Good Food Institute IN

Technological Review of Fungi for Alternative Protein Applications

An overview of technology development across various fungal platforms and value chain, and scope of fungal industry for alternative protein applications in India

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What are Fungi?

Fungi are industrially important organisms due to a wide range of applications including in biotechnology, in the production of food products, organic acids, pharmaceutical products, polysaccharides, and enzymes, and in biological control of pest insects, fungi, and weeds (Ball and Finkelstein 1992). Fungi are known to produce a variety of secondary metabolites and have been exploited for the production of homologous or heterologous gene products due to the efficient protein secretion mechanisms. (Domsch. 1980; Knowles et al. 1987; Saunders et al. 1989; Leong and Berka 1990).

Fungi are eukaryotic and heterotrophic organisms that are most commonly known for their ability to grow on and digest waste organic matter. Fungi range from multicellular macroscopic forms like that of mushrooms to unicellular microscopic forms like that of yeast. With over 100,000 species classified as fungi (Skovgaard 2002), fungi can be divided into three categories based on their morphology -

1. Yeast: these are unicellular organisms with nucleated rounded fungi that reproduce by budding. An example is *Saccharomyces cerevisiae.*
2. Dimorphic fungi: these grow partly as yeasts and partly as filamentous fungi depending on the environmental conditions. An example is *Histoplasma capsulatum.*
3. Molds: these are multicellular organisms that are filamentous in nature with hyphae, and reproduce through spores. An example is *Penicillium chrysogenum.*

Due to their heterotrophic nature, they absorb nutrients from dead material or living matter in a parasitic or mutualistic relationship with a living organism. Fungi secrete hydrolytic enzymes externally, thus initiating digestion followed by ingestion. They possess a cell wall made of chitin and cell membranes that have sterol and ergosterol, unlike mammalian cell membranes which have cholesterol.

Fungi can have varying modes of reproduction depending on species type. Zygomycetes, basidiomycetes, and ascomycetes reproduce through sexual reproduction (as well as asexual reproduction in some cases) through the formation of zygospores, basidiospores, and ascospores respectively. On the other hand, Deuteromycetes or imperfect fungi that used to be a subcategory of ascomycetes, reproduce asexually through spores (conidia) or by budding.

Multicellular fungi have filamentous structures and produce spores. The filamentous structures, also known as mycelium, are a network of connected hyphae or long chains of cells joined end to end divided by cross-walls or septa. This type of fungi has a vegetative mycelium which penetrates through the substrate and is responsible for sourcing nutrition. The reproductive mycelium extends outside the substrate into an open atmosphere where it can release spores.

With more than hundred thousand known species of fungi, few hundreds are pathogenic to human beings and few thousands are pathogenic to plants. However, the application of several species of fungi in food and beverage preparations has been carried out for centuries. Beyond the most commonly

known use as mushrooms and yeast in food products, fungi have been used in creating products like alcoholic beverages, cheese, bread, etc.

Traditional food applications of fungi

Historically fungi have been consumed for both food and medicinal purposes with records of various applications of fungi in ancient Egyptian murals and Greek writings, and documentation in Chinese and Japanese traditional medicine. Fungi-based fermentation products like soy sauces, tempeh, sake, koji, oncom, angkak, miso, and hamanatto have been widely used in Asian cuisines. On the other hand, preparation of mold-ripened cheeses like camembert, brie, roquefort, and blue stilton cheese as well as fermentation of leavened bread using yeast have been common applications of fungi in west countries. Instances of fermentation of cereals using a mixed culture of lactobacillus and yeast to produce beers and fermented porridges can be found in African traditional culture (Moore and Chiu 2001). The production of edible mushrooms including button mushroom, shiitake, Chinese or straw mushroom, winter mushroom, oyster mushroom, and truffle as well as the production of alcoholic beverages using yeast is a common practice observed across the world.

Fermentation as a process can lead to change in flavor, texture, and other organoleptic properties and improve shelf life and nutrition of food products compared to non-fermented foods, in addition to the production of entirely new products as in the case of fermentation of carbohydrates leading to the production of alcoholic beverages. While yeasts can be particularly attributed to modify flavor, nutritive value, alcoholic content, and leavening properties, mycelium fungi contribute significantly to textural properties. Typical food products formed from fungal fermentation include beverages, flavorings, protein-rich meat substitutes, and baking products.

Fermentation using fungi can be divided into three categories:

1. Natural fermentation: Uncooked ingredients undergo fermentation without the addition of any external fungal culture. Commonly known foods include idli and nan. The fermentation occurs due to the presence of microbes in the ingredients or the utensil surfaces.
2. Starter-mediated single-stage fermentation: In this case, the ingredient or substrate is cooked and starter media in the form of spores or mycelium is added to it to initiate the fermentation process. The fermented products are cooked before consumption. Tempeh and oncom are produced by this method.
3. Multi-stage fermentation: In this case, solid-state fermentation leading to the production of digestive enzymes is followed by liquid state or solid-state fermentation involving the breakdown of carbohydrates and proteins. A typical product made from this type of fermentation is rice wine, soy sauce, and vinegar.

With above 20% crude protein content as a percentage of dry matter, fungi as food contain all essential amino acids. The presence of chitin in the cell wall provides a source of dietary fiber. Fungi are also low in fat and lack cholesterol making them a healthy source of nutrition.

Overview of the fungal value chain

As described in the last section, fungi can be used in various food applications through a variety of fermentation processes. The existing use case of fungi in the alternative protein sector is that of filamentous fungi for the production of various meat alternatives under brands like Quorn, Fermotein, and Promyc. Theoretically, yeast can also be used as a protein source for creating alternative protein products, but yeast-based meat alternatives are not in the market yet. Startups like Fumi ingredients and More foods are exploring yeast-derived proteins for creating alternative meat and egg products. While yeast and filamentous fungi have direct applications as protein sources in the alternative protein sector, other fungi-derived ingredients like chitin can also be utilized as scaffolding materials for the production of cultivated meat, and fungal extracts can be used as flavoring and stabilizing agents. Fermentation as a platform can be utilized in three ways for applications of fungi in the alternative protein space. The first route is the traditional fermentation in which fungi are used to ferment a target substrate to improve its sensorial or textural properties for improved product properties. The second route is biomass fermentation to produce whole biomass like filamentous fungi and yeast for proteins or flavors or textured meat analogues. The third route is precision fermentation which uses recombinant DNA technology for exploiting fungi as platforms for production of highly specific compounds. The report focuses on alternative protein applications of fungi using the first two fermentation routes.

The diagram below shows the possible applications of fungi in the alternative protein sector and indication to companies working in each of the application areas.

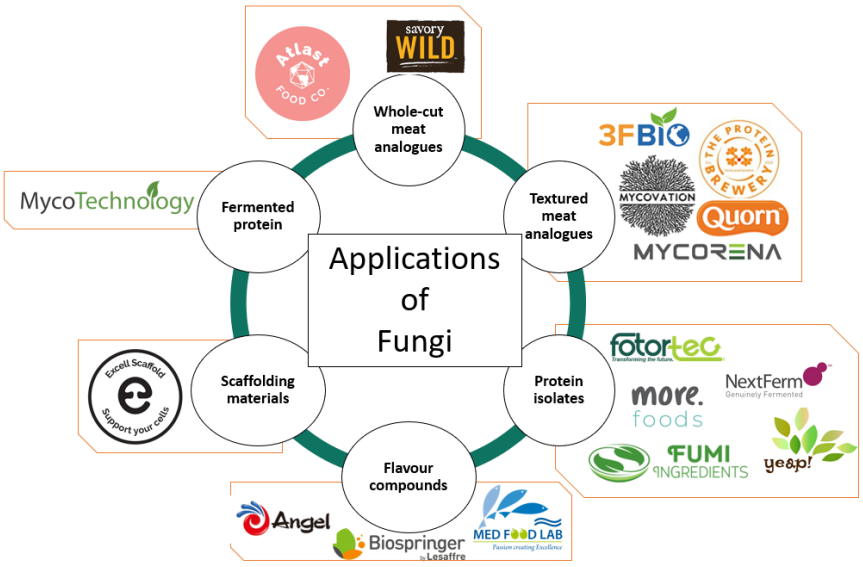

Fig 1. Alternative protein applications of fungi

Depending on the application area, the value chain for utilization of fungi-derived ingredients will defer. However, there are three key steps involved in the production of fungi and subsequent utilization of fungi-based ingredients irrespective of end application. The process can be broken down into three

broad categories: strain selection involving screening of suitable strains, production involving biomass production at manufacturing scale while maintaining sterile conditions, downstream processing involving destruction of RNA or other toxic compounds, harvesting, and concentration of biomass followed by extraction of target compounds. These have been described in figure 2 below.

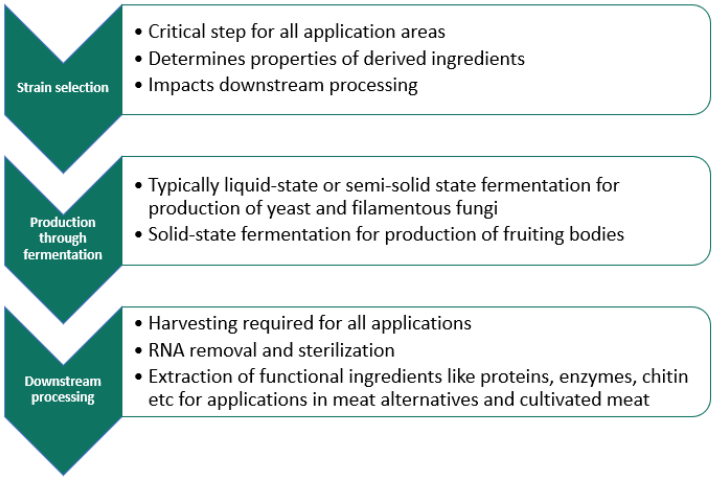

Fig 2. Typical steps involved in the production of fungi-based ingredients

The specific steps involved in the production of yeast and filamentous fungi as sources of protein are similar in nature as depicted in figure 3. Other ingredients like enzymes and chitin can be extracted downstream while the upstream production steps remain the same. The choice of technology at each stage of the value chain will differ due to the differences in the morphological and metabolic properties of the yeast and filamentous fungi. For example, baker's yeast is typically produced in a fed-batch fermentation system running up to 20 hours while filamentous fungi like *Fusarium venenatum* is produced in a continuous fermentation system running for a period of 6 weeks. Even though both species of fungi can be produced in a liquid state fermentation, the configuration and type of bioreactors used may differ.

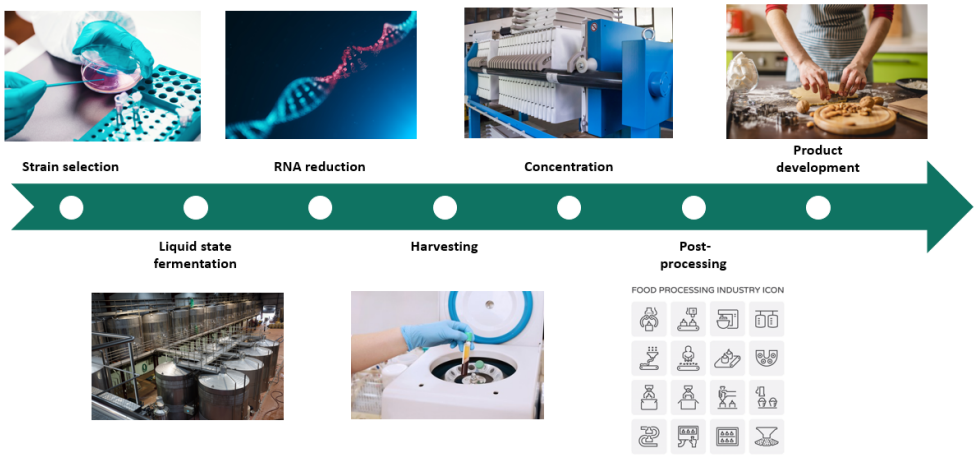

Fig 3. The value chain for fungi production using liquid state or semi-solid state fermentation

Solid-state fermentation used for the production of mushrooms has now found innovative applications in packaging, furniture, textile, and construction industries. Several companies like Ecovative, Bolt Threads, MycoWorks, MycoTechnology, Reshii, and MOGU have been developing materials from fungi using solid-state fermentation. Through appropriate strain selection followed by optimization of fermentation conditions and substrate, companies like Atlast and Fotortech are creating alternative meat products. The complexity in the overall production process is reduced due to ease of harvesting biomass, no requirements to concentrate biomass, and minimal post-processing for whole biomass applications. However, the rules of scaling up or scaling out production systems are not as well-established as in the case of liquid-state fermentation. The value chain for solid-state fermentation has been depicted below in figure 4.

Fig 4. The value chain for fungi production using solid-state fermentation

The key steps of the value chain including strain selection, production, and downstream operations are briefly described in the following sections.

Strain Selection

As with any biological production system, strain selection is the most crucial step as it determines all the subsequent downstream operations required and impacts the costs involved in the production and processing of biomass.

Key characteristics of importance when selecting strains include the following:

1. Productivity - High growth rate of strains is preferred due to lower batch cycle time and hence faster turn around time for the overall process.
2. Genetic stability - The ability of the strain to retain its genetic makeup during the complete production cycle is crucial to ensure a stable production process
3. Morphology - The arrangement in which mycelium structure is organized impacts the mixing process, heat transfer, and local nutrient availability. Mycelium can form pellets or filaments

depending on the processing conditions. The formation of filaments leads to higher viscosity hence higher power requirements to ensure homogenous mixing of nutrients for the continued growth of biomass.

4. Substrate efficiency - The growth rate of the mycelium depends not only on the composition of the growth medium but also on the ability of the strain to efficiently metabolize the nutrients for growth purposes (Ball and Finkelstein 1992).

Other important considerations when selecting appropriate strains for the production of biomass include:

1. History of safe use - It is prudent to use strains that have a history of safe use and are, thus, safe for human consumption. Using a new strain for food applications will require significant investment in nutritional and safety trials to establish safety as per regulatory requirements for human consumption. A list of species that have been historically used in food products has been listed in table 1 below.

Phylum	Genus	Species	Food product
Zygomycetes			
	Actinomucor	Actinomucor elegans, Actinomucor taiwanensis	Sufu
	Amylomyces	Amylomyces rouxii	Ragi
	Mucor	Mucor circinelloides, Mucor rouxii, Mucor indicus	Ragi, Murcha, Tempe, Pehtze
	Rhizopus	Rhizopus microsporus, Rhizopus oligosporus	Temphe
		Rhizopus oryzae	Koji, Nuruk, Chu, Murcha, Tempe
Ascomycetes			
	Monascus	Monascus purpureus, Monascus ruber	Angrak
	Neurospora	Neurospora sitophila, Neurospora intermedia	Oncom
	Aspergillus	Aspergillus oryzae , Aspergillus sojae, Aspergillus niger	Koji, Miso, Soy sauce
		Aspergillus glaucus, Aspergillus melleus, Aspergillus repens, Aspergillus candidus	Katsuobushi
	Penicillium	Penicillium roquefortii	Roquefort, 'blue' Stilton
		Penicillium camembertii	Camembert, Brie, soft ripened cheeses
		Penicillium glaucum	Katsuobushi
	Tuber	Tuber melanosporum	Truffle
	Brettanomyces	Brettanomyces anomalus	Kumiss
	Candida	Candida javanica	Idli, Kombucha, Murcha
	Endomyces	Endomyces fibuliger	Murcha, Ragi
	Hansenula	Hansenula anomala	Sake, Koji
	Hyphopichia	Hyphopichia burtonii	Ragi
	Saccharomyces	Saccharomyces cerevisiae	Nan, Toddy, Murcha, Kombucha
		Saccharomyces dairensis	Temphe
		Saccharomyces globosus	Kumiss
		Saccharomyces kluyveri	Nan
		Saccharomyces sake	Sake
	Torulopsis	Torulopsis versatilis	Idli, Kombucha, Soy sauces and Pastes
	Trichosporon	Trichosporon pullulans	Idli
		Trichosporon beigelii	Temphe
	Zygosaccharomyces	Zygosaccharomyces rouxii, Zygosaccharomyces sojae	Soy sauces, Soya pastes
Basidiomycetes			
	Agaricus	Agaricus bisporus, Agaricus bitorquis	Champignon or Button mushroom
	Lentinula	Lentinula edodes	Shiitake
	Volvariella	Volvariella volvacea	Chinese or straw mushroom
	Flammulina	Flammulina velutipes	Winter mushroom enoki
	Pleurotus	Pleurotus sp.	Oyster mushroom

Table 1. List of fungal species found in historically consumed food products. Adapted from Nout and Aidoo(2002), and Moore and Chiu(2001)

2. Presence of mycotoxins: Fungal species may produce mycotoxins which can cause serious health implications upon consumption. Hence, screening of species that do not produce mycotoxins or identifying a downstream process for selective destruction of mycotoxins would be required to ensure that the fungal biomass is safe for human consumption.
3. RNA content: It is well known that filamentous fungi and yeast have high RNA content upto 10%. Hence, additional downstream processing steps for reduction or removal of RNA need to be implemented. For food applications, the RNA content in the final product needs to be monitored. WHO standards for maximum allowed RNA content should be complied to.

Strains can be obtained from collection centers and screening can be performed to identify the most suitable strains. Strains can also be genetically modified or mutated or selectively bred to get desirable traits. The key challenge with genetic engineering fungi for food applications is the concern of the use of genetically modified organisms(GMOs) for human consumption. In the Indian context, GMO based food products are not acceptable as per regulatory norms.

Production

After the selection of an appropriate strain, the choice of the production system will depend on the end application and desired morphology of the end product. The two most commonly used production systems include submerged liquid fermentation and solid-state fermentation.

Submerged Liquid Fermentation

In submerged liquid fermentation (SLF), the fungi grow in an aqueous solution with dissolved nutrients required for the multiplication of biomass. Microbial fermentation for the production of enzymes is carried out in fermenters or bioreactors with up to 1000 m^3 capacity. For applications in the alternative protein sector, the intracellular components like proteins are of interest in contrast to extracellular components in the fermented broth as in the case of enzyme production. While the fermentation infrastructure can be utilized for growing fungal biomass, parameters like the concentration of nutrients, quality of feedstock, pH, temperature, and oxygen uptake rate need to be optimized to make the process suitable for biomass production and meet safety requirements for subsequent food applications. These parameters can be controlled and monitored continuously during the production process to ensure standard growth conditions for all batches.

The various types of submerged liquid fermentors available are listed below (Behera and Varma 2017):

1. An aerated stirred tank fermentor is a closed cylindrical tank with a mechanical agitation system like an impeller to ensure homogeneous mixing and high mass transfer. High mass transfer rates can be obtained due to the high shear environment created by the agitation system. High shear may not be desirable if maintaining the morphology of the microorganism is critical as the arrangement of mycelium strands can get destroyed due to excessive mixing.
2. A bubble column reactor is a vertically-erected cylindrical column filled with liquid with a gaseous phase supplied from the bottom of the reactor. Mixing takes place due to the upward

movement of the gaseous phase through the column. The shear forces acting in such a reactor are lower than in the case of stirred tank reactors.

3. Airlift reactors operate on a principle similar to that of bubble column reactors. Mixing in the reactor occurs as a result of the circulation of the gaseous phase with a draft tube or in the riser creating a gas-liquid mixture. The reactor provides a low shear environment and requires less energy to operate compared to stirred tank reactors. However, depending on the viscosity of the fluid in the reactor, the cost of circulating the gaseous phase in the reactor can offset the cost and energy saved due to the lack of an agitation system. These reactors don't need internal cooling coils for heat transfer as the external jacket is sufficiently aided by the rapid mixing within the reactor.
4. Fluidized bed reactors consist of small particles to which cells attach and get immobilized. Due to the high surface to volume ratio of particles, the oxygen and nutrient transfer rates to the cell are enhanced. These reactors operate at low shear and require low energy for operation.
5. Packed bed reactors consist of large particles to which cells attach and get immobilized. The particles don't move in the liquid. The mass transfer rates are poor in these reactors because of the low surface area to volume ratio of particles.

Commercial use of stirred tank fermenters for production of baker's yeast and airlift reactor for the production of filamentous fungi have been discussed in detail in later sections of this report.

SLF can be carried out as a batch, fed-batch, or continuous operation. In a batch process, the microorganism grows in a closed system and nutrient media is not added after the inoculation step. Only acid or alkali are added to adjust pH and air is supplied if fermentation needs to be carried out in aerobic conditions. After the nutrient media is depleted, the batch process is completed and the reactor needs to be cleaned to prepare for the next batch process. Alcoholic beverages, enzymes, amino acids, and organic acids are produced through batch fermentation.

Fed-batch fermentation involves the addition of nutrient media as the fermentation progresses with time. Since nutrient media is added either continuously or intermittently but biomass is not removed continuously, this type of fermentation process can be considered as an intermediate process between batch and continuous fermentation. Such a process is used for the production of baker's yeast and penicillin. Fed-batch fermentation is a preferred mode of operation if the substrate or nutrient media is highly viscous causing problems with mixing, or nutrients are growth suppressing at a higher concentration.

The key advantage of batch systems is the ease of terminating operation in case of contamination. However, batch fermentation is less effective if the goal is to produce biomass or growth associated metabolic products due to the limited exponential phase. Other disadvantages include batch-to-batch variation, need for downtime, and man-power requirement for various operations (Ball and Finkelstein 1992).

On the other hand, continuous fermentation involves a system in which nutrient media is added and biomass culture is removed simultaneously. This process is ideal for biomass production as the microorganisms can grow in the exponential phase for prolonged periods at fixed growth rates. As the fermentation process can be carried out for longer periods of time, there is a reduction in overall reactor startup-time and the down-time for sterilization and cleaning, thus lowering the operating costs compared to batch processes. However, continuous fermentation requires R&D investment to screen species and strains which can maintain genetic stability for the duration of the operation.

Solid-state fermentation

As the name suggests, solid-state fermentation(SSF) involves using a solid substrate as a growth medium. Due to the use of the solid substrate, fermentation takes place in a low-moisture environment with limitations of mixing and dispersion of nutrients depending on the reactor design. Classical examples of products produced using solid-state fermentation include tempeh, mold-ripened cheese, and mushroom. This type of fermentation is favorable for growing filamentous fungi as these can penetrate solid substrates through the action of hydrolytic enzymes secreted at the tip of the hyphae. Solid-state fermentation exhibits high volumetric productivity, lower energy consumption, and ease of production and downstream processing. However, the disadvantages include limited nutrient, water, and oxygen diffusion, localized heat generation, and limited overall-process control that can lead to variability in the final product quality. Though SSF can be carried out in closed chambers with temperature and humidity controls in place, controlling localized temperature, and oxygen and moisture availability is challenging. Despite these limitations, SSF can be the preferred mode of production depending on the end applications. For example, the production of certain types of enzymes is only induced in SSF, certain fungi sporulate only during SSF. Also, due to the relatively lower external control required for SSF, low skilled workers can be employed to execute and monitor the production process. It is perhaps for this reason, SSF products such as tempeh, cheeses like Roquefort and Camembert, etc have been traditionally produced in household environments with ease.

Since nutrient, oxygen, water, and heat transfer are non-homogeneous in SSF, various reactor configurations can be utilized to ensure adequate homogeneity, mixing, and oxygen supply. SSF bioreactors can be classified into four groups (Mitchell and Krieger 2006):

1. Bioreactors without agitation and unforced aeration
   Tray Bioreactor: SSF in tray bioreactors is carried out in static conditions without any forced aeration or mixing. Typically trays are stacked on one another, with the tray bottom perforated with mesh to allow aeration. The thickness of solid substrate on the tray impacts heat transfer and gas phase mass transfer efficiency.
2. Bioreactors without agitation but forced aeration
   Packed bed bioreactor: Packed bed bioreactors consist of long columns filled with solid substrate and a perforated base at the bottom. Air is forced into the solid substrate from the perforated base. While forced aeration can ensure homogeneous oxygen and moisture availability in the system, temperature control remains a challenge due to lack of mixing or agitation
3. Bioreactors with agitation and unforced aeration

Rotating drum bioreactor: A rotating drum bioreactor consists of a horizontal aligned cylindrical drum which is partially filled with a solid substrate of appropriate thickness. The rotation of the drum leads to mixing to achieve a homogeneous temperature profile. However, the process of mixing can lead to breakage and damage of long-range mycelium structure. Hence, intermittent mixing is preferred over continuous mixing.

4. Bioreactors with agitation and forced aeration
   Fluidized-bed bioreactor: Fluidized-bed reactor consists of a vertical column with a perforated base plate through which air is forced upwards. The aeration is sufficient to fluidize the particles in a solid substrate. The fluidization leads to homogeneous mixing, temperature control, and efficient mass transfer at the solid-gas interface. Despite these advantages, aeration in a fluidized-bed reactor can lead to damage of the mycelium structure of filamentous fungi.

The choice of bioreactor used for the production of filamentous fungi using SSF will depend on the extent of homogeneous mixing, and temperature, nutrient, water, and oxygen control required while considering the constraints imposed by the fragility of the mycelium structure formed during the biomass production process. For use of fungal biomass for alternative protein applications, bioreactors with low shear environments for SSF will be preferred.

Process parameters

The biomass quantity, growth rate, quality, and nutritional value will depend on the parameters depicted in figure 5, irrespective of the production system used for the production of yeast, the mycelium of the filamentous fungi, or the fruiting body of filamentous fungi (Manan and Webb 2017).

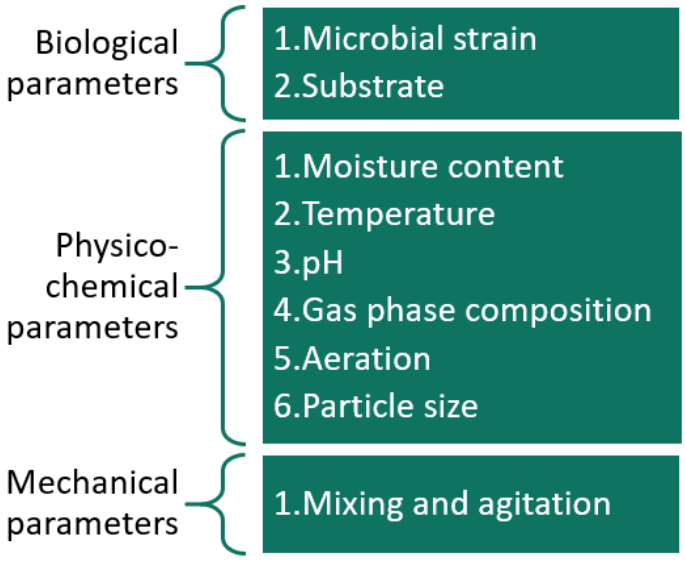

Figure 5. Parameters influencing process design and efficiency of the production system

Downstream operations

The set of downstream operations required after biomass production depends on the end-product of interest. For example, if extracellular components like enzymes are of interest, then the downstream operations would include separation of fungal biomass followed by extraction of enzymes from fermentation broth; however, if intracellular components like proteins are of interest, then separation and concentration of fungal biomass followed by cell disruption and protein extraction would need to be carried out. Figure 6 shows the various steps involved in downstream processing depending on whether the target compound is available as an extracellular or intracellular product.

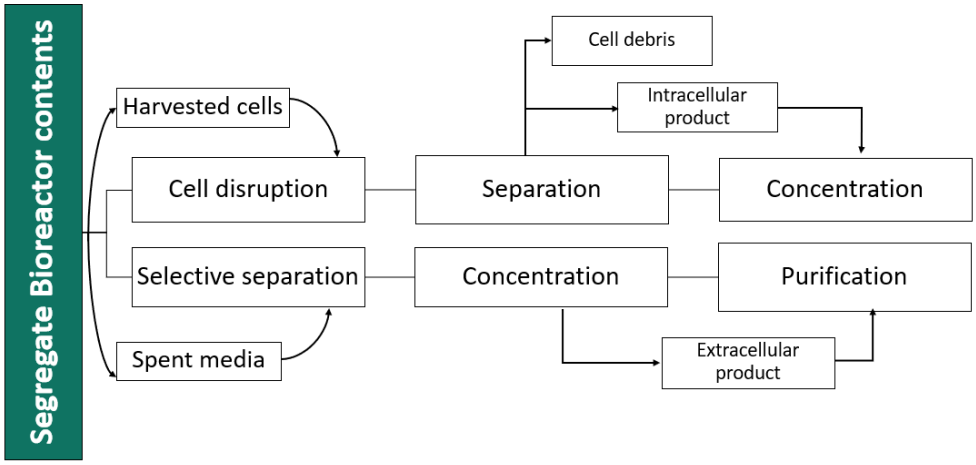

Figure 6. Overview of the downstream value chain

Also, the choice of fermentation technique influences the complexity of downstream processes. In this section, downstream operations relevant for whole biomass utilization and extraction of intracellular components like proteins and chitin will be discussed, which are especially relevant for biomass produced via SLF, keeping in mind the most relevant applications for the alternative protein sector. In the case of SSF, the downstream processing will be simpler if the whole biomass is utilized for creating alternative meat products. However, if the objective is to extract protein isolates from the whole biomass, the downstream operations will include all steps described in this section. Table 2 shows an exhaustive list of techniques available for each of the downstream operations to obtain target compounds from microbial biomass.

Cell harvesting	Cell disruption	Pre-extraction clarification	Selective extraction	High purity extraction
• Centrifugation • Filtration • Sedimentation • Flocculation	• Bead milling • Homogenization • Sonication • Autolysis • Enzymatic or chemical disruption • Osmotic shock	• Centrifugation • Filtration	• Precipitation • Chromatography • Membrane filtration • Distillation • Partition	• Chromatography • Electrophoresis • Dialysis

Table 2. Summary of unit operations involved in downstream processing of fungal biomass. Adapted from Ball and Finkelstein (1992)

The most prominently used unit operations corresponding to each step of the downstream value chain have been discussed below.

Harvesting and concentration

After the desired biomass is produced in the SLF bioreactor, the fungal biomass needs to be concentrated for further processing. The most commonly used methods include centrifugation and filtration.

Centrifugation

Centrifugation utilizes the density difference between microbial biomass and aqueous medium to separate them. Types of centrifuges include tubular centrifuges, multi-chamber bowl centrifuges, disc stack centrifuges, and screw decanter centrifuges. The centrifugation technique has the advantages of processing large volumes in a continuous system with provisions for sterilization and maintaining aseptic conditions throughout the process. Despite readily available centrifugation technologies, the high initial capital cost and operating cost are the key drawbacks of using these technologies

Filtration

Traditional filtration techniques involve the separation of suspended solids using depth filters made of porous material that retains solid components and allows the liquid phase to pass through. This technique is relevant for harvesting filamentous fungi as the morphology of fungi allows for the retention of solids. For batch filtration, filter presses are industrially used to harvest baker's yeast and protein precipitates from microbial biomass. On the other hand, rotary vacuum filters are used in continuous filtration systems industrially for harvesting mycelium used for producing antibiotics.

If the solid content to be separated has a particle size lower than 10µm, membrane filtration can be used. Depending on the particle size to be separated, membrane separation can be classified into particle filtration, microfiltration, ultrafiltration, nanofiltration, and reverse osmosis in order to decrease the pore size hence increasing power requirements. The energy requirements for membrane filtration are driven by pumping costs and pressure or vacuum required to pass the biomass containing fluid through the membrane.

Cell disruption

After getting a concentrated solution of biomass, the next step could be drying or cell disruption. In cases of alternative protein applications of fungi like using filamentous fungi in Quorn products or using biomass produced through SSF as alternatives to whole-cut meat, the biomass can be processed further without cell disruption. On the other hand, if fungi-based proteins need to be extracted for creating desired alternative meat, egg, dairy, and seafood products, then cell disruption is required followed by the extraction process. Before cell disruption of biomass produced through SSF, the biomass needs to be pulverized for efficient cell disruption. Common cell disruption techniques include bead milling, high-pressure homogenization, autolysis, and enzymatic disruption.

Bead milling

Bead milling is one of the most commonly used methods for cell disruption. It is an energy-intensive process in which steel, zirconium, glass, or ceramic beads spinning at high speeds collide, causing mechanical disruption of the microbial cells. The efficiency of disruption is a function of properties of microbial species like cell density and cell wall strength, flow rate, and bead type and size. Bead mill has been used at both lab and industrial scale. Despite high energy requirements for a large scale bead milling operation, bead milling is considered one of the most efficient and practical cell disruption techniques for large scale operations (Suarez et al. 2018; Grossman et al. 2019)

Homogenization

With high cell disruption efficiency and high energy efficiency, homogenization is one of the most commonly used methods for microbial cell disruption. Homogenization involves passing the biomass through small holes in a high shear environment with cavitation and turbulence facilitating cell disruption. It is superior to other methods like ultrasonication, PEF, and bead milling. The efficiency of homogenization depends on pressure, flow rate, the strength of the microbial cell wall, and the number of passes. It has been proven successful in large scale operations but requires high energy inputs.

Enzymatic cell disruption

Enzymatic cell disruption is a mild extraction technique; however, in some cases enzymatic cell disruption technique is not sufficient alone to achieve cell lysis. The composition of the cell wall of fungi determines the choice of enzymes used. Though enzymatic cell disruption requires low energy and mild operating conditions, the cost of the enzyme cocktails can make the cell disruption process cost-prohibitive (Amorim et al. 2020).

Other techniques

Autolysis can also be deployed as in the case of yeast to obtain yeast extracts. Autolysis utilizes the cell's enzymes to release the intracellular components. Thermal shock, osmotic shock and freeze-thaw are other techniques that can be used to rupture the cell wall.

While mechanical disruption techniques like bead milling and high-pressure homogenization are commonly used methods, it is important to ensure cooling systems are built into the system to avoid thermal degradation of intracellular components like proteins due to excessive heat generation during the cell disruption process. The choice of cell disruption technique would depend on the species and strains used, the morphology of the microorganism, target compound of interest, desired purity, economic constraints, operating volumes and scale, and relative efficiency of each of the cell disruption techniques with respect to each other.

Extraction and Purification

The steps involved in the extraction of a target compound from fungi depend on the properties of the target compound and the level of desired purity. For example, for extraction of a high-value compound like a human-growth hormone(HgH) from *Escherichia coli*, the desired purity of HgH is >98% and a particular peptide fraction is of interest. Hence, a series of chromatography techniques and gel filtration

are deployed to obtain a high purity human growth hormone (Ball and Finkelstein 1992). The extraction process will involve utilization of similar techniques if a particular growth factor needs to be extracted from a fungus for cultivated meat applications but since the desired purity is lower for food applications compared to pharmaceutical applications, complexity of extraction and purification processes will be relatively lower. If proteins are extracted for creating alternative meat, egg, dairy, and seafood products, it may be sufficient to obtain the protein fraction by precipitating the protein at the isoelectric point. The efficiency of protein extraction from fungi is impacted by the distribution of protein in the fungal cell. Extracellular proteins and proteins in cell walls will be lost during intracellular protein extraction.

Protein separation is typically based on the principle of solubility of proteins in water. The aqueous phase is enriched with protein, which is then dried or purified. The first step in protein separation is to solubilize protein in water. The solubility of protein depends on the strength of protein-protein interactions and protein-water interactions in the aqueous phase (Amorim et al. 2020). The ionic strength and pH of the aqueous phase impact the protein interactions. The higher the concentration of ions in water, the lower will be the solubility of proteins as salts will compete with proteins to remain solubilized due to common ion effects. It is well known that proteins precipitate at pH equal to their isoelectric point. Hence it is desirable to maintain pH values that are different from the isoelectric point to facilitate aggregate solubilization and hydration. Thus combining pH adjustments with cell disruption can be considered for protein extraction to improve protein yield. However, harsh conditions like high temperature and pH can also cause protein aggregation, degradation, and loss of functional properties which will impact the use of proteins in food applications like alternative meat, egg, dairy products.

Centrifugation is used to concentrate the protein in the supernatant while other insoluble components and insoluble proteins accumulate in the solid phase. At the lab scale, centrifugation is feasible. However, high energy requirements for attaining acceleration in the order of 10,000g and maintaining the low temperature at a large scale are challenging (Amorim et al. 2020). In centrifugation, pigments can adhere to the fine colloidal particles in the aqueous phase, thus imparting color to the protein concentrate. For food applications, this may be undesirable if the protein is used as an ingredient. Hence other techniques like filtration and ultrafiltration can be used in conjunction with centrifugation to obtain cleaner proteins. The protein can be separated out of the supernatant by means of membrane filtration, precipitation at the isoelectric point, ion-exchange chromatography, electrophoresis, or dialysis to get concentrated protein.

Extraction of protein from filamentous fungus *Metarhizium anisopliae* using freeze-drying followed by isoelectric point precipitation, gel electrophoresis or column chromatography has been described by Bridge et al. (2004). Similarly, Oshervor and May(1998) experimented with various buffer solutions to solubilize protein from *Aspergillus nidulans*. Li et al. (2018) evaluated various combinations of media for precipitation of proteins from mycelium and fruiting bodies of *Auricularia auricula*. For extraction of protein using isoelectric precipitation, the critical steps to maximize extraction efficiency include ensuring high protein solubility for preparing the supernatant, reducing protein loss during precipitation and centrifugation, limiting modification of proteins, and separating proteins from other macromolecules.

Yeast as a source of single-cell protein

There are very few companies focusing on using single-cell proteins (SCP) from yeast for alternative protein applications. However, due to the prevalent use of yeast in the alcoholic beverage industry and baking industry, yeast as a microorganism is not only a source of protein but also comes with years of established knowledge on production through fermentation with existing infrastructure. For alternative protein applications, yeast biomass or protein isolates with a neutral taste, color, and odor obtained by choosing the right strains from existing as well as novel strains created through breeding techniques will be a critical step for the development of the fungi value chain. With fairly well-established production systems and downstream operations, the focus on strain selection and process optimization for strains of interest will enable the utilization of yeast-based ingredients for the alternative protein sector.

The several advantages of using single-cell biomass like yeast as a source of protein compared to traditional sources of food especially animal-based sources include(Nasseri et al. 2011) -

1. Ability to manipulate microbial quantity as well as a composition by using different strains, species, nutrient media, and processing parameters
2. High efficiency of substrate conversion
3. High productivity or growth rate of biomass
4. No dependence on seasons and variation in atmospheric conditions

Low cell wall digestibility and high content of nucleic acid are the key areas that need to be addressed for making yeast-based proteins suitable for human consumption. Compared to bacteria, yeast is easier to harvest due to the larger size, has lower nucleic acid content and higher lysine content, and can grow in acidic pH. Yeast also has wider acceptance in food applications for human consumption due to traditional usage in the baking industry.

Overview of yeast production

In the past, petroleum-based hydrocarbon products like gas oil, methane, methanol, and n-alkanes were used as sources of nutrition for yeast and bacteria production for applications in animal feed. As oil prices increased and soy became a cheap source of protein, commercial production of yeast and bacteria using alkanes for feed applications became less lucrative. Methane and methanol continue to be explored as nutrient sources for single-cell protein but the input costs make them less competitive than current protein sources for both animal-feed and regular food applications. Ethanol was explored by the Amoco company for the production of food-grade yeast, *Candida utilis*, which is marketed as Torula for flavor enhancement and meat, egg, and dairy protein replacement. However, the cost of production using ethanol was high, leading to exploration of substrates like molasses, spruce wood and brewing by-products as sources of nutrition for Torula production (Ugalde and Castrillo 2002).

Utilizing organic by-products play a critical role in bringing down the cost of final biomass. Cellulose from agriculture can be utilized as a nutrient source. However, due to the polymeric form and presence of

other compounds like lignin, hemicellulose, and starch, pre-treatment using acid hydrolysis or enzymatic degradation is required to convert them into simpler sugars or monosaccharides and disaccharides. For food applications, the quality of raw material used is critical to prevent any chemical or microbial contamination during the production process. Hence, the source of raw material should be such that it shouldn't have any chemical residue which can be harmful for human consumption. Thus cellulose as a by-product from the paper industry is not suitable for SCP production from yeast.

Starch is one of the most abundantly available and relatively low-cost substrates. Rice, wheat, maize, cereals, cassava, etc. are rich sources of starch. The process developed by Symba in Sweden uses a two-step approach for the production of SCP. In the first step, sterilized starch is hydrolyzed by *Endomycopsis fibuligera* to produce simpler sugars. These simpler sugars are then fermented by *Candida utilis*.

Whey, which is rich in lactose, can also be utilized as a substrate for the production of lactose assimilating yeast like *Kluyveromyces marxianus* (Oura 1983, Moulin et al. 1983). Similarly, molasses which are by-products of the sugar manufacturing process and wastes from coffee-pressing can be utilized for the production of SCP from yeasts as well as multicellular fungi like Trichoderma sp. and Cellulomona (Humphery 1975).

Despite high growth rates during fermentation, the concentration of biomass in the solution is typically less than 5%. Typical concentration techniques include mechanical and membrane filtration, precipitation, and centrifugation. The equipment used for harvesting and dewatering processes can add to significant capital costs making production systems at scale uneconomical.

Cell disruption can be carried by three routes - physical, chemical or biochemical, and mechanical. Cell wall disruption is a critical step to improve the digestibility of biomass as well as make proteins accessible. Mechanical disruption techniques include crushing, grinding, high-pressure homogenization, and ultrasonication. Enzymatic techniques can also be used for partial or complete disruption of cell walls with the advantages that enzymes can be tailored for specific hydrolysis of cell walls of a particular species and that enzymatic hydrolysis is a mild technique not involving high temperature or pressure. However enzymatic hydrolysis can be a relatively slow process and the cost of enzymes can make the cell disruption process more expensive compared to mechanical disruption.

Removal of nucleic acids

Yeast contain up to 10% of nucleic acids in dry weight which is five times more than in an average mammalian organ. Consumption of nucleic acids leads to the formation of purines and pyrimidine bases resulting in the formation of uric acid. Accumulation of uric acid beyond the excretion capacity of the kidney leads to uric acid precipitation which can lead to kidney stones, gout, and liver damage. Hence, a reduction in nucleic acid content in SCP is required before using the SCP for human consumption and food applications. As per WHO recommendation, human ingestion of RNA from single-cell protein sources should be less than 2g RNA per day and less than 4g total nucleic acid per day.

Chemical and enzymatic methods in combination with thermal treatment can be used to degrade the nucleic acid present in SCP. Alkaline hydrolysis destroys RNA but also impacts the nutritional value and techno-functional properties of proteins. While chemical and enzymatic treatments can impact protein functionality and quality, extraction of nucleic acid using acidified alcohol, salt, acid, and alkali can add to an additional step for extraction. Alkaline extraction at high temperatures has been reported to give a high protein yield; however it can lead to the formation of toxic compounds like lysinoalanine. Another route is to utilize endogenous protease which can be activated under optimal conditions and lead to hydrolysis of yeast nucleic acids. However, this process also causes proteolytic degradation of proteins leading to lower protein yield. Alternatively, nucleases like pancreatic ribonuclease or fungal ribonuclease from *Aspergillus candidus* can be added externally to reduce nucleic acids in yeast species (Maul et al. 1970, Kunhi and Rao 1995).

In the next section, a case study of bakers' yeast is discussed in detail highlighting the typical steps involved in the production process including the upstream and downstream operations.

Case study: Production of baker's yeast

The species, Saccharomyces cerevisiae, has been used extensively in the production of ale and bread. Historically, bakers used yeast obtained from breweries which didn't perform well in the dough making process due to poor adaptation to local stressors like high osmotic pressure. Saccharomyces cerevisiae, also found in distiller's yeast, was difficult to separate from distillers' mash. Hence, the commercial production of bakers' yeast was established to meet the needs of the baking industry. Over the 20th century, the production of yeast shifted from anaerobic fermentation leading to a mixture of alcohol and yeast to aerobic fermentation to produce only yeast.

For yeast to divide and grow, nutritional components like carbon as an energy source and nitrogen, phosphorus, minerals (magnesium and trace minerals), and vitamins (biotin and thiamin) are essential. Typically cane or beet molasses are sources of carbon while inorganic compounds like ammonia, ammonium salts, urea, phosphates, or phosphoric acid are sources of nitrogen and phosphorus. The fermentation process is carried out in aerobic conditions through constant aeration and agitation. The fermentation occurs at a pH in the range of 4 to 6, a temperature of 30° C for periods of 8 to 20 hr. The solids concentration in the fermentation media is in the range of 4 to 6%. The downstream processing involves the preliminary concentration of solids to up to 20% through centrifugation followed by cooling and further concentration using mechanical filtration leading up to 30% solids concentration in compressed yeast cakes. Further drying processes can lead to up to 90 to 95% yeast solids.

Strains of bakers' yeast can be found in culture collection centers. While some companies in the alcoholic beverage industry possess proprietary strains, most yeast strains are publicly available for use since most commercially available yeasts don't have patent protection. However, some patents protect hybridized bakers' yeast strains (Reed and Nagodawithana 1991). Strain selection and strain

improvement can be the most research-intensive activity as each strain needs to be evaluated for performance concerning various aspects like yield, growth rate, substrate efficiency, etc.

Critical parameters for production

Nutrient media

Cane and beet molasses with fermentable sugar concentration between 50-55% and Brix of 80 is commonly used as a carbon source while maintaining pH in the range of 6.5 to 8.5. Nitrogen present in cane and beet molasses is not sufficient for the growth of yeast. Hence, ammonium salt, urea, and ammonia are fed additionally in the substrate. Cane molasses have 0.5 to 0.8 ppm of biotin which is sufficient for the growth of yeast. In case other sources like beet molasses are used, the biotin content needs to be adjusted by either blending with cane molasses or addition of synthetic biotin. L(+) - aspartic acid in combination with oleic acid can completely replace biotin. Other vitamins essential for the growth of yeast include pantothenate and inositol, which are already present in molasses. Additionally, the growth medium is supplemented with thiamin which is primarily added to improve yeast activity during baking. Other compounds like phosphates, sodium, sulfur, and calcium are present in sufficient quantities in molasses. However, magnesium needs to be supplemented in the form of magnesium salts. Trace elements like iron, zinc, copper, manganese, and molybdenum are also critical for the growth of yeast and all elements are present in molasses except zinc which can be added in the form of zinc sulfate.

Activators or growth stimulants providing vitamins or trace elements such as flour milling waste and plant growth factors like indolyl acetic acid are reported to improve the growth of yeast, though these are not used commercially. Sulfur dioxide and nitrites or nitrates can act as growth inhibitors above a certain concentration. Hence, the concentration of such inhibitors needs to be monitored to obtain optimal growth rates. Hydrolyzable materials like sugarcane juice, molasses, grape juice, date juice, wood hydrolysates, starch hydrolysates, etc. can serve as raw materials for yeast production. Hydrolysis of these materials results in formation of fermentable sugars like sucrose, maltose, glucose, fructose, and mannose.

Due to the improvement in sucrose extraction from cane juice, the fermentable sugar concentration in molasses has dropped from 50-55% to 40% while the concentration of inorganic components has increased. Coupled with the reduction in fermentable sugar concentration in molasses, the high biological oxygen demand from effluent streams adds to additional costs for waste management. Sources of starch such as corn can be considered but require an additional step of hydrolysis for the conversion of raw material to fermentable sugar which can be carried out by bacterial or fungal amylases.

Aerobic fermentation

Anaerobic batch fermentation of yeast follows a typical microbial growth pattern starting with an early lag phase where growth is followed by an exponential growth phase and final latent phase. This fermentation is used for the preparation of alcoholic beverages. On the other hand, aerobic continuous

fermentation involves exponential growth throughout the fermentation period. The doubling time for yeast biomass, also called generation time, is the specific growth rate constant that can be used to compare the rate of exponential growth under various conditions. Commercially, 'fed-batch' fermentation is commonly used for yeast fermentation where the substrate is added incrementally to prevent anaerobic respiration due to excessive glucose. The biomass is not removed continuously, hence the end-point of the process is achieved when the fermentation tank is full. Typically a 16 hours fermentation period is sufficient to achieve 8-fold biomass production and depending on tank size, the total fermentation period can last between 8 to 20 hours (Fries 1962).

An important parameter that needs to be monitored to ensure the fermentation is aerobic throughout the process is the respiratory quotient (RQ). The respiratory quotient is the ratio of the rate of carbon dioxide evolution and rate of oxygen uptake. RQ should be close to 1 to ensure that there is no ethanol production. Wang et al. (1977) mapped values of RQ corresponding to glucose respiration. The glucose concentration also needs to be optimal as higher concentration can lead to inhibition of respiration resulting in a drop in growth rate and a lower concentration of glucose can lead to lower growth rate. The respiratory quotient is 1 and the preferred growth rate is below 0.2. A growth rate higher than 0.2 would lead to ethanol and CO_2 formation and lower biomass production.

For aerobic fermentation, oxygen can be supplied in pure form or as hydrogen peroxide, or through air. Blowing air is the most common practice with advantages of ease of handling and lower cost. The rate of oxygen transfer from the air depends on the area of the interface between air bubbles and liquid, and the oxygen transfer coefficient. The efficiency of oxygen uptake depends on the extent of mixing and agitation in the system. Mechanical agitation improves the rate of oxygen transfer by reducing the bubble size, thus, increasing the interfacial area available for oxygen transfer. The addition of surface-active agents like anti-foaming compounds can impact the oxygen transfer coefficient as well. The rate of oxygen transfer needs to match the uptake rate of oxygen by yeast cells. Excessive oxygen uptake can lead to a shift in yeast metabolism leading to higher glucose consumption leading to ethanol formation while limited oxygen will lead to a lower growth rate. Hence the oxygen supply needs to be in the optimal range to allow for the aerobic growth of yeast. Typically oxygen concentration of 21-30% in the gaseous phase is optimal while controlling the flow rate such that RQ approaches 1 where ethanol production is negligible and biomass production is highest.

The yield coefficient is the weight of yeast solids produced per unit weight of the substrate used. The typical yield coefficient for bakers' yeast under aerobic conditions is in the range of 0.5 - 0.54 as reported by several investigators (Chen and Gutmanis 1976, Dellweg et al. 1977). Since anaerobic fermentation leads to the formation of ethanol, biomass production is low as indicated by the yield coefficient which is a range of 0.075. The substrate is utilized not only only for biomass production but also for the metabolic activity of cells. Under aerobic conditions, most of the substrate is consumed for biomass production while under anaerobic conditions, most of the substrate is consumed for metabolic activities leading to fermentation of sugar to ethanol. Wang et al. (1977) reported that 0.08g of sugar is utilized for metabolism per gram of yeast cell solids per hour during incremental feeding during fermentation.

pH and Temperature

While bakers' yeast is resilient to a wide hydrogen ion concentration, pH between 3.6-6 is preferred to prevent bacterial growth. The optimal pH range varies from 4 to 5 depending on strains used and growth rate. The growth rate of yeast below 20 ^{0}C is low and it increases up to the temperature of 35^{0}C and decreases at a temperature above 40^{0}C(White 1954). In practice, temperature ranging from 28^{0}C to 30^{0}C is optimal for yeast production.

Osmotic Pressure

High osmotic pressure is known for inhibiting yeast growth and the fermentation process. Hence, incremental feeding of fermentable sugar is better for growth compared to a batch process where sugar is fed in one go. Similarly, additional salts also impact the growth coefficient of bakers' yeast

Concentration of Yeast in the Fermentor

The maximum concentration of yeast solid biomass is a function of various constraints with respect to production in fermentation tanks. Typically lower concentrations are preferred as nutrients in the substrates become limiting factors at high biomass concentration. A concentration of above 20% yeast solid biomass would significantly impact the viscosity of the liquid leading to issues with pumping and transportation for downstream operations. At concentrations in the range of 10-20%, the oxygen transfer rates are likely to be impacted due to the viscosity of the liquid. Thus, energy requirements for achieving homogenous mixing and adequate oxygen transfer will increase. In practice, the final yeast solid concentration is in the range of 4-6%.

Sterilization

Sterilization of substrate is essential for any production process involving products used for food application. Molasses are typically diluted to 40° Brix and clarified to remove insoluble solids through filtration or centrifugation. The clarified molasses undergo sterilization under high-temperature in a heat exchanger and are allowed to cool down. The duration of heat exposure needs to be monitored to prevent the degradation of sugar and darkening of molasses. Other substrates can be fed to the fermenter without special treatments after meeting quality assurance parameters.

Fermentation tanks should be sterilized with appropriate clean-in-place (CIP) equipment and Good Manufacturing Practice (GMP) should be followed to ensure minimal contamination. Auxiliary equipment, pipes, valves, pumps, probes, instruments, sampling devices and air ducts should be also sanitized thoroughly (Reed and Nagodawithana 1991).

Heat transfer and contamination

Fermentation of baker's yeast is carried out at low pH which prevents contamination. Hence, fermentation tanks don't need additional pressurization making the costs of production of yeast lower than that of enzymes or antibiotics. Aerobic fermentation is an exothermic process with 3.5 kcal of heat produced per gram of yeast solids. Hence heat exchangers or internal cooling coils are required to

maintain the optimal temperature of 28-30° C. Edible surface active materials are required to mitigate foam formation due to aeration during aerobic fermentation.

Process control

Pure cultures of desired strains are inoculated from a vial in a lab to a sterile flask of broth which is subsequently transferred to a larger vessel and undergoes several fermentation stages of increasing volume. In fed-batch fermentation, a constant feed rate leads to a diminishing growth rate but productivity increases and is maximum towards the end of the process. Productivity is defined as the yeast solids products per liter of fermentation capacity per unit time. Typically the fermentation lasts between 10 to 20 hours corresponding to a 4 - 8 fold increase in biomass.

The fermentation process requires adequate controls in place to keep parameters like pH, temperature, and feed rate optimal for ensuring the production of biomass. While pH and temperature controls can be directly controlled by adjusting the differential from the desired set point of pH and temperature, the feed rate may need to be adjusted based on the respiration quotient and ethanol concentration in the fermentation broth. To maximize cell yield, the ethanol concentrations need to be kept below 0.1% and the respiratory quotient should be 1.

Harvesting of Yeast Cells

With an initial concentration of 3-4% of yeast solids, the biomass needs to be concentrated for efficient downstream utilization. The separation of yeast cells from the bulk liquid is carried out by centrifugation in a continuous nozzle type centrifuge with 4000-5000 g force leading to 18-20% solids concentration. The fermentation broth needs to undergo several passes to reach up to this concentration. The concentrated yeast solution, also called yeast cream, can be pumped and stored at 1° C - 4° C without impacting the viability of cells.

For further concentration up to 30% solids, filtration can be carried out using existing technologies like plate and frame filter presses, vacuum filters, or rotary filters. More concentrated yeast cakes can be obtained by the addition of salt and then carrying out filtration.

Emulsifiers are added to the yeast solids to allow downstream processing involving extrusion, and cutting for ease of packaging and transportation.

Drying Methods

For ease and low-cost transportation, drying yeast biomass to obtain >90% concentrated yeast solids has been a common practice. To maintain the viability of cells for use in baking, the temperatures during drying are kept under 40° C. However, if protein isolates have to be extracted, then the temperatures can be higher but not so high that there is a possibility of protein degradation.

Continuous belt tunnel dryers involve deposition of a layer of yeast cake on a conveyor belt which passes through several chambers where drying occurs through heat exchange with hot air with temperature ranging from 28° C to 42° C. Batch tunnel drying can also be carried out in a similar fashion if the volumes for drying are low. Fluidized bed dryers, commonly used in the pharmaceutical industry, utilize a perforated bed of wet solid particulates which are suspended in a stream of hot air, with temperature in a range of 100° to 150° C, blowing from the bottom. These dryers can operate in batch, continuous, or plug flow modes depending on the design of the fluidized bed dryer. Typically the drying period is lower for fluidized bed dryers, in the range of several minutes to 2 hours, compared to drying time for tunnel dryers, in the range of 2-4 hours. Drum drying involves blowing hot air through louvers on yeast cakes on the inner surface of a drum. The drying time for yeast is in the order of magnitude of 10 - 20 hours (Pharmapproach 2016).

Other conventional drying techniques can be applied for drying yeast for alternative protein applications as cell viability is not a concern. Most of the drying techniques are either energy-intensive or capital intensive. The most commonly used techniques are briefly described in this section.

Spray drying is a highly efficient, rapid, and energy-intensive method. It is commonly used in the food industry for the production of tea, coffee, soup, and whey proteins. However, since the biomass is exposed to high temperatures for a short period, it is critical to control operating conditions to prevent protein degradation.

Freeze-drying can also be employed to remove water via sublimation at low temperature and pressure. Although freeze-drying can improve protein stability and retain protein activity, it can lead to a higher concentration of salts in biomass or protein extract, which can result in the denaturation of proteins. (Amorim et al. 2020)

Other methods like oven drying, tray drying, vacuum drying, and incinerator drying are other available options. The choice of method used will depend on capital investment, operating costs, end-product application, downstream processes, the impact of the drying method on functional properties of biomass, and shelf life desired for the dried product.

Recent market developments

In this section, the companies focused on creating yeast-based proteins are described. Biospringer Lesaffre, a global supplier of natural yeast extract and yeast-derived ingredients, has launched Springer Proteissimo 101, a yeast-derived protein with applications in alternative meat and cheese products. This product was launched in October 2020 in Europe, the Middle East, and Africa. Springer Proteissimo 101 has 75% protein content with Protein Digestibility Corrected Amino Acid Score (PDCAAS) of 1 and contains all essential amino acids. The key advantages of this product include its clean flavor without any off-notes, favorable melt-behavior for creating cheese analogues, and its ability to improve chewability for creating meat analogues. Biospringer also offers yeast-based products that can impart chicken, beef, and cheese flavors in plant-based meat and cheese analogues. The technical datasheet

comparing the performance of yeast-derived proteins with pea protein and animal protein demonstrates the functional advantages of the yeast as a source of protein for alternative protein applications.

Angel Yeast, a China-based manufacturer, known for its yeast and yeast extract products is exploring new yeast strains to target the emerging alternative protein market. One direct application of yeast extract in the alternative protein sector is that it can regulate the beanie flavor and other off-notes while intensifying the meaty notes in plant-based meat products. The presence of nucleotides, amino-acids, flavor peptides, and small protein molecules that undergo the Maillard reaction to produce nitrogen-rich and sulfur-rich compounds help intensify the meaty flavor. While Angel Yeast is also looking into applications of yeast as a protein source for sports nutrition, there is scope to utilize their expertise in strain selection, yeast production, and flavor development for other applications in the alternative protein sector.

Among the limited companies that are developing yeast-based ingredients for alternative protein applications, More Foods is a unique Israel-based startup focused on product development to create alternative meat products using yeast as a source of protein.

Filamentous Fungi as a source of protein

While filamentous fungi are known to produce a wide range of products like antibiotics, organic acids, enzymes, and heterologous proteins, the most commonly known food application of filamentous fungi is the protein-rich product Quorn marketed by Marlow Foods. The key advantage of using filamentous fungi is the option to utilize the whole biomass for creating an alternative meat product using simple processing techniques that don't rely on traditional texturization technology like extruders.

Another under-exploited application of filamentous fungus is its ability to improve flavor, nutritional value, bio-accessibility, digestibility, and appearance of plant-based proteins through biomass fermentation (Gmoser et al. 2020). Companies like MycoTechnology have created a product called PureTaste which is a proprietary blend of pea and rice protein fermented with Shiitake mycelium to improve the flavor, functionality, bioavailability, and nutrition of the protein blend. This product can be used to create alternative meat and dairy products.

The case study of Quorn products and market developments in the utilization of filamentous fungi for alternative protein applications are discussed in detail in the subsequent sections.

Case study: Production of Filamentous fungi under the Quorn brand

Fusarium venenatum A3/5 was the first filamentous fungi used to create meat alternatives. The development of filamentous fungi as a source of protein by the British company Rank Hovis McDougall

(RHM) began in the 1960s. The strain was screened from a pool of 3000 different fungi over 3 years. It took 12 years of intensive research and testing to establish that the strain was safe for human consumption and get approval from the Ministry of Agriculture for utilization of *Fusarium venenatum* A3/5 as food in 1984. Various testing carried out as part of getting the approval included toxicology testing, animal feeding trials, human trials with 2500 volunteers, and storage stability. No adverse effects on animals or immunological response on humans were found. The UK Food Standards Committee coined the term mycoprotein as a generic name for food products derived from filamentous fungi. The filamentous fungi-based products from Marlow foods are labeled under the brand name 'Quorn'. Additional toxicological testing by AstraZeneca of Quorn products was carried out by the end of 1996 to get approval from the US FDA for the sale of Quorn products in the US (Sutton and Barr 2018).

There was growing interest in single-cell protein sources in the late 1950s due to the anticipated shortage of protein-rich foods by the 1980s. The lower cost associated with manufacturing, ease of harvesting and scale-up, and desirable essential amino acid profile made filamentous fungi an attractive option for exploration when RHM Research Centre was investigating the conversion of starch, coming as a by-product from cereal processing, to protein. Compared to other microorganisms, filamentous fungi were found suitable for further research because of the demonstrated use of *Rhizopus oligosporus* species in foods such as tempeh and the desirable organoleptic properties making it appropriate for food applications.

The manufacturing process of Quorn products involves several steps which in combination help to align and bind the fibers of mycoprotein together. To develop the characteristic texture of Quorn products, a series of steaming, chilling, and freezing processes help obtain the meat-like texture of Quorn products. Mycoprotein is made in several metres (40m+) high fermenters which run continuously for at least five weeks at a time. For the production of filamentous fungi at a large scale, a joint venture between RHM and Imperial Chemical Industries was established under the name of Marlow Foods Limited. While RHM had worked on developing the product, ICI provided the manufacturing facility including the fermenters available with them.

Manufacturing process

From a manufacturing standpoint, several configurations of liquid state fermentation setups were evaluated. Batch and fed-batch processes have already been used at an industrial scale for the production of various high-value products such as enzymes, antibiotics, and other pharmaceutical compounds. However, a continuous flow process was preferred for the production of *Fusarium venenatum* biomass due to the higher productivity and convenience of running the fermentation process up to 6 weeks.

Up until 1994, a 40 m^3 air-lift fermentor designed by ICI for growing bacteria was utilized for the production of *Fusarium venenatum* biomass with a capacity of 1000 tonnes of annual production of Quorn mycoprotein. Subsequently, two 155 m^3 air-lift fermenters were built, increasing the production capacity to 10000 to 14000 tonnes per annum (Wiebe 2002).

To start the process, the fermenter is inoculated with 50 g of biomass in a 5L of batch culture. The continuous production starts 4 days after inoculation. The airlift fermenter as described in earlier sections is designed in such a way that the gaseous phase including ammonia and sterilized air is input through a riser at the bottom of the fermentor. The gaseous phase is dispersed in the culture in the form of bubbles which allow for mass transfer. As the gaseous phase rises with the culture, the oxygen concentration reduces, and CO_2 concentration increases. At the top of the fermenter, CO_2 is released due to low pressure, and culture enters the downcomer where it is redirected to the riser and the circulation of hyphal filaments continues due to the relative difference in densities between the culture in the downcomer and the aerated culture in the rising section. Since this is a continuous process the biomass is removed after every 5 to 6 hours.

Process conditions and nutrient media

The nutrient media required for the growth of filamentous fungi includes nitrogen and oxygen from sterilized compressed air, ammonia, glucose, biotin, minerals, and other trace elements. All nutrients need to be sterilized to prevent contamination. The pH is maintained at 6 and ammonia flow is controlled to maintain this pH. The temperature range of 28-30° C is also maintained through a heat exchanger with external cooling coils to remove heat generated during biomass production. The CO_2 evolution rate indicates the biomass concentration and, thus, can be used to control the nutrient flow rate. The nutrient solution or the liquid phase are fed at a dilution rate in the range of 0.17 to 0.2 hr^{-1} to achieve the target maximum growth rate of 10-15 g/l (Wiebe 2002). Here, glucose is maintained in excess. The growth rate is maintained in this range to prevent the production of toxic secondary metabolites. Since this is a continuous fermentation process, the product is harvested continuously.

Post-processing and harvesting

Before harvesting the biomass, it is necessary to bring down the nucleic acid content of the biomass. As per WHO recommendation, human ingestion of RNA from single-cell protein sources should be less than 2g RNA per day and less than 4g total nucleic acid per day. The RNA content in filamentous fungi is in the range of 8% to 9% which limits the daily consumption of biomass to 20g. To overcome this limitation, the biomass is subjected to thermal shock by raising the temperature to 68° C for 20 to 30 minutes leading to disruption of ribosomes, activation of endogenous ribonuclease, and subsequent breaking down of RNA to nucleotides which get transferred to the culture broth through diffusion. The RNA reduction process leads to 1% (w/w) concentration of RNA and also leads to loss of protein.

Due to the filamentous nature of *Fusarium venenatum*, it is much easier to harvest it compared to other SCP sources like bacteria and yeast. After the thermal shock and diffusion of RNA into culture broth, mycelium suspension with an initial concentration of 1.5% (w/w) solids is heated to 90°C and centrifuged to get to a concentration of 20-30% (w/w) solids. The high-temperature extraction does not impact the fibrous texture or organoleptic properties of the products (Knight et al. 2001).

The pasty material obtained after harvesting is chilled to 4°C and undergoes a mechanical process leading to the alignment of filaments of fungi to form a fibrous structure. Other ingredients for color, flavor, and binding to stabilize the aligned fibers are added and the resulting material is steam-cooked for 30 mins and chilled. Depending on the final product, the material can be shaped using traditional food processing equipment and frozen for storage. Freezing plays an important role as the ice crystals bring the fibers closer to create bundles giving the product a meat-like texture.

After 1000 hours of continuous production which corresponds to close to 100 generations of *Fusarium venenatum* A3/5, the operation needs to be terminated to prevent the build-up of colonial mutants due to loss of genetic stability. While these mutants have the same chemical composition and nutritional value as the parent strain, the texture and morphological properties are different. The crumbly nature of the mutants makes them unsuitable for further production (Sutton and Barr 2018).

Initially, Quorn products were marketed as a healthy food with low fat, no cholesterol, and high dietary fiber. However, owing to the texture of Quorn products and early evidence from consumer surveys in the 1990s indicating that the UK population was moving towards lower red meat consumption, and an emerging vegetarian population in the younger generation, Marlow Foods started positioning Quorn products as meat replacers with products range including mince, chicken-style pieces, sausages, burgers, and ready meals.

Recent market developments

Though Marlow foods have dominated the utilization of filamentous fungi as a technology for providing meat analogues, several companies in this space have emerged in the last decade. One such company is 3F bio which is trying to address the production costs to bring down the final product prices. 3F bio has developed a proprietary patented technology for the production of mycoprotein which brings down the production cost by half by using an integrated, zero-waste fermentation process.

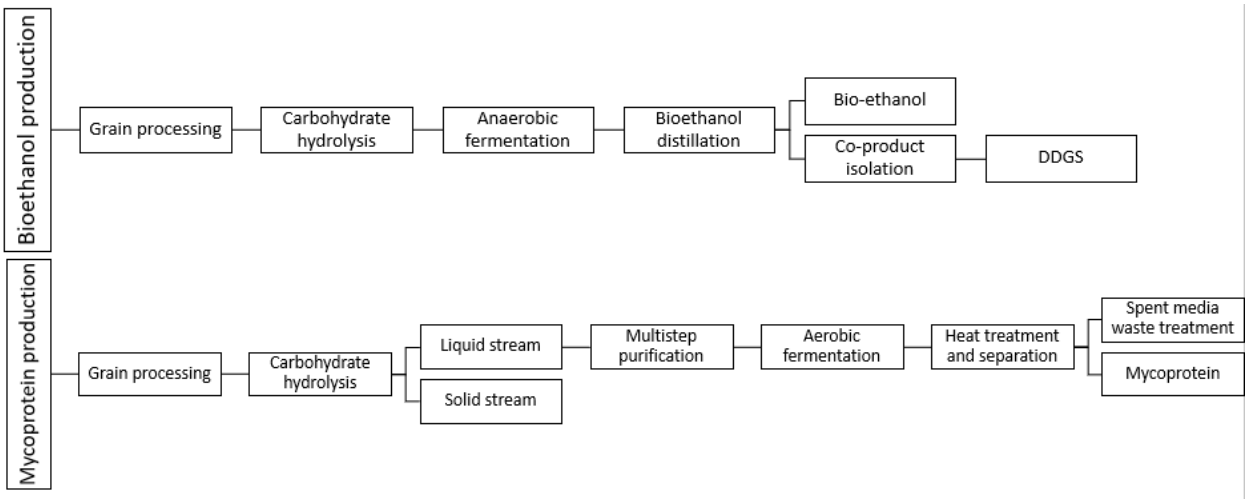

Figure 7. Typical process for bioethanol and mycoprotein production

The integrated technology combines the first generation biorefinery with traditional aerobic fermentation processes. The nutrient-rich byproduct, distiller's dried grains with solubles(DDGS), is produced in the first-generation biorefinery where cereal-based feedstocks are converted to bioethanol. Currently, DDGS is sold as animal feed. 3F bio has demonstrated mycoprotein production using grain starch hydrolysate, derived from cereals, which is used as feedstock for ethanol production. In the integrated process, about 5-10% of the feedstock is segregated from biorefinery feed for use in the production of mycoprotein. Typically the carbohydrate-rich waste stream is discarded after aerobic fermentation. However, in the integrated process, the output stream with glucose and nutrients is recycled back to the feedstock stream creating a zero-waste technology (Ritchie et al. 2017).

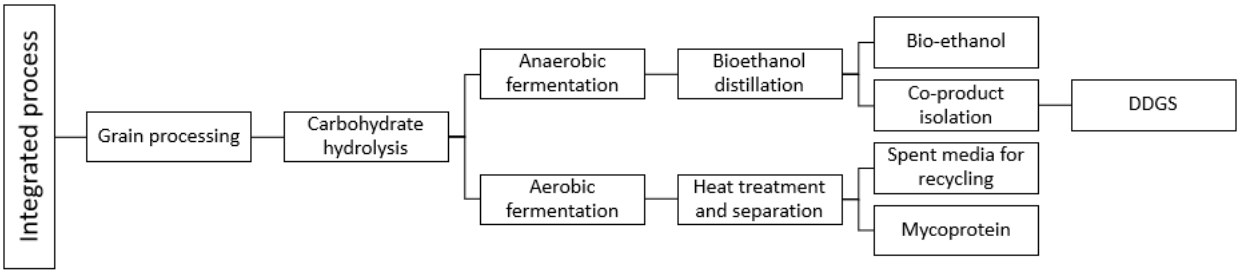

Figure 8. Integrated process for co-manufacturing of bioethanol and mycoprotein. Adapted from Ritchie et al.2017

The integrated process lowers the production cost by utilizing a low-cost feedstock, utilizing the recycled waste streams saving on effluent treatment costs, and allowing proximity to existing biorefinery facilities. Ritchie et al.(2017) offer insights into the sustainability benefits of this integrated process. It is known that greenhouse gas emissions associated with existing mycoprotein products are comparable to chicken, the integration process lowers this further by one third while also lowering the water and land footprint owing to high feed-conversion efficiency and zero-waste process design. The key barrier to the expansion of mycoprotein products has been the limited expansion of production capacity across geographies. The integration of the mycoprotein production process with existing biorefineries reduces the capital costs associated with expansion and diversification.

Another company that is creating meat analogues using filamentous fungi is Mycorena. Mycorena has created a neutral-tasting ingredient with a meat-like texture under the brand name Promyc. Their nutritional reports claim that Promyc has 60% protein and 12% fiber on a dry basis with all essential amino acids and about 26% of the amino-acids are branched-chain amino acids. In addition to this, the technical datasheet provided by Promyc shows that Promyc has a high water holding capacity, comparable to pea and soy protein, and foaming and emulsification capacity. Thus, Promyc can have potential applications in smoothies, cream, mayonnaise, meat and cheese analogues, and cakes. As per publicly available information on Promyc, the production process uses starch-like carbon sources and has the flexibility to use side-streams as feedstocks to enable a sustainable and cost-effective production system.

Protein brewery is another company which is utilizing filamentous fungi for creating proteins and focusing on bringing down costs through feedstock optimization. The company has created the product Fermotein, which has 47% protein content on a dry basis. The key differentiating factors for Fermotein include pleasant and less dry mouthfeel which can be attributed to water-binding properties and unsaturated fat, in-built meat-like flavor due to presence of 10% fat, no off-taste, no chemical treatment or processing, free of allergens, and high nutritive value due to an amino-acid profile close to meat and high fiber content.

Other applications of fungi in the alternative protein sector

While filamentous fungi and yeast have obvious applications in the alternative protein sector, there are emerging applications of fungi in creating whole-cut meats, mycelium-based scaffolding materials, and production of flavoring and stabilization agents.

The approaches for utilizing fungi for the development of scaffolding materials include the use of chitin/chitosan derived from the fungi cell wall and the use of mycelium for creating scaffolding. The use of chitin or chitosan-based scaffolds depicts biocompatibility (Tamura et al. 2011; Croisier and Jérôme 2013), antibacterial properties (Benhabiles et al. 2012), and accelerated healing rate on skin wounds (Tchemtchoua et al. 2011). The use of chitin for creating scaffolding has been reported in academic literature. Chitosan is formed by the alkaline deacetylation of chitin which impacts the physical, chemical, and biological properties of scaffolds (Rodríguez-Vázquez et al. 2015). The degradation of chitosan depends on the degree of deacetylation. This property can be used to customize scaffolding material used as per desired application. Biodegradable chitosan with a controlled degradation rate can act as a temporary matrix for cell adhesion (Berthod et al. 2006; Bitar and Zakhem 2014; Ren et al. 2018). Thus, chitin or chitosan-based scaffolds can be a potential substrate for cultivated meat applications. Rubio et al.(2019) have described chitosan as a promising scaffolding material for cultivated seafood production as it is edible, is well-referenced in tissue engineering research, is an accessible biopolymer, and can be cast as membrane, hydrogels, and sponges (Croisier and Jérôme 2013). Also, fungal chitosan is non-allergenic and approved for human consumption as a food additive or nutrition supplement (Pochanavanich and Suntornsuk 2002; Wu et al. 2004; Nitschke et al. 2011). While growing cells on chitosan is possible, the lack of recognition motifs that signal cell adhesion and migration limits their functionality. However, this limitation can be overcome by modifying the properties of chitosan by blending it with other polymers to resemble native tissue (Campuzano and Pelling 2019).

The majority of chitin production relies on the byproduct from the shellfish industry. Thus, there is an opportunity to leverage fermentation platforms to obtain scalable production of non-animal fungi-based chitin which can then be utilized for cultivated meat applications. Identifying species and strains that can produce high quantities of chitin and developing appropriate downstream processing steps to valorize different fungal components including chitin will play a key role in bringing down the cost of

fungi-derived chitin and demonstrating the viability of animal-free chitin production systems for cultivated meat applications. Chitin derived from *Aspergillus niger* and *Pleurotus ostreatus* is already sold in the market. Fungi-derived chitin has a key advantage of being allergen-free compared to crustacean-derived chitin.

The fungal mycelium can also be utilized due to its textural profile for building the scaffolding matrix. The textural profile of mushroom/mycelium can provide a desired mouthfeel and umami flavor associated with meat. Excell™ Scaffolding, created by Ecovative's Mycelium Foundry One, is an edible cell scaffold that can be used for the development of whole-cut, cultivated meat products. Mycelium is used to grow an edible 3D matrix of microstructure matrices to support the adhesion of cells and the growth of cells into differentiated tissues. Similarly, Myoworks, an India-based startup is developing an edible scaffolding technology from mycelium for cell adhesion, growth, and proliferation for cultivated meat applications. More open access research focused on exploring various species and strains optimized for creating edible scaffolding materials from fungal mycelium would help accelerate the development of cultivated meat and seafood products.

The creation of whole-cut meats from mushrooms is also an interesting application of solid-state fermentation for creating fungi-based alternative meat products. *Laetiporus sulphureus*, also known as the chicken of the woods, is popular for its chicken-like taste when cooked. *Hericium erinaceus*, also known as lion's mane, is known for its close resemblance to various kinds of seafood like lobster and crab. *Hypomyces lactifluorum,* also known as Lobster mushroom, is known for tasting similar to lobster or shrimp. Lobster mushroom is not a mushroom, but a parasitic ascomycete fungus that feeds on other mushroom species and distorts their shape and color. *Pleurotus ostreatus* or oyster mushroom is known for tasting like scallops and bacon upon cooking. *Russula xerampelina*, also known as the crab brittlegill or the shrimp mushroom, and *Entoloma abortivum*, also known as the shrimp of the woods, have a taste profile similar to shrimp. These taste similarities are reported on various food blogs. Mushrooms, in general, have been recommended as vegan substitutes to meat and seafood in various food recipes. Given these taste and textural similarities of various mushroom species with meat and seafood products, there is a scope to scientifically map the various edible mushroom species that mimic meat and seafood products and evaluate the feasibility of using both traditional mushroom cultivation techniques and modified solid-state fermentation techniques to grow whole-cut alternative meat and seafood. Also, leveraging culinary expertise from chefs would aid species selection and development of post-processing techniques to create mushroom-based alternative meat and seafood products. Atlast Food Co., a spinout from Ecovative, is utilizing solid-state fermentation to produce whole-cut meats. Other companies like Savory Wild, Pan's mushroom jerky, Jewels of the Forest, etc. are selling mushroom jerky as vegan substitutes to meat. While the market for mushroom jerky products is relatively small compared to whole-cut meats, these products can help build consumer awareness about the versatile applications of mushrooms in the alternative protein sector. Also, less sophisticated alternative meat products created using mushrooms open up opportunities for local mushroom producers and restaurants to bring these products to consumers at a premium price.

FotorTec, a Chile-based startup, has created several ingredients from oyster mushrooms with different applications in the alternative protein sector. F1 flavor enhancer is one such ingredient made out of dehydrated edible mushroom which amplifies the umami flavor without drastically impacting the sodium content in the food product. Other ingredients that Fotortec is exploring include a texture enhancer and mushroom-derived protein isolate which can be used for creating desired alternative protein products.

Yeast extract, available in the market derived from *Saccharomyces cerevisiae*, is known for its umami flavor due to the presence of umami imparting amino acids, 5′-nucleotides, a large range of proteolytic enzymes, and protein hydrolysates (Alim et al. 2020; Festring and Hofmann 2010; Nagodawithana 1992; Velíšek et al. 1978). The meaty flavor and umami taste from yeast is a result of hydrolysis accelerated by thermal treatment of proteolytic enzymes present in yeast (Ruiz et al. 2016; Ogasawara et al. 2006). Flavor production using yeast has been extensively studied particularly for the alcoholic beverage industry. Consumer preference for natural flavors is favorable for the development of flavors using yeast for alternative protein applications. Identifying strains from a pool of existing as well as new strains created using breeding techniques that can produce desired volatile compounds will be a critical step in the utilization of yeast for targeted flavor development.

Plant-based protein sources like soy and pea protein suffer from the disadvantage of retaining off-flavors which connote beany and green taste due to the presence of aldehydes, ketones, furans, and alcohols. Fermentation of these proteins is one of the approaches to reduce these off-flavors. Youssef et al.(2020) have demonstrated that a combination of lactic acid bacteria with various yeast species including *Kluyveromyces lactis*, *Kluyveromyces marxianus*, and *Torulaspora delbrueckii* to ferment pea proteins led to a significant reduction in green notes of pea proteins as indicated by a sensory evaluation. The case of MycoTechnology using Shiitake mycelium to ferment a blend of pea and rice protein to improve the flavor, functionality, bioavailability, and nutrition of the plant-based protein demonstrates the viability of fungi-based fermentation as a technique for off-flavor reduction for alternative protein applications.

Fungal protein functionality and nutritional properties

For fungi-derived proteins to compete with existing protein sources, the nutritional profile, digestibility, and functionality of these proteins need to be either the same or better than animal or plant-based proteins. A key indicator of protein quality is its amino acid profile. Since not all amino acids are produced in the human body, the essential amino acids (EAA) need to come from external protein sources as part of the food. The protein quality from fungi depends on various intrinsic and extrinsic factors such as choice of fungal strain, production system, growth media, and nutrients supplied, and downstream processing.

Depending on the species used, fungi contain a protein content ranging from 15% to 50% on a dry weight basis, all essential amino acids, high dietary fiber coming from the chitinous cell wall, high

vitamin B content, low fat, and no cholesterol. The crude protein content in edible mushrooms varies depending on species and stage of development (Longvah and Deosthale 1998). Proteins in edible mushrooms are rich in glutamic acid, aspartic acid, and arginine. The reported essential amino acid profiles of edible mushrooms show that mushrooms have a relatively lower content of sulfur-containing amino acids like methionine and cysteine but are rich in threonine and valine (Cheng 2008). Though the free amino acid content in dried edible mushrooms is low, the presence of aspartic acid and glutamic acids is responsible for the umami flavor from mushrooms (Mau et al. 2001.). Mushrooms are a good source of vitamins such as riboflavin (vitamin B2), niacin, and folates. The vitamin B2 content in mushrooms is higher compared to vegetables. Some varieties of *Agaricus bisporus* have a vitamin B2 content as high as in egg and cheese (Mattila et al. 2001). A compilation of the nutritional content of all edible mushroom species will add to an additional data point for the screening of species for SSF to create whole-cut meat products.

Protein source	PDCAAS	Complete EAA
Mycoprotein	0.996	Yes
Chicken	1	Yes
Eggs	1	Yes
Milk	1	Yes
Beef	0.92	Yes
Soy	0.91	Yes
Pea	0.89	Yes
Fish	1	Yes
Hemp	0.61	Yes
Beans	0.68	No
Chickpeas	0.78	No
Cashew nuts	0.9	No
Wheat	0.42	No
Rice	0.44	No

Table 3. Comparison of bioavailability and essential amino acid profiles of various protein sources.

When screening strains and optimizing production systems for fungi, the amino acid profile should be targeted such that it meets the FAO reference standards on the recommendation of essential amino acid content in a protein mix. It would be advantageous to have amino-acid profiles superior to proteins derived from eggs and soy. This can be evaluated using the Essential Amino Acid Index (EAAI), which relates the content of each essential amino acid in a protein to the amino acid content in egg protein. Apart from the amino-acid profile, protein bioavailability and digestibility are crucial parameters that indicate the absorption of nutrients in the human body. The Protein Digestibility Corrected Amino Acid Score (PDCAAS) is an index recommended by FAO in 1989. However, Digestible Indispensable Amino Acid Score (DIAAS) was recommended in 2013 as a more precise digestibility index than PDCASS as DIAAS considers the digestibility of each essential amino acid separately while PDCAAS takes into account protein digestibility in the form of nitrogen. Studies reporting the EAAI and PDCAAS scores of

fungi-based proteins are limited to mycoprotein and few species of edible yeast and mushroom. The nutritional profiles of edible filamentous fungi-based products like Quorn and Promyc are available in the public domain.

The PDCAAS for mycoprotein as reported in scientific literature is 0.996, which is close to PDCAAS for chicken and milk and the mycoprotein contains all essential amino acids making it a complete protein source (Edwards and Cummings 2010). The bioavailability of mycoprotein has been demonstrated to be similar to that of milk protein and significantly better than wheat-based and soy-based protein. According to a recent study conducted on a group of healthy young men, the ingestion of 60g of mycoprotein created an optimal blood amino acid response for muscle protein synthesis (Fairweather-Tait and Southon 2003).

	Promyc	Quorn	
Energy	350	360	kJ
Protein	15.07	11.5	g
Energy	85	86	kcal
Fibre	3.08	6	g
Fat	1.49	2.9	g
Saturated fat	0.34	0.6	g
Monounsaturated fat	0.4	0.5	g
Polyunsaturated fat	0.68	1.8	g
Carbohydrates	0.94	1.7	g
Sugar	<0.30	0.8	g

Table 3. Nutritional composition of mycoprotein per 100 g (wet weight)

Mycoprotein contains dietary high fiber composed of polymeric n-acetyl glucosamine (chitin) and beta 1-3 and 1-6 glucans, in the range of 12-20% by dry weight. Studies have demonstrated that consumption of this type of fiber can provide relief from joint pain in osteoarthritis, stimulate beneficial bacteria in the colon and improve glycemic profile (Müller et al. 2003; Bottin et al. 2016; Turnbull and Ward 1995). In addition to this, mycoprotein fiber can impact satiety and lower cholesterol. According to a study with a test group, individuals who consumed mycoprotein for lunch ingested up to 24% fewer calories in dinner the same day and the following day. This feeling of fullness after a mycoprotein consumption is attributed to the protein and fiber content in mycoprotein. Hence, a mycoprotein diet can impact hunger and total calorie intake making weight loss easier (Turnbull et al. 1990; Turnbull et al. 1992; Bottin et al. 2016; Turnbull et al. 1993).

The availability of detailed nutrition information on mycoprotein is useful to create consumer acceptance, build the credibility of these alternative protein products, and create claims which demonstrate superiority over animal-based food products. More academic research needs to be conducted to generate evidence that incorporating fungal whole biomass or fungi-based proteins for the creation of meat analogues is not only feasible but also beneficial from both a nutritional and

sustainability standpoint. Bioavailability, bioaccessibility, and bioactivity of proteins or proteins in whole biomass need to be studied to evaluate their impact after human consumption. Upcoming studies should also focus on the impact of extrinsic factors on protein quality and evaluate the digestibility of both whole biomass as well as protein extracts from fungi for a fair comparison with plant-based proteins.

The techno-functional properties also determine the quality of protein when used as an ingredient. For ingredient usage, neutral taste, color, and odor profiles are critical for blending into the food product. In addition to the organoleptic properties, the proteins should also be soluble in water and, depending on applications, display functionalities like water-binding capacity, emulsification, structure building, foaming, and gelation. For alternative meat products, functionalities like water holding capacity, gelation, and emulsification are very important whereas, for egg and dairy applications, functionalities like solubility, emulsification, and foaming become the key. Further work needs to be done to evaluate the techno-functional properties of protein isolates and protein concentrates from fungi.

Decentralized fungi production for low-income communities

The traditional applications of fermentation at a household level discussed in earlier sections demonstrate that it is possible to create fungi-derived products at a small scale. Even today, cheese-making and wine-making operate at various scales and small-scale local production is common for these processes. Kombucha preparation using a symbiotic culture of bacteria and yeast (SCOBY) at a household scale is a known practice. While liquid state fermentation may require some degree of process control, solid-state fermentation as in the case of mushroom cultivation can be easily scaled out and facilitate income generation for small-scale producers.

Scaling down and decentralization of fermentation can be leveraged to not only cater to various application areas in the alternative protein sector but also meet nutritional needs in low-income, resource-scarce, isolated regions where protein deficiency is a key concern due to limited access to high-quality affordable protein sources. Fungi is an excellent choice for the production of proteins due to their ability to transform readily available carbohydrates (including simple sugar, of which India produces a glut), into proteins and provide biomass with all essential amino acids, high dietary fiber, high vitamin content, low fat, and no cholesterol.

Local production of fungi through small-scale decentralized units offers the flexibility to utilize a wide variety of feedstocks including those coming from side streams from agriculture and food processing, as well as excess unsold produce. As a spillover effect, this will facilitate income generation for farmers and small-scale producers while also helping meet the needs of the local community.

For such a model to be successful, an upstream strategy needs to be developed to ease the process of technology adoption and knowledge transfer to local producers. Strains need to be optimized for local environmental conditions and available feedstock to obtain maximum productivity. Strains can also be optimized to meet the specific nutritional requirements of the community. To ensure safe operation for a liquid-state fermentation unit, training for operating, maintaining, and cleaning small-scale bioreactors needs to be provided. The design of the setup should be such that it leads to no contamination and minimal human touchpoints for handling the biomass. Operating protocols need to adhere to good manufacturing practices. To ensure food safety and quality, training personnel for microbiological and chemical assessments is also critical. Recipes relevant to the community's culinary preferences should be co-developed to ensure the adoption of the food product by local consumers.

Mushroom cultivation is a technologically mature practice with policy support from the government to not only train farmers but also provide subsidies. While scaling out protein production through mushroom cultivation is viable, such examples for liquid-state fermentation don't exist. Hence, the commercial viability and business model for scaling-down liquid-state fermentation need to be explored further. However, given the immense potential of fermentation as a platform to meet the nutritional needs in remote areas and protein deficit communities, decentralization should be investigated as a preliminary step to make alternative proteins accessible and affordable to the bottom of the pyramid.

Insights from closed door roundtable panel discussion

The utilization of fungi for alternative protein applications in India requires advancements across the value chain from strain selection to end product development. Irrespective of application areas, the ecosystem in India is at a nascent stage. Be it using whole biomass of filamentous fungi to make meat analogues or using yeast-based flavors or using mushroom and yeast-derived proteins, applications of fungi to create alternative meat, egg, dairy and seafood products is an emerging field globally. Hence, there are several opportunities for stakeholders across academia, industry, and startups at each step of the value chain to accelerate the development of alternative protein applications of fungi. The roundtable discussion conducted by GFI India brought together experts from diverse backgrounds ranging from industry experts with rich experience in the Indian biotechnological and pharmaceutical space to innovators working on novel applications of fungi for alternative protein applications.

The panelists shared their insights on several key opportunities identified as a part of our analysis. With more than 6 million species of fungi, strain selection was identified as a crucial step in identifying species by screening through filters such as nutritional value, amino acid profile, RNA content, toxicity, potential to accumulate heavy metals, allergenicity, organoleptic profile, etc. Many strains isolated from food products are available at culture collection centers like CBS-KNAW culture collection at Westerdijk Fungal Biodiversity Institute, VTT Culture Collection in Finland and the Microbial Type Culture Collection and Gene Bank (MTCC) in India. These strains can be procured under material transfer agreements for

producing various food ingredients. One of the panelists shared how they screened several gourmet mushroom species to obtain strains for production of mycelium biomass for creating dairy analogues and highlighted the advantages associated with umami flavor coming from these edible mushroom species and ease of scalability of mycelium production systems.

Panelists agreed on the need for bringing down the cost of production of fungal biomass using liquid-state fermentation. Though filamentous fungi-based meat analogues have existed since the 1990s, the price parity with meat products is yet to be achieved. The cost of end products is not competitive with conventional meat products with key cost barriers including raw material or feedstock costs and the high capex infrastructure required for large-scale production of biomass. The use of side streams from the food industry as feedstock can be instrumental in bringing down end-product costs. Simplifying the production systems required for the manufacturing of biomass is another route to bring down initial capital investment.

The panelists pointed out that the current technological advancements in bioreactor design for the pharmaceutical industry can be adapted to meet the requirements for liquid-state fermentation for food applications. Depending on the desired end product and host microorganism, the existing designs and processes can be modified locally to achieve high growth rates, efficient feedstock utilization, and high protein content. Similar to the challenge in the biopharmaceutical sector, the challenge for food production using microbial hosts is manufacturing target compounds in a cost-effective fashion. Process innovations including the use of perfusion bioreactors have allowed for high productivity in biopharmaceuticals, which can be explored for food applications as well. Also, the quality and purity of target compounds like edible proteins or enzymes need not be as high as desired in pharmaceuticals. Thus, there is potential to lower the relative cost of downstream processing of microbial biomass for food applications. One of the panelists shared that process control, reactor design, and scaling above 50,000L volume are key focus areas for companies exploring novel applications of microbes in the alternative protein sector. The manufacturing capacity for fermentation applications in India is already exhausted because of demands for new vaccines and pharmaceutical products during the pandemic. Even if spare capacity was available for fermentation, the capacity would need to be adapted for food applications making it challenging to directly utilize existing capacity for alternative protein applications. Hence, for food applications, new fermentation capacity needs to be established and made accessible to entrepreneurs to exploit fermentation-derived ingredients for the alternative protein sector.

While there is some academic literature reported on the extraction of proteins from fungi, one of the panelists indicated the need to focus on mild processing techniques such that functionality of fungi-derived proteins is not lost during extraction. Focusing on functionality for specific applications in the alternative protein sector would allow efficient utilization of proteins and other value-added components and help meet the organoleptic properties desired by consumers. The development of downstream processing capability is a crucial step for companies exploring fungi-derived ingredients. Panelists emphasized the need to have accessible downstream processing units which can be utilized on a contract basis by companies involved in upstream production of microbial biomass. The panel members discussed how decentralization of various processing steps across the value chain will enable

companies to operate seamlessly to create end products without the need to heavily invest in entire infrastructure for upstream processing, downstream processing, and product development.

Additionally, one of the panelists mentioned the potential to utilize fungal proteins along with traditional protein sources like soy to extrude proteins under dry and wet conditions to obtain a textured meat analogue. Another panelist described challenges with the extrusion of yeast-derived proteins and the need to access yeast biomass with less strong flavor profiles to enable alternative protein product development. The cost of yeast-derived proteins is currently 3 times more than soy protein isolate. Hence there is a scope to bring down the cost of processing yeast-derived proteins as well as explore combinations with existing protein sources to enhance the organoleptic profiles of end products. One panelist pointed out that the evaluation of fungi or yeast-derived ingredients is currently carried out individually by producers of microbial biomass. However, for the development of microbial ingredients for the alternative protein sector, there is a need to address this duplication on ingredient evaluation. Third-party labs or organizations which can carry out functionality testing assays, ingredient evaluations, and quality testing will free up the capacity of companies focusing on upstream biomass production and act as a bridge between ingredient providers and product developers

Panelists also discussed the potential of fungi-derived ingredients in enhancing flavors, production of growth factors and enzymes. Yeast-derived flavors are already used for enhancing the umami flavor of alternative protein products. There is scope to utilize filamentous fungi for enzyme production to aid hydrolysis and reduce fermentation time. The various textural and organoleptic properties associated with a variety of meat products like beef, chicken, lamb, etc can be mapped to different enzymes and amino acid components which can be further derived from filamentous fungi. Apart from enzymes, growth factors for cultivated meat applications can also be manufactured using fungal host systems.

Safety and regulatory landscape in India

Food Safety and Standards Authority of India (FSSAI) is the apex body that controls food safety and standards in India. This is the autonomous regulatory body that takes requests for pre-market review of products that do not have a history of safe consumption in India. The Food Safety and Standards (Food Product Standards and Food Additives) Regulations 2011, has standardised the uses of edible fungi products. These standardised products come under the broad category of “edible fungi products” and have different sub-categories with general and specific requirements for each of them.

Fungi in food applications as per FSSAI

This regulation covers various products prepared from fresh edible fungi such as-

- freeze-dried fungi,
- fungi grits,
- fungi powder,
- pickled fungi,

- salted fungi,
- fermented fungi,
- fungi in vegetable oils,
- quick-frozen fungi,
- sterilized fungi,
- fungi extract, and
- fungi concentrate and dried fungi concentrate

The terms "fungi" may be replaced by the terms of the genus or species of the fungi, e.g. "mushroom" or "mushrooms" for the genus Agaricus. The method of processing to which the product has been subjected, e.g. "dried", "sterilized" or "quick-frozen", shall be indicated on the label. The regulation has specific sections for the general requirements such as the style (form and presentation of the edible fungi), composition and specific requirements for each product such as water content, mineral and organic impurities and permissible limits of damage. The usage of yeast and baker's yeast glycan is regulated and can be used according to Good Manufacturing Practices (GMP) Limits. Baker's yeast glycan is regulated for use as a gelling agent, stabiliser and thickener in food applications such as bakery products, meat products, seasoning and flavourings, and protein products that are not derived from soy. Fungal alpha-amylase is listed as an 'improver' food additive for bread products with 100 ppm as the limit. There are specific limits placed on the amount of yeast and yeast mould spores that can be found in food products concerning the hygiene and safety of that product. Pimaricin (natamycin) can be used as a preservative in dairy and dairy analogue products upto 40mg per kg.

Innovating with other forms of fungi

Under the Food Safety and Standards (Health Supplements, Nutraceuticals, Food for Special Dietary Use, Food for Special Medical Purpose, Functional Food and Novel Food) Regulations, 2016, *Grifola frondosa* extract from Maitake mushroom and Shiitake mushroom's extract is recognised as a nutraceutical and may be used as such in food applications.

For food manufacturers or food importers who want to manufacture or import articles of food or food ingredients consisting of or isolated from microorganisms- yeast or fungi, such as using filamentous fungi, mycoprotein or use fermentation derived foods must first apply for pre-market product approval for the non-specified food product. The food manufacturer or importer must apply under the Food Safety and Standards (Approval for Non-Specified Food and Food Ingredients) Regulation, 2017 with the required documentation for the authorisation of the non-specified food. The application made under Form 1 requires general details such as name of the organisation, nature of business, name of the food product, name of ingredients used, the proposed category which the product will fall under, source of the food ingredient, genus and species of the fungi used, functional use and intended use of the product, a certificate of analysis from a nationally accredited laboratory (the list of accredited laboratories can be found here), manufacturing process in brief, regulatory status of the food product or food ingredient globally such as the Generally Recognised As Safe (GRAS) or approval from the European Food Safety Authority & if previously obtained, safety information and toxicity studies. Additional information is required as 'specific details' for the application with details such as the nature of microbe,

genus/species/strain of the microbe, if it is locally sourced or imported, if it is collected and stored in any national collection centre and details regarding the collection centre and the GRAS status of the ingredient which can be furnished to expedite the non-specified food pre-market approval process. After getting this approval only can entrepreneurs apply for the FSSAI license.

Other Authorities

The National Biodiversity Authority (NBA) controls the access to biological resources for commercial utilization. The NBA or the State Authority is established under the Biological Diversity Act 2002. Indian companies interested in commercialising species under the NBA need to get prior permission from the NBA. The applications for approvals under NBA depends on the purpose of the application, and the entity who seeks permission for the access to the biological resources. The forms with the fees can be found here. The Act also mandates that the commercial activity's earnings be shared with the local community as a part of the Access Benefit Sharing system (ABS). Prior information may have to be provided to the State Authority for commercial utilization including bio-survey and bio-utilization of biological resources by Indians (individuals/entities).

Testing and Safety

For food testing and analysis FSSAI recognizes and notifies National Accreditation Board for Testing and Calibration Laboratories (NABL) accredited food laboratories and also recognises foreign laboratories to reduce the time in clearance of food consignments at ports. FSSAI approved notified laboratories with updated NABL accreditation validity and a list of referral labs in case of appeals can be found here.

The FSSAI regulations prescribe that the edible fungi products must be clean, undamaged, free, as far as possible, of maggot damage and possess the flavour and taste appropriate to the species. Additionally, there are limits placed on the amount of damage that is permissible in these products. It is stated that Good Agricultural Practices (GMP) certification and NABL compliance is to be availed to assure compliance with regard to pesticide/chemical residue for the National Horticulture Board.

Import and Export

Most items are freely exportable unless they fall into the restricted/prohibited list. Under the Agricultural & Processed Food Products Export Development Authority (APEDA), mushrooms are freely exportable. Food Products that are meant for 100% export only, must be prepared as per standard/specification and the labelling requirements of the importing country and cannot be sold/supplied for domestic consumption. A No Objection Certificate (NOC) is imperative to export food, and it will be issued after providing the following information:

- Company Information;
- IEC that is issued by the Directorate General of Foreign Trade;
- Food Business License by FSSAI State/Central Authority ;
- Requisite food safety tests and labelling compliances as laid down by the importing country for the goods to be imported after which NOC will be issued.

- Depending on the individual products, other documents may be required. Notices and regulations regarding the export of agricultural products can be found here.

The general requirements to import food and food ingredients to India are to register as an importer and gain the import-export code from the Directorate General of Foreign Trade. FSSAI's Import Regulation 2017 also lays down specific rules for shelf life, packaging and labelling requirements, storage of imported food, safety requirements and sampling of imported food. If the Authorising Officer is satisfied, a NOC is assigned.

The following species of edible truffle fungi have been approved for import: *Tuber melanosporum Vittadini, Tuber aestivum Vittadini, Tuber uncinatum Chatin, Tuber mezsentericum Vittadini, Tuber magnatum Pico, Tuber macrosporum Vittadini, Tuber gibbosum Gilkey, Tuber borchii Vittadini, Tuber brumale Vittadini, Tuber indicum* and *Geopora cooperi*.

National Duties

Mushroom spawn, mushrooms of the genus *Agaricus* which are fresh or chilled and other mushrooms & Truffles are regularly exported with a low GST Rate. But mushrooms prepared or preserved by vinegar or other preservatives face 12-18% GST which may be a financial burden for entrepreneurs looking to export prepared mushroom products and the end consumers looking to try out other mushroom products. The HSN code for edible fungi products is covered under 2102, 210210, 21021010, 21021020, 21021090, 21022000.

Global Overview

In the United States, the Food and Drug Administration (FDA) provides approvals for food ingredients that may be food additives for specific uses as GRAS (Generally Recognised as Safe) substances. Food additives and ingredients are given based on a history of safe use after being determined as safe by qualified experts. Mycoprotein is recognised by the FDA as GRAS and seven mycoprotein fermentation based products were launched in the United States under the trade name Quorn (Denny et al. 2008). Other companies using fermentation technology include 3F Bio, Protein Brewery & Mycorena.

The following is a list* of food additives derived from fungi have attained the FDA's GRAS approval and can be referred to during pre-market approval required in India -

- *Alpha-galactosidase* derived from *Mortierella vinacea var. raffinose utilizer* for use in the production of sucrose from sugar beets
- A solvent extraction process for recovery of citric acid from *Aspergillus niger* fermentation liquor
- Bakers Yeast extract from *Saccharomyces cerevisiae*
- *Bakers yeast glycan* from *Saccharomyces cerevisiae*
- Bakers yeast protein derived from *Saccharomyces cerevisiae*
- *Candida guilliermondii* as the organism for fermentation production of citric acid
- *Candida lipolytica* for fermentation production of citric acid

- *Carbohydrase* and *cellulase* derived from *Aspergillus niger* for use in clam and shrimp processing
- *Carbohydrase* derived from *Rhizopus oryzae* for use in the production of dextrose from starch
- Dried yeasts*, Saccharomyces cerevisiae, Saccharomyces fragilis,* and *dried torula yeast, Candida utilis*
- *Esterase-lipase* derived from *Mucor miehei var. Cooney et Emerson* as a flavor enhancer in cheeses, fats and oils, and milk products
- Fermented microbial protein (FMP) from the fungal microorganism *Fusarium novum yellowstonensis*
- Flour may contain *alpha-amylase* obtained from the fungus *Aspergillus oryzae*
- *Gibberellic acid* derived by fermentation from *Fusarium moniliforme*
- *Invertase* from edible baker's yeast or brewer's yeast (*Saccharomyces cerevisiae)*
- Lactase enzyme preparation from *Kluyveromyces lactis* (previously called *Saccharomyces lactis*)for use in hydrolyzing lactose in milk
- Milk-clotting enzymes, microbial for use in the production of cheese (Milk-clotting enzymes are derived from *Endothia parasitica, Bacillus cereus, Mucor pusillus Lindt* and *Mucor miehei* and *Aspergillus oryzae* modified to contain the gene for aspartic proteinase from *Rhizomucor miehei var Cooney et Emerson*
- *Mycoprotein*
- Natamycin derived from *Streptomyces natalensis* and *Streptomyces chattanoogensis*
- Riboflavin biosynthesized by *Eremothecium ashbyii*
- Vitamin D, produced by ultraviolet irradiation of *ergosterol* isolated from yeast and related fungi
- Yeast-malt sprout extract*,* derived from *Saccharomyces cerevisiae, Saccharomyces fragilis, Candida utilis*

In Europe, the Novel Food classification is given by the European Food Safety Authority (EFSA) to foods that have not been widely consumed before 1997. Since the regulatory process can be long, many entrepreneurs gather proof that certain fungal strains they are innovating with have been consumed before 1997 to avoid the Novel Food classification. Within the Novel Foods classification, there is a subcategory of “traditional foods” which are foods consumed outside of the European Union countries and have a history of safe consumption. These traditional foods are regulated slightly differently and are not required to go through rigorous health and safety approvals required for the broader category of Novel Foods. Fungi derived food consumed widely outside the European Union can be applied as Traditional Foods due to the history of safe consumption. Under EFSA, a scientific panel studied the possibility of awarding a ‘Qualified Presumption of Safety’ (QPS) assessment to selected microorganisms and its application in food requests reviewed by EFSA, which would streamline the novel food approval processes. This scientific panel found that for certain species of yeast and filamentous fungi, a blanket approval or QPS status could not be given due to the regular occurrence of infestations and toxins even though the regulator gets regular approval requests for these applications.

Current Policy Landscape in India

Major government bodies and policy initiatives

Under the Government of India, The Ministry of Agriculture's **National Horticulture Board** (NHB) has been instrumental in extending financial support for mushroom production with credit-linked, back-ended subsidy schemes for commercial horticulture which has been the impetus for mushroom production in India. Under the 'Development of Commercial Horticulture through Production and Post-Harvest Management' scheme by the NHB, there is financial support for a credit linked back-ended subsidy @ 20% of the total project cost limited to Rs 25 lakh per project in the general area and Rs 30 lakh in the Northeast region, hilly and scheduled areas for mushroom cultivation.

Mission for Integrated Development of Horticulture (MIDH) is a centrally sponsored scheme for the holistic growth of the horticulture sector which includes mushrooms under its ambit. Under MIDH, Government of India (GOI) contributes 60%, of total outlay for developmental programmes in all the states except states in north east and Himalayas, 40% share is contributed by State Governments. In the case of north eastern states and Himalayan States, GOI contributes 90%. The other schemes also provide assistance for post-harvest management covering collection, pre-cooling units, cold storage units, refer vans, marketing infrastructure etc., which can be availed for the mushroom sector.

Under the **Ministry Of Food Processing Industries** there is a scheme for 'Creation of Backward and Forward Linkages' where the objective of the scheme is to provide effective and seamless backward and forward integration for the processed food industry by plugging the gaps in the supply chain in terms of availability of raw material and linkages with the market. Under the scheme, financial assistance is provided for setting up of primary processing centers/ collection centers at farm gates and modern retail outlets at the front end along with connectivity through insulated/ refrigerated transport. This scheme which is applicable to mushrooms would enable linking of farmers to processors and the market for ensuring remunerative prices for agriculture produce.

The **National Bank for Agriculture and Rural Development** (NABARD) is an apex financial institution that has tried to promote mushroom production through refinancing programmes for export oriented mushroom production and also supports the establishment of Mushroom Spawning Units.

Under the Indian Council for Agricultural Research (ICAR), the **Directorate of Mushroom Research** (DMR) is a research organisation present in Solan (which is called the 'Mushroom City of India') has prepared a "Vision 2050" which has plans to promote mushroom cultivation in the agro-industry, as well as developing components of the farming system for addressing the issues like nutritional security, unemployment, environmental sustainability, total recycling of agri-residues, etc. ICAR's Mushroom Lab conducts 'Entrepreneurship Training On Mushroom Spawn Production And Mushroom Cultivation' and holds regular online training programmes for Mushroom Cultivation Technology.

The **Ministry of Skill Development and Entrepreneurship** under the Skill Development Initiative has created a competency based curriculum for vocational training for those looking to become skilled mushroom cultivators. The central government facilitates and promotes the training while Vocational Training Providers (VTP) under the government provide training. The **National Centre for Mushroom**

Research and Training (NCMRT) holds a 15-day training programme each year, training about 50 people in each capsule.

Specific states that cultivate mushrooms offer additional support to mushroom cultivation. State Agricultural Universities in particular states, a few Krishi Vigyan Kendras (KVK) and District Agricultural offices through the Agricultural Technology Management Agency, conduct training on mushrooms and also facilitate spawn of particular mushrooms. A study found that the mushroom training conducted by the KVKs were found to be useful and the trainees found these training programmes highly effective in meeting their expectations and they had gained knowledge in different mushroom cultivation practices. Problems of poor quality of spawn, lack of assured marketing and non-remunerative prices and labour-intensive nature of the enterprise were the major hindrances in adoption of mushroom cultivation enterprises by the trainees (Dharminder et al. 2016).

India's FDI (Foreign Direct Investment) policy aims to attract investment in technology to development and production of vegetables and mushrooms under controlled conditions (Annual report 2017-2018). Efforts are being made by the Indian government to improve mushroom research and development and to encourage mushroom growers to develop advanced R&D technologies and policies (Jagdeesh et al. 2018).

Policy and Regulatory Recommendations

Since research and literature reiterates the nutritional and dietary value of edible fungi products, mushroom growing can be a potentially valuable crop in the coming years due to the various functionalities of fungi and its usage in alternative protein products. Below are a set of recommendations that can help improve mushroom cultivators and manufacturers of edible fungi food preparations to gain profit across the value chain and help India become a hub of mushroom production and exports for the growing alternative protein industry.

Food Regulations:

- FSSAI should list more macro-fungi and micro-fungi species apart from *Agaricus* under the standardised regulations which will ease the regulatory burden on FSSAI for pre-market product approvals for products created from fungi. Listing more macro-fungi and micro-fungi species in the The Food Safety and Standards (Food Product Standards and Food Additives) Regulations, 2011 will give clarity to entrepreneurs to innovate using the listed species, and to understand which species are non-standardised and therefore will fall under the non-specified food regulation process (and hence require pre-market approval). Many more species have been used widely and have a safe history of human consumption in various food applications.

Government support for R&D:

- There is a large scope for crop diversification through cultivation of other edible mushrooms like oyster, shiitake, milky and other medicinal mushrooms are additional opportunities for Indian growers (Nishi et al. 2018). The Central Indian region has a rich mycodiversity that is yet to be fully explored (Jagdeesh et al. 2018). A majority of fungal species including many promising

edible species have not been successfully cultivated because it is not feasible to re-create their growing conditions in isolation from their normal environment (Alka and Mahendra 2010). Cultivation of mushrooms like *Morchella*, *Cordyceps*, *Podaxis*, *Tuber*, *Phellorina* mushroom require additional modern techniques such as the provision of a balanced nutrition through a hydroponics system or similar systems. To scale the cultivation of *Paddy Straw mushroom*, it is important to set up and adopt indoor cultivation technology using alternative agro-residues procured at an industrial level. Additional government support in subsidizing these technologies would be beneficial for mushroom cultivators. This support can be extended by the National Horticulture Board.

Creation of Mushroom Development Board:

- NABARD recommends in one of its reports that "State Government may consider constituting an independent agency like the Mushroom Development Board on the lines of Coffee Board and Coir Board for holistic development of mushroom processing and marketing industry in the state" (Alka and Mahendra 2010). This Board must come under the NHB. This board may be empowered to generate awareness about mushroom cultivation, mushroom consumption, supplying information about spawn and post harvesting technologies, developing market chains for supply of mushrooms, promoting cultivation of different species and production of indigenous mushroom products.

Promoting mushroom cultivation:

- Mushroom cultivation is a profitable business since it requires low initial investment and less space, and it generates employment opportunities due to the large labour resource required throughout the cultivation process, making it a viable employment venture. Thus, policy makers must try to incentivise mushroom growers and farmers through credit and electricity provisions equivalent to general agriculture and agricultural products. Proper training to mushroom growers and enabling smooth availability of inputs till the industry attains the take-off stage is very crucial. Further government support and attention can help promote mushroom cultivation to make India's mushroom cultivation globally competitive.

Support for the Spawn Industry:

- Spawn, which acts as the starter' or seed, is the most crucial input for successful cultivation of mushrooms. In India, the spawn industry is an unorganized venture and needs research support in the years to come so that it may attain quality standards and competitiveness comparable to multinational companies (Jagdeesh et al. 2018). Collaborations between research institutes and the upcoming spawn industry will help the production with a knowledge base of genetic purity and the quality of spawn is continually ensured.
- Involvement of cooperatives and other marketing organizations for providing the required inputs as well as help in viable marketing of mushrooms can help promote mushroom cultivation. Ensuring liberal financial support by the government agencies and financial institutions by ensuring minimum support-price for mushrooms and provisions for insurance coverage for mushroom cultivation are additional recommendations listed in the ICAR Vision 2015 Report.

Improving Mushroom exports:

- The mushroom cultivators and exporters must develop new technologies for attaining maximum yields without using pesticides and chemicals. Mushroom exporters must procure the mushroom from quality mushroom cultivators to maintain their standard and brand in the overseas market to avoid the reverse logistics of mushroom cargo. The Government of India through APEDA can propose initiatives to educate mushroom cultivators through job training about importing countries' food safety control and export-import policy. To increase the production, productivity, and quality of the mushroom, APEDA must conduct a periodical visit to the mushroom cultivator's locality for checks. APEDA must do research and development related to mushroom cultivation and its allied area to enrich the volume of production as well as quality to meet future demand and compete with developed countries (Guruswamy and Vignesh 2020).
- APEDA can establish a separate unit for quality control and certification that standardize the products for the national and international market (Jay 2019).

Post-harvest management practices:

- Currently, mushroom products face a challenge of short shelf life therefore post-harvest management practices must be developed and implemented by mushroom cultivators and post-harvest infrastructures must be developed to increase shelf life and marketability. Since mushrooms are highly perishable goods, especially during peak season, suitable post-harvest management/practices must be followed to increase the shelf life and marketability of mushrooms. The mushroom cultivators would be benefited by the establishment of cold warehouse facilities that can help them store the mushroom and avoid the shrinkage as well as weight loss of mushrooms during the storage time leading to better export quality mushrooms (Guruswamy and Vignesh 2020.
- NHB or the Ministry of Skill Development and Entrepreneurship may add courses on improving the knowledge and skills regarding post harvest management such as knowledge on the right stage of picking, grading quality, preservation, cold storage, refrigerated transportation, proper processing, eye appealing packaging, labeling that maybe useful to promote standards of products.

Transfer of Technology:

- DMR being at the apex, a 2-tier system for transfer of technology is envisaged under the ICAR Vision 2050. DMR will act primarily as trainers training centre, where R&D workers from various States and Central Organizations including NGOs will be imparted training in the latest technical know-how on mushrooms, who, in turn, will train the prospective growers and entrepreneurs. DMR will also impart training to personnel from big commercial units and international agencies. The Krishi Vigyan Kendras (KVKs), State Agricultural Universities and State development departments will directly train the growers. DMR may also train the managers and senior technicians to be employed in the big mushroom units. The training will benefit mushroom

cultivators if it additionally includes a course on post harvest management and marketing different species of mushrooms.

Talent pool in India and opportunities

Fungi play an important role in our food systems. Historically, edible mushrooms (such as Agaricus) have been used worldwide for direct consumption. Yeasts and filamentous fungi were traditionally employed in the production of alcoholic beverages and fermented foods. Advances in food technology have drawn attention to fungi's inherent functionality, and are being used to produce functional ingredients (via precision fermentation), biomass (e.g. mycoprotein), or to modulate plant proteins (traditional fermentation) for enabling meat, egg, dairy, and seafood replacements.

Diversification of fungi's role in food production is dependent not only on advances in research and development of food biotechnology but also in engineering and downstream processing, beyond the laboratory research level. This involves a disciplinary crossover of biochemistry, genetics, microbiology, nutritional sciences, and engineering (Khachatourians 2003). Outside the natural sciences, food biotechnology also requires the convergence of several disciplines from production strategies, process engineering, commerce, and international law (Phillips and Khachatourians 2001).

Overview of the fungal value chain

As per the value chain for the production of fungi and subsequent utilization of fungi-based ingredients for meat, egg, and dairy products, the specific skill sets required are described below -

1. Fungal strain selection
    a. Microbiology
    b. Biochemistry/ Lifesciences
2. Biomass production through fermentation
    a. Industrial Biotechnology
    b. Bioprocess Engineering
    c. Chemical Engineering
3. Downstream operations
    a. Food Technology
    b. Biotechnology

The skills required across each step of the value chain including strain selection, production, and downstream operations are described in the following sections.

Strain selection

Among the 1.5 million fungal species, less than 5% have been described (Alam et al. 2017). Although fungi have so many potential uses, research on their potential applications is in general poorly funded and much of the research that is being carried out in academia is fundamental (Hyde et al. 2019). Globally we have some knowledge of about 10,000 fungal species in various collections and the public

domain literature to cross connect to biotechnology for exploitation of fungi for food and ingredients manufactured for human benefit (Arora 2003). Strain banks of the likes of the CBS-KNAW culture collection at Westerdijk Fungal Biodiversity Institute, is the largest one in the world with more 100,000 strains of fungi (including yeasts) and bacteria, and VTT Culture Collection in Finland collect, identify and study fungal specimens, making available a culture, ITS barcode sequence, and genetic analysis for many species. In India, the Microbial Type Culture Collection and Gene Bank (MTCC) housed at the Institute of Microbial Technology (IMTECH), Chandigarh, has established itself as a culture collection centre for microbial resources in India. Strains can be obtained from collection centres and screened for suitable applications to develop commercial products. Strain banks usually distribute material under the terms and conditions as specified in the Material Transfer Agreement (MTA), which prevents any authorised licensee from developing a private collection.

New strain identification of fungi has been focussed on providing strains for biological control, environmental remediation, and industrial processes. Although, recent advances in diagnostic biotechnology have revolutionized the procedures used in the identification of food fungi, these molecular techniques need to be robust, reliable and inexpensive if they are to be used to identify new strains and species of edible fungi. Presently new strain identification and optimization techniques are costly, time-consuming and require high R&D investment.

Owing to nutritional value, growth properties, toxicity profile and regulatory status of various species, food biotechnologists are currently limited to work with a few well-studied fungi including strains of yeast, filamentous fungi and edible mushrooms. When selecting an appropriate strain, it is essential to screen for key characteristics. Molecular techniques can be applied to predict protein structures and determine gene function, identify genes with particular properties and modify the genes in target fungi (Hodkinson and Parnell 2006).

Microbiology covers fundamental research on the biochemistry, physiology, cell biology, ecology, evolution and clinical aspects of microorganisms, including fungi. A number of microbiology departments within the current ecosystem traces back to pharmacology laboratories with a focus on infectious diseases and vaccine and drug development. As per the All India Survey on Higher Education (AISHE) 2018-19 survey, an annual web-based survey conducted by the Ministry of Education, there are approximately 7721 graduates from M.Sc Microbiology (non- Medical Science) programmes annually across India. The table below is a non-exhaustive list of training infrastructure/ universities/ academic institutions with expertise in developing, optimizing, and characterizing various microbial strains using advanced molecular biology methods -

Name of institute	Courses available related to microbiology	Key research areas
Banaras Hindu University (BHU)	MSc Applied Microbiology	Mycology
Indian Institute of Science	MSc Microbiology	Plant biology, Regulation of

(IISc), Bangalore		gene expression
Institute of Microbial Technology (IMTECH), Chandigarh	Industrial & Environmental Microbiology	Microbial Type Culture Collection & Gene Bank (MTCC), Bioinformatics & big data analytics
ICAR - Indian Agricultural Research Institute (IARI)	MSc Applied Microbiology	Agricultural microbiology, Techniques in microbiology

Table 4. Key microbiology degree programs in India

The fungal strain selected for production and processing of biomass will impact downstream products and processes. When evaluating proteins or flavors or textured meat analogues it is critical to to understand protein structure, function, and interactions. to reduce downstream processing. Biochemical methods can be applied for detecting proteins, for isolating and purifying proteins, and for characterizing the structure and function of proteins.

There are approximately 3571 graduates annually from M.Sc Biochemistry programmes across India (AISHE 18-19). The table below is a non-exhaustive list of training infrastructure/ universities/ academic institutions with expertise in biochemistry -

Name of institute	**Courses available related to biochemistry**	**Key research areas**
Banaras Hindu University (BHU)	M.Sc Biochemistry	Plant biochemistry, Enzyme technology
IIT Madras	M.Sc Biosciences	Nanoparticles, Recombinant Enzymes, Developmental Biology
IISc Bangalore	B.Sc Biochemistry	Proteins - structure and function
IIT Bombay	M. Tech Biosciences & Bioengineering	Proteomics, Molecular cell biology

Table 5. Key biochemistry degree programs in India

A new avenue for applied research is the application of genomics, proteomics, and bioinformatics towards intervention with fungal developmental genes for enhanced functionality. In the Indian context, using genetically modified organisms for food products are not favored as per regulatory norms.

Biomass production through fermentation

As compared to other biological sources, in particular plants, fungi have the great advantage that they can be grown in large bioreactors at an industrial scale, and suitable processes for their cost-efficient

fermentation have been available for many decades (Hyde et al. 2019). Over time, these fermentation techniques were scaled up and made more efficient with respect to engineering theories and practices.

In India, the infrastructure available for fermentation is well-established owing to its strengths in the biopharmaceutical manufacturing industry. The Indian pharmaceutical industry is world renowned for producing good quality and cost-effective generic drugs backed by structural cost-advantages due to innovative approaches in manufacturing. As such, industrial yeast manufacturing units - specifically baker's yeast and distiller's yeast, have been set-up with robust production systems and downstream operations. Raw materials for feed-stock are available in plenty owing to the large quantum of sugar cultivation in the country.

The two most commonly used techniques for fermentation include submerged liquid fermentation and solid-state fermentation. Each of these methods requires a diverse range of skill sets to enable manufacturing and scale-up.

1. Submerged Liquid Fermentation (SLF):
   The SLF process involves the cultivation of fungi in a liquid medium. This process is carried out in bioreactors optimised for pH, temperature, dissolved oxygen, and stirring. Different reactors are able to carry out the fermentation in different modes of operation (batch, fed-batch, and continuous) as per standard growth conditions (Confortin et al. 2019).

   However, this technology will need to be adapted for alternative protein applications. Fungi, because of their unique mycelial structures, have challenged bioengineers to reinvent fermenters and downstream processing instruments (Arora 2003). Production of fungal biomass or protein isolates in this manner require equipment to be repurposed to control physical–chemical variables in the process. This would necessarily involve interdisciplinary engineering and food processing concepts such as, better equipment design, heat and mass transfer systems, feedstock supplementation system, product recovery, effluent and waste management, computerization and automation, and hazard analysis critical control points (HACCP) and quality assurance (Arora 2003). Bioreactors would have to be designed for 10x the scale of existing bioreactors used for the pharmaceutical and fermentation industries, and optimized for the growth of microorganisms.

   The substrate or feedstock used for liquid-state fermentation would need to be optimized for the production of filamentous fungus. Although there is a scope to utilize side streams from agricultural produce as well as food processing industries for feedstock, it would be essential to conduct bioprocess modelling strategies for achieving high-production process efficiency in yield, productivity or component availability within the desired output.

2. Solid-State Fermentation (SSF):
   The SSF process involves the cultivation of fungi in a solid substrate, such as straw, in a low-moisture environment, requiring no arable land. For efficient and large-scale production,

operations are highly mechanized, automatized and even computerized, using bioreactors to maintain homogeneous growing conditions. By the selection of appropriate design and operating variables, most of the negative factors can be minimized in various SSF bioreactors. With advancement in engineering, more viable and efficient SSF processes will be available in the near future.

Initiatives by Central Food Technological Research Institute (CSIR-CFTRI), Mysore's R&D Department on Microbiology and Fermentation Technology and Institute of Chemical Technology (ICT), Mumbai's Food and Fermentation Technology Department, have significantly contributed to the development of a talent pool with expertise in fermentation with incremental training and re-skilling required for alternative protein applications.

Biotechnological engineering focuses on an integrated approach of fundamental biological sciences and traditional engineering principles. It includes diverse disciplines such as biochemical engineering, biomedical engineering, bioprocess engineering, biosystem engineering, and so on.

There are approximately 1173 graduates annually from M.Tech Chemical Engineering programmes across India (AISHE 18-19). The table below is a non-exhaustive list of training infrastructure/ universities/ academic institutions with expertise in design, optimization, and implementation of fermentation technology and manufacturing processes:

Name of institute	Courses available related to bio-manufacturing	Key research areas
ICT Mumbai	M.Tech Biochemical Engineering	Development of novel reactors, reactions and separation processes
IIT Madras	M.Tech Bioprocess Engineering	Centre of Excellence in Bioprocess Engineering
Manipal Institute of Technology	B.Tech Industrial Biotechnology	Computational analysis, process engineering principles in biotechnology
IIT Kharagpur	M.Tech Biochemical Engineering	Bioprocess and bioproduct development

Table 6. Key biomanufacturing degree programs in India

A number of vocational training programs to promote edible mushroom cultivation technology are being run by State Governments and Agriculture Universities. These usually involve low-tech engineering of specific cultivation techniques, spawn and mushroom production as a means to diversify agricultural income during the sowing season and encourage mushroom

entrepreneurship. The table below shows the training infrastructure/ universities/ academic institutions with expertise in mushroom cultivation:

Name of institute	Courses available related to mushroom cultivation	Key research areas
ICAR-DMR Solan	Training on mushroom cultivation technology for entrepreneurs	Farm design, mushroom cultivation, crop management
ICAR-IIHR Bangalore	Entrepreneurial and basic orientation trainings for mushroom cultivation	Mushroom cultivation technologies, Spawn production
Institute of Horticulture Technology, Noida	Certificate in mushroom cultivation	Mushroom culture, spawn production and spawning, Making and casing beds
Dr. Rajendra Prasad Central Agricultural University	Mushroom production technology	Production, protection and crop management of mushrooms, preparation of mushroom products

Table 7. Mushroom cultivation training programs in India

Downstream processing

Fungi in food technology have benefited from intellectual property rights associated with process patents. Depending on the choice of fermentation technique and the specific end-product application of the fungal biomass, the set of downstream operations differ. Broadly these can be categorised into two application areas - whole biomass utilization for creating alternative meat products and extraction of intracellular components, like protein, from microbial biomass.

1. Whole biomass utilization:
   Owing to the fibrous nature of mycelial structures, whole biomass may be utilized for creating an alternative meat product that doesn't require traditional texturization technologies like extruders. Simple processing techniques may be employed to bind fibres to create a meat-like texture. Food technologists can lead the product development process including working with fungal biomass, formulation & process development, and pilot & manufacturing trials.

   Food science and technology programs deal with the techniques involved in the production, processing, preservation, packaging, labeling, quality management, and distribution of food products. There are approximately 470 graduates annually from M.Tech Food Technology programmes across India (AISHE 18-19). The table below is a non-exhaustive list of training infrastructure/ universities/ academic institutions with expertise in food technology:

Name of institute	Courses available related to food processing	Key research areas
ICT Mumbai	M. Tech Food Engineering & Technology	Fermentation Technology & Food Biotechnology
National Institute of Food Technology Entrepreneurship and Management (NIFTEM)	B. Tech in Food Science and Technology M. Tech in Food Technology and Management	
IIT Kharagpur	M. Tech Food Process Engineering	3D printing of food, Design of food processing machineries, Novel Food Product & Process Development
CSIR-Central Food Technological Research Institute	M.Sc Food Technology	
Indian Institute of Food Processing Technology (IIFPT)	M. Tech Food Process Engineering M. Tech Food Process Technology	Food engineering, Food product development, Food biotechnology

Table 8. Key food processing and technology degree programs in India

2. Extraction of intracellular components:
   In order to extract intracellular components like protein and chitin, expertise and experience is needed in cell disruption, protein extraction, concentration, and drying technologies. This involves a multidisciplinary approach involving chemical and mechanical engineering techniques. Specializations involving computational biology, biomaterials, and bioprocess engineering are, especially, of relevance to carry out these processes.

   At present, while there is some academic research focused on purification of protein from fungi, these processes are not at the industrial or commercial scale. Cost has traditionally been a prohibitive factor for companies and hence often they look to outsource this process vertically to a specialised lab or firm. It is important to note that this process differs in one key aspect from the pharmaceutical industry, where functionality rather than purity remains the goal to be pursued. Hence, for cell separation, concentration and extraction more economical alternatives to pharmaceutical processes may be developed. These could be mild processing technologies keeping protein intact for use as single-cell protein products for end- product formulation in the alternative protein industry.

Biotechnology follows a multidisciplinary approach in which biological processes, organisms, cells or cellular components are exploited to develop new technologies. Specializations emerging from this field including tissue engineering, bioinformatics and computational biology, genomics, biomaterials, and bioprocess engineering are especially of relevance within the alternative protein industry. There are approximately 7869 graduates annually from M.Sc Biotechnology programmes across India (AISHE 18-19). The table below is a non-exhaustive list of training infrastructure/ universities/ academic institutions with expertise in food technology:

Name of institute	Courses available related to biotechnology	Key research areas
IIT Kharagpur	M.Sc Biotechnology	Biotechnology of Plants, Fungi and Algae, Microbial genomics and metagenomics, Bioprospecting of Endophytic Microbes, Food Biotechnology
IIT Madras	M. Sc Biotechnology	Industrial metabolite production, plant cell bioprocessing, tissue engineering
IIT Kanpur	M.Sc in Biological Sciences & Bioengineering	Molecular/cell biology, Structural biology, Computational biology, Tissue engineering, Biomaterials
IISc Bangalore	M. Sc Biotechnology	

Table 9. Key biotechnology degree programs in India

Emerging application areas within the alternative protein landscape

Beyond these broad application areas there are specific use-cases for fungi including creating whole-cut meats, mycelium-based scaffolding materials, and production of flavoring and stabilization agents. These are discussed below -

1. Scaffolding: Tissue engineering techniques first developed in the medical field for tissue regeneration and transplantation are currently being used within the food sector to produce cultured meat products suitable for human consumption. Chitin/chitosan derived from the fungi cell wall and the use of mycelium are currently being pursued for creating scaffolding materials to form meat-like 3D structures. Similar to applications within biomedical engineering, development of scaffolds for the alternative meat sector would require an understanding of

microstructure of mycellia, tissue culture and strain selection with the objective to optimise functionality of mycelia. IIT-Bombay's Cell and Tissue Engineering Laboratory is working to develop 3D cell culture platforms for culturing cells that could have direct relevance to the cultivated meat industry.

2. Flavoring and stabilization agents: By leveraging the flavours of different strains of edible mushrooms, fungi can be exploited for the development of natural flavors for alternative protein applications. Application of data-driven strategies for investigating gastronomic data, such as precepts of flavor compounds, has opened up an all-new field of 'Computational Gastronomy'. This emerging interdisciplinary science, using methods of statistics, machine learning, natural language processing, pattern mining, and chemo-informatics, has the potential to transform the food landscape by effectively leveraging data-driven food innovations for better health and nutrition. This emerging field has found roots in India within the Complex Systems Laboratory, IIIT-Delhi, a research-oriented state university focused on computer science and related areas.

3. Whole-cut meats: By using modified solid-state fermentation techniques, various mushroom species with taste and textural similarities of meat and seafood products can be grown for whole-cut alternative meat and seafood products. This would involve a relatively low-technology approach and can open up opportunities for local mushroom producers and restaurants to bring these products to consumers at a premium price.

Developing a talent pipeline for alternative protein

Stakeholders need to assess skill sets across specific areas of the value chain and perform targeted outreach to universities, student groups, research centers and vocational institutions, to draw in expertise that can contribute to leveraging fungi's role in food production. To foster talent, stakeholders must be willing to dedicate significant resources for knowledge building and research and development.
Based on the analysis of the current state of talent pool in India, several initiatives that would contribute directly towards directing and building talent across the value chain have been identified. These opportunities have been collated in the opportunities database. Details about the opportunities database have been discussed in the last section.

Conclusion

Fungi are potentially the most versatile microorganism which have applications across plant-based, fermentation-based and cultivated meat, egg, dairy, and seafood products. Not only do the applications span across the three pillars of the alternative protein sector but also across the fungal value chain from the fermentation of plant-based protein to the production of yeast-derived protein isolates. This breadth of options available for the utilization of fungi opens up opportunities for existing companies as well as startups to explore new application areas and create innovative technology platforms as well as products focused on the alternative protein sector. Companies like Angel Yeast and Biospringer Lesaffre have already launched products focused on the alternative protein sector while leveraging decades of expertise in the production of yeast. A similar approach can be taken by indigenous companies in India

to cater to the needs of the evolving alternative protein landscape in India. Startups like Myoworks, Food Myco Lab and Mycovation are emerging players in India driving innovation in this sector using fungi as a technology platform. Application of fungi-based fermentation technologies to create fungi-based meat analogues, yeast-based proteins, fungi-based flavoring agents, and whole-cut meats using solid-state fermentation are relatively more mature compared to other microbial fermentation technologies utilizing microbes like bacteria and microalgae. With this technological maturity coupled with India's unique advantage as a global bio-manufacturing hub, India can be an industry leader in fungi-based products with the right balance of academic research and funding from the government, corporate sector, and venture capital firms. With the help of existing technical expertise and channeling investments in technological development and academic research across the application areas, India can lead the way to cost-efficient, functional applications of fungi in the alternative protein sector, and a new generation of delicious, nutritious, sustainable meat, egg, and dairy replacement applications.

Next steps: Maturity of the fungi-derived ingredient industry in India

Based on the literature review of the current state of technology development across the fungal application areas and insights garnered from stakeholder interviews, the maturity of each fungal application area has been determined. This will help current and potential stakeholders understand the current state of the fungal applications in India. The maturity of each application area can be interpreted based on the combination of the technology readiness level and manufacturing readiness level. Based on a scale of 0 to 5, the technology or manufacturing readiness level indicates the level of technology or manufacturing development of a particular application area. The technology readiness level ranges from 0, indicating the technology development has not begun, to 5, indicating the presence of product lines in the market by many companies. Similarly, the manufacturing readiness level ranges from 0, indicating the absence of any infrastructure or manufacturing scale facility where the technology relevant to the application area is implemented, to 5, indicating readily available infrastructure or manufacturing facilities where one or multiple technologies relevant to the application area have been scaled in a cost-effective way. Both the technology readiness level and manufacturing readiness level are determined keeping in mind the Indian as well as the global market. The table below describes the technology readiness levels and manufacturing readiness levels in detail.

Scale/Type of readiness	0	1	2	3	4	5
Technology Readiness Level	No academic research	Only academic research	Proof of concept validated at lab scale/experimental prototypes/technologies reported by companies/startups	Prototypes/technology demonstrated to be economically viable	Few companies have established product lines for this application area	Many companies have established product lines for this application area

Manufacturing Readiness Level	No existing infrastructure capacity to support this activity	No infrastructure capacity to support this activity but infrastructure available for other applications	Infrastructure established by few companies but cost is prohibitive, no scalable solutions exist	Infrastructure established by few companies but cost is prohibitive, scalable solutions demonstrated to bring down costs	Infrastructure established by few companies and cost is no longer prohibitive	Infrastructure is common-place, high scalability of production and processing techniques

Table 10: Technology readiness scale and manufacturing readiness scale

The table below shows the ratings for each application area using fungi-derived ingredients.

	Whole-cut meats	Textured meats	Protein isolates	Flavor compounds	Scaffolding materials	Fermented protein
Technological readiness level	3	5	3	5	3	5
Manufacturing readiness level	4	3	2	5	0	4

Table 11: Maturity ratings for application area globally

	Whole-cut meats	Textured meats	Protein isolates	Flavor compounds	Scaffolding materials	Fermented protein
Technological readiness level	0	3	2	5	2	0
Manufacturing readiness level	1	1	1	5	0	1

Table 12: Maturity ratings for application areas in India

As the report demonstrates, based on the developments under various application areas, there is significant progress in terms of technological readiness for most application areas globally. Manufacturing readiness for most application areas is relatively lower due to high scale-up costs. In India particularly, both technology and manufacturing readiness levels are lower than global readiness levels. This can partly be explained by the absence of indegenious companies that are exploring novel food applications from fungi.

While the maturity rating presents the potential for fungi-derived ingredients in India to accelerate the alternative protein sector, the key white space opportunities and interventions which will help accelerate the application of fungi have been elaborated in detail in our opportunities database. The opportunities database lists out opportunities across value chain segments and categories including R&D, manufacturing, regulatory, investment, end product optimization, and demand generation. The opportunities have also been rated for the extent of their impact on the alternative protein sector, the difficulty of implementation, and the maturity of the intervention in the Indian context. The opportunities database can serve as a guide for various stakeholders working on food applications of fungi as well as the alternative protein sector to help accelerate the development of alternative protein products using fungi-based proteins or fungal biomass.

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