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**Types of fermentation (i.e.
submerged, surface and solid state
fermentation)**



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- a) Fermentation preserves and enriches food, improves digestibility, and enhances taste and flavour of foods.
- b) Fermentation systems may be liquid (submerged) or solid state (surface).
- c) Most industries used submerged type, because it saves space and is more amenable to engineering control and design.





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Submerged Liquid Fermentations

- Used for production of microbially derived enzymes.
- Involves submersion of microorganism in an aqueous solution containing all nutrients needed for growth.
- Fermentation takes place in large vessels (fermenter) with volumes of up to 1,000 cubic metres.
- Fermentation media sterilizes nutrients based on renewable raw materials (maize, sugars and soya).
- Most industrial enzymes are secreted by microorganisms into fermentation medium to break down carbon and nitrogen sources.
- In the batch-fed process, sterilized nutrients are added to fermenter during growth of biomass.
- In continuous process, sterilized liquid nutrients are fed into fermenter at same flow rate as fermentation broth leaving the system.



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- Temperature, pH, oxygen consumption and carbon dioxide formation are measured and controlled to optimize fermentation process.
- Next in harvesting enzymes from the fermentation medium one must remove insoluble products, e.g. microbial cells (done by centrifugation).
- As most industrial enzymes are extracellular (secreted by cells into the external environment), they remain in the fermented broth after the biomass has been removed.
- Enzymes in remaining broth are then concentrated by evaporation, membrane filtration or crystallization depending on their intended application.
- Pure enzyme preparations are usually isolated by gel or ion exchange chromatography.

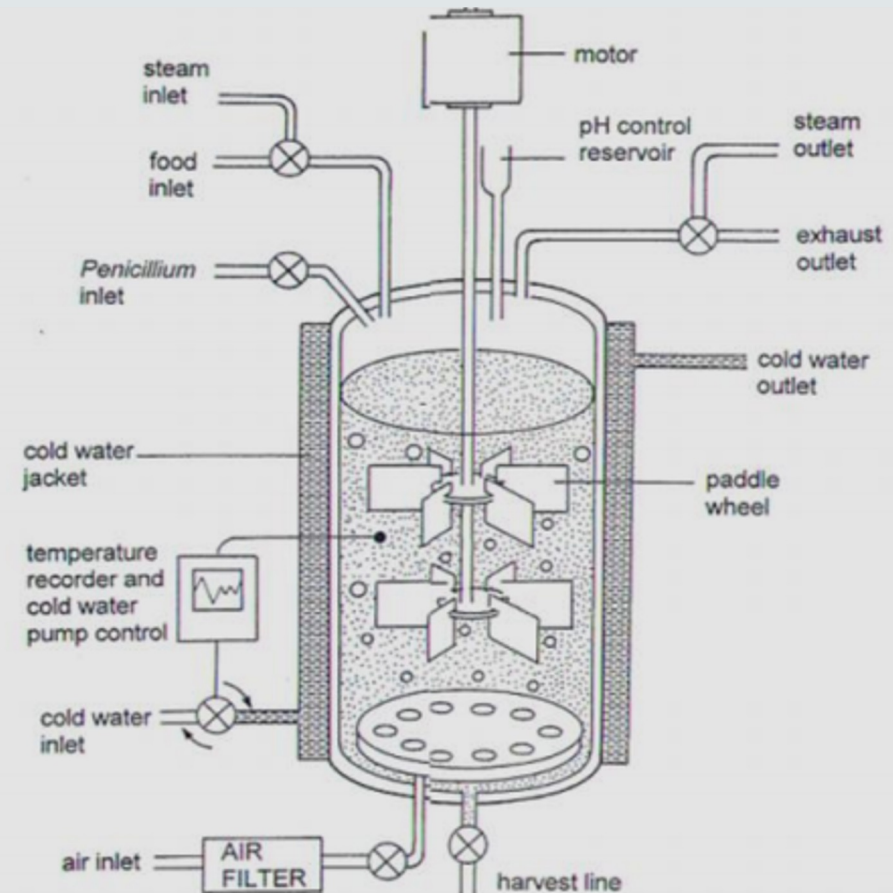


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Aerated Stirred Tank Batch Fermentor

1. Upright closed cylindrical tank fitted with one or more baffles attached to side of wall, water jacket or coil for heating and/ or cooling, device for forcible aeration (known as sparger), mechanical agitator usually carrying pair/more impellers, means of introducing organisms and nutrients and of taking samples, and outlets for exhaust gases.
2. Modern fermentors are automated and have continuously monitoring, controlling pH, oxidation reduction potential, dissolved oxygen, effluent O₂ and CO₂.



1.lid, 2 air-outlet, 3 fermenter, 4 module base permeable for air, 5 cultivation substrate, 6 cooling device, 7 air-inlet, 8 water supply, 9 water discharge, 10 heat resistant seal, 11 support device for the module base, 12 edge of the module base, 13 quick coupling, 14 pipe for the inflow and outflow of the cooling liquid, 15 heat resistant seal, 16 exterior ring, 17 coupling



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Aeration System (Sparger):

- Device for introducing air into fermenter.
- Aeration provides sufficient oxygen for organism in fermenter.
- Fine bubble aerators must be used.
- Large bubbles will have less surface area than smaller bubbles which will facilitate oxygen transfer.
- Agitation is not required when aeration provides enough agitation which is case Air lift fermenter.
- For aeration to provide agitation, vessel height/diameter ratio should be 5:1.
- Air supply to sparger should be supplied through filter.



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Porous sparger

- Made of sintered glass, ceramics or metal.
- Used only in lab scale-non agitated vessel.
- Size of bubble is 10-100 times larger than pore size.
- There is a pressure drop across sparger and holes tend to be blocked by growth (limitation of porous sparger).

Orifice sparger

- Used in small stirred fermenter.
- Perforated pipe kept below impeller in form of crosses/rings.
- Size should be $\sim \frac{3}{4}$ of impeller diameter.
- Air holes drilled on under surfaces of tubes and holes should be at least 6mm diameter.
- Used mostly with agitation.

Nozzle sparger

- Mostly used in large scale.
- Single open/partially closed pipe positioned centrally below impeller.
- When air is passed through this pipe there is lower pressure loss and does not get blocked.



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Important criteria in choice of organism:

- Nutritional characteristics of the organism
- Optimum temperature of the organism
- Reaction of organism with equipment to be employed and suitability of organism to type of process to be used
- Stability of organism and its amenability to genetic manipulation
- Productivity of the organism, measured in its ability to convert substrate into product and to give high yield of product per unit time
- Ease of product recovery from the culture
- Irrespective of origins of an industrial microorganism



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Surface Fermentation

- Microorganisms are cultivated on surface of liquid or solid substrate.
- *A. niger* forms a mycelium layer on liquid surface of the aluminum/stainless steel trays.
- Trays are stacked in fermentation rooms supplied with filtered air which supply oxygen and control temperature of fermentation.
- Easy to control and implement.
- Needs no aeration/agitation of fermentation broth.
- Fermentation broth is concentrated due to high evaporation rate during fermentation. So, expenses and losses during recovery and purification are low.
- **Disadvantages: Building investment costs are high, Personnel expenses are high, Fermentation time is long and productivity is low.**



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Submerged fermentation is favored over surface fermentation:

**Lower total
investment
costs**

**Improved
process
control**

**Reduced
fermentation
time**

**Reduced
floor space
requirements**

**Lower labour
costs**

**Simpler
operations**



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Solid State Fermentation

- Include well known microbial processes such as soil growth, composting, silage production, wood rotting and mushroom cultivation.
- Cheese, bread, sausage, miso, tempeh and soy sauce are produced using SSF.
- Beverages derived from SSF processes include kaoliang (sorghum) liquor in China and sake in Japan.
- Production of bioproducts from microorganisms under conditions of low moisture content for growth.
- Medium used is usually a solid substrate (rice bran, wheat bran, or grain) which requires no processing.
- It is necessary to take into account water sorption properties of solid substrate during fermentation.
- Power requirements are lower than submerged fermentation.
- Inadequate mixing, limitations of nutrient diffusion, metabolic heat accumulation and ineffective process control renders SSF applicable for low value products with less control.



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- There exists potential for conducting SSF on inert substrate supports impregnated with defined media for production of high value products.
- It involves growth of microorganisms on moist solid particles, in situations in which spaces between particles contain continuous gas phase and minimum of visible water.
- Although droplets of water may be present between particles and there may be thin films of water at particle surface, inter-particle water phase is discontinuous and most of inter-particle space is filled by gas phase.
- **Type of fermentation process that involves solids, including suspensions of solid particles in continuous liquid phase.**



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Solid state fermentation is favored over submerged fermentation:

- **Higher volumetric productivity**
- **Usually simpler with lower energy requirements**
- **Easier to meet aeration requirements**
- **Resembles natural habitat of some fungi and bacteria**
- **Fungal hyphae are bathed in liquid medium and do not run risk of desiccation**
- **Organism is exposed to constant temperature throughout its growth cycle**
- **Availability of O₂ to biomass can be controlled at a particular level of saturation of medium**
- **Availability of nutrients to organism can be controlled within relatively narrow limits.**



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- Provide low-shear environment, if organism is highly susceptible to shear damage, such as bubble columns
- pH control is relatively easy to provide.

Traditional SSF processes:
Tempeh, which involves cultivation of
***Rhizopus oligosporus* on cooked**
soybeans.

Koji step of soy sauce manufacture,
which involves cultivation of *Aspergillus*
***oryzae* on cooked soybeans.**



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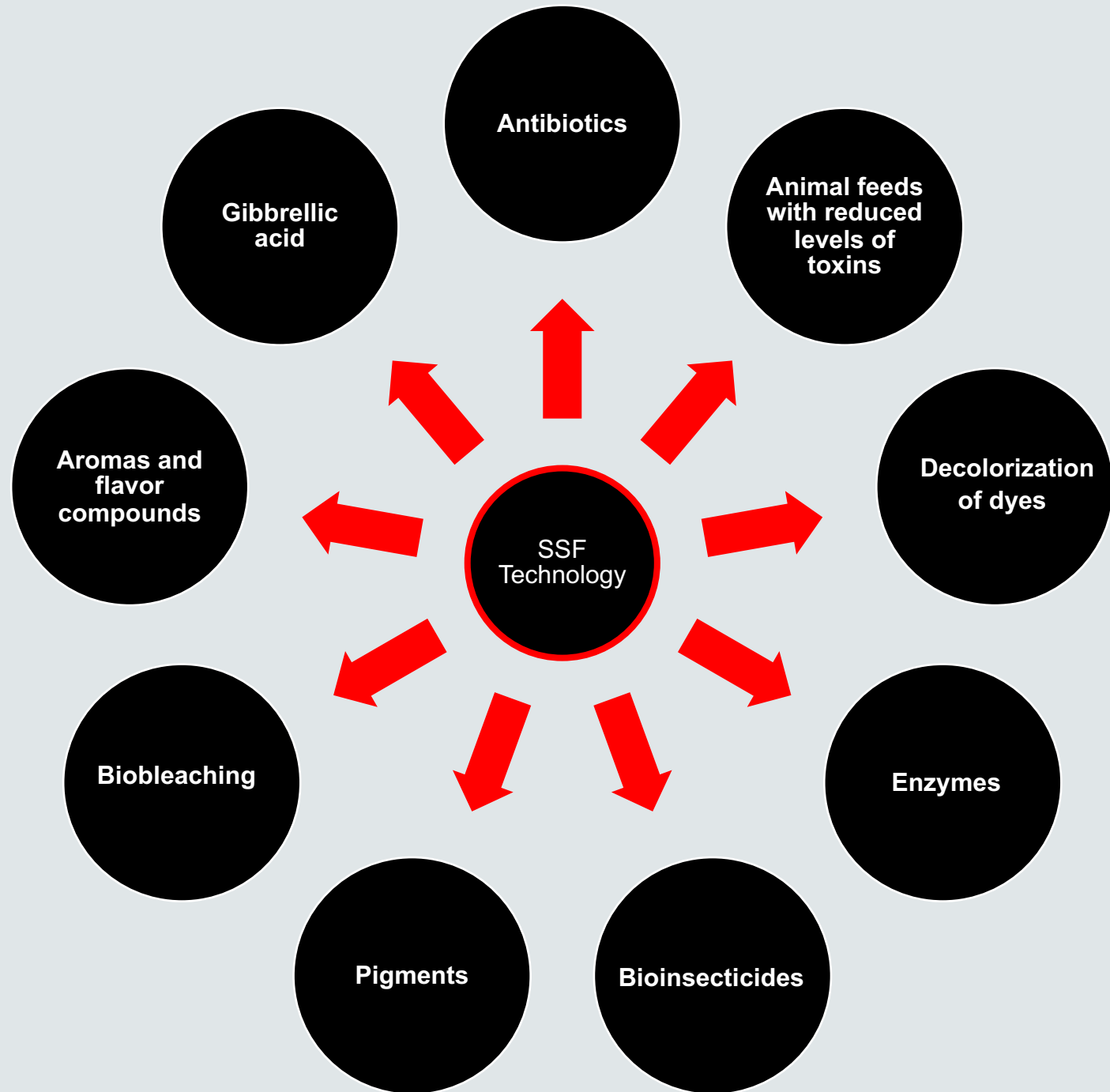
Despite being more problematic, SSF may be appropriate:

- **When product needs to be in a solid form (e.g., fermented foods).**
- **When a particular product is only produced under conditions of SSF. Eg., some fungi only sporulate when grown in SSF, in which hyphae are exposed directly to an air phase.**
- **If desired to use genetically unmodified organisms for production of product, then SSF is only option.**
- **Some SSF processes can be relatively resistant to being overtaken by contaminants**
- **Spore-based fungal biopesticides produced in SSF are usually more resistant to adverse conditions than those produced in SLF.**



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- **Filamentous fungi are best adapted species for SSF (enzyme production).**
- **Hyphal mode of growth gives major advantage to filamentous fungi over unicellular microorganisms in colonization of solid substrates and utilization of available nutrients.**
- **Filamentous fungi have power to penetrate solid substrates.**
- **Hydrolytic enzymes are excreted at hyphal tip, without large dilution. This allows hydrolytic enzymes penetration into most solid substrates.**
- **Fungi cannot transport macromolecular substrates across cell wall, so macromolecule hydrolyzed externally into soluble units that can be transported into cell.**



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Types of solid-state fermenters:

**Tray
bioreactor**

**Packed bed
bioreactor**

**Rotary drum
bioreactor**

**Swing solid
state
bioreactor**

**Stirred
vessel
bioreactor**

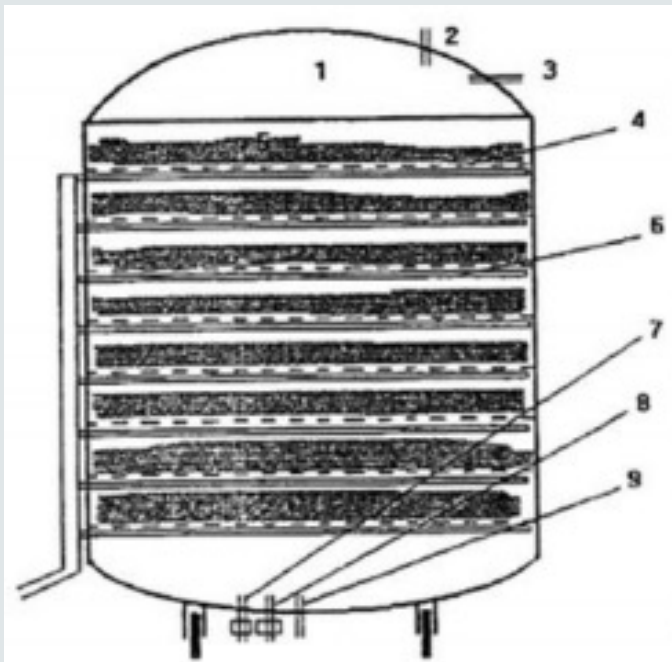
**Air solid
fluidized bed
bioreactor**



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- **Simplest SSF reactor is the tray.**
- **In a tray bioreactor, a relatively thin layer of substrate is spread over large horizontal area.**
- **There is no forced aeration, although base of tray may be perforated and air forced around tray.**
- **Mixing done by simple automatic devices/manual.**
- **Internal temperature may vary with ambient temperature; or tray may be placed in temperature-controlled room.**
- **Tray bioreactors used successfully at laboratory, pilot, semi-commercial and commercial scale.**





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Factors affecting enzyme production in solid state fermentation systems:

- **Selection of suitable substrate and microorganism**
- **Pre-treatment of substrate**
- **Particle size (inter -particle space and surface area) of substrate**
- **Water content of substrate**
- **Relative humidity**
- **Type and size of inoculum**
- **Control of temperature of fermenting matter/removal of metabolic heat**
- **Period of cultivation**
- **Gaseous atmosphere, i.e. oxygen consumption rate and carbon dioxide evolution rate.**



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Current applications of SSF:

- Development of bioprocess such as bioremediation and biodegradation of hazardous compounds
- Biological detoxification of agro-industrial residues
- Biotransformation of crops and crop-residues for nutritional enrichment
- Production of value-added products such as biologically active secondary metabolites, including antibiotics, alkaloids, plant growth factors, enzymes, organic acids, biopesticides, including mycopesticides and bioherbicides, biosurfactants, biofuel, aroma compound.