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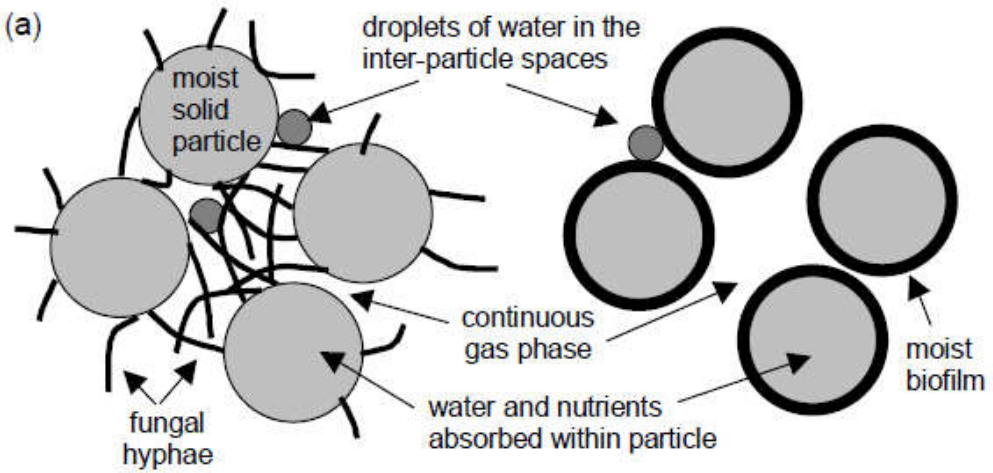
Solid State Fermentation

What is SSF?

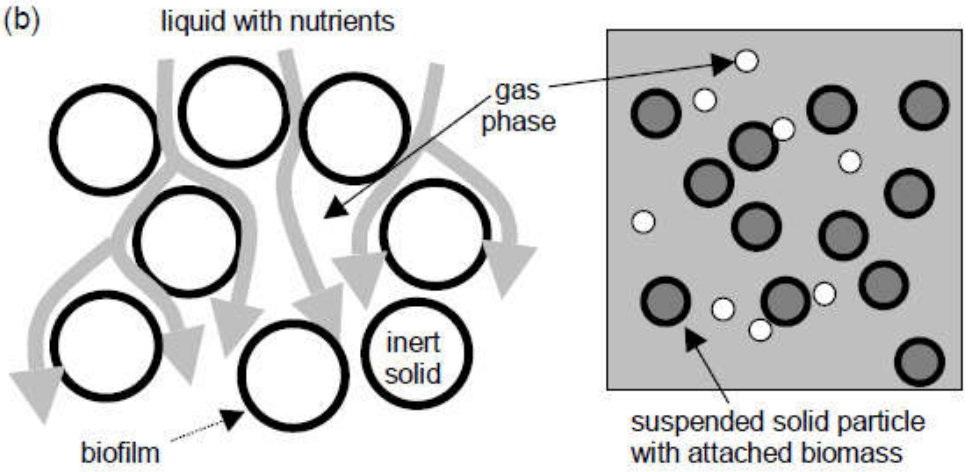
- Solid-state fermentation (SSF) involves the growth of microorganisms on moist solid particles, in situations in which the spaces between the particles contain a continuous gas phase and a minimum of visible water.
- The more general term “solid-substrate fermentation” is used to denote any type of fermentation process that involves solids, including suspensions of solid particles in a continuous liquid phase and even trickling filters

- The majority of SSF processes involve **filamentous fungi**, although some involve bacteria and some involve yeasts.
- SSF processes may involve the pure culture of organisms, or the culture of several pure strains inoculated simultaneously or sequentially, while in some processes a “self-selected” microflora arises from the original microflora (e.g., in composting) or from a specially prepared traditional inoculum.
- The majority of SSF processes involve aerobic organisms.
- The substrates used in SSF processes are often products or byproducts of agriculture, forestry or food processing. Typically the source of nutrients comes from within the particle, although there are some cases in which nutrients are supplied from an external source. Usually a polymer gives the solid structure to the particle and this polymer may or may not be degraded by the microorganism during the fermentation. There are also some cases in which artificial or inert supports are used, with a nutrient solution absorbed within the matrix.

(a) The arrangement of moist solid particles and the continuous gas phase in SSF systems involving a filamentous fungus (left-hand side) and a unicellular organism (right-hand side).



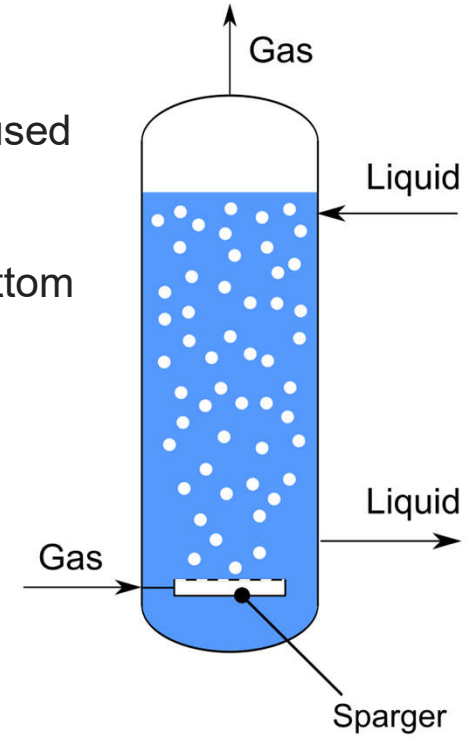
(b) Other systems that involve growth on solids, but which are not defined as SSF due to the large amount of water in the inter-particle spaces. The left-hand diagram represents a trickling-filter type system while the right-hand diagram represents a suspension or slurry system



SLF vs SSF

- In Submerged Liquid Fermentation (SLF) it is relatively easy to control the conditions to which the process organism is exposed:
  1. the fungal hyphae(long branching filaments) are bathed in a liquid medium and do not run the risk of desiccation(drying)
  2. temperature control is typically not overly difficult, such that the organism is exposed to a constant temperature throughout its growth cycle
  3. the availability of O2 to the biomass can be controlled reasonably well at a particular level of saturation of the medium (although this can become very challenging in high density cultures);
  4. the availability of the nutrients to the organism can be controlled within relatively narrow limits if desired, through the feeding of nutrient solutions (at least in those processes in which soluble carbon and energy sources are provided);
  5. although shear forces do occur within mechanically stirred bioreactors, the nature and magnitude of these forces are well understood and it is possible to use bioreactors that provide a low-shear environment, if the organism is highly susceptible to shear damage, such as bubble columns or air lift bioreactors;
  6. pH control is relatively easy to provide.

A **bubble column reactor** is an apparatus used to generate and control gas-liquid chemical reactions. It consists of a vertically-arranged cylindrical column filled with liquid, at the bottom of which gas is inserted



SLF vs SSF contd...

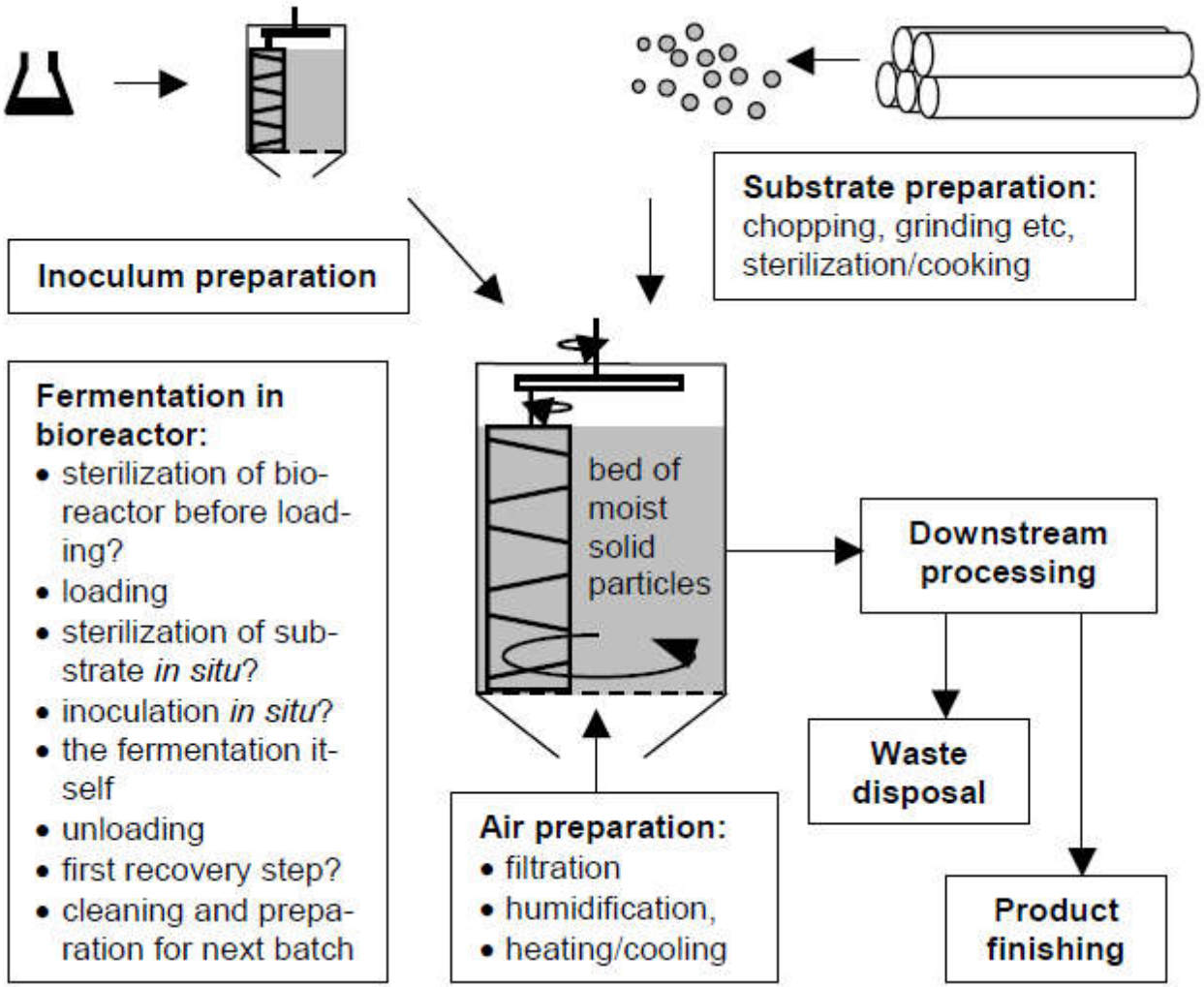
- The environment in SSF can be quite stressful to the organism. For example:
  1. fungal hyphae are exposed to an air phase that can desiccate them;
  2. temperatures can rise to values that are well above the optimum for growth due to the inadequate removal of waste metabolic heat. In other words, the temperature to which the organism is exposed can vary during the growth cycle;
  3. O2 is typically freely available at the surface of the particle, however, there may be severe restrictions in the supply of O2 to a significant proportion of the biomass that is within a biofilm at the surface or penetrating into the particle;
  4. the availability of nutrients to the organism may be poor, even when the average nutrient concentration within the substrate particle, determined after homogenizing a sample of fermenting solid particles, is high. In other words, there tend to be large concentration gradients of nutrients within the particles;
  5. movement of the particles of the solid substrate can cause impact and shear damage. In the case of fungal processes the hyphae can suffer severe damage;
  6. it may be difficult to provide pH control.

- The ease of using SLF is greater still when substrate handling is considered. For example, it is much simpler and cheaper to pump liquids from one place to another than to move solids and it is easier to sterilize a large volume of liquid than a large volume of solids.
- SLF is the method of choice in the majority of cases.
- However, despite being more problematic SSF may be appropriate in many instances for eg:
    1. when the product needs to be in a solid form (e.g., fermented foods);
    2. when a particular product is only produced under the conditions of SSF or, if produced in both SLF and SSF, is produced in much higher levels in SSF. For example, certain enzymes are only induced in SSF and some fungi only sporulate when grown in SSF, in which the hyphae are exposed directly to an air phase. If it is desired to use genetically unmodified organisms in a process for the production of such a product, then SSF may be the only option;
    3. when the product is produced in both SLF and SSF, but the yield is much higher in SSF. For example, *Monascus* pigment and many fungal spores are produced in much higher yields in SSF;

4. when socio-economic conditions mean that the fermentation process must be carried out by relatively unskilled workers. Some SSF processes can be relatively resistant to being overtaken by contaminants;
5. when the product is produced in both SSF and SLF, but the product produced in SSF has desirable properties which the product produced in SLF lacks. For example, spore-based fungal biopesticides produced in SSF processes are usually more resistant to adverse conditions than those produced in SLF, and are therefore more effective when spread in the field;
6. when it is imperative to use a solid waste in order to avoid the environmental impacts that would be caused by its direct disposal. This is likely to become an increasingly important consideration as the ever-increasing population puts an increasing strain on the environment.

General Steps in SSF Process

- Inoculum preparation
- Substrate preparation
- Bioreactor preparation
- Inoculation and loading
- Bioreactor operation
- Unloading
- Downstream processing
- Waste disposal


Substrate preparation:
chopping, grinding etc,
sterilization/cooking
Inoculum preparation
Fermentation in bioreactor:
• sterilization of bio-reactor before load-ing?
• loading
• sterilization of sub-strate *in situ*?
• inoculation *in situ*?
• the fermentation it-self
• unloading
• first recovery step?
• cleaning and prepa-ration for next batch
bed of moist solid particles
Downstream processing
Waste disposal
Product finishing
Air preparation:
• filtration
• humidification,
• heating/cooling

- **Substrate preparation**
  - The substrate may need to be cut, milled, cracked, or granulated in order to obtain particles of an appropriate size. It may be necessary to add water and nutritional supplements or to cook or pre-treat the substrate to increase the availability of nutrients. The substrate might be sterilized, or at least pasteurized, outside the bioreactor. Alternatively, it may be possible and preferable to do this step with the substrate inside the bioreactor.
- **Inoculum preparation.**
  - The type and method of inoculum preparation depends on the microorganism involved. Many SSF processes involve filamentous fungi and therefore spore-based inocula may be used. The aim of this step is to develop an inoculum of sufficient size and high viability. The inoculum can often be prepared in one of various forms. For a fungal fermentation it may be possible to produce a suspended mycelial inoculum by SLF, or to undertake a solid-state fermentation followed either by suspension of spores in a liquid or by drying and grinding of the solid to produce a powder than can be used as the inoculum.

- **Bioreactor preparation**
  - The bioreactor must be cleaned after the previous fermentation, and may need to be sterilized before addition of the substrate, although, as noted above, in some cases it might be appropriate to sterilize the substrate inside the bioreactor.
- **Inoculation and loading**
  - The inoculation step may occur either prior to loading or after loading. If the substrate bed cannot be mixed within the bioreactor, inoculation must be done outside the bioreactor. If the bed can be mixed, then the best method of inoculation might be to spray the inoculum as a mist over the bed as it is being mixed. If the substrate is pasteurized or sterilized and inoculated outside the bioreactor, it may be necessary to undertake the loading step quite carefully in order to prevent or at least minimize the entry of contaminants. At large scale, loading will need to be mechanically assisted.

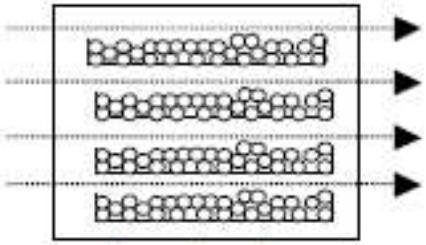
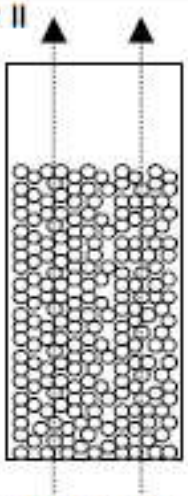
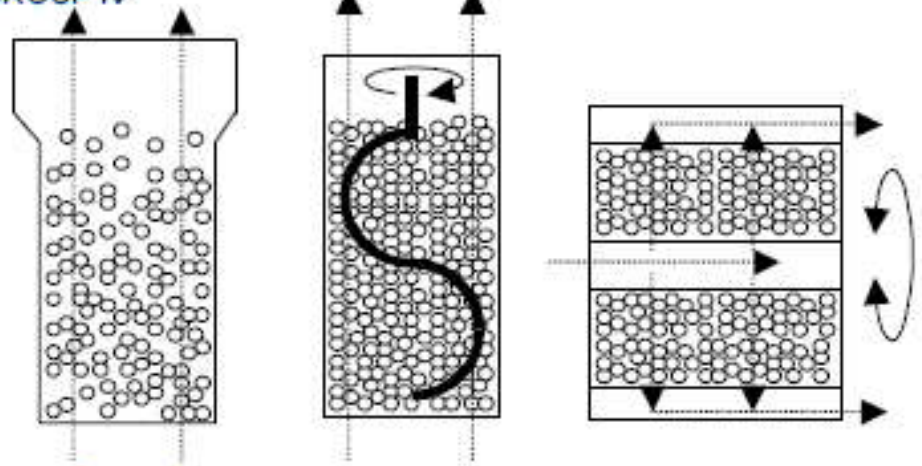
- **Bioreactor operation**
  - Much attention will be paid to this step later in the book. The details will depend on the specific bioreactor design, however, the general task is to manipulate various operating variables, such as the flow rate and temperature of the inlet air, the bed mixing speed, and the cooling water temperature, in order to control key fermentation parameters, such as bed temperature and water activity, at the optimum values for growth and product formation.
- **Unloading**
  - In some cases a leaching or drying step is undertaken within the bioreactor, in other cases the product recovery steps are undertaken outside of the bioreactor. In any case, solids must eventually be removed from the bioreactor. At large scale, unloading will need to be mechanically assisted.

- **Downstream processing**
  - Depending on the process, either the whole of the fermented solids represents the product or a specific product is recovered from the solids and then purified. In the latter case, the extraction of the product from the solids represents a step in SSF processes that is not necessary in SLF processes. However, after extraction, the general principles of downstream processing are similar for both SSF and SLF.
- **Waste disposal**
  - SSF is often suggested as a means of minimizing the impact of waste solid organic materials by preventing their being dumped in the environment. In some cases the whole solid is used as the product, for example, as a food or animal feed, but in others there will be a solid residue that must be disposed of adequately.

Types of Bioreactors

- SSF bioreactors can be divided into four groups on the basis of how they are mixed and aerated
  - Group I: Bioreactors in which the bed is static, or mixed only very infrequently (i.e., once or twice per day) and air is circulated around the bed, but not blown forcefully through it. These are often referred to as “tray bioreactors”.
  - Group II: Bioreactors in which the bed is static or mixed only very infrequently (i.e., once per day) and air is blown forcefully though the bed.(packed bed)
  - Group III: Bioreactors in which the bed is continuously mixed or mixed intermittently with a frequency of minutes to hours, and air is circulated around the bed, but not blown forcefully through it. Two bioreactors that have this mode of operation, using different mechanisms to achieve the agitation, are “stirred drum bioreactors” and “rotating drum bioreactors”. “packed-bed bioreactors”.

- Group IV: Bioreactors in which the bed is agitated and air is blown forcefully through the bed. This type of bioreactor can typically be operated in either of two modes, so it is useful to identify two subgroups. Group IVa bioreactors are mixed continuously while Group IVb bioreactors are mixed intermittently with intervals of minutes to hours between mixing events. Various designs fulfill these criteria, such as “gas-solid fluidized beds”, the “rocking drum”, and various “stirred-aerated bioreactors”.

Mixing → / ↓ Aeration	No mixing (or very infrequent)	Continuous mixing, or frequent intermittent mixing
No forced aeration (air passes around bed)	GROUP I  Tray chamber	GROUP III  Rotating drum Stirred drum
Forced aeration (air blown forcefully through the bed)	GROUP II  Packed bed	GROUP IV  Gas-solid fluidized bed Stirred bed Rocking drum

Applications of SSF

- SSF technology has been used for many centuries. Some examples of traditional SSF processes are:
  1. Tempe involves the cultivation of the fungus *Rhizopus oligosporus* on cooked soybeans. The fungal mycelium binds the soybeans into a compact cake, which is then fried and eaten as a meat substitute. This fermented food is quite popular in Indonesia;
  2. The *koji* step of soy sauce manufacture, which involves the cultivation of the fungus *Aspergillus oryzae* on cooked soybeans. During the initial SSF process of 2 to 3 days, the fungal mycelium not only covers the beans but also secretes a mixture of enzymes into them. The fermented beans are then transferred into brine, in which, over a period of several months, the enzymes slowly degrade the soybeans, leaving a dark brown sauce.
  3. Ang-kak, or "red rice", which involves the cultivation of the fungus *Monascus purpureus* on cooked rice. The fungus produces a dark red pigment. At the end of the fermentation the red fermented rice is dried and ground, with the powder being used as a coloring agent in cooking.

- Interest in SSF technology over past decades has led to the production of a myriad of different products like:
  - ❑enzymes such as amylases, proteases, lipases, pectinases, tannases, cellulases, and rennet
  - ❑pigments
  - ❑aromas and flavor compounds
  - ❑"small organics" such as ethanol, oxalic acid, citric acid, and lactic acid
  - ❑gibberellic acid (a plant growth hormone)
  - ❑protein-enriched agricultural residues for use as animal feeds
  - ❑animal feeds with reduced levels of toxins or with improved digestibility
  - ❑antibiotics, such as penicillin and oxytetracycline
  - ❑biological control agents, including bioinsecticides and bioherbicides
  - ❑spore inocula (such as spore inoculum of *Penicillium roqueforti* for blue cheese production).
- There is also research into the use of microorganisms growing in SSF conditions to mediate processes such as:
  - ✓decolorization of dyes;
  - ✓biobleaching;
  - ✓biopulping;
  - ✓bioremediation.

Questions

- What is solid state fermentation? Describe two applications using SSF?
- Describe the different types of bioreactors used in SSF?
- What are the general steps involved in SSF? Briefly describe each?
- Compare SSF vs SLF?