

Advances in Fermentation Technology

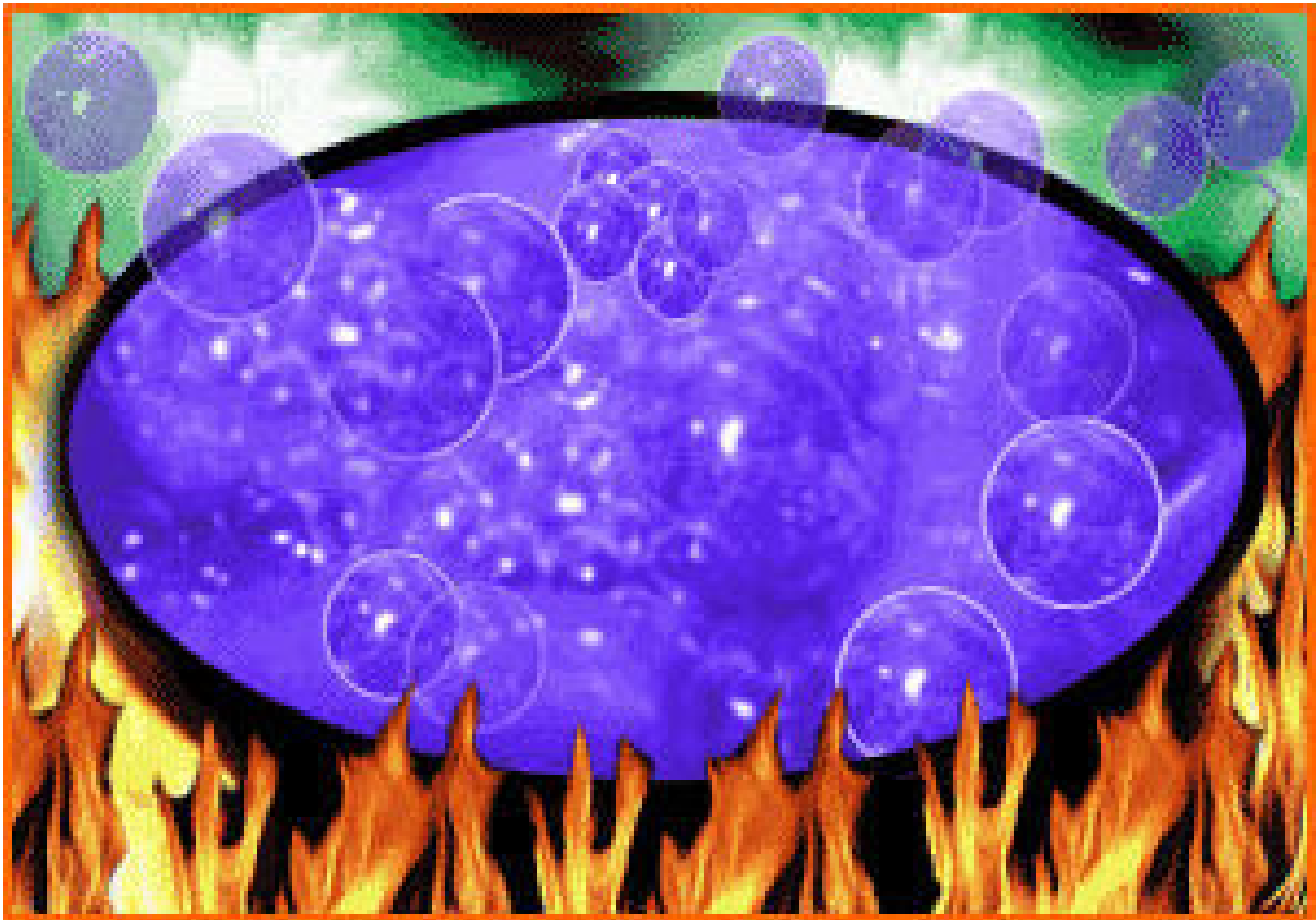
Overview of Fermentation Technology-1

Overview of Fermentation Technology

WHAT IS FERMENTATION?

- Fermentation actually comes from Latin word “**fervere**” which means “**to boil**”
- So the word “**Fermentation**” is referred to physical state of boiling/bubbling.

Overview of Fermentation Technology



Overview of Fermentation Technology



Overview of Fermentation Technology

The bubbling appearance in fermentation is due to the production of CO₂ bubbles caused by anaerobic catabolism of sugars present in the extract by the action of yeast cells

Overview of Fermentation Technology

Definition of Fermentation

In the words of
BIOCHEMIST

**“Fermentation
relates to the
generation of energy
by catabolism of
organic compounds”**

Overview of Fermentation Technology

**But in strict
biochemical sense**

***“the fermentation is
Energy-generation
process in which
organic compounds
act as both electron
donors and electron
acceptors”***

Overview of Fermentation Technology

**FERMENTATION IN
TERMS OF A
MICROBIOLOGIST:**

*INDUSTRIAL
MICROBIOLOGISTS*
have extended the
term fermentation
much broader than
CHEMISTS

Overview of Fermentation Technology

***Microbiologists
describe it as:***

***"any process for
the production of
product by the
mass culture of
micro-organism"***

Overview of Fermentation Technology

FERMENTATION IN TERMS OF A BIOTECHNOLOGIST

The term
fermentation
becomes more
broader than Mass
culturing of the
cells for the sake of
desired product.

Overview of Fermentation Technology

Fermentation in biotechnology can be referred to the

“Use of Biological system for the sake of development / improvement / facilitation in a process by mass culture of cells”

END OF
MODULE 1

Advances in Fermentation Technology

Overview of Fermentation Technology-2

Overview of Fermentation Technology

*As the
Fermentation is a
process of mass
culturing of the
cells for the sake
of desired
product.*

Overview of Fermentation Technology

*By this definition,
fermentation
involves*

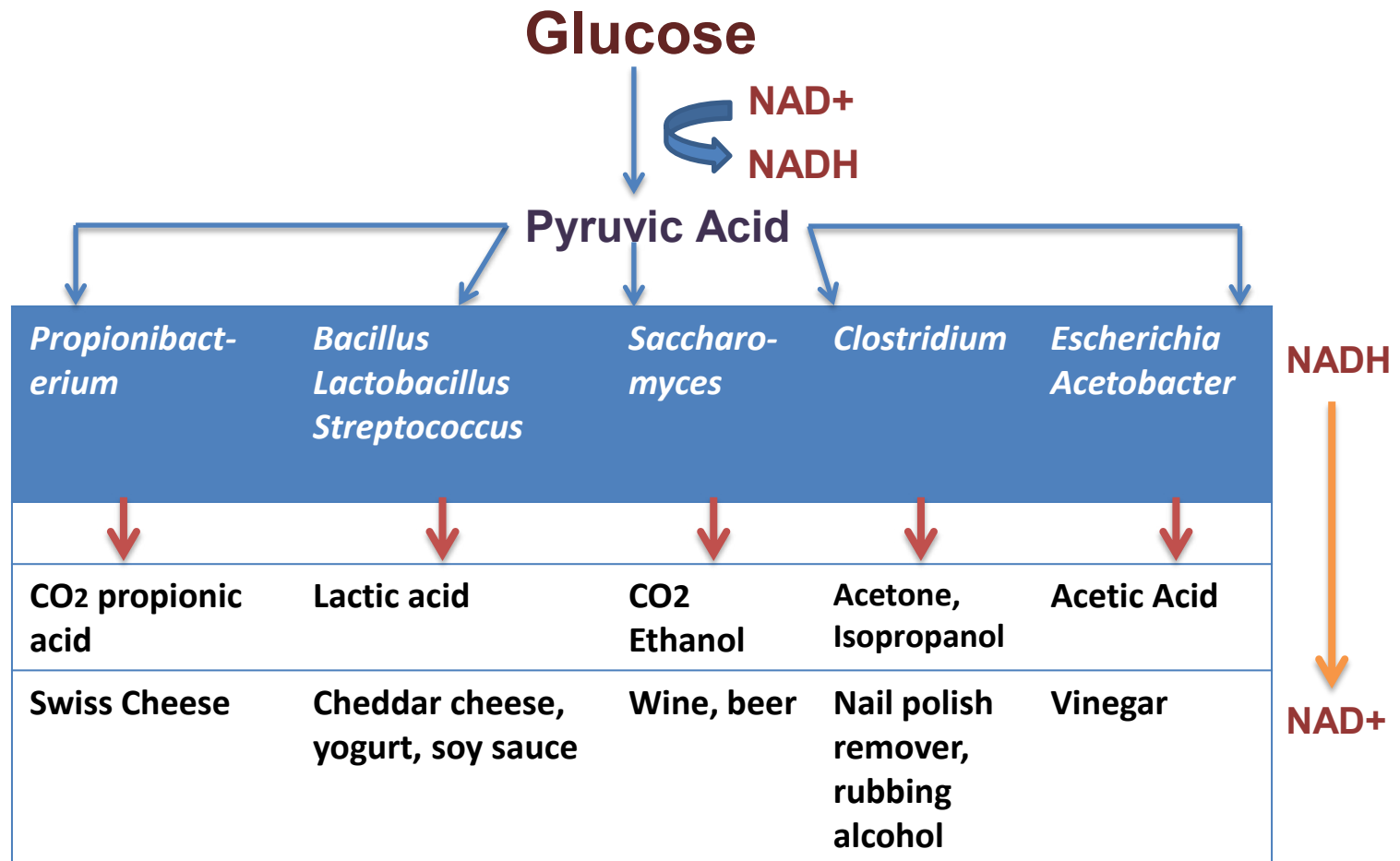
- 1. Mass culturing
techniques*
- 2. Types of cells
and organisms*
- 3. Fermentation
Products*

Overview of Fermentation Technology

Products of Fermentation Process

- What **possible products** we can made by fermentation process?
- What is the **range** of capacity of **fermentation products**?

Overview of Fermentation Technology



Overview of Fermentation Technology

Common Fermentation Products

**Acids, Enzymes,
Gasses, Alcohols,
Hormones, Single
Cell Protein,
Yogurt, Cheese,
Wines, Bread &
Pickles, etc.**

**END OF
MODULE
2**

Advances in Fermentation Technology

Types of Fermentation

Types of Fermentation

Fermentation can be classified into different types **on the basis of followings:**

- 1.Oxygen (O_2) Need*
- 2.Moisture/Water (H_2O) content in medium*
- 3.Production Scale*
- 4.Process*
- 5.State/Mixing*
- 6.Product*
- 7.Organism*

END

Advances in Fermentation Technology

Types of Fermentation on the basis of **Oxygen (O₂) Need**

Types of Fermentation

The O₂ supply must be according to need of the process of fermentation:

- ☐ **Aerobic Fermentation**
- ☐ **Semi-anaerobic Fermentation**
- ☐ **Anaerobic Fermentation**

Types of Fermentation

❑ Aerobic Fermentation

- Aerobic fermentation is carried out in the presence of oxygen.
- Organisms use oxygen for the conversion of complex organic compounds.

Types of Fermentation

☐ Aerobic Fermentation

Oxygen here is actually required for the reproduction and growth of microorganisms.

Types of Fermentation

❑ Semi-anaerobic Fermentation

Process in which organism makes ATP by aerobic respiration , but capable of switching to anaerobic respiration if oxygen is absent.

Types of Fermentation

☐ **Semi-anaerobic
Fermentation**

**Oxygen is
needed, but
not much
more.**

Types of Fermentation

□ Anaerobic Fermentation

Anaerobic fermentation is carried out in the **strict absence of oxygen**; means presence of oxygen may be toxic.

Types of Fermentation

□ Anaerobic Fermentation

Involves
Conversion of
complex organic
compounds into
simpler ones in
the absence of
Oxygen.

END

Advances in Fermentation Technology

**Types
of
Fermentation
on the basis of
Moisture/Water
(H₂O) content in
medium**

Types of Fermentation

On the basis of
**water/ moisture
requirement ,**
process can be:

- ☐ **Solid State**
- ☐ **Submerged/
Liquid**
- ☐ **Surface Culture/
Jelly**

Types of Fermentation

❑ Solid-State Fermentation (SSF)

In solid-state fermentation, the **microorganisms grow on a moist solid with little or no 'free' water**, although capillary water may be present.

Examples of this type of fermentation are seen in mushroom cultivation, bread-making and the processing of cocca, and in the manufacture of some traditional foods, e.g. miso (soy paste), soy sauce, tempeh (soybean cake) and gari (cassava), which are now produced in large industrial operations. SSF is also called as **Koji Fermentation**.

Types of Fermentation



Types of Fermentation

Some food fermentations involving SSF:

- i. Wheat by *Aspergillus spp.*
- ii. Soybean by *Rhizopus spp.*
- iii. Soybean by *Aspergillus spp.*



Types of Fermentation

❑ Submerged/Liquid Fermentation (SmF)

Fermentation process wherein

microorganisms
employed grows in
submerged state
within the
fermentation media.

Types of Fermentation

❑ Submerged/Liquid Fermentation (SmF)

Submerged fermentation may **use a dissolved substrate**, e.g. sugar solution, **or a solid substrate**, suspended in a large amount of water to form a slurry.

Types of Fermentation



❑ Submerged/Liquid Fermentation (SmF)

➤ Organism is grown in a liquid medium which is vigorously aerated & agitated.

➤ **Used** for pickling vegetables, brewing beer and producing wine and soy sauce.

Types of Fermentation

❑ Surface Culture/ Jelly Fermentation

It refers to those fermentations in which the microorganisms utilized to grow on the surface of the fermentation media.

Types of Fermentation

❑ Surface Culture/ Jelly Fermentation

➤ Organism is allowed to grow on the surface of a liquid medium without agitation. After an appropriate incubation period the culture filtrate is separated from the cell mass and is processed to recover the desirable product.

➤ **Citric acid** is commonly produced by surface fermentation process wherein the microorganism *Aspergillus niger* grows as a thick floating mycelial mat over the surface of the media used.

Types of Fermentation



Surface Culture Fermentation

END

Advances in Fermentation Technology

**Types
of
Fermentation
on the basis of
Production
Scale**

Types of Fermentation

On the basis of **scale**, there are following types of fermentation:

- ☐ **Laboratory scale fermentation**
- ☐ **Pilot scale fermentation**
- ☐ **Industrial scale fermentation**

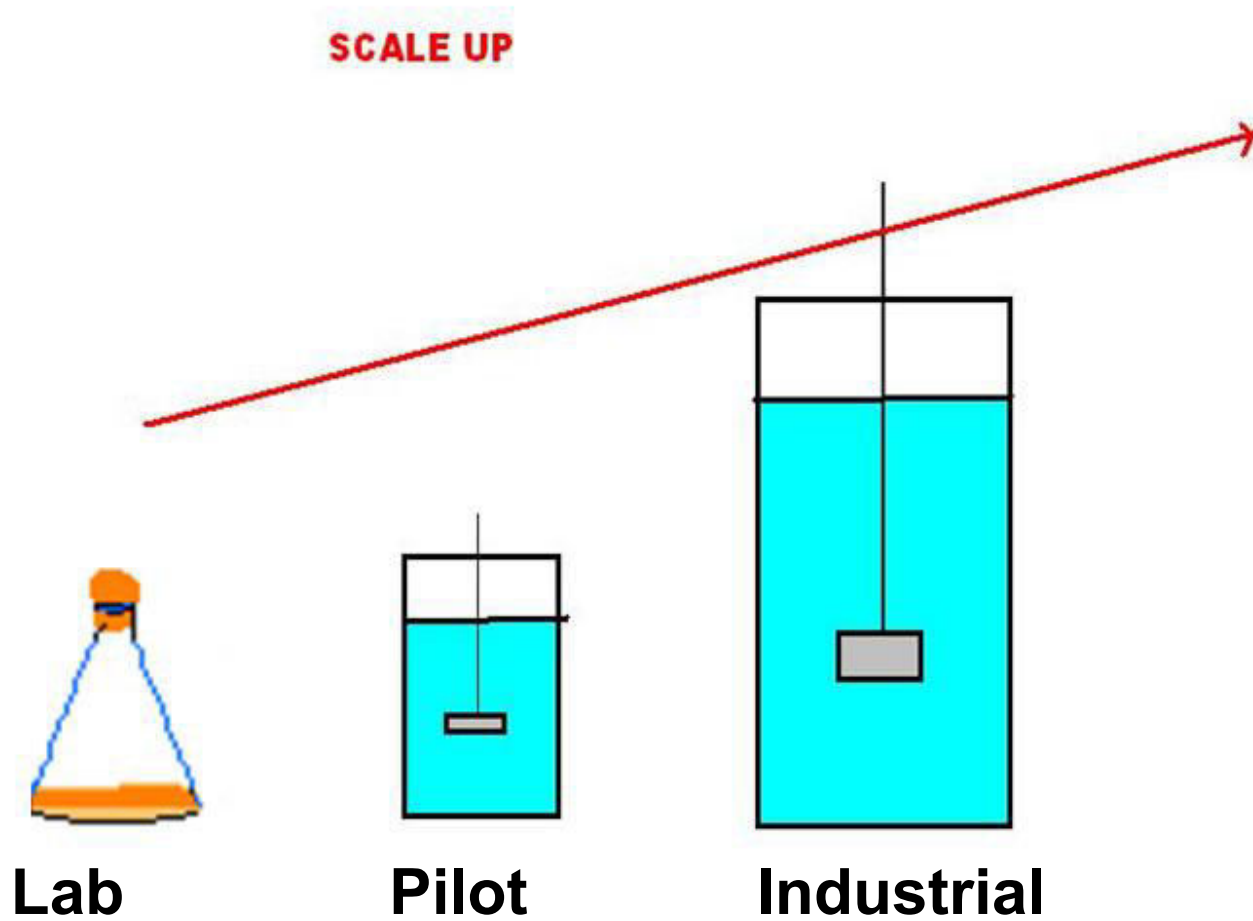
Types of Fermentation

➤ **Laboratory scale fermentation** is carried out in flasks, and cultures may be mixed by means of shaking in a controlled environment chamber.

➤ **Pilot and industrial scale fermentations** are normally carried out in stirred, aerated vessels, termed as fermenters.

Scale-up means increasing the production scale, for example from the laboratory scale to the pilot plant scale or from the pilot plant scale to industrial scale.

Types of Fermentation



Types of Fermentation

This is actually done on the basis of two factors:

➤ **Mode of sterilization**

In-situ → means sterilization is done on the spot/ inside fermenter maintaining high temperature by steam

Ex-situ → means sterilization is done outside e.g. in autoclave.

➤ **Inoculum Level/quantity**

An increase in scale also vary in the inoculum development .

Types of Fermentation

Ex-situ Sterilization in Autoclave



Types of Fermentation

In-situ Sterilization (Inside the Fermenter)



Types of Fermentation

❑ Lab Scale Fermentation

This is small scale fermentation mostly run at lab scale either in flask of different quantity or fermenter.

Types of Fermentation

❑ Lab Scale Fermentation

- Sterilization mode at this scale is always **ex-situ**.
- Inoculum that runs fermentation process is prepared in **slants/small flasks**.

Types of Fermentation



Types of Fermentation

❑ Pilot Scale Fermentation

A pilot scale plant offers flexibility, versatility and a broad range of equipment to rapidly convert laboratory results into industrial processes.

Types of Fermentation

□ Pilot Scale Fermentation

In fact **pilot plant fermentation** 'mimic' the **industrial scale fermentation process** by carrying out the industrial process on a smaller scale.

Types of Fermentation

➤ We carry out fermentation plant studies based on '**uncertainties**' of what will the industrial scale fermentation plant will be like in reality.

AND

➤ How will the processes be similar or different from the smaller lab scale studies and how much changes will need to be executed at the various processes.

Types of Fermentation

Type of fermentations at inoculum level and mode of sterilization:

Inoculum			Sterilization	
Fermentation type	Slant/flask	Seed Fermenter	Ex-situ	In-situ
Lab Scale	✓		✓	
Pilot Scale	✓			✓
Industrial		✓		✓

Types of Fermentation

□ Industrial Fermenters



END

Advances in Fermentation Technology

**Range
of
Fermentation
Products**

Range of Fermentation Products

There are five major groups of commercially important fermentations:

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

Range of Fermentation Products

□ Biomass Production

- Baker's Yeast
- SCP (Single Cell Protein)
- Probiotics
- Bio fertilizers
- Vaccines
- Bioweapons

Advances in Fermentation Technology

Range of Fermentation Products-2

Range of Fermentation Products

☐ Biofertilizers

Biofertilizers are low cost, renewable sources of plant nutrients which supplement chemical fertilizers.

Range of Fermentation Products

❑ **Biofertilizers**

These are nothing but selected strains of beneficial soil microorganisms cultured in the laboratory and packed in a suitable carrier. They can be used either for seed treatment or soil application.

Range of Fermentation Products

❑ Bioweapons

➤ **Biological weapons** (often termed "bio-weapons", "biological threat agents", or "bio-agents") are living organisms or replicating entities (viruses, which are not universally considered "alive") that reproduce or replicate within their host victims.

➤ The **North Atlantic Treaty Organization (NATO)** defines a biological weapons as "the provision of any infectious agent or toxin by any means of delivery in order to cause harm to humans, animals, or plants."

Range of Fermentation Products

❑ Top 5 Examples of Bioweapons



1. Smallpox
2. Anthrax
3. Botulinum toxin
4. Aflatoxin
5. *Clostridium perfringens*

Range of Fermentation Products

❑ Smallpox

If you took a gram of smallpox, which is **highly contagious and lethal**, and for which there's **no vaccine available globally now**, and released it in the air and created about a hundred cases, the chances are excellent that the virus would go global in six weeks as people moved from city to city.....the death toll could easily hit the hundreds of millions.....in scale, that's **like a nuclear war**.



Range of Fermentation Products

☐ Anthrax

Another Biological Weapon, *B. anthracis*, is an aerobic spore forming, gram positive bacterium that is highly infectious and lethal to man and many of his domestic animals.

Range of Fermentation Products

☐ Anthrax

Inhaled spores germinate in the lungs and produce a **pulmonary anthrax** which is **rapidly fatal** in 80% of the cases.



END

Advances in Fermentation Technology

Range of Fermentation Products-3

Range of Fermentation Products

☐ Metabolites Production

- Intermediates and products of metabolism.
- All the compounds and substances that are stops of metabolic reaction are metabolites.

Range of Fermentation Products

☐ Metabolites Production

Metabolites are classified as:

i. Primary

Metabolites

ii. Secondary

Metabolites

iii. Tertiary

Metabolites

Range of Fermentation Products

❑ Types of Metabolites

- Primary Metabolites are those which are directly involved in the process.
- Secondary Metabolites are those which are not directly involved in those metabolic processes, but usually have an important ecological function.

Metabolites may involve Acids e.g. Citric acid, Oxalic acid, Glutamic acid and Amino acid.

Range of Fermentation Products



❑ Aflatoxins

This is a class of **biological carcinogens**, product by certain molds, that **induce liver cancer**. Molds that produces this material grows well on grain, peanuts and other rich nutrients.

Range of Fermentation Products

❑ Botulinum Toxin

- Often touted as the **most toxic substance** in the world or at least in the biological world.
- Produced from *Clostridium botulinum*, an **obligate anaerobe**, which makes it a bit difficult to grow.

END

Advances in Fermentation Technology

Range of Fermentation Products-4

Range of Fermentation Products

☐ Enzymes

- Enzymes are Functional Proteins, with few exceptions.
- Enzymes may be extracellular or intracellular.

Range of Fermentation Products

☐ Enzymes

Intracellular enzymes are those which remain within the cell and are responsible of catalyzing all those processes need in cells.

Range of Fermentation Products

☐ Enzymes

Extracellular enzymes are those which are released by the cells to perform a job external to the cell e.g. digestive enzymes.

Range of Fermentation Products

☐ Enzymes

Enzymes can also be classified as constitutive enzymes or inducible enzymes.

- **Constitutive enzyme:** An enzyme that is synthesized continually regardless of growth conditions.
- **Inducible enzyme:** An enzyme that is synthesized only in the presence of the substrate that acts as an inducer.

Range of Fermentation Products

As **example** of inducible enzyme serves pyruvate carboxylase (enzyme which turns pyruvate into oxaloacetate) which is synthesized on demand and the process is activated by cortisol.

END

Advances in Fermentation Technology

Range of Fermentation Products-5

Range of Fermentation Products

□ Biotransformation

The conversion of molecules from one form to another within an organism.

Examples:

Starch → Maltose

Starch → Glucose

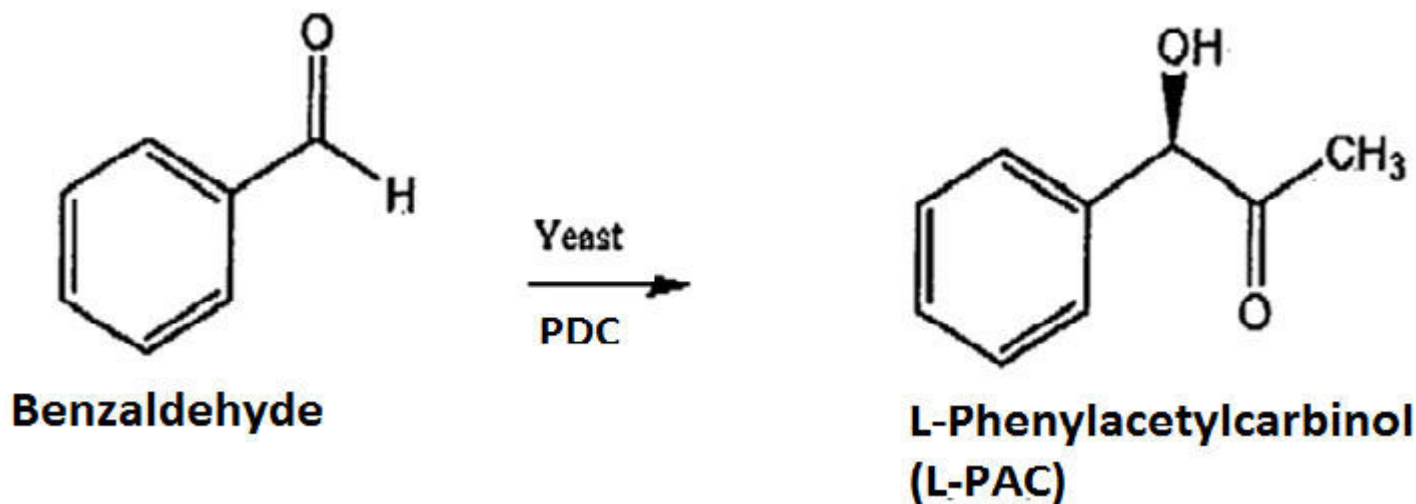
Benzaldehyde →

LPAC

Range of Fermentation Products

Biotransformation of Benzaldehyde to LPAC

Biotransformation of benzaldehyde to L-phenylacetylcarbinol (L-PAC) as a key intermediate for L-ephedrine synthesis has been synthesized by the bioconversion by yeast cells.



Range of Fermentation Products

Biotransformation

For most microbial fermentations, glucose dissimilation occurs through the glycolytic pathway. A Simple organic compound most commonly generated is pyruvate, or a compound derived enzymatically from pyruvate, such as acetaldehyde, etc.

Range of Fermentation Products

❑ Biotransformation

Acetaldehyde can then be reduced by $\text{NADH} + \text{H}^+$ to ethanol, which is excreted by the cell.

END

Advances in Fermentation Technology

Range of Fermentation Products-6

Range of Fermentation Products

❑ Recombinant Products

The advent of rDNA technology has extended the range of fermentation products. Genes from higher organisms may be introduced into microbial cells with suitable expression systems.

Range of Fermentation Products

☐ Recombinant Products

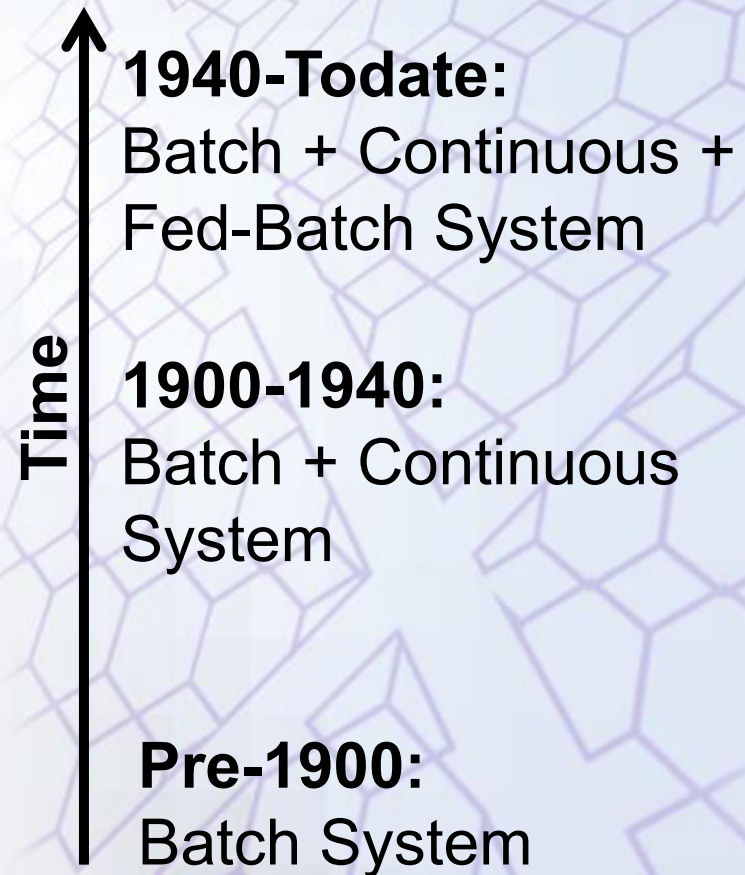
- Interferons
- Insulin
- Human Serum Albumin
- Clotting Factors VIII and IX
- Epidermal Growth Factor
- Calf Chymosin
- Bovine Somatostatin

Range of Fermentation Products

Stages in the chronological development of fermentation industry

Stage	Main Products
(1) Pre-1900	Alcohol and Vinegar.
(2) 1900-1940	Baker's Yeast, Glycerol, Citric acid, Lactic acid, Acetone/Butanol.
(3) 1940-date	Penicillin, Streptomycin, Gibberelin, aminoacids, nucleotides, enzymes.
(4) 1964-date	Single-cell Protein using hydrocarbons and other feed stocks.
(5) 1979-date	Production of heterologous proteins by microbial and animal cells, Mabs production by animal cells.

Range of Fermentation Products



END

Advances in Fermentation Technology

Types of Fermentation on the basis of Process

Types of Fermentation

➤ On the basis of **process, fermentation** can be

- 1) **Batch**
fermentation
- 2) **Fed-Batch**
Fermentation
- 3) **Continuous**
Fermentation

Types of Fermentation

❑ Batch Fermentation

- It is a **closed system**.
- In batch processing, a batch of culture medium in a fermenter is inoculated with a microorganism (the 'starter culture'). The fermentation proceeds for a certain duration (the 'fermentation time' or '**batch time**'), and the **product is harvested at the end**.
- In the course of the entire fermentation, **nothing is added** except oxygen (in the form of air, if the process is aerobic), an antifoam agent, and acid or base to control the pH. The culture is allowed to grow until no more of the product is being made, at which point the reactor is "harvested" and cleaned out for another run.

Types of Fermentation

❑ Fed-Batch Fermentation

- An **enhancement of the closed batch process** is the fed-batch fermentation.
- In fed-batch fermentations, **sterile culture medium is added either continuously or periodically** to the inoculated fermentation batch.
- Nutrients **in the medium are added when needed** and can't be added in bulk amount at start because they can act as toxin in bulk amounts.
- The **volume of fermented broth increases** with each addition of the medium, and the fermenter is harvested after the batch time.

Types of Fermentation

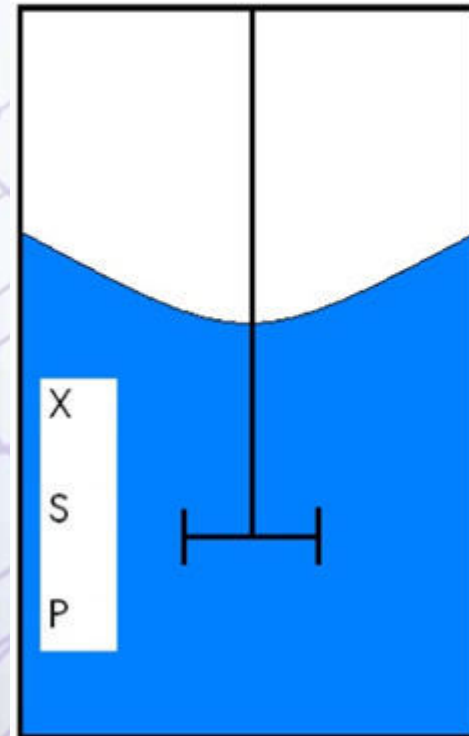
❑ Continuous Fermentation

➤ In continuous fermentations, **sterile medium is fed continuously into a fermenter** and the **fermented product is continuously withdrawn**, so the **fermentation volume remains unchanged**.

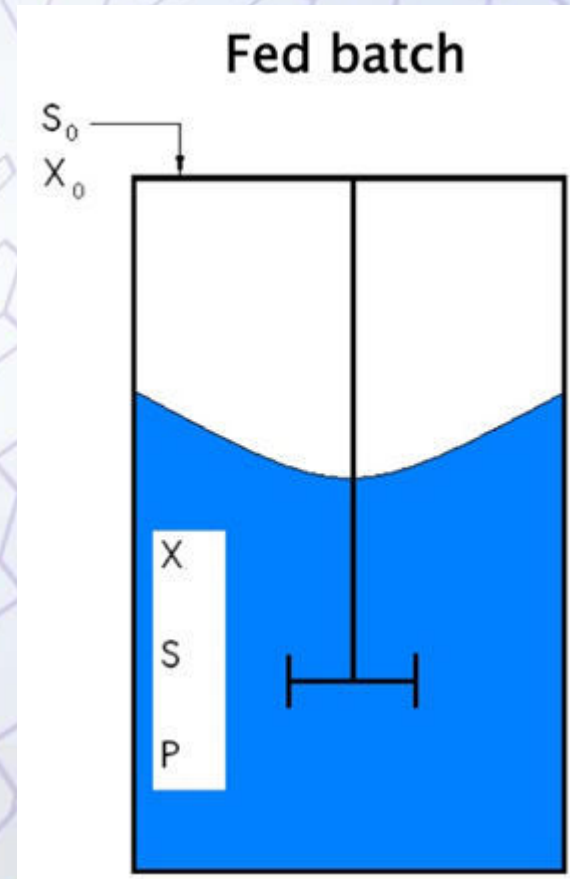
➤ Typically, continuous fermentations are started as batch cultures and feeding begins after the microbial population has reached a certain concentration.

Types of Fermentation

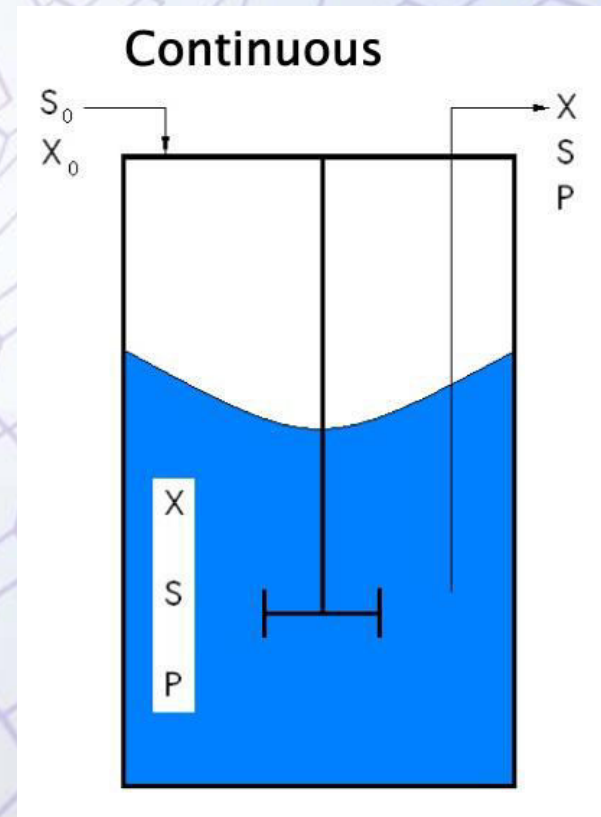
Batch



Types of Fermentation



Types of Fermentation



END

Advances in Fermentation Technology

Types of Fermentation on the basis of State of Mixing

Types of Fermentation

➤ Mixing in Fermentation is done:

- 1) To disperse the air bubbles.
- 2) To suspend the cells.
- 3) To enhance heat and mass transfer in the medium.

Types of Fermentation

➤ On the basis of state or mixing fermentation can be

- 1) Static Fermentation
- 2) Shake-Flask Fermentation
- 3) Stirred Fermentation

Types of Fermentation

❑ Static Fermentation

➤ In this type of fermentation there is **no mixing** during the process.

➤ Surface culture

➤ Un-aerated and unmixed bioreactor can be used here.

Types of Fermentation

❑ Static Fermentation

Un-aerated and unmixed bioreactor is the one in which the bed is **static**, or mixed only very occasionally (i.e., once or twice per day), and air is circulated around the bed but not blown forcefully.

Types of Fermentation

☐ Shake-Flask Fermentation

On **small level**, simple shaking procedure can be applied during fermentation process, hence termed as shake flask fermentation.

Shake culture flasks are usually applied to **aerobic processes**.

Types of Fermentation

☐ Shake-Flask Fermentation

Shake cultures are a convenient method of growing microorganisms in submerged cultures under aerobic conditions created by shaking; it is a small scale equivalent of stirred tank bioreactor.

Types of Fermentation

Shake-Flask Fermentation



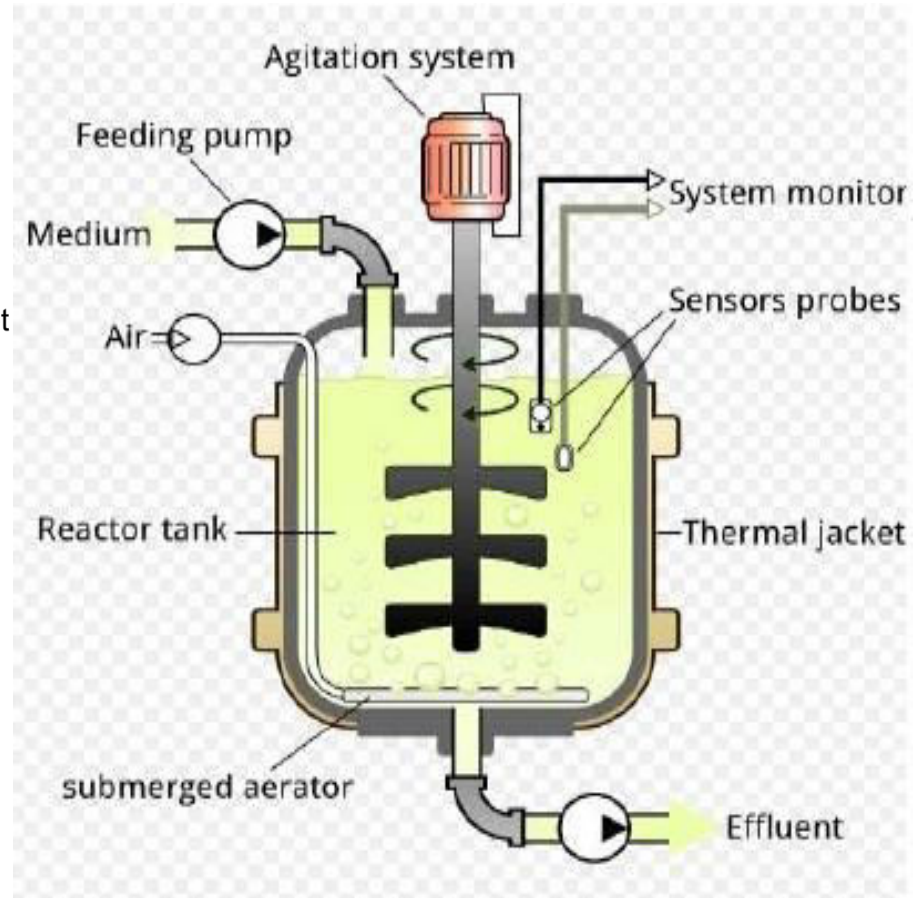
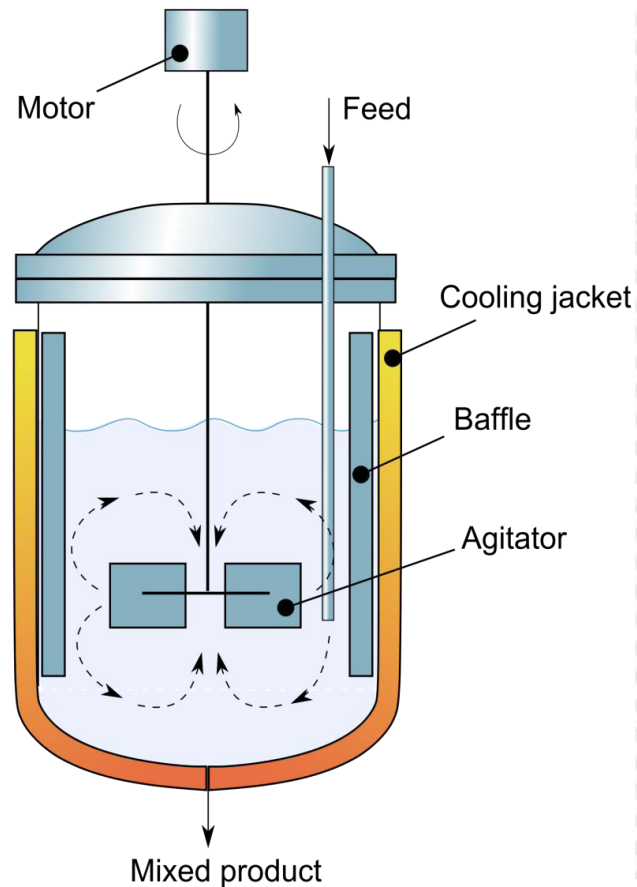
Types of Fermentation

☐ Stirred Fermentation

This type of fermentation involves **proper mixing during the process of fermentation** which is done by: **Agitator/ Impeller.**

Types of Fermentation

Stirred Fermenters



Types of Fermentation

☐ Stirred Fermentation

The medium must be stirred to keep the cells in suspension

Various types of stirrers range from simple magnetic stirrers, flat blade turbine impellers, to marine impellers.

END

Advances in Fermentation Technology

**Types
of
Fermentation
on the basis of
Product &
Organisms**

Types of Fermentation

Following are the types of fermentation on the basis of the **products that formed:**

1) **Beer**

Fermentation

2) **Yogurt**

Fermentation

3) **Citric acid**

Fermentation

4) **Pickle**

Fermentation

and many more

Types of Fermentation

Fermentation can also be named on the basis on the **organisms nomenclature**. For example:

1) **Yeast**

Fermentation

2) **Bacterial**

Fermentation

3) ***Aspergillus niger***

Fermentation

and many more

END

Advances in Fermentation Technology

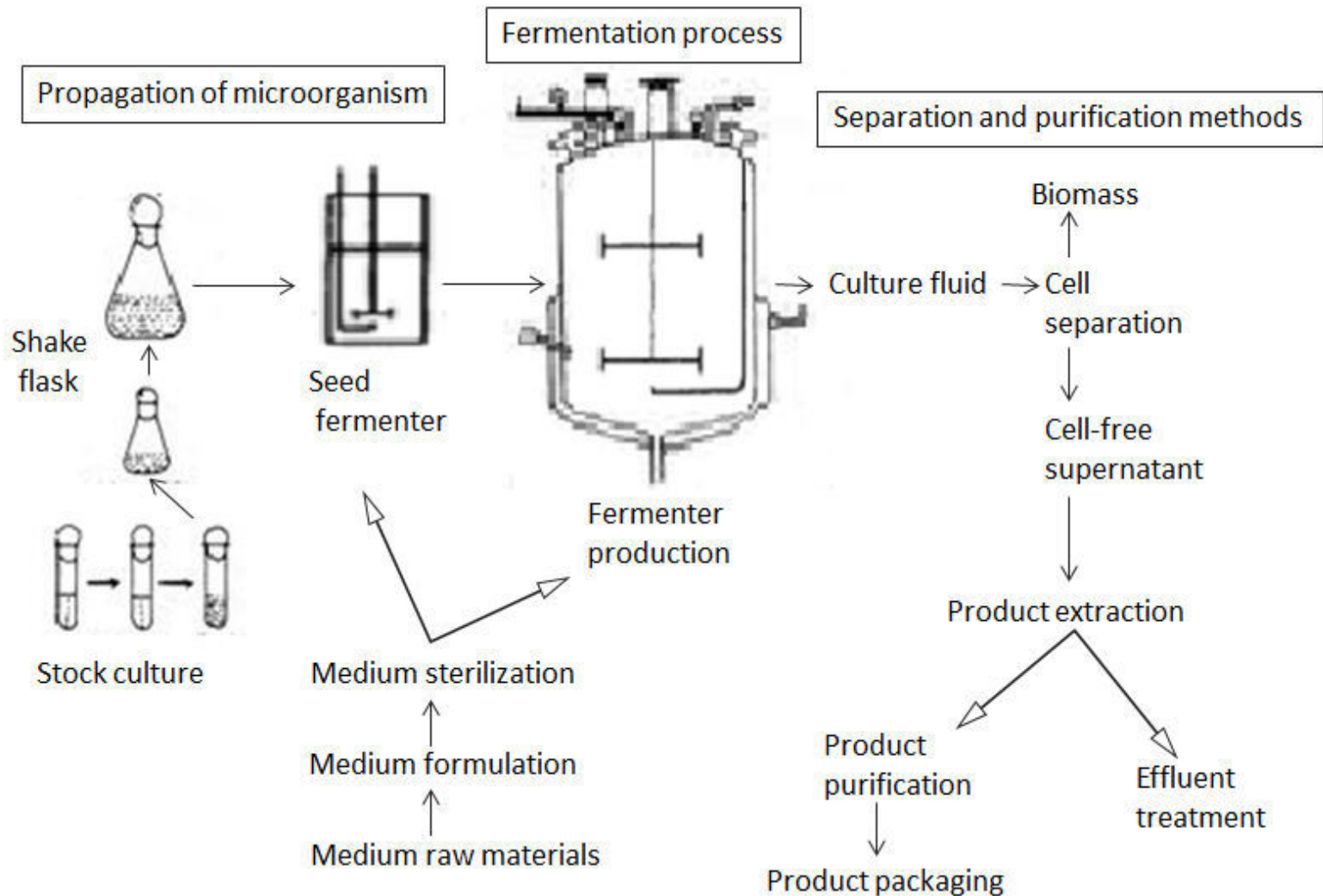
Organization of Fermentation Process

Organization of Fermentation Process

Regardless of the type of fermentation, an established process may be divided into following **basic component parts**:

1. **Organism** (Hero of Fermentation process).
2. **Fermenter** (stage on which microorganism act/grow).
3. **Media** (Formulation).
4. **Sterilization.**
5. **Monitoring/Control** (Environmental conditions).
6. **Recovery of the product** (Isolation, purification etc).
7. **Effluent treatment** (processing of materials that remained after fermentation process).

Organization of Fermentation Process



Organization of Fermentation Process

Fermentation process has two sub-processes that are:

1) Up-streaming Process

(Step 1-5)

2) Down-streaming Process

(Step 6-7)

Organization of Fermentation Process

□ Up-streaming Process

Upstream processing is an industrial term describing the **steps followed for the production of a specific product** by industrial means.

Organization of Fermentation Process

❑ Down-streaming Process

Downstream Processing includes the **recovery of the products** in a pure state and the **effluent treatment**.

Organization of Fermentation Process

□ Down-streaming Process

Product recovery is carried out through a series of operations including *cell separation* by settling, centrifugation or filtration.

Organization of Fermentation Process

□ Down-streaming Process

Product recovery by disruption of cells (if the product intracellular);
Extraction and ***purification*** of the product.

Organization of Fermentation Process

❑ Down-streaming Process

Finally, the *effluents are treated* by chemical, physical or biological methods.

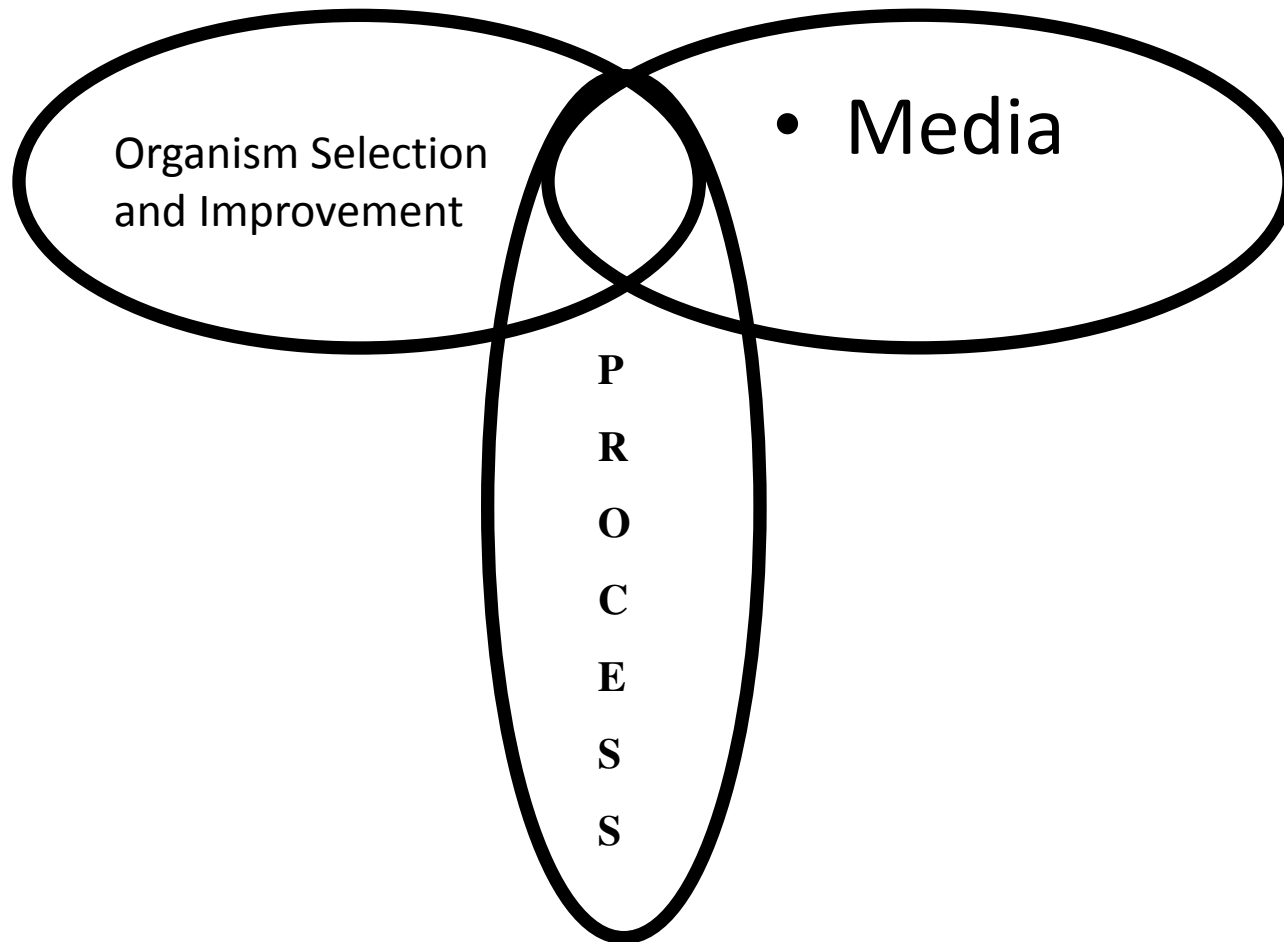
END

Advances in Fermentation Technology

Media for Industrial Fermentations: Introduction-1

Media for Industrial Fermentations

Overview



Media for Industrial Fermentations

Medium

The intervening substances through which metabolic requirements are conveyed or physical forces are transmitted.

Media for Industrial Fermentations

- What does the medium need to do?

Grow the microorganism so it produces biomass and product and should not interfere with down stream processing

Media for Industrial Fermentations

Detailed investigation is needed to establish the most suitable medium for an individual fermentation process, but certain basic requirements must be met by any such medium.

All micro-organisms require

- **Water,**
 - **Sources of energy,**
 - **Carbon,**
 - **Nitrogen,**
 - **Mineral elements,**
- and possibly
- **Vitamins,**
- plus
- **Oxygen** (if aerobic) or other gasses such as CO₂ etc.

Media for Industrial Fermentations

It must be remembered that the medium will affect the design of fermenter to be used.

END

Advances in Fermentation Technology

Media for Industrial Fermentations: Introduction-2

Media for Industrial Fermentations

On a small scale it is relatively simple to devise a medium containing pure compounds, but the resulting medium, although supporting satisfactory growth, may be unsuitable for use in a large scale process.

On a large scale one must normally use sources of nutrients to create a medium which will meet as many as possible of the following criteria:

Media for Industrial Fermentations

1. It will produce the maximum yield of product or biomass per gram of substrate used.
2. It will produce the maximum concentration of product or biomass.
3. It will permit the maximum rate of product formation.
4. There will be the minimum yield of undesired products.
5. It will be of a consistent quality and be readily available throughout the year.
6. It will cause minimal problems during media making and sterilization.
7. It will cause minimal problems in other aspects of the production process particularly aeration and agitation, extraction, purification and waste treatment.

Media for Industrial Fermentations

- Crude and defined media:
 - Crude media is made up of unrefined agricultural products e.g. containing barley.
 - Defined media are like those we use in the lab e.g. minimal salts medium.
 - Crude media is cheap but composition is variable.
 - Defined media is expensive but composition is known and should not vary.
 - Crude media is used for large volume inexpensive products e.g. biofuel from whey.
 - Defined media is used for expensive low volume products e.g. anticancer drugs.

Media for Industrial Fermentations

The use of cane molasses, beet molasses, cereal grains, starch, glucose, sucrose and lactose as carbon sources, and ammonium salts, urea, nitrates, corn steep liquor, soya bean meal, slaughter-house waste and fermentation residues as nitrogen sources, have tended to meet most of the above criteria for production media because they are cheap substrates.

Media for Industrial Fermentations

However, other more expensive pure substrates may be chosen if the overall cost of the complete process can be reduced because it is possible to use simpler procedures.

END

Advances in Fermentation Technology

Media for Industrial Fermentations: **Introduction-3**

Media for Industrial Fermentations

- The problem of developing a process from the laboratory to the pilot scale, and subsequently to the industrial scale, must also be considered for selecting a suitable medium.
- A laboratory medium may not be ideal in a large fermenter with a low gas-transfer pattern. A medium with a **high viscosity** will also need a higher power input for effective stirring. Besides meeting requirements for growth and product formation, the medium may also influence **pH variation, foam formation, the oxidation-reduction potential, and the morphological form of the organism.**
- It may also be necessary to provide precursors or metabolic inhibitors. The medium will also affect product recovery and effluent treatment.

Media for Industrial Fermentations

Historically, **undefined complex natural materials** have been used in fermentation processes because they are much cheaper than pure substrates. However, there is often considerable batch variation because of variable concentrations of the component parts and impurities in natural materials which cause unpredictable biomass and/or product yields.

As a consequence of these variations in composition small yield improvements are difficult to detect. Undefined media often make **product recovery and effluent treatment more problematical** because not all the components of a complex nutrient source will be consumed by the organism.

The residual components may **interfere with recovery** and contribute to the **BOD** (Biological Oxygen demand) of the **effluent**.

Media for Industrial Fermentations

Thus, although manufacturers have been reluctant to use defined media components because they are more expensive, pure substrates give more predictable yields from batch to batch and recovery.

Media for Industrial Fermentations

Purification and effluent treatment are much simpler and therefore cheaper.

Process improvements are also easier to detect when pure substrates are used.

END

Advances in Fermentation Technology

Media for Industrial Fermentations: Introduction-4 (General Types of Media)

Media for Industrial Fermentations

Selective media are designed to suppress the growth of unwanted bacteria and encourage the growth of the desired microbes. For example, **bismuth sulfite** is one medium used to isolate the typhoid bacterium, the gram-negative *Salmonella typhi*. Bismuth sulfite inhibits gram-positive bacteria and most gram-negative intestinal bacteria (other than *S. typhi*), as well. **Sabouraud's dextrose**, which has a pH of 5.6, is used to isolate fungi that outgrow most bacteria at this pH. **Starch** is used for selective and differential isolation of amylase/starch degrading enzymes producing microorganisms.

Media for Industrial Fermentations

Differential media make it easier to distinguish colonies of the desired organism from other colonies growing on the same plate. Similarly, pure cultures of microorganisms have identifiable reactions with differential media in tubes or plates. **Blood medium** (which contains red blood cells) is a medium that microbiologists often use to identify bacterial species that destroy red blood cells. These species, such as *Streptococcus pyogenes*, the bacterium that causes strep throat, show a clear ring around their colonies (beta-hemolysis) where they have lysed the surrounding blood cells

Media for Industrial Fermentations

Enrichment culture is used when bacteria present in small numbers can be missed, especially if other bacteria are present in much larger numbers, it is sometimes necessary to use an enrichment culture. This is often the case for soil samples. The medium (enrichment medium) for an enrichment culture is usually liquid and provides nutrients and environmental conditions that favour the growth of a particular microbe but not others. In this sense, it is also a selective medium, but it is designed to increase very small numbers of the desired type of organism to detectable levels.

Media for Industrial Fermentations

The cultivation of anaerobic bacteria poses a special problem.

Because anaerobes might be killed by exposure to oxygen, special media called **Reducing media** must be used.

Media for Industrial Fermentations

Reducing media contain ingredients, such as **sodium thioglycolate**, that chemically combine with dissolved oxygen and deplete the oxygen in the culture medium.

END

Advances in Fermentation Technology

Media for Industrial Fermentations: **Introduction-5**

Media for Industrial Fermentations

Collins (1990) has given an excellent example of a process producing recombinant protein from *S. cerevisiae* instead of just biomass. The range of growth conditions which can be used is restricted because of factors affecting the stability of the recombinant protein.

The control of pH and foam during growth in a fermenter were indentified as two important parameters. Molasses would normally be used as the cheapest carbohydrate to grow yeast biomass in a large scale process. However, this is not acceptable for the recombinant protein production because of the difficulties, and incurred costs caused in subsequent purification which result from using crude undefined media components.

Media for Industrial Fermentations

Collins and co-workers therefore used a defined medium with **glucose, sucrose** or another suitable carbon source of reasonable purity plus **minimal salts, trace elements, pure vitamins** and **ammonia as the main nitrogen source** and for **pH control**. Other impurities in molasses might have helped to stabilize foams and led to the need to use antifoams.

Tables in next slides gives the recipes for some typical media for submerged culture fermentations.

Media for Industrial Fermentations

Itaconic acid (Nubel and Ratakak, 1962)

Cane Molasses (as sugar)	150 gdm ⁻³
ZnSO ₄	1.0 gdm ⁻³
ZnSO ₄ .7H ₂ O	3.0 gdm ⁻³
CuSO ₄ .5H ₂ O	0.01 gdm ⁻³

Amylase (Underkofler, 1966)

Ground soybean meal	1.85%
Autolysed Brewers yeast fractions	1.50%
Distilled dried solubles	0.76%
Enzymatic casein hydrolysate	0.65%
Lactose	4.75%
MgSO ₄ .7H ₂ O	0.04%
Hodag KG-1 antifoam	0.05%

Media for Industrial Fermentations

Glutamic acid (*Gore et al.*, 1968)

Dextrose	270 gdm ⁻³
NH ₄ H ₂ PO ₄	2.0 gdm ⁻³
(NH ₄) ₂ HPO ₄	2.0 gdm ⁻³
K ₂ SO ₄	2.0 gdm ⁻³
MgSO ₄ .7H ₂ O	0.5 gdm ⁻³
MnSO ₄ .H ₂ O	0.04 gdm ⁻³
FeSO ₄ .7H ₂ O	0.02 gdm ⁻³
Polyglycol 2000	0.3 gdm ⁻³
Biotin	12 µgdm ⁻³
Penicillin	11 µgdm ⁻³

Media for Industrial Fermentations

These examples of different media (in previous slides) are used to illustrate the range of media in use, but are not necessarily the best media in current use.

END

Advances in Fermentation Technology

Media for Industrial Fermentations: Medium Formulation-1

Media for Industrial Fermentations

Medium Can Be a Significant Proportion of
Total Product Cost

Elements of total product cost (%)

Item	Product					
	Beer (Pratten, 1971)	Alcohol (batch) (Maiorella, 1984)	Acetic acid (Pape, 1977)	Citric acid (Schierholt, 1977)	SCP (Anon, 1974)	Penicillin (Swartz 1979)
Raw materials	38.4	76.7	42.2	39.7	62	58.0
Utilities	*	11.7	23.1	35.3	10	20.3
Labour and supervision	24.5	2.9	19.5	25.0	9	5.4
Fixed charges	7.2	4.8	10.5	—	19	—
Maintenance	29.9	—	—	—	†	14.9
Operating supplies	—	—	—	—	—	1.4‡

Raw materials costs range from 38-77% in the examples shown

Media for Industrial Fermentations

Medium formulation is an essential stage in the design of successful laboratory experiments, pilot-scale development and manufacturing processes. The constituents of a medium must satisfy the elemental requirements for cell biomass and metabolite production and there must be an adequate supply of energy for biosynthesis and cell maintenance. The first step to consider is an equation based on the stoichiometry for growth and product formation. Thus for an anaerobic fermentation:

$C + N + O_2 + \text{other req} \rightarrow \text{biomass} + \text{product} + CO_2 + H_2O + \text{Heat}$

Media for Industrial Fermentations

The above equation should be expressed in quantitative terms which is important in the economical design of media component wastage is to be minimal.

Thus, it should be possible to calculate the minimal quantities of nutrients which will be needed to produce a specific amount of biomass.

Knowing that a certain amount of biomass is necessary to produce a defined amount of product, it should be possible to calculate substrate concentrations necessary to produce required product yields.

There may be medium components which are needed for product formation which are not required for biomass production. Unfortunately, it is not always easy to quantify all the factors very precisely.

Media for Industrial Fermentations

A knowledge of the elemental composition of a process microorganism is required for the solution of the elemental balance equation. Table given in next slide will serve as a guide to the absolute minimum quantities of **N**, **S**, **P**, **Mg** and **K** to include in an initial medium recipe.

Trace elements (Fe, Zn, Cu, Mn, Co, Mo, B) may also be needed in smaller quantities.

The concentration of **P** is deliberately raised in many media to increase the **buffering capacity**. These points emphasize the need for considerable attention to be given to medium design.

Media for Industrial Fermentations

TABLE *Element composition of bacteria, yeasts and fungi (% by dry weight)*

Element	Bacteria (Luria, 1960; Herbert, 1976; Aiba <i>et al.</i> , 1973)	Yeasts (Aiba <i>et al.</i> , 1973; Herbert, 1976)	Fungi (Lilly, 1965; Aiba <i>et al.</i> , 1973)
Carbon	50-53	45-50	40-63
Hydrogen	7	7	
Nitrogen	12-15	7.5-11	7-10
Phosphorus	2.0-3.0	0.8-2.6	0.4-4.5
Sulphur	0.2-1.0	0.01-0.24	0.1-0.5
Potassium	1.0-4.5	1.0-4.0	0.2-2.5
Sodium	0.5-1.0	0.01-0.1	0.02-0.5
Calcium	0.01-1.1	0.1-0.3	0.1-1.4
Magnesium	0.1-0.5	0.1-0.5	0.1-0.5
Chloride	0.5	—	—
Iron	0.02-0.2	0.01-0.5	0.1-0.2

Media for Industrial Fermentations

Some micro-organisms cannot synthesize specific nutrients, e.g. amino acids, vitamins or nucleotides. Once a specific growth factor has been identified it can be incorporated into a medium in adequate amounts as a pure compound or as a component of a complex mixture.

END

Advances in Fermentation Technology

Media for Industrial Fermentations: Medium Formulation-2

Media for Industrial Fermentations

- Typical medium ingredients:
 - Water
 - Carbon sources and energy sources
 - Nitrogen sources
 - Vitamins and growth factors
 - Minerals and trace elements
 - Buffers
 - Inducers
 - Precursors
 - Chelators
 - Inhibitors e.g. KMS in beer medium
 - Antifoams

Media for Industrial Fermentations

The carbon substrate has a dual role in biosynthesis and energy generation. The carbon requirement for biomass production under aerobic conditions may be estimated from the cellular yield coefficient (Y) which is defined as:

$$\text{cellular yield coefficient } (Y) = \frac{\text{Quantity of cell dry matter produced}}{\text{Quantity of carbon substrate utilized}}$$

An adequate supply of the carbon source is essential for a product-forming fermentation process.

Media for Industrial Fermentations

TABLE *Cellular yield coefficients (Y) of bacteria on different carbon substrates (data from Abbott and Clamen, 1973)*

Substrate	Cellular yield coefficient (g biomass dry wt. g ⁻¹ substrate)
Methane	0.62
n-Alkanes	1.03
Methanol	0.40
Ethanol	0.68
Acetate	0.34
Malate	0.36
Glucose (molasses)	0.51

Media for Industrial Fermentations

According to the values from Table in previous slide, for bacteria with a Y for glucose of 0.5, which is $0.5 \text{ g cells g}^{-1} \text{ glucose}$, the conc. of glucose needed to obtain 30 g dm^{-3} cells will be $30/0.5=60 \text{ g dm}^{-3}$ glucose. One litre of this medium would also need to contain approximately 3.0 g N , 1.0 g P , 1.0 g K , 0.3 g S and 0.1 g Mg .

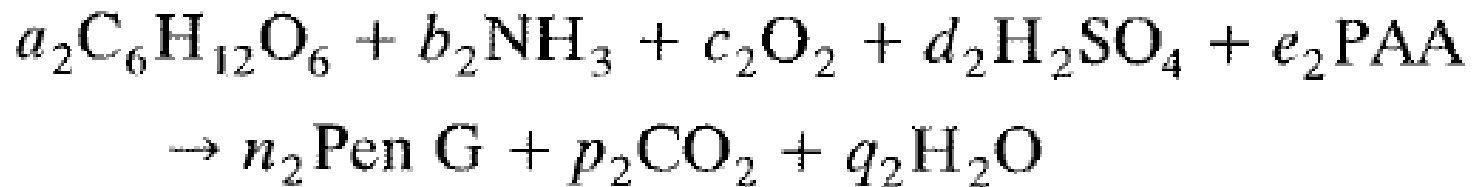
END

Advances in Fermentation Technology

Media for Industrial Fermentations: Medium Formulation-3

Media for Industrial Fermentations

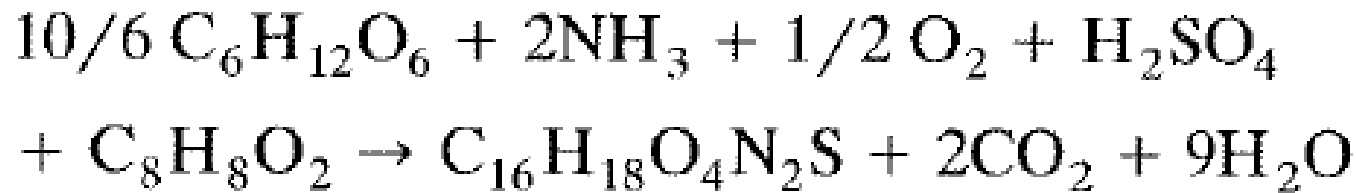
Cooney (1979) has calculated theoretical yields for Penicillin G biosynthesis on the basis of material and energy balances using a biosynthetic pathway based on reaction stoichiometry. The stoichiometry equation for the overall synthesis is:



where a_2 , b_2 , c_2 , d_2 , e_2 , n_2 , p_2 and q_2 are the stoichiometric coefficients and PAA is phenylacetic acid.

Media for Industrial Fermentations

Solution of above equation yields:



In this instance it was calculated that the theoretical yield was 1.1 g penicillin G g⁻¹ glucose (1837 units mg⁻¹).

The other major nutrient which will be required is **oxygen** which is provided by aerating the culture.

Media for Industrial Fermentations

Medium design will influence the oxygen demand of a culture in that the **more reduced carbon sources** will result in a **higher oxygen demand**.

END

Advances in Fermentation Technology

Media for Industrial Fermentations: Medium Components

Media for Industrial Fermentations

Water is the major component of all fermentation media, and is needed in many of the ancillary services such as

- ✓ heating
- ✓ cooling
- ✓ cleaning
- ✓ rinsing.

When assessing the suitability of a water supply it is important to consider pH, dissolved salts and effluent contamination.

The mineral content of the water is very

Media for Industrial Fermentations

Energy for growth comes from either the oxidation of medium components or from light. Most industrial microorganisms are chemo-organotrophs, therefore the commonest source of energy will be the carbon source such as carbohydrates, lipids and proteins.

Media for Industrial Fermentations

Some micro-organisms can also use hydrocarbons or methanol as carbon and energy sources.

END

Advances in Fermentation Technology

Media for Industrial Fermentations: CARBON SOURCES

Media for Industrial Fermentations

□ Factors influencing the choice of carbon source

The rate at which carbon source is metabolized influence the formation of biomass or production of primary or secondary metabolites. Fast growth due to high concentrations of rapidly metabolized sugars is often associated with low productivity of secondary metabolites.

The main product of a fermentation process will often determine the choice of carbon source, particularly if the product results from the direct dissimilation of it.

In fermentations such as ethanol or single-cell protein production where raw materials are 60 to 77% of the production cost, the selling price of the product will be determined largely by the cost of the carbon source.

Media for Industrial Fermentations

❑ Factors influencing the choice of carbon source

The method of media preparation, particularly sterilization, may affect the suitability of carbohydrates for individual fermentation processes. It is often best to sterilize sugars separately because they may react with ammonium ions and amino acids to form black nitrogen containing compounds which will partially inhibit the growth of many micro-organisms.

Starch suffers from the handicap that when heated in the sterilization process it gelatinizes, giving rise to very viscous liquids, so that only concentrations of up to 2% can be used without modification.

The choice of substrate may also be influenced by government legislation.

Media for Industrial Fermentations

The purity of the carbon source may also affect the choice of substrate. For example, metallic ions must be removed from carbohydrate sources used in some citric acid processes.

END

Advances in Fermentation Technology

Media for Industrial Fermentations: EXAMPLES OF COMMONLY USED CARBON SOURCES-1

Media for Industrial Fermentations

□ Carbohydrates

It is common practice to use carbohydrates as the carbon source in microbial fermentation processes. The most widely available carbohydrate is starch obtained from maize grain. It is also obtained from other cereals, potatoes and cassava.

Maize and other cereals may also be used directly in a partially ground state, e.g. maize chips. Starch may also be readily hydrolysed by dilute acids and enzymes to give a variety of glucose preparations (solids and syrups).

Hydrolysed cassava starch is used as a major carbon source for glutamic acid production in Japan.

Media for Industrial Fermentations

❑ Carbohydrates

Syrups produced by acid hydrolysis may also contain toxic products which may make them unsuitable for particular processes.

Barley grains may be partially germinated and heat treated to give the material known as malt, which contains a variety of sugars besides starch. Malt is the main substrate for brewing beer. Malt extracts may also be prepared from malted grain.

Sucrose is obtained from sugar cane and sugar beet. It is commonly used in fermentation media in a very impure form as beet or cane molasses, which are the residues left after crystallization of sugar solutions in sugar refining.

Media for Industrial Fermentations

Carbohydrate composition of barley malt (expressed as % dry weight of total)

Starch	58-60
Sucrose	3-5
Reducing sugars	3-4
Other sugars	2
Hemicellulose	6-8
Cellulose	5

Analysis of beet and cane molasses (expressed as % of total w/v)

Sucrose	48.5	33.4
Raffinose	1.0	0
Invert sugar	1.0	21.2

Media for Industrial Fermentations

Molasses is used in the production of high-volume/low-value products such as **ethanol**, **SCP**, **organic** and **amino acids** and some **microbial gums**. In 1980, 300,000 tons of cane molasses were used for amino acid production in Japan (Minoda, 1986).

Molasses or sucrose also may be used for production of higher value/low-bulk products such as **antibiotics**, speciality **enzymes**, **vaccines** and **fine chemicals**.

The cost of molasses will be very competitive when compared with pure carbohydrates. However, molasses contains many impurities and molasses-based fermentations will often need a more expensive and complicated extraction/purification stage to remove the impurities and effluent treatment will be more expensive because of the unutilized waste materials which are still present.

Media for Industrial Fermentations

The use of **lactose and crude lactose** (milk whey powder) in media formulations is now extremely limited since the introduction of continuous-feeding processes utilizing glucose.

END

Advances in Fermentation Technology

Media for Industrial Fermentations: EXAMPLES OF COMMONLY USED CARBON SOURCES-2

Media for Industrial Fermentations

□ Oils and Fats

Oils were first used as carriers for antifoams in antibiotic processes. Vegetable oils (**olive, maize, cotton seed, linseed, soya bean**, etc.) may also be used as carbon substrates, particularly for their content of the fatty acids, **oleic, linoleic** and **linolenic acid**, because costs are competitive with those of carbohydrates.

Bader *et al.*, (1984) discussed factors favouring the use of oils instead of carbohydrates. A typical oil contains approximately 2.4 times the energy of glucose on a per weight basis. Oils also have a volume advantage as it would take 1.24 dm³ of soya bean oil to add 10 kcal of energy to a fermenter, whereas it would take 5 dm³ of glucose or sucrose assuming that they are being added as 50% w/w solutions.

Media for Industrial Fermentations

□ Oils and Fats

Ideally, in any fermentation process, the maximum working capacity of a vessel should be used. Oil based fed-batch fermentations permit this procedure to operate more successfully than those using carbohydrate feeds where a larger spare capacity must be catered for to allow for responses to a sudden reduction in the residual nutrient level. Oils also have antifoam properties which may make downstream processing simpler, but normally they are not used solely for this purpose.

Media for Industrial Fermentations

Soyabean oil or **Rapeseed oil** and **Glycerol trioleate** is known to be used in some fermentations.

Methyl oleate has been used as the sole carbon substrate in cephalosporin production.

END

Advances in Fermentation Technology

Media for Industrial Fermentations:

**EXAMPLES
OF
COMMONLY USED
CARBON SOURCES-3**

Media for Industrial Fermentations

□ Hydrocarbons and their derivatives

Significant research work has been done using n-alkanes for production of organic acids, amino acids, vitamins and co-factors, nucleic acids, antibiotics, enzymes and proteins. Methane, methanol and n-alkanes have all been used as substrates for biomass production.

There are some advantages and disadvantages of hydrocarbons and their derivatives as fermentation substrates, particularly with reference to cost, process aspects and purity. In processes where the feedstock costs are an appreciable fraction of the total manufacturing cost, cheap carbon sources are important.

On a weight basis n-alkanes have approximately twice the carbon and three times the energy content of the same weight of sugar.

Media for Industrial Fermentations

□ Hydrocarbons and their derivatives

Although petroleum-type products are initially impure they can be refined to obtain very pure products in bulk quantities which would reduce the amount of effluent treatment and downstream processing.

At this time the view was also held that hydrocarbons would not be subject to the same fluctuations in cost as agriculturally derived feedstocks because it would be a stable priced commodity and might be used to provide a substrate for conversion to microbial protein (SCP) for economic animal and/or human consumption.

Cost of hydrocarbons does not make them economically attractive bulk feedstocks for the production products where feedstock costs are an appreciable fraction of manufacturing costs of low-value bulk products.

Media for Industrial Fermentations

It was considered that hydrocarbons and their derivatives might have a potential role as feedstocks in the microbial production of higher value products such as intermediates, pharmaceuticals, fine chemicals and agricultural chemicals.

END

Advances in Fermentation Technology

Media for Industrial Fermentations: NITROGEN SOURCES

Media for Industrial Fermentations

❑ Factors influencing the choice of N₂ source

Control mechanisms exist by which nitrate reductase, an enzyme involved in the conversion of nitrate to ammonium ion, is repressed in the presence of ammonia. For this reason ammonia or ammonium ion is the preferred nitrogen source.

In fungi that have been investigated, ammonium ion represses uptake of amino acids by general and specific amino acid permeases. In *Aspergillus nidulans*, ammonia also regulates the production of alkaline and neutral proteases. Therefore, in mixtures of nitrogen sources, individual nitrogen components may influence metabolic regulation so that there is preferential assimilation of one component until its concentration has diminished.

Media for Industrial Fermentations

□ Factors influencing the choice of N_2 source

It has been shown in many studies that antibiotic production by many micro-organisms is influenced by the type and concentration of the nitrogen source in the culture medium.

Antibiotic production may be inhibited by a rapidly utilized nitrogen source (NH_4^+ , NO_3^- , certain amino acids). The antibiotic production only begins to increase in the culture broth after most of the nitrogen source has been consumed.

In shake flask media experiments, salts of weak acids (e.g. ammonium succinate) may be used to serve as a N_2 source and eradicate the source of a strong acid pH change due to chloride or sulphate ions which would be present if NH_4Cl or sulphate were used as the N_2 source.

Media for Industrial Fermentations

□ Factors influencing the choice of N₂ source

The use of complex nitrogen sources for antibiotic production has been common practice. They thought to help create physiological conditions in trophophase which favour antibiotic production in idiophase.

For example, in the production of polyene antibiotics, soybean meal has been considered a good nitrogen source because of the balance of nutrients, the low phosphorus content and slow hydrolysis.

It has been suggested that this gradual breakdown prevents the accumulation of ammonium ions and repressive amino acids. These are probably some of the reasons for the selection of ideal nitrogen sources for some secondary metabolites.

Media for Industrial Fermentations

Some of the complex nitrogenous material may not be utilized by a micro-organism and create problems in downstream processing and effluent treatment. This can be an important factor in the final choice of substrate.

END

Advances in Fermentation Technology

Media for Industrial Fermentations: **EXAMPLES OF COMMONLY USED NITROGEN SOURCES**

Media for Industrial Fermentations

Most industrially used micro-organisms can utilize **inorganic** or **organic sources** of **nitrogen**. Inorganic nitrogen may be supplied as ammonia gas, ammonium salts or nitrates. Ammonia has been used for pH control and as the major nitrogen source in a defined medium for the commercial production of human serum albumin by *Saccharomyces cerevisiae*. Ammonium salts such as ammonium sulphate will usually produce acid conditions as the ammonium ion is utilized and the free acid will be liberated.

On the other hand nitrates will normally cause an alkaline drift as they are metabolized. Ammonium nitrate will first cause an acid drift as the ammonium ion is utilized, and nitrate assimilation is repressed. When the ammonium ion has been exhausted, there is an alkaline drift as the nitrate is used as an alternative nitrogen source.

Media for Industrial Fermentations

One exception to this pattern is the metabolism of *Gibberella fujikuroi* (Borrow *et al.*, 1961, 1964). In the presence of nitrate the assimilation of ammonia is inhibited at pH 2.8-3.0. Nitrate assimilation continues until the pH has increased enough to allow the ammonia assimilation mechanism to restart.

Organic nitrogen may be supplied as **amino acid**, **protein** or **urea**. In many instances growth will be faster with a supply of organic nitrogen, and a few microorganisms have an absolute requirement for amino acids. Amino acids are more commonly added as complex organic nitrogen sources which are non-homogeneous, cheaper and readily available. In lysine production, methionine and threonine are obtained from soybean hydrolysate since it would be too expensive to use the pure amino acids.

Media for Industrial Fermentations

Best nitrogen sources for some secondary metabolites

Product	Main nitrogen source(s)	Reference
Penicillin	Corn-steep liquor	Moyer and Coghill (1946)
Bacitracin	Peanut granules	Inskeep <i>et al.</i> (1951)
Riboflavin	Pancreatic digest of gelatine	Malzahn <i>et al.</i> (1959)
Novobiocin	Distillers' solubles	Hoeksema and Smith (1961)
Rifomycin	Pharmamedia	Sensi and Thiemann (1967)
	Soybean meal, $(\text{NH}_4)_2\text{SO}_4$	
Gibberellins	Ammonium salt and natural plant nitrogen source	Jefferys (1970)
Butirosin	Dried beef blood or haemoglobin with $(\text{NH}_4)_2\text{SO}_4$	Claridge <i>et al.</i> (1974)
Polyenes	Soybean meal	Martin and MacDaniel (1977)

Media for Industrial Fermentations

**Other
proteinaceous
nitrogen
compounds serving
as sources of amino
acids include corn-
steep liquor, soya
meal, peanut meal,
cotton-seed meal.**

END

Advances in Fermentation Technology

**Media for
Industrial
Fermentations:
MINERALS-1**

Media for Industrial Fermentations

All micro-organisms require certain mineral elements for growth and metabolism. In many media, magnesium, phosphorus, potassium, sulphur, calcium and chlorine are essential components, and because of the concentrations required, they must be added as distinct components. Others such as cobalt, copper, iron, manganese, molybdenum and zinc are also essential but are usually present as impurities in other major ingredients.

When synthetic media are used the minor elements will have to be added deliberately. The form in which the minerals are usually supplied and the concentration ranges are given in Table (in next slide).

Media for Industrial Fermentations

TABLE *The range of typical concentrations of mineral components (g dm⁻³)*

Component	Range
*KH ₂ PO ₄	1.0–4.0 (part may be as buffer)
MgSO ₄ ·7H ₂ O	0.25–3.0
KCl	0.5–12.0
CaCO ₃	5.0–17.0
FeSO ₄ ·4H ₂ O	0.01–0.1
ZnSO ₄ ·8H ₂ O	0.1–1.0
MnSO ₄ ·H ₂ O	0.01–0.1
CuSO ₄ ·5H ₂ O	0.003–0.01
Na ₂ MoO ₄ ·2H ₂ O	0.01–0.1

*Complex media derived from plant and animal materials normally contain a considerable concentration of inorganic phosphate.

Media for Industrial Fermentations

The concentration of phosphate in a medium, particularly laboratory media in shake flasks, is often much higher than that of other mineral components. Part of this phosphate is being used as a buffer to minimize pH changes when external control of the pH is not being used.

In specific processes the concentration of certain minerals may be very critical. Some secondary metabolic processes have a lower tolerance range to inorganic phosphate than vegetative growth. This phosphate should be sufficiently low as to be assimilated by the end of trophophase.

Media for Industrial Fermentations

The inorganic phosphate concentration also influences production of bacitracins, citric acid (surface culture), ergot, monomycin, novobiocin, oxytetracycline, polyenes, ristomycin, rifamycin Y, streptomycin, vancomycin and viomycin.

END

Advances in Fermentation Technology

Media for Industrial Fermentations: MINERALS-2

Media for Industrial Fermentations

Weinberg (1970) has reviewed the nine trace elements of biological interest (Atomic numbers 23-30, 42). Of these nine, the concentrations of manganese, iron and zinc are the most critical in secondary metabolism.

In every secondary metabolic system in which sufficient data has been reported, the yield of the product varies linearly with the logarithmic concentration of the 'key' metal. The linear relationship does not apply at concentrations of the metal which are either insufficient, or toxic, to cell growth. Some of the primary and secondary microbial products whose yields are affected by concentrations of trace metals greater than those required for maximum growth are given in Table (in next slide).

Media for Industrial Fermentations

TABLE 1 *Trace elements influencing primary and secondary metabolism*

Product	Trace element(s)
Bacitracin	Mn
Protease	Mn
Gentamicin	Co
Riboflavin	Fe, Co
	Fe
Mitomycin	Fe
Monensin	Fe
Actinomycin	Fe, Zn
Candididin	Fe, Zn
Chloramphenicol	Fe, Zn
Neomycin	Fe, Zn
Patulin	Fe, Zn
Streptomycin	Fe, Zn
Citric acid	Fe, Zn, Cu
Penicillin	Fe, Zn, Cu
Griseofulvin	Zn

Media for Industrial Fermentations

Chlorine does not appear to play a nutritional role in the metabolism of fungi. It is, however, required by some of the **halophilic bacteria**. Obviously, in those fermentations where a chlorine-containing metabolite is to be produced the synthesis will have to be directed to ensure that the non-chloro-derivative is not formed. The most important compounds are **chlorotetracycline** and **griseofulvin**.

In **griseofulvin** production, adequate available chloride is provided by the inclusion of at least 0.1% KCl as well as the chloride provided by the complex organic materials included as nitrogen sources.

Media for Industrial Fermentations

Some of the other chlorine containing metabolites are **caldriomycin**, **nornidulin** and **mollisin**.

END

Advances in Fermentation Technology

**Media for
Industrial
Fermentations:
CHELATORS**

Media for Industrial Fermentations

Many media cannot be prepared or autoclaved without the formation of a visible precipitate of insoluble metal phosphates.

Gaunt *et al.* (1984) demonstrated that when the medium of Mandels and Weber (1969) was autoclaved, a white precipitate of metal ions formed, containing all the iron and most of the calcium, manganese and zinc present in the medium.

Media for Industrial Fermentations

The problem of insoluble metal phosphate(s) may be eliminated by incorporating low concentrations of chelating agents such as **ethylene diamine tetraacetic acid (EDTA)**, **citric acid**, **polyphosphates**, etc., into the **medium**.

These chelating agents preferentially form complexes with the metal ions in a medium. **The metal ions then may be gradually utilized by the microorganism**. Gaunt *et al.* were able to show that the precipitate was eliminated from Mandel and Weber's medium by the addition **EDTA at 25 mg dm⁻³**. **It is important to check that a chelating agent does not cause inhibition of growth the micro-organism which is being cultured.**

Media for Industrial Fermentations

In many media, particularly those commonly used in large scale processes, there may not be a need to add a chelating agent as complex ingredients such as yeast extracts or proteose peptones will complex with metal ions and ensure gradual release of them during growth.

END

Media for Industrial Fermentations

The types of antifoams mentioned in previous slides are generally added when foaming occurs during the fermentation. **Because many antifoams are of low solubility** they need a carrier such as lard oil, liquid paraffin or castor oil, which may be metabolized and affect the fermentation process.

END

Advances in Fermentation Technology

**Media for
Industrial
Fermentations:
GROWTH FACTORS**

Media for Industrial Fermentations

Some micro-organisms cannot synthesize a full complement of cell components and therefore require preformed compounds called **growth factors**. The growth factors most commonly required are **vitamins**, but there may also be a need for specific **amino acids, fatty acids or sterols**. Many of the natural carbon and nitrogen sources used in media formulations contain all or some of the required growth factors. When there is a vitamin deficiency it can often be eliminated by careful blending of materials. It is important to remember that if only one vitamin is required it may be occasionally more economical to add the pure vitamin, instead of using a larger bulk of a cheaper multiple vitamin source.

Media for Industrial Fermentations

Calcium pantothenate has been used in one medium formulation for vinegar production. In processes used for the production of glutamic acid, limited concentrations of **biotin** must be present in the medium. Some production strains may also require **thiamine**.

END

Advances in Fermentation Technology

**Media for
Industrial
Fermentations:
BUFFERS**

Media for Industrial Fermentations

The control of pH may be extremely important if optimal productivity is to be achieved. A compound may be added to the medium to serve specifically as a buffer, or may also be used as a nutrient source. Many media are buffered at about pH 7.0 by the incorporation of calcium carbonate (as chalk). If the pH decreases the carbonate is decomposed. Obviously, phosphates which are part of many media also play an important role in buffering. However, high phosphate concentrations are critical in the production of many secondary metabolites.

Media for Industrial Fermentations

The balanced use of the carbon and nitrogen sources will also form a basis for pH control as **buffering capacity can be provided by the proteins, peptides and amino acids, such as in corn-steep liquor.** The pH may also be controlled externally by addition of ammonia or sodium hydroxide and sulphuric acid.

END

Advances in Fermentation Technology

**Media for
Industrial
Fermentations:
PRECURSORS**

Media for Industrial Fermentations

Some components of a fermentation medium help to regulate the production of the product rather than support the growth of the micro-organism.

Such additives include **precursors, inhibitors and inducers**, all of which may be used to manipulate the progress of the fermentation.

Media for Industrial Fermentations

Some chemicals, when added to certain fermentations, are directly incorporated into the desired product. Probably the earliest example is that of improving penicillin yields. A range of different side chains can be incorporated into the penicillin molecule.

The significance of the different side chains was first appreciated when it was noted that the addition of corn-steep liquor increased the yield of penicillin from 20 units cm^{-3} to 100 units cm^{-3} .

Corn-steep liquor was found to contain phenylethylamine which was preferentially incorporated into the penicillin molecule to yield benzyl penicillin (Penicillin G).

Media for Industrial Fermentations

Some studies showed that addition of **phenylacetic acid** and its derivatives to the medium were **capable of both increasing penicillin production threefold and to directing biosynthesis towards increasing the proportion of benzyl penicillin from 0 to 93% at the expense of other penicillins.**

Phenylacetic acid is still the most widely used precursor in penicillin production. Some important examples of precursors are given in Table (in next slide).

Media for Industrial Fermentations

Precursors used in fermentation processes

Precursor	Product	Microorganism
Phenylacetic-acid related compounds	Penicillin G	<i>Penicillium chrysogenum</i>
Phenoxy acetic acid	Penicilin V	<i>Penicillium chrysogenum</i>
Chloride	Chlortetracycline	<i>Streptomyces aureofaciens</i>
	Griseofulvin	<i>Penicillium griseofulvin</i>
Propionate	Riboflavin	<i>Lactobacillus bulgaricus</i>
Cyanides	Vitamin B12	<i>Proprianobacterium</i> , <i>Streptomyces spp.</i>
β -Iononones	Carotenoids	<i>Phycomyces blakesleeanus</i>
α -Amino butyric acid	L-Isoleuchine	<i>Bacillus subtilis</i>
D-Threonine	L-Isoleuchine	<i>Serratia marcescens</i>

Media for Industrial Fermentations

Precursors used in fermentation processes (Cont..)

Precursor	Product	Microorganism
Anthranilic acid	L-Tryptophan	<i>Hansenula anomala</i>
Nucleosides and bases	Nikkomycins	<i>Streptomyces tendae</i>
Dihydronovobionic acid	Dihydronovobiocin	<i>Streptomyces sp.</i>
<i>p</i> -Hydroxycinnamate	Organomycin A and B	<i>Streptomyces organonensis</i>
DL- α -Amino butyric acid	Cyclosporin A	<i>Tolypocladium inflatum</i>
L-Threonine	Cyclosporin c	
Tyrosine or <i>p</i> -hydroxy-phenylglycine	Dimethylvancomycin	<i>Nocardia orientalis</i>

Media for Industrial Fermentations

Having established that the **activity of penicillin lay in the side chain, and that the limiting factor was the synthesis of the side chain**, it became standard practice to add side-chain precursors to the medium, in particular phenylacetic acid.

END

Advances in Fermentation Technology

**Media for
Industrial
Fermentations:
INHIBITORS**

Media for Industrial Fermentations

When certain inhibitors are added to fermentations, more of a specific product may be produced, or a metabolic intermediate which is normally metabolized is accumulated. One of the earliest examples is the microbial production of glycerol. Glycerol production depends on modifying the ethanol fermentation by removing acetaldehyde. The addition of sodium bisulphite to the broth leads to the formation of the acetaldehyde bisulphite addition compound (sodium hydroxy ethyl sulphite). Since acetaldehyde is no longer available for re-oxidation of NADH_2 , its place as hydrogen acceptor is taken by dihydroacetone phosphate, produced during glycolysis. The product of this reaction is glycerol-3-phosphate, which is converted to glycerol.

Media for Industrial Fermentations

The application of general and specific inhibitors are illustrated in Table (in next slide). In most cases the inhibitor is effective in increasing the yield of the desired product and reducing the yield of undesirable related products.

A number of studies have been made with potential chlorination inhibitors, e.g. **bromide**, to minimize chlortetracycline production during a tetracycline fermentation.

Media for Industrial Fermentations

Specific and General Inhibitors used in fermentations

Product	Inhibitor	Main effect	Micro-organism
Glycerol	Sodium bisulphate	Acetaldehyde production repressed	<i>Saccharomyces cerivisiae</i>
Tetracycline	Bromide	Chlortetracycline formation repressed	<i>Streptomyces aureofaciens</i>
Glutamic acid	Penicillin	Dell wall permeability	<i>Micrococcus glutamicus</i>
Citric acid	Alkali metal/ phosphate, pH below 2.0	Oxalic acid repressed	<i>Aspergillus niger</i>
Valine	Various inhibitors	Various effects with different inhibitors	<i>Brevibacterium roseum</i>
Rifamycin B	Di-ethyl barbiturate	Other rifamycins inhibited	<i>Nocardia mediterranel</i>

Media for Industrial Fermentations

Inhibitors have also been used to affect cell-wall structure and increase the permeability for release of metabolites. **The best example is the use of penicillin and surfactants in glutamic acid production.**

END

Advances in Fermentation Technology

**Media for
Industrial
Fermentations:
INDUCERS-1**

Media for Industrial Fermentations

The majority of enzymes which are of industrial interest are inducible. Induced enzymes are synthesized only in response to the presence in the environment of an inducer. Inducers are often substrates such as starch or dextrans for amylases, maltose for pullulanase and pectin for pectinases.

Some inducers are very potent, such as isovaleronitrile inducing nitrilase. Substrate analogues that are not attacked by the enzyme may also serve as enzyme inducers. Examples of industrially important enzyme inducers are shown in Table given in next slide.

Media for Industrial Fermentations

Some examples of industrially important enzyme inducers

Enzyme	Inducer	Micro-organism
α-Amylase	Starch Maltose	<i>Aspergillus spp.</i> <i>Bacillus subtilis</i>
Pullulanase	Maltose	<i>Aerobacter aerogenes</i>
α-Mannosidase	Yeast mannans	<i>Streptomyces griseus</i>
Penicillin acylase	Phenylacetic acid	<i>Escherichia coli</i>
Proteases	Various proteins	<i>Bacillus spp.</i> <i>Streptococcus spp.</i> <i>Streptomyces spp.</i> <i>Aspergillus spp.</i>
Cellulase	Cellulose	<i>Trichoderma viridae</i>
Pectinases	Pectin (beet pulp, apple pomace, citrus peel)	<i>Aspergillus spp.</i>
Nitralase	Isovaleronitrile	<i>Rhodococcus rhodochrous</i>

Media for Industrial Fermentations

One unusual application of an inducer is the use of yeast mannan in streptomycin production. During the fermentation varying amounts of streptomycin and mannosidostreptomycin are produced.

Since mannosidostreptomycin has only 20% of the biological activity of streptomycin, the former is an undesirable product.

The production organism *Streptomyces griseus* can be induced by yeast mannan to produce β -mannosidase which will convert mannosidostreptomycin to streptomycin.

Media for Industrial Fermentations

Most inducers which are included in microbial enzyme media are substrates or substrate analogues, but intermediates and products may sometimes be used as inducers. **For example, maltodextrins will induce amylase and fatty acids induce lipase. However, the cost may prohibit their use as inducers in a commercial process.**

END

Media for Industrial Fermentations

The types of antifoams mentioned in previous slides are generally added when foaming occurs during the fermentation. **Because many antifoams are of low solubility** they need a carrier such as lard oil, liquid paraffin or castor oil, which may be metabolized and affect the fermentation process.

END

Advances in Fermentation Technology

**Media for
Industrial
Fermentations:
INDUCERS-2**

Media for Industrial Fermentations

It is now possible to produce a number of heterologous proteins in yeasts, fungi and bacteria. These include proteins of viral, human, animal, plant and microbial origin. However, heterologous proteins may show some degree of toxicity to the host and have a major influence on the stability of heterologous protein expression. As well as restricting cell growth as biomass the toxicity will provide selective conditions for segregant cells which no longer synthesize the protein at such a high level.

Media for Industrial Fermentations

Therefore, optimum growth conditions may be achieved by not synthesizing a heterologous protein continuously and only inducing it after the host culture has grown up in a vessel to produce sufficient biomass. In cells of *Saccharomyces cerevisiae* where the *Gal1* promoter is part of the gene expression system, product formation may be **induced by galactose** addition to the growth medium which contains glycerol or low non-repressing levels of glucose as a carbon source.

Methylotrophic yeasts such as *Hansenula polymorpha* and *Pichia pastoris* may be used as alternative systems because of the presence of an alcohol oxidase promoter.

Media for Industrial Fermentations

During growth on **methanol, which also acts as an inducer**, the promoter is induced to produce about 30% of the cell protein. In the presence of glucose or ethanol it is undetectable.

END

Advances in Fermentation Technology

**Media for
Industrial
Fermentations:
OXYGEN
REQUIREMENTS,
TRACE ELEMENTS,
pH and OSMOLALITY**

Media for Industrial Fermentations

It is sometimes forgotten that oxygen, although not added to an initial medium as such, is nevertheless a very important component of the medium in many processes, and its availability can be extremely important in controlling growth rate and metabolite production.

Media for Industrial Fermentations

- 1. *Fast metabolism.*** *The culture may become oxygen limited because sufficient oxygen cannot be made available in the fermenter if certain substrates, such as rapidly metabolized sugars which lead to a high oxygen demand, are available in high concentrations.*
- 2. *Rheology.*** The individual components of the medium can influence the viscosity of the final medium and its subsequent behaviour with respect to aeration and agitation.
- 3. *Antifoams.*** Many of the antifoams in use will act as surface active agents and reduce the transfer rate.

Media for Industrial Fermentations

□ *Trace Elements*

The role of trace elements in medium formulation can be significant. Cultured cells normally require **Fe, Zn, Cu, Se, Mn, Mo** and **V**. These are often present as impurities in other media components. Cleveland *et al.* (1983) found that if the number of trace elements were increased, insulin, transferrin, albumin and liposomes were not needed in a serum-free hybridoma medium. They included **Al, Ag, Ba, Br, Cd, Co, Cr, F, Ge, J, Rb, Zr, Si, Ni** and **Sn** as well as those previously mentioned.

Media for Industrial Fermentations

□ *pH*

The normal buffer system in tissue culture media is the CO₂-bicarbonate system. This is a weak buffering system and can be improved by the use of a **zwitterinic buffer** such as **Hepes**, either in addition to or instead of the **CO₂-bicarbonate buffer**. Continuous pH control is achieved by the addition of **sodium bicarbonate** or **sodium hydroxide** (with fast mixing) when too acid. The pH does not normally become too Alkaline so acid additions are not required but provision may be made for CO₂ additions.

Media for Industrial Fermentations

☐ *Osmolality*

The optimum range of osmotic pressure for growth is often quite narrow and varies with the type of cell and the species from which it was isolated. It maybe necessary to adjust the concentration of NaCl when major additions are made to a medium.

END

Advances in Fermentation Technology

**Media for
Industrial
Fermentations:
ANTIFOAMS-1**

Media for Industrial Fermentations

- In most microbiological processes, foaming is a problem may be due to a component in the medium or some factor produced by the micro-organism.
- The most common cause of foaming is due to proteins in the medium, such as corn-steep liquor, meal, soybean meal, yeast extract or meat extract.
- These proteins may denature at the air-broth interface and form a skin which does not rupture readily.
- The foaming can cause removal of cells from the medium which will lead to autolysis and the further release of microbial cell proteins will probably increase the stability of the foam.

Media for Industrial Fermentations

- If uncontrolled, then numerous changes may occur and physical and biological problems may be created. These include reduction in the working volume of the fermenter due to oxygen exhausted gas bubbles circulating in the system changes in bubble size, lower mass and heat transfer rates, invalid process data due to interference at sensing electrodes and incorrect monitoring and control.
- The biological problems include deposition of cells in upper parts of the fermenter, problems of sterile operation with the air filter exits of the fermenter becoming wet, and there is danger of microbial infection and the possibility of siphoning leading to loss of product.

Media for Industrial Fermentations

Hall *et al.* (1973) have recognized five patterns of foaming in fermentations:

1. Foaming remains at a constant level throughout the fermentation. Initially it is due to the medium and later due to microbial activity.
2. A steady fall in foaming during the early part of the fermentation, after which it remains constant. Initially it is due to the medium but there are no later effects caused by the micro-organism.
3. The foaming falls slightly in the early stages of the fermentation then rises. There are very slight effects caused by the medium but the major effects are due to microbial activity.
4. The fermentation has a low initial foaming capacity which rises. These effects are due solely to microbial activity.

Media for Industrial Fermentations

5. A more complex foaming pattern during the fermentation which may be a combination of two or more of the previously described patterns.

END

Advances in Fermentation Technology

**Media for
Industrial
Fermentations:
ANTIFOAMS-2**

Media for Industrial Fermentations

If excessive foaming is encountered there are three ways of approaching the problem:

1. To try and avoid foam formation by using a defined medium and a modification of some of the physical parameters (pH, temperature, aeration and agitation). This assumes that the foam is due to a component in the medium and not a metabolite.
2. The foam is unavoidable and antifoam should be used. This is the more standard approach.
3. To use a mechanical foam breaker.

Media for Industrial Fermentations

Antifoams are surface active agents, reducing the surface tension in the foams and destabilizing protein films by

- (a) hydrophobic bridges between two surfaces,
- (b) displacement of the absorbed protein, and
- (c) rapid spreading on the surface of the film.

Media for Industrial Fermentations

An ideal antifoam should have the following properties:

1. Should disperse readily and have fast action on an existing foam.
2. Should be active at low concentrations.
3. Should be long acting in preventing new foam formation.
4. Should not be metabolized by the microorganism.
5. Should be non-toxic to the micro-organism.
6. Should be non-toxic to humans and animals.
7. Should not cause any problems in the extraction and purification of the product.
8. Should not cause any handling hazards.
9. Should be cheap.

Media for Industrial Fermentations

10. Should have no effect on oxygen transfer.

11. Should be heat sterilizable.

END

Advances in Fermentation Technology

**Media for
Industrial
Fermentations:
ANTIFOAMS-3**

Media for Industrial Fermentations

The following compounds which meet most of ideal antifoam requirements (as discussed in previous module) have been found to be most suitable in different fermentation processes:

1. **Alcohols; stearyl and octyl decanol.**
2. **Esters.**
3. **Fatty acids and derivatives, particularly glycerides, which include cottonseed oil, linseed oil, soy-bean oil, olive oil, castor oil, sunflower oil, rapeseed oil and cod liver oil.**
4. **Silicones.**
5. **Sulphonates.**
6. **Miscellaneous; Alkaterge C, oxazoline, poly-propylene glycol.**

Media for Industrial Fermentations

Unfortunately, the concentrations of many antifoams which are necessary to control fermentations will reduce the oxygen-transfer rate by as much as 50%; therefore antifoam additions must be kept to an absolute minimum. There are also other antifoams which will increase the oxygen-transfer rate. If the oxygen-transfer rate is severely affected by antifoam addition then mechanical foam breakers may have to be considered as a possible alternative. Various studies concluded that foam control in industry is still an empirical art. The best method for a particular process in one factory is not necessarily the best for the same process on another site. The design and operating parameters of a fermenter may affect the properties and quantity of foam formed.

Media for Industrial Fermentations

The types of antifoams mentioned in previous slides are generally added when foaming occurs during the fermentation. **Because many antifoams are of low solubility** they need a carrier such as lard oil, liquid paraffin or castor oil, which may be metabolized and affect the fermentation process.

END

Advances in Fermentation Technology

**Media for
Industrial
Fermentations:
MEDIUM
OPTIMIZATION**

Media for Industrial Fermentations

When considering the biomass growth phase in isolation it must be recognized that efficiently grown biomass produced by an 'optimized' high productivity growth phase is not necessarily best suited for its ultimate purpose, such as synthesizing the desired product.

Different combinations and sequences of process conditions need to be investigated to determine the growth conditions which produce the biomass with the physiological state best constituted for product formation. There may be a sequence of phases each with a specific set of optimal conditions.

Media for Industrial Fermentations

Medium optimization by the classical method of changing one independent variable (nutrient, antifoam, pH, temperature, etc.) while fixing all the others at a certain level can be extremely time consuming and expensive for a large number of variables.

Media for Industrial Fermentations

To make a full factorial search which would examine each possible combination of independent variable at appropriate levels could require a large number of x^n experiments, where

x is the number of levels and

n is the number of variables.

This may be quite appropriate for three nutrients at two concentrations ($2^3 = 8$ trials) but not for

six nutrients at three concentrations ($3^6 = 729$)

Industrially the aim is to perform the minimum number of experiments to determine optimal conditions.

Media for Industrial Fermentations

Other alternative strategies can therefore be considered which allow more than one variable to be changed at a time.

END

Advances in Fermentation Technology

**Media for
Industrial
Fermentations:
MEDIUM
OPTIMIZATION-
Plackett-Burman Design-1**

Media for Industrial Fermentations

When more than five independent variables are to be investigated, the Plackett-Burman design may be used to find the most important variables in a system, which are then optimized in further studies (Plackett and Burman, 1946). These authors give a series of designs for up to one hundred experiments using an experimental rationale known as balanced incomplete blocks. This technique allows for the evaluation of $X-1$ variables by X experiments. X must be a multiple of 4, e.g. 8, 12, 16, 20, 24, etc.

Media for Industrial Fermentations

Normally one determines how many experimental variables need to be included in an investigation and then selects the Plackett-Burman design which meets that requirement most closely in multiples of 4.

Any factors not assigned to a variable can be designated as a dummy variable.

Alternatively, factors known to not have any effect may be included and designated as **dummy variables**. Table in the next slide shows a Plackett-Burman design for **seven variables (A -G)** at high and low levels in which **two factors, E and G**, are designated as '**dummy**' variables.

Media for Industrial Fermentations

TABLE *Plackett–Burman design for seven variables*

Trial	Variables							Yield
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>	<i>G</i>	
1	H	H	H	L	H	L	H	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

H denotes a high level value; L denotes a low level value.

Each horizontal row represents a trial and each vertical column represents the H (high) and L (low) values of one variable in all the trials.

Media for Industrial Fermentations

Consider the variable **A**; for the trials in which **A** is high, **B** is high in two of the trials and low in the other two. Similarly, **C** will be high in two trials and low in two, as will all the remaining variables.

For those trials in which **A** is low, **B** will be high two times and low two times. This will also apply to all the other variables.

Thus, the effects of changing the other variables cancel out when determining the effect of **A**. The same logic then applies to each variable.

However, no changes are made to the high and low values for the **E** and **G** columns.

Media for Industrial Fermentations

The effects of the dummy variables are calculated in the same way as the effects of the experimental variables.

If there are **no interactions** and no errors in measuring the response, the **effect shown by a dummy variable should be 0**. If the effect is not equal to 0, it is assumed to be a measure of the lack of experimental precision plus any analytical error in measuring the response. This procedure will identify the important variables and allow them to be ranked in order of importance to decide which to investigate in a more detailed study to determine the optimum values to use.

Media for Industrial Fermentations

This procedure will identify the important variables and allow them to be ranked in order of importance to decide which to investigate in a more detailed study to determine the optimum values to use.

END

Advances in Fermentation Technology

**Media for
Industrial
Fermentations:
MEDIUM
OPTIMIZATION-
Plackett-Burman Design-2**

Media for Industrial Fermentations

The stages in analyzing the data (mentioned in Table in previous module; and in the Table given in next slide) using Nelson's (1982) example are as follows:

1. Determine the difference between the average of the **H (high)** and **L (low)** responses for each independent and dummy variable. Therefore the difference =

$$\Sigma A (H) - \Sigma A (L)$$

Media for Industrial Fermentations

TABLE *Plackett–Burman design for seven variables*

Trial	Variables							Yield
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>	<i>G</i>	
1	H	H	H	L	H	L	H	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

H denotes a high level value; L denotes a low level value.

Each horizontal row represents a trial and each vertical column represents the H (high) and L (low) values of one variable in all the trials.

Media for Industrial Fermentations

Analysis of the Yields

	Factor						
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>	<i>G</i>
$\Sigma(H)$	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	-11.0	10.2	0.2	0.0	-0.6	9.2	-0.4
Effect	-2.75	2.55	0.05	0.00	-0.15	2.30	-0.10
Mean square	15.125	13.005	0.005	0.000	0.045	10.580	0.020

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

Media for Industrial Fermentations

The effect of an independent variable on the response is the difference between the average response for the four experiments at the high level and the average value for four experiments at the low level. Thus the effect of

$$A = \frac{\sum A(H)}{4} - \frac{\sum A(L)}{4}$$
$$= \frac{2(\sum A(H) - \sum A(L))}{8}$$

This value should be near zero for the dummy variables.

Media for Industrial Fermentations

2. Estimate the mean square of each variable (the variance of effect). For A the mean square will be =
$$\frac{(\sum A(H) - \sum A(L))^2}{8}$$

3. The experimental error can be calculated by averaging the mean squares of the dummy effects of *E* and *G*. Thus, the mean square for error =
$$\frac{0.045 + 0.020}{2} = 0.0325$$

This experimental error is not significant.

Media for Industrial Fermentations

4. The final stage is to identify the factors which are showing large effects. In the example this was done using an *F*-test for

$$\frac{\text{Factor mean square.}}{\text{error mean square.}}$$

This gives the following values:

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

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

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

$$D = \frac{0.0000}{0.0325} = 0.00$$

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

Media for Industrial Fermentations

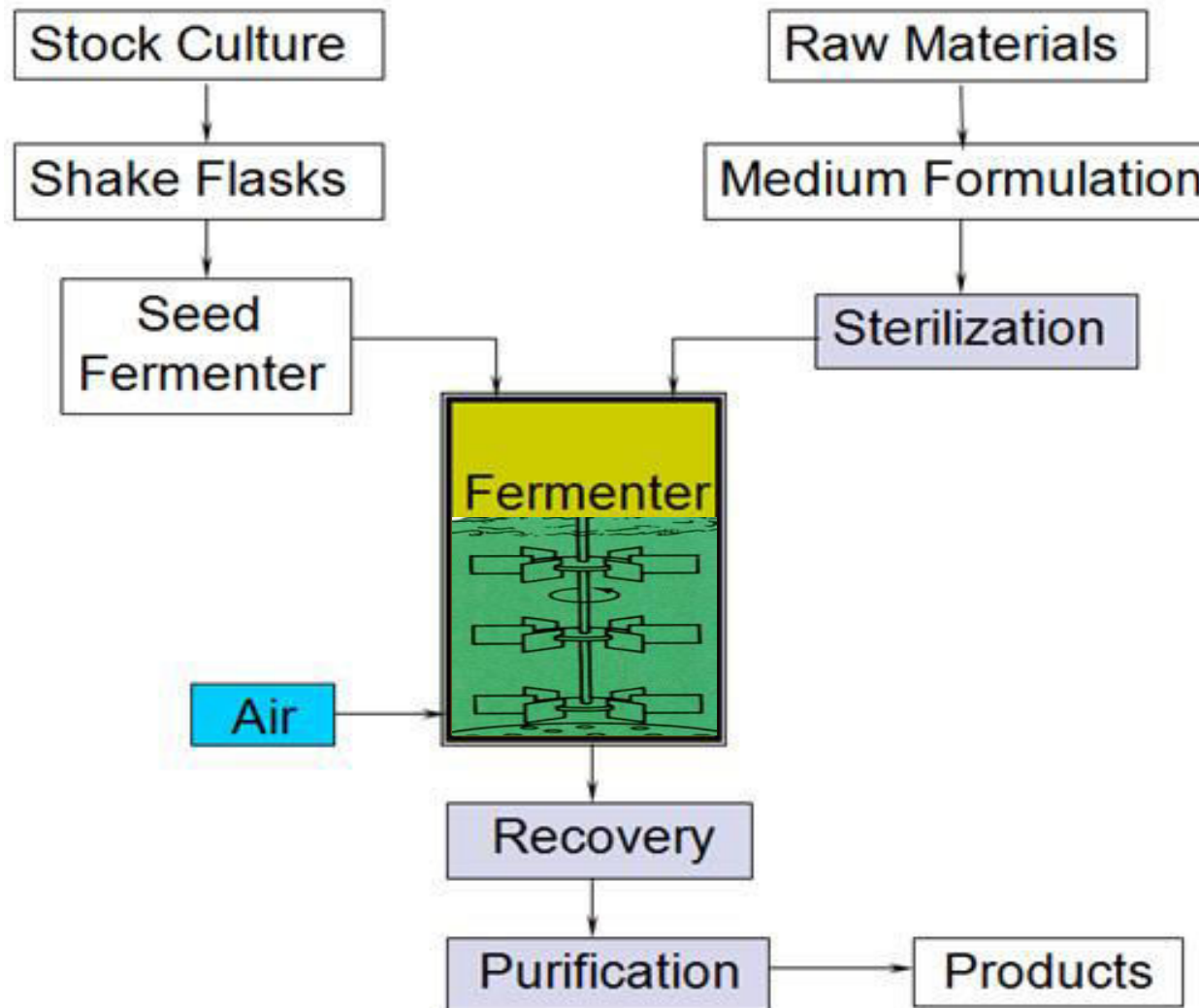
When Probability Tables are examined it is found that Factors **A**, **B** and **F** show large effects which are very significant, whereas **C** shows a very low effect which is not significant and **D** shows no effect. **A**, **B** and **F** have been identified as the most important factors.

END

Advances in Fermentation Technology

Aeration & Agitation: Introduction-1

Aeration & Agitation



Aeration & Agitation

The majority of fermentation processes are aerobic and, therefore, require the provision of oxygen. If the stoichiometry of respiration is considered, then the oxidation of glucose may be represented as:



1 mole	+	6mole
180g		192g

However, both components must be in solution before they are available to a micro-organism and oxygen is approximately **6000 times** less soluble in water than is glucose.

Aeration & Agitation

A fermentation medium saturated with oxygen contains approximately

7.6 mg dm⁻³ of oxygen at 30°C

Thus, it is not possible to provide a microbial culture with all the oxygen it will need for the complete oxidation of the glucose (or any other carbon source) in one addition.

Therefore, a microbial culture must be supplied with oxygen during growth at a rate sufficient to satisfy the organisms' demand.

Aeration & Agitation

The oxygen demand of an industrial fermentation process is normally satisfied by **aerating and agitating** the fermentation broth.

END

Advances in Fermentation Technology

Aeration & Agitation: Introduction-2

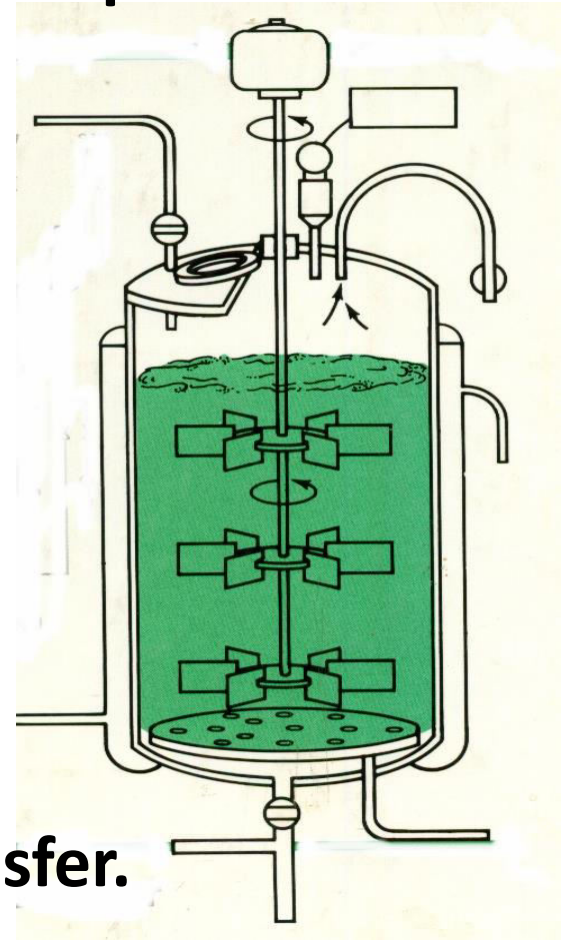
Aeration & Agitation

Important factor in a fermenters is provision for adequate mixing of its contents.

Mixing in fermentation

- **to disperse the air bubbles**
- **to suspend the cells**
- **to enhance heat and mass transfer in the medium**

All relate to Gas-liquid mass transfer.



Aeration & Agitation

Aeration refers to the process of introducing air to increase oxygen concentration in liquids. Aeration may be performed by bubbling air through the liquid, spraying the liquid into the air or agitation of the liquid to increase surface absorption.

Supplying oxygen to aerobic cells is a significant challenge.

The problem is that oxygen is poorly soluble in water . The solubility of oxygen in pure water is 8 mg/L at 4°C. The solubility of oxygen decreases as with increasing temperature and concentration of solutes in the solution.

Aeration & Agitation

- Supply of oxygen is the rate limiting step in an aerobic fermentation
- Satisfy oxygen demands constitute a large proportion of the operating and capital cost of a industrial scale fermentation system.

END

Advances in Fermentation Technology

**Aeration
&**

Agitation:

**Oxygen Requirements
of
Industrial
Fermentations-1**

Aeration & Agitation

Although a consideration of the stoichiometry of respiration gives an appreciation of the problem of oxygen supply, it gives no indication of an organism's true oxygen demand as it does not take into account the carbon that is converted into biomass and products.

A number of workers have considered the overall stoichiometry of the conversion of oxygen, a source of carbon and a source of nitrogen into biomass and have used such relationships to predict the oxygen demand of a fermentation.

Aeration & Agitation

Stoichiometric equations describing oxygen demand in a fermentation



Terms used

$\text{C}_{3.92}\text{H}_{6.5}\text{O}_{1.94}$ is 100 g (dry weight) of yeast cells; CH_2O is carbohydrate

CH_2 is hydrocarbon

Reference: Darlington (1964)

Aeration & Agitation

Stoichiometric equations describing oxygen demand in a fermentation (Cont.....)

$$C = (A/Y) - B$$

A = Amount of oxygen for combustion of 1 g of substrate to CO_2 , H_2O and NH_3 , if nitrogen is present in the substrate

B = Amount of oxygen required for the combustion of 1 g cells to CO_2 , H_2O and NH_3

Y = Cell yield (g cells g^{-1} substrate)

C = g oxygen consumed for the production of 1 g of cells

Reference: Johnson (1964)

Aeration & Agitation

Stoichiometric equations describing oxygen demand in a fermentation (Cont.....)

$$(dx/dt)/Y + mx + p = (dCO_2/dt) = -(dO_2/dt)RQ$$

x = biomass concentration

t = time

Y = g biomass g⁻¹ carbon substrate

m = maintenance

p = allowance for antibiotic production

Reference: Righelato et al., (1968)

Aeration & Agitation

Stoichiometric equations describing oxygen demand in a fermentation (Cont.....)

$$Y_{O/P} = (0.53/Y_{P/G}) - (0.6 X/P) - 0.43$$

$Y_{O/P}$ = g oxygen consumed g^{-1} glucose

$Y_{P/G}$ = g sodium penicillin G produced g^{-1} glucose

X = g cells (dry weight) produced

P = g sodium penicillin G produced

Reference: Cooney (1979)

Aeration & Agitation

**Stoichiometric equations describing oxygen demand
in a fermentation (Cont.....)**

$$Y_O = \{(32C + 8H - 16O)/YM\} - 1.58$$

Y_O = g oxygen consumed g^{-1} cells produced

Y = Cell yield (g cells g^{-1} substrate)

M = Molecular weight of the carbon source

C, H and O = Number of atoms of carbon,
hydrogen and oxygen per molecule of carbon
source

Reference: Mateles (1971)

Aeration & Agitation

From Darlington's and Johnson's equations (in above mentioned Table) it may be seen that the **production of 100 grams of biomass from hydrocarbon requires approximately three times the amount of oxygen to produce the same amount of biomass from carbohydrate.**

END

Advances in Fermentation Technology

**Aeration
&**

Agitation:

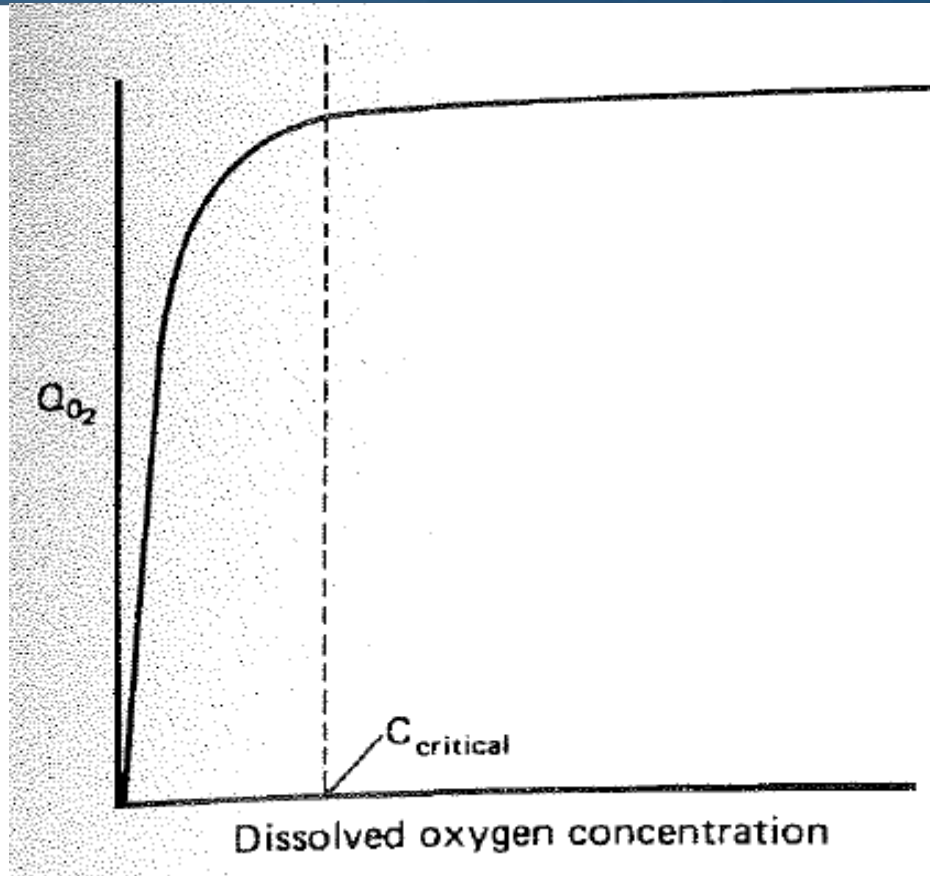
**Oxygen Requirements
of
Industrial
Fermentations-2**

Aeration & Agitation

It must be remembered that the high carbon content of hydrocarbon substrates means that high yield factors (g biomass g^{-1} substrate consumed) are obtained and the decision to use such substrates is based on the balance between the advantage of high biomass yield and the disadvantage of high oxygen demand and heat generation.

However, it is inadequate to base the provision of oxygen for a fermentation simply on an estimation of overall demand, because the metabolism of the culture is affected by the concentration of dissolved oxygen in the broth. The effect of dissolved oxygen concentration on the specific oxygen uptake rate (Q_{O_2} mmoles of oxygen consumed per gram dry weight of cells per hour) has been shown to be of the **Michaelis-Menten type**, as shown in Fig. (in next slide).

Aeration & Agitation



The effect of dissolved oxygen concentration on the Q_{O_2} of a microorganism

It may be seen that the specific oxygen uptake rate increases with increase in the dissolved oxygen concentration up to a certain point (referred to as $C_{critical}$) above which no further increase in oxygen uptake rate occurs.

Aeration & Agitation

Critical dissolved oxygen concentrations for a range of microorganisms

Organism	Temperature	Critical dissolved oxygen concentration (mmoles dm ⁻³)
<i>Azotobacter</i> sp.	30	0.018
<i>Escherichia coli</i>	37	0.008
<i>Saccharomyces</i> sp.	30	0.004
<i>Penicillium chrysogenum</i>	24	0.022

Aeration & Agitation

Oxygen requirements of a range of microorganisms grown on a range of substrates

Substrate	Organism	Oxygen requirement (g O ₂ g ⁻¹ dry wt.)
Glucose	<i>Escherichia coli</i>	0.4
Methanol	<i>Pseudomonas C</i>	1.2
Octane	<i>Pseudomonas</i> sp.	1.7

Aeration & Agitation

Thus, maximum biomass production may be achieved by satisfying the organism's maximum specific oxygen demand by maintaining the dissolved oxygen concentration greater than the critical level. *If the dissolved oxygen concentration were to fall below the critical level then the cells may be metabolically disturbed.*

END

Advances in Fermentation Technology

**Aeration
&
Agitation:
Henry's Law
and
Oxygen Supply**

Aeration & Agitation

Henry's Law describes the solubility of O₂ in nutrient solution in relation to the O₂ partial pressure in the gas phase

$$C^* = \frac{P_o}{H}$$

C* is the oxygen saturation concentration of the nutrient solution,

P_o is the partial pressure of the gas in the gas phase

H is Henry's constant, which is specific for the gas and the liquid phase.

Aeration with **air** 9 mg O₂/L dissolves in water, with **pure oxygen** 43 mg O₂/L.

Aeration & Agitation

❑ Oxygen Supply

Oxygen is normally supplied to microbial cultures in the form of air, this being the cheapest available source of the gas. The method for provision of a culture with a supply of air varies with the scale of the process:

- Laboratory-scale cultures may be aerated by means of the shake-flask technique where the culture (50 to 100 cm³) is grown in a conical flask (250 to 500 cm³) shaken on a platform contained in a controlled environment chamber.
- Pilot- and industrial-scale fermentations are normally carried out in stirred, aerated vessels (fermenters).

Aeration & Agitation

Bartholomew *et al.* (1950) represented the transfer of oxygen from air to the cell, during a fermentation, as occurring in a number of steps:

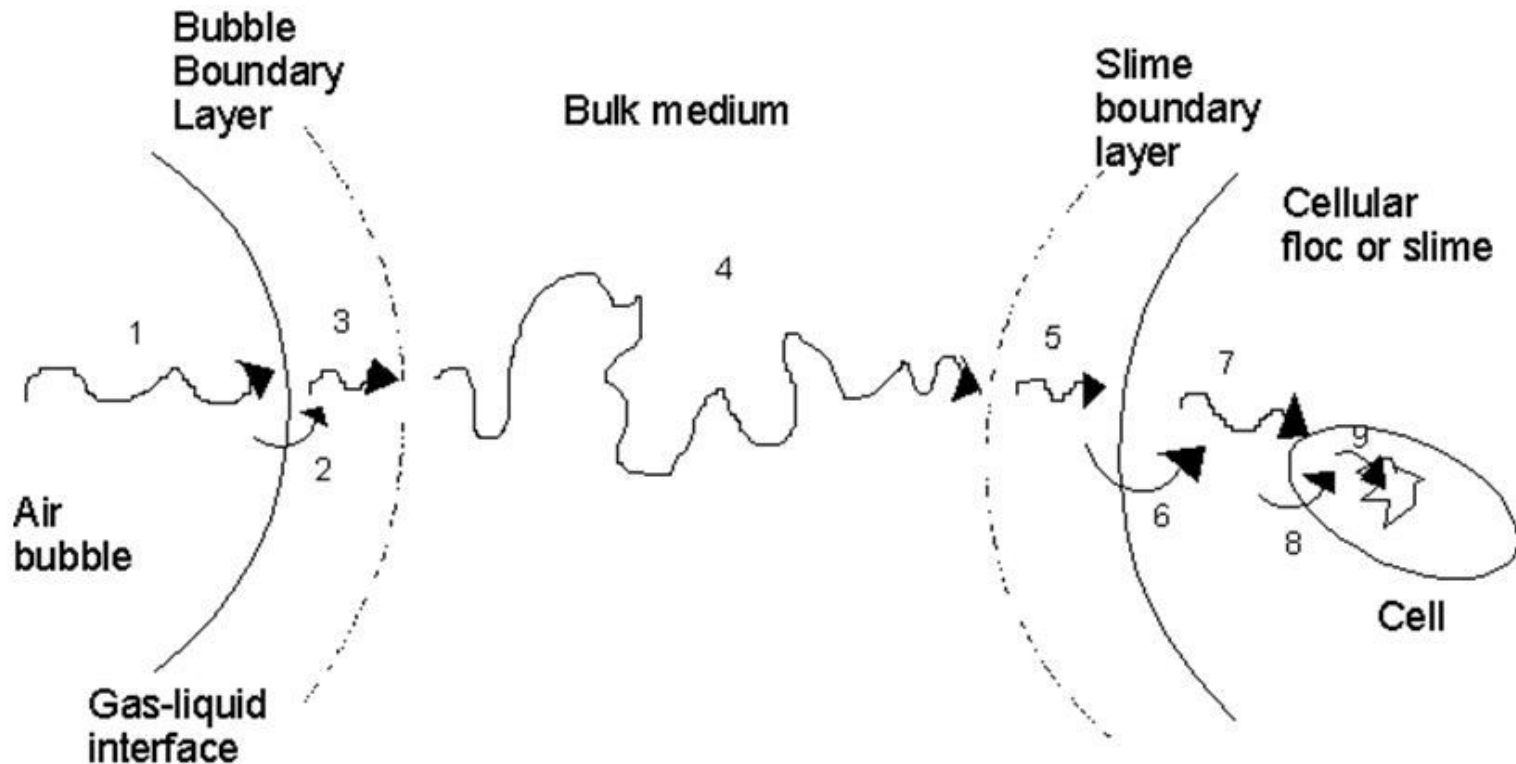
- i. The transfer of oxygen from an air bubble into solution.
- ii. The transfer of the dissolved oxygen through the fermentation medium to the microbial cell.
- iii. The uptake of the dissolved oxygen by the cell.

These findings have been shown to be correct for non-viscous fermentations but it has been demonstrated that transfer may be limited by some other stages in certain highly viscous fermentations.

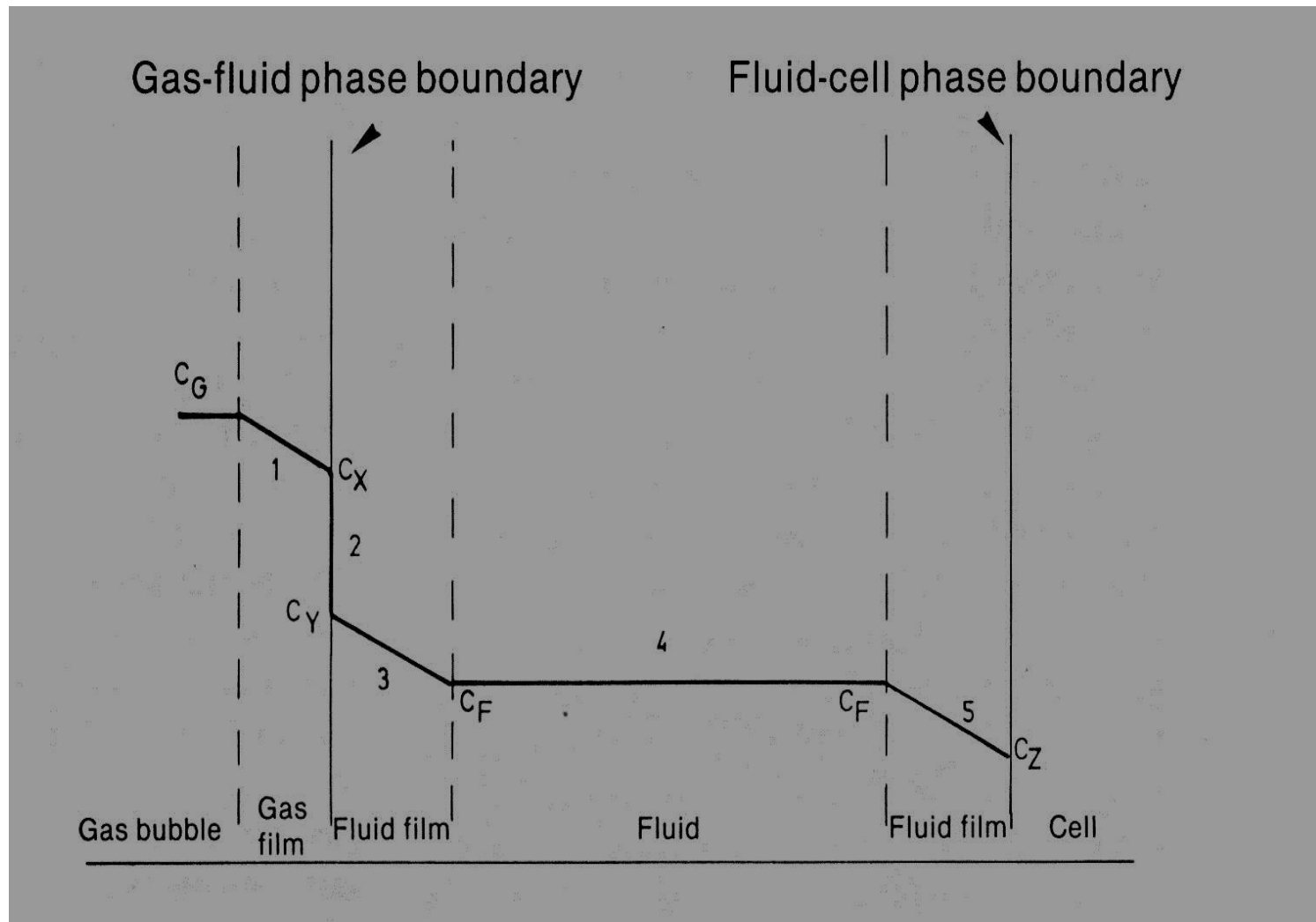
Aeration & Agitation

Oxygen Path

(From A Bubble To An Immobilized Cell System)



Aeration & Agitation



Aeration & Agitation

The requirement for a high dissolved oxygen concentration by many fermentations has resulted in the development of process techniques to ensure that the fermentation does not exceed the oxygen-supply capabilities of the fermentation vessel.

END

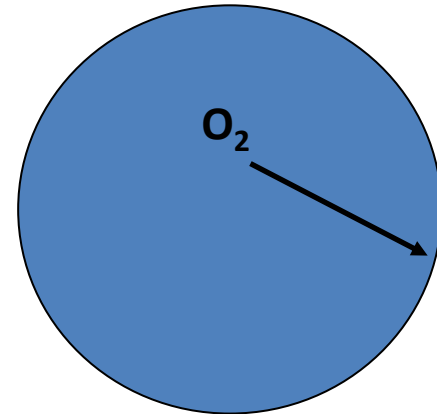
Advances in Fermentation Technology

Aeration & Agitation: Oxygen Transfer Process-1

Aeration & Agitation

Step 1

Diffusion through the bubble to the gas liquid interface. Gas molecules move quickly, they are evenly distributed throughout the bubble.



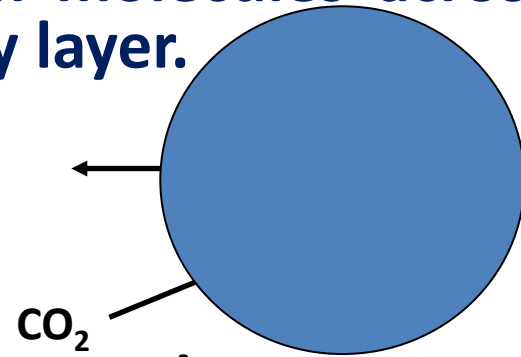
Aeration & Agitation

Step 2

Diffusion across the gas-liquid interface. This step will be very rapid if the concentration of oxygen in the bubble high. High oxygen concentrations in the bubble (as measured in terms of partial pressure) will push the oxygen molecules across the interface, into the boundary layer.

If the medium is rich in CO_2 , then the carbon dioxide will be pushed into the bubble.

The bubble contains a low concentration of oxygen, then the rate of oxygen transfer out of the bubble will be slow or even zero.



Aeration & Agitation

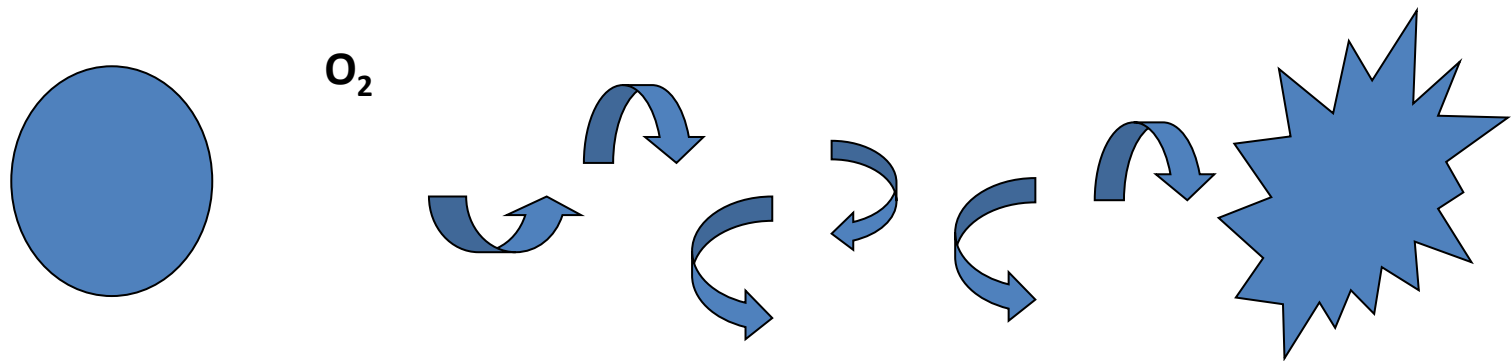
Step 3

Diffusion through the bubble boundary layer. The movement of solutes through the boundary layer is slow. Solutes move through the liquid by diffusion. The movement of the molecule will be driven by the concentration gradient across the boundary layer. Factors affect the rate of diffusion of oxygen through the boundary layer, including: temperature, concentration of oxygen in the bulk liquid, saturation concentration of oxygen in the liquid, concentration of oxygen in the bubble, size of the molecule and viscosity of the medium.

Aeration & Agitation

Step 4

Movement through the bulk liquid by forced convection and diffusion. The rate of movement of an oxygen molecule through the bulk liquid is dependent on the **degree of mixing** (relative to the volume of the reactor) and **viscosity of the medium**.



Aeration & Agitation

Step 5-9: Movement through the floc

Step 5 Movement through the boundary layer surrounding the microbial slime

Step 6 Entry into the slime

Step 7 Movement through the slime

Step 8 Movement across the cell membrane

Step 9 Reaction

Step 5 and Step 7 are slow processes.

Aeration & Agitation

If only, suspended cells are involved the level of mixing in the bulk liquid is sufficiently high;

Then, the rate limiting step in the oxygen transfer process is the movement of the oxygen molecules through the bubble boundary layer.

END

Advances in Fermentation Technology

**Aeration
&
Agitation:
Oxygen Transfer
Process-2**

Aeration & Agitation

The rate of oxygen transfer from air bubble to the liquid phase may be described by the equation:

$$dC_L/dt = K_L a (C^* - C_L)$$

where C_L is the concentration of dissolved oxygen in the fermentation broth (mmoles dm^{-3}),
 t is time (hours),
 dC_L/dt is the change in oxygen concentration over a time period, i.e. the oxygen-transfer rate (mmoles $\text{O}_2 \text{ dm}^{-3} \text{ h}^{-1}$),
 K_L is the mass transfer coefficient ($\text{cm} \text{ h}^{-1}$),
 a is the gas/liquid interface area per liquid volume ($\text{cm}^2 \text{ cm}^{-3}$),
 C^* is the saturated dissolved oxygen concentration (mmoles dm^{-3}).

Aeration & Agitation

It is extremely difficult to measure both ' K_L ' and ' a ' in a fermentation and, therefore, the two terms are generally combined in the term K_La , known as the **volumetric mass-transfer coefficient**. The units of K_La , are reciprocal time (h^{-1}).

The volumetric mass-transfer coefficient is used as a measure of the aeration capacity of a fermenter. **The larger the K_La , the higher the aeration capacity of the system.**

The K_La value will depend upon the design and operating conditions of the fermenter and will be affected by the variables such as aeration rate, **agitation rate** and **impeller design**.

Aeration & Agitation

Oxygen transfer rate (OTR) is the critical criterion in a fermentation and, as may be seen from equation in previous slide, it is affected by both K_La and dissolved oxygen concentration.

The dissolved oxygen concentration reflects the balance between the supply of dissolved oxygen by the fermenter and the oxygen demand of the organism. If the K_La of the fermenter is such that the oxygen demand of the organism cannot be met, the dissolved oxygen concentration will decrease below the critical level (C_{crit}).

If the K_La is such that the oxygen demand of the organism can be easily met the dissolved oxygen concentration will be greater than C_{crit} and may be as high as 70 to 80% of the saturation level.

Aeration & Agitation

Thus, the K_La of the fermenter must be such that the optimum oxygen concentration for product formation can be maintained in solution throughout the fermentation.

END

Advances in Fermentation Technology

**Aeration
&
Agitation:**
Determination of
 K_La values-
*Sulphite Oxidation
Technique*

Aeration & Agitation

Determination of the K_La of a fermenter is essential in order to establish its **aeration efficiency** and **to quantify the effects of operating variables on the provision of oxygen**. It is important to remember at this stage that dissolved oxygen is usually monitored using a dissolved oxygen electrode which records dissolved oxygen activity or dissolved oxygen tension (DOT) whilst the equations describing oxygen transfer are based on dissolved oxygen concentration.

The solubility of oxygen is affected by dissolved solutes so that pure water and a fermentation medium saturated with oxygen would have different dissolved oxygen concentrations yet have the same DOT, i.e. an oxygen electrode would record 100% for **both**. Thus, to translate DOT into concentration the solubility of oxygen in the fermentation medium must be known and this can present difficulties.

Aeration & Agitation

□ Sulphite Oxidation Technique

This technique does not require the measurement of dissolved oxygen concentrations but relies on the rate of conversion of a **0.5 M solution of sodium sulphite to sodium sulphate in the presence of a copper or cobalt catalyst:**



As oxygen enters solution it is immediately consumed in the oxidation of sulphite, so that the sulphite oxidation rate is equivalent to the oxygen-transfer rate. **Since the dissolved oxygen concentration, is zero then the $K_L a$ may then be calculated from the equation:**

$$\text{OTR} = K_L a \cdot C^*$$

(where OTR is the oxygen transfer rate)

Aeration & Agitation

The volumes of the thiosulphate titrations are plotted against sample time and the **oxygen transfer rate** may be calculated from the **slope of the graph**.

END

Advances in Fermentation Technology

**Aeration
&
Agitation:**
Determination of
 $K_L a$ values-
Gassing-out
Techniques-1

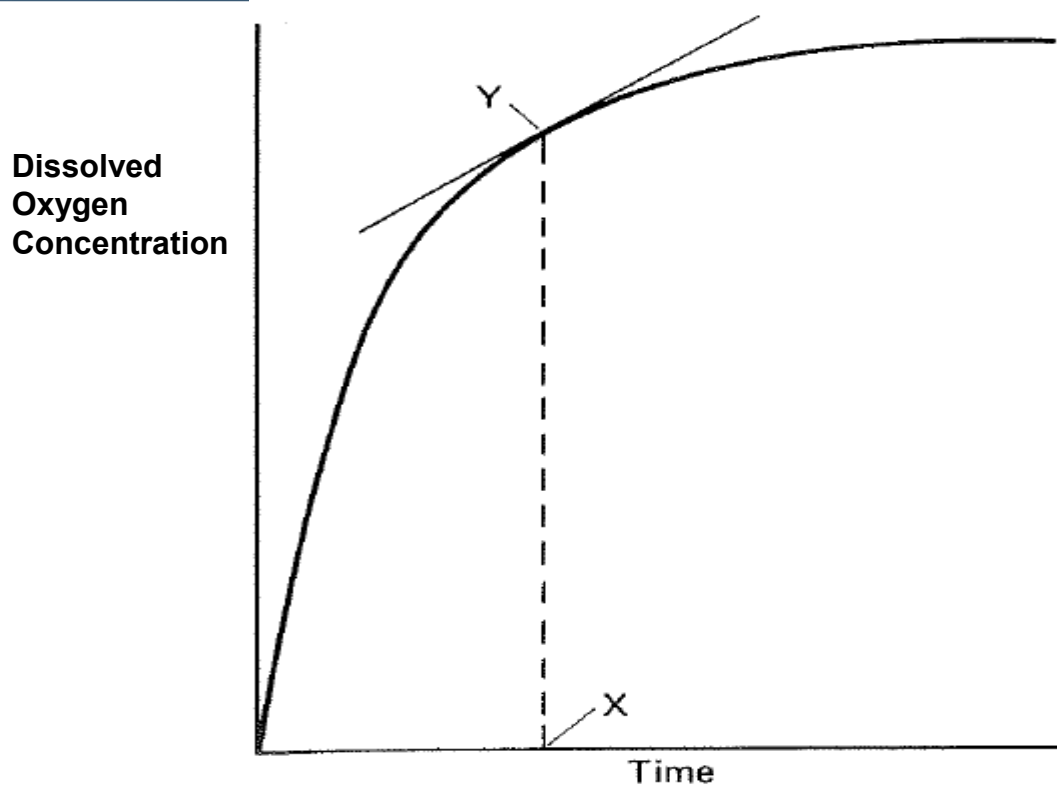
Aeration & Agitation

□ Gassing-out Techniques

The estimation of the $K_L a$ of a fermentation system by gassing-out techniques depends upon monitoring the increase in dissolved oxygen concentration of a solution during aeration and agitation.

The oxygen transfer rate will decrease during the period of aeration as C_L approaches C^* due to the decline in the driving force ($C^* - C_L$). The oxygen transfer rate, at anyone time, will be equal to the slope of the tangent to the curve of values of dissolved oxygen concentration against time of aeration, as shown in Figure on next slide.

Aeration & Agitation



The increase in dissolved oxygen concentration of a solution over a period of aeration. The oxygen transfer rate at time X is equal to the slope of the tangent at point Y.

Aeration & Agitation

To monitor the increase in dissolved oxygen over an adequate range it is necessary first to decrease the oxygen level to a low value.

Two methods have been employed to achieve this lowering of the dissolved oxygen concentration - **the static method** and **the dynamic method**.

Aeration & Agitation

i. Static Method of Gassing-out

In this technique, the **oxygen concentration** of the solution **is lowered by gassing the liquid out with nitrogen gas**, so that the solution is 'scrubbed' free of oxygen. The deoxygenated liquid is then aerated and agitated and the increase in dissolved oxygen monitored using some form of dissolved oxygen probe. The increase in dissolved oxygen concentration has already been described by equation:

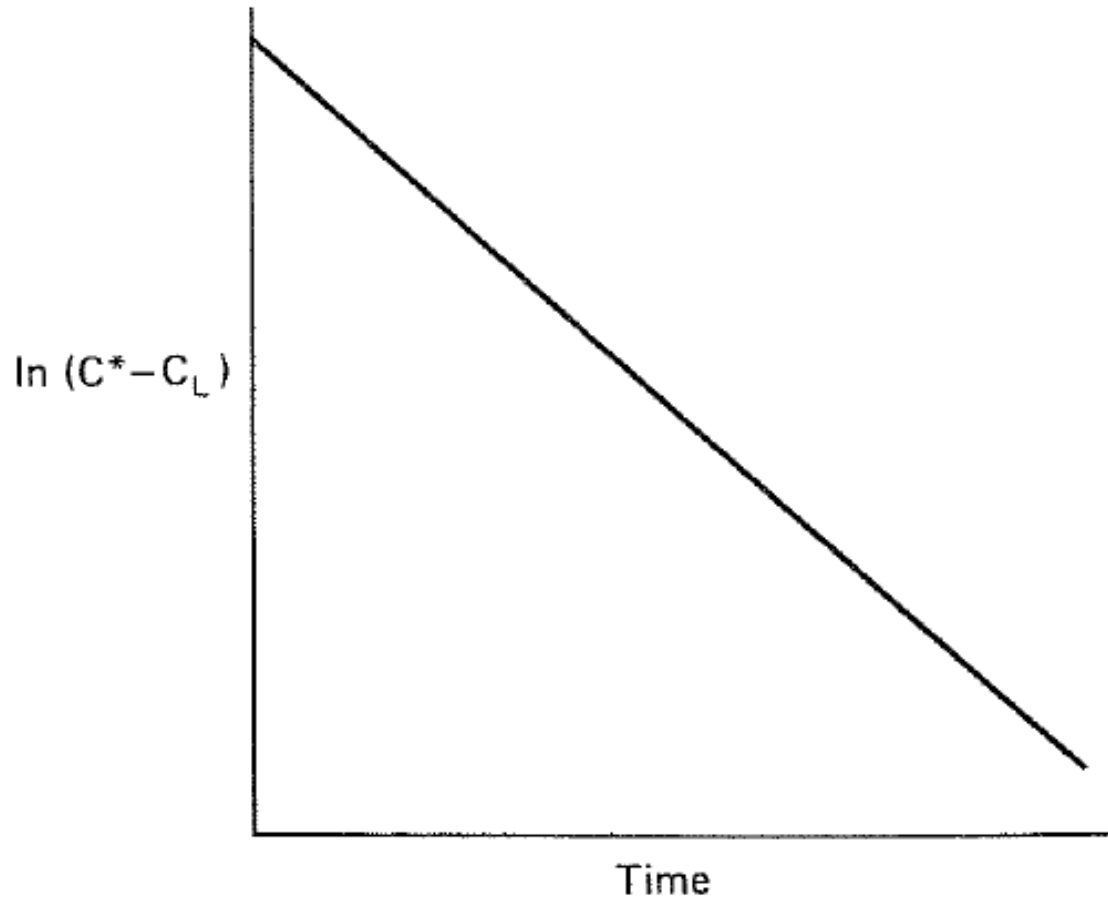
$$dC_L/dt = K_L a(C^* - C_L)$$

Integration of above equation yields:

$$\ln(C^* - C_L) = -K_L at.$$

Aeration & Agitation

Thus, a **plot of $\ln (C^* - C_L)$** against time will yield a straight line of slope $K_L a$, as shown in following figure:



Aeration & Agitation

This method is acceptable for small scale vessels, there are severe limitations to its use on large scale fermenters which have high gas residence times.

Aeration & Agitation

When the air supply to such a vessel is resumed after deoxygenation with nitrogen, the oxygen concentration in the gas phase may change with time as the nitrogen is replaced with air. Thus, C^* will no longer be constant.

END

Advances in Fermentation Technology

**Aeration
&
Agitation:**
Determination of
 $K_L a$ values-
Gassing-out
Techniques-2

Aeration & Agitation

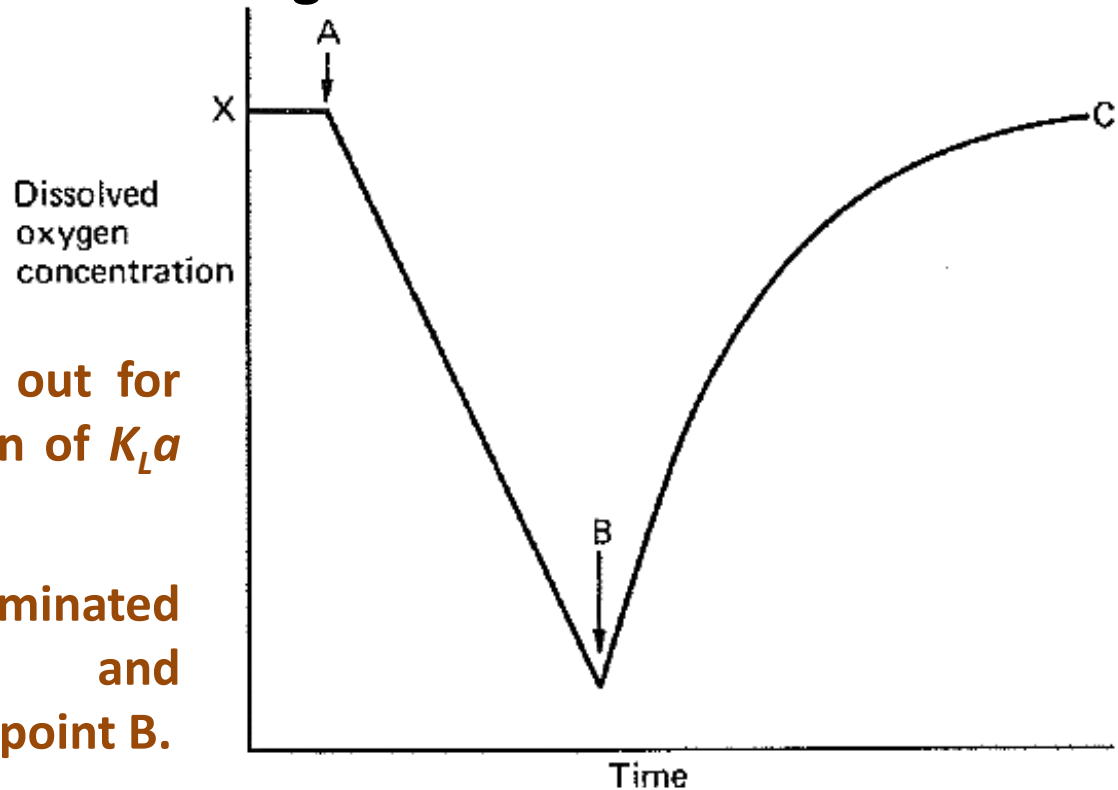
ii. Dynamic Method of Gassing-out

Respiratory activity of a growing culture in the fermenter is utilized to lower the oxygen level prior to aeration. Therefore, the estimation has the advantage of being carried out during a fermentation which should give a more realistic assessment of the fermenter's efficiency.

Because of the complex nature of fermentation broths the probe used to monitor the change in dissolved oxygen concentration must be of the membrane-covered type which may necessitate the use of the response-correction factors.

Aeration & Agitation

The procedure involves stopping the supply of air to the fermentation which results in a linear decline in the dissolved oxygen concentration due to the respiration of the culture, as shown in Figure:



Dynamic gassing out for the determination of $K_L a$ values.

Aeration was terminated at point A and recommenced at point B.

Aeration & Agitation

The slope of the line AB in above Figure a measure of the respiration rate of the culture. At point B the aeration is resumed and the dissolved oxygen concentration increases until it reaches concentration X. Over the period, BC, the observed increase in dissolved oxygen concentration is the difference between the transfer of oxygen into solution and the uptake of oxygen by the respiring culture as expressed by the equation:

$$dC_L/dt = K_L a(C^* - C_L) - xQ_{O_2}$$

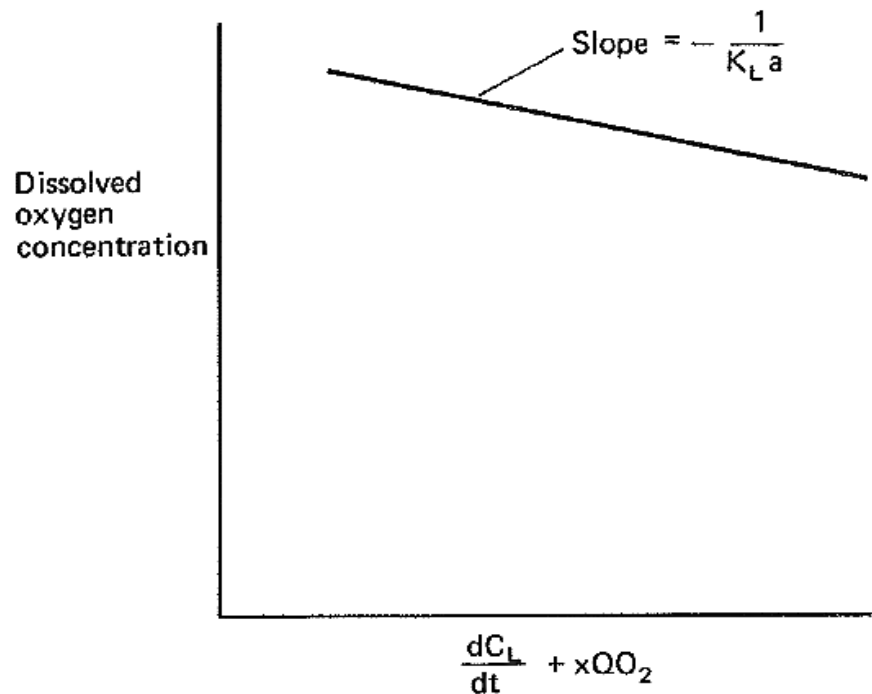
where x is the concentration of biomass and
 Q_{O_2} is the specific respiration rate (mmoles of oxygen g^{-1} biomass h^{-1}).

Aeration & Agitation

The term xQ_{O_2} is given by the slope of the line AB in previous referred Figure. Equation in previous slide may be rearranged as:

$$C_L = -1/K_L a \{ (dC_L/dt) + xQ_{O_2} \} + C^*$$

Now, from this equation, a plot of C_L versus $(dC_L/dt) + xQ_{O_2}$ will yield a straight line, the slope of which will equal $-1/K_L a$, as shown in Figure:



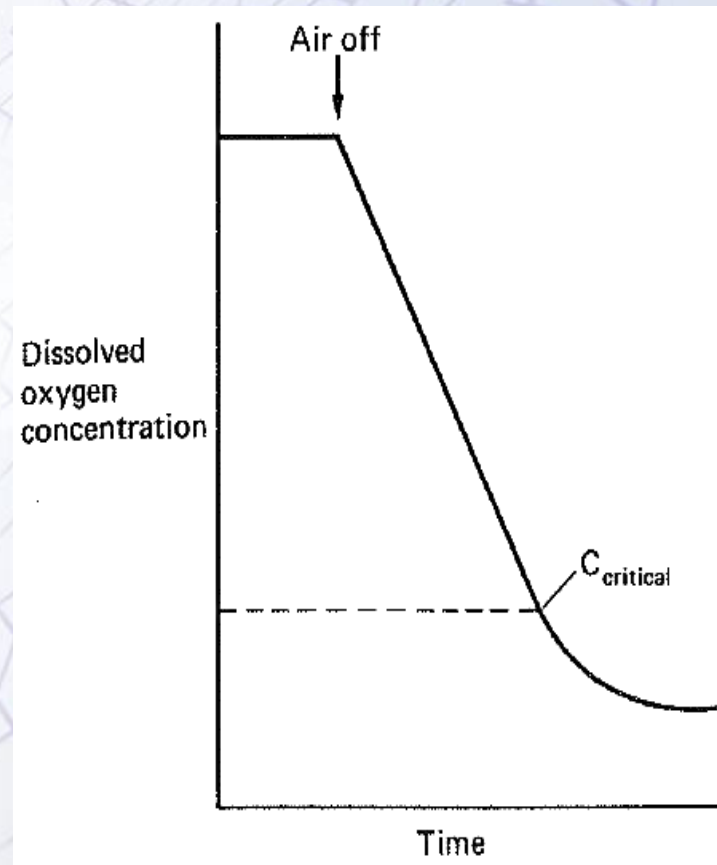
Aeration & Agitation

The dynamic gassing-out method has the advantage over the previous methods of determining the K_La during an actual fermentation and may be used to determine K_La values at different stages in the process. The technique is also rapid and only requires the use of a dissolved-oxygen probe, of the membrane type.

A major limitation in the operation of the technique is the range over which the increase in dissolved oxygen concentration may be measured. It may be difficult to apply the technique during a fermentation which has an oxygen demand close to the supply capacity of the fermenter.

Both the dynamic and static methods are also unsuitable for measuring K_La values in viscous systems.

Aeration & Agitation



The occurrence of oxygen limitation during the dynamic gassing out of a fermentation.

END

Advances in Fermentation Technology

**Aeration
&
Agitation:**
Determination of
 $K_L a$ values-
*The Oxygen Balance
Technique*

Aeration & Agitation

The K_La of a fermenter may be measured during a fermentation by the oxygen balance technique which determines, directly, the amount of oxygen transferred into solution in a set time interval. The procedure involves measuring the following parameters:

- i. The volume of the broth contained in the vessel, V_L (dm³).
- ii. The volumetric air flow rates measured at the air inlet and outlet, Q_i and Q_o respectively (dm³ min⁻¹).
- iii. The total pressure measured at the fermenter air inlet and outlet, P_i and P_o , respectively (atm. absolute).
- iv. The temperature of the gases at the inlet and outlet, T_i and T_o , respectively (K).
- v. The mole fraction of oxygen measured at the inlet and outlet, Y_i and Y_o respectively.

Aeration & Agitation

The oxygen transfer rate may then be determined from the following equation:

$$\text{OTR} = (7.32 \times 10^5 / V_L) (Q_i P_i y_i / T_i - Q_o P_o y_o / T_o)$$

Where, 7.32×10^5 is the conversion factor equaling $(60 \text{ min h}^{-1}) [\text{mole}/22.4 \text{ dm}^3 (\text{STP})] (273 \text{ K}/1 \text{ atm})$.

These measurements require accurate flow meters, pressure gauges and temperature-sensing devices as well as gaseous oxygen analyzers. The ideal gaseous oxygen analyzer is a mass spectrometer analyzer which is sufficiently accurate to detect changes of 1 to 2%. The $K_L a$ may be determined, provided that C_L and C^* are known, from equation

$$dC_L/dt = K_L a (C^* - C_L) :$$

$$\text{OTR} = K_L a (C^* - C_L) \text{ or } K_L a = \text{OTR} / (C^* - C_L)$$

Aeration & Agitation

The oxygen-balance technique appears to be the simplest method for the assessment of K_La and has the advantage of measuring aeration efficiency during a fermentation.

Aeration & Agitation

Factors affecting $K_L a$
the air-flow rate
employed in vessels,
—the degree of
agitation inside
vessels,
— the rheological
properties of the
culture broth and
— the presence of
antifoam agents

END

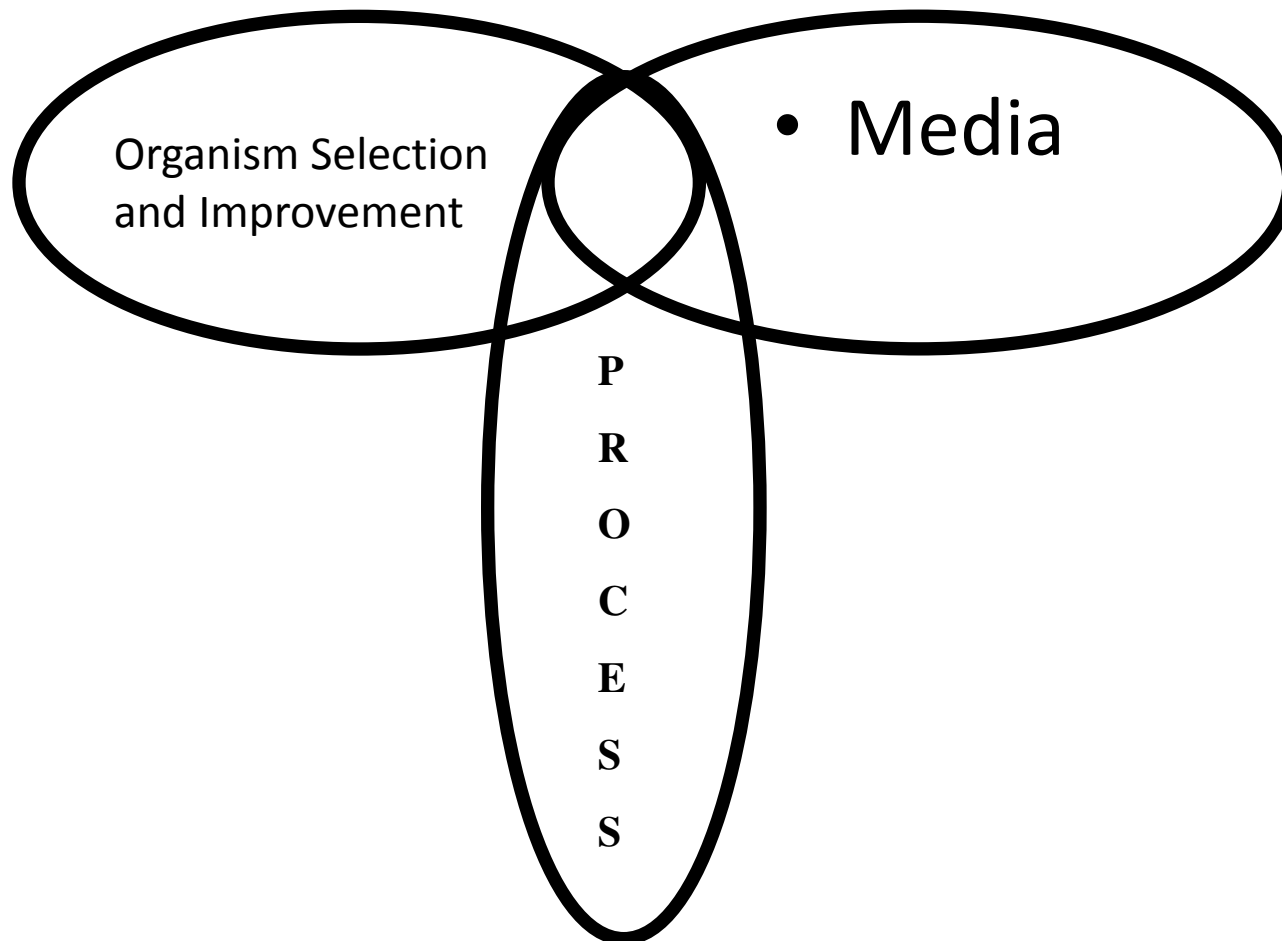
Advances in Fermentation Technology

**Process
Organism:**

Selection criteria

Process Organism

Overview



Process Organism

Microorganisms are ubiquitous in nature.

Thus, the diversity of micro-organisms may be exploited by searching for strains from the natural environment able to produce products of commercial value.

Process Organism

The selection of the culture to be used is a compromise between the **productivity** of the organism and the **economic constraints** of the process.

Process Organism

Bull *et al.* (1979) cited a criteria as being important in the choice of process organism:

- 1. The nutritional characteristics of the organism. It is frequently required that a process be carried out using a very cheap medium or a pre-determined one, e.g. the use of methanol as an energy source. These requirements may be met by the suitable design of the isolation medium.**
- 2. The optimum temperature of the organism. The use of an organism having an optimum temperature above 40° considerably reduces the cooling costs of a large-scale fermentation and, therefore, the use of such a temperature in the isolation procedure may be beneficial.**

Process Organism

3. The reaction of the organism with the equipment to be employed and the suitability of the organism to the type of process to be used.
4. The stability of the organism and its amenability to genetic manipulation.
5. The productivity of the organism, measured in its ability to convert substrate into product and to give a high yield of product per unit time.
6. The ease of product recovery from the culture.

Process Organism

The Question is?

How we get organism who meet the Bull *et al.*, (1979) criteria.

Answer will be:

Isolate from the nature or collect from the stock cultures.

Process Organism

The first stage in the screening for microorganisms of potential industrial application is their **isolation**. Isolation involves obtaining either pure or mixed cultures followed by their assessment to determine which carry out the desired reaction or produce the desired product.

Process Organism

In some cases it is possible to design the isolation procedure in such a way that the growth of producers is encouraged or that they may be recognized at the isolation stage, whereas in other cases, organisms must be isolated and producers recognized at a subsequent stage. However, it should be remembered that the isolate must eventually carry out the process economically.

Process Organism

However, before the process may be put into commercial operation the **toxicity** and **product potential** of the organism has to be assessed.

END

Advances in Fermentation Technology

**Process
Organism:**

**Collection
of
Industrially Important
Microorganisms-1**

Process Organism

As pertained to the discussion in previous module, it implies that cultures must be isolated, in some way, from natural environments.

but

It can be collected from culture banks/stocks.

Process Organism

List of Some of the Major Culture Collections

Culture Collection	Address
National Collection of Type cultures (NCTC)	PHLS Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, UK
National Collections of Industrial and Marine Bacteria Ltd. (NCIB, NCMB)	23 St Machar Drive, Aberdeen AB2 1RY, UK
National Collection of Yeast Cultures (NCYC)	AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA, UK
American Type Culture Collection (ATCC)	12301 Parklawn Drive, Rockville, MD 20852, USA

Process Organism

List of Some of the Major Culture Collections

Culture Collection	Address
Collection of International Mycological Institute (IMI)	Culture Collection and Industrial Service Division, Ferry Lane, Kew, Surrey TW9 3AF, UK
Culture Collection of the Institute for Fermentation (IFO)	Institute for Fermentation, 17-85 Jugo-Honchmachi, 2-chome, Yodogawa-ku, Osaka, Japan
Agricultural Research Service Culture Collection (ARS/NRRL)	ARS Culture Collection National Center for Agricultural Utilization Research 1815 N. University Street Peoria, IL 61604, USA

Process Organism

Most of the Culture collection facilities as mentioned in last slide provide pure prokaryotic and eukaryotic microbial cultures free of cost for research studies only. Some of them might charge shipping costs accordingly.

Such collections may provide organisms of known characteristics but may not contain those possessing the most desirable features, whereas the environment contains a myriad of organisms, very few of which may be satisfactory. It is certainly cheaper to buy a culture than to isolate from nature, but it is also true that a superior organism may be found after an exhaustive search of a range of natural environments.

Process Organism

However, it is always worthwhile to purchase cultures demonstrating the desired characteristics, however weakly, as they may be used as model systems to develop culture and assay techniques which may then be applied to the assessment of natural isolates.

END

Advances in Fermentation Technology

**Process
Organism:
Collection
of
Industrially Important
Microorganisms-2**

Process Organism

The ideal isolation procedure commences with an environmental source (frequently soil) which is highly probable to be rich in the desired types, is so designed as to favour the growth of those organisms possessing the industrially important characteristic (i.e. the industrially useful characteristic is used as a selective factor) and incorporates a simple test to distinguish the most desirable types.

Selective pressure may be used in the isolation of organisms which will grow on particular substrates, in the presence of certain compounds or under cultural conditions adverse to other types.

Process Organism

INDUSTRIAL STRATEGY FOR USAGE OF MICROORGANISMS

Isolation of microbes from natural source



Identification of desired microbes



Characterization of microbes



Screening of desired microbes



Strain Improvement



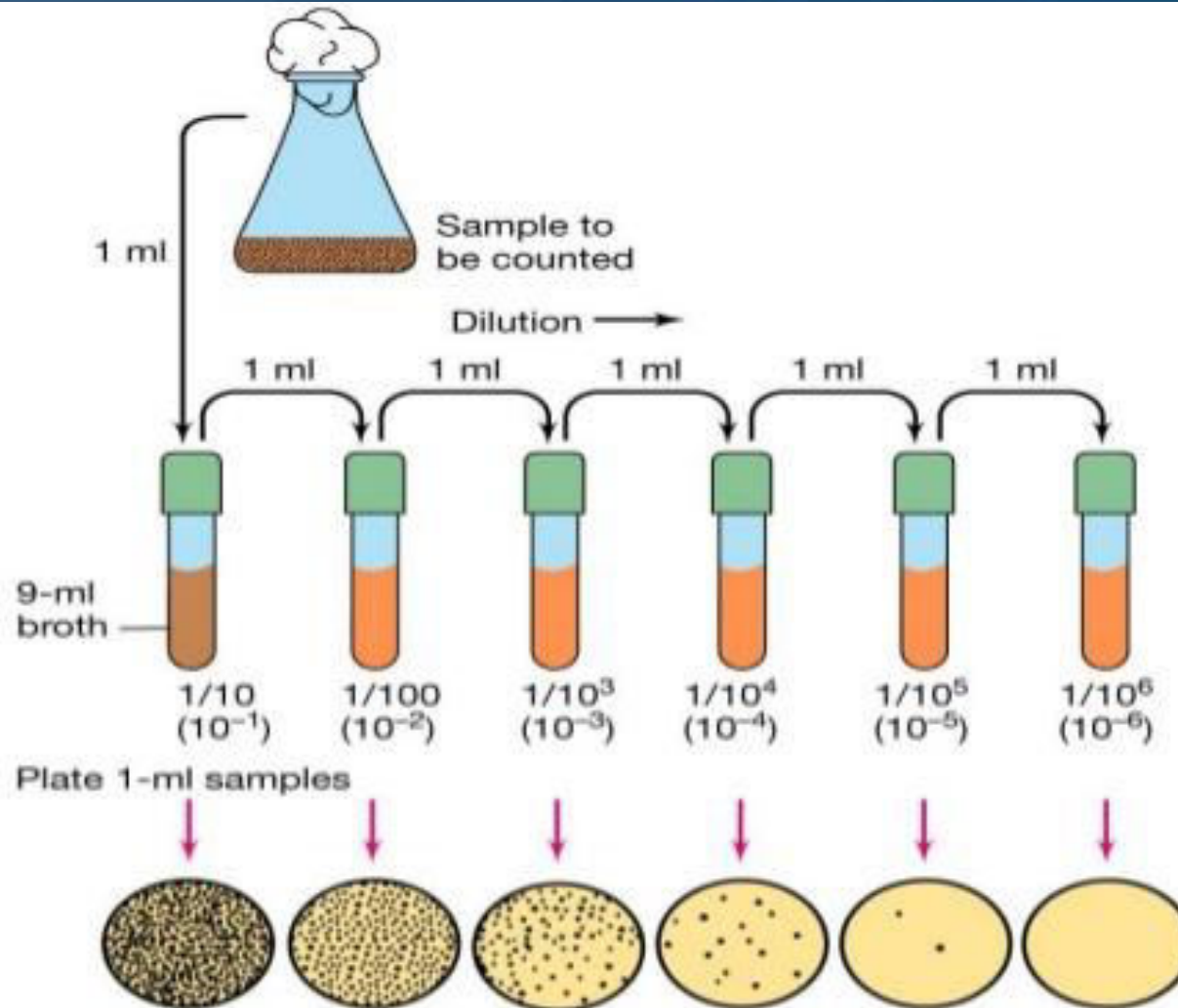
Fermentation

Process Organism

Key strategy to isolate a target microorganism from a specific environmental sample/site

Thermophiles	Hot spring, thermophilic vents, compost
Alkalophiles	High salinity soils and wells
Acidophiles	Pine growing sites, fruits
Osmophiles	Seas and oceans
Yeast	Wine yards
Phosphate solubilizers	Agricultural soil, rhizosphere
Protease producers	Dairy industry, meat processing units.
Enzymes like cellulase, pectinase, xylanase etc.	Agricultural soil, paper and pulp industry, waste disposal area of fruit juice plants, grain market.

Process Organism



Process Organism

10-folds serial dilution of natural or enriched sample and its inoculation on selective medium is one of the most practically applicable procedure for initial isolation of desired or all types of microbes from different samples, which gives a mixed microbial population most of the times.

END

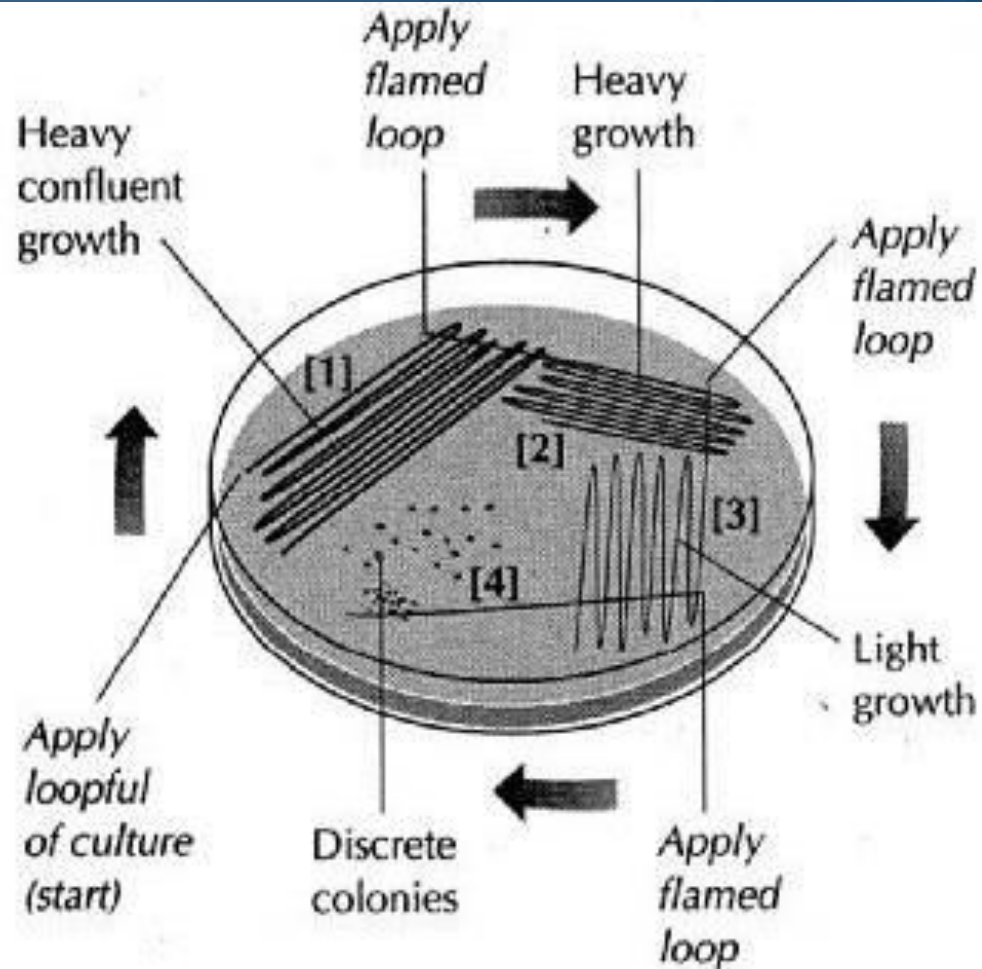
Advances in Fermentation Technology

**Process
Organism:**
collection
of
Industrially Important
Microorganisms-3

Process Organism

The isolation method most commonly used to get pure cultures is the **streak plate method** (Figure on next slide). A sterile inoculating loop is dipped into a mixed culture that contains more than one type of microbe and is streaked in a pattern over the surface of the nutrient medium. As the pattern is traced, bacteria are rubbed off the loop onto the medium. The last cells to be rubbed off the loop are far enough apart to grow into isolated colonies. These colonies can be picked up with an inoculating loop and transferred to a test tube of nutrient medium to form a pure culture containing only one type of bacterium.

Process Organism



Streak plate method for pure-culturing of required microorganism is done for non-filamentous unicellular microorganisms only.

Process Organism



Process Organism

The most desirable isolation medium would be one which selects for the desired types and also allows maximum genetic expression. Cultures grown on such media could then be used directly in a screen.

Process Organism

**However,
it is more common
that, once isolated,
the organisms
are grown on a
range of media
designed to
enhance
productivity.**

END

Advances in Fermentation Technology

**Process
Organism:
Screening Methods
for
Isolated Microbes**

Process Organism

Early screening strategies tended to be empirical, labour intensive and had relatively low success rates. As the number of commercially important compounds isolated increased, the success rates of such screens decreased further.

Thus, new screening methods have been developed which are more precisely targeted to identify the desired activity. The evolution of antibiotic screens serves as an excellent illustration of the development of more precise, targeted systems.

Process Organism

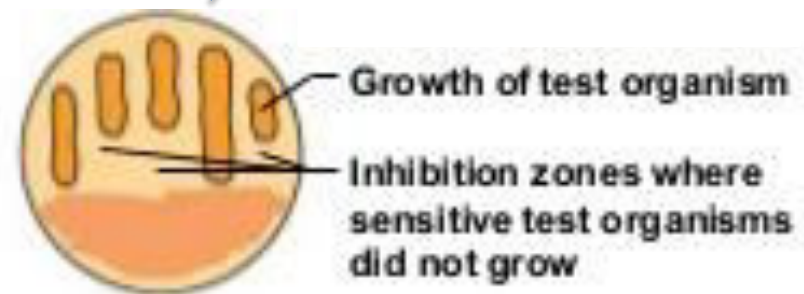
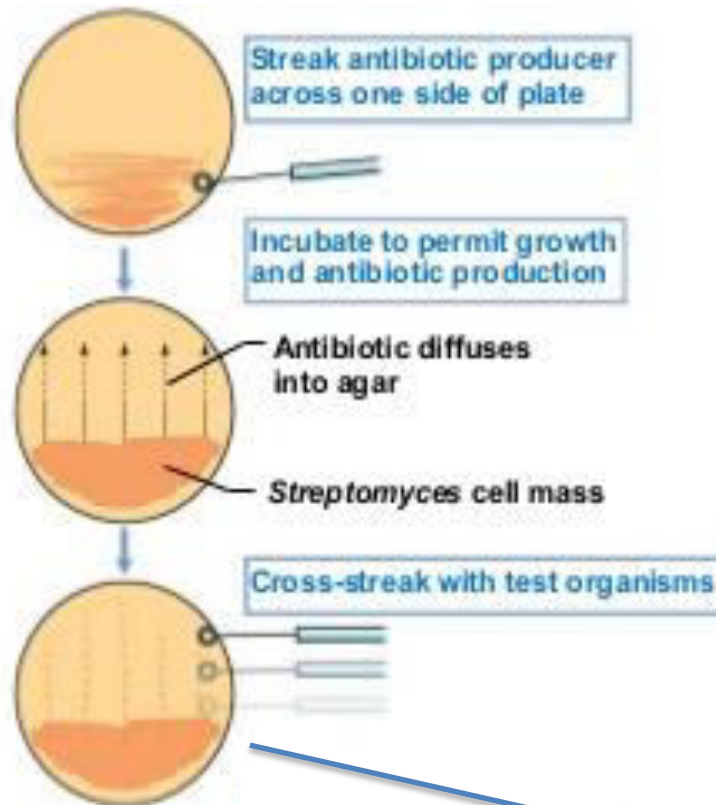
Antibiotics were initially detected by growing the potential producer on an agar plate in the presence of an organism (or organisms) against which antimicrobial action was required.

Production of the antibiotic was detected by inhibition of the test organism(s).

Alternatively, the microbial isolate could be grown in liquid culture and the cell-free broth tested for activity.

This approach was extended by using a range of organisms to detect antibiotics with a defined antibacterial spectrum.

Process Organism



Process Organism

The progress in molecular biology, genetics and immunology has also contributed extensively to the development of innovative screens, by enabling the construction of specific detector strains, increasing the availability of enzymes and receptors and constructing extremely sensitive assays.

Process Organism

Bull (1992) summarized the major contributions as follows:

- 1. The provision of test organisms that have increased sensitivities, or resistances, to known agents. For example, the use of super-sensitive strains for the detection of β -lactam antibiotics.**
- 2. The cloning of genes coding for enzymes or receptors that may be used in inhibitor or binding screens makes such materials more accessible and available in much larger amounts.**
- 3. The development of reporter gene assays. A reporter gene is one which codes for an easily assayable product so that it can be used to detect the activation of a control sequence to which it is fused.**

Process Organism

4. Molecular probes for particular gene sequences may enable the detection of organisms capable of producing certain product groups.

5. The development of immunologically based assays such as ELISA.

END

Advances in Fermentation Technology

**Process
Organism:
Identification
of
Microorganisms**

Process Organism

Various techniques are employed to characterize and identify microorganisms:

- **Phenotypic characteristics**

- Microscopic morphology
- Metabolic differences (Biochemical Testing)
- Serology
- Fatty acid analysis

- **Genotypic characteristics**

- Nucleic acid probes
- DNA amplification
- Ribotyping

Process Organism

Genotypic Characterization (Nucleic acid probes)

- Used to locate unique sequences
 - Single-stranded DNA (or RNA)
- Generally labeled (radioactive or fluorescent)
 - Complementary to the sequence of interest
 - Observe and identify intact microorganisms
- Fluorescence in situ hybridization (FISH)
 - Observe and identify samples
- DNA amplification by PCR
 - Specific primers are used

Process Organism

- Sequencing rRNA genes (16S rRNA for bacteria)
 - Evolutionarily highly conserved genes Variable regions are used to identify an organism. Particularly useful in identifying microbes that are difficult to culture.

END

Advances in Fermentation Technology

Process
Organism:
Storage/Preservation
of
Microbial Cultures

Process Organism

- The isolation of a suitable organism for a commercial process may be a long and very expensive procedure and it is therefore essential that it retains the desirable characteristics that led to its selection.
- Also, the culture used to initiate an industrial fermentation must be viable and free from contamination. Thus, industrial cultures must be stored in such way as to eliminate genetic change, protect against contamination and retain viability.

Process Organism

- An organism may be kept viable by repeated sub-culture into fresh medium, but, at each cell division, there is a small probability of mutations occurring and because repeated sub-culture involves very many such divisions, there is a high probability that strain degeneration would occur.
- Also repeated sub-culture carries with it the risk of contamination. Thus, preservation techniques have been developed to maintain cultures in a state of 'suspended animation' by storing either at reduced temperature or in a dehydrated form.

Process Organism

❑ Storage at Reduced Temperature

▪Storage on Agar Slants

Cultures grown on agar slopes may be stored in a refrigerator (5°C) or a freezer (- 20°C) and sub-cultured at approximately 6-monthly intervals. The time of subculture may be extended to 01 year if the slopes are covered with sterile medicinal grade mineral oil.

▪Storage under Liquid Nitrogen

The metabolic activities of micro-organisms may be reduced considerably by storage at the very low temperatures (-150° to -196°) which may be achieved using a liquid nitrogen refrigerator.

Process Organism

Fungi, bacteriophage, viruses, algae, yeasts, animal and plant cells and tissue cultures can all be successfully preserved by using this method. The technique involves growing a culture to the maximum stationary phase, resuspending the cells in a **cryoprotective agent** (such as 10% glycerol) and freezing the suspension in sealed ampoules before storage under liquid nitrogen.

Process Organism

❑ Storage in a Dehydrated Form

▪Dried Cultures

Dried soil cultures have been used widely for culture preservation, particularly for sporulating mycelial organisms. Moist, sterile soil may be inoculated with a culture and incubated for several days for some growth to occur and then allowed to dry at room temperature for approximately 2 weeks. The dry soil may be stored in a dry atmosphere or, preferably, in a refrigerator. The technique has been used extensively for the storage of fungi and actinomycetes and Pridham *et al.* (1973) observed that of 1800 actinomycetes dried on soil about 50% were viable after 20-years storage.

Process Organism

❑ Storage in a Dehydrated Form

▪Lyophilization

During lyophilization (freeze-drying), a suspension of microbes is quickly frozen at temperatures ranging from -54°C to -72°C , and the water is removed by a high vacuum (sublimation). While under vacuum, the container is sealed by melting the glass with a high-temperature torch. The remaining powder like residue that contains the surviving microbes can be stored for years. The organisms can be revived at any time by hydration with a suitable liquid nutrient medium.

Process Organism

Lyophilization is very convenient for service culture collections because, once dried, the cultures need no further attention and the storage equipment (a refrigerator) is cheap and reliable.

END

Advances in Fermentation Technology

**Process
Organism:
Improvement
of
Industrial Important
Microorganisms-1**

Process Organism

Natural isolates usually produce commercially important products in very low concentrations and therefore every attempt is made to increase the productivity of the chosen **organism**. Increased yields may be achieved by optimizing the culture medium and growth conditions, but this approach will be limited by the organism's maximum ability to synthesize the product.

The potential productivity of the organism is **controlled by its genome** and, therefore, the genome must be modified to **increase the potential yield**. The cultural requirements of the modified organism would then be examined to provide conditions that would fully exploit the increased potential of the culture, while further attempts are made to beneficially change the genome of the already improved strain.

Process Organism

Thus, the process of **strain improvement** involves the continual genetic modification of the culture, followed by reappraisals of its cultural requirements.

Process Organism

Improvement of strains can be put down in simple term as follows:

- ❖ regulating the activity of the enzymes secreted by the organisms;
- ❖ in the case of metabolites secreted extra-cellularly, increasing the permeability of the organism so that the microbial products can find these way more easily outside the cell;
- ❖ selecting suitable producing strains from a natural population;
- ❖ manipulation of the existing genetic apparatus in a producing organism;
- ❖ introducing new genetic properties into the organism by recombinant DNA technology or genetic engineering.

Process Organism

Methods of manipulating the genetic apparatus of industrial organisms

A. Methods not involving foreign DNA

1. Conventional mutation/Random Mutagenesis

B. Methods involving DNA foreign to the organism (i.e. recombination)

2. Transduction

3. Conjugation

4. Transformation

6. Protoplast fusion

7. Genetic engineering

8. Metabolic engineering

9. Site-directed mutagenesis

Process Organism

Detail of some of the above mentioned methods will be discussed in next modules.

END

Advances in Fermentation Technology

**Process
Organism:
Improvement
of
Industrial Important
Microorganisms-2**

Process Organism

❑ Mutation and selection

- Mutations; can occur spontaneously or can be induced by chemical and physical agents involves change in the genetic material, might cause reduction, enhancement or loss of gene activity.
- In order to isolate mutants selection system is required.
- Nutritionally defective, resistant, temperature sensitive and similar types of mutations that are used in basic research are relatively simple.

The establishment of selection system for mutations which aim to improve the yield in the production of given primary and secondary metabolites or certain enzymes is more complicated.

Process Organism

Chemical mutagens (alkylating agents, base analogues, deaminating agents) or **Physical mutagens** (UV and the ionizing radiations: X-rays, gamma rays, alpha-particles and fast neutrons) could be used for increasing the mutation rate of bacteria. Then we can select the ones with desired properties.

Process Organism

❑ Choice of a Mutagen

Mutagenic agents are numerous but not necessarily equally effective in all organisms. Other factors besides effectiveness to be borne in mind are:

a) the safety of the mutagen: many mutagens are carcinogens, b) simplicity of technique, and c) ready availability of the necessary equipment and chemicals.

Among physical agents, UV is to be preferred since it does not require much equipment, and is relatively effective and has been widely used in industry. The disadvantage of UV is that it is absorbed by glass; it is also not effective in opaque or colored organisms. Chemical methods other than NTG (nitrosoguanidine) are probably best used in combination with UV.

Process Organism

❑ Random Mutagenesis

Early approaches to mutagenesis rely on methods which are entirely random in the mutations produced. Cells or organisms may be exposed to mutagens such as UV radiation or mutagenic chemicals, and mutants with desired characteristics are then selected. **For *Escherichia coli*, mutants may be selected first by exposure to UV radiation, then plated onto agar medium.** The colonies formed are then replica-plated, one in rich medium, another in minimal medium, and mutants that have specific nutritional requirements can then be **identified by their inability to grow in minimal medium.** Similar procedures may be repeated with other types of cells and with different media for selection.

Process Organism

Alkylating agents such as ***N*-ethyl-*N* nitrosourea (ENU)** have been used to generate mutant mice. **Ethyl methanesulfonate (EMS)** is also often used to generate animal and plant mutants.

END

Advances in Fermentation Technology

**Process
Organism:
Improvement
of
Industrially Important
Microorganisms-3**

Process Organism

❑ Site-Directed Mutagenesis

Many researchers seek to introduce selected changes to DNA in a site-specific manner. Analogs of nucleotides and other chemicals were first used to generate localized point mutations. Such chemicals include aminopurine, which induces AT to GC transition, while nitrosoguanidine, bisulfite, and N⁴-hydroxycytidine may induce GC to AT transition.

These techniques allow specific mutations to be engineered into a protein; however, they are not flexible with respect to the kinds of mutants generated, nor are they as specific as later methods of site-directed mutagenesis and therefore have some degree of randomness.

Process Organism

- Current techniques for site-specific mutation commonly involve using mutagenic oligonucleotides in a primer extension reaction with DNA polymerase.
- This methods allows for point mutation, or deletion or insertion of small stretches of DNA to be introduced at specific sites.
- Advances in methodology have made such mutagenesis now a relatively simple and efficient process.

Process Organism

❑ Protoplast Fusion

Protoplasts are formed from bacteria, fungi, yeast and actinomycetes when dividing cells are caused to lose their cell walls. Fusion from mixed populations of protoplasts is greatly enhanced by the use of Polyethyleneglycol (PEG). The method has great industrial potential and has experimentally been used to achieve higher yields of antibiotics through fusion with protoplasts from different fungi. Protoplast fusion has been demonstrated as an efficient way to induce heterokaryon formation and recombination with high frequency.

Process Organism

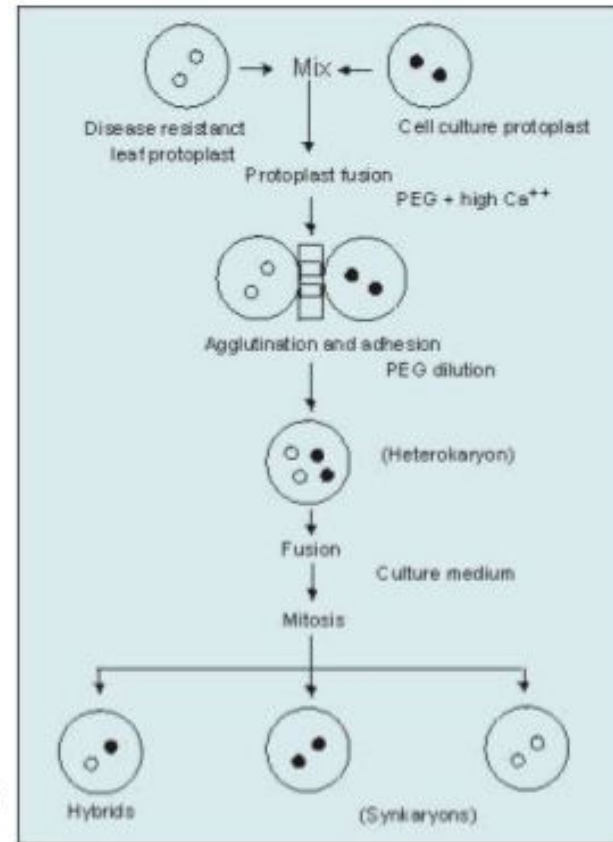
Protoplast fusion has been successfully done with *Bacillus subtilis* and *Bacillus megaterium*; among several species of *Streptomyces spp.* like *S. coeli-color*, *S. acrimycini*, *S. olividans*, *S. pravulies*; between the fungi *Geotrichum* and *Aspergillus*; and Yeasts.

Kim *et al.* (1998) did a comparative study on strain improvement of *Aspergillus oryzae* for protease production by both mutation and protoplast fusion.

- UV radiation = 14 times higher yield
- EMS = 39 times higher yield
- Protoplast fusion using PEG and CaCl_2 = 82 times higher yield

Process Organism

- Bacteria-grown plasmids are introduced into animal cells via fusion with bacterial protoplasts
- Bacterial cell walls are removed using lysozyme treatment
- Protoplasts fuse to the mammalian cells
- DNA purification not required
- may introduce bacterial components into recipient cells, cultures may be overgrown with bacteria
- *Bacteria are used to produce plasmids containing the human gene of interest. The cell walls are removed using lysozyme, resulting in the formation of protoplasts. The protoplasts are brought in contact with the mammalian cells, leading to protoplast fusion to mammalian cells, using Polyethylene glycol (PEG).*



Process Organism

Auxotrophy is the inability of an organism to synthesize a particular organic compound required for its growth. An auxotroph is an organism that displays this characteristic.

Process Organism

Prototrophy is characterized by the ability to synthesize all the compounds needed for growth.

Protrophic organism is a wild-type organism expressing its natural phenotype.

END

Advances in Fermentation Technology

Process
Organism:

Mutant Selection-1

Process Organism

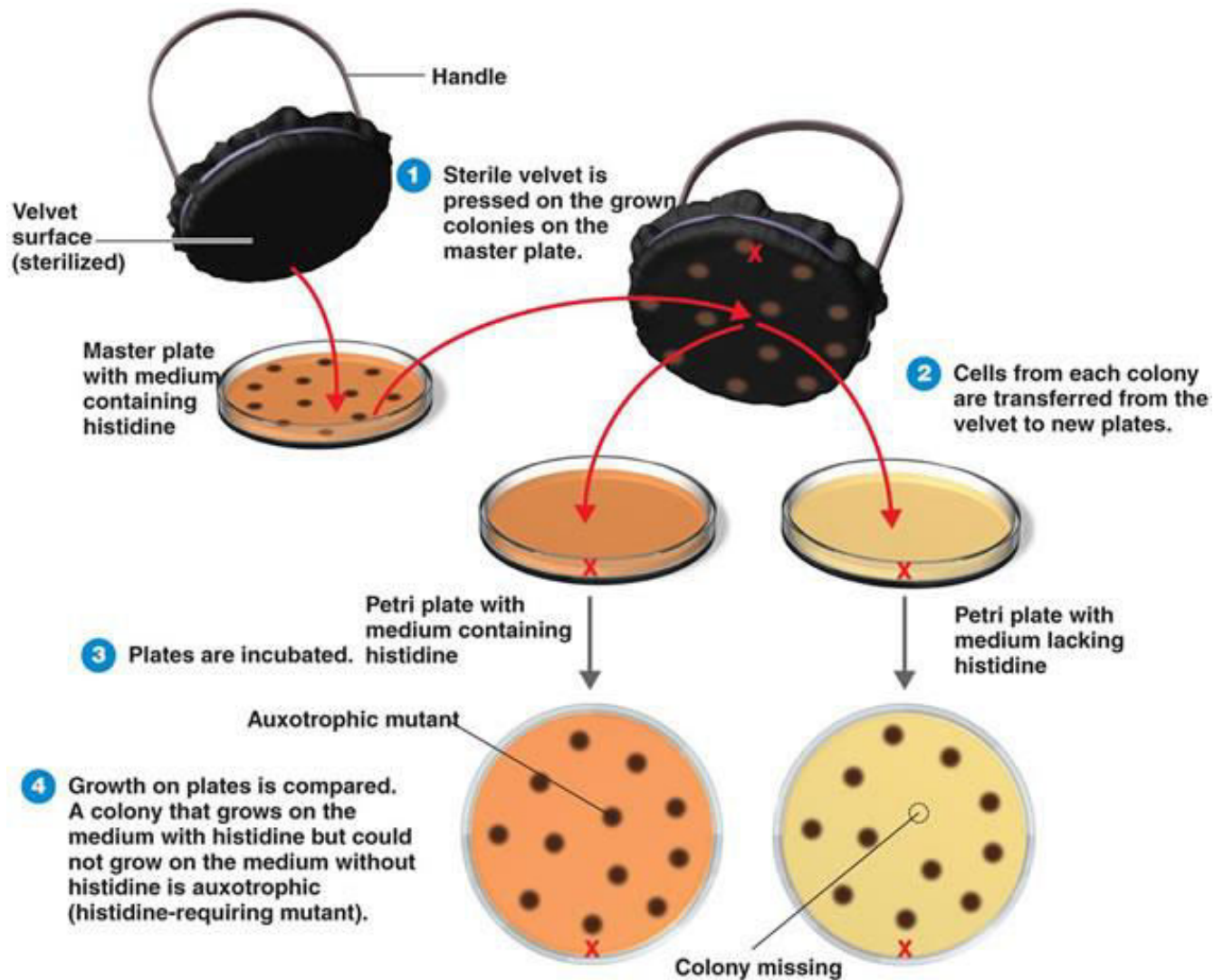
Following exposure to the mutagen the cells should be suitably diluted and plated out to yield 50 – 100 colonies per plate. The selection of mutants is greatly facilitated by relying on the morphology of the mutants or on some selectivity in the medium. When morphological mutants are selected, it is in the hope that the desired mutation is pleotropic (i.e., a mutation in which change in one property is linked with a mutation in another character). The classic example of a pleotropic mutation is to be seen in the development of penicillin-yielding strains of *Penicillium chrysogenum*. It was found in the early days of the development work on penicillin production that after irradiation, strains of *Penicillium chrysogenum* with smaller colonies and which also sporulated poorly were better producers of penicillin.

Process Organism

❑ Isolation/Selection of Auxotrophic Mutants by Replica Plating Method

Mutants can be detected by selecting or testing for an altered phenotype. Positive selection involves the selection of mutant cells and rejection of non-mutated cells. To identify mutants that have gained some physical trait, **Replica plating is used for negative selection.** Negative selection identifies mutants that have lost some physical trait - for example, auxotrophs that have nutritional requirements not possessed by the parent (nonmutated) cell.

Process Organism



Process Organism

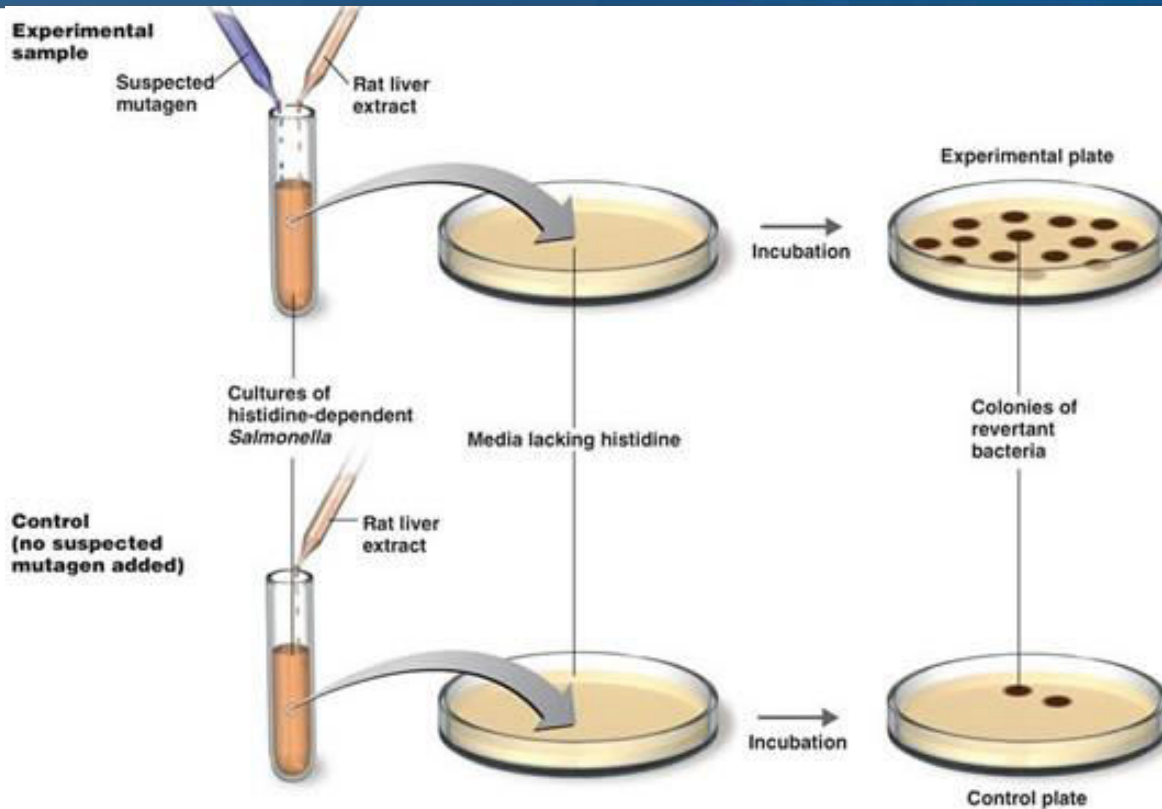
❑ Ames Test

The Ames test is a relatively inexpensive and rapid test for identifying possible chemical carcinogens.

The test assumes that a mutant cell can revert to a normal cell in the presence of a mutagen and that many mutagens are carcinogens.

Histidine auxotrophs (require histidine in the media) of *Salmonella* are exposed to an enzymatically treated potential carcinogen, and reversions to the nonmutant state are selected.

Process Organism



- 1 Two cultures are prepared of *Salmonella* bacteria that have lost the ability to synthesize histidine (histidine- dependent).
- 2 The suspected mutagen is added to the experimental sample only; rat liver extract (an activator) is added to both samples.
- 3 Each sample is poured onto a plate of medium lacking histidine. The plates are then incubated at 37°C for two days. Only bacteria whose histidine-dependent phenotype has mutated back (reverted) to histidine-synthesizing will grow into colonies.
- 4 The numbers of colonies on the experimental and control plates are compared. The control plate may show a few spontaneous histidine-synthesizing revertants. The test plates will show an increase in the number of histidine-synthesizing revertants if the test chemical is indeed a mutagen and potential carcinogen. The higher the concentration of mutagen used, the more revertant colonies will result.

Process Organism

Before considering the methods used for the selection of methods producing improved levels of primary metabolites it is necessary to study the mechanisms of control of their biosynthesis such that the 'blueprints', may be drawn accurately.

END

Advances in Fermentation Technology

Process Organism:

**Development
of
Inocula
for
Industrial
Fermentations
Introduction 1**

Development of Inocula

It is essential that the culture used to inoculate a fermentation satisfies the following criteria:

1. It must be in a healthy, active state thus minimizing the length of the lag phase in the subsequent fermentation.
2. It must be available in sufficiently large volumes to provide an inoculum of optimum size.
3. It must be in a suitable morphological form.
4. It must be free of contamination.
5. It must retain its product-forming capabilities.

Development of Inocula

The quantity of inoculum normally used is between 3 and 10% of the medium volume. A relatively large inoculum volume is used to minimize the length of the lag phase and to generate the maximum biomass in the production fermenter in as short a time as possible, thus increasing vessel productivity. Thus, starting from a stock culture, the inoculum must be built up in a number of stages to produce sufficient biomass to inoculate the production-stage fermenter. This may involve two or three stages in shake flasks and one to three stages in fermenters, depending on the size of the ultimate vessel. Throughout this procedure there is a risk of contamination and strain degeneration necessitating stringent quality-control procedures.

Development of Inocula

The greater the number of stages between the master culture and the production fermenter the greater the risk of contamination and strain degeneration. Therefore, a compromise may be reached regarding the size of the inoculum to be used and the risk of contamination and strain degeneration.

Another factor to be considered in the determination of the inoculum volume is the economics of the process. A seed fermenter 10% of the size of the production fermenter represents a considerable financial investment and must be justified in terms of productivity.

Development of Inocula

A large-scale continuous fermentation for the production of biomass would be expected to operate at steady state in excess of 100 days.

Thus, the number of times that the fermenter inoculated should be very few compared with batch or fed-batch systems. In such circumstances it may be more economic to compromise on the size of the inoculum and to tolerate a relatively lengthy period of growth up to maximum biomass than to invest a large seed vessel which would be used on very few occasions.

Development of Inocula

This is particularly relevant for biomass continuous processes where one very large fermenter may be used and, thus, any seed vessel would only be servicing the one production vessel.

END

Advances in Fermentation Technology

Development of Inocula

FOR INDUSTRIAL
FERMENTATIONS-
Introduction-2

Development of Inocula

❑ Description of Typical inoculum-development

The master culture is reconstituted and plated on to solid medium; approximately ten colonies of typical morphology of high producers are selected and inoculated on to slopes as the sub-master cultures, each sub-master culture being used for a new production run.

At this stage, shake flasks may be inoculated to check the productivity of these cultures, the results of such tests being known before the developing inoculum eventually reaches the production plant. A sub-master culture is used to inoculate a shake flask (250 or 500 cm³ containing 50 or 100 cm³ medium) which, in turn, is used as inoculum for a larger flask, or a laboratory fermenter, which may then be used to inoculate a pilot-scale fermenter.

Development of Inocula

Culture purity checks are carried out at each stage to detect contamination as early as possible. Although the results of these tests may not be available before the culture has reached the production plant, at least it is known at which stage in the procedure contamination occurred. **For a sporulating organism the process may be modified to facilitate the use of a spore suspension as inoculum.**

Lincoln (1960) suggested a more elaborate procedure for the development of inoculum for bacterial fermentations which, with minor modifications, is applicable to any type of culture.

Development of Inocula

The procedure involved the use of one sub-master culture to develop a bulk inoculum which was subdivided, stored in a frozen state and used as inocula for several months. A single colony, derived from a sub-master culture, was inoculated into liquid medium and grown to maximum log phase. **This culture was then transferred into nineteen times its volume of medium and incubated again to the maximum log phase, at which point it was dispensed in 20 cm³ volumes, plug frozen and stored at below –20°C.** At least 3% of the samples were tested for purity and productivity in subsequent fermentation and, provided these were suitable, the remaining samples could be used as initial inocula for subsequent fermentations.

Development of Inocula

To use one of the stored samples as inoculum it was thawed and used as a 5% inoculum for a seed culture which, in turn, was used as a 5% inoculum for the next stage in the programme.

END

Advances in Fermentation Technology

Development of Inocula

FOR INDUSTRIAL
FERMENTATIONS-
Criteria for the Transfer
of Inoculum

Development of Inocula

The physiological condition of the inoculum when it is transferred to the next culture stage can have a major effect on the performance of the fermentation. The optimum time of transfer must be determined experimentally and then procedures established so that inoculation with an ideal culture may be achieved routinely.

These procedures include the standardization of cultural conditions and monitoring the state of an inoculum culture so that it is transferred at the optimum time, i.e. in the correct physiological state. The most widely used criterion for the transfer of vegetative inocula is biomass and such parameters as packed cell volume, dry weight, wet weight, turbidity, respiration, residual nutrient concentration and morphological form have been used.

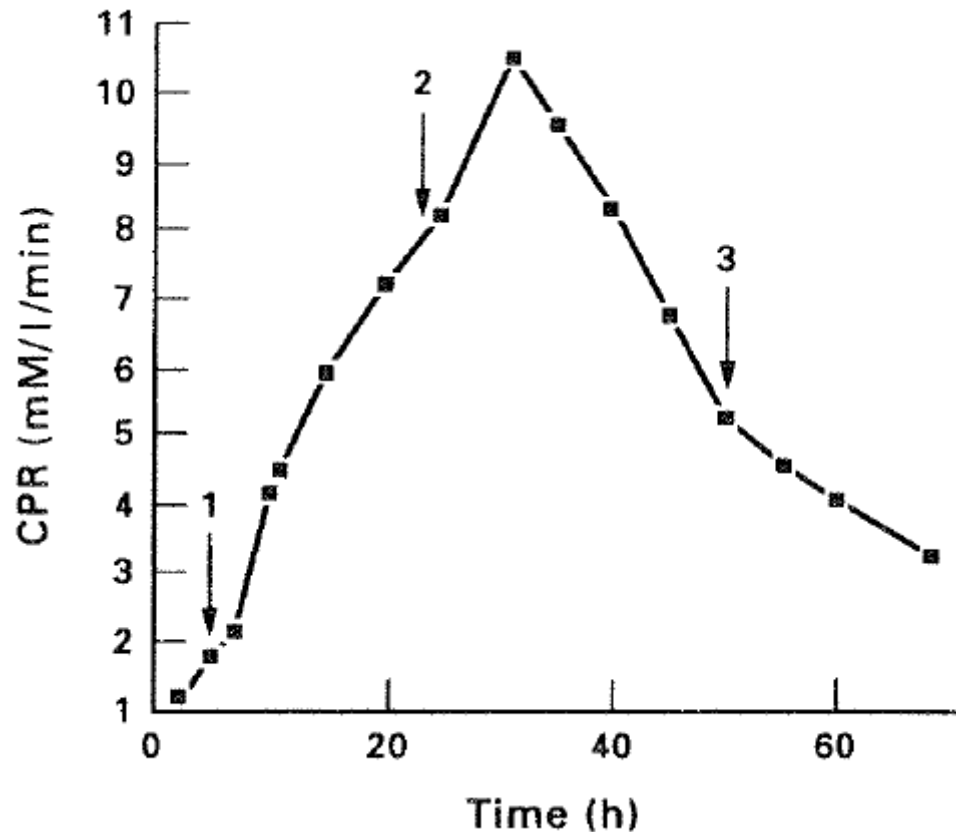
Development of Inocula

Criteria which may be monitored on-line are the most convenient parameters to use as indicators of inoculum quality and these would include:

- dissolved oxygen, pH (although pH would normally be controlled in seed fermentations) and oxygen or carbon dioxide in the effluent gas.

Parton and Willis (1990) advocated the use of the carbon dioxide production rate (CPR) as a transfer criterion which requires analysis of the fermenter effluent air. This approach is suitable only when transfer is being made from a fermenter. These workers provide an excellent example of the effect of inoculum transfer time on the productivity of a streptomycete secondary metabolite, as shown in Figures in next slides.

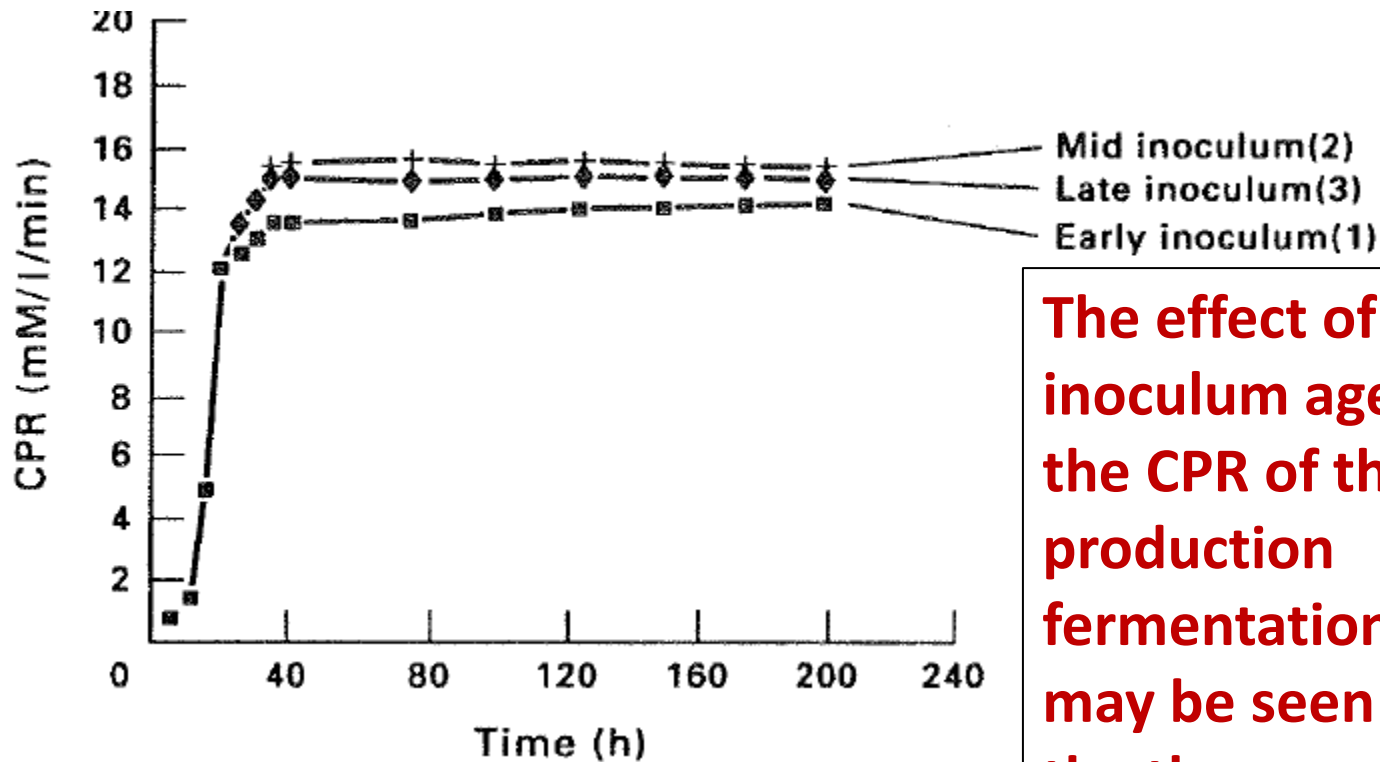
Development of Inocula



The carbon dioxide production rate (CPR) profile of the inoculum culture showing the points 0, 2 and 3) at which inocula were removed.

The effect of inoculum age on growth and productivity in a streptomycete fermentation.

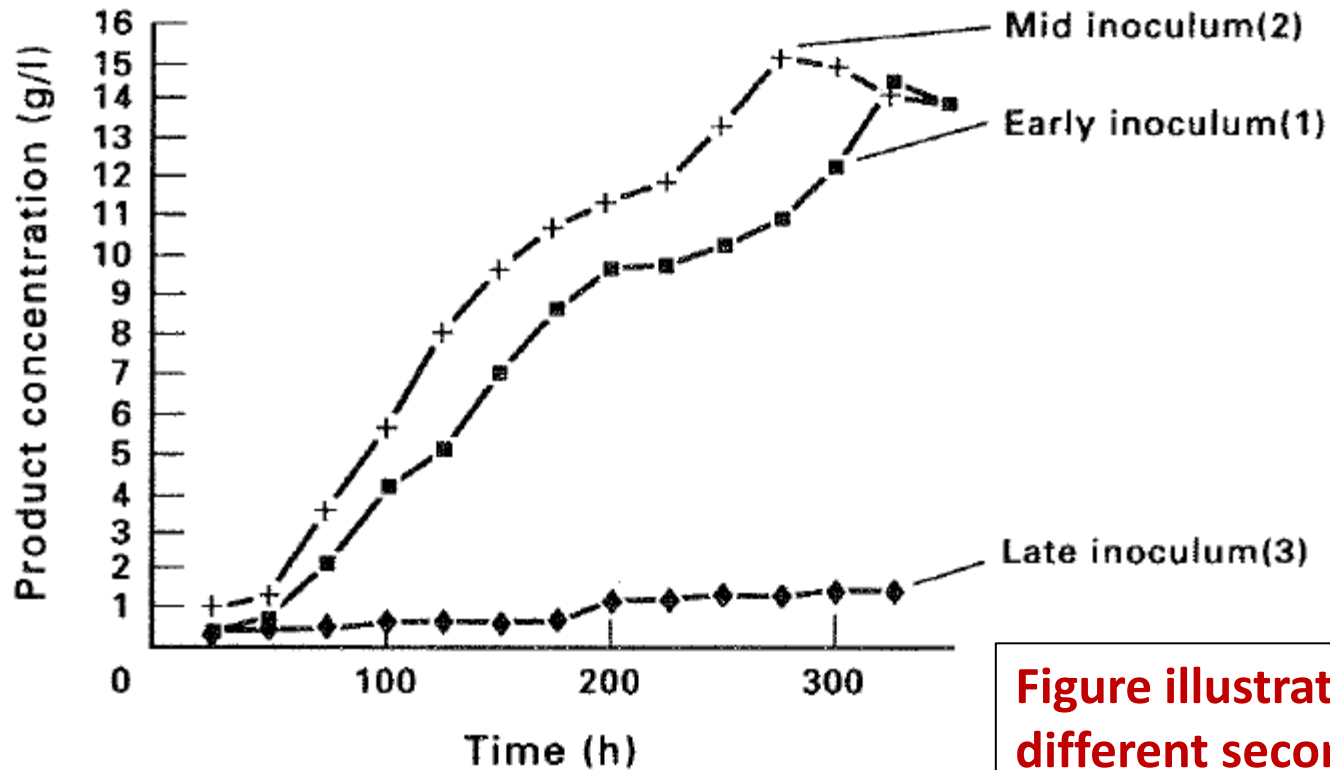
Development of Inocula



The effect of inoculum age on growth and productivity in a streptomycete fermentation.

The effect of inoculum age on the CPR of the production fermentation, it may be seen that the three fermentations performed similarly.

Development of Inocula



The effect of inoculum age on growth and productivity in a streptomycete fermentation.

Figure illustrates very different secondary metabolite production of the three fermentations.

Development of Inocula

Thus, although the time of transfer had only a marginal influence on biomass in the production fermentation the effect on product formation was critical.

END

Advances in Fermentation Technology

Development of Inocula Examples

Development of Inocula

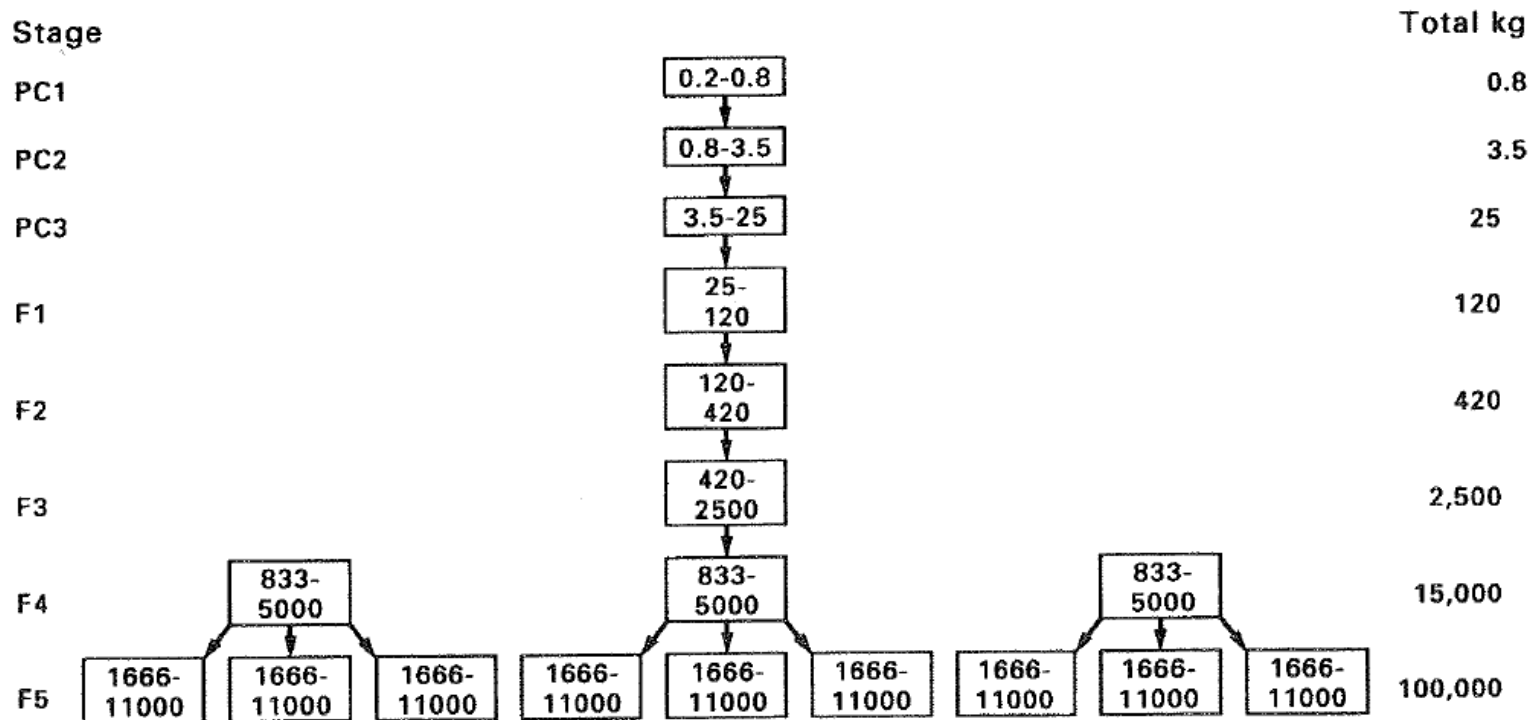
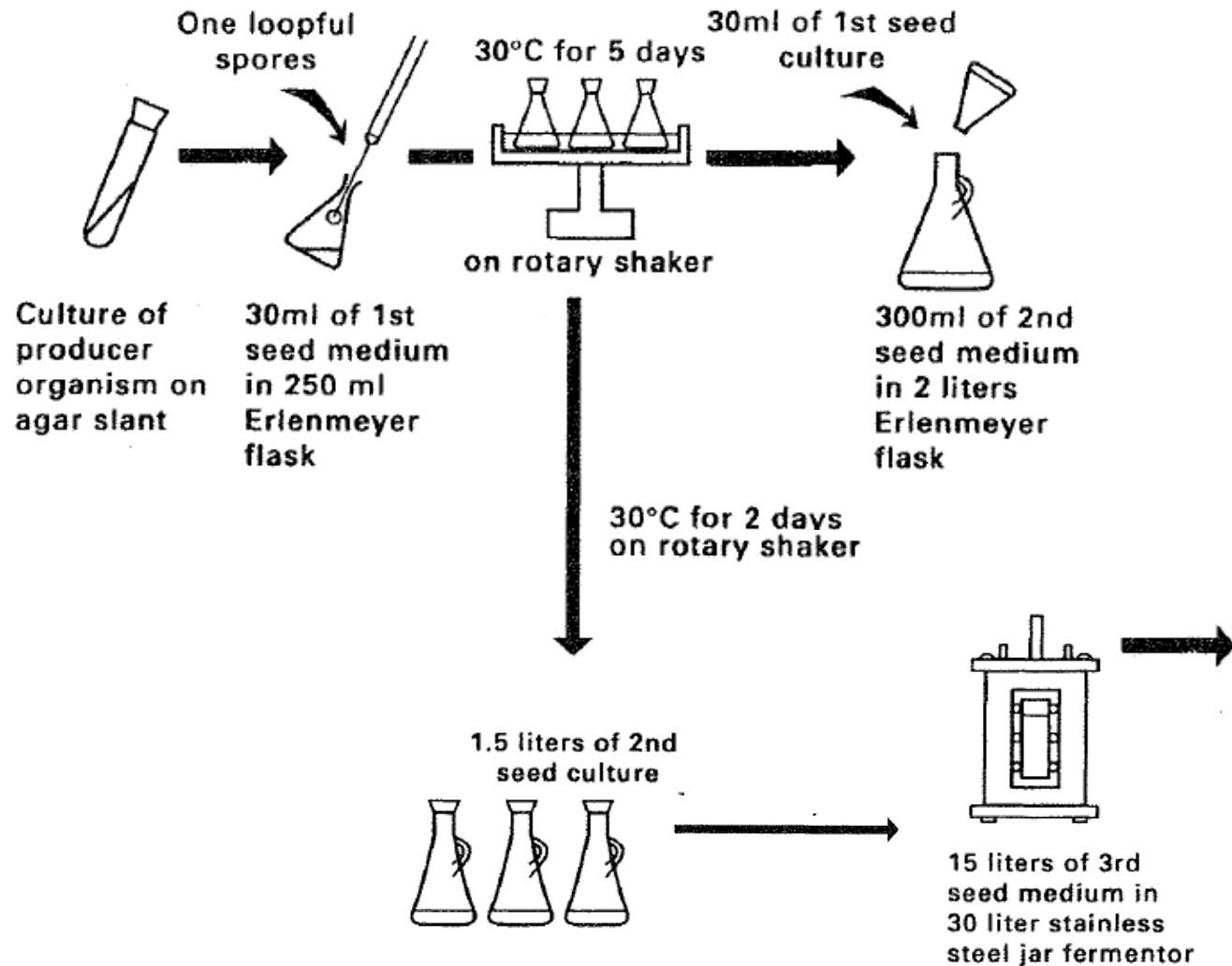
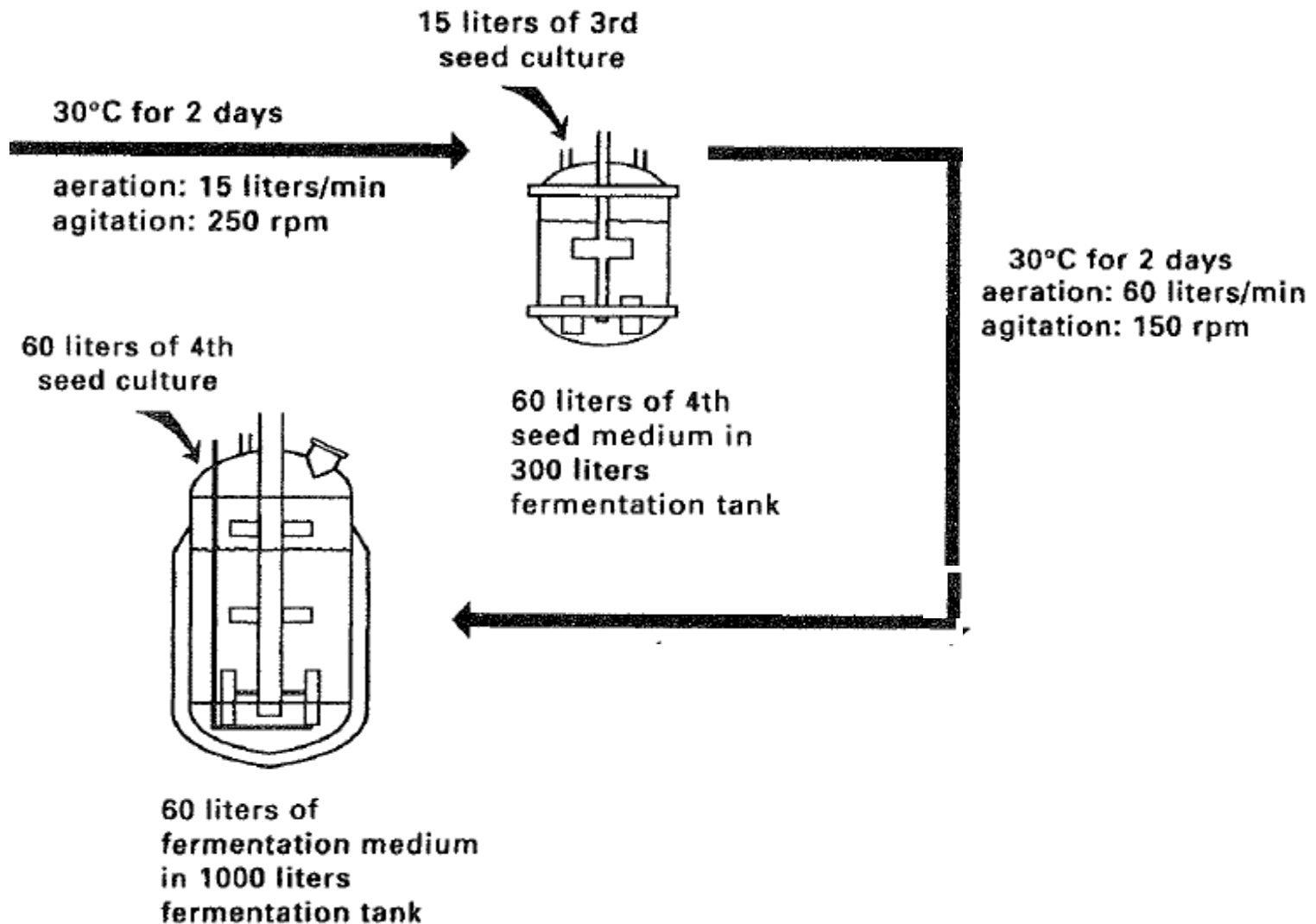


Figure showing the development of inoculum for the commercial production of bakers' yeast. PC 1, 2 and 3 are pure culture batch fermentations. F1 and 2 are non-aseptic batch fermentations. F3 and 4 are fed-batch fermentations and F5 is the final fed-batch fermentation

Development of Inocula



Development of Inocula



Development of Inocula

The choice of inoculum for the production stage depends on various factors such as the length of the cycle of the fermentation process, plant size and the availability and cost of labour.

END