Solid State Fermentation (SSF)

What Is “Solid State Fermentation”?

- Solid-state fermentation (SSF) involves the growth of microorganisms on moist solid particles
What Is “Solid State Fermentation”?

- “solid-substrate fermentation” is used to denote any type of fermentation process that involves solids.

Main point:

1. The majority of SSF processes involve filamentous fungi

2. The majority of SSF processes involve aerobic organisms.
Main point:

3. 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.

Main point:

4. The water content of a typical submerged fermentation is >95%
5. The water content of a typical solid state fermentation is typically between 40-80%
Why Should We Be Interested in SSF?

- The environment that the organism experiences in SSF is different from that experienced in SLF.
- In SLF it is relatively easy to control the conditions to which the process organism is exposed:

  **Temperature control**
  - The availability of O2
  - The availability of the nutrients
  **Shear forces**
  - PH control is relatively easy to provide.

These, and other differences, mean that SLF is an "easier" system with which to work.

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.

THEN WHY SSF???
WHY SSF?

- when the product needs to be in a solid form (e.g., fermented foods);

- 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.

- when the product is produced in both SLF and SSF, but the yield is much higher in SSF.

WHY SSF?

- 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;

- 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.

- 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.
Advantages of SSF

- The major advantages of SSF’s over conventional submerged fermentations are:
  - The small volume of fermentation mash or rector volume results in lower capital and operating costs
  - A lower chance of contamination due to low moisture levels
  - Ease of product separation
  - Energy efficiency

Applications of SSF:

1. **Tempe**, which involves the cultivation of the fungus *Rhizopus oligosporus* on cooked soybeans.

1. The **koji** step of soy sauce manufacture, which involves the cultivation of the fungus *Aspergillus oryzae* on cooked soybeans.

1. **Ang-kak**, or “red rice”, which involves the cultivation of the fungus *M. purpureus* on cooked rice. The fungus produces a dark red pigment.
Applications ....

- Enzymes
- Pigments; aromas and flavor compounds;
- “Small organics” such as ethanol, oxalic acid
- Gibberellic acid (a plant growth hormone);
- Antibiotics, such as penicillin and oxytetracycline;
- Spore inocula

(spore inoculum of Penicillium roqueforti for blue cheese production).

Applications ....

- There is also research into the use of microorganisms growing in SSF conditions to mediate processes such as:
  - Biobleaching;
  - Biopulping;
  - Bioremediation
The General Steps of an SSF Process:

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

Fermentation in bioreactor:
- sterilization of bioreactor before loading?
- loading
- sterilization of substrate in situ?
- inoculation in situ?
- the fermentation itself
- unloading
- first recovery step?
- cleaning and preparation for next batch

Substrate preparation: chopping, grinding etc, sterilization/cooking

Air preparation:
- filtration
- humidification,
- heating/cooling

Downstream processing

Waste disposal

Product finishing
The Physical Structure of SSF Bioreactor Systems

In order to understand the phenomena occurring within an SSF bioreactor, it is necessary to understand the physical arrangement of the various phases within the system, since the various phenomena occur within and between these phases. We can choose two different levels of detail to examine the physical structure of the system:

a. macroscale
b. microscale
Solid-State Fermentation Bioreactors

- Many different bioreactors have been used in SSF processes, however, based on similarities in design and operation, SSF bioreactors can be divided into groups on the basis of how they are mixed and aerated.
**Group I**

These typically consist of a chamber containing a large number of individual trays, stacked one above the other with a gap in between.

**Group II**

- A typical packed-bed bioreactor consists of a column of cylindrical or rectangular cross section, oriented vertically, with a perforated base plate on the bottom which supports a bed of substrate. Air is blown up through the base plate.
Group III

- These typically consist of a drum of cylindrical cross section lying horizontally.

- The drum is partially filled with a bed of substrate, and air is blown through the headspace. In rotating drums, the whole drum rotates around its central axis to mix the bed.

- In stirred drums, the bioreactor body remains stationary and paddles or scrapers mounted on a shaft running along the central axis of the bioreactor rotate within the drum.
Group IV

- Stirred-bed bioreactors.
- Rocking-drum bioreactors.
- Air-solid fluidized beds (ASFBs).

Stirred-bed bioreactors:

- are similar to the static packed bed in that a bed of substrate sits on a perforated base plate and air is forcefully blown through the bed, but rather than being static, an agitator is inserted and provides continuous or intermittent mixing. Such stirred beds are typically aerated from the bottom, and have the agitator inserted from the top.
**Rocking-drum bioreactors:**

- consist of three concentric cylinders: an inner perforated cylinder, an outer perforated cylinder, and an outer solid cylinder. The two outer cylinders rotate in relation to the inner cylinder, thereby mixing the substrate bed, although not very effectively.

**Air-solid fluidized beds (ASFBs):**

In this bioreactor, air is blown upwards through a perforated base plate at sufficient velocity to fluidize the substrate bed, which then behaves as though it were a fluid.
A simple mathematical model for predicting the temperature within a well-mixed SSF bioreactor: The model equations, showing the kinetic and balance/transport submodels and their interrelations

**KINETIC SUB-MODEL**
Parameters within the kinetic equation are expressed as functions of the environmental conditions. For example, $\mu = \frac{a \cdot e^{-Ea1/(RT+273)}}{1 + b \cdot e^{-Ea2/(RT+273)}}$.

**Growth kinetic equation**
\[ \frac{dX}{dt} = \mu(X) \left(1 - \frac{X}{X_{\text{max}}} \right) \]

**BALANCE/TRANSPORT SUB-MODEL**
Energy balance:
\[ MC_{\text{pa}} \frac{dT}{dt} = Y_Q \frac{dX}{dt} \]
Metabolic heat production
Equations that have been used to describe growth profiles or parts of growth profiles in SSF systems

<table>
<thead>
<tr>
<th>Name</th>
<th>Integrated Kinetic equation</th>
<th>Differential form</th>
<th>Parameters to be found by regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear</td>
<td>( C_t = C_i + C_{xt} )</td>
<td>( \frac{dC_{xt}}{dt} = k )</td>
<td>( k )</td>
</tr>
<tr>
<td>Exponential</td>
<td>( C_t = C_{xt} e^{\mu t} )</td>
<td>( \frac{dC_{xt}}{dt} = \mu C_{xt} )</td>
<td>( \mu )</td>
</tr>
<tr>
<td>Logistic</td>
<td>( C_t = \frac{C_{\text{max}}}{1 + C_{\text{max}}^{-1}} e^{-\mu t} )</td>
<td>( \frac{dC_{xt}}{dt} = \mu C_{xt} \left( 1 - \frac{C_{xt}}{C_{\text{max}}} \right) )</td>
<td>( C_{\text{max}}, \mu )</td>
</tr>
<tr>
<td>Deceleration</td>
<td>( C_t = C_{\text{ao}} \exp \left[ \frac{\mu}{k} \left( 1 - e^{-t} \right) \right] )</td>
<td>( \frac{dC_{xt}}{dt} = k A C_{xt} e^{-\mu t} )</td>
<td>( k, A )</td>
</tr>
</tbody>
</table>

KINETIC EQUATION

**e.g., logistic equation**

\[
\frac{C_{xt}}{C_{\text{max}}} = \frac{C_{xt}}{C_{\text{ao}}} + \frac{C_{\text{ao}}}{C_{\text{ao}}} e^{-\mu t}
\]

- \( C_{\text{max}}, C_{\text{ao}}, \) and \( \mu \) by regression
- Also need estimates of \( Y_{X_S} \) and \( m_s \)

### Equations

1. \[
\frac{dC_{xt}}{dt} = \mu C_{xt} \left( 1 - \frac{C_{xt}}{C_{\text{max}}} \right)
\]

2. \[
\frac{dD}{dt} = D_o \left( 1 - \frac{1}{Y_{X_S}} \right) \frac{dC_{xt}}{dt} - m_s C_{xt}
\]

3. \[
\frac{dC_{XR}}{dt} = D_o \frac{dC_{xt}}{dt} \frac{C_{XR}}{D} \frac{dD}{dt}
\]

Numerical integration of these three equations gives predicted profile for \( C_{xr} \).
Incorporating the Effect of the Environment on Growth

- Temperature
  
  Saucedo-Castaneda et al. (1990)

\[ \mu_T = A \exp\left(\frac{-E_{a1}}{R(T + 273)}\right) / \left[1 + B \exp\left(\frac{-E_{a2}}{R(T + 273)}\right)\right] \]
**Water Activity**

von Meien and Mitchell (2002)

\[ \mu_w = \mu_{opt} \exp \left( D_1 a_{w5}^3 + D_2 a_{w5}^2 + D_3 a_{w5} + D_4 \right) \]

- **Aspergillus niger**
  \[ \frac{\mu_w}{\mu_{opt}} = \exp \left( 618.92a_{w5}^3 - 1863.53a_{w5}^2 + 1865.10a_{w5} - 620.67 \right) \]

- **Rhizopus oligosporus**
  \[ \frac{\mu_w}{\mu_{opt}} = \exp \left( -131.60a_{w5}^3 + 94.00a_{w5}^2 + 214.22a_{w5} - 177.67 \right) \]

**Temperature & Water Activity**

\[ f = \frac{\mu_{measured}}{\mu_{opt}} \quad \mu = \mu_{opt} \sqrt{f_T f_W} \]
Modeling Death Kinetics

- The simplest assumption is that death is a first order process, giving the equation:

\[
\frac{dC_{XAD}}{dt} = r_d = k_d C_{XAV}
\]

- In the case in which growth follows logistic kinetics then the equation for total biomass production might be:

\[
\frac{dC_{XAT}}{dt} = \mu C_{XAV} \left(1 - \frac{C_{XAT}}{C_{XAM}}\right)
\]

The simplest assumption is that death is a first order process, giving the equation:

\[k_d = A_d \exp\left(\frac{-E_{od}}{R(T + 273)}\right)\]

\[\mu = A_g \exp\left(\frac{-E_{og}}{R(T + 273)}\right)\]
Modeling of the Effects of Growth on the Local Environment

High intensity light

Various effects that we may wish to include in a bioreactor model are:

- the liberation of waste metabolic heat;
- the consumption of substrate (i.e., overall residual substrate) or particular nutrients;
- the consumption of O₂ and production of CO₂;
- the production of water;
- the formation of products

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- **Metabolic Heat Production**
  \[ r_O = Y_{OX} \frac{dX}{dt} + m_O X \]

- **Water Production**
  \[ r_W = Y_{WX} \frac{dX}{dt} + m_w X \]

- **Substrate & Nutrient Consumption**
  \[ r_N = \frac{1}{Y_{XN}} \frac{dX}{dt} + m_N X \]

- **Oxygen Consumption**
  \[ r_O = OUR = \frac{1}{Y_{XO}} \frac{dX}{dt} + m_o X \]

- **Carbon Dioxide Production**
  \[ r_C = CER = Y_{CX} \frac{dX}{dt} + m_c X \]