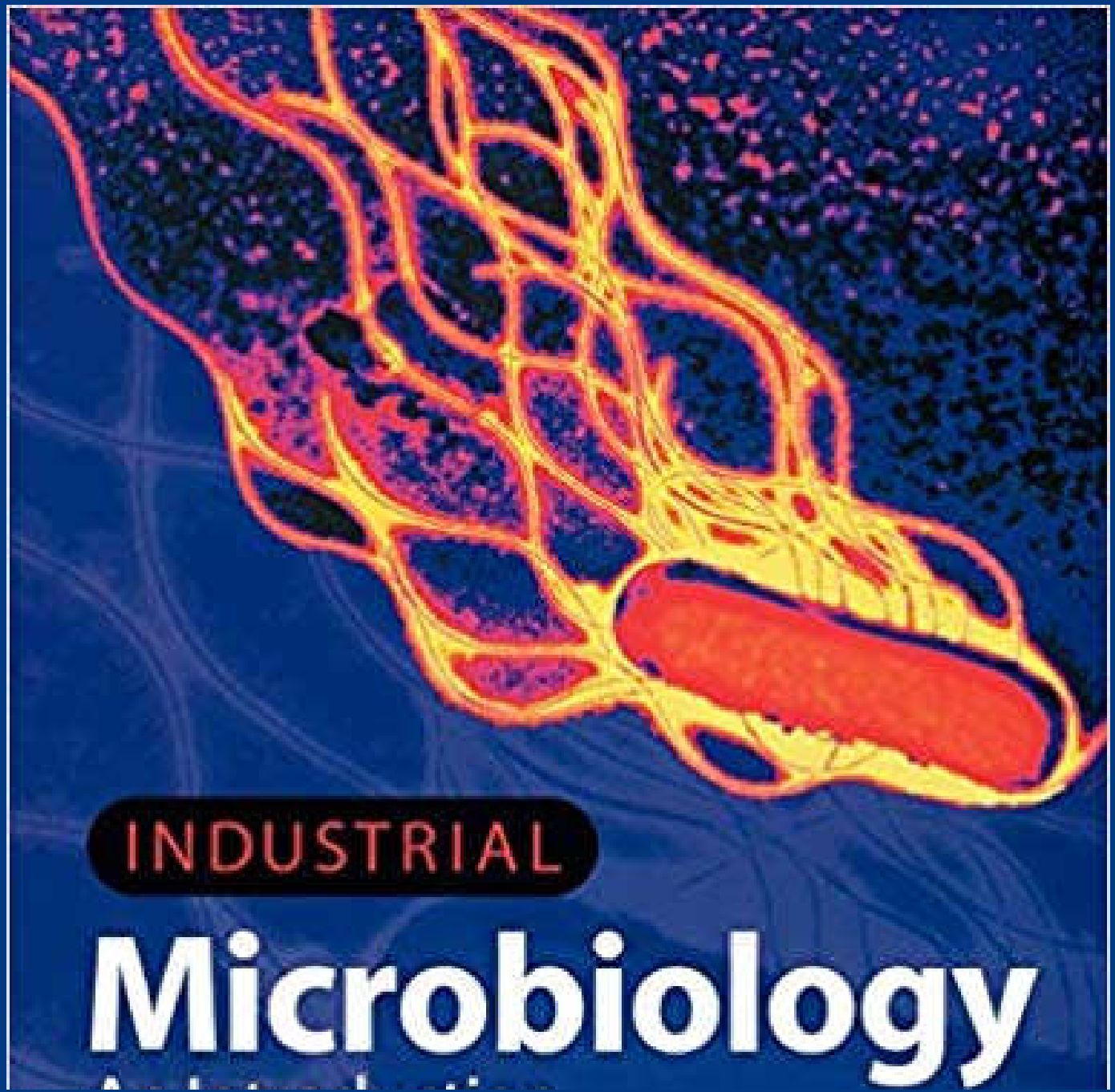
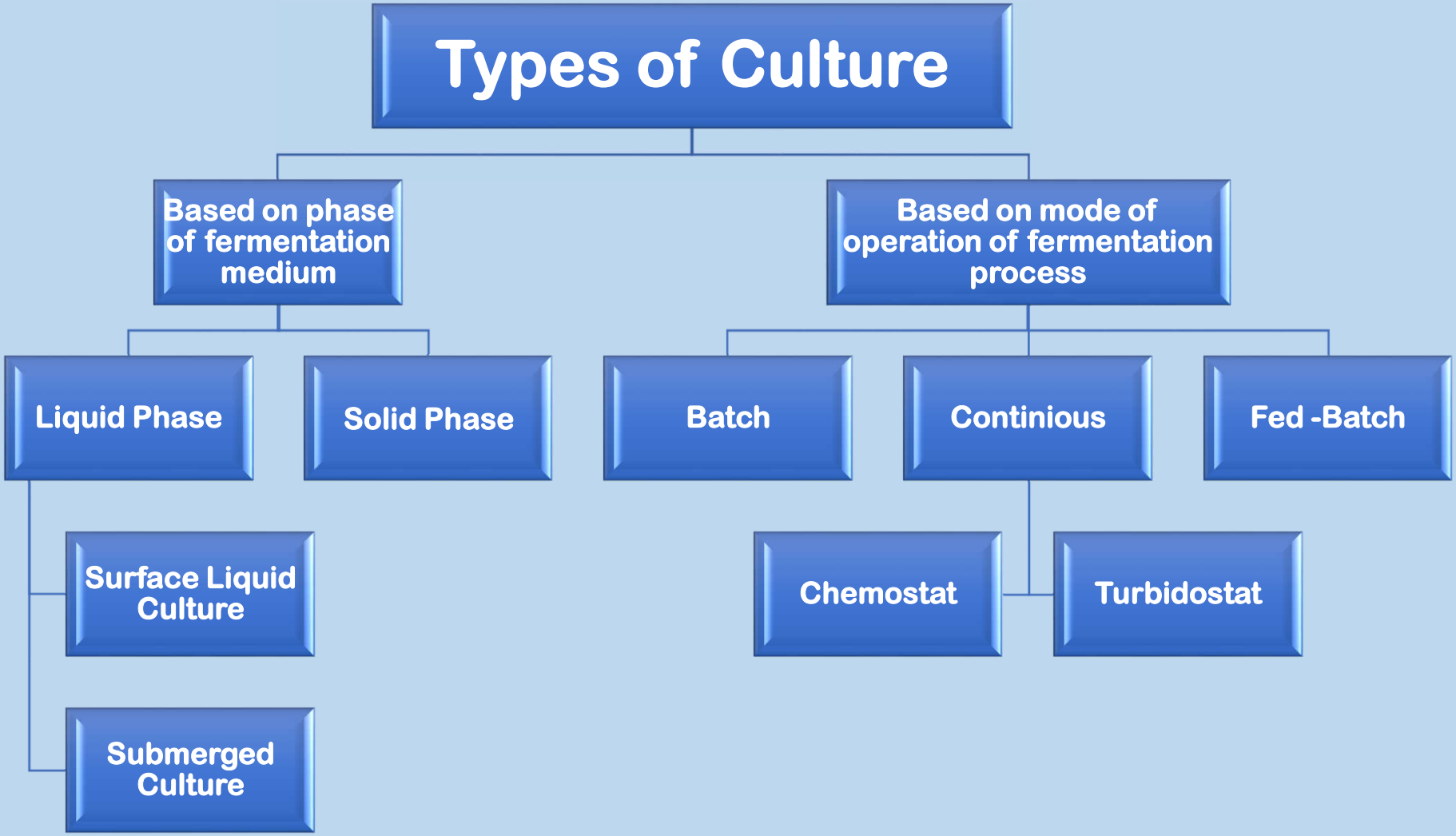
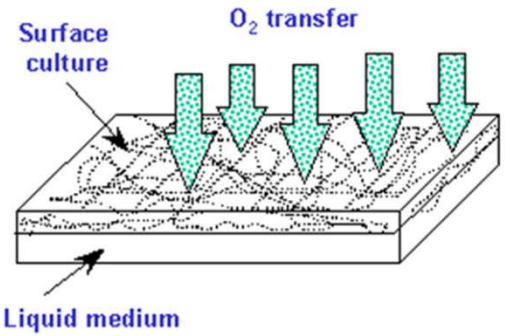

INDUSTRIAL
Microbiology


Types of Culture
Based on phase of fermentation medium
Based on mode of operation of fermentation process
Liquid Phase
Solid Phase
Batch
Continious
Fed -Batch
Surface Liquid Culture
Submerged Culture
Chemostat
Turbidostat

SURFACE CULTURE METHOD

Liquid surface culture process: -Generally preferred for fungus, especially mold based production process. Alluminium or stainless steel shallow pans (5-20 cms deep) or trays are used. The sterilized medium usually contains media and salts. The fermentation is carried out by blowing the fungal spores of production strain over the surface of the solution. Surface culture is used for production of citric acid and Penicillin. Spore germination occurs within 24 hours and a white mycelium grows over the surface of the solution within 4 days. This process is carried out to maximize the surface of substrate exposed to the air. The liquid can be drained off and any portion of mycelial mat left becomes submerged and inactivated. Required products are obtained by suitable downstream processing of the media.

***Aspergillus niger* mycelia**



Submerged fermentation:

- In the submerged process, the substrate used for fermentation is always in liquid state which contains the nutrients needed for growth.
- The fermentor which contains the substrate is operated continuously and the product biomass is continuously harvested from the fermenter by using different techniques then the product is filtered or centrifuged and then dried.
- Submerged fermentation is a method of manufacturing biomolecules in which enzymes and other reactive compounds are submerged in a liquid such as alcohol, oil or a nutrient broth.

Submerged fermentation / liquid fermentation

- Submerged fermentation is the techniques of cultivation of microorganism in liquid broth which breaks down the nutrient to release the desired bio-active compound into solution.
- In this method, selected microorganism are grown in closed vessels containing a broth rich in nutrients and high concentration of oxygen.
- In SmF substrate are utilized quite rapidly hence need to be constantly replaced or supplemented with nutrients
- Bacteria that requires high moisture content or high water activity are best suited for submerged fermentation.

The process is used for a variety of purposes, mostly in industrial manufacturing

Applications:

- Submerged Fermentation (SmF)/Liquid Fermentation (LF) SmF utilizes free flowing liquid substrates, such as molasses and broths. The bioactive compounds are secreted into the fermentation broth.
- The substrates are utilized quite rapidly; hence need to be constantly replaced/supplemented with nutrients.
- This fermentation technique is best suited for microorganisms such as bacteria that require high moisture.
- An additional advantage of this technique is that purification of products is easier.
- SmF is primarily used in the extraction of secondary metabolites that need to be used in liquid form

What is Solid State Fermentation??

- Solid-state (substrate) fermentation (SSF) has been defined as the fermentation process occurring in the absence or near-absence of free water.
- SSF employs natural raw materials as carbon source such as cassava, barley, wheat bran, rice bran, sugarcane bagasse, cassava bagasse, various oil cakes (e.g. coconut oil cake, palm kernel cake, soybean cake, ground nut oil cake, etc), fruit pulps (e.g. apple pomace), corn cobs, saw dust, seeds (e.g. tamarind, jack fruit), coffee husk and coffee pulp, tea waste, spent brewing

Advantages of solid-state fermentation

- Low-cost media / Simple technology / Low capital costs / Reduced energy requirements / Low waste-water output
- Higher and reproducible product yields / Concentrated substrate / Smaller fermentation vessels
- Seed tanks are unnecessary, and spore inocula may be used
- Low moisture reduces the problem of contamination
- Conditions for fungal growth are similar to those in natural habitats
- Can be used to provide low-shear environments for shear-sensitive mycelial organisms
- Products may be incorporated directly into animal feeds

Solid state fermentation

- Solid state fermentation has been defined as "the fermentation process occurring in the absence or near absence of free water utilizing the solid substrate".
- It is a biomolecule manufacturing process used in the food, pharmaceutical, cosmetic, fuel and textile industries. These biomolecules are mostly metabolites generated by microorganisms grown on a solid support selected for this purpose.
- This technology for the culture of microorganisms is an alternative to liquid or submerged fermentation, used predominantly for industrial purposes

Disadvantages of solid-state fermentation

- Slower microbial growth
- Problems with heat build-up
- Continuous agitation or rotation may involve high power requirements
- The addition of water in early fermentation stages may increase the risk of bacterial contamination
- Difficult scale-up and considerable developmental work
- Difficult control of the environment within the bioreactors, particularly the simultaneous maintenance of optimal temperature and moisture
- Agricultural substrates may require some kind of mechanical, chemical or biological pretreatment processing

ADVANTAGES OF SOLID STATE FERMENTATION OVER SUBMERGED FERMENTATION

- 1. HIGHER VOLUMETRIC PRODUCTIVITY.
- 2. USUALLY SIMPLER WITH LOWER ENERGY REQUIREMENTS
- 3. MIGHT BE EASIER TO MEET AERATION REQUIREMENTS
- 4. RESEMBLES THE NATURAL HABITAT OF SOME FUNGI AND BACTERIA
- 5. EASIER DOWNSTREAM PROCESSING.

APPLICATION OF SOLID STATE FERMENTATION

- **A. Production of Enzymes by Solid State fermentation-**
  protease, lipase, cellulase, pectinase
- **B. Production of Organic Acids under Solid State Fermentation-**
  citric acid, lactic acid.

C. Secondary Metabolites production Under SSF Condition- antibiotics, quinolines, growth factors, terpenoides

D. Production of Biocontrol Agents under Solid State Fermentation- eg. Biocontrol agent- microbial agent, fungal agent. ***Liagenidium giganteum*** a fungal agent used for control of Mosquitoes.

E. Production of Biofuel by Solid State Fermentation
eg. ethanol

Submerged fermentation	Solid state fermentation
1. Fermentation may be carried out as batch or continuous 2. Medium is added in large vessel 3. Surface area to volume height ratio is very less 4. 5-10% of inoculums is added 5. Inoculum is usually in liquid form 6. Product used are usually high as compared to input cost	1. Fermentation may be carried out as batch 2. Medium is added in flat vessel or trays 3. Surface area to volume height ratio is very high 4. Less inoculum is added 5. Inoculum is usually sprayed on surface of medium 6. Product yield is comparatively less
7. Lesser space is required 8. Less contamination 9. If a batch get contaminated there is a loss of entire batch 10. Entire fermentation media is utilized by microorganism for growth and product fermentation 11. Aeration and agitation of system is possible by use of sparger and impeller	7. More space is required 8. More contamination 9. If a tray gets contaminated then there is a loss of only tray but not the batch 10. There is wastage of fermentation media 11. Aeration is usually carried out by passing sterile air and no agitation
12. Power consumption is high 13. Controlling parameters like temperature, pH is easy 14. Foaming occurs 15. Automation and use of computer is easy 16. Less labor required 17. eg penicillin, streptomycin production etc.	12. Power consumption is less 13. Controlling parameters like temperature, pH is difficult 14. Foaming doesn't occurs 15. Automation and use of computer is difficult 16. More labor required 17. eg Veniger, amylase production etc.

SUBTRATES

Table.1

Submerged fermentation (SmF)	Solid state fermentation (SSF)
➢ Soluble sugar ➢ Molasses ➢ Liquid media ➢ Fruit and vegetable juices ➢ Sewage / waste water	➢ Wheat bran ➢ Rice and wheat straw ➢ Fruit and vegetable waste ➢ Paper pulp ➢ Bagasses ➢ Coconut coir ➢ Synthetic media

Batch Cultures

Clip slide

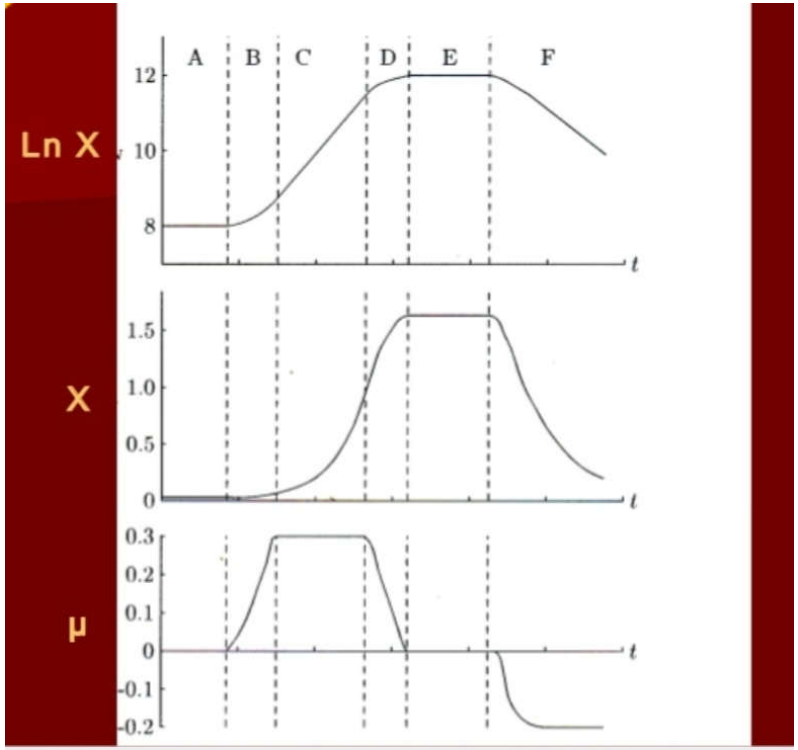
- In a batch operation, all necessary medium components and the inoculum are added at the beginning and not during period of fermentation.
- Therefore, their concentrations are not controlled but are allowed to vary as the living cells take them up.
- The products, be they intra- or extracellular, are harvested only at the end of the run.
- Basic controls for pH, temperature, dissolved oxygen, and foam are applied during the course of batch culture.
- The pH, dissolved oxygen, and temperature are normally held constant during the course of batch reactor operation.
- The only optimization parameters are the initial medium composition.
- However, profile optimizations of temperature and pH may lead to improved performance over the operations carried out at constant temperature and constant pH.

- Batch cultivation is closed system where there is no interaction between the system and the surrounding during the process. Except air during the aerobic cultivation.
- In Batch cultivation we prepare medium, sterilize it and inoculate the culture into the bioreactor.
- Allow the cells to grow and produce the product.
- Once the product formation reaches maximum harvest the fermentation broth.

How cells grow during Batch cultivation

After inoculating the medium and start measuring the biomass at different time intervals, you may find six different phases. They are

1. Lag phase
2. Accelerated growth phase
3. Exponential growth phase
4. Decelerated growth phase
5. Stationary phase
6. Death phase



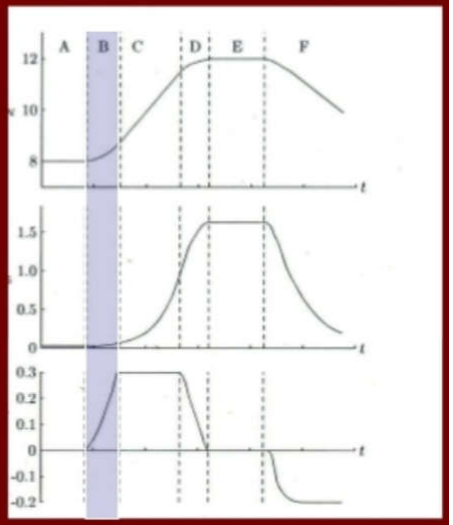
How to avoid lag phase

Lag phase is non productive period in the industrial fermentations. Hence minimizing it is essential.

- **The stage of culture from where the inoculum is drawn is important. Exponentially growing cells will have adequate concentrations of intermediates and intracellular pool of compounds. Hence if the inoculum is drawn from this stage they will not suffer dilution effect.**
- Size of the inoculum – If the size of the inoculum is large then the lag phase can be minimized. Generally 10% are used for yeast and mold and 5% for bacteria.
- Medium of inoculum should be same of that production medium.
- In certain cases such as recombinant *E.coli* cultivation to minimize plasmid loss higher percentage of inoculum will be used.

Accelerated growth phase

- At the end of Lag phase, when growth begins the division rate increase gradually and reaches a maximum value.
- The sp growth rate increases to maximum during this phase.



Exponential growth phase

- Cell division occurs in this phase.
- Often cell dry weight is used for cell concentration. During exponential phase we write as

$$\frac{dX}{dt} = \mu X$$

Where μ - Specific growth rate

X- cell dry weight

Rearranging and integrating the eqn

$$\int_{X_o}^{X} \frac{dX}{X} = \mu \int_{0}^{t} dt$$

$$\ln X \Big|_{X_o}^{X} = \mu t \Big|_{0}^{t}$$

$$\ln\left(\frac{X}{X_o}\right) = \mu t$$

$$X = X_o e^{\mu t}$$

Other phases of growth

- The end of the exponential phase occur when any of the essential nutrients is depleted or toxic metabolite accumulated in the system. During this phase the growth rate declines.
- Stationary phase will follow this phase. The length of stationary phase may vary with cell type, previous growth conditions etc., In certain cases the product formation will occur during this phase
- Following this is the death phase where the cells will start to lyse and the cell density decreases.

5.7. GROWTH YIELDS

When microbial growth is limited by low concentration of required nutrient, the final net growth or yield of cells increases with the initial amount of the limiting nutrient present. The rate of growth also increases with nutrient concentration but in a hyperbolic manner. The shape of the curve reflects the rate of nutrient uptake by microbial transport proteins.

When the nutrient quantity limits the production of bacteria, it is possible to define a growth yield constant (Y). The growth yield constant is the amount of dry weight of cells produced per weight of nutrient used

$$Y = \frac{\text{mass of microorganism formed}}{\text{mass of substrate consumed}}$$

The molar growth yield constant is Y_m, which is the dry weight of the cells produced (in grams) per mole of substrate used. For example, the $Y_{glucose}$ for aerobically growing cells is about 0.5, which means about 50% of the sugar is converted to cell material and 50% oxidised to CO_2. For certain sugars and bacteria, the efficiency of conversion of cell material can be much lower (e.g, 20%). In dilute media, some bacteria are able to increase their efficiency and assimilate upto 80% of the sugar acquired.