to improve the growth of plant crops. When given directly to the soil without composting, organic waste with a high C:N ratio (such as straw) can give rise to a temporary nitrogen deficiency which will then result in a reduction in yield of the plant crop.

The second type of composting is also a process of microbial fermentation, but in this case the substrate is used for the cultivation of edible mushrooms. Through composting, a mixture of rich organic materials is converted into a stable medium which is selective for the growth of a particular mushroom but is not suitable, or is less favourable, for the growth of competing micro-organisms. The competitors exist in uncomposted materials, and often in partially composted materials, but they are far less active in well-composed mushroom substrates. Actually, this type of composting is derived mainly from the *Agaricus* mushroom-growing industry, in which a composting technique which renders wheat straw with horse manure specific for the growth of the *Agaricus* mushroom has been developed. It should be noted that the treatment of substrates for growing other mushrooms can be regarded as "composting", but the procedures followed in composting and the nature of the product can be quite different. This is because the starting materials and the lengths of time accompanying the various changes in the substrates vary from mushroom to mushroom.

As stated above, the role of composting is the production of a selective substrate that will preferentially support the growth of the mycelium of the mushroom. The basis of this selectivity, however, cannot be attributed to one factor or one aspect of the entire system. The physical, chemical and biological aspects of composting are fundamentally interrelated, but can be artificially separated for the convenience of investigation and discussion.

Mushroom growers like to practise the liberal use of the sense of sight, smell and touch to evaluate the progression of the composting process and the quality of the final product. The gross characteristics of compost, usually referred to as "structure", result from a number of complex physical, chemical and microbial processes.

The overall goal of composting is to produce selective nutrient media for the growth of the mushroom. These selected nutrient-rich substrates should support a high yield of good quality mushrooms. The general aspects of the achievement of composting are summarised as involving:

1) straw softening and other structural changes;
2) modification of plant materials so that nutrients are made available to mushroom growth and development;
3) building up of an appropriate biomass and a variety of microbial products(some of these can serve as nutrient sources for the mushroom);
4) establishment of selectivity, i.e. the compost promotes the growth of the mushroom over competitor organisms;
5) modification of compost structure so that it holds more water; and
6) building up of compost moisture content to serve as a water reservoir for the mushroom crop.

Composting is prepared in accordance with well-documented commercial procedures (van Griensvan, 1988, Chang and Hayes, 1978, and Kaul and Dhar, 2007). In Phase I of the process (outdoor composting), locally available raw materials are arranged into piles which are periodically turned and watered. The initial breakdown of the raw ingredients by micro-organisms takes place in Phase I. This phase is usually complete within 9-12 days, when the materials have become pliable, dark brown in colour and capable of holding water. There is normally a strong smell of ammonia. Phase II (indoor fermentation) is pasteurisation, when undesirable organisms are removed from the compost. This is carried out in a steaming room where the air temperature is held at 60°C for at least 4 hours. The temperature is then lowered to 50° C for 8 to 72 hours depending upon the nature of the compost. CO_2 is maintained at 1.5 to 2% and the ammonia level drops below 10 PPM. Following Phase II composting, the substrate is cooled to 30° C for *A. bitorquis* and to 25° C for *A. bisporus* for spawning. Production of Phase III or Phase IV composts for growing *Agaricus* mushrooms has been an advanced technological development in recent years in Western countries. The production of Phase III compost is Phase II compost spawn run in a bulk tunnel, and ready for casing when delivered to the grower. If the Phase III compost is then cased and spawn developed into casing layer before dispatching to the growing unit or delivering to growers, it is named as Phase IV compost. The successes of bulk Phase III and Phase IV depend a lot on the quality of Phase I and Phase II processes. Phase II on shelves produce an average of 4.1 crops per year. Since 1999, growers using Phase III production enjoyed an average of 7.1 crops per year. In recent years, Phase IV can generate 10-12 crops per year (Dewhurst 2002, Lemmers 2003).

3.4.2 *Lentinula edodes* (Xiang-gu, Shiitake, Oak mushroom) as shown in Figure 8

Figure 8. *Lentinula edodes* grown on sawdust synthetic logs.

Lentinula edodes (Berk) Sing., (common name: black forest/oak mushroom; Chinese name: Xiang-gu; Japanese name: shiitake) is the most important edible mushroom in the world from the standpoint production; and it is the most popular fungus cultivated in China, and in other Asian countries. For a long time, this mushroom has been valued for its unique taste and flavour and as a medicinal tonic. It can be cultivated either on wood log or on synthetic substrate logs (Quimio, et al., 1990, Stamets, 2000, Chang and Miles, 2004).

(1). Biological nature: *Lentinula edodes* is a heterothallic mushroom. Its sexuality is controlled by two mating factors, A and B, with multiple alleles, and therefore, its life history is a tetrapolar or bifactorial mating system (Chang and Miles, 1984). Its life cycle starts with the germination of basidiospores. After selected mating between two compatibility germinative mycelium, the dikaryon mycelium or fruiting culture is established. From the fruiting culture, the stock culture, mother spawn and commercially planting spawn can be made. When the spawn is planted on a suitable substrate, under good climatic conditions the fruiting bodies of the mushroom are developed. Then when the mature stage is reached, the spores are discharged and its life cycle is completed.

Lentinula edodes is a kind of wood rot fungus. In nature, it grows on dead tree trunks or stumps. In general, the wood for the mushroom growth consists of crude protein 0.38%, fat 4.5%, soluble sugar 0.56%, total nitrogen 0.148%, cellulose 52.7%, lignin 18.09% and ash 0.56%. Generally speaking, the C:N ratio in the substrate should be in the range from 25 to 40: 1 in the vegetative growth stage and from 40 to 73: 1 in the reproductive stage. If nitrogen source is too rich in the reproductive phase, fruiting bodies of the mushroom are usually not formed and developed.

The optimum temperature of spore germination is 22-26^{o}C. The temperature for mycelial growth ranges from 5-35^{o} C, but the optimum temperature is 23-25^{o} C. Generally speaking, *Lentinula edodes* belongs to low temperature mushrooms, the initial and development temperature of fruiting body formation is in the range of 10-20^{o} C and the optimum temperature of fructification for most varieties of the mushroom is about 15^{o}C. Some variety can fruit in higher temperatures, e.g. 20-23^{o} C. These high temperature mushrooms usually grow faster and have a bigger and thinner cap (pileus), thin and long stalk (stipe). Their fruiting bodies are easily opened and become flat grade mushrooms, which are considered to be low quality. The optimum pH of the substrate used in making the mushroom bag/log is about 5.0-5.5.

(2) Culture media and preparation: The mushroom can grow on a variety of culture media and on different agar formulations, both natural and synthetic, depending on the purpose of the cultivation. Synthetic media are often expensive and time-consuming in preparation; hence they are not commonly used for routine purposes.

The potato dextrose agar, or PDA, is the simplest and the most popular medium for growing the mycelium of the mushroom. It can be prepared following the instructions in Section 3.1.2.

(3). Examples of the different formulas for spawn substrates are described below.
Mother grain spawn: (i) Wheat/rye grain + 1.5% gypsum or slaked lime. (ii) Cotton seed hull 40%, sawdust 38%, wheat bran 20%, sugar 1% and gypsum 1%. (iii) Sugar cane bagasse 40%, sawdust 38%, wheat bran 20%, sugar 1% and gypsum 1%. Planting spawn: A number of materials, mostly agricultural and forest wastes can be used to prepare mushroom planting spawn. Three of them are given here as examples: (i) Sawdust 78%, rice/wheat bran 16%, sugar 1.5%, corn flour 1.7%, ammonium sulphate 0.3%, Calcium superphosphate 0.5% and gypsum 2%; (ii) Sawdust 64%, wheat bran 15%, spent coffee grounds 20% and gypsum/lime 1%; and (iii) Sawdust 78%, sucrose 1%, wheat bran 20% and Calcium carbonate 1%.

The *Lentinula edodes* mushroom is produced both on a cottage and a commercial scale. The following section outlines some of the issues associated with the methodologies for the different cultivation styles.

(1). Cottage scale cultivation: There are many formulas for the composition of the substrate. The ingredients can be variable from place to place and country to country depending upon the raw materials available and local climatic conditions. In general, after mixing the dry ingredients by hand or with a mechanical mixer, water is added to the mixture so that the final moisture content of the substrate is between 55 and 60%, depending on the capacity of the sawdust to absorb water. The ingredients are then packed into autoclavable polypropylene or high-density polyethylene bags. Although they are more expensive, polypropylene bags are the most popular since polypropylene provides greater clarity than polyethylene. After the bags have been filled with the substrate (1.5 to 4 kg wet weight, w/w), the end of the bag can be closed either by strings or plugged with cottonwool stopper.

Four formulas in the preparation of the substrate for the cultivation of the mushroom are given here as reference. (i) Sawdust 82%, wheat bran 16%, gypsum 1.4%, Potassium phosphate, dibasic 0.2%, and lime 0.4%. (ii) Sawdust 54%, spent coffee grounds 30%, wheat bran 15%, and gypsum 1%. (iii) Sawdust 63%, corncob powder 20%, wheat bran 15%, Calcium superphosphate 1% and gypsum 1%. (iv) Sawdust 76%, wheat bran 18%, corn powder 2%, gypsum 2%, sugar 1.2% Calcium superphosphate 0.5% and urea 0.3%.

(2). Commercial scale cultivation: In general, the operation can use oak or other hard wood sawdust medium to grow the mushroom. The basic steps are (i) to mix the sawdust, supplements, and water; (ii) bag the mixture; (iii) autoclave the bags to 121^{o}C and cool the bags; (iv) inoculate and seal the bags; (v) incubate for 90 days to achieve full colonisation of the sawdust mixture, in other words, to allow the mycelium to be established for ready fructification; (vi) fruit the colonised and established sawdust logs/bags/blocks 6 times using a 21 days cycle at 16 to 18^{o} C; and (vii) harvest, clip steps, grade, box, and cold store for fresh market, or harvest, dry, cut steps, grade and dry again before box for dry market.

Major equipment used in production consists of mixer/conveyor, autoclave, gas boiler, cooling tunnel, laminar-flow cabinet, bag sealer, air compressor for humidification, shelves to incubate. Incubation can be done in two rooms and in two shipping containers. The two shipping containers can be installed near the fruiting rooms. Temperature during incubation is held between 18 to 25^{o} C.

Fruiting can be done in 6 rooms so that the blocks/logs can be moved as a unit. With compartmentalization, blocks in each room can be subjected to a cycle of humid cold, humid heat, and dry heat.

3.4.3 *Pleurotus sajor-caju* (Grey oyster mushroom, Phoenix-tail mushroom, Indian oyster) as shown in Figure 9

Figure 9. *Pleurotus sajor-caju* grown on cereal straw substrates.

Pleurotus sajor-caju (grey oyster mushroom) is comparable to the high temperature species in the group of *Pleurotus* (oyster) mushrooms, with high temperatures required for fructification. This mushroom has a promising prospect in the tropical/subtropical areas. Its cultivation is easy with relatively less complicated procedures (Chang and Miles, 2004, Kaul and Dhar, 2007).

(1). <u>Biological nature</u>: The temperature for growth of mycelium is 10-35^{o}C. The optimum growing temperature of the mycelium is 23-28^{o}C. The optimum developmental temperature of the fruiting body is 18-24^{o}C. The optimum pH of the substrate used in making the mushroom bag/bed is 6.8-8.0. The C:N ratio in the substrate is in the range of 30-60: 1. A large circulation of air and reasonable light are required for the development of the fruiting bodies.

(2). <u>Examples of spawn substrates</u>: (i) Wheat grain + 1.5% gypsum or lime. (ii) Cotton seed hull 88%, wheat bran 10%, sugar 1% and gypsum 1%. (iii) Sawdust 78%, wheat bran 20%, sugar 1% and gypsum 1%. (vi) Sawdust 58%, spent coffee grounds/spent tea leaves 20%, water hyacinth/cereal straw 20%, sugar 1% and gypsum 1%.

(3). <u>Examples of cultivation substrates</u>: (i) Cotton seed hull 95%, gypsum 2%, lime 1% and Calcium superphosphate 2%. (ii) Rice straw 80%, cotton waste 18%, gypsum 1% and lime 1%. (iii) Water hyacinth 80%, cereal straw 17%, gypsum 2% and lime 1 %.

For demonstration purposes, this mushroom can be nurtured to grow into a tree-like shape (Chang and Li, 1982). The cultivation method, which has been tested to be successful, is as follows: Cotton waste or rice straw mixed with water hyacinth is used as the substrate. Tear large pieces of cotton waste into small parts or cut the straw and water hyacinth into small segments. Add 2 per cent (w/w) lime and mix with sufficient water to get moisture content of about 60-65 per cent. Pile the materials up, cover with plastic sheets and leave to stand overnight. Load the substrate into small baskets or on shelves for pasteurisation or cook the substrate with boiled water for 15 minutes. After cooling to approximately 25^{o}C, mix around 2 per cent (w/w) spawn thoroughly with the substrate and pack into columns of 60 cm long tubes which have hard plastic (PVC) tubing of 100 cm (4 cm in diameter) as central support, and with plastic sheets as outside wrapping.

Incubate these columns at around 24-28^{o}C, preferably in the dark. When the mycelium of the mushroom has ramified the entire column of substrate after three to four weeks, remove the plastic wrapping and switch on white light. Watering occasionally is needed to keep the surface from drying. In around three to four days white primordia start to appear over the whole surface. After another two to three days, the *Pleurotus* mushrooms are ready for harvesting. During the cropping period watering is very important if many flushes are required.

3.4.4 *Volvariella volvacea* (Patty straw mushroom, Chinese mushroom) as shown in Figure 10

Figure10. Different stages of fruiting bodies of the Straw mushroom (*Volvariella volvacea)* grown on cotton waste as substrate.

The edible straw mushroom, *Volvariella volvacea* is a fungus of the tropics and subtropics and has been traditionally cultivated in rice straw for many yeas in China and in South East Asian countries. In 1971, cotton wastes were first introduced as heating material for growing the straw mushroom (Yau and Chang 1972), and in 1973, cotton wastes had completely replaced the traditional paddy straw to grow the mushroom (Chang 1974). This was a turning point in the history of straw mushroom cultivation, because the cotton-waste compost through pasteurisation process brought the cultivation of the mushroom into an industrial scale first in Hong Kong and then in Taiwan, Thailand, and China. Several techniques are adopted for the cultivation of the mushroom, which thrives in the temperature range of 28-36° C and a relative humidity of 75-85%. Detailed descriptions of the various methods are given by Chang and Quimio (1982), Chang and Miles (2004), Kaul and Dhar (2007) and Quimio, et al. (1990). Choice of technology usually depends on personal preference, and on the availability of substrates and the amount of resources available. While the more sophisticated indoor technology is recommended for an industrial-scale production of the mushroom, most of the other technologies are low-cost and appropriate for rural area development, especially when production is established at the community level.

3.4.5 *Agaricus brasiliensis* (Royal Sun Agaricus, Himematuatake) as shown in Figure 11.

Figure 11. Different stages of Agaricus brasiliensis mushroom grown in straw compost with case soil.

In recent years, *Agaricus brasiliensis*, formerly called *Agaricus blazei* Murill (Wasser et al., 2002) has rapidly become a popular mushroom. It has been proved to be not only a good tasting and highly nutritious mushroom, but also an effective medicinal mushroom, particularly for anti-tumour active polysaccharides.

Agaricus brasiliensis was a wild mushroom in south eastern Brazil, where it was consumed by the people as a part of their diet. The culture of the mushroom was brought to Japan in 1965 and an attempt to cultivate this mushroom commercially was made in 1978. In 1992, this mushroom was introduced to China for commercial cultivation (Chang and Miles, 2004).

(1) Biological nature: *A. brasiliensis* belongs to middle temperature mushrooms. The growth temperature for mycelium ranges from 15 to 35°C and the optimum growth temperature ranges from 23°C to 27°C. The temperature for fruiting can be from 16°C to 30°C and the optimum developmental temperature of fruiting bodies is 18 to 25°C. The ideal humidity for casing soil is 60-65%. The preferred air humidity in a mushroom house is 60-75% for mycelium growth and 70-85% for fruiting body formation and development. The optimum pH of the compost used in making the mushroom bed is 6.5-6.8. The optimum pH of the casing soil is 7.0. A good circulation of air is required for the development of the fruiting bodies. These conditions are similar to those needed for the cultivation of *Agaricus bisporus*. Under natural conditions, the mushroom can be cultivated for two crops each year. Each crop can harvest three flushes. According to the local climate, the farmer can decide the spawning time in the year in order to have mushrooms for harvest within 50 days after spawning.

(2). Preparation of mushroom bed (Stamets, 2000): *A. brasiliensis* is a kind of mushroom belonging to straw-dung fungi and prefers to grow on substrate rich in cellulose. The waste/by-productive agro-industrial materials, e.g. rice straw, wheat straw, bagasse (squeezed residue of sugar cane), cotton seed hull, corn stalks, sorghum stalks and even wild grasses, can be used as the principal component of the compost for cultivation of the mushroom. It should be noted that these materials have to be air dried first and then mixed with cattle dung, poultry manure and some chemical fertilisers. The following formulas for making compost are for reference only.

They should be modified according to the local available materials and climatic conditions. (i) Rice straw 70%, air-dry cattle dung 15%, cottonseed hull 12.5%, gypsum 1%, calcium superphosphate 1% and urea 0.5%. (ii) Corn stalks 36%, cottonseed hull 36%, wheat straw 11.5% dry chicken manure 15%, calcium carbonate 1% and ammonium sulphate or urea 0.5%. (iii) Rice straw 90.6%, rice bran 2.4%, fowl droppings 3.6%, slaked lime 1.9%, superphosphate 1.2% and ammonium sulphate/urea 0.3%. (iv) Bagasse 75%, cottonseed hull 13%, fowl droppings 10%, superphosphate 0.5% and slaked lime 1.5%.

3.4.6 *Ganoderma lucidum* (ling Zhi, Reishi) as shown in Figure 12.

Figure 12. The fruiting bodies of *Ganoderma lucidum* as shown growing on short-wood segments which then were buried in the soil base for fruiting.

Although the medicinal value of *G. lucidum* has been treasured in China for more than two thousand years, the mushroom was found infrequently in nature. This lack of availability was largely responsible for the mushroom being so highly cherished and expensive. During ancient times in China, any person who picked the mushroom from the natural environment and presented it to a high-ranking official was usually well-rewarded (Chang and Miles, 2004).

Artificial cultivation of this valuable mushroom was successfully achieved in the early 1970s and, since 1980 and particularly in China, production of *G. lucidum* has developed rapidly. Currently, the methods most widely adopted for commercial production are the wood log, short wood segment, tree stump, sawdust bag and bottle procedures (Hsu 1994, Mizuno et al. 1996, Hung 1996, Mayzumi et al. 1997, Chang and Buswell, 1999, Stamets, 2000).

Log cultivation methods include the use of natural logs and tree stumps which are inoculated with spawn directly under natural conditions. The third alternative technique involves the use of sterilized short logs about 12cm in diameter and approximately 15 cm long which allow for good mycelial running. This method provides for a short growing cycle, higher biological efficiency, good quality of fruiting bodies, and, consequently, superior economical benefit. However, this production procedure is more complex and the production costs much higher, than natural log and tree stump methods. For this production procedure, the wood logs should be prepared from broad-leaf trees, preferably from oak. Felling of the trees is usually carried out during the dormant period, which is after defoliation in autumn and prior to the emergence of new leaves the following spring. The optimum moisture content of the log is about 45-55% .The flow-chart for the short-log cultivation method is as follows : selection and felling of the tree---sawing/cutting the log into short segments---transfer segments to plastic bags---sterilization---inoculation---spawn running--- burial of the log in soil---tending the fruiting bodies during development from the pinhead stage to maturity---harvesting of the fruiting bodies---drying of the fruiting bodies by electrical driers--packaging. It should be noted that the prepared logs/segments are usually buried in soil inside a greenhouse or plastic shed. The soil should allow optimum conditions of drainage, air permeability and water retention, but excessive humidity should be avoided.

Examples of cultivation substrates, using plastic bags or bottles as containers, include the followings (please note that these examples are for reference purposes only and can be modified according to the strains selected and the materials available in different localities): (i) sawdust 78%, wheat bran 20%, gypsum 1% and soybean powder 1%; (ii) bagasse 75%, wheat bran 22%, cane sugar 1%, gypsum 1% and soybean powder 1%; (iii) cotton seed hull 88%, wheat bran 10%, cane sugar 1% and gypsum 1%; (iv) sawdust 70%, corn cob powder 14%, wheat bran 14%, gypsum 1% and cereal straw ash 1%; (v) corn cob powder 78%, wheat/rice bran 20%, gypsum 1% and straw ash 1%. After sterilisation, the plastic bags can be laid horizontally on beds or the ground for fruiting.

4. ENVIRONMENTAL IMPACT

4.1 Reducing Environmental Pollution by Bioconversion of Vast Quantities of Organic Wastes into Mushrooms

Organic solid wastes are a kind of biomass, which are generated annually through the activities of the agricultural, forest and food processing industries. They consist mainly of three components: cellulose, hemicellulose and lignin. The general term for these organic wastes is lignocellulose.

It is common knowledge that lignocellulosic wastes are available in abundance both in the rural and urban areas. They have insignificant or less commercial value and certainly no food value, at least in their original form. **When carelessly disposed of in the surrounding environment by dumping or burning, these wastes are bound to lead to environmental pollution and consequently health hazards.** It should be recognised that the wastes are resources out of place and their proper management and utilization would lead to further economic growth as well.

Huge quantities of lignocellulosic and other organic waste residues are generated annually through the activities of agricultural, forest and food processing industries. In 1999, more than 3,000 million tons of cereal straws were available in the world, and about half of these residues remain unused. In addition, the world produced 952 million tons of bagasse; 6,476 thousand tons of coffee pulps; 6,152 thousand tons of coffee wastes; 9,386 thousand of cottonseed hulls; 14,073 thousand tons of sunflower seed hulls; and 325 thousand tons of sisal wastes. Million tons of sawdust, wood chips, and water hyacinth are also available worldwide. All these lignocellulosic waste residues can be used as substrate growing mushrooms; otherwise, they would cause health hazards. Mushroom enzymes can break down lignin, cellulose and hemicellulose present in these organic materials into simpler molecules, which the mushrooms then use for their growth and metabolism.

Lignocellulosic compounds are complex and insoluble. They can be treated by various chemical methods, e.g. with dilute hydrochloric acid and calcium chloride to increase the digestibility and nutritional qualities, and even to form sugars to serve as carbon sources. However, these chemical methods are tedious and costly. Furthermore, treatments to eliminate adverse side effects of the chemicals are also very complex. In contrast, mushroom cultivation techniques have become significantly important in recent years in improving nutritional quality and upgrading the economic value of the solid organic wastes. Mushrooms with other fungi are presently only organisms that can synthesize and excrete the relevant hydrolytic and oxidative enzymes that enable them to degrade complex organic substrates into soluble substances which can then be absorbed by the mushrooms for their nutrients,

The ability of the different mushroom species to utilize various substrates will depend on both mushroom-and substrate-associated factors. For example, examination of the lignocellulolytic enzymes profiles of the three important commercially cultivated mushrooms exhibit varying abilities to utilise different lignocellulosics as growth substrate. *Lentinula edodes* is cultivated on highly lignified substrates such as wood or sawdust, produces two extracellular enzymes (manganese peroxidase and laccase) which have been associated with lignin depolymerisation. Conversely, *Volvariella volvacea* prefers high cellulose- low lignin-containing substrates such as paddy straw and cotton wastes which have relatively low lignin content, and produces a family of cellulolytic enzymes including at least five endoglucanases, five cellobihydrolases and two ß-glucosidases, but none of the recognised lignin-degrading enzymes. *Pleurotus sajor-caju* is the most adaptable of the three species and can be grown on a wide variety of agricultural waste materials of differing composition in terms of polysaccharide/lignin ration, because it is able to excrete both kinds of cellulose- and lignin-degrading enzymes.

4.2 Recycling of Organic Wastes into Mushrooms, Biofertilizer and Biogas

The ultimate aim in the applied aspects of any scientific endeavour is to integrate wherever possible the various disciplines of science as well as the technological processes in order that maximum benefits accrue from such efforts. Combined production of mushrooms, biogas and biofertilizer from the rural and urban organic wastes should be one of the aims of such integrated schemes that can eventually be put into profitable operation. Though the conventional and established approaches towards the production of food, fertilizer and fuel exist, the explosive growth of the population vis-à-vis the rapid depletion of conventional fuel resources leads mankind to look for alternative sources for food, fertilizer and fuel.

Even though man has been harvesting mushrooms as food from wild sources from times immemorial, their nutritive value was not assessed and their production under controlled conditions was not undertaken until recent decades. The lignocellulosic substrate used for mushroom production and which is left after harvesting of the mushrooms can be used as compost for soil conditioning. It should be noted that this compost besides being rich in nitrogenous material contains partly degraded lignocellulosic components, which when combined with animal dung or human excreta in a biogas digest would yield not only biogas but also a good quality organic nitrogenous fertilizer in the form of sludge. The sludge from the biogas plant as a nitrogenous fertilizer is far more beneficial than the compost from which it has been derived. Part of the biogas that is produced in the vicinity of the mushroom house can also be conveniently used for pasteurization of the mushroom bed material and maintenance of the optimal temperature in the mushroom house as well.

It is therefore suggested that an integrated approach in the production of mushroom, biofertilizer and biogas should be considered as a feasible approach for the rural and urban lignocellulosic waste utilization and disposal. This is the "**Zero Emission or Total Productivity"** concept. It is said "The earth can not produce more: Man has to do more with what the earth produces" (Pauli, 1996; Chang, 2007).

4.3 Restoration of Damaged Environment by Mushroom Mycelia

Mushroom cultivation technology is friendly to the environment. Mushroom mycelia can produce a group of complex extracellular enzymes which can degrade and utilize the lignocellulosic wastes in order to reduce pollution. It has been revealed recently that mushroom mycelia can play a significant role in the restoration of damaged environments. Saprotrophic, endophytic, mycorrhizal, or even parasitic fungi/mushrooms can be used in mycorestoration, which can be performed in four different ways: mycofiltration (using mycelia to filter water), mycoforestry (using mycelia to restore forests), mycoremediation (using mycelia to eliminate toxic waste, and mycopesticides (using mycelia to control insect pests). These methods represent the potential to create the clean ecosystem, where no damage will be left after fungal implementation (Stamets 2005).

5. ECONOMIC AND SOCIAL IMPACTS

Since mushroom cultivation can be a labour-intensive agro-industrial activity, it could have great economic and social impact by generating income and employment for both women and youth, particularly in rural areas in developing countries. Using China as an example, in 1978 the total production of mushrooms in China was only 60,000 tonnes, which accounted for less than 6 percent of total world mushroom production. In 2006, however, total production of mushrooms in China reached 14 million tonnes and accounted for over 70 per cent of total world mushroom production. According to recent statistics, in 2006 the value of total mushroom production in China was US$6billion and the export value of mushrooms was US$1.1billion. It is estimated that in 2007, the export value of mushrooms is US$1.4billion and it is expected to grow to US$1.6billion in 2008. Total employment in the mushroom industry in China was over 30 million people in 2006, with only 10 percent of the employed being actual mushroom farmers, other employment fall within sectors such as food, beverage manufacturing, trading and management, transport, marketing, wholesaling, retailing, export etc. The mushroom industry can also have even broader positive spill-overs, generating complementary employment in areas such as accommodation, restaurant services etc. Further, it is interesting to note that in some counties in China with a population of just under 200,000 people, 60 per cent of the population were engaged in mushroom production and management. The local mushroom industry can also be the main source of revenue for local government.

Mushrooms, like all other fungi, lack chlorophyll and are non-green organisms. They cannot convert solar energy through the process of photosynthesis to organic matters as green plants do, but they can produce extensive enzymes that can degrade lignocellulosic materials for their own nutrients for growth and fruiting. Different mushrooms have different lignocellulolytic enzyme profiles (Buswell and Chang, 1994, Buswell et al., 1996a). This demonstrates the impressive capacities of mushrooms for 'biosynthesis', which is different from 'photosynthesis' by green plants. The species of mushroom fungi not only can convert the agricultural and forestry lignocellulosic wastes through solid fermentation technology into the high quality protein consumed directly in the form of the mushroom fruiting body, but also can convert food processing biomass wastes, e.g., soybean wastes using submerged culture, into fungal protein (Buswell and Chang 1994) or "mycomeat" (Miles and Chang 1988). Soybean waste materials (slurries) are generated in large quantities during the processing of soybean mild and "tofu" (bean curd), which are popular foods in many countries now and are, in some places, discarded without treatment thereby constituting an environmental pollutant. In addition, mushrooms and their mycelia can provide nutriceutical and pharmaceutical products. As outlined in above sections, by blending the advances in basic biological knowledge with that of practical technology, a mushroom-related industry based on utilization of the lignocellulosic waste materials that are abundantly available in rural and urban areas can have positive global impacts on long-term food nutrition, health, environmental conservation and regeneration, and economic and social change. Therefore, the significant impact of APPLIED MUSHROOM BIOLOGY on human welfare has been named as a "Non -green revolution" (Chang 1999).

The following statements summarise the significance of mushrooms in our drive towards alleviating poverty, enhancing human health, and arresting environmental degradation:

(1). Mushrooms can convert lignocellulosic waste materials into a wide diversity of products, which have multi-beneficial effects to human beings, e.g., as food, health tonic, and medicine, as feed, as fertilisers, and for protecting and regenerating the environment. In addition, mushroom cultivation can positively generate equitable economic growth. The tropical regions, particularly, have a wet and warm climate and have an abundant supply of agricultural wastes. These materials are resistant to natural biological degradation because they contain mainly cellulose, hemicellulose and lignin. Mycelia of mushrooms can excrete enzyme complexes which can directly attack/degrade these components of lignocellulosic materials. Therefore, mushrooms can use these wastes as nutrients for their growth and in the process become food and medicine for human consumption.

(2). Mushrooms are relatively fast growing organisms. Some tropical mushrooms can be harvested and consumed within 10 days after spawning. By the use of different varieties, mushrooms can be cultivated year round. They can be cultivated by using primitive farming techniques in rural areas or by using highly industrialised technologies in the urban and peri-urban communities.

(3). Mushroom cultivation can be labour intensive. Thus the activity can generate new jobs, especially in tropical or less developed countries.

(4). While land availability is usually a limiting factor in most types of primary production, mushroom cultivation requires relatively little space. Actually they can be stacked using shelf-like culture systems.

(5). Mushrooms have been accepted as human food from times immemorial, and can immediately supply additional protein to human food. Other sophisticated and unconventional sources of food protein, such as yeast, uni-algal cultures and single-cell proteins have relatively more complicated requirements, and need to be processed before they can be consumed.

(6). Edible mushrooms should be treated as healthy vegetables. After improving the cultivation techniques, they should be cultivated as widely and as cheaply as other common vegetables, which will thus be beneficial to the general public.

(7). In view of their pleasing flavour, their high protein, and tonic and medicinal values, mushrooms no doubt represent one of the world's greatest untapped resources of nutritious and palatable food for the future.

6. CONCLUDING REMARKS

From what has been presented in the preceding pages, the following summary can serve as concluding remarks:

---Mushrooms can serve as food, as tonic, and as medicine. A regular intake of mushrooms can make you healthier, fitter, and happier. They can make you live longer, and always look younger.

---Mushrooms are biota characterized by wonder. They rise up from lignocellulosic wastes, yet they become so bountiful and nourishing.

---Mushrooms are environmentally very friendly. They biosynthesise their own food from agricultural crop residues, which would otherwise cause health hazards. And their spent composts/substrates can be used as animal feed, biofertilizers and biogas.

---Mushrooms can serve as agents for promoting equitable economic growth in society. They are a unique group of fungi through which we can pilot a non-green revolution in less developed countries, and in the world at large. They demonstrate great potential for generating a great socio-economic impact in human welfare, at local, national and regional levels.

Therefore, the aims of the discipline of applied mushroom biology are to tackle the three basic problems: **shortage of food, diminishing quality of human health and pollution of the environment,** which human beings still face, and will continue to face, due to the continued increase of the world population. On the other hand, it has been observed that over 70% of agricultural and of forest products have not been put to total productivity, and have been discarded as waste. Applied mushroom biology not only can convert these huge lignocellulosic biomass wastes into human food, but also can produce notable nutriceutical products, which have many health benefits. Another significant aspect of applied mushroom biology is using the biota in creating a pollution-free and beneficial environment. These three components of applied mushroom biology are closely associated with three aspects of wellbeing – food shortage, human health and environmental pollution. **One of the most significant benefits of mushroom cultivation is their ability to create a pollution free and friendly environment.**

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8. SUGGESTED FURTHER READINGS

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9. ANNEX: RECOMMENDATIONS FOR FOLLOW-UP ACTIVITIES

Cultivated mushrooms have now become very popular all over the world. This is because mushrooms can adapt to a wide range of climatic conditions, and also because they have immense nutritional and medicinal values.

The production of mushrooms in the Asian and Pacific area accounted for over 75% of the total world production in 2005. Those amounts were mainly contributed by the three north-eastern countries, China, South Korea and Japan. China's success in the development of its mushroom industry during the past three decades should serve as an example on what is also possible for other developing countries.

9.1 Problems for Cultivation of Mushrooms in Some Countries in the Region

In practical application, the cultivation of *Agaricus* mushrooms in developed countries has become a high technology industry, and in fundamental research, it has become a branch of science which is derived from the disciplines of microbiology, fermentation and environmental engineering. In the last two decades, millions of dollars have been spent in western countries to develop the industry. The mushroom farms are usually furnished with heavy equipment. The support from industry and governments for *Agaricus* mushroom research is equally impressive in some developed countries such as the USA (Mushroom Research Centre at the Pennsylvania State University), UK (Mushroom Research Section at Horticultural Research International).

In Asia, there are also national and local government support mushroom research centres, particularly in China, Japan, South Korea and India.

However, in other Asian and Pacific countries, there appears to be a lack of such infrastructure for the mushroom industry. There are several reasons/ problems, which may be directly or indirectly related to the slow development of mushroom cultivation in tropical regions:

(1). Social concept. Mushrooms are usually eaten for their culinary properties, providing a flavouring and garnish for other foods. They are cultivated with special technique and usually consumed by the rich people because the price of mushroom is usually much higher than that of the most common vegetables. This may give one the impression that mushrooms constitute a luxury food and that their promotion would only benefit relatively rich people. Actually, mushrooms are rich in protein and contain several vitamins and mineral salts and should thus be considered as high protein vegetables to enrich all human diets.

(2). Lack of government and industrial support for research. Research on tropical mushrooms is relatively inexpensive (requiring neither huge research establishments nor massive, highly complex equipment). As far as I know, no government supported mushroom research centre or institute has been established in tropical regions.

(3). Inadequate interest on the part of academic scientists in the fundamental biological studies of edible mushrooms. This contributes to the delay in the massive production of edible mushrooms in the tropical regions. Therefore, the knowledge on the biological nature of mushrooms cultivated in tropical regions is very meagre indeed. Without such basic knowledge, the development of any mushroom industry is rather difficult. Since the cultivation of mushrooms, by its nature, requires very strong regional and local adaptation, the greatest needs should be the creation of national and regional laboratories /centres of fundamental biological and applied technical research on edible mushrooms in tropical/ subtropical countries, and also the development of bright, young, and highly motivated people who will be attracted work in these laboratories / centres.

(4).Those three problems mentioned above are the most basic and important problems. There are several other problems, such as shortage of technical expertise, lack of appropriate equipment, inadequate regional cooperation, lack of organised marketing strategies, etc that would require particular attention.

(5). Further, due to the prevailing hot and humid climate, mushroom cultures are more easily contaminated; the shelf life of the mushrooms is difficult to be prolonged; and the preparation and preservation of the mushroom pure culture spawn is also a common problem to mushroom growers, since they do not have basic knowledge in microbiology.

There should be a concerted effort to lobby the governments and international/national agents, reminding them that research and development of mushroom industry is not a luxury but a national necessity for human welfare.

9.2 The Strategies for Developing Mushroom Industries in the Asian and Pacific Region

(1). At the beginning, the strategy is not to use highly mechanized technologies as in the large mushroom farms in industrialized countries, but to promote cottage style enterprise for the rural poor in thousands of small mushrooms sheds, constructed using locally available materials (like those used in China at the beginning of its mushroom industry).
(2). Then more towards gradual introduction and familiarization of the art of large scale commercial cultivation techniques. This was the path China followed from its humble beginnings to its current status as the world's leading mushroom production powerhouse.
(3). Select appropriate target strains of different mushrooms grown on seasonal basis so that an attempt could be made to obtain yields all year-round.
(4). Make use of existing lignocellulosic residues and waste from agricultural activities and agro-industries.
(5). Create employment opportunities, particularly for women and the youth in rural areas, and control/reduce pollution.
(6). Emphasize quick-investment-return mushrooms, and select relatively fast growing species that can be harvested within 3 to 4 weeks after spawning, thus generating immediate benefits.
(7). Promote mushrooms species demonstrated to generate potent nutriceuticals with superior immune-enhancing attributes: species whose natural products include unique bioactive compounds that can make people healthier and fitter.

The following ideas also need to be emphasized. Although science and farming practice have led to the development of some universal or general concepts concerning mushroom cultivation, the diverse biological nature of the process (in which large numbers of mushroom species and natural organic substrates are involved) also means that a wide spectrum of variations in farming methods must be employed. Thus the transfer of mushroom cultivation techniques from one region or country to another cannot be treated in the same way as the transfer of non-biological industrial technology, such as that of a complete complex of factory equipment for textile or chemical fertilizer industries. Since the cultivation of mushrooms deals with living organisms, one should consider, not only the unique attributes of the mushroom itself, and of the various micro-organisms growing with it (including both the harmful and beneficial ones), but also the biochemical nature of the local available substrates. Therefore, the specific methods must be tailored in accordance with the prevailing unique natural resources, heritage, local climate, and socio-economic conditions of the farming community. All these consideration call for a critical mass of well trained mushroom scientists. Thus the training activities of the UN-APACEM Project of Asian member countries need to be further supported.

9.3 Appropriate Training for Regional Mushroom Scientists and Mushroom Farmers

(1) A one-week mushroom training workshop can be held first at a convenient time covering the topics in Section 2. The number of participants can be around 18-24. Two to three can be nominated from each of the selected regional members, and the host country can have four. They

should have a general knowledge of microbiology/ plant pathology/and mushroom cultivation. It is hoped that by the end of the Workshop, the selected participants would be equipped with new knowledge and basic skills that will enable them to do further research, training and farming mushrooms, and eventually to generate robust and high quality edible and medicinal products from the local biota.

(A). Content of the Workshop: The Training Workshop is designed to cover the basic science and technologies involved in the production of mushrooms using agricultural and other organic wastes, as well as other categories of biomass (e.g., water hyacinth, which many communities often conceive as environmental tragedies).

(B) Methodology: The training Workshop would involve Lecture Sessions in the morning, and Discussion and Question Sections in the Afternoon.

(C) Resource Person: The Key Resource Person for the Training Workshop can be a world known mushroom scientist.

(2). Then there should be a two- or three -week mushroom training course, which provides more detailed and practical lessons on cultivation technology, similar to the topics in Section 3.

(A) Contents**:** Establishment of a mushroom spawn facility;

- Appropriate training for national scientists;
- Identification of local mushrooms for farming development; and
- Construction of model mushroom farm for demonstration and training purposes.
- Establishment of a mushroom spawn facility: An old Chinese saying stipulates that if you wish to get the work done well, you must first have proper facilities or the correct tools. The isolation of pure cultures of mushrooms and the preparation of mushroom spawns are microbial manipulations. Therefore, mushroom spawn workers need not only the knowledge and appropriate techniques in microbiology, but also require a suitable working place (laboratory), where proper equipment and facilities should be provided, in order to achieve the production of good quality spawns.

(B). Methodology: In addition to the Lecture and Discussion Sessions, there should be Laboratory Exercise Sessions.

(C). Resource Person: At least, two resource persons should be invited.

9.4 Consideration on the Formation of a Regional Network on Mushroom Development

It may be considered in due course that the establishment of a Network as an integral part of the sustainable development of edible/medicinal mushroom industry aims to improve the capability, productivity and business development of the mushroom industry in the Region. The justifications of this suggestion are in responding to different needs of member countries in the development of mushroom industries, particularly in capacity building, different socio-economic and environmental aspects, exchange of information on the development of edible and medicinal mushroom industry; emerging need to share differences and similarities in experiences and knowledge, and program and policy regarding the development of mushroom industry within the regional countries; growing demand in solving multilateral constraints on marketable products and marketing of edible and medicinal mushrooms in global market; and developing strategies to promote the consumption of edible and medicinal mushrooms.