

Lecture Notes
Bioprocess Engineering
by
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Lecture No 1

Fermentation: Introduction

①

Fermentation (tum) → derived from Latin verb

Fervere

(to boil)

describe the appearance of action of yeast on extracts of fruits or malted grain.

Boiling action is due to → Production of CO_2 bubbles caused by anaerobic catabolism of sugar present in the extract.

Meaning of fermentation to Biochemist & Industrial microbiologist

Biochemical meaning → generation of energy by catabolism of organic compounds.

Catabolism of Sugar:-

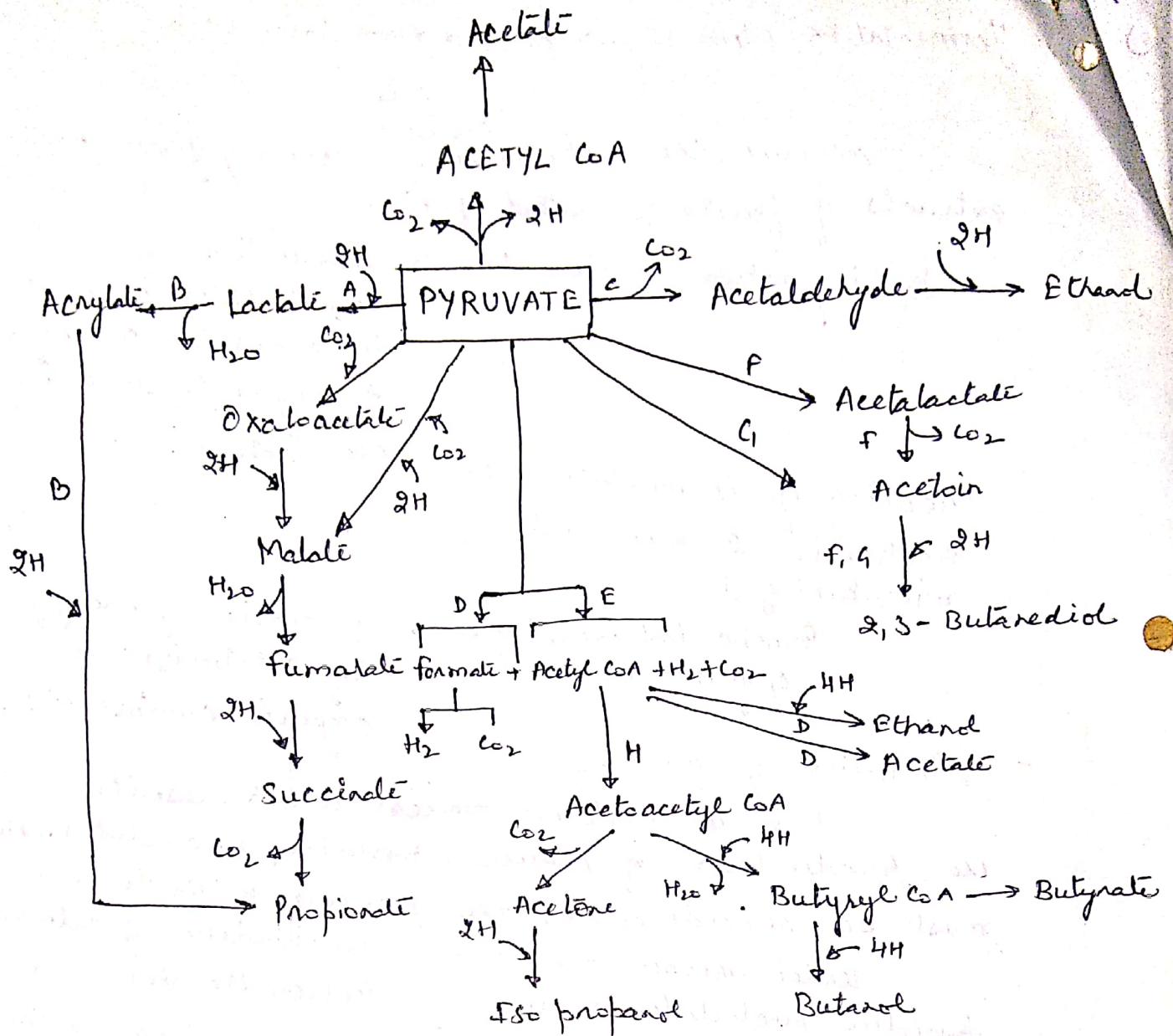
It is an oxidative process which results in the production of reduced pyridine nucleotides which must be reoxidized for the process to continue.

Under aerobic conditions, reoxidation of reduced pyridine nucleotide occurs by electron transfer via the cytochrome system, with oxygen acting as the terminal electron acceptor.

Under anaerobic conditions, reduced pyridine nucleotide oxidation is coupled with the reduction of an organic compound, which is often a subsequent product of the catabolic pathway.

In case of the action of yeast on fruit or grain extract, NADH is regenerated by the reduction of pyruvic acid to ethanol.

Different microbial taxa are capable of reducing pyruvate to a wide range of end products.



Bacterial fermentation product of pyruvate

Pyruvate formed by the catabolism of glucose is further metabolized by pathways which are characteristic of particular organism & which serve as a biochemical aid to identification.

- A → Lactic acid bacteria (Streptococcus, Lactobacillus)
- B → Clostridium propionicum
- C → Yeast, Acetobacter, Zymomonas
- E → Clostridia
- F → Klebsiella
- G → Yeast
- H → Clostridia (butyric, butylic organisms)
- I → Propionic acid bacteria

Microbiologist definition of fermentation →
Any process for the production of product
by the mass culture of a micro-organism. ②

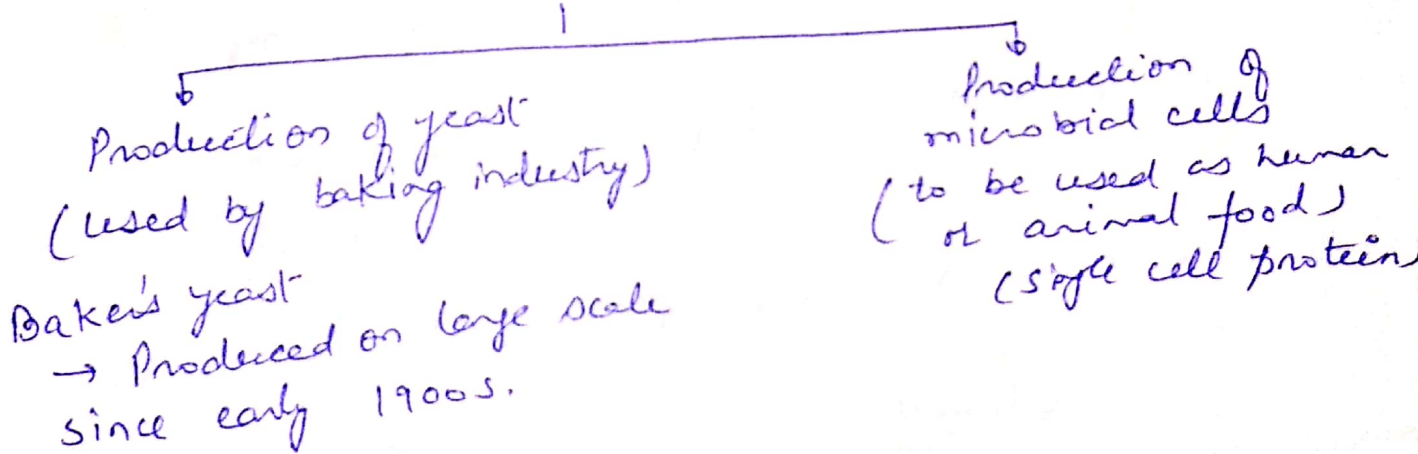
Range of fermentation processes :-

5 groups of commercially important fermentation

- (1) Those that produce microbial cells as product
- (2) Those that produce microbial enzymes
- (3) Those that produce microbial metabolites
- (4) Those that produce recombinant products
- (5) Those that modify a compound which is added to the fermentation → the transformation process.

Microbial Biomass :-

Commercial production of biomass

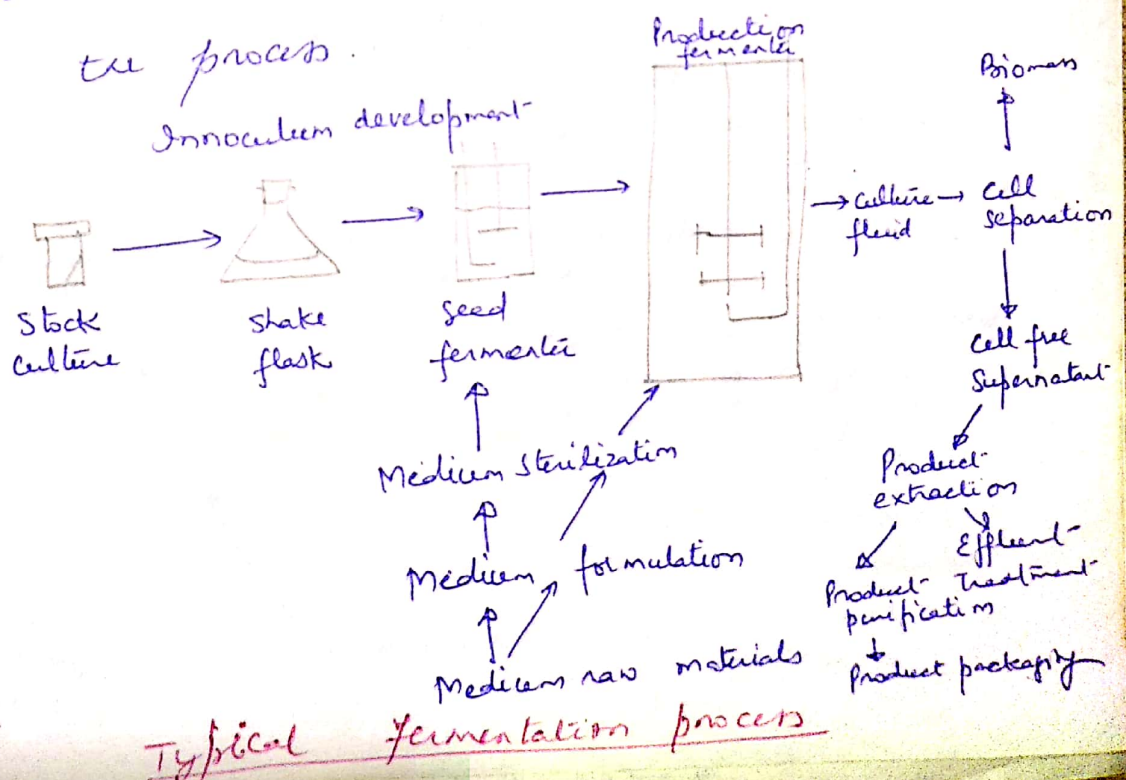


Lecture No 2

The component parts of a fermentation process - ③

fermentation process may be divided into six basic component parts -

- (1) The formulation of media to be used in culturing the process organism during the development of inoculum & in the production fermenter.
- (2) The sterilization of the medium, fermenters & ancillary equipment.
- (3) The production of an active, pure culture in sufficient quantity to inoculate the production vessel.
- (4) The growth of the organism in the production fermenter under optimum condition for product formation.
- (5) The extraction of the product & its purification.
- (6) The disposal of effluents produced by the process.



Metabolic reactions tends to organise in sequences

→ These sequences are called metabolic pathways

→ There is some connections between the pathways

→ Arrows in the diagram shows one or more enzyme-catalysed reactions which converts cell constituents (metabolites) to different products.

→ Cell produces order from its disorderly surroundings

During this process the energy from the environment is used to derive it.

Some cells (obligate or strict anaerobes) do not use free O_2

(obligate aerobes) → Require O_2

Some cells can grow in either environment (eg. Yeast. These are termed as facultative anaerobes)

Energy utilization pattern by microbes

→ Microbes utilizes two different types of energy

→ Light

→ Chemical

Micro Organism → which rely on light energy are called phototrophs

→ which rely on certain nutrients breakdown process are called chemotrophs

Chemotrophs are further classified on the basis of energy yielding nutrients

Lithotrophs

oxidize inorganic materials

Organotrophs

uses organic nutrients for energy production.

→ Energy obtained from environment is stored & shuttled in the form of ATP.

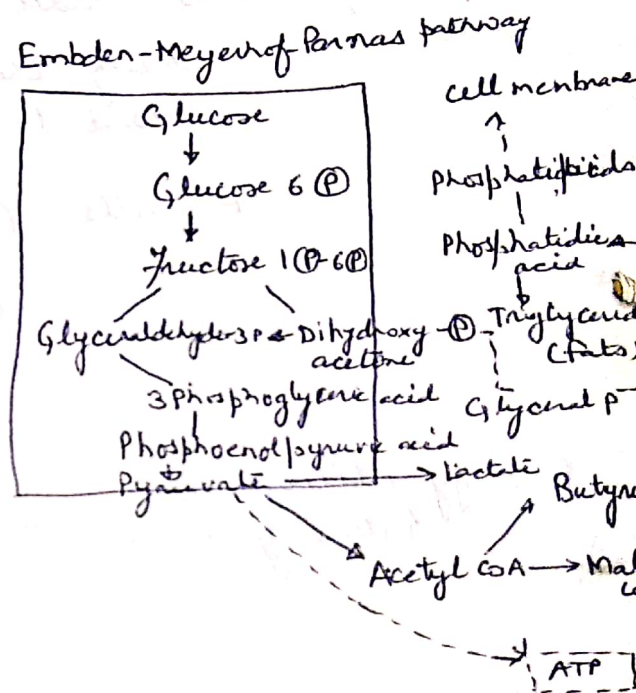
→ Cell uses this energy to perform three types of work

→ Chemical synthesis of large or complex molecules (growth)

→ Transportation of ionic & neutral substances into or out of the cell & its internal organelles

→ Mechanical work required for cell division & motion

- Living cell is a complex reactor.
 - More than 1000 independent reactions catalyzed by enzymes takes place in the cell.
 - All the reactions follow the material & energy balance principles along with the thermodynamic principles during the reactions.
 - All the chemical reaction occurring in the cell is called metabolism.
- The diagram given below depicts the metabolism of E. coli



- All these processes are nonspontaneous & results in increase in free energy of the cell.
- All these process occur when simultaneously coupled to another process which has a negative-free energy change of greater magnitude.
- Biosynthesis work is performed with relatively high efficiency of free energy utilization usually greater than 20%.
- Transportation work also require ATP
(small molecules & ions can be moved through membranes against the concentration gradient)
after the movement the ratio of concentration gradient is 10⁵.
- Mechanical work is done by the cell during cell division of bacteria & protozoa.

unique property of living system

Animal muscles activity - sperm movement also uses ATP.
(i.e. conversion of chemical free energy to mechanical work with out intermediaries like electricity or heated gases)

During chemical energy conversion heat generation takes place.

Growth of microbes :-

→ In order to grow & reproduce

> cells must ingest the materials necessary to manufacture membranes, proteins, walls, chromosomes & other components

from anotic point of view

4 major requirements of life

Carbon, Nitrogen, Sulphur, Phosphorus

H & O → may be taken from medium component & H₂O

C → CO₂, Sugars, proteins, fats

N → Proteins, NH₃, NO₃⁻

S → Proteins, SO₄²⁻

P → PO₄³⁻

Lecture No 3

CELL NUTRIENTS

→ cells composition → different from its environment.
In order to survive

cell must selectively remove the ~~undesirable~~ undesirable compounds from its external environment & retaining other compounds within itself.

Nutrient Selection mechanism → Semi permeable membrane (plasma membrane) → used for the select for cells thermodynamic equilibrium & death are same. Therefore cell tries to maintain distance from thermodynamic equilibrium by expending energy.

Composition of living organisms :-

All organisms except viruses contains

large amount of water (about 80%)

50% of dry weight of cells is protein

Proteins are largely enzymes

Nucleic acid content of the cells varies from

10-20% of the dry weight.

→ viruses may contain nucleic acids upto 50% of their dry weight.

→ lipid content of the cells varies from 5% to 15% of the dry weight.

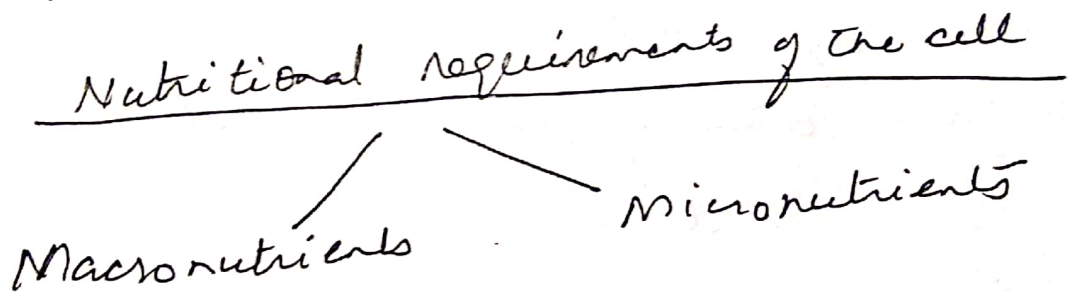
Intracellular composition of cells varies depending upon the type & age of the cells & their composition of the nutrient media.

Organism	Composition (% dry weight)		
	Protein	Nucleic acid	Lipids
Viruses	50-90	5-50	<1
Bacteria	40-70	13-34	10-15
Filamentous fungi	10-25	1-3	2-3
Yeast	40-50	4-10	1-6
Unicellular algae	10-60	1-5	4-80

→ Most of the products formed by organisms are produced as a result of their response to environmental conditions, such as nutrients, growth hormones & ions.

To optimize the growth & product-formation *(qualitative & quantitative nutrient requirement prediction)*.

Qualitative & quantitative nutritional requirements of the cells are needed to be determined.



Macronutrients - are needed in concentrations larger than 10^{-4} M.
Carbon, nitrogen, oxygen, hydrogen

Macronutrients - are needed in concentrations of 100 times
more than
Trace elements such as
 Fe^{++} , Zn^{++} , Cu^{++} , Mn^{++} , Ca^{++} , Mg^{++}
Vitamins, growth hormones & metabolic
enzymes are micronutrients.

Macronutrients - Carbon Compounds
Carbon compounds are major source of
cellular carbon & energy.

Micro organisms - classified on the basis
of their Carbon source -

(1) Heterotrophs → use organic compounds
such as
Carbohydrates
Lipids
Hydrocarbons as Carbon
& energy source.

(2) Autotrophs -
use CO_2 as the source of Carbon

→ Mixotrophs → grow under both autotrophic
& heterotrophic conditions.

Chemoautotrophs → utilize CO_2 as a
Carbon source

&
obtain energy from the
oxidation of inorganic
compounds.

→ Photoautotrophs → use CO_2 as the source
of Carbon

&
utilize light as the
source of energy.

→ for industrial fermentation.

most common source of carbon are

Molasses (sucrose)

Starch (glucose & dextrin)

Corn Syrup

Waste sulfite liquor

→ For laboratory fermentation

glucose

Sucrose

Fructose

are not common sources

of carbon.

Methanol, ethanol & methane are also cheap sources for some fermentation.

Aerobic fermentation
 50% of substrate carbon is incorporated into cells & 50% is used as energy source

Anaerobic fermentation → large part of the substrate carbon is used as an energy source.

→ smaller fraction (<3%) is used to convert into cell mass.

It constitutes Nitrogen about 10% to 14% of cell dry weight.

Most widely used nitrogen compounds

are ① ammonia & ammonium salts
(NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$, NH_4NO_3)

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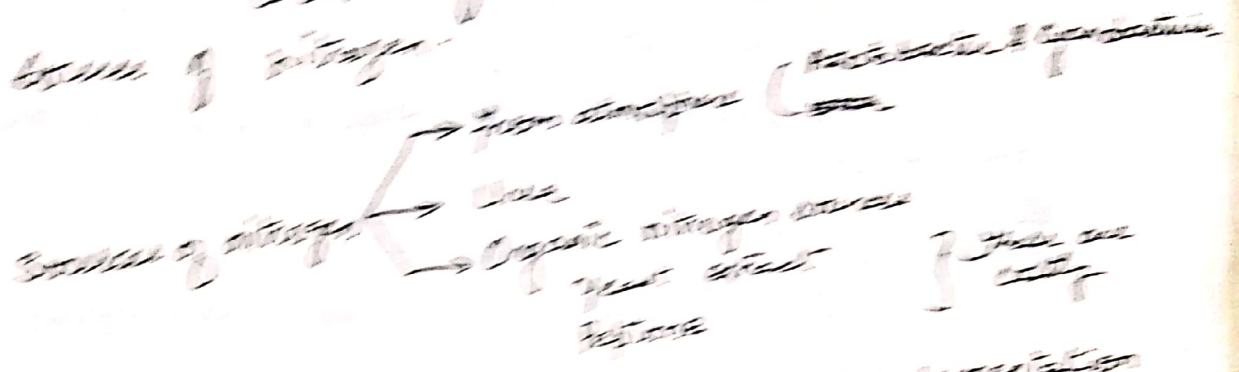
Lecture No 4

- ③ Nitrogen
- ③ Phosphorus
- ③ Potassium

Nitrogen is incorporated into cell mass in the form of proteins & nucleic acids

Some organisms and as substrates & components for nitrogen for the synthesis of for example

Some may also be used as the source of nitrogen



<u>Types (Sources & Nitrogen sources)</u>	<u>(related by concentration)</u>
<u>Inorganic sources</u>	<u>Nitrogen sources</u>
Soil water (Nitrate & Nitrite)	Leaf water
Airborne (Nitrate & Nitrite)	Plant exudates
Water	Cellular exudates
Atmosphere	Water and exudates
Leaf litter	Dried wood
Soil organic matter (Nitrogen)	Leaf litter
Soil organic matter	Plant exudates & exudates
Cellular waste	Groundnut meal
Animal waste	

Diffusion of nutrients 2% of the dry weight of the cell. Nutrient uptake is required as a nutrient. Diffusion is the main mechanism of water uptake. Water is introduced into growth media in a buffer solution.

Hydrogen :- 8% of cell dry weight
Derived primarily from carbon compounds
such as carbohydrates.

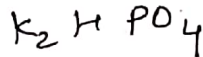
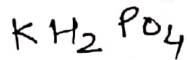
Some bacteria such as methanogens can
utilize hydrogen as energy source.

Phosphorus :- 3% of the cell dry weight

Present in nucleic acids

Present in cell wall of some gram positive bacteria
such as teichoic acids.

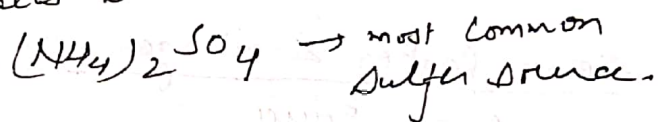
Inorganic salts



Phosphorus is the key element in cell
metabolism.

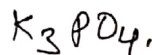
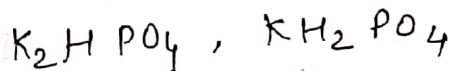
Sulfur : 1% of cell dry weight
It is present in proteins & co-enzymes.

Sulfate salts such as

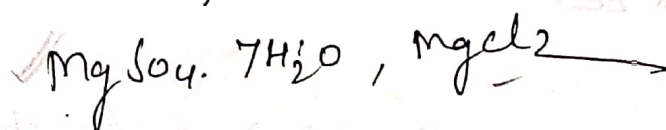


Potassium :- is a cofactor for some enzymes
It is required for carbohydrate metabolism.

Commonly used potassium salts are



Magnesium - is a co-factor for some enzymes
It is present in cell walls & membrane



Micronutrients -

Trace elements are essential for microbial nutrition. Lack of essential trace elements increases the lag phase (the time from inoculation to active cell replication in batch culture) & may decrease the specific growth rate & the yield.

Major Categories of trace elements -

(i) Widely Needed trace elements
Fe, Zn, Mn.

Fe - is an important cofactor

Iron plays a regulatory role in some fermentation processes

Zn → is a cofactor for some enzymes and it regulates some fermentation such as penicillin fermentation.

Mn → enzyme cofactor

(ii) Trace elements needed under specific growth conditions -

Cu, Co, Mo, Ca, Na, Cl, Ni, Se

(iii) Trace elements that are rarely required
B, Al, Si, Cr, V, Sn, Be, F, Ti, Ga, Ge.

Br,

Growth factors -

Stimulate the growth & synthesis of some metabolites.

Vitamins, Hormones & amino acids are major growth factors.

Commonly used vitamins are

- thiamine (B₁)

Riboflavin (B₂)

Pyridoxine (B₆)

Cyano cobalamin (B₁₂)

Vitamin K

Conc range required -
 10^{-6} M to 10^{-12} M

Lecture No 5

CULTURE OF MICROORGANISMS

Microorganisms → Very small in size
Individual study of physical & physiological characters
of microorganisms is not possible.

Microbial morphology
Biochemistry
Physiology
Reproduction

all are studied
in collective form
by
artificially culturing them.

→ Pure cultures of the micro-organisms are prepared
by providing them with all the necessary optimum
conditions required for growth
eg. Nutrients
pH
temp etc.

Culture media -

Earliest culture media were liquid
Problem with liquid media → isolation &
development of pure
culture of bacteria
was very difficult.

Procedure → Introduction of bacteria in medium
↓
Subsequently diluting the media
till only one microorganism
remained in the media

Process → tedious & problems of contamination.

Briefeld → introduced pure culture
He developed fungal cultures
used gelatin to solidify the medium.
Lister 1878 → developed 1st pure culture using
dilution method.

J. Schroeter → suggested growth on potato starch,
starch paste, bread, egg albumin etc.

Robert Koch 1881 → culture bacteria on solid medium
& efficiently develop pure culture

sterile cut surface of potato (potato was ^{cut} with a knife in plane) Walter Koch

↓
Inoculated with bacteria with needle

↓
Streaked out over the surface

↓
Slices were incubated under bell jars (to prevent contamination)

→ This method was not a big success

① as potato was not a good nutrient medium.

Many bacteria did not grow well on potato slices.

② It was difficult to distinguish between individual colonies of each bacterium as bacteria would soon spread all over the moist surface.

Frederick Loeffler → developed a medium containing meat extract & pepton.

↓
Koch tried to solidify this medium by adding gelatin to it.

Koch developed streak plate method —

He spread meat extract, peptone medium containing gelatin on a glass slide

→ streaked inoculum on the plate.

He repeatedly streaked → finally transferring the culture to tubes containing solid medium.

Plugged the tubes with cotton & placed them in slanting position.

This led to development of slant culture.

① Microbes degrade gelatin (it is proteinaceous)

② Gelatin liquefied at 28°C .

(∴ it can't be incubated at 37°C)

Required temp for the culturing of pathogenic microbes)

Walter Hesse → suggested the use of Agar (Solidifying agent - to prepare Jem & Jellies)

Koch → replaced gelatin by agar. (Polysaccharide isolated from red alga Gelidium)

Agar is a good solidifying agent -

- (1) It remains liquid till 44°C , hence it is easier to use
- (2) Requires heating to 100°C for melting
- (3) Solidify at room temp $\therefore$ there is no need to refrigerate it.
- (4) Cannot be degraded by microbes.

Isolation → Purification → Cultivation.

Culture Media
To grow bacteria in lab we need to provide all those conditions required for growth by bacteria in nature.

Most important thing is to provide all nutrients.

Culture medium → is an aqueous solution to which all the necessary nutrients have been added.

Basic medium contained 0.5% peptone, 0.3% meat extract
0.8% NaCl.

Different categories of media can be made depending upon the type & combination of nutrients. The mixture of necessary nutrients can be used as liquid medium, or a solidifying agent can be added. 'Agar Agar' is commonly used solidifying agent added to media.

Solid media are useful for identifying bacteria by colony characteristics.

Isolation of pure culture can be done by using solid media whereas liquid media yield mixture of all type of bacteria present in the sample.

Components of media

1. Nutrients → Carbohydrates, serum, whole blood, yeast extract, peptone, beef extract
2. Solidifying agent → Agar
3. Water → Tap water with low mineral content or demineralised water
4. Additives or Supplements → Dyes, vitamins, amino acids, growth factors & antibiotics

Role of substances added to the media

① Carbohydrate → Provide energy, acts as a carbon source also indicate fermentation reactions.

② Serum, whole blood, ascitic fluid → Promote growth of less hardy organisms.

③ Dyes → indicate pH change due to acid formation.

Phenol red → Red in alkaline medium
Yellow in acidic medium

④ Agar → Solidifies medium.

It is made from red algae Gelidium (Rhodophyta)

Chemically it is mostly galactose

Very few microbes can degrade it.

It remains liquid at 100°C . (easy to pour)

Solidifies at 40°C .

⑤ Yeast extract → Rich source of B vitamins
Organic nitrogen & carbon compounds.

- (1) Slant - Test tubes filled with agar & allowed to solidify at an angle.
Used to maintain pure cultures.
- (2) Deep - Test tubes filled with agar & allowed to solidify within barrel.
Often used to identify gas production.
- (3) Plate - Petri plates filled with agar.
Provides large surface area for microbial growth.
Used to isolate pure cultures.
Plates should be incubated in an inverted position to prevent condensation from dripping on media surface.
(Water droplets on the surface can spread bacteria & ruin the streak).
- (4) Complex - Support growth of most heterotrophic organisms.
- (5) Defined - Support growth of specific heterotrophs mandatory for microbiological assays.
- (6) Reducing - Support growth of obligate anaerobes.

(7) Simple or Basal media -

Nutrient broth is an example of basal media.

Contains 1% pepton
1% meat extract
0.5% NaCl

& distilled water

pH - 7.5 to 7.4

When 2-3% agar is added to nutrient broth it is called nutrient agar.

(8) Natural or empirical medium -

Media in which concentration of various nutrients is not known. e.g. milk, urine, veg. juice.

Essential nutrients of Potato Dextrose agar

- (10) Synthetic media -
Media made up of entirely synthetic substances
- (11) Dehydrated media -
These are commercially available
Powdered, ready to use media.
Sp. amount of water & sp. amount of food
- (12) Living culture media -
obligate parasites are cultured in living
organs or animals or plants.
eg chick embryo is used to culture
- (13) Minimal Media -
A defined media, which has just essential
ingredients to support growth.
Types of ingredients vary with species
- (14) Special media -
When certain ingredients are added
to basal media to study special characteristics
or to provide special nutrients required for
growth of the organism, it is called special
media.

Lecture No 6

Medium Optimization

→ Detailed investigation is required to find out most suitable medium of any individual fermentation process.

Any designed medium must fulfil some basic requirements.

eg. All microbes require

- Water
- Source of energy
- Source of Carbon, nitrogen, mineral elements, vitamins
- O₂ for aerobic fermentation process.

Medium design optimization

- Small scale (Relatively easy) as the medium contains pure substances
- Complex for large scale process

Criteria required to be followed by a medium for fermentation process -

1. It will yield maximum product from per gm of substrate used
2. Medium should produce max^m conc. of product or biomass.
3. Should permit max^m rate of product formation
4. Min^m yield of undesirable products
5. Medium should be of
 - consistent quality
 - Must be available through out the year
6. Easy medium preparation method
Easy to sterilize
7. Other aspects of production
 - aeration
 - agitation
 - Extraction
 - Purification
 - Waste treatment

Carbon sources →

Cane molasses
beet molasses
cereals
starch
glucose
lactose

Nitrogen sources →

Ammonium salts
urea
Nitrates
Corn steep liquor
Soybean meal
Slaughter house waste
Fermentation residues

Tended to meet
the established
criteria

Cheap substrate

Other medium containing pure substrate
may be carefully chosen in order to reduce the
overall cost of fermentation

Medium selection affects the design of fermenter vessel.

eg. use of methanol & ammonia
for the production of SCP

— Required novel design.

Microbial oxidation of hydrocarbon is highly
aerobic & exothermic.

∴ the fermenter is required to have
high O_2 transfer rate
& excellent cooling capacity.

Design of fermenter also affects the medium to be used.

In designing the medium

Problems of Laboratory → Pilot scale → Industrial
Scale

should also be considered.

As lab scale medium may not be suitable for
large scale production (because of low O_2 transfer
rate)

→ Highly viscous medium → more power input for effective agitation. ②

Medium may also affect

pH variation
foam formation
Oxidation-reduction potential
Morphology of the organism.

Medium also effect

product recovery & effluent treatment process.

Historical developments -

undefined natural materials → were used in fermentation
(Because they were cheap)

Problem → Variation in product quality in different batches
(Due to ① Variation in composition of substrate in different batches)
② Presence of impurities.

This cause unpredictable product yield
(Qualitatively/quantitatively)

Undefined medium causes

→ Product recovery } difficult-
→ Effluent treatment }

(because → Not all the components of media are consumed by m.o.)

Residual component may interfere with the product.

Pure Defined media → Higher yield of product-
But manufacturers are reluctant to use these.
(more expensive)

Better product yield

Medium formulation :-

Essential step in the design of a successful laboratory experiment, pilot scale development & manufacturing process.

→ Constituents of the media must meet the elemental requirement of m.o.

Provide → Necessary energy for cell metabolism.
(i.e. for biosynthesis cell maintenance)

For aerobic fermentation.

C & Energy source + Nitrogen source + O_2 + other requirements

→ Biomass + Product + CO_2 + H_2O + heat

This equation is based on stoichiometry of growth & product formation.

Quantitative analysis of above eqⁿ is essential to ensure the minimum quantity of required nutrient for the max^m yield of product.

For a specific amount of product formation certain amount of biomass is necessary

→ Biomass must be present in desired concentration.

Knowledge of elemental composition of process m.o. is required

→ Experimental data are available to ascertain the elemental requirement of different m.o.

Fe, Zn, Cu, Mn, Co, Mo, B

may also be added to the medium

Some nutrients are frequently added in excess amount than that required

eg. P K

(P is added in excess to have buffering capacity)

Other are added only to limiting value.

Some microbes can't produce $\rightarrow$ some specific nutrients

eg amino acids

Vitamins

Nucleotides

Sp. Growth factor is added to the media.

Carbon substrates are used for
Biosynthesis
Energy generation.

Carbon requirement (under aerobic condition)
is ascertained by

$$\text{Cellular yield coeff}^n = \frac{\text{Quantity of dry matter produced}}{\text{Quantity of carbon substrate utilised.}}$$

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Lecture No 7

Medium optimization

An optimized medium fulfills all the 7 criteria for fermentation process.

For optimization process different combinations & sequences of process conditions needs to be investigated to determine growth conditions which produces the biomass with the physiological state best constituted for product formation.

Classical method :-

Changing one variable (eg nutrient, antifoam, pH, temp etc) & fixing all other parameters at certain predetermined level.

Time consuming
expensive.

large no. of experiments required
 2^n

x = no. of levels

n = no. of variables

eg. 3 nutrients at 2 conc. level

$$2^3 = \text{No. of trials}$$

eg. 6 nutrients at 3 conc. level

$$3^6 = 729 \text{ trials}$$

Our target is to perform minimum no. of experiments to determine optimum level.

When no. of ^{independent} variables are more than 5

Plackett-Burman Design is used to find out the most important variables in the system; these variables are further optimized for the studies.

→ This technique allows for the evaluation of $(X-1)$ variables by X experiments

X must be a multiple of 4

eg 4, 8, 12, 16, 20, 24 etc.

→ One determines how many experimental variables need to be included in an investigation & then select the Plackett-Burman design

→ Any factors not assigned to a variable is considered as dummy variable.

→ Dummy variables are included in experiment to estimate the variation of an effect.

Plackett Burman Design 7 Variables.

Variables are at high & low levels

E & G are dummy variables

(used to measure the error)

3 dummy variables → adequate for estimation of error

→ Each horizontal row represents a trial

→ Each vertical column represent level
(H) High & (L) low of variable in a trial.

Trial	Variables							Yield
	A	B	C	D	E	F	G	
1	H	H	H	L	H	L	H L	1.1
2	L	H	H	H	L	H	L	6.3
3	L	L	H	H	H	L	H	1.2
4	H	L	L	H	H	H	L	0.8
5	L	H	L	L	H	H	H	6.0
6	H	L	H	L	L	H	H	0.9
7	H	H	L	H	L	L	H	1.1
8	L	L	L	L	L	L	L	1.4

→ Frequency of each level of a variable in a given column should be equal & that in each test the no. of high & low variable should be equal

Variable A.

For trials in which A is High
B is high in 2 trials
low in 2 trials

C is high in 2 trials
low in 2 trials

applicable for all variables.

→ Effect of changing of other variable comes out when determining the effect of A.

Trials are carried out in randomized manner.

Effect of change in dummy variable = 0

- This method identifies the important variables
- Allow them to be ranked in order of importance

Steps → Determine the difference between the average of the H(H) & (L) low responses for each independent & dummy variable

$$\sum ACH - \sum ACL$$

4 high level & 4 low level

$$A = \frac{\sum ACH}{4} - \frac{\sum ACL}{4}$$

$$= \frac{2(\sum ACH - \sum ACL)}{8}$$

This value should be nearly 0 for dummy variable.

- ② Estimate the mean square of each variable
- Mean square for A

$$= \frac{(\sum ACH - \sum ACL)^2}{8}$$

- ③ Experimental error can be calculated by taking average of mean squares of the dummy effects of E & G

$$\frac{0.045 + 0.020}{2} = 0.0325$$

- ④ Identify the factors which are showing large effect

f test

$$\frac{\text{factor mean square}}{\text{error mean square}}$$

	Factors				✓	✓	✓	6
	A	B	C	D	E	F	G	
$\Sigma(H)$	3.9	14.5	9.5	9.4	9.1	14.0	9.2	
$\Sigma(L)$	14.9	4.3	9.3	9.4	9.7	4.8	9.6	
Difference	<u>-11.0</u>	10.2	0.2	0.0	-0.6	9.2	-0.4	
Effect	-2.25	2.55	0.05	0.00	-0.15	2.30	-0.10	
Mean Square	15.125	13.005	0.005	0.00	0.045 (0.34) 8	10.580	0.020 (0.4) 2	

$$\text{Mean square for 'error'} = \frac{0.045 + 0.020}{2} = 0.0325$$

$$A = \frac{15.125}{0.0325} = 465.4$$

$$B = \frac{13.005}{0.0325} = 400.2$$

$$C = \frac{0.0500}{0.0325} = 1.538$$

$$D = \frac{0.000}{0.0325} = 0.00$$

$$F = \frac{10.580}{0.0325} = 325.6$$

When probability tables are examined it is found that factors A, B, F show large effects which are very significant.

C shows a very low effect.

D shows no effect.

By this technique it is identified that A, B, F are the most important factors.

Next stage of optimization —
optimization of conc. of each factor

Determination of optimum level of key independent variable as identified by Plackett Burman tech.

→ This is done with the help of Response surface optimization tech.

→ This tech. was introduced by Box & Wilson (1951)

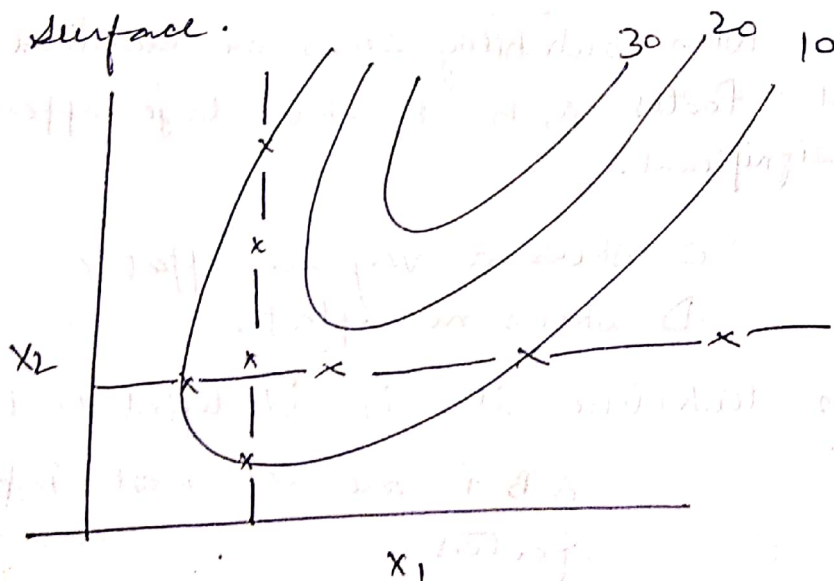
Response surface :- These are similar to contour plots or topographical maps.

Contours → Line joining the places of same elevation.

Contours on the response surface — represents the points having same values of response (production).

→ Axes of the contour plot are experimental variables

→ Area within the axes is called response surface.



Optimal point of response surface by one factor at a time.

Construction procedure of response surface

- A series of experiments using different combination of variables are inserted on the surface of the plot.
- Points giving the same response (results) are joined together to make a contour line.
- Two dimensional plot (simplest)
(In this case both the variables vary) it is to ensure that the data is distributed over the response surface.
- This technique is used at different levels of sophistication.
- More sophisticated applications of RSM use mathematical models to predict the relationships betⁿ the response & the variables.

Lecture No 8

Sterilization

A fermentation product is produced by the culture of a certain organism, or organisms, in a nutrient medium.

If the fermentation is invaded by a foreign microorganism, following consequences may occur -

- (1) The media would have to support the growth of both the production organism & contaminant resulting in loss of productivity.
- (2) If the fermentation is a continuous one then the contaminant may 'outgrow' the production organism & displace it from the fermentation.
- (3) The foreign organism may contaminate the final product eg SCP where cells separated from the broth, constitute the product.
- (4) The contaminant may produce compound which make subsequent extraction of the final product difficult.
- (5) The contaminant may degrade the desired product.
- (6) Contamination of a bacterial fermentation with phage could result in the lysis of the culture.

How Contamination can be avoided -

- (1) Using a pure inoculum to start the fermentation.
- (2) Sterilizing the media to be employed.
- (3) Sterilizing the fermenter vessel.
- (4) Sterilizing all materials to be added to the fermentation during the process.
- (5) Maintaining aseptic conditions during fermentation.

Categories of fermentation

Protected

The media may be utilized by only a very limited range of microorganisms.

eg. Brewing of Beer

Brewing worts are boiled, but not necessarily sterilized, and the fermenters are thoroughly cleaned via disinfectant solution but not necessarily sterilized.

Not protected

If these are contaminated then these would suffer some of the consequences of contamination.

Medium Sterilization

Media may be sterilized by

- (1) Filtration
- (2) Radiation
- (3) Ultrasonic treatment
- (4) chemical treatment
- (5) Heat

Steam is almost universally used for the sterilization of fermentation media.

For animal cell culture → filtration method is used
(Such media are completely soluble & contains heat labile components)

Kinetics of sterilization process

Destruction of micro-organism by steam (moist heat) may be described as a first-order chemical reaction.

$$\boxed{-\frac{dN}{dt} = kN} \quad \text{--- (1)}$$

N = No. of viable organisms present-

t = Time of the sterilization treatment-

k = Reaction rate const of the reaction or

Specific death rate

Here we are considering
Total no. of organisms present in the
volume of the media to be sterilized, not the
concentration.

→ Minimum no of organisms to contaminate a
batch is One, regardless the volume of the
batch.

$$\boxed{\frac{N_t}{N_0} = e^{-kt}}$$

Lecture No 9

For microbes $\rightarrow$ growth is most essential response to their physico-chemical environment.

Growth $\leftarrow$ Replication
change in cell size

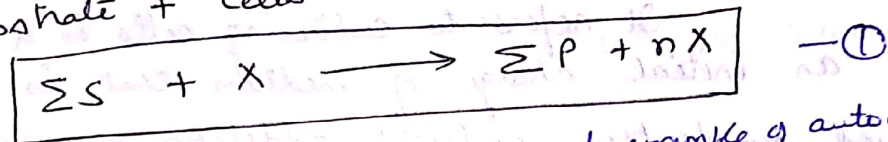
Microorganisms can grow under variety of physical, chemical, Nutritional conditions.

In suitable nutrient-media organisms extract nutrients from the medium & convert them into biological compounds.

Part of these nutrients are used for energy production
Part are used for biosynthesis & product formation

Microbial mass increases with time.

Substrate + Cells $\rightarrow$ Extracellular products + more cells



Microbial growth is a good example of autocatalytic reaction.

$\rightarrow$ Rate of growth is directly related to cell conc.

$\rightarrow$ Cellular reproduction is the normal outcome of this reaction.

Specific growth rate :-

Rate of microbial growth is characterized by net specific growth rate.

$$\boxed{\mu_{net} = \frac{1}{X} \frac{dX}{dt}} \quad - (2)$$

$$\boxed{\mu_{net} = \mu_g - K_d}$$

X = cell mass concentration g/l

t = time, h

μ_g = gross sp growth rate h^{-1}

μ_{net} = Net sp. growth rate, h^{-1}

K_d = Rate of loss of cell mass due to cell death or endogenous metabolism h^{-1}

Net specific growth rate is the difference between a gross specific growth rate (μ_g) & the rate of loss of cell mass due to cell death (k_d)

Microbial growth in terms of cell no. conc. (N)

$$\mu_R = \frac{1}{N} \frac{dN}{dt}$$

μ_R = Net specific replication rate h^{-1}

If the cell death is unimportant -

$$\mu_R = \mu_g$$

Specific growth rate & Environment

Batch Growth

It refers to culturing cells in a vessel with an initial charge of medium that is not altered by further nutrient addition or removal.

This method is simple

widely used in labs & industries.

Cell concentration :-

Quantification of cell conc. in a culture media is essential for determining kinetics & stoichiometry of microbial growth.

Quantification methods

Direct

Indirect

(In some cases direct method can't be applied because of the presence of suspended solids or other interfering compds in the media)

Determination of cell number density :-

Petroff-Hauser slide
or
Hemocytometer

used for direct cell counting.

A calibrated grid is placed over a culture chamber, and the no. of cells per grid square is counted using microscope.

At least 20 grid squares must be counted & take average.

Culture media should be clear & free of particles that hide the cells or be confused with cells.

Stains can be used to distinguish between dead & live cells.

This method is suitable for nonaggregated cultures.

It is difficult to count mold under microscope because of their mycelial nature.

Plate Count :- Plates containing appropriate growth medium gelled with agar (petri plates) are used for counting viable cells. Culture samples are diluted & spread on the agar surface & the plates are incubated. Colonies are counted on the agar surface following the incubation period. The results are expressed in terms of colony-forming units (CFU).

If cells form aggregates, even a single colony may not be formed from a single cell.

This method is suitable for bacteria & yeast but less suitable for molds. A colonies must be

unless correct media & culture conditions are chosen, some cells that are metabolically active may not form colonies.

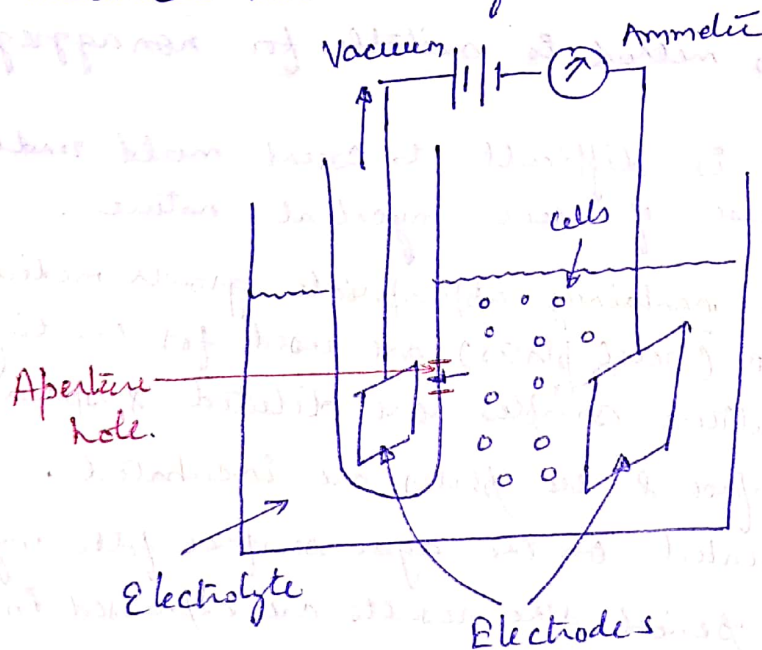
An agar-gel medium is placed in a small ring mounted on microscopic slide, the cells are spread on this miniature culture dish.

After a incubation period of few doubling times, the slide is examined with a microscope to count cells.

This method is rapid.

Particle Counter

It is based on the relatively high electrical resistance of cells.



Particle counter

It employ two electrodes & an electrolyte solution. One electrode is placed in a tube containing an orifice. A vacuum is applied to the inner tube, which cause an electrolyte solution containing cells to be sucked through the orifice. An electric potential is applied across the electrodes. As cells pass through the orifice,

the electrical resistance increases & causes pulses in electrical voltage.

The no. of pulses is a measure of the no. of particles.

The height of the pulse is a measure of the cell size.

Nephelometry:-

The no. of particles in a solution can be determined from the measurement of scattered light intensity with the aid of a phototube.

Light passes through the culture sample, & a phototube measures the light scattered by cells in the sample. Intensity of light is proportional to cell concentration.

Determination of cell mass concentration -

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Lecture No 10

Sterilization

Modern fermenter → consumes?
large amounts of media & gas.

eg. → Tens of thousands of litres of medium & millions of litres of air is used in a typical antibiotic fermentation.
(In successful fermentation process the fermentation process must be devoid of any contaminating organisms)

→ Economic penalty of contamination is high.

→ Exit air (steam) must also be treated to prevent the discharge of any living cells.

Analysing sterilization process:-
Fermentation product.

↓ Produced by culture
of certain organism / or organisms
in a nutrient medium. (Pure cultures) (Mixed cultures)

Consequences of presence of foreign (undesirable) or contaminating organisms

(1) Starts on resources resulting in loss of productivity → Media would have to support the growth of both
Production organism / Contaminant

✓ This results in loss of productivity

(2) Outgrowth of contaminant in comparison to the desired m.o.

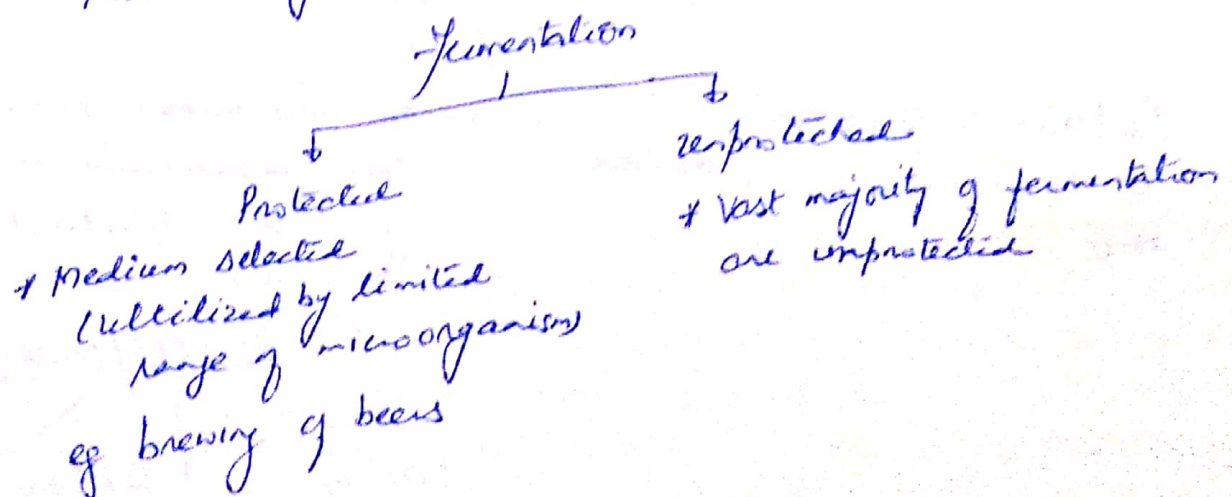
→ In continuous fermentation the contaminant may outgrow the production organism

Results / consequences
↓
Loss of productivity Higher downstream processing cost

- (3) Contamination of final product
 ↓ Resulting in
 poor product quality (Poor microbial quality)
- (4) Difficult downstream process —
 due to production of some compounds (chemicals)
 by contaminant making subsequent extraction of final
 product difficult —
- (5) Degradation of final product →
 ↓ contaminant / or the products of contaminants
 product & degrade it
- (6) If the fermentation process is contaminated by phage
 → result lysis of cell culture.

How to avoid contamination :-

- * Using pure culture at the start of fermentation
- * Sterilization of medium
- * Sterilization of fermenter vessel
- * Sterilization of materials to be added in fermentation during the process
- * Maintaining aseptic condition during fermentation process.



Medium Sterilization

Methods of medium sterilization

- (1) Filtration
- (2) Radiation
- (3) Ultrasound treatment
- (4) Chemical treatment
- (5) Heat treatment

Most widely used method $\rightarrow$ Heat sterilization
i.e.
use of moist heat
i.e.
use of steam

For animal cell culture $\rightarrow$ Filtration method is used.
(medium is completely soluble
& contains heat labile labile components)

Kinetics of sterilization

Destruction of micro-organism by steam is a
 $\rightarrow$ 1st Order chemical reaction

$$-\frac{dN}{dt} = KN \quad \text{--- (1)}$$

N = No. of viable organisms present

t = time of sterilization treatment

K = Reaction rate const. or
Specific death rate

We consider only the ^{total} no. of organisms present in
medium to be sterilized or concentration.

$\rightarrow$ Minimum no. of microbes required for contamination = 1
Regardless the volume of the batch.

Integrate eq (1)

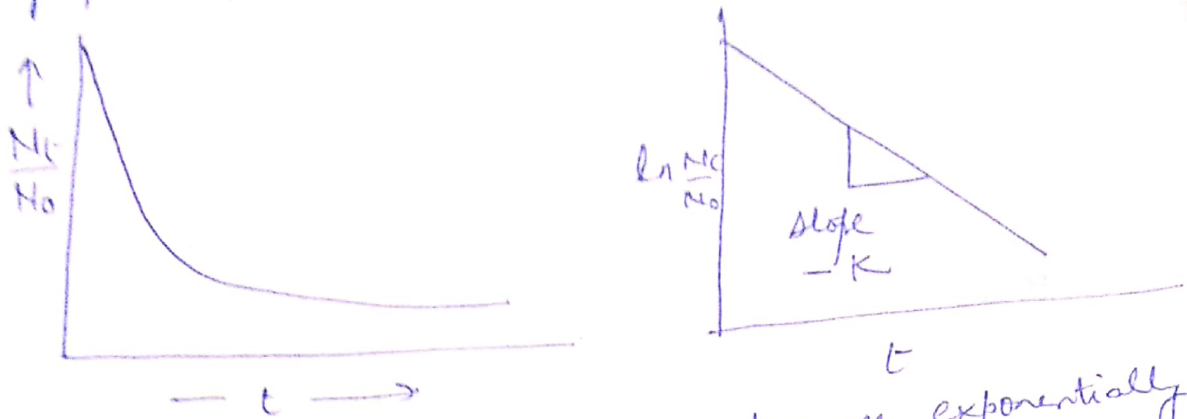
$$\frac{N_t}{N_0} = e^{-Kt} \quad \text{--- (2)}$$

N_0 = No. of viable organisms present at the
start of sterilization treatment

N_t = No. of viable organisms present after
the treatment period, t —

$$\ln(N_t/N_0) = -Kt \quad \text{--- (3)}$$

graphical presentation of eq (1) & eq (3)



No. of Viable organisms decrease exponentially over the treatment period.

From the graphs it may be concluded

- * An infinite time is required to achieve complete sterile conditions (i.e. $N_t = 0$)

- * After a certain time there will be less than one viable cell present.

Note

→ Such graphs would be observed only with sterilization of a

- * Pure culture in one physiological form under ideal sterilization condition.

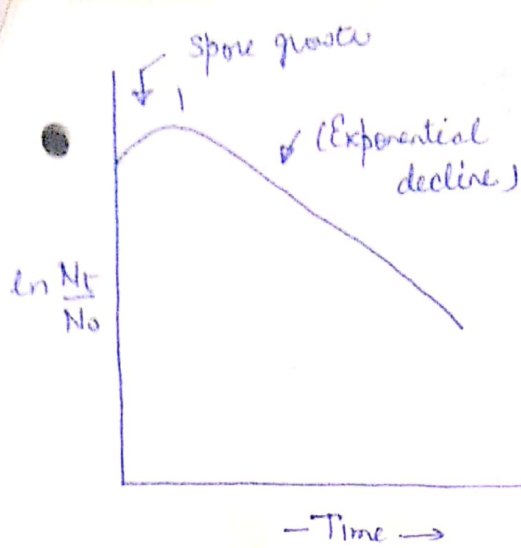
Value of K → depends upon the species of the m.o.

→ depends on the physiological form of the cell.

Example-

Endospores of Bacillus are far more resistant to heat than vegetative cells.

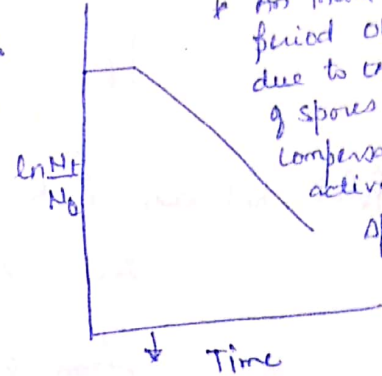
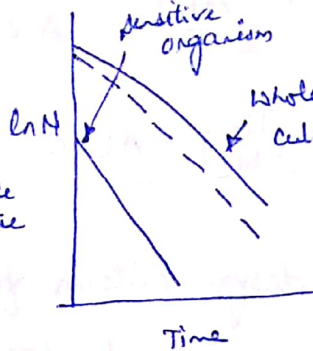
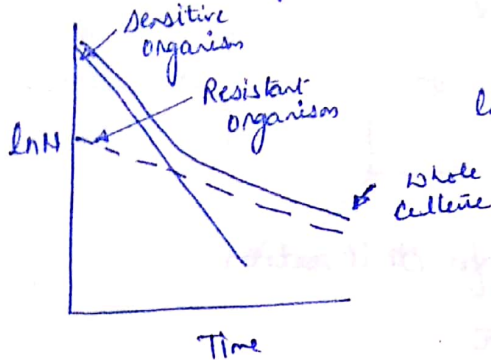
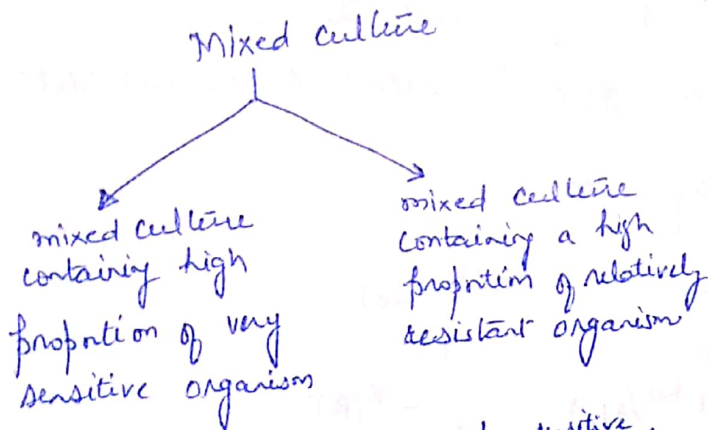
Deviation from the ideal relationship between the No. of microbes & time of sterilization were noted in practice.



observations —
 * There was an increase in the no. of population due to heat activation of spores in the early stages of sterilization process.
 (Spore germination is stimulated by heat & moisture)

* An initial stationary period observed due to the death of spores being compensated by activation of spores.

Sterilization of mixed cultures



* Initial population decline at sub maximum rate during a sterilization treatment due to death of spore being compensated by heat activation of spores

In first order reaction →

Reaction rate increases with increase in temperature due to increase in reaction rate constant. (sp death rate)

k is const only at const temp conditions.

Relationship between temp & sp death rate given by Arrhenius

$$\frac{d \ln k}{dT} = \frac{E}{RT^2}$$

E = Activation energy

R = gas constⁿ

T = absolute temp

$$K = A e^{-E/RT}$$

A = Arrhenius const.

$$\ln K = \ln A - E/RT$$

$$\ln K = \frac{E}{R} \cdot \frac{dT}{T^2}$$

$$\ln K \cdot T^2 = \frac{E}{R} \cdot T^{-1}$$

$$-\frac{1}{K} = \frac{E}{R} \cdot \frac{1}{T}$$

$$\ln K = -\frac{E}{RT} + c$$

Plot between $\ln K$ & $\frac{1}{T}$ will be straight line
And this plot is called Arrhenius plot.

from eq ③

$$K = - \frac{\ln(N_t/N_0)}{t}$$

$$- \frac{\ln(N_t/N_0)}{t} = A e^{-E/RT}$$

$$\text{or } \ln\left(\frac{N_0}{N_t}\right) = A t e^{-E/RT}$$

$\frac{N_0}{N_t}$ = design criteria for sterilization
= del factor

∇ (del factor) is a measure of the fractional reduction in viable organism count produced by a certain heat & time regime.

$$\nabla = \ln\left(\frac{N_0}{N_t}\right)$$

But from eq ③

$$\ln\left(\frac{N_0}{N_t}\right) = K t$$

$$K t = A t \cdot e^{-E/RT}$$

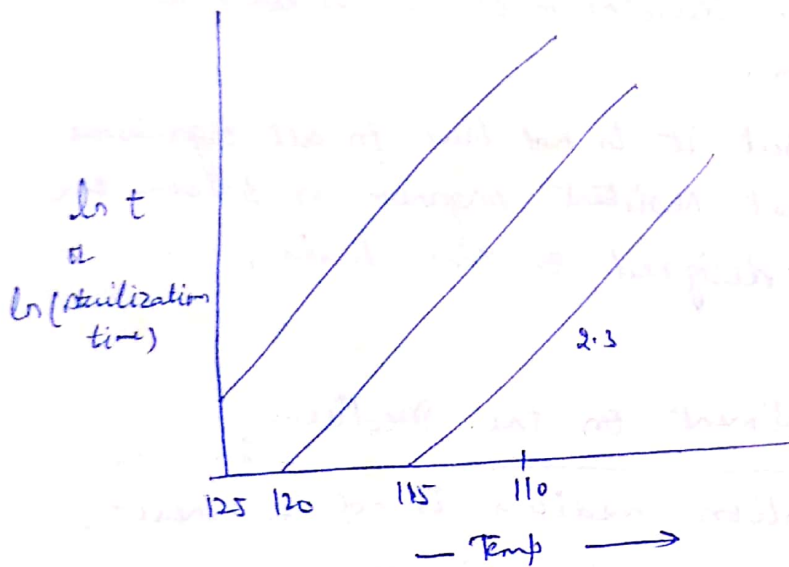
$$\nabla = A t e^{-E/RT}$$

$$\frac{\nabla}{A} = t e^{-E/RT}$$

$$\frac{\left(\frac{\nabla}{A}\right)}{e^{-E/RT}} = t$$

$$\ln(e^{E/RT}) + \ln\left(\frac{\nabla}{A}\right) = \ln t$$

$$\text{or } \boxed{\frac{E}{RT} + \ln\left(\frac{\nabla}{A}\right) = \ln t}$$



graph between
ln(sterilisation
time) &
reciprocal
of temp

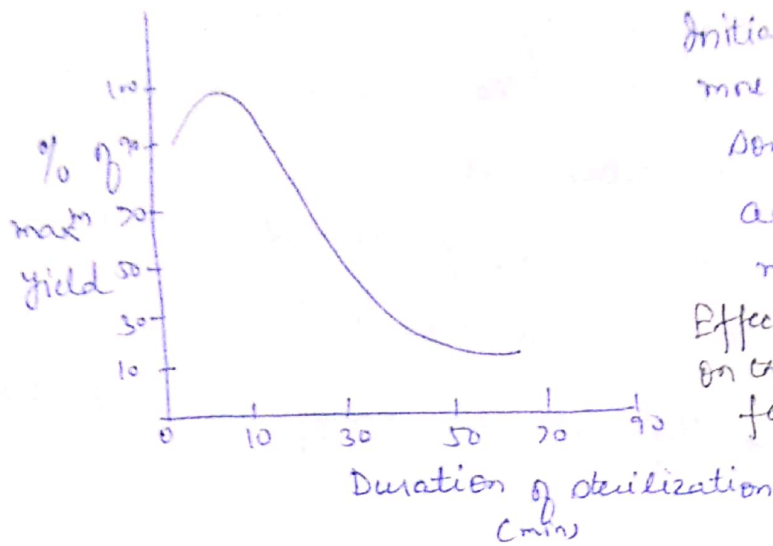
Slope of the line
depends upon
activation energy

For same degree of sterilization →
wide range of time & temp combination
can be obtained.

or
Same degree of sterilization can be achieved
by

- (1) High temp short time (HTST)
- (2) Low temp high time (LTHT)

Lecture No 12



Initial rise is due to more availability of some components made available to process microorganisms.
Effect of time of sterilization on the yield of a subsequent fermentation.

Deleterious effect of medium sterilization

Reactions Contributing to the loss of Nutrients

Reactions between nutrient components of medium.

(commonly occurring reaction is

Millard-Reaction

or

Browning reaction)

Non Enzymatic browning
Reaction between reducing sugars & amino group of amino acids & protein)

→ How to check it

* sterilizing the sugars separately & recombining the two after cooling.

Degradation of heat labile components

certain vitamins
amino acids
proteins

may be denatured during steam sterilization process.

→ How to resolve
* judicious choice of steam sterilization regime.

Thermal destruction of nutrients also follow

1st order reaction kinetics -

$$\frac{x_t}{x_0} = e^{-kt}$$

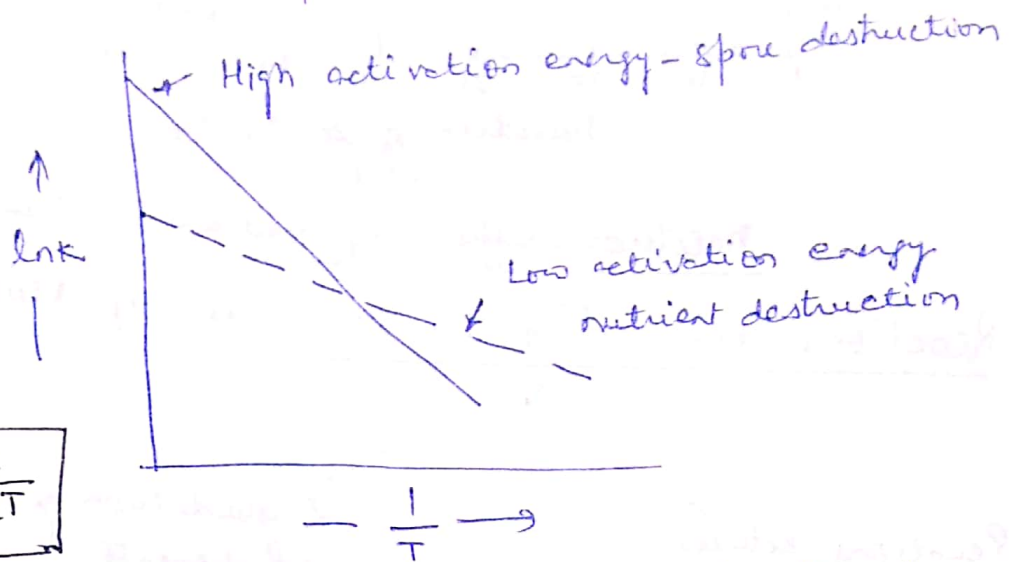
x_t = conc. of nutrient after sterilization

x_0 = Original conc. of nutrient at the start of sterilization

k = Reaction rate const.

→ The activation energy for the thermal destruction of *B. Stearothermophilus* spore
= 67.7 Kcal/mol

→ The activation energy for the destruction of nutrients = 10-30 Kcal/mol



$$\ln K = \ln A - \frac{E}{RT}$$

An increase in temp will accelerate spore destruction more than medium denaturation.

→ Short time, high temperature treatment is used to sterilize the medium in continuous stream.

Advantages of continuous sterilization over batch sterilization :-

- (1) Superior maintenance of medium quality
- (2) Easy to scale up
- (3) Easier automatic control
- (4) The reduction in sterilization cycle time
- (5) Reduction in fermenter corrosion.

Advantage of batch sterilization over continuous sterilization -

- (1) Lower capital equipment cost
- (2) Lower risk of contamination.
- (3) Easier manual control
- (4) Easier to use with media containing a high proportion of solid matter.

Batch Sterilization

Batch Sterilization → less effective in avoiding destruction of nutrients compared with continuous sterilization process

then the objective remains the same i.e. achieve the required probability of obtaining sterility with minimum loss of nutrients.

Highest temp feasible for batch sterilization — 121°C

this temp is quite high
∴ process must be designed such that the material is exposed for minimum time at this temperature.

Time includes $\left\{ \begin{array}{l} \text{Heating time} \\ \text{Cooling time} \end{array} \right.$ → during the batch process.

For designing of batch sterilization →

Following parameters must be considered -

* Temperature profile → increasing temp — during heating
decreasing temp → during cooling

* No. of microbes originally present in the medium

* Thermal death characteristics of the 'design' organism

eg. *Bacillus Stenothermophilus*

or
Some other organism.

Del factor is calculated $\left\{ \begin{array}{l} \text{Original No. of organisms} \\ \text{after ascending} \\ \text{acceptance level of} \\ \text{the contamination.} \end{array} \right.$

Frequently adopted risk factor
1 in 1000

$N_0 = 10^{-3}$ of viable cells

We are concerned with no. of organisms present in the system we are not concerned with the concentration
if suppose sterile broth contains 10^{11} viable cells, then the Del factor.

$$\Delta = \ln \left(\frac{10^{11}}{10^{-3}} \right)$$

$$\Delta = \ln 10^{14}$$

$$= 32.2$$

$\therefore$ Required Δ factor = 32.2

destruction of cells occurs during the heating & cooling & holding time.

$$\Delta_{\text{overall}} = \Delta_{\text{heating}} + \Delta_{\text{holding}} + \Delta_{\text{cooling}}$$

After knowing the heating & cooling profile next step is to calculate the contribution made to overall Del factor by these periods.

↓
Then holding time is calculated

Calculation of Del factor using heating & cooling -

$$\Delta = At e^{-E/RT} \quad \text{--- (1)}$$

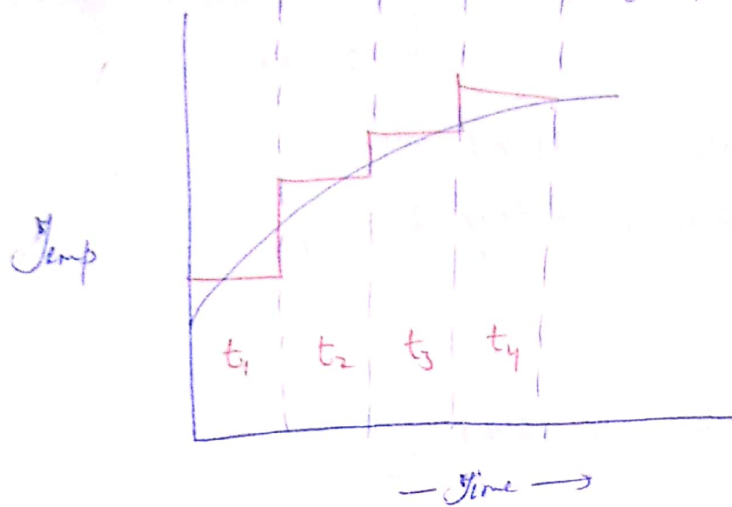
During heating & cooling $\rightarrow$ temp is not const. & therefore calculation of Δ would require an integration of eq (1) for time-temp regime observed.

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Lecture No 13

Temp response may be $\rightarrow$ linear
exponential
Hyperbolic } Theoretical

No. specific pattern } Practical



Time axis divided into equal no. of increment
 $t_1, t_2, t_3, \dots$ etc.

Increment may be around 30

For each increment temp corresponding to mid-point time is recorded.

Total del factor for heating = Sum of del factor of mid point temp for each time increment.

$$\nabla_1 = K_1 t_1$$

$$\nabla_2 = K_2 t_2$$

$$\nabla_3 = K_3 t_3$$

!

!

K_1, K_2, K_3 values are obtained from Arrhenius Equation

$$\nabla_{\text{heating}} = \nabla_1 + \nabla_2 + \nabla_3 + \dots$$

Calculation of holding time at constant temperature -

∇_{heating} - Determined

∇_{cooling} - Determined

$$\nabla_{\text{holding}} = \nabla_{\text{overall}} - \nabla_{\text{heating}} - \nabla_{\text{cooling}}$$

$$\text{let } \nabla_{\text{overall}} = 32.2$$

$$\nabla_{\text{heating}} = 9.6$$

$$\nabla_{\text{cooling}} = 10.1$$

$$\Delta_{\text{holding}} = 32.2 - 9.8 - 10.1$$

$$= 12.3$$

$$\Delta = kt$$

from data k for *S. aureus*
at 121°C
 $= 2.54 \text{ min}^{-1}$

$$t = \frac{\Delta}{k}$$

$$= 12.3 / 2.54 = 4.84 \text{ min.}$$

If contribution of heating & cooling is
considered nil
then

$$t = \frac{32.2}{2.54} = 12.68 \text{ min}$$

Batch Sterilization — (Methods)

Design of Continuous Sterilization Process

* Design considerations are same as the batch sterilization system.

→ Continuous process includes

Time during which the medium is heated
+

Holding time at the desired temp
+

Cooling time to restore the medium to the fermentation temperature.

Temp elevation takes place in continuous heat exchanger

Holding of media takes place in holding tubes
(length of the tube + discharge rate will decide the holding period)

Cooling of the medium takes place in two sequential heat exchangers.

(i) First heat exchanger → utilizes the incoming medium as cooling medium.

(2) Second heat exchanger → uses cooling water.

Advantage of continuous sterilization process:-

* Much higher temp^t can be utilized

* Reduction in nutrient loss

Time & temperature combinations can be selected for a given acceptable level of nutrient decay

eg.

For sterilization D_{121} factor is 45.7

Time temp regimes

Time of holding

2.44 min

51.9 sec

18.9 sec

2.7 sec.

Temp

130°

135°

140°

150°

Heating up & cooling down time is less

Types of steriliser :-

Indirect heat exchanger

Direct heat exchanger (Steam injector).

Indirect heat exchanger :-

Double-spinal type

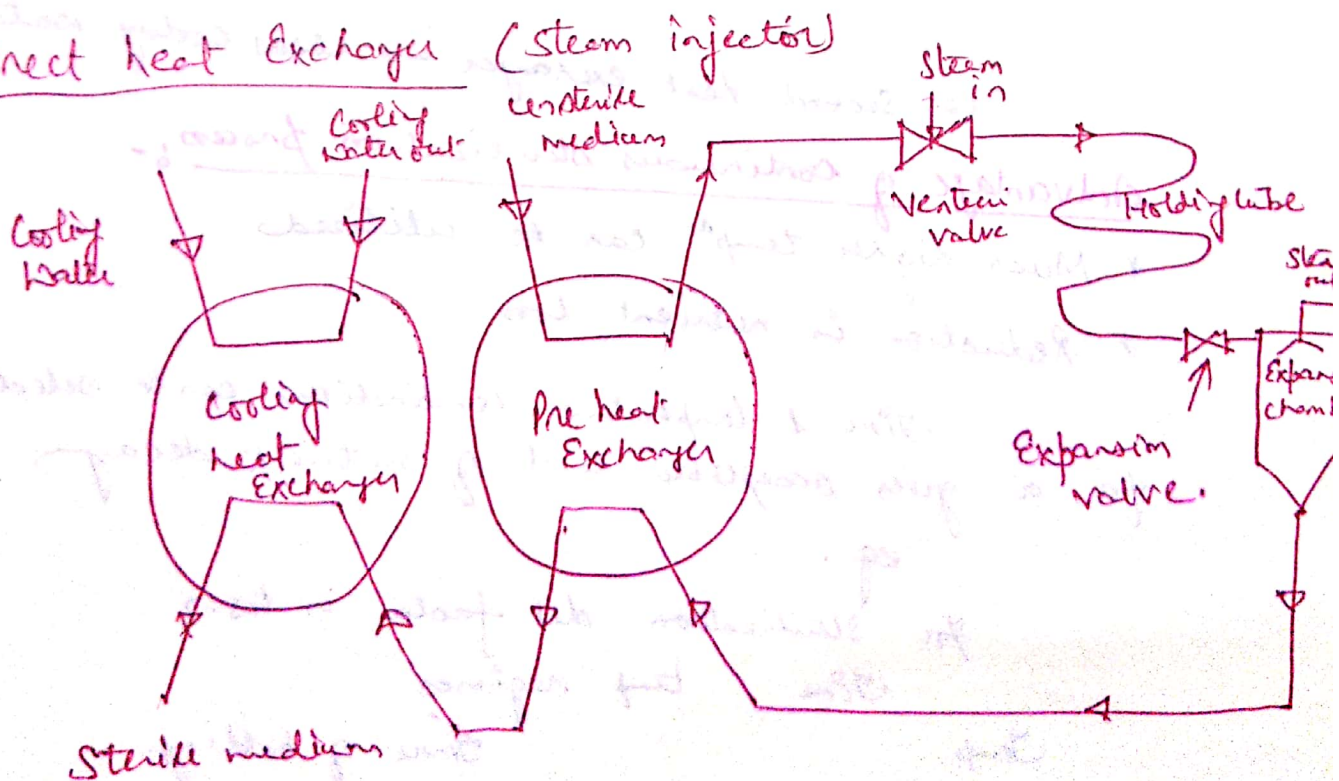
consisting of two sheets of high grade stainless steel which has been curved around a central axis to form a double spiral. The ends of the spiral are sealed by covers. Steam is passed through one spiral & media through the other in countercurrent direction.

Spiral heat exchangers are also used to cool the media after passing through the holding coil.

Advantages :

- (1) Unlikely cross contamination
- (2) Sufficient clearance to be incorporated for the system to cope with the suspended solids.

Direct heat Exchanger (Steam injector)



→ Del factor to be achieved in a continuous sterilizer has to be increased with an increase in scale,

If volume to be sterilize is increased from 1000 dm^3 to $10,000 \text{ dm}^3$ the risk of fluidists remain at 1 in 1000 then Del factor must be increased from 34.5 to 36.8.

Sterilization of fermenter

If medium is sterilized in separate batch cooker or is sterilized continuously, then fermenter has to be sterilized separately before sterile medium is added.

→ Sparge steam into the vessel.

Steam pressure 15 psi for 20 min.

Sterilization of liquid waste

Waste biomass → must be sterilized before disposal

Batch sterilization → sparge of steam into holding.

Sterilization of air

Aerobic fermentation require → sterile air

Heat treatment is not possible

most commonly used method → Filtration.

Air sterilization

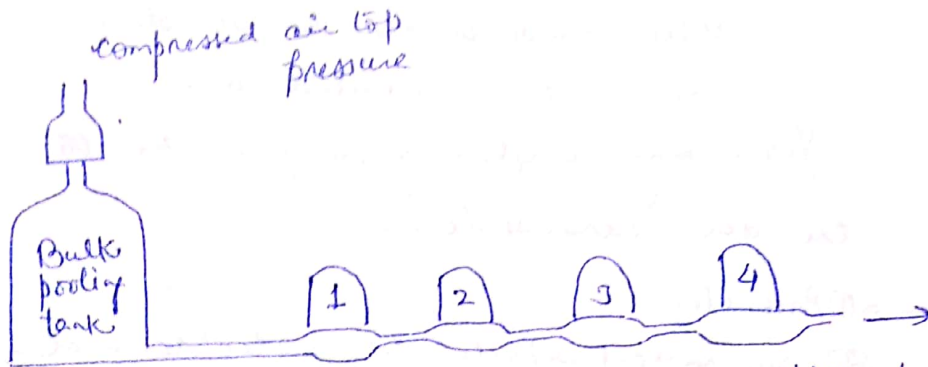
Aerobic fermentation $\rightarrow$ continuous addition of considerable quantity of sterile air

sterilization of air by heat treatment $\rightarrow$ not possible
 $\rightarrow$ therefore filtration of air for sterilization is commonly practiced.

Fixed pore filters are commonly used in fermentation industry.

Filtration systems are also commonly used.

consisting of pleated membrane cartridges designed in such a way so that these can be fixed in stainless steel modules.



Filter 1 $\rightarrow$ 5 μ m absolute rated prefilter for removal of coarse precipitates

2 $\rightarrow$ 0.5 μ m absolute rated prefilter for bulk bioburden removal

3 $\rightarrow$ 0.1 μ m absolute rated single layer prefilter for further bioburden & endotoxin removal

4 $\rightarrow$ 0.1 μ m absolute rated double layer final filter for absolute sterility, mycoplasma removal & further endotoxin removal.

Common material of construction for pleated member $\rightarrow$ PTFE. (hydrophobic $\therefore$ resistant to wetting)

PTFE filter can be steam sterilized
Resistant to ammonia (may be injected to air for pH control).

Prefilters are fixed at upstream side of absolute filters.

Prefilters traps $\rightarrow$ dust, oil & rust

$\rightarrow$ Many traditional fermentation exhaust gas from fermenter as vented without sterilization.

$\rightarrow$ Recombinant organisms & strict effluent disposal norms (allergic organisms)

Fixed pore membrane filters are used (Filter must be able to withstand the water saturated air)

Foam may overflow from fermenter to the air exhaust line.

$\rightarrow$ ① Prefilter

② mechanical separator for water removal

③ Pretreated air is passed through 0.2 μ m hydrophobic filter.

→ If a particle touches a fiber it remains attached to it

→ ∴ there is a uniform concentration of particles at any given depth in the filter.

→ Each layer of unit thickness of filter should reduce the population entering it by the same proportion.

$$\frac{dN}{dx} = -KN$$

$N \rightarrow$ conc. of particles in the air at a depth x in the filter

$K = \text{const}$

$$\frac{N}{N_0} = e^{-Kx}$$

$N_0 =$ No. of particles entering the filter

$N =$ No. of particles leaving the system.

$$\ln\left(\frac{N}{N_0}\right) = -Kx$$

log penetration relationship.

Efficiency of filter —

Ratio of the no. of particles removed to the original no. present.

$$E = \frac{(N_0 - N)}{N_0}$$

$E =$ efficiency of filter

$$\frac{N_0 - N}{N_0} = 1 - \frac{N}{N_0}$$

Substituting $\frac{N}{N_0} = e^{-KX}$

$$E = 1 - e^{-KX}$$

X_{90} concept.

Depth of the filter required to remove 90% of the total no. of particles entering the filter

If $N_0 = 10$

$X = X_{90}$

$N = 1$

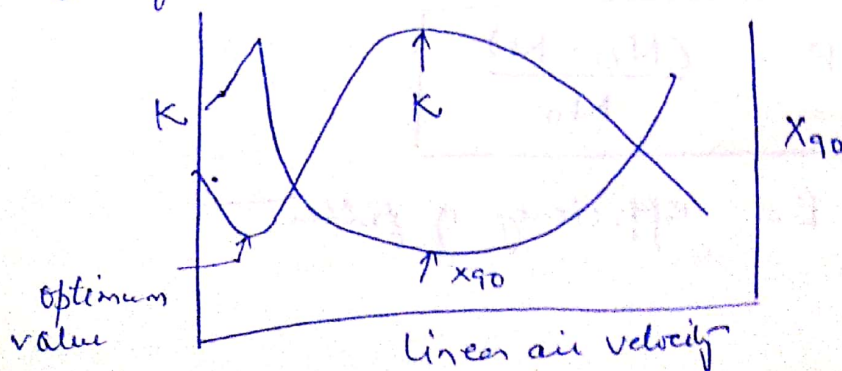
$$\ln \frac{1}{10} = -KX_{90}$$

$$2.303 \log_{10} \left(\frac{1}{10} \right) = -KX_{90}$$

$$= 2.303 (-1) = -KX_{90}$$

$$X_{90} = \frac{2.303}{K}$$

Value of K is affected by nature of filter material, linear velocity of air passing through the filter.



Effect of increasing linear air velocity on K & X_{90} of filter system

- Increase in K with increasing air velocity is due to increase in impact.
- decrease in K at high air velocity is due to disruption of the filter, allowing channels to develop & fibers to vibrate, resulting in release of previously captured organisms.

- log penetration relationships → same as that of thermal death kinetics
- Thermal death → infinite long period of time is required to reduce population to zero level.
- depth filter → infinitely long filter is required to reduce population to zero level.

Probability of one fermentation in 1000 being contaminated is used.

Design parameter → filter material
→ Depth of filter.

known quantity → volume of air

20 m³ air to be filtered

rate of flow 10 m³/min

duration of fermentation 100 hrs

optimum linear velocity = 0.15 m/s

$K = 1.535 \text{ cm}^2$

calculate dimensions of the filter.

$$\ln \left(\frac{N}{N_0} \right) = -Kx$$

The air in fermentation plant contains 300 m³/min

N_0 = total amount of air provided 1200

$$= \frac{10}{\text{air flow rate}} \times \frac{60 \times 100}{\text{total min}} \times 1200$$

$$= 12 \times 10^6 \text{ organisms}$$

acceptable level of contamination

$$N = 10^3$$

$$\ln \left[\frac{10^3}{12 \times 10^6} \right] = -Kx$$

$$\ln 8.33 \times 10^{-11} = -Kx$$

$$\ln 8.33 \times 10^{-11} = -1.535x$$

$$x = \frac{-23.21}{-1.535} = 15.12 \text{ m}$$

Cross sectional area of filter

$$\pi R^2 = \frac{10}{60 \times 1.5}$$

$$R = 0.59 \text{ m}$$

$$Q = A \cdot V$$

$$A = \frac{Q}{V}$$

$$Q = 10 \frac{\text{m}^3}{\text{min}}$$

$$= \frac{10}{60} \frac{\text{m}^3}{\text{sec}}$$

Now consider that if the air velocity changes K will decrease

$$V = 0.15 \frac{\text{m}}{\text{sec}}$$

if linear velocity drops to 0.03 m/sec
 $K = 0.2 \text{ cm}^2$

Control of micro-organisms

Control means → Reduction in number/A activity of total microbial flora.

Why control

- Prevent transmission of diseases
- Infection prevention
- Contamination prevention.
- Control of growth of undesirable m.o.
- Prevent deterioration or spoilage of materials

Controlling methods

Physical agents Physical processes Chemical agents

Understanding death:-

microbiologically → It is irreversible loss of the ability to reproduce.

Viable m.o.
 → Multiply
 → do not multiply

Failure of a m.o. to grow when inoculated into an appropriate medium indicates that the m.o. is no longer able to reproduce. i.e. dead.

Response of m.o. for different media may be different.

Rate of death of m.o.:-

○ One drop of bacterial suspension + bottle of hot acid

→ Bacteria will be killed death rate is very high.
 Can't be measured.

less drastic method → cells being killed over a longer period of time.

Const. exponential rate of death
 just
 inverse
 of exponential growth rate

Understanding Exponential death -

Model:-

Each cell is target

Large No. of bullets are fired at random manner.
(Physical & chemical agents) with a machine gun.

→ One is aiming the gun at a target.
assume → single hit kills a bacterium.

Probability of hitting the target —

it is proportional to no. of targets
(i.e. no. of bacteria present)

→ No. of targets that may not ^{yet} hit decrease steadily.
→ It becomes harder to hit the remaining ones.

eg. Initial microbial population — 1 million target
Shooen them with bullets for 1 min
manage to hit 90%

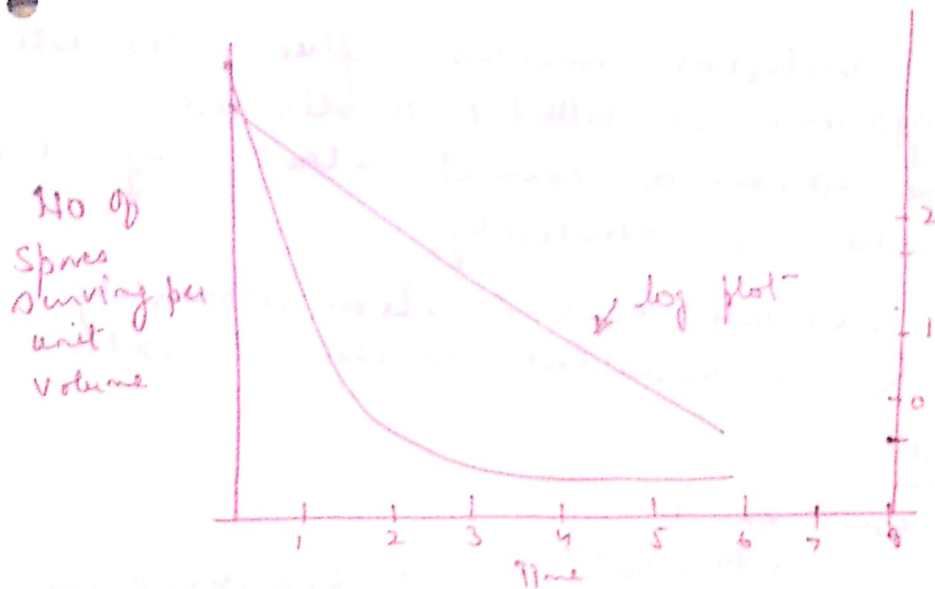
No. of survivors — 100,000 survivors left
→ Shooen for 1 more min
No. of survivors will be 10,000

→ Shooen for 1 more min
No. of survivors 1,000

Repeat until No survivors is left

Time	Survivors	Death per unit time	Total death
0	1,000,000	0	0
1	100,000	900,000 = 90%	900,000
2	10,000	90,000 = 90%	990,000
3	1,000	9000 = 90%	999,000
4	100	90 = 90%	999,900
5	10	9 = 90%	999,990
6	1		999,999

It is just as hard to kill last 1 bacterium as to kill the first 200,000.



Same portion of the population dies during any given unit of time.

log plot tells the death rate is const.

→ Probability of hitting the target also proportional to no. of bullets shot.

(i.e. conc. of chemical & intensity of physical agent)

more bullets fire → more chances of target to be hit.

m.o. kills faster if we increase the intensity of radiation is increase.

→ Period of killing (shooting).
increase in period → more target will be hit

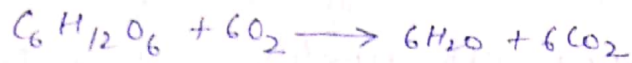
Lecture Notes
Bioprocess Engineering
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Lecture No 16

Aeration & Agitation

- * Majority of industrial fermentation processes are aerobic.
- * Requires provision of oxygen.

Glucose oxidation reaction -



For complete oxidation of 180 g of glucose 192 g of O_2 is required.

- * Both the components (i.e. glucose & O_2) must be in solution before they are available to microorganisms.
- * O_2 is 6000 times less soluble in water in comparison to glucose.
- * It is not possible to supply O_2 in one turn for complete oxidation of glucose (or any other carbon source).
- * Therefore O_2 must be supplied to microbial culture during growth at a rate sufficient to satisfy the organism's demand.

O_2 is supplied to industrial fermentation $\rightarrow$ by aeration & agitation of fermentation broth.

$\rightarrow$ In some fermentation process productivity is limited by O_2 availability.

Oxygen requirements of industrial fermentation :-

Stoichiometry of respiration gives an overview of problem of O_2 supply.

It gives no indication of organism's true O_2 demand, It does not take into account the carbon that is converted into biomass & product.

$\rightarrow$ Several stoichiometric equations are available for the prediction of O_2 demand in a fermentation process.

$\rightarrow$ These equations indicates that the cultures demand for O_2 is very much dependent on the source of carbon in the medium.

→ If carbon is more from more O_2 will be required for oxidation.

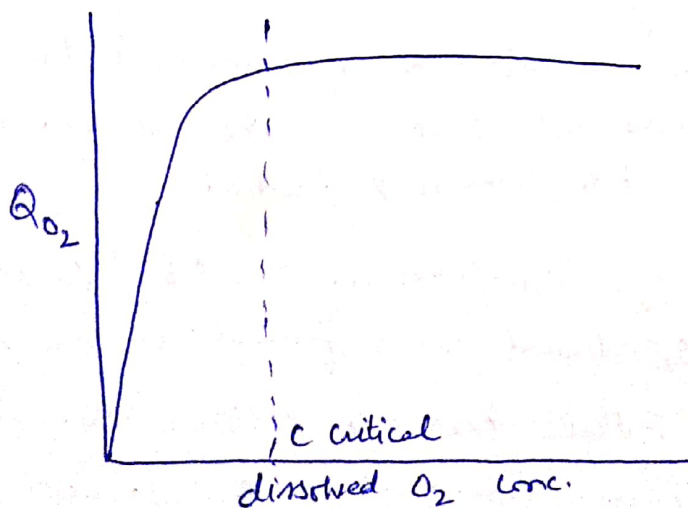
eg production of 100g of biomass from hydrocarbon require approximately 3 times the amount of O_2 to produce the same amount of biomass from Carbohydrate.

decision factors $\left\{ \begin{array}{l} \text{optimum combination of} \\ \text{① High carbon content} \\ \text{of hydrocarbon} \\ \text{Substrate} \\ \text{(giving higher yield} \\ \text{factor)} \\ \text{② High } O_2 \text{ demand} \\ \text{disadvantage \& heat generation.} \end{array} \right.$

Balance betⁿ the
adv. & disadv

→ Metabolism of the culture is affected by the dissolved O_2 in broth.

The effect of dissolved O_2 conc. on the specific O_2 uptake rate (Q_{O_2} , mmol of O_2 consumed per g dry weight of cell per hr) follows Michaelis-Menten type relationship.



specific O_2 uptake rate increases with increase in dissolved O_2 conc. upto a certain point (critical) above which no further increase in O_2 uptake rate occurs.

effect of dissolved O_2 conc. on Q_{O_2} of a microorganism

The max^m biomass production may be achieved by satisfying organisms max^m specific O_2 demand by maintaining the dissolved O_2 conc. greater than the critical level.

A If O_2 conc. is less than critical value the cells may be metabolically disturbed.

Main target is to  produce the product - $\therefore$ some times O_2 starvation is favoured for the production of some products.

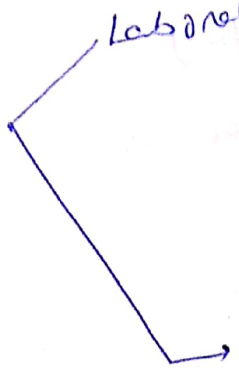
$\rightarrow$ Aeration conditions necessary for the optimum production of a product may be different from those favouring biomass production.

Oxygen Supply :-

Oxygen is supplied to microbial culture $\xrightarrow{\text{in form of AIR}}$

AIR is cheapest available resource.

O_2 supply method varies with the scale of process.

 Laboratory scale culture (may be averted by means of shake flask technique. Culture is grown in a conical flask (250-500 cm³) shaken on a platform)

$\rightarrow$ Pilot or industrial level fermentation. & carried out in stirred, aerated vessel

Process of transfer of O_2 from air to cell :-

Steps involved

- (1) Transfer of O_2 from an air bubble into solution
- (2) Transfer of dissolved O_2 through the fermentation medium to the microbial cell.
- (3) The uptake of the dissolved O_2 by the cell.

from
amount -
from

The max^m biomass production may be achieved by (3)
satisfying organisms max^m specific O_2 demand by maintaining
the dissolved O_2 conc. greater than the critical level.
A If O_2 conc. is less than critical value the cells
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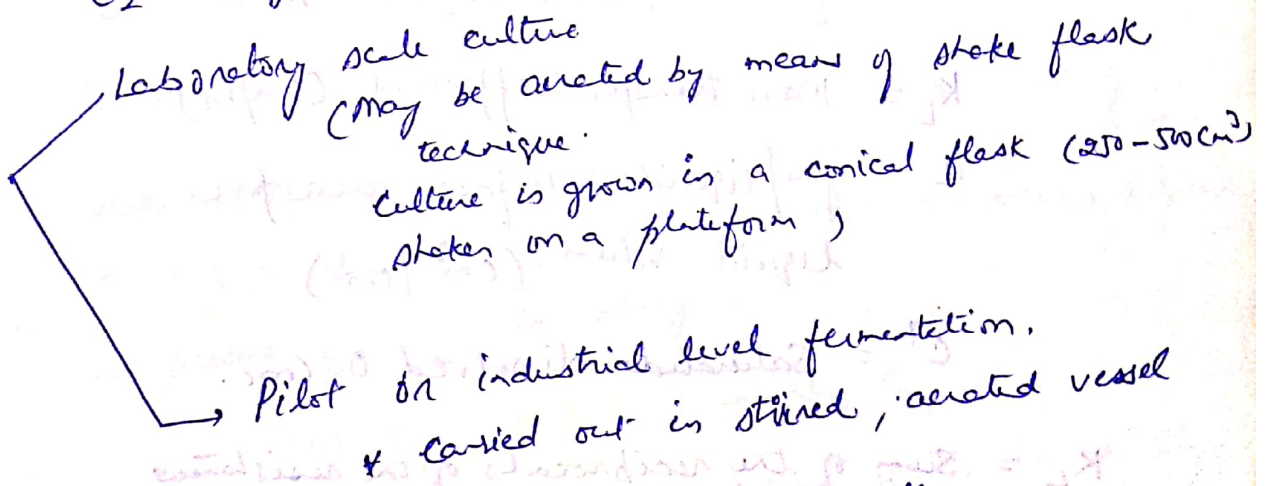
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- (3) The uptake of the dissolved O_2 by the cell.

Lecture No 17

limiting step in the process is transfer of O_2 into the solution. (In case of non viscous medium)

In case of viscous medium
limiting step may be $\rightarrow$ uptake of O_2 into the cell.

Rate of transfer of O_2 from air bubble to the liquid phase $\rightarrow$ it may be described by the following equation

$$\frac{dC_L}{dt} = K_L a (C^* - C_L)$$

C_L = conc. of dissolved O_2 in the fermentation broth (m moles dm^{-3})

$\frac{dC_L}{dt}$ = change in O_2 conc. over a period of time ($\text{m moles } O_2 \text{ dm}^{-3} \text{ h}^{-1}$)

K_L = Mass transfer coefficient (cm/m)

a = gas/liquid interface area per liquid volume (cm^2/cm^3)

C^* = Saturated dissolved O_2 conc.

K_L = Sum of the reciprocals of the resistances to the transfer of O_2 from gas to liquid
 $A(C^* - C_L)$.

= It is the driving force across the resistance

K_L & a are difficult to measure in the fermentation process and are therefore expressed combinedly.

$K_L a$ = Volumetric mass transfer coeffⁿ. (h^{-1})

It is used to measure the aeration capacity of a fermenter.

If $K_L a$ is higher $\rightarrow$ higher aeration capacity of the system.

Factors affecting $K_L a$

- * design of fermenter
- * operating condition
- * aeration rate
- * agitation rate
- * Impeller design.

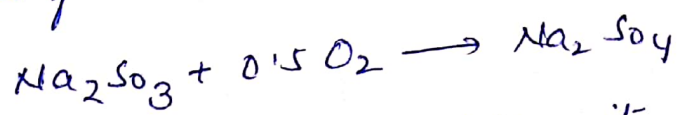
Determination of $K_L a$ value

Essential in order to establish its aeration efficiency.

The Sulphite Oxidation Technique:-

It is used to determine the Oxygen transfer rates in aerated vessels.

It does not depend upon the measurement of dissolved O_2 concentration, but relies on rate of conversion of 0.5 M solution of Sodium Sulphite to sodium sulphate in presence of Cu or Co catalyst.



As the O_2 enters solution it is immediately consumed in the oxidation of sulphite.

$\therefore$ Sulphite oxidation rate is equivalent to the Oxygen transfer rate.

Dissolved O_2 concentration will be zero

$K_L a$ will be calculated by eq.

$$OTR = K_L a \cdot C^*$$

OTR = Oxygen transfer rate.

⑥

Procedure:-

- * Fermenter is batched with 0.5 M solution of Sulphite containing 10^{-3} M Ce^{+2} and aerated & agitated at fixed rate.
- * Samples are removed at set time intervals
- * Samples are added to excess iodine solution, which reacts with unconsumed Sulphite.
- * Level of unconsumed Sulphite is determined by the titration with standard sodium thiosulphate solution.
- * The volume of the thiosulphate titration are plotted against sample time.
- * OTR may be calculated from the slope of the graph.

Oxygen uptake rate; Volumetric Oxygen transfer coefficient; Carbon dioxide evolution rate

The rate of change, $\frac{d\bar{C}}{dt}$, in dissolved oxygen concentration at a particular point in the fermenter vessel is given by

$$\frac{d\bar{C}}{dt} = K_L a (C^* - \bar{C}) - Q_{O_2} X$$

C^* = conc. of dissolved O_2 which is in equilibrium with partial pressure $\bar{p}$ in bulk gas phase

$\bar{C}$ = conc. of dissolved O_2 in bulk liquid

$K_L a$ = Volumetric oxygen transfer coeffⁿ

Q_{O_2} = specific rate of O_2 uptake (microbial respiration)

X = cell mass concentration.

$C^* - \bar{C} \rightarrow$ average value

logarithmic mean $(C^* - \bar{C})$ mean with respect to the fermenter bottom & top may be taken in place of $(C^* - \bar{C})$.

$Q_{O_2} X \rightarrow$ Evaluated from the monitoring of O_2 partial pressure in inlet & exit gas around the fermenter

$$Q_{O_2} X = \frac{F}{V} \left(\frac{P_{in}}{P - P_{in}} - \frac{P_{out}}{P - P_{out}} \right)$$

= OUR (Oxygen uptake rate)

P_{in} = Oxygen partial pressure in inlet gas

P_{out} = Oxygen partial pressure in exit gas

P = Total pressure

V = Batch volume in vessel

F = inlet gas flow rate

In steady state, the value of K_{La} can be determined by

$$K_{La} = \frac{Q_{O_2} \times}{(C^* - \bar{C})_{mean}}$$

$$= \frac{OUR}{(C^* - \bar{C})_{mean}}$$

Lecture No 18

Commonly used instruments in Biochemical Expts

Laminar Air Flow Hood (LF Hood)

It is required for aseptic handling and thus prevents the contamination of the sterile materials.

In this device, air within a confined area moves with uniform velocity along parallel flow lines in a single direction. This device consists of pre filters and high efficiency particulate air (HEPA) filters which removes all dust and airborne particles (all $0.3 \mu m$) from the air. Room air is filtered before entering the working chamber. The filter of LF hood should be tested at the time of installation and regularly at least twice in a year.

The LF hood is fitted with UV lamp in order to provide aseptic environment.

pH meter

pH meter is used to measure and setting the pH of the solution. The working of pH meter depends on the development of membrane potential by glass electrode. This potential is proportional to the pH of the solution.

Centrifuge

Centrifuge is used for the separation of particulate matter from a suspension at higher speed. The particulate matter gets settled at the bottom of the centrifuge tubes depending upon the density, under the influence of centrifugal force.

Incubator

Incubators are used for the culturing of micro-organisms, cells of plants and animals at constant temp which is maintained to the required level by thermostat. Incubators are provided with double door. The inner door is made up of glass that material present inside can be seen without

fermenter :-

fermenter is used for culturing of m.o., cell under controlled condition of pH, temp, air, stirring etc. Facilities are also provided in the instrument to add nutrients, cultures and to remove the product under aseptic conditions.

Colony counter:-

The Quebec colony counter is used to count the number of colonies in a plate. A magnifying lens is provided in the instrument which magnifies the object 1.5 times. A petridish is kept on the platform provided in the instrument for keeping the petriplate. The colonies are counted with the help of touching the pen provided with the instrument on the lid of the petriplate corresponding to the colony, on an illuminated screen.

Autoclave:-

Autoclave is an instrument in which saturated steam under pressure is used to sterilize the materials. The pressure increases the boiling point of water and hence steam under pressure provides the temp above than those obtainable by boiling

Temp of steam at various pressures

Pressure (psi)	temp °C
0	100.00
10	115.60
15	121.00
20	126.00
25	130.00
30	135.00

Saturated steam heats the material about 2500 times more efficiently than the hot air at the same temp. Moist heat has more penetration power as compared to dry heat. Most of microbes get killed at 121°C , 15 psi and within 15 min. Microbial cells get killed due to high temp and not due to pressure.

Autoclave is the backbone of the microbiology lab. It is used for sterilizing media, heat resistant equipment, glassware, rubber products and contaminated materials, discarded cultures. Autoclaving is not recommended for heat sensitive materials, powders and oils.

An autoclave is double walled vessel, made of aluminium, copper or stainless steel. Autoclave lid is provided with pressure gauge for noting the pressure, safety valve to avoid explosion, controls for adjusting the pressure & temp and exhaust valve for air exhaustion of the vessel. The materials to be sterilized are kept loosely in the perforated containers. Then close the lid of the autoclave. Switch on the instrument. Set the temp & pressure with the knobs. Open the exhaust valve so that air could get released otherwise desired temp required to achieve sterilization would not be attained. After sometime, the steam starts flowing freely from the exhaust valve, which is allowed to continue for about 5 min and then the valve is closed. Temp & pressⁿ will now start rising till the controlled conditions are attained. Holding time is noted from this stage and continued for the desired period & then it is allowed

to cool. The valve is opened only after the autoclave has attained the atmospheric conditions. The lid is opened at the sterilized material is removed.

M.O are destroyed by moist heat due to coagulation of proteins in the cell.

Inoculating loop or needle :

They are used for aseptic transfer. An inoculating needle is used to transfer m.o. culture growing on solid medium. The loop is mainly used to transfer the liquid microbial culture and the liquid drop is retained in the loop area by surface tension. They are sterilized by heating in the blue portion of the flame until red hot.

Lecture Notes
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Lecture No 19

Control of physical, chemical & Biological Environment

Success of a fermentation depends upon the existence of a definite environmental conditions for biomass & product formation.

To achieve → We must know

① What is happening to the fermentation process

How to control it to obtain optimum operating conditions.

There are some factors which are controlled strictly & regularly

Temp

pH

degree of agitation

Oxygen concentration in medium

} These factors must be kept const during the process.

For controlling these factors → careful monitoring is required

Monitoring means { data acquisition
data analysis

↓
Any deviation in system parameters can be corrected timely.

Criteria which are frequently monitored -

Process sensors & their possible control functions

Category	Sensor	Possible control function
Physical	Temp	Heat/Cool
	Pressure	
	Agitator shaft power	
	Temp	
	Foam height	foam control
	flow rate	change flow rate
Chemical	pH	Acid/Alkali addition
	Redox	Additive to change redox potential
	Oxygen	change feed rate
	Exit-gas analysis	change feed rate
	Medium Analysis	change in medium composition.

Monitoring { controlling system parameters
Information regarding the progress of process.

Such informations are necessary to determine → optimum time to harvest
or if the fermentation is contaminated it can be corrected.

Monitoring equipments are required.

Instrumentation

Sensors
&

associated control system to interface with a computer.

There are large no. of process variables requiring monitoring during fermentation.

Sensors are used to monitor them

Three main classes of sensors

- (1) Sensors which penetrate into the interior of the fermenter
eg. pH electrode
dissolved O_2 electrode
- (2) Sensors which operate on the samples which are continuously withdrawn from the fermenter
eg. exhaust gas analyser
- (3) Sensors which do not come into contact with the fermentation broth or gas
tachometers
load cells.

Sensors may be classified in relation to its application for process control.

- (1) In line sensor $\rightarrow$ Integral part of the fermentation equipment & measured values obtained from it is used directly for process control.
- (2) On line sensor - Integral part of the fermentation equipment - measured values obtained can't be used directly for control.
- (3) Off line sensor - The sensor is not part of fermentation equipment; measured values can't be used directly for process control.

Methods of measuring process variables

Temperature :-

Most important parameter to be monitored & controlled.

Temperature measurement :-

Mercury in glass thermometer

Bimetallic thermometer

Pressure bulb thermometer

Metal resistance thermometer

Metal resistance thermometer or thermistors are most commonly used in industry.

Mercury in glass thermometer :-

- * may be used in small bench fermenter
- * Fragile in nature / restricted use.
- * In large fermenter thermometer is inserted into the pocket provided
- * Large time lag
- * Only indicative, No controlling or recording possible

Electrical Resistance thermometer :-

Electrical resistance changes with change in temp.

- * The bulb of the instrument contains the resistance element, a mica framework around which a sensing element is bound. A platinum wire of 100 Ω resistance is normally used. Lead coming from the bulb are connected to measuring element. The reading is ^{obtained by} used by wheatstone bridge.

- * These type of thermometer are highly accurate
- * fast response in detecting temp change.

Thermistors :-

These are semiconductors made from specific mix of pure oxides of iron, nickel & other metals.

There is a large change in resistance with change in temp.

Change in resistance is a function of absolute temp. Temp change reading is measured with the help of Wheatstone bridge.

- * These are relatively cheap

- * Can be installed at far off place from read out point.

Temperature Control :-

Use of water jackets or pipe coils within furnace
300-400 W capacity small system for 10 dm³ furnace
& system for cooling water.

Pressure measurement :-

Purpose of pressure measurement -

Industrial & laboratory equipments are designed to withstand a specific working pressure.

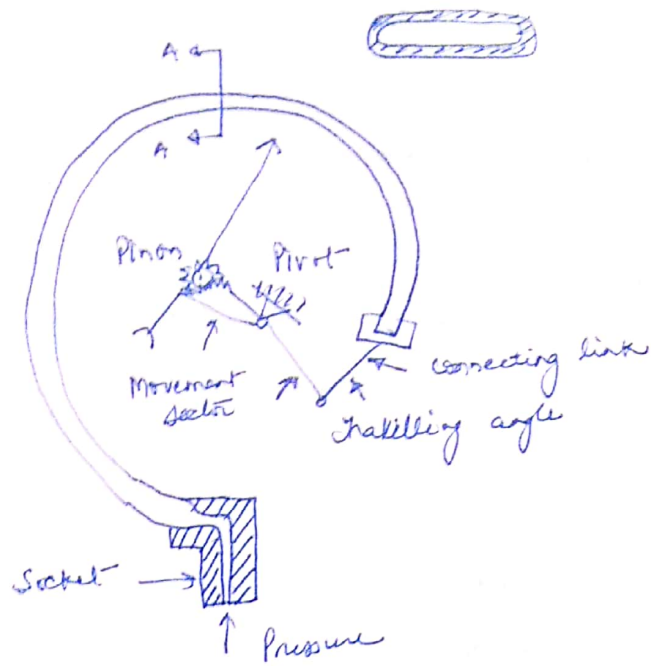
It is important that the device that senses pressure, indicate & record & control it.

Pressure influences the solubility of gases

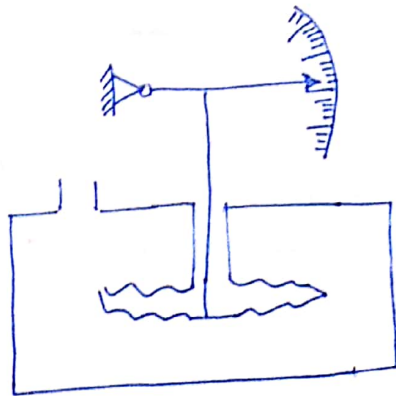
Bourdon tube pressure gauge -

The partial coil has an elliptical cross section, which tends to become circular with increasing pressure, and because of the difference between the internal & external radius gradually straightens out. The process pressure is connected to the fixed socket end of the tube while the sealed tip of the other end is connected by a geared sector & pinion movement which actuates an indicator pointer to show linear rotational response.

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Bourdon tube pressure gauge



Nested diaphragm type pressure sensor

When a vessel or pipe is to be operated under aseptic conditions a diaphragm gauge can be used. Changes in pressure cause movement of the diaphragm capsule which are monitored by a mechanically levered pointer.

- Pressure can be measured remotely using pressure bellows to the core of a variable transformer. The movement of the core generates a corresponding output.
- Pressure can be measured with the help of strain gauge.

of a wire is subjected to strain its resistance changes. The amount can be measured distance.

→ Piezo electric transducers

Certain solid crystals such as quartz have an asymmetrical electric charge distribution. Any strain in shape of the crystal produces equal, unlike electric charges on the opposite faces of the crystal. This is called piezoelectric effect.

Pressure can be measured by means of electrode attached to the opposite surfaces of the crystal.

Pressure Control —

- + Different working pressures are required in different parts of a fermentation plant.
- + Normal operating pressure head 1.2 atmosphere is maintained to achieve aseptic conditions.
- + Pressure rises during steam sterilization.
- + Correct pressure is maintained by regulating valves.

Safety valves —

- + located at suitable places of the vessel.
- + valves should be set to release pressure as soon as it increases markedly above the specified working pressure.

Agitator shaft power :-

Measure of the power consumption of a fermenter.

Large Scale / Watt meter attached to agitator of fermenter provides the measure of power consumed.

for small scale measurement —

Torsion dynamometer can be used. It also accounts for the friction losses.

Strain gauges are used to avoid friction.

Rate of stirring :-

For fermentation process measurement & control of rpm is necessary.

Tachometer is used to measure rpm.

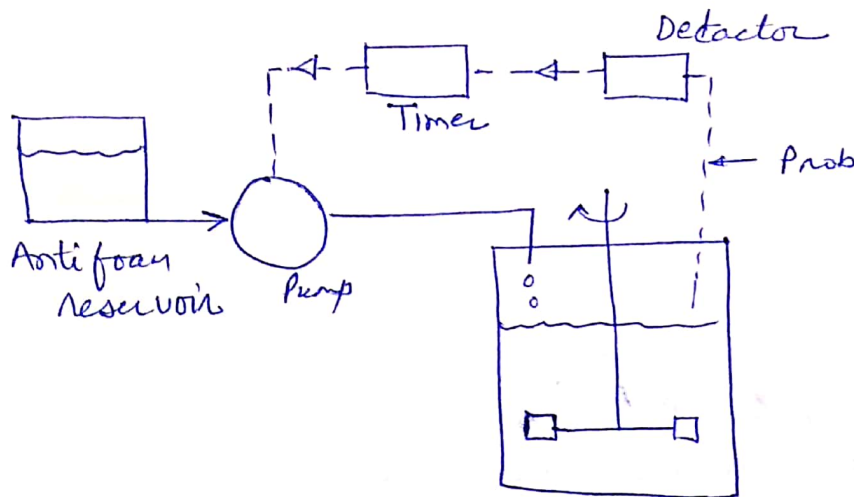
For controlling rpm of slip motors are used.

Gear box, size modification of wheels & drive belt or by changing the drive motor the rpm can be changed.

Foam Sensing & Control :-

Foam production causes problems in fermentation process.

antifoam is added to fermenter to control foam.



A prob is inserted through the top plate of fermenter. Prob is a stainless steel rod which is insulated except at the tip & set at a defined level above the broth surface. When the foam rises & touches the prob tip, a current is passed through the circuit of the prob. The current actuates a pump or valve & antifoam is released into the fermenter for a few seconds.

Mechanical antifoam devices

discs, propellers, brushes or hollow cones attached to the agitator shaft above the surface of the broth.

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foam is broken down when it is thrown against the wall of the fermenter.

Other devices include
horizontal rotating shafts
centrifugal separators
Jet spraying
deflector plates.

Most of the devices are used in conjunction with antifoam.

7 RATE
A number
gas flow rate.
from



LOW RATE :- (Gas & liquid)

A number of devices can be used to measure & control gas flow rate.

From economy point of view variable area flow meter such as rotameters are preferred. The position of the float in the meter is converted to an electrical signal through either a capacitance or resistance principle. This signal is then used, with the aid of a controller, to regulate a flow valve in the gas line.

Large number of systems are used to monitor & control liquid feed rate. An electromagnetic flow meter can be used to monitor liquid addition. In general this system is expensive & unsatisfactory for small flow rates. Mounting the addition vessels on load-cell systems permits continuous weighing of the contents, and hence, measure the additions.

Turbidity :-

Measurement of fermentation broth turbidity inside the vessel, although technically possible, gives many operational difficulties. This measurement is most important because it is a rapid means of following cell growth. At present turbidity measurements are limited to grab-sampling technique with all the problems of obtaining a representative sample & then determining the sample turbidity in a turbidimeter or spectrophotometer.

The primary obstacle to 'in tank' measurement is the tendency of culture debris to be deposited on the measuring cell. It has been recognized that to overcome this, one needs to make differential (dual) measurements from a single source. Newly developed light pipes containing split bundles of random fibers may be useful to circumvent this problem.

Viscosity:-

→ It can be used as an indicator of cell growth & / or cell morphology.

→ It also can yield insight into mycelial development processes that occur in the vessel.

There is no satisfactory system for the measurement of viscosity monitoring in a fermenter vessel. The viscosity depends upon both the character & shape of the flow field in the device used to measure the viscosity. As a result, different viscosity values for the same broth can be obtained in vessels differing in size & shape. Different flow rates & mycelial shapes give different viscosity readings.

As the broth exhibits marked non-Newtonian characteristics, it becomes more difficult to disperse nutrients uniformly and to supply oxygen to the microbial population. Therefore, it may be possible to improve the performance of a fermentation by controlling its viscosity.

pH measurement is readily accomplished by combined glass reference electrodes.

The device is placed directly into the fermenter. The half cell of the glass electrode is composed of Ag/AgCl saturated with solid KCl.

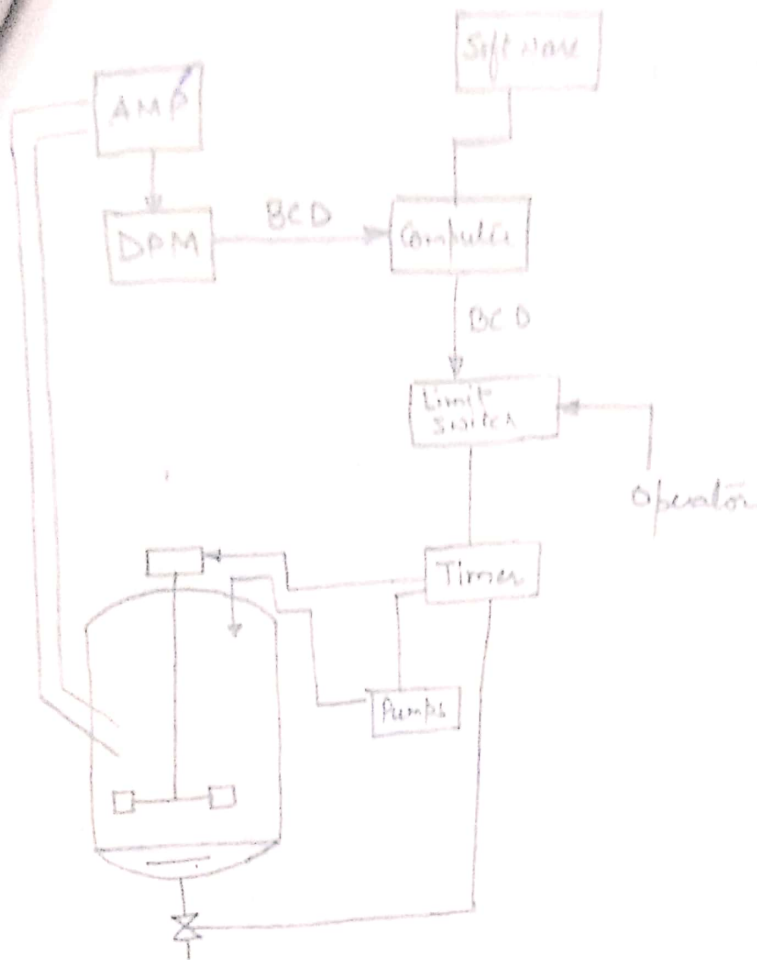
The half cell of the reference electrode consisted of the same material as the glass electrode, as asbestos or porcelain cylinder being used as the junction material.

To ensure good insulation, both the glass & reference electrodes are mounted in Teflon gasket & silicon washer. The internal resistance of the electrode is 300 - 500 M Ω . Both electrodes are protected by steel sleeves provided with several holes to allow free passage of the broth.

Lecture Notes
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Lecture No 22

Basic Concepts of Computer Control of fermentation



Sensor signals from the fermenter are amplified (AMP) & displayed on digital panel meter (DPM). The latter converts the analog values to digital forms (BCD) and transmits them to the computer.

The computer has access to the fermenter via limit switches or set points on the individual controller. The controller actuates pumps or valves on the real time basis to alter the controlled variables.

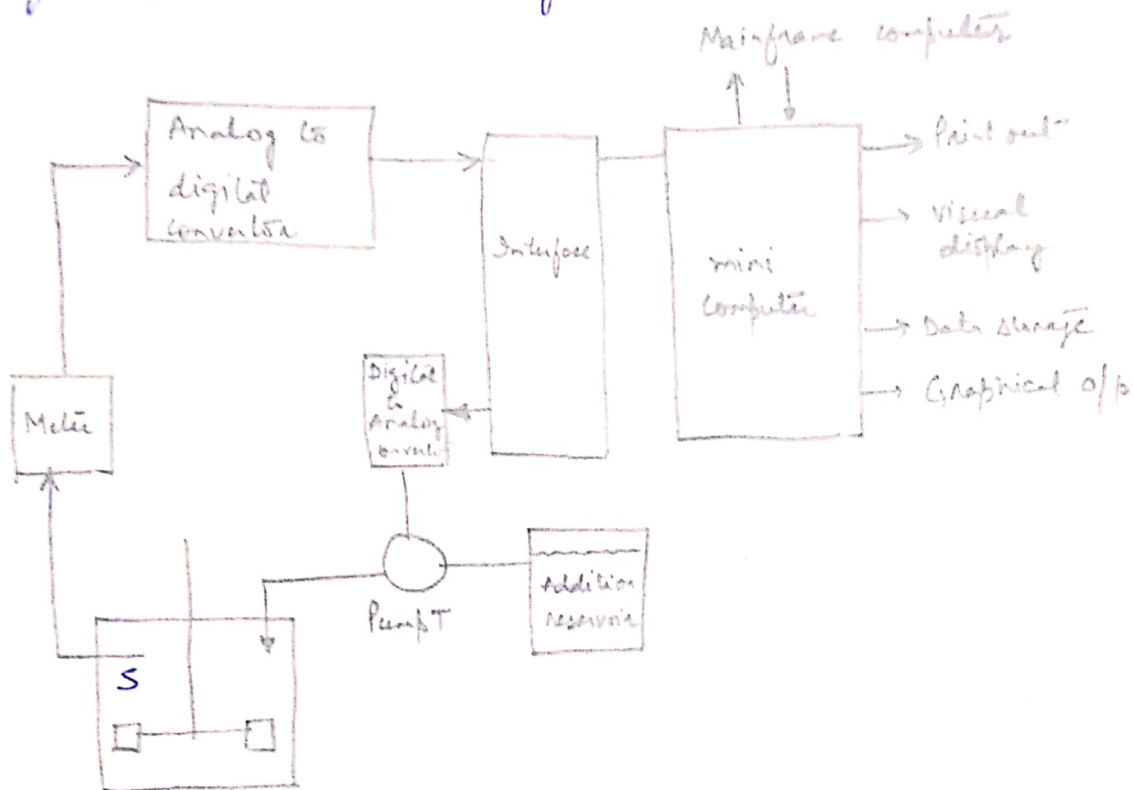
This concept requires a highly instrumented fermenter with perfected operation performance both from microbiological & instrumentation points of view.

Approaches to computer control fermentation

- ↓
- Fermentor is under direct control of computer software
 - Independent controller to manage all control functions
computer communicates via controller
SSC (supervisory set point control)

Components of computer linked system

DDC system is used to control addition of liquid from a reservoir to a fermenter.



- Sensor S in the fermenter produces a signal
- Signal may require amplification
- Signal is conditioned in correct analog form
- Analog signal is converted to digital signal
- Digital signal is sent to computer

Interface — It serves as a junction point for the inputs from the fermenter sensors to the computer & the output signals from the computer to the fermenter controls such as pump attached to the pump T.

ADC (Analog to digital converter)
converts analog signal to digital

DAC (Digital to Analog converter)

converts digital signal to electric voltage

which is used to drive stirrer pump etc.

Computer:-

The computer performs on line operation in three basic fields of function

1. logging of data obtained through the data acquisition system
2. Analysis of data on the basis of algorithms
3. control of the function of pumps & valves which results in the control of certain process variables.

Data logging:- It is performed by data acquisition system having hardware & software components. There is an interface between the sensor & computer. The software should include the computer program for sequential scanning of the sensor signals & procedure of data storage.

Data Analysis:-

Data reduction is performed by the data analysis system (computer program based on a series of selected mathematical equations).

The analysed data may be fed into a data bank or utilized for process control.

Process Control:-

Performed using computer.

Signals from the computer are fed to the pumps, valves or switches via the interface.

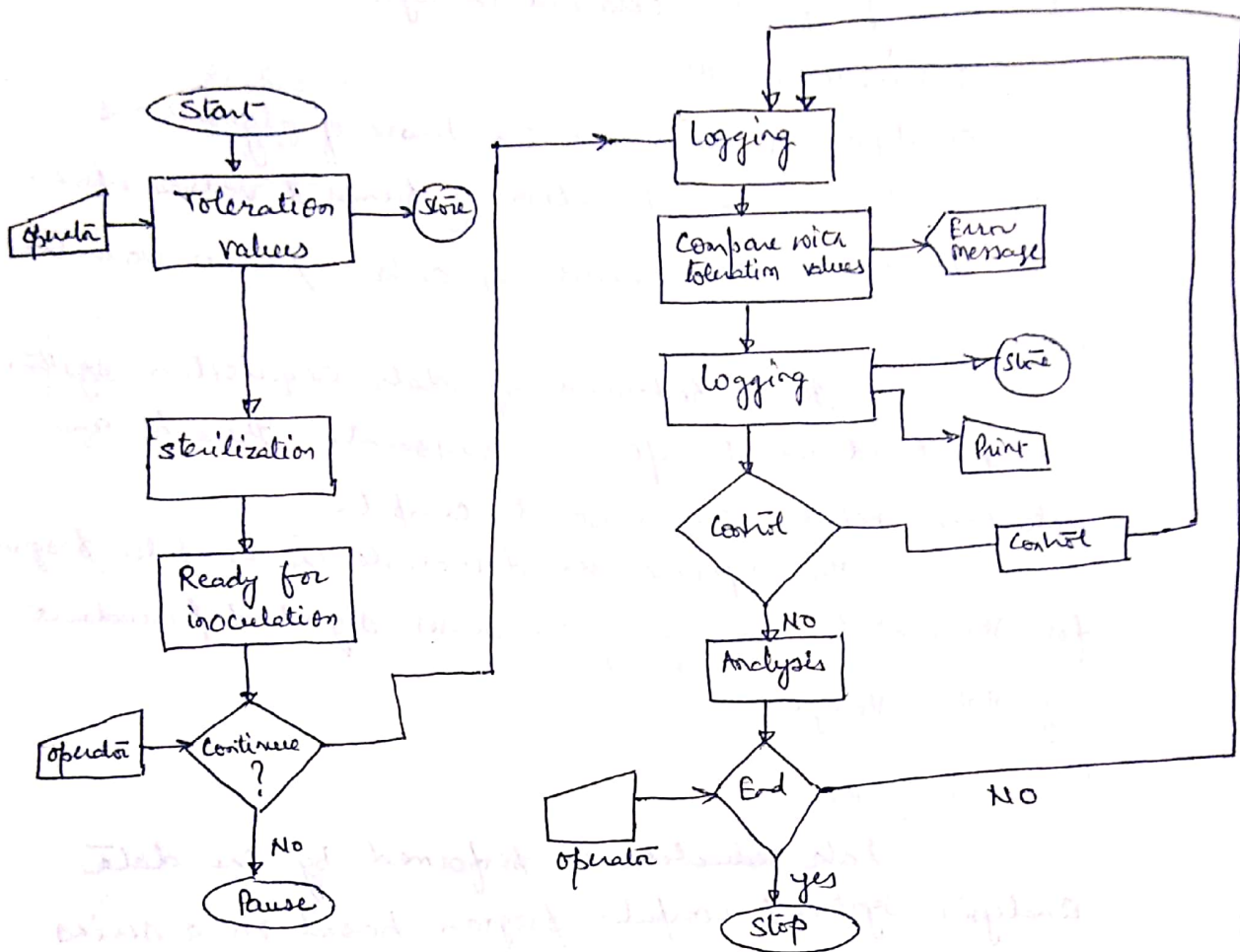
Considerations for the choice of a particular computer:-

1. A suitable word capacity which assures the handling requirements of the whole system.
2. An expandable memory capacity which can accommodate future needs.

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3. A large selection of peripheral equipments
4. Adequate field service ability.

Computer program



Preparatory

Executive

Essentials of a computer program for fermentation

A program to operate the computer must consider the hardware & software capabilities.

A program package consists of major portions i.e.

1. Preparatory phase for fermentation
2. Executive phase for the fermentation

In preparatory phase two basic functions are performed.

calculation of toleration values of the process variables which are introduced through the operator's console

2. Sterilization.

Data Analysis :-

Objective of computer application in fermentation process → To provide an insight into the physico-chemical, physiological & biochemical conditions of the process.

Grouping of Algorithm for data analysis

Physical	Physico-chemical	Physiological	Biochemical
Agitator shaft power	Apparent viscosity	Oxygen uptake	Carbon balance
Rate of addition of ingredients	Power number	Carbon dioxide evolution	Organic energy yield
Broth volume	Reynolds no.	RQ	Cell mass based on acid/base titration
	Flow character		Carbon dioxide evolution
	Volumetric Oxygen transfer coefficient		

→ Application of reliable gas analysers (Oxygen & Carbon dioxide) makes it possible to construct programs for detailed, real time study of CO_2 evolution & O_2 uptake of microbial cultures.

→ Oxygen uptake & CO_2 evolution by a culture can be used to obtain information on actual respiratory activity of the culture.