UNIT -4

Kinetics of Substrate Utilisation, Product Formation and Biomass Production in Cell Cultures

1.The kinetics of substrate consumption in cellular growth and enzyme-catalysed reaction and their relationship with bioreactor modeling

- unstructured batch growth models
- structured kinetic models
  - compartmental models
  - metabolic models

2. The kinetics of product formation

- Unstructured models
- Structured product formation kinetics models

3. Microbial and enzyme kinetics models and their applications in bioreactor design

- Continuous-stirred-tank bioreactor
- Plug-flow-tubular bioreactor with immobilised enzyme

The kinetics of substrate consumption in cellular growth and enzyme-catalysed reaction and their relationship with bioreactor modeling

The growth of cell with the presence of enough substrate can be described using other form of model equations. These models are proposed in such a way that they could give better fits to experimental data points.

Model equation

Tessier equation: $\mu = \mu_m [1 - \exp(-KC_s)]$

Moser equation: $\mu = \dfrac{\mu_m C_s^n}{K_s + C_s^n}$

Contois equation: $\mu = \dfrac{\mu_m C_s}{K_{sx} C_x + C_s}$

Unstructured Batch Growth Models

$dx/dt = f(x)$

Such a model does not need to neglect any changes occurring in the medium during growth. Inhibition was than introduced assuming that it is proportional to the squared of the amount of cells;

$dx/dt = kx(1 - \beta x)$

Integrating gives,

$x = x0e^{kt}/1 - \beta x\ 0(1 - e^{kt})$

At a stationary state when $dx=dt = 0$, the curve representing the amount of cells would give $xs = 1/\beta$

If the production rate of an inhibitor/toxin is proportional to the population growth rate, therefore;

$dctox/dt = \alpha\ dx/\ dt$

with α as the constant of proportionality, with the initial condition given as $ctox(0) = 0$, integrating the above differential form would give;

$ctox = \alpha\ (x - x0)$

Most of the equations described above failed to describe the declining phase after the stationary period of nutrient exhaustion. A form of integral function has been introduced as an addition to μx which is given by;

$dc/dt = \| K(t)x(t) \|$

Comparing equations

$dx/dt = kx(1 - \beta x) + ko\|\int x(r)\, dr \|$

The disadvantage of the model are it doesn't account for lag phase and it doesn't give detailed information into the variables which influence the growth.

Compartmental models

- the simplest structured models
- compartmentalisation of components/section into small sizes
- synthetic components such as RNA and precursors
- structural components such as DNA and proteins
- this could also be defined as the assimilatory component and a synthetic component
- class of transport and reaction occurring in a cell population include;
    - molecular collision
    - chemical reactions

Compartment models are lumped models. The cell is compartmentalized into a small no. of components related to the main functions of cell.The concept of the compartmental model assumes that the system can be divieded into a number of homogenous well mixed componenets caleed compartment.

Metabolic models

- this type of models incorporate some aspects cell metabolism
- more biological detail incorporated in a model, it will become more specific to a particular organism/process
- the more detailed the model becomes, the more one should know a prior about the organism

Product Formation Kinetics

Unstructured model

Simplest type of product formation kinetics arise when there is a simple stoichiometric connection between product formation and substrate utilisation or cell growth. The rate of product formation can be written as;

$rfs = -Y_{P/S}\, rfs$

it can also be expressed as;

$rfp = Y_{P/X}\, rfx$

For anaerobic fermentation such as Lactobacillus delbrukii, the kinetics of product formation is famously expressed by the Leudeking-Piret kinetics given by;

$rfp = \alpha rfx + \beta x$

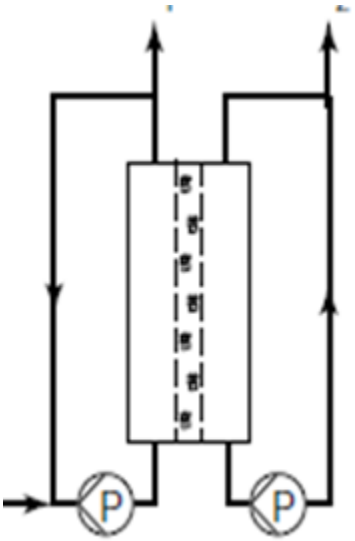
Such a form is normally used in fitting product formation data. Microbial and enzyme kinetic models and their applications in bioreactor design

Plug-flow-tubular bioreactor with immobilised enzyme

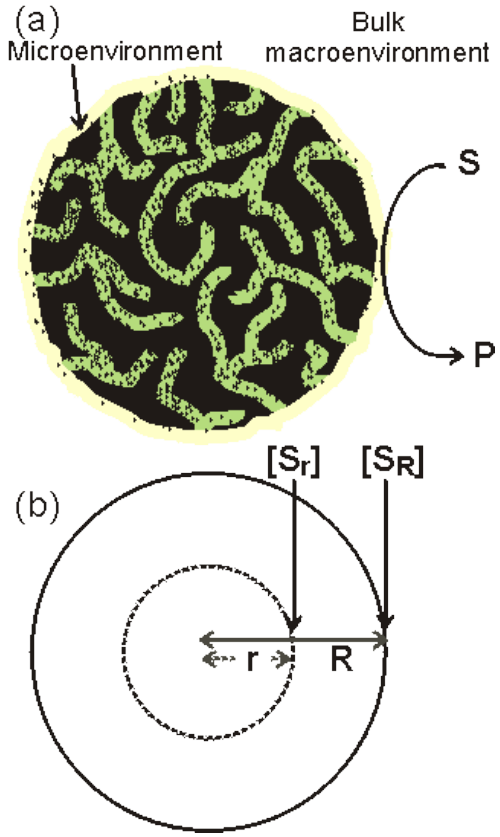
Plug Flow Turbular (Bio)Reactor

- it is an alternative to CSTR system
- no mixing required
- fluid entering the reactor passes through as a discrete "plug" and it does not interact with neighbouring fluid element
- can be achieved by supplying high fluid flowrate which could minimise back-mixing and variation of fluid velocity

the diagram of a plug-flow-tubular reactor is given in Figure



Immobilised enzymes



This is one type of heterogeneous reaction which considers only the concentration gradient. Temperature effect in for biological based reactions is relatively low and the effect can be generally neglected.Reactions normally involve solid-phase catalysts that consist of macroscopic flocs, clumps and pellets.If cells or enzymes do not produce such clumps of solid surfaces, they can be induced to do so using immobilisation technique. Immobilisation can be done in various methods such as;

- entrapment within gels (agarose, alginate and carrageenan)
- porous solid material (ceramics, porous glass and resin beads)

In both methods above, site of reaction are distributed throughput the particles, catalyst particle of higher activity can be formed by increasing the loading of cells or enzyme per volume of matrix.

Advantages:

- continuous operation with the same material (recycle of catalyst) enhance stability of certain enzymes by increasing their half-life

- each enzyme/cell responds to substrate concentration at its location with rate of reaction determined by the kinetic parameters of the catalyst. This is known as intrinsic rate of reaction.
- the actual (true) rate of reaction is difficult to measure in solid catalyst without altering the reaction conditions{but the overall reaction rate for the entire catalyst can be measured.
- in a closed system, rate of disappearance of substrate from bulk liquid must equal the overall rate of conversion, this is known as the observed rate.

Sterilisation

Sterilisation refers to any process that effectively kills or eliminates transmissible agents such as fungi, bacteria, viruses, prions and spores forms from a surface, equipment, foods, medications or biological culture medium. Sterilization can be achieved through application of heat, chemicals, irradiation, high pressure or filtration.

There are four types of sterilizations:

1. **physical sterilisation**
- heat sterilisation
- radiation sterilisation

2. **chemical sterilisation**
- ethylene oxide
- ozone
- chlorine bleach
- glutaraldehyde
- formaldehyde
- hydrogen peroxide
- peracetic acid
- prions

3. Sterilization by radiation
4.Sterilization by mechanical methods

Batch sterilization

The objective is to achieve the required probability of obtaining a minimum loss of nutritive quantity. The highest temperature which is feasible in batch sterilization is 121ºC.Heat is provided by external jacket and cooling is carried by sparging cooling medium.

Continuous sterilization

The continuous system includes the time period during which the medium is heated to the sterilization temperature , a holding time at the desired temperature and cooling period to restore the medium to fermentation temperature. Temperature as high as 140ºC can be achieved .Heating is achieved by heating coils and cooled by heat exchange with the remaining media.

Del factor

▼= ln (No/Nt)

▼ total =▼ heating+▼ holding +▼ cooling

No- no. of viable organism before the sterilization treatment

Nt-number of viable organism after the sterilization treatment

Applications

Foods

The first application of sterilisation was through a thorough cooking to affect the partial heat sterilisation of foods and water. Cultures that practice heat sterilisation of food and water have longer life expectancy and lower rates of disability. Canning of foods by heat sterilisation was an extension of the same principle. Ingestion of contaminated food and water remains a leading cause of illness and death in the developing world, particularly for children.

Medicine and Surgery

Generally, surgical instruments and medications that enter an already sterile part of the body such as blood or beneath the skin must have a high sterility assurance level. Examples of such instruments include scalpels, hypodermic needles and artificial pacemakers. This is also essential in the manufacture of parenteral pharmaceuticals. Heat sterilization of medical instruments is known to have been used in ancient Rome, but mostly disappeared throughout the Middle ages resulting in significant increases in disability and death following surgical procedures. Preparation of injectable medications and intravenous solutions for fluid replacement therapy requires not only a high sterility assurance level, but well-designed containers to prevent entry of adventitious agents after initial sterilization.

Heat Sterilisation

Steam sterilisation

A widely used method for heat sterilization is the autoclave. Autoclaves commonly use steam heated to 121±ºC or 134±ºC. To achieve sterility, a holding time of at least 15 minutes at 121±ºC or 3 minutes at 134±ºC is required. As items such as liquids and instruments packed in layers of cloth may take longer to reach the required temperature than the steam solid instruments additional sterilizing time is usually required. After sterilization, autoclaved liquids must be cooled slowly to avoid boiling over when the pressure is released. Proper autoclave treatment will inactivate all fungi, bacteria, viruses and also bacterial spores, which can be quite resistant. It will not necessarily eliminate all prions. For prion elimination, various recommendations state 121-132±ºC (270±F) for 60 minutes or 134±ºC (273±F) for at least 18 minutes.

This is the most common method of sterilization. The heat used kills the microbes in the substance. The temperature of the heat and duration of heating are the factors that affect the extent of sterilization. In heat sterilization process, the longer the exposure to heat the better is the sterilization at a given temperature. As the temperature of heat raises the time span required for sterilization decreases. Further, the sterilization time increases with a decrease in temperature and vice-versa. But one needs to maintain minimum sterilization time or minimum contact time for the heat to be in touch with microbes or bacteria and thereby kill them.

The heat method of sterilization is again of two types based on the type of heat used.

A) Moist heat methods.
B) Dry heat methods.
Moist heat method of sterilization: Here heat is applied in the form of steam or just boiling. This method includes techniques like

- Boiling.
- Pasteurization.
- By use of steam (Autoclave).

Boiling is preferred for metallic devices like surgical scissors, scalpels, needles, etc. Here substances are boiled to sterilize them. Pasteurization is the process of heating the milk at a temperature of 60 degrees or 72 degrees 3 to four times. Here alternative heating and cooling kills all the microbes and molds without boiling the milk.
Using Steam (autoclaving): Here the substances are subjected to sterilization in an autoclave sterilization equipment. The process is carried out at a temperature of 115 degrees for 60 min or 121 degrees for 20 min at 15psi pressure.The saturated steam is formed at boiling temperature of water, i.e.,100 degrees.This steam condenses on the material and relieves the latent heat repeatedly to convert back into the water.Further, the saturated steam under pressure penetrates all the narrow spaces leaving no microbes alive thereby making the sterilization very efficient.It is the most common method used for drugs as it is powerful enough even to kill bacterial spores.Bacterial spores are the forms of bacteria which are inert. They form a rigid cover over the cell wallduring harsh climate. This cover prevents any damage to cell and drying of the cell. By steam sterilization, these forms of bacteria are also killed as steam destroys the cell wall.

Dry heat methods: Here the substances are subjected to dry heat like

- Flaming
- Incineration
- Hot air oven.
- Radiation sterilization

Flaming is the process of exposing metallic device like the needle, scalpels, scissors to flame for few minutes. The fire burns the microbes and other dust on the instrument directly.
Incineration is done especially for inoculating loops used in microbe cultures. The metallic end of the loop is heated to red hot on the flame. This exposure kills all the germs.

Hot air oven is suitable for dry material like powders, metal devices, glassware, etc. Here thermostable materials on the racks inside the hot air oven. Then in the closed oven, hot air is circulated at particular temperature and time.

Radiation Sterilization

Radiation method involves exposing the packed materials to radiation for sterilization. There are two types of radiations available for sterilization i.e. non-ionic and ionic radiation.

- Non-ionic radiations are safe to the operator of sterilization, and they are like Ultra Violet radiations, they can be used even at the door entrances to prevent entry of live microbes through the air.
- Ionizing radiation sterilization. They are powerful radiation and very useful for sterilization. The operator needs to protect himself from exposure from these radiations by use of special clothing. Ex: X-rays, γ-rays, etc.

Chemical method of sterilization

Here the articles are subjected to sterilization by using toxic gasses. The gas penetrates quickly into the material like steam so, the sterilization is effective. But the chances of explosion and cost factors are to be considered. The gasses used for sterilization are very poisonous. The commonly used gas is ethylene oxide with a combination of carbon-dioxide. Carbon dioxide is added to minimize the chances of an explosion.

Filtration Sterilization

Here the liquids are filtered through bacterial filters to remove any microbes present. This method is very effective for sterilization of heat sensitive liquids. The chances of clogging and long time duration for the process to happen are drawbacks.
For sterlization three types of filters are used viz.

A) Membrane filters: These are thin filters which are made of cellulose. They can be employed for online sterilization during injection by placing the membrane between the syringe and needle. Used for sterilization of solvents, gasses.
The disadvantage is there are chances of rupture of membrane leading to improper sterilization.

B) Seitz filters: These are made of asbestos or other material. They are pad like and thicker than membrane filters. They do not rupture during filtration. But the solution might get absorbed by the filter pad itself.
An alternative type of filter is sintered glass filters. These are made of glass and hence do not absorb liquids during filtration. The disadvantage is that they are very brittle and break easily.
c) Candle filters: These are made of clay like diatomous mud. This special mud has minute pores made by algae. The filters have many minute lengthy pores. The microbes get stuck during their travel through the pore in the candle.
So of the available methods,

1. Methods of sterilization of surgical instruments are Boiling, Incineration, Autoclave.

2. Methods of sterilization of glass ware are autoclave, boiling and also the hot-air oven.

3. Methods of sterilization of water we use filtration and for other moist liquid material autoclave.

4. For powders and other dry forms, it is hot air oven if thermostable or gaseous methods and radiation.

5. Methods of Sterilization in hospitals are for surgical metallic instruments boiling, autoclave, incineration can be done. To prevent microbial contamination due to air,UV radiation lamps for sterilization can be arranged at the doors.Also, ultra sound waves are being tested for sterilization. Though it is not as effective as other methods, it was found to be useful in tissue cultures. Here the aim is to sterilize or even prevent the growth of bacteria during culturing of tissue.