VU MEDICAL ZONE

ADMIN:

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INDUSTRIAL BIOTECHNOLOGY

BT 604 FINAL TERM PPT

Lecture 91 to 167

METABOLIC REGULATION II

- **CONTROL OF ENZYME SYNTHESIS**
- The concentration of any enzyme is determined by its rate of synthesis, rate of dilution (where growth proceeds when synthesis of the enzyme ceases), and the rate of inactivation and degradation by proteases.
- However, it is enzyme synthesis that generally plays the most important role in regulation, albeit providing a slower means of regulating flux through metabolic pathways than does modification of enzyme activity.

METABOLIC REGULATION II

- Some enzymes are constantly synthesized and are **constitutive components of the cell.**
- **Others are** synthesized only when required.
- Even the constitutive enzymes are produced at different concentrations, as some genes are more highly expressed than others.

METABOLIC REGULATION II

- Many catabolic enzymes are regulated by **induction,** as it is efficient for them to be synthesized only when their substrates are present.
- Synthesis of anabolic enzymes is mostly controlled through **repression.**
- When a cell accumulates excess end-product of an anabolic pathway, it responds by slowing or stopping the synthesis of enzymes in the biosynthetic pathway for this product.

METABOLIC REGULATION II

- Studies on the induction of b-galactosidase in *E. Coli have provided* a considerable amount of information regarding the mechanisms involved.
- This enzyme catalyses the hydrolysis of lactose to glucose and galactose, and is not actively synthesized if lactose is not present.
- In the absence of lactose, a repressor protein, produced under the direction of regulatory genes, binds to the operator region of the DNA, upstream from the structural gene for b-galactosidase, thereby blocking the synthesis of MRNA/ by RNA polymerase.

METABOLIC REGULATION II

- When a metabolite is catabolized or synthesized by a series of enzymes, it is usually advantageous for all enzymes of the pathway to be switched on or off simultaneously.
- In bacteria this coordinated action is more easily achieved as the genes for these enzymes are often located together as a cluster on the chromosome called an **operon.**

METABOLIC REGULATION II

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METABOLIC REGULATION II

- Enzyme synthesis may also be controlled by a mechanism called **attenuation, where regulation of gene** expression is via termination of transcription, rather than control of initiation.
- This involves termination sites within the operon that result in truncated MRNA.

METABOLIC REGULATION II

- Tryptophan biosynthesis, which involves an operon of five enzymes, is coordinated through both repression, as mentioned above, and attenuation.

METABOLIC REGULATION III

- **MECHANISMS OF GENERAL REGULATION**

- **REGULATION OF RESPIRATION AND FERMENTATION**

- Aerobic respiration and fermentation can be regulated by environmental conditions, which include the availability of oxygen and sugars.
- Under aerobic conditions, many organisms exhibit a slower rate of sugar catabolism via glycolysis than when maintained under anaerobic conditions.

METABOLIC REGULATION III

- Under aerobic conditions, many organisms exhibit a slower rate of sugar catabolism via glycolysis than when maintained under anaerobic conditions.
- This is because fewer carbon units have to be metabolized to obtain the same quantity of ATP, as aerobic respiration and associated oxidative phosphorylation is considerably more efficient than fermentation.

METABOLIC REGULATION III

- This suppression of glycolysis by oxygen is a regulatory phenomenon referred to as the **pasteur effect,** which is apparent only at low sugar concentrations (less than 5mmol/L for some yeasts).
- The flux through glycolysis appears to be primarily controlled by the allosteric enzyme phosphofructokinase, which is regulated by the relative concentrations of ATP and AMP.

METABOLIC REGULATION III

- Several yeasts exhibit a further phenomenon, the **crabtree effect, where at high sugar concentrations,** even in the presence of oxygen, fermentation overrides respiration.
- Consequently, nadh generated via the emp pathway is mainly oxidized by fermentative means rather than by mitochondrial respiration.

METABOLIC REGULATION III

- **CARBON CATABOLITE REPRESSION;**
- In many microorganisms the enzymes for glucose catabolism are constitutive,
- whereas additional enzymes must usually be produced for the catabolism of any other carbon and energy source.
- If glucose is present in the medium, along with other sugars, it is always the first to be used.

METABOLIC REGULATION III

- The result is sequential utilization that produces a diauxic (biphasic) growth pattern.
- This is because the production of enzymes for the uptake and utilization of the other sugars, and several other enzymes, is repressed by glucose or an initial product of its metabolism.

METABOLIC REGULATION III

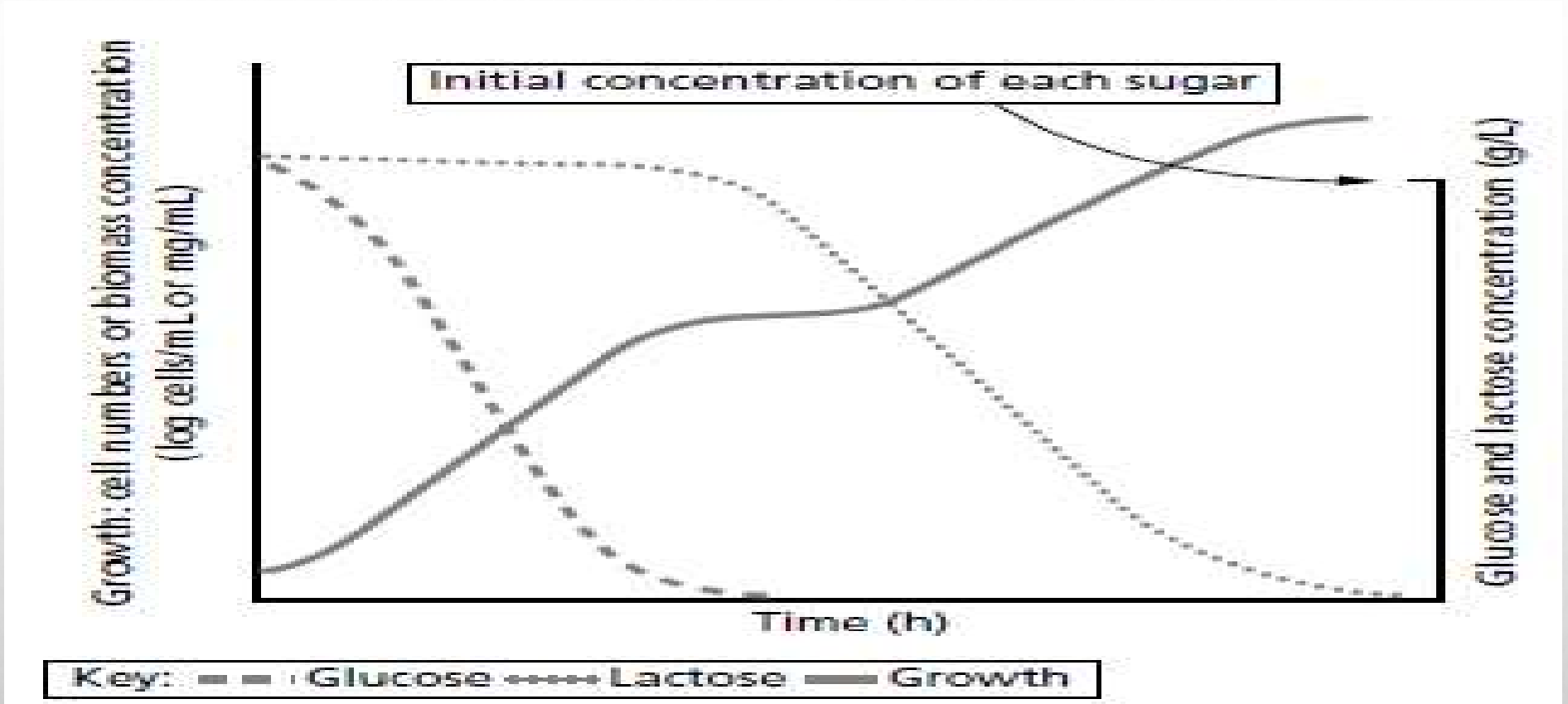
- In some cases, it is due to glucose-induced inactivation of key enzymes; for others, repression results from rapid glucose uptake, which causes the intracellular level of cyclic adenosine monophosphate (camp) to fall.
- This important compound is involved in the positive control of many catabolic enzymes, e.g. the lac operon

METABOLIC REGULATION III

- Camp is the activator of a protein called catabolite activator protein (CAP), which in the active state binds to a site within the promoter region of the gene.
- Without it, rna polymerase is unable to bind and start gene transcription.
- Therefore, in the absence of camp only inactive CAP molecules are present and transcription does not take place.

METABOLIC REGULATION III

- Levels of camp rise only when the rate of glucose transport into the cell falls below a certain level.
- Carbon catabolite regulation of primary metabolism provides a selective advantage for the organism, as it ensures the economical use of metabolic machinery.
- It also plays a role in controlling secondary metabolism.
- For example, glucose has a repressive effect on the production of many secondary metabolites, including antibiotics(b-lactams and tetracylines) and some alkaloids.



Diauxic growth of a microorganism on a mixture of glucose and lactose.

INDUSTRIAL MICROORGANISMS

- Microoorganisms are used extensively to provide a vast range of products and services.
- They have proved to be particularly useful because of the ease of their mass cultivation, speed of growth, use of cheap substrates and the diversity of potential products.
- Their ability to readily undergo genetic manipulation has also opened up almost limitless further possibilities for new products and services from the fermentation industries.

INDUSTRIAL MICROORGANISMS

- Traditional fermentations were originally performed (and still are in some cases) by a mixture of wild microorganisms emanating from the raw materials or the local environment, e.G. Some food and alcoholic beverage fermentations.

INDUSTRIAL MICROORGANISMS

- Initial attempts to improve the microorganisms involved occurred little more than 120 years ago, when they were first isolated from these processes as pure cultures from which the most useful strains were then selected.
- Those fermentation processes developed during the first 80 years of the 20th century have mostly used monocultures.

INDUSTRIAL MICROORGANISMS

- The specific microorganisms employed were often isolated from the natural environment, which involved the random screening of a large number of isolates.
- Alternatively, suitable microorganisms were acquired from culture collections.

INDUSTRIAL MICROORGANISMS

- Most of these microorganisms, irrespective of their origins, were subsequently modified by conventional strain improvement strategies, using mutagenesis or breeding programmes, to improve their properties for industrial use.
- Several processes developed in the last 20 years have involved recombinant microorganisms and genetic engineering technology has increasingly been used to improve established

  industrial strains

INDUSTRIAL MICROORGANISMS

- In most cases, regulatory considerations are of major importance when choosing microorganisms for industrial use.
- Fermentation industries often prefer to use established gras (generally regarded as safe) microorganisms, particularly for the manufacture of food products and ingredients.

INDUSTRIAL MICROORGANISMS

- Where pathogens and some genetically manipulated microorganisms (gmms) are used as the producer organism, additional safety measures must be taken.
- Special containment facilities are employed and it may be possible to use modified ('crippled') strains that cannot exist outside the fermenter environment.

ISOLATION OF SUITABLE MICROORGANISMS FROM THE ENVIRONMENT I

- Strategies that are adopted for the isolation of a suitable industrial microorganism from the environment can be divided into two types, '**shotgun' and objective** approaches.
- In the shotgun approach, samples of free living microorganisms, biofilms or other microbial communities are collected from animal and plant material, soil, sewage, water and waste streams, and particularly from unusual man-made and natural habitats.

ISOLATION OF SUITABLE MICROORGANISMS FROM THE ENVIRONMENT I

- These isolates are then screened for desirable traits. The alternative is to take a more objective approach by sampling from specific sites where organisms with the desired characteristics are considered to be likely components of the natural microflora.
- For example, when attempting to isolate an organism that can degrade or detoxify a specific target compound, sites may be sampled that are known to be contaminated by this material.

ISOLATION OF SUITABLE MICROORGANISMS FROM THE ENVIRONMENT I

- These environmental conditions may select for microorganisms able to metabolize this compound.
- Once the samples have been collected a major problem is deciding on the growth media and cultivation conditions that should be used to isolate the target microorganisms.
- An initial step is often to kill or repress the proliferation of common organisms and encourage the growth of rare ones.

ISOLATION OF SUITABLE MICROORGANISMS FROM THE ENVIRONMENT I

- **Enrichment cultures may then** be performed in batch culture, or often more suitably in continuous systems. This encourages the growth of those organisms with the desired traits and increases the quantity of these target organisms, prior to isolation.

Examples of industrial fermentation products and their producer microorganisms

	Bacteria	Yeasts and filamentous fungi
Traditional products		
Bread, beer, wine and spirits		Mainly *Saccharomyces cerevisiae*
Cheeses, other dairy products	Lactic acid bacteria	
Ripening of blue and Camembert-type cheeses		*Penicillium* species
Fermented meats and vegetables	Mostly lactic acid bacteria	
Mushrooms		*Agaricus bisporus*, *Lentinula edodes*
Soy sauce		*Aspergillus oryzae* *Zygosaccharomyces rouxii*
Sufu (soya bean curd)		*Mucor* species
Vinegar	*Acetobacter* species	
Agricultural products		
Gibberellins		*Fusarium moniliforme*
Fungicides		*Coniothyrium minitans*
Insecticides	*Bacillus thuringiensis*	
Silage	Lactic acid bacteria	
Amino acids		
L-Glutamine	*Corynebacterium glutamicum*	
L-Lysine	*Brevibacterium lactofermentum*	
L-Tryptophan	*Klebsiella aerogenes*	

Enzymes		
Carbohydrases		
α-amylase	*Bacillus subtilis*	
β-amylase		*Aspergillus niger*
amyloglucosidase		*Aspergillus niger*
glucose isomerase	*Streptomyces olivaceus*	
invertase		*Kluyveromyces* species
lactase (β-galactosidase)		*Kluyveromyces lactis*
Cellulases		*Trichoderma viride*
Lipases		*Candida cylindraceae*
Pectinases		*Aspergillus wentii*
Proteases		
subtilisin (alkaline)	*Bacillus licheniformis*	
neutral		*Aspergillus oryzae*
microbial rennet (acid)		*Rhizomucor miehei*
Fuels and chemical feedstocks		
Acetone	*Clostridium* species	
Butanol	*Clostridium acetobutylicum*	
Ethanol	*Zymomonas mobilis*	*Saccharomyces cerevisiae*
Glycerol		*Zygosaccharomyces rouxii*
Methane	Methanogenic archaeans	

ISOLATION OF SUITABLE MICROORGANISMS FROM THE ENVIRONMENT II

- Examples of industrial fermentation products and their producer microorganisms.

Nucleotides		
5′-Inosine monophosphate	*Bacillus subtilis*	
5′-Guanosine monophosphate	*Brevibacterium ammoniagenes*	
Organic acids		
Acetic	*Acetobacter xylinum*	
Citric		*Aspergillus niger*
		Yarrowia lipolytica
Fumaric		*Rhizopus* species
Gluconic	*Acetobacter suboxydans*	
Itaconic		*Aspergillus itaconicus*
Kojic		*Aspergillus flavus*
Lactic	*Lactobacillus delbrueckii*	

continued

	Bacteria	Yeasts and filamentous fungi
Pharmaceuticals and related compounds		
Alkaloids		
ergotamine		*Claviceps purpurea*
ergometrine		*Claviceps fusiformis*
D-lysergic acid		*Claviceps paspali*
Antibiotics		
Aminoglycosides		
streptomycin	*Streptomyces griseus*	
β-Lactams		
penicillins		*Penicillium chrysogenum*
cephalosporins		*Acremonium chrysogenum*
clavulanic acid	*Streptomyces clavuligerus*	
Lantibiotics		
nisin	*Lactococcus lactis*	
Macrolides		
erythromycin	*Saccharapolyopora erythraea*	
Peptides		
bacitracin	*Bacillus licheniformis*	
gramicidin	*Bacillus brevis*	
Tetracyclines		
chlortetracycline	*Streptomyces aureofasciens*	
Hormones		
Human growth hormone	Recombinant *Escherichia coli*	Recombinant *Saccharomyces cerevisiae*
Insulin	Recombinant *Escherichia coli*	Recombinant *Saccharomyces cerevisiae*

Immunosuppressants		
Cyclosporin		*Trichoderma polysporum*
Interferon	Recombinant *Escherichia coli*	Recombinant *Saccharomyces cerevisiae*
Steroids	*Arthrobacter* species	*Rhizopus* species
Vaccines	*Bacillus anthracis*	
	Clostridium tetani	
	Recombinant *Escherichia coli*	
	Salmonella typhi	
Vitamins		
B_{12} (cyanocobalamin)	*Pseudomonas denitrificans*	
β-Carotene (provitamin A)		*Blakeslea trispora*
Ascorbic acid (vitamin C)	*Acetobacter suboxydans*	
Riboflavin	Recombinant *Bacillus subtilis*	*Ashbya gossypii*
Polymers		
Alginates	*Azotobacter vinelandii*	
Cellulose	*Acetobacter xylinum*	
Dextran	*Leuconostoc mesenteroides*	
Gellan	*Sphingomonas paucimobilis*	
Polyhydroxybutyrate	*Ralstonia eutropha*	
Pullulan		*Aureobasidium pullulans*
Scleroglucan		*Sclerotium rolfsii*
Xanthan	*Xanthomonas campestris*	
Single cell protein	*Methylococcus capsulatus*	*Candida utilis*
	Methylophilus methylotrophus	*Fusarium venenatum*
		Kluyveromyces marxianus
		Paecilomyces variotii
		Saccharomyces cerevisiae

Examples of microorganisms classified as GRAS (generally regarded as safe)

Bacteria
- *Bacillus subtilis*
- *Lactobacillus bulgaricus*
- *Lactococcus lactis*
- *Leuconostoc oenos*

Yeasts
- *Candida utilis*
- *Kluyveromyces marxianus*
- *Kluyveromyces lactis*
- *Saccharomyces cerevisiae*

Filamentous fungi
- *Aspergillus niger*
- *Aspergillus oryzae*
- *Mucor javanicus (Mucor circinelloides* f. *circinelloides)*
- *Penicillium roqueforti*

ISOLATION OF SUITABLE MICROORGANISMS FROM THE ENVIRONMENT II

- Subsequent isolation as pure cultures on solid growth media involves choosing or developing the appropriate selective media and growth conditions.
- Once isolated as pure cultures, each must be screened for the desired property; production of a specific enzyme, inhibitory compound, etc.

ISOLATION OF SUITABLE MICROORGANISMS FROM THE ENVIRONMENT II

- However, at this stage the level of activity or concentration of the target product *per se is not of* major concern, as strain development can normally be employed to vastly improve performance. Selected isolates must also be screened for other important features, such as stability and, where necessary, non-toxicity.

ISOLATION OF SUITABLE MICROORGANISMS FROM THE ENVIRONMENT III

- These isolation and screening procedures are more easily applied to the search for a single microorganism.
- However, it is much more difficult to isolate consortia which together have the ability/characteristic that is sought and whose composition may vary with time.

ISOLATION OF SUITABLE MICROORGANISMS FROM THE ENVIRONMENT III

- Such groups can be more efficient, particularly where the ability to degrade a complex recalcitrant compound is involved.

CULTURE COLLECTIONS

- Microbial culture collections provide a rich source of microorganisms that are of past, present and potential future interest.
- There are almost 500 culture collections around the world;
- Most of these are small, specialized collections that supply cultures or other related services only by special agreement.

CULTURE COLLECTIONS

- Others, notably national collections, publish catalogues listing the organisms held and provide extensive services for industrial and academic organizations.
- In the uk for example, the national culture collection (ukncc) is made up of several collections.

CULTURE COLLECTIONS

- They are housed in separate institutions and tend to specialize in bacteria, yeasts, filamentous fungi or algae of either industrial or medical importance;
- Whereas in the USA there is a main centralized collection,
- The american type culture collection (ATCC), which holds all types of microorganisms.

CULTURE COLLECTIONS

- The prime functions of a culture collection are to maintain the existing collection, to continue to collect new strains
- And to provide pure, authenticated culture samples of each organism.
- Problems of culture maintenance have been aided by the development and use of cryopreservation and freeze-drying (lyophilization) techniques, along with miniaturized storage methods.

CULTURE COLLECTIONS

- One convenient method involves adsorption of cells to glass beads (2 mm diameter)
- that may be placed in frozen storage,
- from which individual beads may be removed without thawing the whole sample.

CULTURE COLLECTIONS

- Use of microorganisms selected from a culture collection obviously provides significant cost savings compared with environmental isolation and has the advantage that some characterization of the microorganism will have already been performed.
- However, the disadvantage is that competitors have access to the same microorganism.

INDUSTRIAL STRAINS AND STRAIN IMPROVEMENT

- Irrespective of the origins of an industrial microorganism, it should ideally exhibit:

1. **Genetic stability;**
2. **Efficient production of the target product, whose** route of biosynthesis should preferably be well characterized;

INDUSTRIAL STRAINS AND STRAIN IMPROVEMENT

3. **Limited or no need for vitamins and additional growth** factors;
4. **Utilization of a wide range of low-cost and readily** available carbon sources;
5. **Amenability to genetic manipulation;**

INDUSTRIAL STRAINS AND STRAIN IMPROVEMENT

6. **Safety, non-pathogenicity and should not produce** toxic agents, unless this is the target product;
7. **Ready harvesting from the fermentation;**
8. **Ready breakage, if the target product is intracellular;** and
9. **Production of limited byproducts to ease subsequent** Purification problems.

Examples of some important culture collections useful to industrial microbiologists*

Culture collection	Type of microorganisms held
American Type Culture Collection (ATCC) Manassa, Virginia, USA	All
Centraalbureau voor Schimmelcultures (CBS) Baarn, The Netherlands	Filamentous fungi and yeasts
Collection Nationale de Cultures de Microorganismes (CNCM) Paris, France	All
Deutsche Sammlung von Mikroorganismen und Zellkulture (DSMZ) Braunscheig, Germany	All
UK microbial culture collections	
Culture Collection of Algae & Protozoa (Marine) (CCAP), Dunstaffnage Marine Laboratory, Oban	Algae and protozoa (marine)
Culture Collection of Algae & Protozoa (Freshwater) (CCAP), Institute for Freshwater Ecology, Ambleside	Algae and protozoa (freshwater)
European Collection of Animal Cell Cultures (ECACC), Centre for Applied Microbiological Research (CAMR), Porton Down	Animal cell cultures
CABI Bioscience UK Centre, Egham (formerly International Mycological Institute)	Filamentous fungi

National Collection of Food Bacteria (NCFB), Aberdeen	Food bacteria
National Collection of Industrial & Marine Bacteria (NCIMB), Aberdeen	Bacteria (general, industrial and marine)
National Collection of Pathogenic Fungi (NCPF), Public Health Laboratory, Bristol	Pathogenic fungi
National Collection of Plant Pathogenic Bacteria (NCPPB), Central Science Laboratory, Sand Hutton, York	Plant pathogenic bacteria
National Collection of Type Cultures (NCTC), Central Public Health Laboratory, Colindale	Medical microorganisms
National Collection of Wood Rotting Fungi (NCWRF), Building Research Establishment, Watford	Wood-rotting fungi
National Collection of Yeast Cultures (NCYC), Institute for Food Research, Norwich	Yeasts (other than known pathogens)

* For a comprehensive list see World Data Centre for Microorganisms.

INDUSTRIAL STRAINS AND STRAIN IMPROVEMENT

- Other features that may be exploited are thermophilic or halophilic properties, which may be useful in a fermentation environment.
- Also, particularly for cells grown in suspension,

INDUSTRIAL STRAINS AND STRAIN IMPROVEMENT

- They should grow well in conventional bioreactors to avoid the necessity to develop alternative systems.
- Consequently, they should not be shear sensitive, or generate excessive foam, nor be prone to attachment to surfaces.

STRAIN IMPROVEMENT

- Further strain improvement is a vital part of process development in most fermentation industries.
- It provides a means by which production costs can be reduced through increases in productivity or reduction of manufacturing costs.

STRAIN IMPROVEMENT

- In many cases strain improvement has been accomplished using natural methods of genetic recombination,
- which bring together genetic elements from two different genomes into one unit to form new genotypes.

Examples of targets for strain improvement

Rapid growth
Genetic stability
Non-toxicity to humans
Large cell size, for easy removal from the culture fluid
Ability to use cheaper substrates
Modification of submerged morphology
Elimination of the production of compounds that may
  interfere with downstream processing
Catabolite derepression
Phosphate deregulation
Permeability alterations to improve product export rates
Metabolite resistance
Production of
  additional enzymes
  compounds to inhibit contaminant microorganisms
  heterologous proteins that may also be engineered
  with downstream processing 'aids', e.g. polyarginine tails

STRAIN IMPROVEMENT

- An alternative strategy is via mutagenesis.
- Those recombinants and mutants are then subjected to screening and selection to obtain strains whose characteristics are more specifically suited to the industrial fermentation process.

STRAIN IMPROVEMENT

- However, such strains are unlikely to survive well in nature, as they often have altered regulatory controls that create metabolic imbalances.
- Also, they must then be maintained on specific media that select for, and help retain, the special characteristic(s).

NATURAL RECOMBINATION

- Bacterial DNA is usually in the form of a single chromosome and plasmids; the latter are autonomous selfreplicating accessory pieces of DNA.
- Each plasmid carries up to a few hundred additional genes and there may be as many as 1000 copies of a plasmid per cell.
- They contain supplemental genetic information coding for traits not found in the bacterium's chromosomal DNA.

NATURAL RECOMBINATION

- Unlike most eukaryotic organisms, bacteria have no form of sexual reproduction conjugation, transduction and transformation.
- However, they are able to exchange some genetic material via the processes of

NATURAL RECOMBINATION

- **Conjugation involves cell-to-cell contact, where the** donor contacts the recipient with a filamentous protein structure called a sex pilus,
- which draws the two cells close together.
- The donor copies all or a part of its plasmid or chromosomal DNA and passes it through the pilus to the recipient.

NATURAL RECOMBINATION

- In **transduction, a bacterial virus** (bacteriophage) acts as a vector in transferring genes between bacteria.
- The bacteriophage attaches to a bacterial cell and injects its dna into the host to become incorporated into the host chromosome.
- During bacteriophage replication the phage may acquire pieces of the adjacent host DNA.

NATURAL RECOMBINATION

- If the phages go on to enter new hosts, they are able to integrate their original DNA, and The genes picked up from their previous host, into the New host's chromosome.
- Bacteriophages, like plasmids, May also acquire transposons, which are pieces of DNA That can 'jump' from one piece of DNA to another, e.g. From a plasmid to a chromosome and vice versa.

NATURAL RECOMBINATION

- The bacteriophages can carry transposons on to new host bacterial cells, where they are able to 'jump' onto a plasmid or the host chromosomal DNA.
- The third process, **transformation, involves cellular uptake of a naked** piece of DNA from the surrounding medium, which then becomes incorporated into the cell.
- In natural environments this is a totally random process, the DNA fragments available for uptake being derived from cells that have lysed.

NATURAL RECOMBINATION

- The DNA fragments can be relatively large and may contain several genes.
- However, they are capable of entering and thus transforming only so-called '**competent' cells, which are in a specific** physiological state rendering them permeable to DNA.
- In eukaryotes, genetic recombination naturally occurs during sexual reproduction.

NATURAL RECOMBINATION

- New genotypes result from the combination of parental chromosomes and as a consequence of crossing-over events during meiosis.
- The latter involves breakage of sections of chromosomal DNA and the exchange of these segments between homologous chromosomes to form new combinations.
- Some industrially important fungi, including *penicillium* and *aspergillus, do not have a true sexual phase*.

NATURAL RECOMBINATION

- However, a **parasexual cycle has provided a route by** Which new strains can be produced.
- This is promotedwhen two genetically different haploid strains are Grown together, allowing fusion of their hyphae.
- These Events result in the formation of a heterokaryon, composed Of mycelium containing nuclei derived from each Strain.

NATURAL RECOMBINATION

- Direct formation of heterokaryons can now be Performed *in vitro by fusing protoplasts, which are cells* That have had their walls removed.
- Also, certain eukaryotes, Including some yeasts and filamentous fungi, possess Autonomous plasmids, such as the 2µm plasmid of *Saccharomyces cerevisiae, which have proved useful as* Vectors in genetic engineering

MUTAGENESIS: A CONVENTIONAL TOOL FOR STRAIN IMPROVEMENT

- Mutations result from a physical change to the DNA of a cell, such as deletion, insertion, duplication, inversion and translocation of a piece of DNA, or a change in the number of copies of an entire gene or chromosome.
- Subjection of microorganisms to repeated rounds of mutagenesis, followed by suitable selection and screening of the survivors, has been a very effective tool in improving many industrial microorganisms.

MUTAGENESIS: A CONVENTIONAL TOOL FOR STRAIN IMPROVEMENT

- As mutants can arise naturally or be induced, they are considered to be the product of natural events.
- Consequently, there are fewer problems in gaining approval from the regulatory authorities than when recombinant DNA technology is used to develop an industrial microorganism.

MUTAGENESIS: A CONVENTIONAL TOOL FOR STRAIN IMPROVEMENT

- Spontaneous mutation rates are low; in most bacterial genes for example, the rate is approximately 10–10 per generation per gene.
- The mutation rate can be greatly increased by using mutagens, which are of two types.
- Physical mutagens include ultraviolet, g and X radiation; and chemical mutagens are compounds such as ethane methane sulphonate (EMS), nitroso methyl guanidine (NTG), nitrous acid and acridine mustards.

MUTAGENESIS: A CONVENTIONAL TOOL FOR STRAIN IMPROVEMENT

- Mutants are formed when the mutagens induce modifications of the base sequences of DNA that result in basepair substitutions, frame-shift mutations or large deletions that go unrepaired.
- Mutagenesis can also be induced using transposons delivered by a suitable vector.
- They produce insertion mutants whose normal nucleotide sequence is interrupted by the transposon sequence.

MUTAGENESIS: A CONVENTIONAL TOOL FOR STRAIN IMPROVEMENT

- Mutagenesis methods generally have rather limited use as they primarily achieve either loss of an undesirable characteristic or increasing production of a product, due to impairment of a control mechanism.
- These traditional methods have been successfully employed in removing the yellow colour of early penicillin preparationspreparations caused by chrysogenin, a yellow pigment produced by *penicillium chrysogenum*.

MUTAGENESIS: A CONVENTIONAL TOOL FOR STRAIN IMPROVEMENT

- Mutagenesis programmes have also been highly effective in increasing the yield of penicillin in industrial strains of the same organism.
- Other notable examples of impairment of control processes, resulting in greater product yields, are seen in several microorganisms used for amino acid production.

MUTAGENESIS: A CONVENTIONAL TOOL FOR STRAIN IMPROVEMENT

- More recently, methods have been developed to enhance both the overall mutability and mutation rate of specific genes, in order to obtain the maximum frequency of desired mutant types.
- This **directed mutagenesis** obviously requires a knowledge of the genes that control the target product and often a genetic map of the organism.

MUTAGENESIS: A CONVENTIONAL TOOL FOR STRAIN IMPROVEMENT

- In addition, *in vitro mutagenesis is now used in* combination with genetic engineering to modify isolated genes or parts of genes.

GENETIC ENGINEERING OF MICROORGANISMS

- Over the last 20 years the development of recombinant DNA technology and methods of cell fusion, such as hybridoma formation for monoclonal antibody production, have had a major impact on industrial microbiology.
- In contrast to natural recombination processes, modern recombinant DNA technology provides almost unlimited opportunities for the production of novel combinations of genes.

GENETIC ENGINEERING OF MICROORGANISMS

- These methods are also highly specific and well controlled, and a vast range of genetic information is available from almost any living and even extinct organisms.
- Recombinant DNA technology has allowed specific gene sequences to be transferred from one organism to another and allows additional methods to be introduced into strain improvement schemes.

GENETIC ENGINEERING OF MICROORGANISMS

- This can be used to increase the product yield by removing metabolic bottlenecks in pathways and by amplifying or modifying specific metabolic steps.
- Overall, genetic engineering procedures allow totally new properties to be added to the capabilities of industrial microorganisms.

GENETIC ENGINEERING OF MICROORGANISMS

- Microorganisms may be manipulated to synthesize and often excrete enhanced ranges of enzymes, which may facilitate the production of novel compounds or allow the utilization of cheaper complex substrates.
- As there is no restriction to the origins of the genes that microorganisms express, the production of plant and animal proteins is made possible.

GENETIC ENGINEERING OF MICROORGANISMS

- Valuable products already produced include human growth hormone, insulin and interferons.
- Nevertheless, these methods have not totally replaced traditional mutatagenesis methods and the two approaches should be viewed as complementary strategies for strain improvement.

STRATEGIES FOR THE GENETIC ENGINEERING OF BACTERIA

- Genetic engineering involves manipulation of DNA outside the cell.
- It necessitates the initial isolation and recovery of the gene(s) of interest from the donor organism's genome.
- Isolated DNA sequences may then be modified and the regulation of their expression altered, before insertion into host organisms via a suitable easily manipulated vector system.

STRATEGIES FOR THE GENETIC ENGINEERING OF BACTERIA

- The first step requires total DNA extraction from the donor organism, which is then cut into smaller sequences using a specific **restriction endonuclease.**
- **Many of these restriction enzymes,** found in various species of bacteria, make a staggered cut through a double-stranded DNA molecule at a specific sequence or **palindrome.**

STRATEGIES FOR THE GENETIC ENGINEERING OF BACTERIA

- As a result, the ends of cut molecules have complementary single-stranded sequences.
- The small sections of DNA (restriction fragments) can then be joined or spliced into vector DNA molecules that have been cut with the same restriction enzyme.
- Splicing is performed by an enzyme, DNA ligase, and creates a synthetic DNA molecule.

Enzyme	Source	Restriction site
Eco RI	*Escherichia coli* RY13	5' – G A A T T C – 3' 3' – C T T A A G – 5'
Bam HI	*Bacillus amyloliquefaciens* H	5' – G G A T C C – 3' 3' – C C T A G G – 5'
Pst I	*Providencia stuartii* 164	5' – C T G C A G – 3' 3' – G A C G T C – 5'

STRATEGIES FOR THE GENETIC ENGINEERING OF BACTERIA

- Plasmids and bacteriophages have been the most useful cloning vectors.
- They play an important role as delivery systems to introduce the recombinant molecules into host cells via transformation or transduction.
- Once inside they are capable of autonomous replication, which maintains the recombinant DNA within the host cell.

STRATEGIES FOR THE GENETIC ENGINEERING OF BACTERIA

- Introduction of recombinant plasmids into bacterial cells can be achieved following calcium chloride treatment, which renders the cell membranes more permeable to DNA.
- After introduction the plasmids replicate autonomously.
- In some cases, numerous copies are produced within the host cell to increase the amount of the recombinant DNA per cell.

STRATEGIES FOR THE GENETIC ENGINEERING OF BACTERIA

- Plasmids can be designed to contain selectable genetic markers, such as antibiotic resistance, vitamin requirement, etc.
- These markers may be used to select only those host cells that have incorporated the plasmid during transformation, e.g. The 4.3kb plasmid pbr322, carrying ampicillin and tetracycline resistance markers.

STRATEGIES FOR THE GENETIC ENGINEERING OF BACTERIA

- Bacteriophages are particularly useful cloning vectors as up to half of their genome can be removed and replaced with foreign DNA.
- This is achieved *in vitro using* restriction enzymes in a similar manner to plasmid manipulation.
- Suitable DNA fragments are then packaged into phage particles, which are able to infect a selected host.

STRATEGIES FOR THE GENETIC ENGINEERING OF BACTERIA

- The mixture of restriction fragments, originating from a whole DNA extract, once packaged within phages or plasmids, is used to transform or transduce host cells.
- This generates a **DNA library consisting of** individual clones that contain different recombinant DNA molecules, representing all DNA sequences/genes of the donor genome.

STRATEGIES FOR THE GENETIC ENGINEERING OF BACTERIA

- Once the library has been established, the clones are allowed to form colonies on solid selective media.
- At this stage, the specific clone containing the recombinant dna molecule of interest can be identified.
- If the foreign gene is successfully expressed in the host Bacterium and a heterologous protein is made, Detection can be achieved by use of a specific antibody Reaction with the protein.
- Alternatively, if the recombinant Protein is an enzyme that is not normally produced By the host, the enzyme activity can be detected

LIMITATIONS OF PROKARYOTIC HOSTS I

- Often, the whole purpose of cloning a gene is to obtain large quantities of its product.
- If *escherichia coli is used as the host and the gene introduced* is not from an e. *Coli strain, i.E. A heterologous* gene, it may not be expressed.

LIMITATIONS OF PROKARYOTIC HOSTS I

- This problem can be overcome using expression vectors in which the foreign gene is inserted in a configuration that puts it under regulatory controls recognized by the host.
- Additionally, tomaximize production of the foreign protein, the expression vector must replicate to a high copy number and be stable.

LIMITATIONS OF PROKARYOTIC HOSTS I

- The foreign gene should ideally be linked to a strong promoter that has a high affinity for RNA polymerase and the transcribed mrna should be efficiently translated.
- Sometimes, it may be advantageous for the expression of the cloned gene to be manipulated by placing it under the control of a regulatory switch, in order that production of the recombinant protein does not occur until required.

LIMITATIONS OF PROKARYOTIC HOSTS I

- Generally, gram-negative bacteria are able to express genes from gram-positive bacteria.
- However, the converse is not always as readily achievable.
- Additional problems also arise if the objective is to clone and express genes from a eukaryotic organism in a bacterium such as *E. Coli*.

LIMITATIONS OF PROKARYOTIC HOSTS I

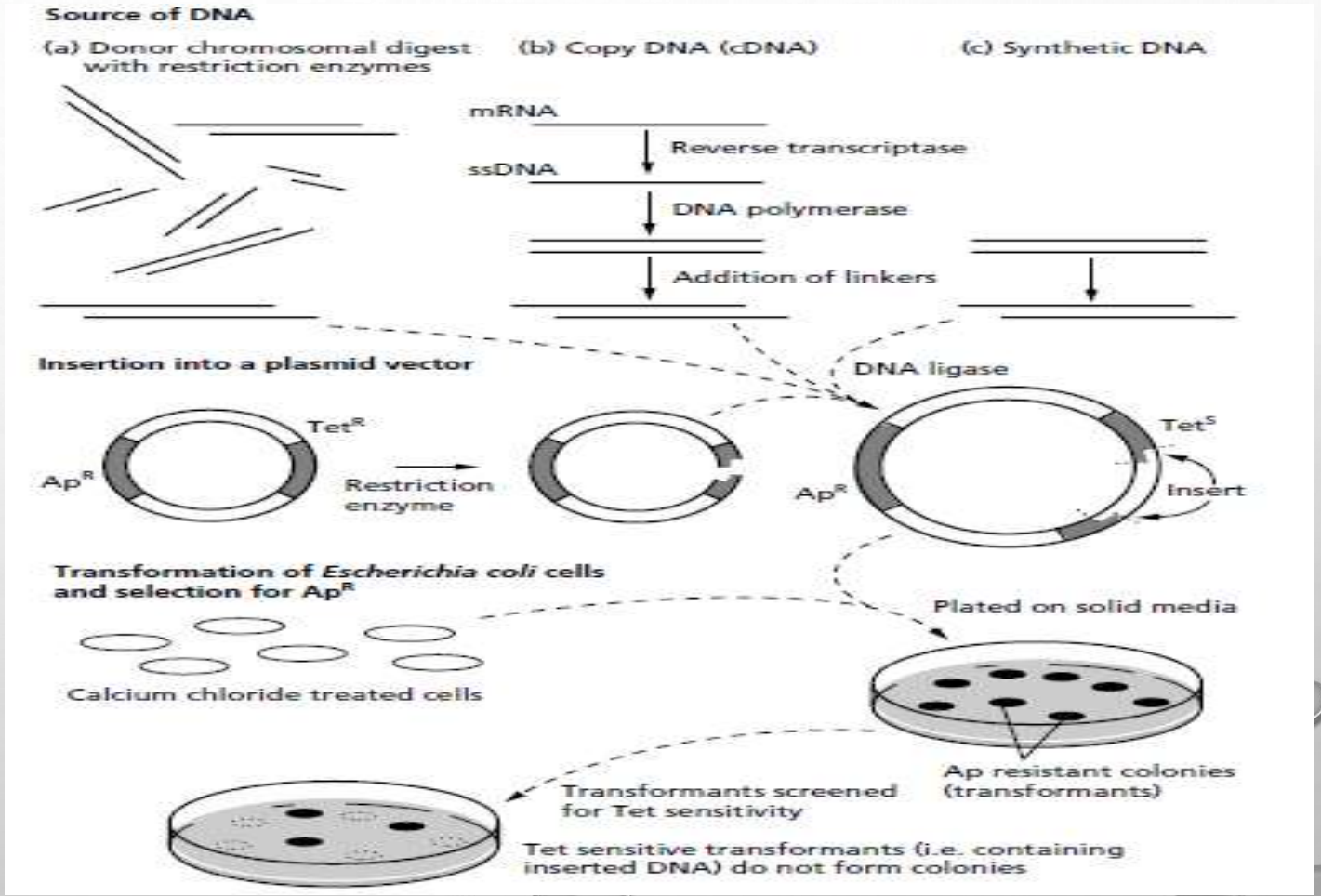
- Here the differences between prokaryotic and eukaryotic gene expression must be taken into account.
- Eukaryotic genes contain non-coding regions, introns, which would obviously cause problems if the gene was directly transferred to a prokaryotic system.
- However, introns are excised during normal rna processing.

LIMITATIONS OF PROKARYOTIC HOSTS I

- Consequently, eukaryotic mRNA can be used to synthesize a gene that can function within a prokaryote.
- This requires the use of reverse transcriptase to generate a complementary DNA copy or cDNA from the RNA.
- Alternatively, if the gene nucleotide sequence or amino acid sequence of the product protein is known, a synthetic gene can be synthesized

LIMITATIONS OF PROKARYOTIC HOSTS I

Outline of a strategy for cloning DNA in *E. coli* (after Brown *et al.* (1987)). The source DNA is usually DNA fragment from bacteria, or, for eukaryotic sources, synthetic or cDNA (derived via reverse transcription of mRNA) is used. The DNA fragment, from whatever source, is ligated into a plasmid vector containing suitable markers; in this case, resistance to ampicillin (Ap^R) and tetracycline (Tet^R). Ligation is into the tetracycline resistance gene thus inactivating it (Tet^S). The vector is then used to transform the host cells. All cells successfully transformed exhibit ampicillin resistance. However, of those, only cells containing foreign DNA are tetracycline sensitive. Those containing foreign DNA can then be screened for the desired gene or gene product.



LIMITATIONS OF PROKARYOTIC HOSTS I

- Secretion of recombinant proteins is often preferred for product stability and it may make downstream processing to recover the product less problematical.
- However, it presents a further challenge as some organisms excrete more efficiently than others.

LIMITATIONS OF PROKARYOTIC HOSTS II

- In gram-negative bacteria, secretion is often into the periplasmic space rather than directly into the medium, as proteins cannot readily traverse their outer membrane.
- For some purposes, this may be beneficial as it often simplifies downstream processing.

LIMITATIONS OF PROKARYOTIC HOSTS II

- Where secretion into the medium is necessary, it may be achieved by using cell-wall-less bacteria (l-forms).
- Secretion of proteins is further complicated by the fact that they must be synthesized with an extra amino acid sequence at their n-terminal end.

LIMITATIONS OF PROKARYOTIC HOSTS II

- SIGNAL SEQUENCE OF 20–25 AMINO ACIDS AIDS THE PASSAGE OF THE PROTEIN ACROSS THE CELL MEMBRANE AND IS REMOVED DURING SECRETION.

LIMITATIONS OF PROKARYOTIC HOSTS II

- Other general problems in the expression of heterologous proteins, which may also be encountered with some eukaryotic hosts (see below) include:
- **1 instability of certain gene products within the host**
- Microorganism, leading to their rapid degradation by proteases;

LIMITATIONS OF PROKARYOTIC HOSTS II

- **2 incorrect folding of the polypeptide that generates an**
- Inactive molecule, which may accumulate to form inclusion bodies within the cell; and
- **3 difficulties in achieving post-translational modification**
- Of proteins, such as cleavage, glycosylation or amidation.

EUKARYOTIC HOSTS

- A preferred alternative strategy for the expression of heterologous eukaryotic genes is often the employment of a suitable eukaryotic host
- that will naturally perform any necessary post-translational protein modification and secretion.

EUKARYOTIC HOSTS

- Initially, *saccharomyces cerevisiae was a* popular choice because it is safe and a vast amount of information has been accumulated about its genetics, physiology and performance in industrial fermentation processes.
- Also, it has a relatively rapid growth rate and readily undergoes genetic manipulation,
- but unliken higher eukaryotes (animal and plant cells) this yeast is Easily and cheaply grown on an industrial scale.

EUKARYOTIC HOSTS

- Several heterologous eukaryotic proteins have been successfully mass produced by *S. Cerevisiae*.
- *However, product yields* are relatively low at 1–5% of total protein and some proteins are retained within the periplasm.
- Other yeasts may be better hosts, particularly the methylotrophs, *pichia angusta (formerly hansenula polymorpha) and pichia pastoris, which have a number of advantages* over *S. Cerevisiae*.

EUKARYOTIC HOSTS

- They have strong inducible promoters and are capable of generating post-translational protein modification similar to those performed by human cells.
- Downstream processing is also less problematical as they do not secrete many of their own proteins into the medium.

EUKARYOTIC HOSTS

- *P. Angusta has often been preferred for industrial* applications due to greater versatility;
- examples of established heterologous products from this yeast include hepatitis B vaccine, the feed additive enzyme phytase and the antithrombotic hirudin.

EUKARYOTIC HOSTS

- Developments in the field of recombinant DNA technology are progressing at a rapid rate and gene cloning in animal and plant cells is now relatively routine.
- For example, vectors based on viruses, such as bovine papilloma virus, retroviruses and simian virus 40 are used to stably transform mammalian cells.

EUKARYOTIC HOSTS

- Transformation of dicotyledonous plant cells can be performed using plasmids derived from the soil bacteria *agrobacterium tumefaciens and A. Rhizogenes.*
- *In vivo, A. Tumefaciens* induces tumours that involves its TI plasmid, and *A. Rhizogenes induces formation of hairy roots, which is* mediated by the RI plasmid.

EUKARYOTIC HOSTS

- Individual proteins can now be engineered by changing a few component amino acids in order to modify their properties, and there are opportunities to 'buildin' features that aid downstream processing.
- In addition, the complete sequencing of many microbial genomes, including several food grade organisms, is stimulating further advances in metabolic engineering.

EUKARYOTIC HOSTS

- Relatively minor metabolic engineering has already been implemented to improve the production of existing metabolites, allow the production of new metabolites, impart new catabolic activities and improve fermentation performance.
- However, whole metabolic networks within a microorganism may now be restructured and such extensive metabolicengineering has major implications within industrial microbiology.

STRAIN STABILITY

- A key factor in the development of new strains is their stability.
- An important aspect of this is the means of preservation and storage of stock cultures so that their carefully selected attributes are not lost.

STRAIN STABILITY

- This may involve storage in liquid nitrogen or lyophilization.
- Strains transformed by plasmids must be maintained under continual selection to ensure that plasmid stability is retained.
- Instability may result from deletion and rearrangements of recombinant plasmids, which is referred to as **structural instability, or complete loss of a** plasmid, termed **segregational stability.**

STRAIN STABILITY

- Some of these problems can be overcome by careful construction of the plasmid and the placement of essential genes within it.
- Segregational instability can also be overcome by constructing so-called suicidal strains that require specific markers on the plasmid for survival.

STRAIN STABILITY

- Consequently, plasmid-free cells die and do not accumulate in the culture. These strains are constructed with a lethal marker in the chromosome and a repressor of this marker is located on the plasmid.

STRAIN STABILITY

- Cells express the repressor as long as they possess the plasmid, but if it is lost the cells express the lethal gene.
- However, integration of a gene(s) into the chromosome is normally the best solution, as it overcomes many of these instability problems.

MEDIA FORMULATION I

- Most fermentations require liquid media, often referred to as broth, although some solid-substrate fermentations are operated.
- Fermentation media must satisfy all the nutritional requirements of the microorganism and fulfil the technical objectives of the process.

MEDIA FORMULATION I

- The nutrients should be formulated to promote the synthesis of the target product, either cell biomass or a specific metabolite.
- In most industrial fermentation processes there are several stages where media are required.
- They may include several inoculum (starter culture) propagation steps, pilot-scale fermentations and the main production fermentation.

MEDIA FORMULATION I

- The technical objectives of inoculum propagation and the main fermentation are often very different, which may be reflected in differences in their media formulations.
- Where biomass or primary metabolites are the target product, the objective is to provide a production medium that allows optimal growth of the microorganism.

MEDIA FORMULATION I

- For secondary metabolite production, such as antibiotics, their biosynthesis is not growth related.
- Consequently, for this purpose, media are designed to provide an initial period of cell growth, followed by conditions optimized for secondary metabolite production.

MEDIA FORMULATION I

- At this point the supply of one or more nutrients (carbon, phosphorus or nitrogen source) may be limited and rapid growth ceases.
- Most fermentations, except those involving solid substrates, require large quantities of water in which the medium is formulated.

MEDIA FORMULATION I

- General media requirements include a carbon source, which in virtually all industrial fermentations provides both energy and carbon units for biosynthesis, and sources of nitrogen, phosphorus and sulphur.
- Other minor and trace elements must also be supplied, and some microorganisms require added vitamins, such as biotin and riboflavin.

MEDIA FORMULATION I

- Aerobic fermentations are dependent on a continuous input of molecular oxygen, and even some anaerobic fermentations require initial aeration of media,
- For example beer fermentationsusually, media incorporate buffers, or the PH is controlled by acid and alkali additions, and antifoam agents may be required.
- For some processes, precursor, inducer or inhibitor compounds must be introduced at certain stages of the fermentation.

MEDIA FORMULATION I

- The initial step in media formulation is the examination of the overall process based on the stoichiometry for growth and product formation.
- This primarily involves consideration of the input of the carbon and nitrogen sources, minerals and oxygen, and their conversion to cell biomass, metabolic products, carbon dioxide, water and heat.

MEDIA FORMULATION I

- From this information it should be possible to calculate the minimum quantities of each element required to produce a certain quantity of biomass or metabolite.
- Typically, the main elemental formula of microbial cells is approximately $C_4H_7O_2N$, which on a basis of dry weight is 48% C, 7% H, 32% O and 14% N.

MEDIA FORMULATION II

- Elemental composition varies slightly with growth rate, but the range is relatively small compared with interspecies differences, particularly between bacteria and fungi.
- Ideally, a knowledge of the complete elemental composition of the specific industrial microorganism allows further media refinement.

MEDIA FORMULATION II

- This ensures that no element is limiting, unless this is desired for a specific purpose.
- Once the elemental requirements of a microorganism have been established, suitable nutrient sources can be incorporated into the media to fulfil these demands.
- However, it is important to be aware of potential problems that can arise when using certain compounds.

MEDIA FORMULATION II

- For example, those that are rapidly metabolized may repress product formation.
- To overcome this, intermittent or continuous addition of fresh medium may be carried out to maintain a relatively low concentration that is not repressive.
- Certain media nutrients or environmental conditions may affect not only the physiology and biochemistry, but also the morphology of the microorganism.

MEDIA FORMULATION II

- In some yeasts the single cells may develop into pseudo-mycelium or flocculate, and filamentous fungi may form pellets.
- This may or may not be desirable, as such morphological changes can influence product yield and other fermentation properties.
- The media adopted also depend on the scale of the fermentation.
- For small-scale laboratory fermentations pure chemicals are often used in well-defined media.

MEDIA FORMULATION II

- However, this is not possible for most industrial-scale fermentation processes, simply due to cost, as media components may account for up to 60–80% of process expenditure.
- Industrial-scale fermentations primarily use cost-effective complex substrates, where many carbon and nitrogen sources are almost undefinable.
- Most are derived from natural plant and animal materials, often byproducts of other industries, with varied and variable composition.

MEDIA FORMULATION II

- The effects of such batchto- batch variations must be determined.
- Small-scale trials are usually performed with each new batch of substrate, particularly to examine the impact on product yield and product recovery.
- The main factors that affect the final choice of individual raw materials are as follows.

1. **cost and availability: ideally, materials should be** Inexpensive, and of consistent quality and year round Availability.

MEDIA FORMULATION II

2. **Ease of handling in solid or liquid forms, along with** associated transport and storage costs, e.g. Requirements for temperature control.
3. **Sterilization requirements and any potential denaturation** problems.
4. **Formulation, mixing, complexing and viscosity** characteristics that may influence agitation, aeration and foaming during fermentation and downstream processing stages.

MEDIA FORMULATION II

- **5 the concentration of target product attained, its rate** of formation and yield per gram of substrate utilized.
- **6 the levels and range of impurities, and the potential** for generating further undesired products during the process.
- ***7* overall health and safety implications.**

MEDIA FORMULATION II

- The final composition of industrial media is not solely the concern of the fermentation stage.
- Crude substrates provide initial cost savings, but their higher levels of impurities may necessitate more costly and complex recovery and purification steps downstream, and possibly increased waste treatment costs.
- Also, the physical and chemical properties of the formulated medium can influence the sterilization operations employed.

MEDIA FORMULATION II

- A medium that is easily sterilized with minimum thermal damage is vitally important.
- Thermal damage not only reduces the level of specific ingredients, but can also produce Potentially inhibitory byproducts that may also Interfere with downstream processing.
- Other media Characteristics can affect product recovery and purification, And the ease with which the cells are separated From the spent medium.

CARBON SOURCES I

- A carbon source is required for all biosynthesis leading to reproduction, product formation and cell maintenance.
- In most fermentations it also serves as the energy source.
- Carbon requirements may be determined from the biomass yield coefficient (*Y*), *an index of the efficiency* of conversion of a substrate into cellular material.

CARBON SOURCES I

- *Ycarbon g/g* =biomass produced (g) /carbon substrate utilized (g)
- For commercial fermentations the determination of yield coefficients for all other nutrients is usually essential.
- Each may be determined by conducting a series of batch culture experiments where the specific substrate is the only growth-limiting media component and all other nutrients are in excess.

CARBON SOURCES I

- By varying the initial concentration of the growth-limiting substrate and then plotting total growth against substrate concentration for each batch, the growth yield (*Y*) *can* be estimated.
- However, the value obtained relates to a specific set of operating conditions; varying ph, temperature, etc., Can alter the yield coefficient.

CARBON SOURCES I

- Various organisms may exhibit different yield coefficients for the same substrate, due primarily to the pathway by which the compound is metabolized.
- Differences can also be seen within an individual.
- For example, *saccharomyces cerevisiae grown on glucose* has biomass yield coefficients of 0.56 and 0.12 g/g under aerobic and anaerobic conditions, respectively.

CARBON SOURCES I

- As most carbon substrates also serve as energy sources, the organism's efficiency of both adenosine triphosphate (ATP) generation and its utilization are obviously additional key factors.
- Often, it is very useful, although rather difficult, to estimate how much ATP is required for growth.

	$Y_{glucose}$	$Y_{ethanol}$	$Y_{methanol}$	Y_{octane}
Aerobic growth				
Aspergillus nidulans	0.61			
Candida utilis	0.51	0.68		
Escherichia coli	0.52			
Pichia angusta			0.36	
Penicillium chrysogenum	0.43			
Pseudomonas aeruginosa	0.43			
Pseudomonas species			0.54	1.07
Saccharomyces cerevisiae	0.56	0.63		
Anaerobic growth				
Moorella thermacetica	0.11			
Escherichia coli	0.13			
Klebsiella pneumoniae	0.12			
Saccharomyces cerevisiae	0.12			

Growth yield (Y_{carbon}) on minimal medium plus various carbon and energy sources

CARBON SOURCES I

- However, estimates of *YATP (yield* of cells per mole of ATP generated during growth) can be calculated if the metabolism of the organism has been fully elucidated.
- Carbohydrates are traditional carbon and energy sources for microbial fermentations, although other sources may be used, such as alcohols, alkanes and organic acids.
- Animal fats and plant oils may also be incorporated into some media, often as supplements to the main carbon source.

CARBON SOURCES I

- MOLASSES

- Pure glucose and sucrose are rarely used for industrialscale fermentations, primarily due to cost.
- Molasses, a byproduct of cane and beet sugar production, is a cheaper and more usual source of sucrose.
- This material is the residue remaining after most of the sucrose has been crystallized from the plant extract.

CARBON SOURCES I

- It is a dark coloured viscous syrup containing 50–60% (w/v) carbohydrates, primarily sucrose, with 2% (w/v) nitrogenous substances, along with some vitamins and minerals.
- Overall composition varies depending upon the plant source, the location of the crop, the climatic conditions under which it was grown and the factory where it was processed.
- The carbohydrate concentration may be reduced during storage by contaminating microorganisms.
- A similar product, hydrol molasses, can also be used.
- This byproduct of maize starch processing primarily contains glucose.

CARBON SOURCES II

- **MALT EXTRACT**

- Aqueous extracts of malted barley can be concentrated to form syrups that are particularly useful carbon sources for the cultivation of filamentous fungi, yeasts and actinomycetes.
- Extract preparation is essentially the same as for malt wort production in beer brewing.

CARBON SOURCES II

- The composition of malt extracts varies to some extent, but they usually contain approximately 90% carbohydrate, on a dry weight basis.
- This comprises 20% hexoses (glucose and small amounts of fructose), 55% disaccharides (mainly maltose and traces of sucrose), along with 10% maltotriose, a trisaccharide.

CARBON SOURCES II

- In addition, these products contain a range of branched and unbranched dextrins (15–20%), which may or may not be metabolized, depending upon the microorganism.
- Malt extracts also contain some vitamins and approximately 5% nitrogenous substances, proteins, peptides and amino acids.

CARBON SOURCES II

- Sterilization of media containing malt extract must be carefully controlled to prevent over-heating.
- The constituent reducing sugars and amino acids are prone to generating maillard reaction products when heated at low ph.

CARBON SOURCES II

- These are brown condensation products resulting from the reaction of amino groups of amines, amino acids and proteins with the carbonyl groups of reducing sugars, ketones and aldehydes.
- Not only does this cause colour change, but it also results in loss of fermentable materials and some reaction products may inhibit microbial growth.

CARBON SOURCES II

- **STARCH AND DEXTRINS**

- These polysaccharides are not as readily utilized as monosaccharides and disaccharides, but can be directly metabolized by amylase-producing microorganisms, particularly filamentous fungi.

CARBON SOURCES II

- Their extracellular enzymes hydrolyse the substrate to a mixture of glucose, maltose or maltotriose to produce a sugar spectrum similar to that found in many malt extracts.
- Maize starch is most widely used, but it may also be obtained from other cereal and root crops.

CARBON SOURCES II

- To allow use in a wider range of fermentations, the starch is usually converted into sugar syrup, containing mostly glucose.
- It is first gelatinized and then hydrolysed by dilute acids or amylolytic enzymes, often microbial glucoamylases that operate at elevated temperatures.

CARBON SOURCES III

- **SULPHITE WASTE LIQUOR**

- Sugar containing wastes derived from the paper pulping industry are primarily used for the cultivation of yeasts.
- Waste liquors from coniferous trees contain 2–3% (w/v) sugar, which is a mixture of hexoses (80%) and pentoses (20%).
- Hexoses include glucose, mannose and galactose, whereas the pentose sugars are mostly xylose and arabinose.

CARBON SOURCES III

- Those liquors derived from deciduous trees contain mainly pentoses.
- Usually the liquor requires processing before use as it contains sulphur dioxide.
- The low ph is adjusted with calcium hydroxide or calcium carbonate, and these liquors are supplemented with sources of nitrogen and phosphorus.

CARBON SOURCES III

- **CELLULOSE**

- Cellulose is predominantly found as lignocellulose in plant cell walls, which is composed of three polymers: cellulose, hemicellulose and lignin.
- Lignocellulose is available from agricultural, forestry, industrial and domestic wastes.

CARBON SOURCES III

- Relatively few microorganisms can utilize it directly, as it is difficult to hydrolyse.
- The cellulose component is in part crystalline, encrusted with lignin, and provides little surface area for enzyme attack.
- At present it is mainly used in solid-substrate fermentations to produce various mushrooms.
- However, it is potentially a very valuable renewable source of fermentable sugars once hydrolysed, particularly in the bioconversion to ethanol for fuel use.

CARBON SOURCES III

- **WHEY**

- Whey is an aqueous byproduct of the dairy industry.
- The annual worldwide production is over 80 million tonnes, containing over 1 million tonnes of lactose and 0.2 million tonnes of milk protein.
- This material is expensive to store and transport.
- Therefore, lactose concentrates are often prepared for later fermentation by evaporation of the whey, following removal of milk proteins for use as food supplements.

CARBON SOURCES III

- Lactose is generally less useful as a fermentation feedstock than sucrose, as it is metabolized by fewer organisms. *S. Cerevisiae, for example,* does not ferment lactose.
- This disaccharide was formerly used extensively in penicillin fermentations and it is still employed for producing ethanol, single cell protein, lactic acid, xanthan gum, vitamin B12 and gibberellic acid.

CARBON SOURCES III

- **ALKANES AND ALCOHOLS**

- *N-alkanes of chain length C10–C20 are readily metabolized* by certain microorganisms.
- Mixtures, rather than a single compound, are usually most suitable for microbial fermentations.
- However, their industrial use is dependent upon the prevailing price of petroleum.
- Methane is utilized as a carbon source by a few microorganisms, but its conversion product methanol is often preferred for industrial fermentations as it presents fewer technical problems.

CARBON SOURCES IV

- High purity methanol is readily obtained and it is completely miscible with water.
- Methanol has a high per cent carbon content and is relatively cheap, although only a limited number of organisms will metabolize it.
- Also, unlike many other carbon sources, only low concentrations, 0.1–1% (v/v), are tolerated by microorganisms, higher levels being toxic.
- During fermentations on methanol, the oxygen demand and heat of fermentation are high, but this is even more problematic when growing on alkanes.

CARBON SOURCES IV

- Several companies used methanol in microbial protein production in the 1970s and early 1980s, but these processes are currently uneconomic.
- Ethanol is less toxic than methanol and is used as a sole or cosubstrate by many microorganisms, but it is too expensive for general use as a carbon source.
- However, its biotransformation to acetic acid by acetic acid bacteria remains a major fermentation process

CARBON SOURCES IV

- **FATS AND OILS**

- Hard animal fats that are mostly composed of glycerides of palmitic and stearic acids are rarely used in fermentations.
- However, plant oils (primarily from cotton seed, linseed, maize, olive, palm, rape seed and soya) and occasionally fish oil, may be used as the primary or supplementary carbon source, especially in antibiotic production.

CARBON SOURCES IV

- Plant oils are mostly composed of oleic and linoleic acids, but linseed and soya oil also have a substantial amount of linolenic acid.
- The oils contain more energy per unit weight than carbohydrates.

CARBON SOURCES IV

- In addition, the carbohydrates occupy a greater volume, because they are usually prepared as aqueous solutions of concentrations no greater than 50% (w/w).
- Consequently, oils can be particularly useful in fed-batch operations, as less spare capacity is needed to accommodate further additions of the carbon source.

NITROGEN SOURCES I

- Most industrial microbes can utilize both inorganic and organic nitrogen sources.
- Inorganic nitrogen may be supplied as ammonium salts, often ammonium sulphate and diammonium hydrogen phosphate, or ammonia.
- Ammonia can also be used to adjust the ph of the fermentation.

NITROGEN SOURCES I

- Organic nitrogen sources include amino acids, proteins and urea.
- Nitrogen is often supplied in crude forms that are essentially byproducts of other industries, such as corn steep liquor, yeast extracts, peptones and soya meal.
- Purified amino acids are used only in special situations, usually as precursors for specific products.

NITROGEN SOURCES I

- CORN STEEP LIQUOR corn steep liquor is a byproduct of starch extraction from maize and its first use in fermentations was for penicillin production in the 1940s.
- The exact composition of the liquor varies depending on the quality of the maize and the processing conditions.

NITROGEN SOURCES I

- Concentrated extracts generally contain about 4% (w/v) nitrogen, including a wide range of amino acids, along with vitamins and minerals.
- Any residual sugars are usually converted to lactic acid (9–20%, w/v) by contaminating bacteria.
- Corn steep liquor can sometimes be replaced by similar liquors, such as those derived from potato starch production.

NITROGEN SOURCES I

- YEAST EXTRACTS yeast extracts may be produced from waste baker's and brewer's yeast, or other strains of *S. Cerevisiae*.
- *Alternate* sources are *kluyveromyces marxianus (formerly classified* as *K. Fragilis) grown on whey and candida utilis cultivated* using ethanol, or wastes from wood and paper processing.
- Those extracts used in the formulation of fermentation media are normally salt-free concentrates of soluble components of hydrolysed yeast cells.

NITROGEN SOURCES I

- Yeast extracts with sodium chloride concentrations greater than 0.05% (w/v) cannot be used in fermentation processes due to potential corrosion problems.
- Yeast cell hydrolysis is often achieved by autolysis, using the cell's endogenous enzymes, usually without the need for additional hydrolytic enzymes.

NITROGEN SOURCES I

- Autolysis can be initiated by temperature or osmotic shock, causing cells to die but without inactivating their enzymes.
- Temperature and ph are controlled throughout to ensure an optimal and standardized autolysis process.
- Temperature control is particularly important to prevent loss of vitamins. Autolysis is performed at 50–55°C for several hours before the temperature is raised to 75°C to inactivate the enzymes.

NITROGEN SOURCES I

- Finally, the cells are disrupted by plasmolysis or mechanical disruption.
- Cell wall materials and other debris are removed by filtration or centrifugation and the resultant extract is rapidly concentrated.
- Extracts are available as liquids containing 50–65% solids, viscous pastes or dry powders.
- They contain amino acids, peptides, watersoluble vitamins and some glucose, derived from the yeast storage carbohydrates (trehalose and glycogen).

NITROGEN SOURCES II

- **PROTIENS**

- Proteins are usually too expensive for large-scale industrial fermentations.
- They are prepared by acid or enzyme hydrolysis of high protein materials: meat, casein, gelatin, keratin, peanuts, soy meal, cotton seeds, etc.

NITROGEN SOURCES II

- Their amino acid compositions vary depending upon the original protein source.
- For example, gelatinderived peptones are rich in proline and hydroxyproline, but are almost devoid of sulphur-containing amino acids;
- whereas keratin peptone is rich in both proline and cystine, but lacks lysine.
- Peptones from plant sources invariably contain relatively large quantities of carbohydrates.

Protein and vitamin composition of yeast extract

Total proteins, peptides & amino acids (%, w/v)	73–75
free amino acids	35–40
peptides less than 600 Da	10–15
material above 600 Da	20–30
Vitamins (µg/g)	
thiamin	30
riboflavin	120
niacin	700
pyridoxine	20
folic acid	30
calcium pantothenate	300
biotin	2.5

Note: mineral content varies with the processing steps used.

NITROGEN SOURCES II

- SOYA BEAN MEAL residues remaining after soya beans have been processed to extract the bulk of their oil are composed of 50% protein, 8% non-protein nitrogenous compounds, 30% carbohydrates and 1% oil.

NITROGEN SOURCES II

- This residual soya meal is often used in antibiotic fermentations because the components are only slowly metabolized, thereby eliminating the possibility of repression of product formation.

WATER, PRECURSORS, INDUCERS AND ELICITORS

- **WATER**

- All fermentation processes, except solid-substrate fermentations, require vast quantities of water.
- In many cases it also provides trace mineral elements. Not only is water a major component of all media, but it is important for ancillary equipment and cleaning.

WATER, PRECURSORS, INDUCERS AND ELICITORS

- A reliable source of large quantities of clean water, of consistent composition, is therefore essential.
- Before use, removal of suspended solids, colloids and microorganisms is usually required.
- When the water supply is ‘hard’, it is treated to remove salts such as calcium carbonate.

WATER, PRECURSORS, INDUCERS AND ELICITORS

- Iron and chlorine may also require removal.
- For some fermentations notably plant and animal cell culture, the water must be highly purified.
- Water is becoming increasingly expensive, necessitating its recycle/reusage wherever possible.
- This minimizes water costs and reduces the volume requiring waste-water treatment.

WATER, PRECURSORS, INDUCERS AND ELICITORS

- **MINERALS**
- Normally, sufficient quantities of cobalt, copper, iron, manganese, molybdenum, and zinc are present in the water supplies, and as impurities in other media ingredients.
- For example, corn steep liquor contains a wide range of minerals that will usually satisfy the minor and trace mineral needs.
- Occasionally, levels of calcium, magnesium, phosphorus, potassium, sulphur and chloride ions are too low to fulfil requirements and these may be added as specific salts.

WATER, PRECURSORS, INDUCERS AND ELICITORS

➢ VITAMINS AND GROWTH FACTORS

- Many bacteria can synthesize all necessary vitamins from basic elements.
- For other bacteria, filamentous fungi and yeasts, they must be added as supplements to the fermentation medium.
- Most natural carbon and nitrogen sources also contain at least some of the required vitamins as minor contaminants.

WATER, PRECURSORS, INDUCERS AND ELICITORS

- Other necessary growth factors, amino acids, nucleotides, fatty acids and sterols, are added either in pure form or, for economic reasons, as less expensive plant and animal extracts.

- **PRECURSORS**

- Some fermentations must be supplemented with specific precursors, notably for secondary metabolite production.
- When required, they are often added in controlled quantities and in a relatively pure form.

WATER, PRECURSORS, INDUCERS AND ELICITORS

- Examples include phenylacetic acid or phenylacetamide added as side-chain precursors in penicillin production.
- Dthreonine is used as a precursor in l-isoleucine production by *serratia marsescens, and anthranillic acid* additions are made to fermentations of the yeast *hansenula anomala during l-tryptophan production.*

WATER, PRECURSORS, INDUCERS AND ELICITORS

➢ INDUCERS AND ELICITORS

- If product formation is dependent upon the presence of a specific inducer compound or a structural analogue, it must be incorporated into the culture medium or added at a specific point during the fermentation.
- In plant cell culture the production of secondary metabolites, such as flavonoids and terpenoids, can be triggered by adding elicitors.
- These may be isolated from various microorganisms, particularly plant pathogens.

WATER, PRECURSORS, INDUCERS AND ELICITORS

- Inducers are often necessary in fermentations of genetically modified microorganisms (gmms).
- This is because the growth of gmms can be impaired when the cloned genes are ‘switched on’, due to the very high levelsn of their transcription and translation.
- Consequently, inducible systems for the cloned genes are incorporated that allow initial maximization of growth to establish high biomass density,
- whereupon the cloned gene can then be ‘switched on’ by the addition of the specific chemical inducer.

INHIBITORS AND CELL PERMEABILITY MODIFIERS

- **INHIBITORS**

- Inhibitors are used to redirect metabolism towards the target product and reduce formation of other metabolic intermediates;
- others halt a pathway at a certain point to prevent further metabolism of the target product.
- An example of an inhibitor specifically employed to redirect metabolism is sodium bisulphite, which is used in the production of glycerol by *S. Cerevisiae*.

INHIBITORS AND CELL PERMEABILITY MODIFIERS

- Some gmms contain plasmids bearing an antibiotic resistance gene, as well as the heterologous gene(s).
- The incorporation of this antibiotic into the medium used for the production of the heterologous product selectively inhibits any plasmid-free cells that may arise.

INHIBITORS AND CELL PERMEABILITY MODIFIERS

- **CELL PERMEABILITY MODIFIERS**

- These compounds increase cell permeability by modifying cell walls and/or membranes, promoting the release of intracellular products into the fermentation medium.

INHIBITORS AND CELL PERMEABILITY MODIFIERS

- Compounds used for this purpose include penicillins and surfactants.
- They are frequently added to amino acid fermentations, including processes for producing lglutamic acid using members of the genera *corynebacterium* and *brevibacterium*.

OXYGEN AND ANTIFOAMS

- Depending on the amount of oxygen required by the organism, it may be supplied in the form of air containing about 21% (v/v) oxygen, or occasionally as pure oxygen when requirements are particularly high.

OXYGEN AND ANTIFOAMS

- The organism's oxygen requirements may vary widely depending upon the carbon source.
- For most fermentations the air or oxygen supply is filter sterilized prior to being injected into the fermenter.

OXYGEN AND ANTIFOAMS

- **ANTIFOAMS**
- Antifoams are necessary to reduce foam formation during fermentation.
- Foaming is largely due to media proteins that become attached to the air–broth interface where they denature to form a stable foam.
- If uncontrolled the foam may block air filters, resulting in the loss of aseptic conditions; the fermenter becomes contaminated and microorganisms are released into the environment.

OXYGEN AND ANTIFOAMS

- Of possibly most importance is the need to allow ‘freeboard’ in fermenters to provide space for the foam generated.
- If foaming is minimized, then throughputs can be increased.
- There are three possible approaches to controlling foam production: modification of medium composition, use of mechanical foam breakers and addition of chemical antifoams.

OXYGEN AND ANTIFOAMS

- Chemical antifoams are surfaceactive agents which reduce the surface tension that binds the foam together. The ideal antifoam should have the following properties:

1. **readily and rapidly dispersed with rapid action;**
2. **high activity at low concentrations;**
3. **prolonged action;**
4. **non-toxic to fermentation microorganisms, humans** or animals;

OXYGEN AND ANTIFOAMS

5. **Low cost;**
6. **Thermostability; and**
7. **Compatibility with other media components and the**

- Process, i.E. Having no effect on oxygen transfer rates or downstream processing operations.

OXYGEN AND ANTIFOAMS

- Natural antifoams include plant oils (e.g. From soya, sunflower and rapeseed), deodorized fish oil, mineral oils and tallow.
- The synthetic antifoams are mostly silicon oils, poly alcohols and alkylated glycols.
- Some of these may adversely affect downstream processing steps, especially membrane filtration.

ANIMAL AND PLANT CELL CULTURE MEDIA

- Animal cell culture media are normally based on complex basal media, such as eagle's cell culture medium, which contains glucose, mineral salts, vitamins and amino acids.
- For mammalian cells a serum is usually added, such as fetal calf serum, calf serum, newborn calf serum or horse serum.

ANIMAL AND PLANT CELL CULTURE MEDIA

- Sera provide a source of essential growth factors, including initiation and attachment factors, and binding proteins.
- They also supply hormones, trace elements and protease inhibitors.
- The highly complex composition of sera makes substitution with lower cost ingredients very difficult.

ANIMAL AND PLANT CELL CULTURE MEDIA

- Sterilization of formulated animal culture media and media constituents is also more problematic as many components are thermolabile, requiring filter sterilization.
- Normally, sera constitute 5–10% (v/v) of the medium, but attempts have been made to reduce and ultimately eliminate its use.

ANIMAL AND PLANT CELL CULTURE MEDIA

- This is necessary due to its high cost and the fact that it is a potential source of prions and viruses.
- In some circumstances levels have now been lowered to 1–2% (v/v) and some cell lines have been developed that grow in serum-free media.

ANIMAL AND PLANT CELL CULTURE MEDIA

- **PLANT CELL CULTURE MEDIA**
- In contrast to most animal cell culture media, those used for plant cell culture are usually chemically defined.
- They contain an organic carbon source (as most plant cells are grown heterotrophically), a nitrogen source, mineral salts and growth hormones.

ANIMAL AND PLANT CELL CULTURE MEDIA

- Sucrose is frequently incorporated as the carbon source, particularly for secondary metabolite production, but glucose, fructose, maltose and even lactose have been used.
- Nitrate is the usual nitrogen source, often supplemented with ammonium salts. However, some species may require organic nitrogen, normally in the form of amino acids.

ANIMAL AND PLANT CELL CULTURE MEDIA

- The combination and concentration of plant hormones provided depend upon the specific fermentation.
- Auxins are usually supplied, along with cytokinins to promote cell division. A two-phase culture has often proved to be useful in increasing productivity, particularly for producing secondary metabolites such as shikonin.
- The first phase uses a medium optimized for growth, the second promotes product formation.

ANIMAL AND PLANT CELL CULTURE MEDIA

- **CULTURE MAINTENANCE MEDIA**
- These media are used for the storage and subculturing of key industrial strains.
- They are designed to retain good cell viability and minimize the possible development of genetic variation.

ANIMAL AND PLANT CELL CULTURE MEDIA

- In particular, they must reduce the production of toxic metabolites that can have strain-destabilizing effects.
- If strains are naturally unstable, they should be maintained on media selective for the specific characteristic that must be retained.

FERMENTER DESIGN AND CONSTRUCTION

- The main function of a fermenter is to provide a suitable environment in which an organism can efficiently produce a target product that may be cell biomass, a metabolite or bioconversion product.
- Most are designed to maintain high biomass concentrations, which are essential for many fermentation processes, whereas control strategies largely depend on the particular process and its specific objectives.

FERMENTER DESIGN AND CONSTRUCTION

- The performance of any fermenter depends on many factors, but the key physical and chemical parameters that must be controlled are agitation rate, oxygen transfer, ph, temperature and foam production.
- Laboratory fermentations may be performed in bottles or conical flasks that can be shaken to provide aeration where necessary.

FERMENTER DESIGN AND CONSTRUCTION

- These vessels are normally plugged with cotton wool or a styrofoam bung to prevent microbial contamination, but this can lead to evaporation losses and restricted exchange of gases.
- Consequently, even on a laboratory scale, vessels specifically constructed for fermentations are usually preferred.
- For industrial processes, fermenters with capacities up to several hundred thousand litres are used.

FERMENTER DESIGN AND CONSTRUCTION

- These are mostly purpose- built and designed for a specific process, although some flexibility may be necessary in certain instances.
- Their design, quality of construction, mode of operation and the level of sophistication largely depend upon the production organism,
- the optimal operating conditions required for target product formation, product value and the scale of production.

Examples of aseptic and non-aseptic fermentations

Aseptic		Non-aseptic	
Aerobic	Anaerobic	Aerobic	Anaerobic
Animal and plant cell cultures	Acetone	Acetification of ethanol in vinegar production*	Alcoholic beverages; beer, wine, etc.*
Alkaloids	Butanol	Ripening of some cheeses	Primary dairy fermentations*
Amino acids	Ethanol	Mushroom production	Silage production
Most antibiotics	Glycerol	Aerobic waste-water treatment	Anaerobic waste-water treatment
Most biomass (SCP) production	Lactic acid		
Most enzymes	Some toxins		
Most organic acids			
rDNA proteins			
Steroid biotransformations			
Some toxins			
Most vaccines			
Most vitamins			
Xanthan gum			

* Usually a clean operation often referred to as 'commercially sterile'.

FERMENTER DESIGN AND CONSTRUCTION

- Large volume, low value products that include many of the traditional fermentation products, such as alcoholic beverages, are usually produced using relatively simple fermenters and may not operate under aseptic conditions.
- There are often fewer risks when operating such non-aseptic fermentations at extreme ph or high temperatures, or where protected substrates are used.

Classification of industrial fermentations according to the organization of the biological phase

Suspended mode		Supported mode	
Individual cells	Flocs and aggregates	Fixed film	Films on fluidized supports
Cylindroconical vessels *Saccharomyces cerevisiae* (beer) Airlift fermenter *Methylophilus methylotrophus* (biomass) Stirred tank reactor *Bacillus subtilis* (enzymes)	Activated sludge reactor mixed culture (waste-water treatment) Stirred tank reactor *Aspergillus niger* (citric acid production) Stirred tank reactor *Penicillium chrysogenum* (penicillin)	Trickling film generator acetic acid bacteria (vinegar) Trickle filters mixed culture (waste-water treatment) Hollow fibre fermenter animal cells (monoclonal antibodies)	Fluidized bed reactor mixed culture (waste-water treatment) Fluidized bed reactor animal cells (monoclonal antibodies)

FERMENTER DESIGN AND CONSTRUCTION

- These are substrates that few microorganisms will utilize.
- Nevertheless, strict adherence to good manufacturing practices reduces the risk of microbial contamination of pure culture (axenic) fermentations.
- Other fermentations do not involve pure culture inoculum and activelyencourage the development of indigenous microorganisms, e.g. Some food fermentations and waste-water treatment.

FERMENTER DESIGN AND CONSTRUCTION

- Conversely, fermentations producing high value, relatively low volume products, especially pharmaceuticals, invariably demand more elaborate systems and operate under strict aseptic conditions.
- Traditionally, fermenters have been open cylindrical or rectangular vessels made from wood or stone.
- Some of these are still used, particularly for certain food and beverage fermentations.

FERMENTER DESIGN AND CONSTRUCTION

- However, most fermentations are now performed in closed vessels designed to exclude microbial contamination.
- These fermenters must with-stand repeated sterilization and cleaning, and should be constructed from non-toxic, corrosion-resistant materials.
- Small fermentation vessels of a few litres capacity are constructed from glass and/or stainless steel.

CONTROL OF CHEMICAL AND PHYSICAL CONDITIONS

- The basic concept of a bioreactor is to separate, by use of boundaries, the internal fermentation environment from the external environment.
- Therefore, anything entering or leaving the fermentation can be monitored and this introduces the basic notion of ‘energy and mass balances’.

CONTROL OF CHEMICAL AND PHYSICAL CONDITIONS

- However, some parameters in a system cannot be balanced.
- These are **intensive properties (temperature,** concentration, pressure and specific heat) whose properties are independent of the size of the system,
- whereas **extrinsive properties (mass, volume, entropy and energy)** can be balanced.
- For example, if 10 g of water at 30°C is added to 35 g of water at 30°C, the resulting water has a temperature (intensive property) of 30°C not 60°C, but the mass of water (extrinsive property) is additive at 45 g.

CONTROL OF CHEMICAL AND PHYSICAL CONDITIONS

- The key extrinsive parameters are **mass and energy,** consequently the number of atoms of carbon, nitrogen, oxygen, etc.,
- And the energy present in the system at the start of the operation, and any further input during the fermentation, must all be accounted for at the end of the process.
- If the inventory for mass and energy in the system at the start and finish balance, then the system is understood.

CONTROL OF CHEMICAL AND PHYSICAL CONDITIONS

- This provides a powerful analytical tool, especially when combined with the determination of thermodynamic properties (heat transfer, density, rheology and temperature)
- and rate equations (biomass production, nutrient utilization and waste product formation).

CONTROL OF CHEMICAL AND PHYSICAL CONDITIONS

- Together this information can be used to build mathematical and computer models that may be used to monitor and control future fermentations.

AGITATION

- Agitation of suspended cell fermentations is performed in order to mix the three phases within a fermenter.
- The liquid phase contains dissolved nutrients and metabolites, the gaseous phase is predominantly oxygen and carbon dioxide,
- and the solid phase is made up of the cells and any solid substrates that may be present.

AGITATION

- Mixing should produce homogeneous conditions and promote nutrient, gas and heat transfer.
- Heat transfer is necessary during both sterilization and for temperature maintenance during operation.
- Efficient mixing is particularly important for oxygen transfer in aerobic fermentations, as microorganisms can take up oxygen only from the liquid phase.

AGITATION

- Transfer into liquid from the gaseous phase is enhanced by agitation. It prolongs retention of air bubbles in suspension,
- reduces bubble size to increase the surface area for oxygen transfer,
- prevents bubble coalescence and decreases the film thickness at the gas-liquid interface.

AGITATION

- However, maintenance of suitable shear conditions during the fermentation is very important, because certain agitation systems develop high shear that may damage shear-sensitive cells.
- Conversely, low shear systems can lead to cell flocculation or unwanted growth on surfaces, such as on the vessel walls, stirrer and electrodes.

AGITATION

- Fermenter agitation requires a substantial input of energy and there are three principal mechanisms that may be used.

STIRRED TANK REACTORS (STRS)

- **STIRRED TANK REACTORS (STRS) HAVE MECHANICALLY MOVING**
- Agitators or impellers within a baffled cylindrical vessel.
- Baffles are usually flat vertical plates whose width is about one-tenth of the vessel diameter.
- Normally, 4–6 baffle plates are fitted to the inside vessel walls to aid mixing and mass transfer by increasing turbulence, preventing vortex formation and eliminating ‘dead spaces’.

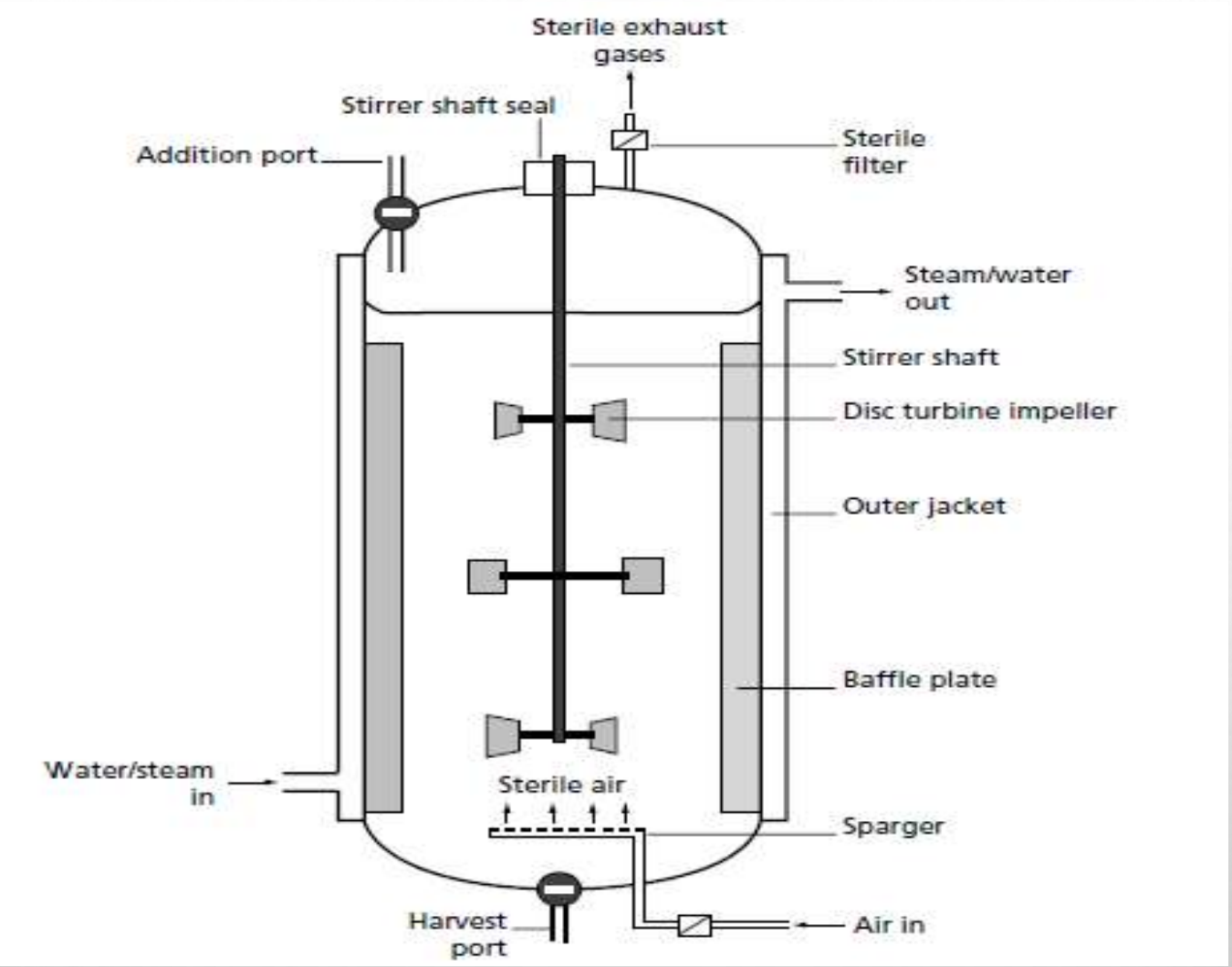


Diagram of a stirred tank reactor.

STIRRED TANK REACTORS (STRS)

- Strs are the most commonly used vessels and have been adopted for a wide range of fermentation processes.
- Within each vessel the impeller is connected to an external motor which drives the stirrer system. The agitator assembly, including the seal, is often a potential route of contamination.
- To prevent this problem, the shaft has to pass into the fermenter through a set of aseptic seals.

STIRRED TANK REACTORS (STRS)

- There are specific regulations regarding the numbers and types of seals.
- For certain fermentations, two or three seals are required to minimize the risk of fermenter contamination, and release of microorganisms and their products into the environment.

STIRRED TANK REACTORS (STRS)

- The effectiveness of agitation depends upon the design of the impeller blades, speed of agitation and the depth of liquid.
- Most strs have height–diameter aspect ratios of 3 : 1 or 4 : 1. Strs must create high turbulence to maintain transfer rates,
- but this also generates considerable shear force that is detrimental to certain cells.

STIRRED TANK REACTORS (STRS)

- For instance, many animal and plant cells are shearsensitive and excessive stirring may result in cell disruption.
- In these cases strs may be unsuitable without modification, and airlift or supported biofilm reactors may be preferred.

PNEUMATIC SYSTEMS

- **PNEUMATIC SYSTEMS,**

- **Such as airlift fermenters, have no** Moving parts and use the expansion of compressed gas to bring about the mixing These systems have Lower energy requirements and create less shear than STRs.

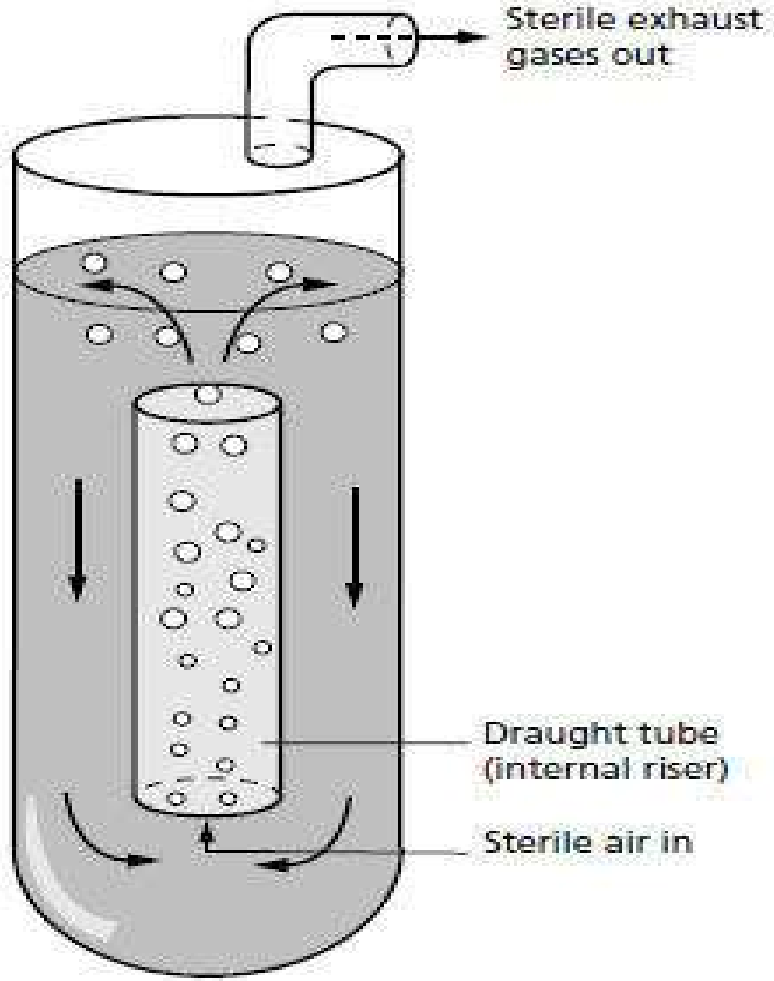
- Liquid movement is initiated by the injection of Compressed air at the bottom of the internal or external Riser column and the air bubbles expand in the rise Causing the upward movement of liquid and initiating Its cycling within the fermenter.

PNEUMATIC SYSTEMS

- Liquid movement is initiated by the injection of compressed air at the bottom of the internal or external riser column and
- the air bubbles expand in the riser causing the upward movement of liquid and initiating its cycling within the fermenter.

PNEUMATIC SYSTEMS

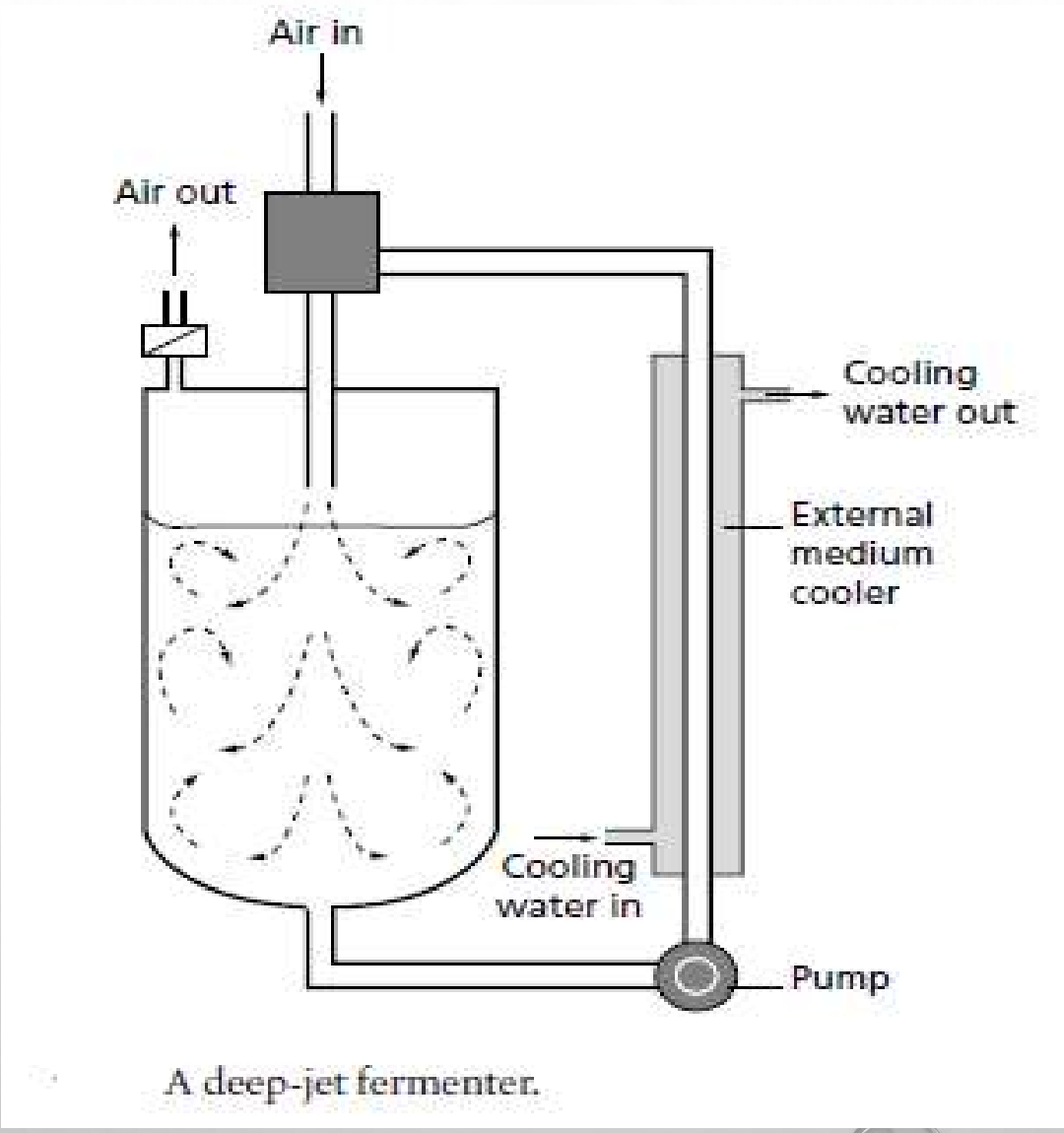
- Even large fermenters do not require internal cooling coils as a jacket can normally provide sufficient heat transfer, due to the rapid movement of fluid within the vessel.



A diagram illustrating the principle of an airlift fermenter.

HYDRODYNAMIC MECHANISMS

- Use liquid kinetic energy to mix the fermenter contents, which is achieved by using an external liquid pump for external circulation and reinjection, e.g. **Deep-jet fermenters**
- . the mixing of nutrients and gaseous exchange within any fermenter is complex.



A deep-jet fermenter.

HYDRODYNAMIC MECHANISMS

- It is influenced by medium density and rheology, size and geometry of the vessel, and the amount of power used in the system.
- SSTRs have agitators with multiple impellers to give a well-mixed homogeneous environment.
- Nevertheless, in reality, non-uniform conditions normally prevail in vessels greater than 500 L capacity.

HYDRODYNAMIC MECHANISMS

- The flow patterns of fluid motion in these stirred tanks can be of two types, laminar and turbulent flow, as a function of the **reynolds.**
- **Number (re) of the impeller**re = inertial forces/ viscous forces

HYDRODYNAMIC MECHANISMS

- Laminar and turbulent flow are characterized by high and low re, respectively.
- The re value that marks the transition between the two regimes depends on the geometry of the impeller and vessel.
- The re is an example of a natural variable or dimensionless number, whose magnitude, unlike substantial variables (length, mass, volume, temperature, etc.), Does not require units.

HYDRODYNAMIC MECHANISMS

- This is because the dimensions of the numerator exactly cancel those of the denominator, such as ratios of substantial variables, e.g. The aspect ratio of a cylinder (length divided by diameter).
- Dimensionless analysis is used extensively for solving problems in chemical/biochemical engineering and is particularly useful here,
- where such complex hydrodynamics are associated with physical transfer processes operating within a fermenter.

HYDRODYNAMIC MECHANISMS

- In liquid culture the rheological behaviour, fluid flow properties, are vitally important as they have a major impact on mixing and mass transfer, particularly for oxygen transfer in aerobic fermentations.
- When fluids are stirred they may behave as follows.

1. **Newtonian fluids, which obey newton's law of viscosity.**

- Their viscosity does not vary with shear or agitation rate.

HYDRODYNAMIC MECHANISMS

- Fluid viscosity or fluid resistance to flow = shear stress or force per unit area /shear rate or velocity gradient

2. non-newtonian fluids, whose viscosity varies with Shear or agitation rates.

- For example, **pseudoplastic** Fluids exhibit decreasing apparent viscosity with increasing Shear or agitation rate, whereas in **dilatant solutions** The opposite occurs.

HYDRODYNAMIC MECHANISMS

- For **bingham-plastic behaviour,** flow does not occur unless a stress is first imposed.

3. **Viscoelastic fluids, which do not observe normal liquidstate** properties when stirred, such as some polymers. Many bacterial and yeast fermentations exhibit newtonian fluid characteristics.

HYDRODYNAMIC MECHANISMS

- Mycelial cultures, and those involving polymeric substrates and products, particularly polysaccharide gels (e.g. Xanthan),
- often display non-newtonian properties that inhibit high flow dynamics within the fermenter.
- However, few fermentations behave as viscoelastic solutions.

HEAT AND MASS TRANSFER

- **HEAT TRANSFER**
- In fermenter design, efficient heat transfer is important in controlling the temperature during sterilization operations and maintaining the required operating temperature throughout the fermentation run.
- Heat generated in the fermentation is primarily due to metabolic activity of microorganisms and mechanical agitation processes.

HEAT AND MASS TRANSFER

- For most fermentations this heat needs to be dissipated by cooling.
- Conversely, for fermentations that operate above ambient temperature, such as those involving thermophilic organisms, there needs to be an input of heat.

HEAT AND MASS TRANSFER

- Heat transfer is primarily achieved using an outer jacket surrounding the internal phase or via internal coils.
- No direct contact exists between the cooling/ heating system and the fermentation medium.
- The heat is conducted through the vessel wall, coils and baffles.

HEAT AND MASS TRANSFER

- These systems are also used to sterilize the vessel and contents before inoculation, by the injection of pressurized steam.
- Automatic temperature control during the fermentation is accomplished by injecting either cold or hot water into the outer jacket and/or internal coils.
- In some circumstances alternative cooling media may be used, e.g. Glycol.

HEAT AND MASS TRANSFER

- **MASS TRANSFER**
- Transfer of nutrients from the aqueous phase into the microbial cells during fermentation is relatively straightforward as the nutrients are normally provided in excess.
- However, oxygen transfer in aerobic fermentations is rather more complex.

AERATION I

•Some fermentations operate an aerobically, but the majority are aerobic and require the provision of large quantities of normally sterile air or oxygen that must be dispersed throughout the fermenter.
•Compressed air entering a fermenter is usually stripped of moisture and any oil vapors that may originate from the compressor.
•To promote aeration in stirred tanks, the sparger is usually located directly below the agitator.

AERATION I

•Oxygen is only sparingly soluble in aqueous solution and the solubility decreases as the temperature rises.
•When high biomass concentrations are used to increase productivity it also creates an enormous demand for oxygen.

AERATION I

•To prevent the risk of contamination, gases introduced into the fermenter should be passed through a sterile filter.
•A similar filter on the air exhaust system avoids environmental contamination.

AERATION I

•Oxygen transfer is complex, as it involves a phase change from its gaseous phase to the liquid phase, and is influenced by the following factors.

1. The prevailing physical conditions; temperature, pressure and surface area of air/oxygen bubbles;
2. The chemical composition of the medium;
3. The volume of gas introduced per unit reactor volume per unit time;
4. The type of sparger system used to introduce air into the fermenter;
5. The speed of agitation; or
6. A combination of these factors.

AERATION II

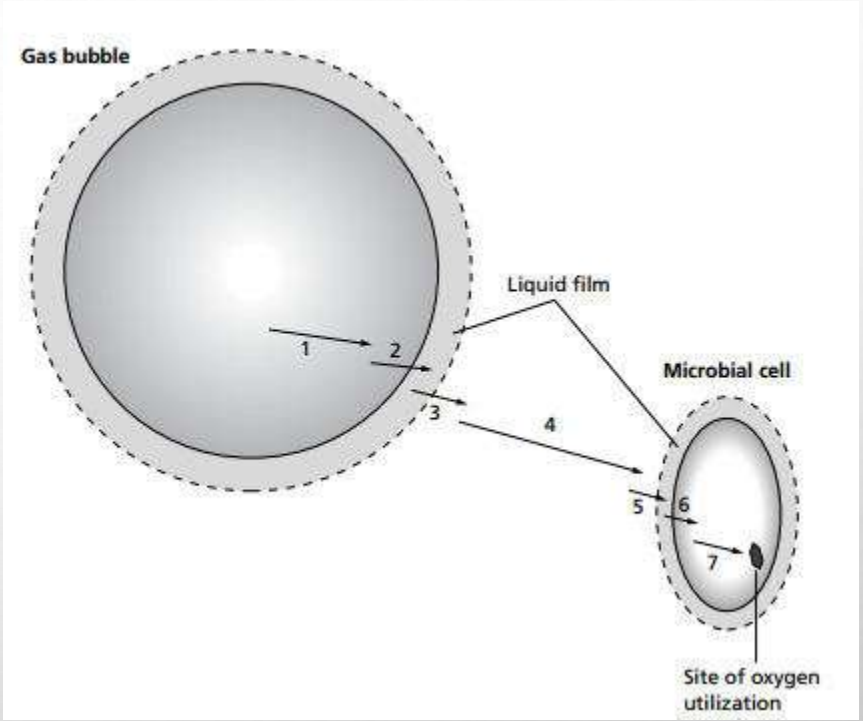
•The two steps associated with an oxygen mass balance are the rate at which oxygen can be delivered to the biological system (oxygen transfer rate, OTR) and the rate at which it is utilized by the microorganisms (critical oxygen demand).

AERATION II

Transfer through/across:

1 The bulk gas phase in the bubble.
2 The gas–liquid interface.
3 The liquid film around the bubble.
4 The bulk liquid culture medium.
5 The liquid film around the microbial cell*
6 The cell–liquid interface*
7 The intracellular oxygen transfer resistance*

*minor resistance



AERATION II

•Determination of K_la is relatively straightforward and is used to compare fermenters in both scale-up and scale-down.

•Movement in the bulk liquid is aided by good mixing.
•The rate of use by the biological system will be determined by the affinity and saturation characteristics of the terminal oxidase.

AERATION II

As microorganisms exhibit different oxygen requirements, the level of aeration necessary will vary from fermentation to fermentation.

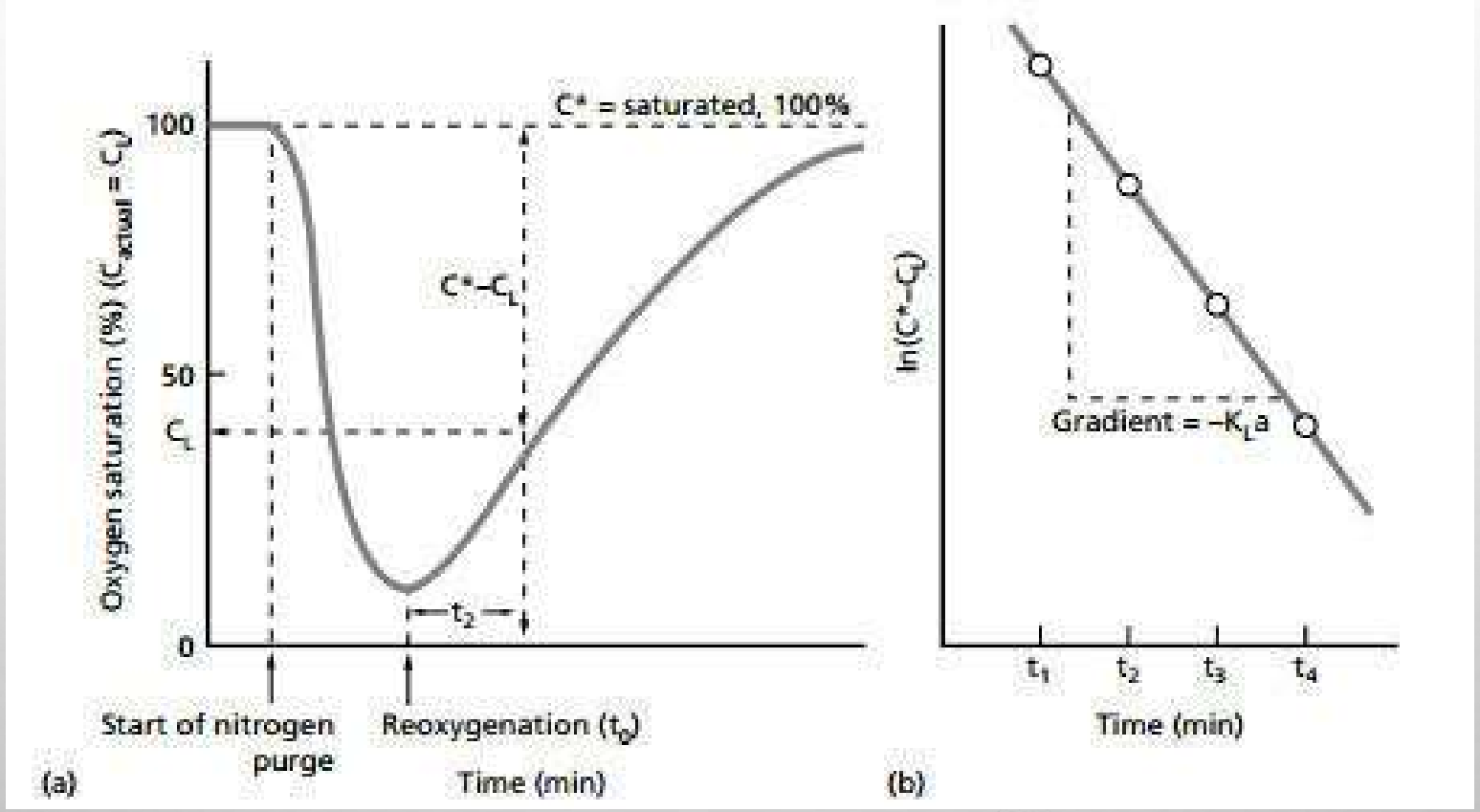
FERMENTER CONTROL AND MONITORING I

- Fermentation systems must be efficiently controlled inorder to optimize productivity and product yield, and ensure reproducibility.
- Control and maintenance at optimum levels inside the reactor is mediated by sensors (electrodes), along with compatible control systems and data logging.

FERMENTER CONTROL AND MONITORING I

	Measurement		
Sensor	Physical	Chemical	Biological
Electrodes	Temperature (thermistor, resistance thermometer, thermometer)	Dissolved oxygen Dissolved carbon dioxide Nutrients (biosensors, e.g. for glucose) pH Metal ions Foam level detection	Biosensors for biologically active products
Meters	Air flow rate in and out Agitation shaft power Speed of agitation, e.g. impeller tachometer	Acid/alkali addition	
Transducers	Pressure Liquid flow		
Mass spectra		Directly on-line or off-line nutrients and in flow and exhaust gases	Products
Spectrophotometers (determination on-line and off-line)			Biomass

FERMENTER CONTROL AND MONITORING I



FERMENTER CONTROL AND MONITORING I

•Samples can be taken off-line for various analyses, such as cell counts and determination of DNA, RNA, lipids, specific proteins, carbohydrates and other key metabolites and substrates.

FERMENTER CONTROL AND MONITORING I

•Fermentation media often contain buffering salts, usually phosphates, but their capacity to control pH can be exceeded and addition of acid or alkali may be required.

FERMENTER CONTROL AND MONITORING I

- Many fermentations produce acid and adjustment of the pH can be made with ammonium hydroxide, which may also act as a nitrogen source
- Levels of dissolved O_2 and CO_2 are determined using O_2 and CO_2 electrodes.

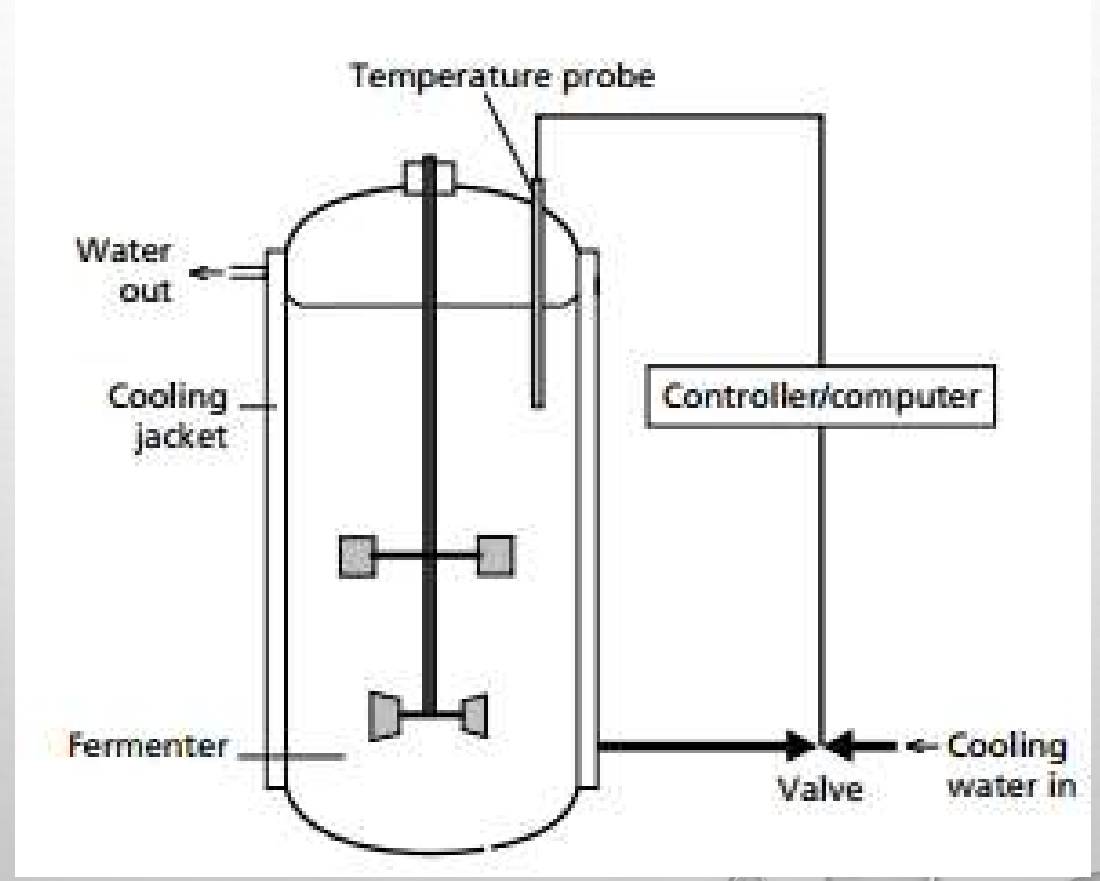
FERMENTER CONTROL AND MONITORING I

•Here are basically three methods used to control foam production: media modification, mechanical foam-breaking devices or the automatic addition of chemical antifoam agents

FERMENTER CONTROL AND MONITORING I

- The basic principle of control involves a sensory system linked to a control system and feedback loop
- Overall control can be manual or automated; newer systems have integral and derivative control systems

FERMENTER CONTROL AND MONITORING I



FERMENTER CONTROL AND MONITORING I

• If a deviation is discovered, appropriate alarm and correctional systems are activated, giving greater control of the fermentation

.

•However, any control system needs to be regularly calibrated when first installed and then regularly checked to conform to good manufacturing practices (GMP).

OPERATING MODES

•Industrial fermentations are operated as batch, fedbatch or continuous cultures

•A fermenter is loaded, sterilized and inoculated, and the organism is grown through a typical batch profile

•Variations include fed-batch systems that have been successfully used for producing baker's yeast and penicillin.

OPERATING MODES

•Fed-batch operation can extend the product formation phase and may overcome problems associated with the use of epressive, rapidly metabolized, substrates

•Fed-batch with recycle of cells (biomass) can also be used for specific purposes, e.g. some ethanol fermentations and waste-water treatment processes.

OPERATING MODES

•Only a small fraction of each batch fermentation cycle is roductive, as there may be a considerable lag period and it is only in the later stages of the exponential phase that large uantities of the product are generated

•The increased frequency of sterilization may also cause greater stress on instruments and probes.

OPERATING MODES

•Furthermore, the system has the property of reaching a steady state in which the concentration of limiting nutrient and the cell number do not vary with time.

•Problems associated with continuous culture processes, other than waste-water treatment, include the fact that throughout their 20–50 days or longer operation, sterility must be maintained and a continuous supply of media of constant composition provided.

OPERATING MODES

STERILIZATION I

Air sterilization

To prevent contamination of either the fermentation by air-borne microorganisms or the environment by aerosols generated within the fermenter, both air input and air exhaust ports have air filters attached.

STERILIZATION I

- MEDIA AND VESSEL STERILIZATION
- For pilot-scale and industrial aseptic fermentations the fermenter can be sterilized empty.
- The vessel is then filled with sterile medium, prepared in a batch or continuous medium ‘cooker’ that may supply several fermentations.

STERILIZATION I

•Some industrial fermentations are not aseptic, but microbial contamination is still maintained at a minimum level by boiling or pasteurization of the media.

STERILIZATION I

•Normally, the aim is to provide sterilization conditions that give an acceptable probability of contamination of 0.1% (1 in 000). Conse-104 Chapter 6 quently, if the original number of microbial cells (No) is known, the Del factor (—=ln No /Nt) can be calculated

STERILIZATION II

The overall Del factor may be represented as

$$\nabla_{\text{total}} = \nabla_{\text{heating}} + \nabla_{\text{holding}} + \nabla_{\text{cooling}}$$

STERILIZATION II

1heating and cooling between 0°C and 100°C is unimportant for sterilization;
2heating between 100°C and 121°C is at 1°C per minute, i.e. 20 min; and
3cooling from 121°C to 100°C is at 1°C per minute, i.e. 20 min

STERILIZATION III

•Steam may also be injected directly into the head space above the fermentation medium.

•certain media constituents may be heat labile and destroyed by excessive heat, e.g. glucose, some vitamins and components of animal cell culture media.

STERILIZATION II

Usually a continuous operation where the holding time is controlled by the flow rate through the sterilizer and the material is then rapidly cooled in a heat exchanger.

SOLID SUBSTRATE FERMENTATION

•Solid-substrate fermentations have been used for producing various fermented foods in Asia for thousands of years, but this method is rarely used in Europe and North America.

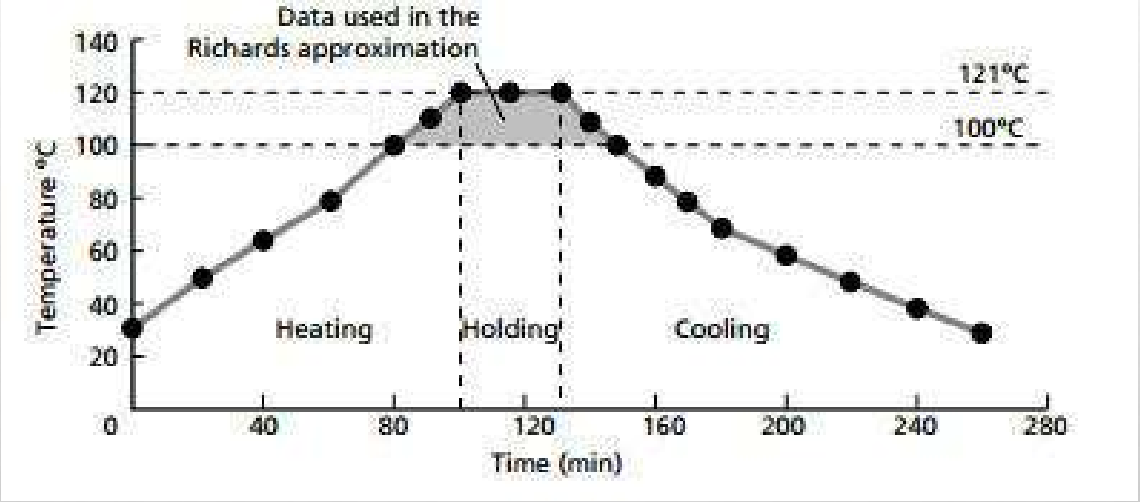
•The substrates used are often cereal grains, bran, legumes and lignocelluloses materials, such as straw, wood chippings, etc.

SOLID SUBSTRATE FERMENTATION

•Enzymes, organic acids and ethanol are now produced by solidsubstrate fermentations, particularly in areas where modern fermentation equipment is unavailable

SOLID SUBSTRATE FERMENTATION

•Control of the environment within the bioreactors is also difficult to achieve, particularly the simultaneous maintenance of optimal temperature and moisture.



SOLID SUBSTRATE FERMENTATION

Advantages	Disadvantages
Potentially provide superior productivity	Slower microbial growth
Low-cost media	Problems with heat build-up
Simple technology	Bacterial contamination can be problematic
Low capital costs	Difficulties often encountered on scale-up
Reduced energy requirements	Substrate moisture level difficult to control
Low waste-water output	
No problems with foaming	

SOLID SUBSTRATE FERMENTATION

•The microorganisms associated with solid-substrate fermentations are those that tolerate relatively low water activity down to Aw values of around 0.7

ENVIRONMENTAL PARAMETERS

•Water is lost during fermentation through evaporation and metabolic activity.

•If moisture levels are too low, the substrate is less accessible, as it does not swell and microbial growth is reduced

ENVIRONMENTAL PARAMETERS

TEMPERATURE

Heat generation can be more problematic than in liquid fermentations and has a major influence on relative humidity within a fermentation.

ENVIRONMENTAL PARAMETERS

AERATION

Most solid-substrate fermentations are aerobic, but the particular requirements for oxygen depend upon the microorganism(s) used and the specific process.

The rate of oxygen transfer is greatly influenced by the size of the substrate particles, which determines the void space.

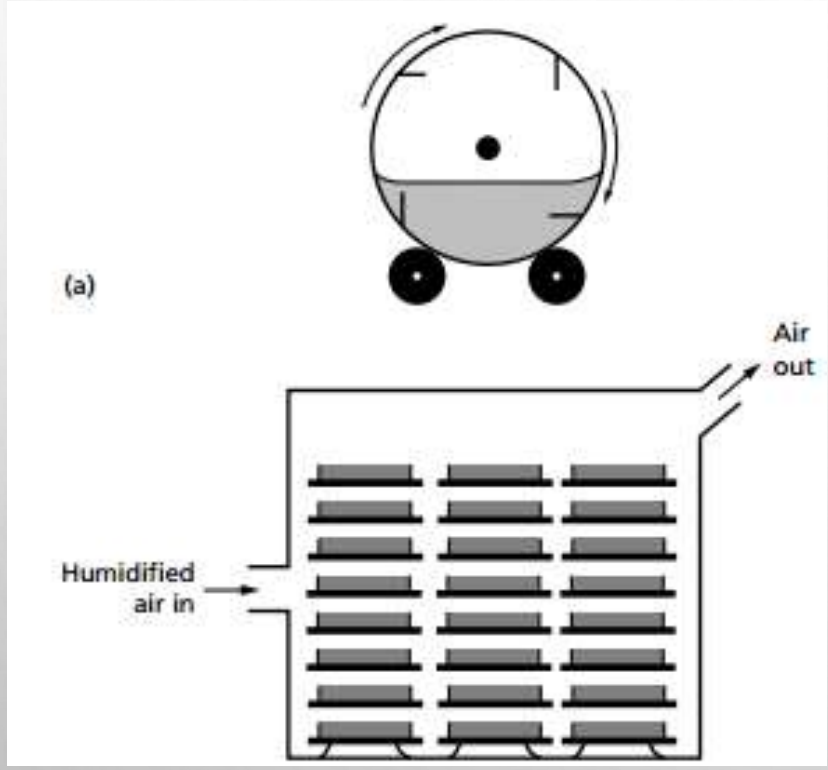
BIOREACTORS USED FOR SOLID-SUBSTRATE FERMENTATION

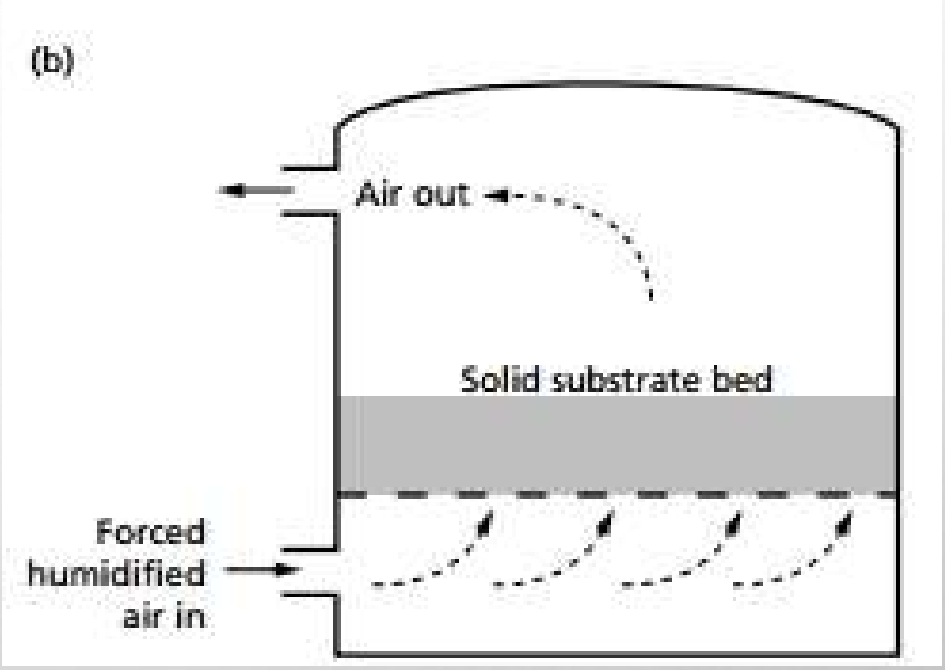
- Some processes do not require bioreactors, they simply involve spreading the substrate onto a suitable floor.

BIOREACTORS USED FOR SOLID-SUBSTRATE FERMENTATION

Few anaerobic processes, such as silage production, require no mechanisms for agitation or aeration.

BIOREACTORS USED FOR SOLID-SUBSTRATE FERMENTATION





BIOREACTORS USED FOR SOLID-SUBSTRATE FERMENTATION

Rotating drum fermenters, comprising a cylindrical vessel of around 100 L capacity mounted on its side onto rollers that both support and rotate the vessel

BIOREACTORS USED FOR SOLID-SUBSTRATE FERMENTATION

Tray fermenters, which are used extensively for the production of fermented oriental foods and enzymes.

BIOREACTORS USED FOR SOLID-SUBSTRATE FERMENTATION

- Bed systems, as used in commercial koji production

- Column bioreactors, consisting of a glass or plastic column, into which the solid substrate is loosely packed, surrounded by a jacket that provides a means of temperature control.

- Fluidized bed reactors, which provide continuous agitation with forced air to prevent adhesion and aggregation of substrate particles.

FERMENTATION PROCESS DEVELOPMENT

Once a microorganism has been selected as the producer rganism for a particular process, research is initially performed under laboratory-scale conditions using 1–10 L fermenters.

FERMENTATION PROCESS DEVELOPMENT

Other factors that are considered include reactor configuration, and control of pH, dissolved oxygen, foam and temperature.

FERMENTATION PROCESS DEVELOPMENT

Key factors that influence yield during the scale-up process are as follows.

1Inoculum propagation procedures adopted, and the quality and quantity of inoculum used to start the fermentation.

2Choice of medium; cheaper nutrient sources are often employed for large-scale operations due to cost constraints.

FERMENTATION PROCESS DEVELOPMENT

3Industrial-scale sterilization protocols may result in greater degradation of heat-labile compounds, which affects the final quality of the medium.
4Larger fermenters are often subject to the development of nutrient, emperature, pH and oxygen gradients, which were not experienced in smaller, wellmixed, fermenters.

FERMENTATION PROCESS DEVELOPMENT

In scale-down studies, the opposite approach can be implemented, where the conditions in the largescale fermenter are mimicked, as far as possible, in the small-scale systems.

DOWN STREAMING PROCESSING I

USP involves all factors and processes leading to, and including, the fermentation, and consists of three main areas.

DOWN STREAMING PROCESSING I

DSP encompasses all processes following the fermentation. It has the primary aim of efficiently, reproducibly and safely recovering the target product to the required specifications (biological activity, purity, etc.)

DOWN STREAMING PROCESSING I

Each stage in the overall recovery procedure is strongly dependent on the protocol of the preceding fermentation. Fermentation factors affecting DSP include the properties of microorganisms, particularly morphology, flocculation characteristics, size and cell wall rigidity.

DOWN STREAMING PROCESSING I

The whole process, both upstream and downstream factors, needs to be considered.
For example, the choice of fermentation substrate influences subsequent DSP.

DOWN STREAMING PROCESSING I

The physical and chemical properties of the product, along with its concentration and location, are obviously key factors as they determine the initial separation steps and overall purification strategy.

DOWN STREAMING PROCESS II

DSP can be divided into a series of distinct unit processes linked together to achieve product purification

DOWN STREAMING PROCESS II

The specific unit steps chosen will be influenced by the economics of the process, the required purity of the product, the yield attainable at each step and safety aspects.

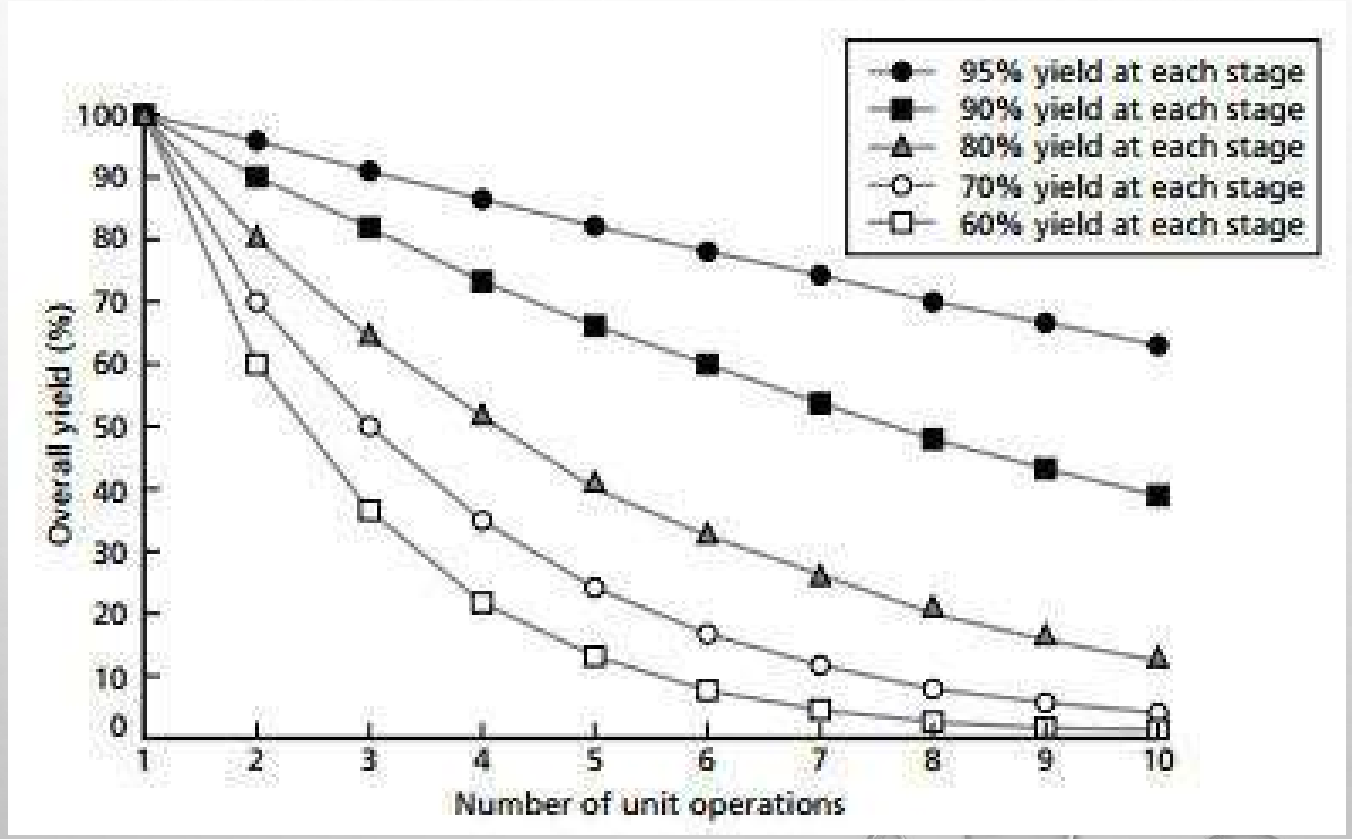
DOWN STREAMING PROCESS II

Physical process integration may be achieved by placing separation units inside the fermenter or by directly linking the two systems together.

DOWN STREAMING PROCESS II

Early product extraction can also enhance the yield for those products sensitive to prolonged exposure to the fermentation environment, where shear, proteolytic enzymes, oxidation, etc., may be destructive.

DOWN STREAMING PROCESS II



CELL HARVESTING I

Broth conditioning

Broth conditioning techniques are mostly used in association with sedimentation and centrifugation for the separation of cells from liquid media.

CELL HARVESTING I

Some organisms naturally flocculate, which can be enhanced by chemical, physical and biological treatments. Such treatments can also be effective with cells that would not otherwise form flocs.

CELL HARVESTING I

Sedimentation

Sedimentation is extensively used for primary yeast separation in the production of alcoholic beverages, and in waste-water treatment.

CELL HARVESTING I

Centrifugation

If instead of simply using gravitational force to separate suspended particles, a centrifugal field is applied, the rate of solid–liquid separation is significantly increased and much smaller particles can be separated.

CELL HARVESTING I

Higher-speed centrifuges are required for the separation of smaller microorganisms, such as bacteria, compared with yeasts.

CELL HARVESTING I

Advantages of centrifugation include the availability of fully continuous systems that can rapidly process large volumes in small volume centrifuges.

CELL HARVESTING I

Disadvantages of centrifugation are the high initial capital costs, the noise generated during operation and the cost of electricity.

CELL HARVESTING II

Centrifuges can be divided into small-scale laboratory units and larger pilot- and industrial-scale centrifuges.

CELL HARVESTING II

1 Tubular centrifugesusually produce the highest centrifugal force of 13 000–17 000g. They have hollow tubular rotor bowls providing a long flow path for the suspension, which is pumped in at the bottom and flows up through the rotor

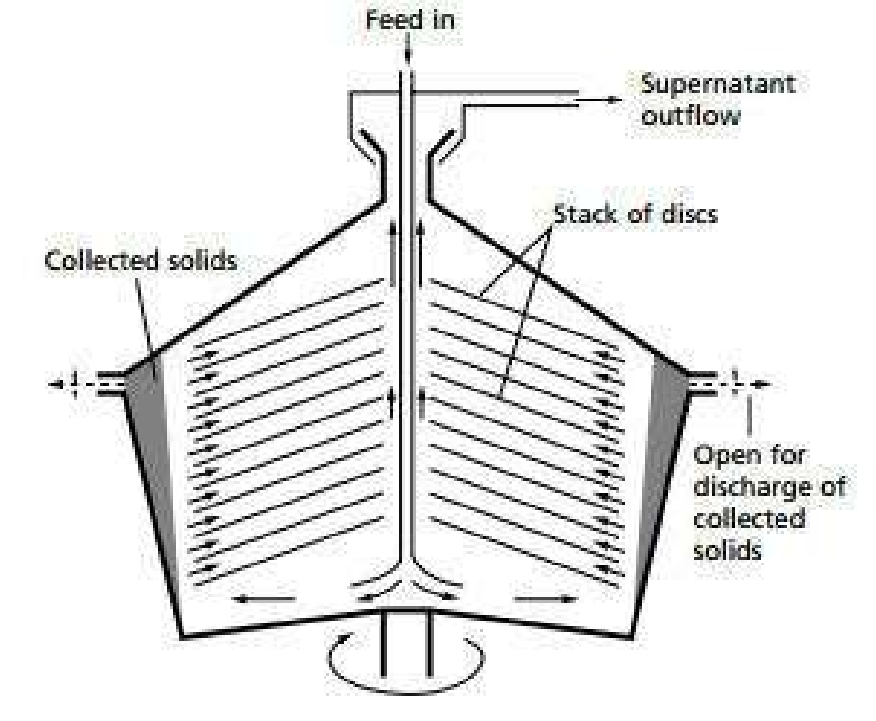
CELL HARVESTING II

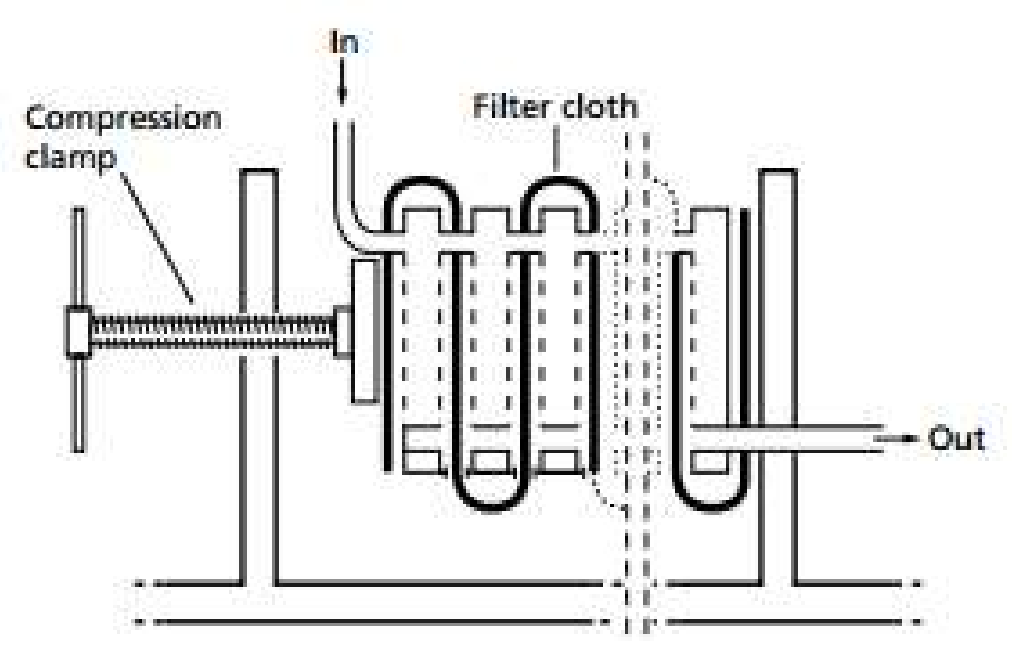
Multichamber bowl centrifugesconsist of a bowl that is divided by vertically mounted interconnecting cylinders and are capable of operating at 5000–10 000g

CELL HARVESTING II

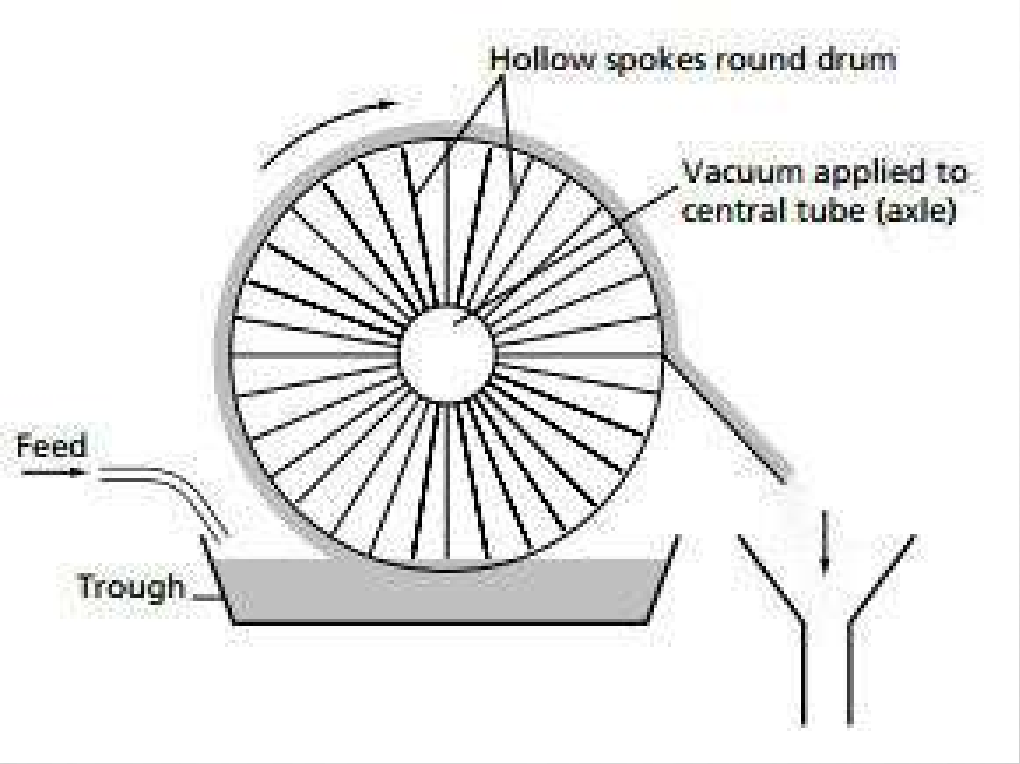
Disc stack centrifugescan operate at 5000–13 000g.
The centrifuge bowl contains a stack of conical discs whose close packing aids separation

CELL HARVESTING II





CELL HARVESTING II



CELL HARVESTING II

filtration proceeds collected solids accumulate above the filter medium, resistance to filtration increases and flow through the filter decreases.

CELL HARVESTING III

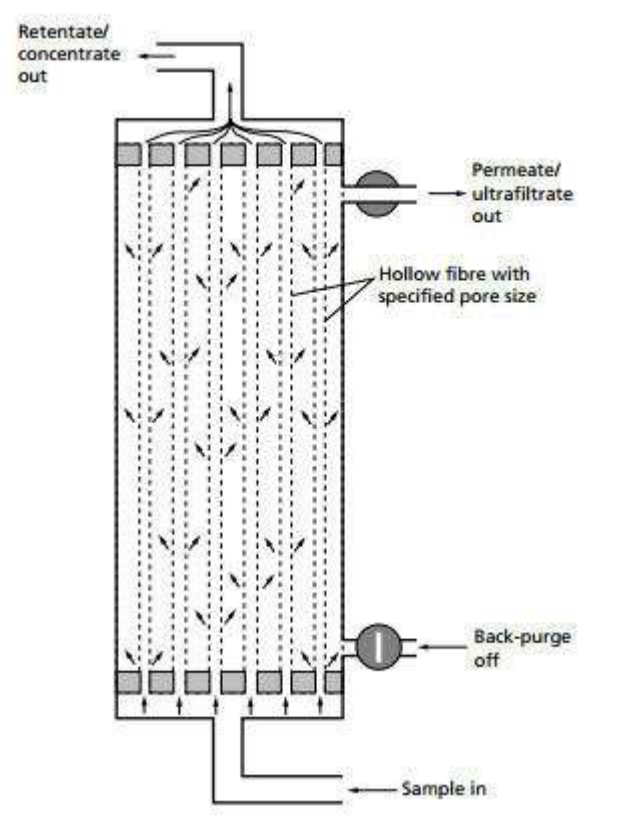
Solids accumulate on the filter medium as liquid filtrate is drawn, under vacuum, through the filter medium into the hollow drum to a receiving vessel.

CELL HARVESTING III

The presence of silicon antifoams may have a similar negative effect

non-spherical proteins may exhibit different exclusion reactions to the membrane. Flat membranes are available, but for larger-scale operations hollow-fibre systems are usually preferred

CELL HARVESTING III



CELL HARVESTING III

Ultrafiltration is also effective in removing pyrogens (bacterial cell wall lipopolysaccharides), cell debris and viruses from media, and for whey processing.

CELL HARVESTING III

Reverse osmosisis used for dewatering or concentration steps and has been employed to desalinate sea water for drinking

CELL HARVESTING III

if pressure is applied to the ‘salt side’ then reverse osmosis will occur, and water will be driven across the membrane from the salt side.

CELL DISRUPTION I

The breaching of the cell wall/envelope and cytoplasmic membrane can pose problems, particularly where cells possess strong cell walls.

CELL DISRUPTION I

General problems associated with cell disruption include the liberation of DNA, which can increase the viscosity of the uspension

CELL DISRUPTION I

damage can be reduced by the addition of enzyme inhibitors, cooling the cell extract and rapid processing.

Alternatively, attempts may be made to produce mutant strains of the producer microorganism lacking the damaging enzymes

CELL DISRUPTION I

The French press (pressure cell) is often used in the laboratory and the high-pressure homogenizers, such as the Manton and Gaulin homogenizer (APV-type mill), are employed for pilot- and production-scale cell disruption.

CELL DISRUPTION I

As a result, the product of interest must be separated from a complex mixture of proteins, nucleic acids and cell wall fragments.

CELL DISRUPTION II

On a small scale, manual grinding of cells with abrasives, usually alumina, glass beads, kieselguhr or silica, can be an effective means of disruption, but results may not be reproducible.

CELL DISRUPTION II

he efficiency of cell breakage is a function of agitation speed, concentration of beads, bead density and diameter, broth density, flow rate and temperature.

CELL DISRUPTION II

Ultrasonic disruption of cells involves cavitations, microscopic bubbles or cavities generated by pressure waves.

CELL DISRUPTION II

Cell disruption is a somewhat neglected area of bio processing, as there has been relatively little innovation and progress.

CELL DISRUPTION II

A wide range of other techniques have been developed for small-scale microbial disruption using various chemicals and enzymes.

CELL DISRUPTION II

he antibiotics penicillin and cycloserine may be used to lyse actively growing bacterial cells, often in combination with an osmotic shock.

PRODUCT RECOVERY I

Following cell disruption, soluble proteins are usually separated from cell debris by centrifugation.

PRODUCT RECOVERY I

The solubility of
the salt varies with temperature, so strict temperature control is required.

PRODUCT RECOVERY I

Many alkaloids, antibiotics, steroids and vitamins are recovered by liquid–liquid extraction methods using organic solvents.

PRODUCT RECOVERY I

Chromatographic techniques are usually employed for higher-value products.

These methods, normally involving columns of chromatographic media (stationary phase), are used for desalting, concentration and purification of protein preparations.

PRODUCT RECOVERY I

The order and choice of technique will depend on the particular product, but the following chromatographic parameters should be considered: capacity, recovery andresolving power (selectivity).

PRODUCT RECOVERY I

Affinity chromatography is a particularly powerful and highly selective purification technique

PRODUCT RECOVERY II

Ligands are covalently linked to the matrix material, e.g. agarose, via a spacer arm to avoid steric hindrance.

PRODUCT RECOVERY II

The loading capacity of affinity columns is large, as the volume of the sample is unimportant and high resolution can be attained

For industrial-scale operations, it is often necessary to sterilize the chromatographic media in order to comply with various regulatory requirements.

PRODUCT RECOVERY II

Gel filtration chromatography essentially involves separation on the basis of molecular size (molecular sieving), although molecular shape can also influence separation performance.

PRODUCT RECOVERY II

The initial choice of stationary phase material is also a key factor, as some may interact with the target product, e.g. carbohydrate-based matrices interact with glycoprotein's.

PRODUCT RECOVERY II

High-performance liquid chromatography(HPLC) was originally developed for the separation of organic molecules in non-aqueous solvents, but is now used for proteins in aqueous olution.

PRODUCT RECOVERY II

The protein to be purified must have an affinity for this ion and binds to it by forming coordination complexes with groups such as the imidazole of histidine residues.

PRODUCT RECOVERY II

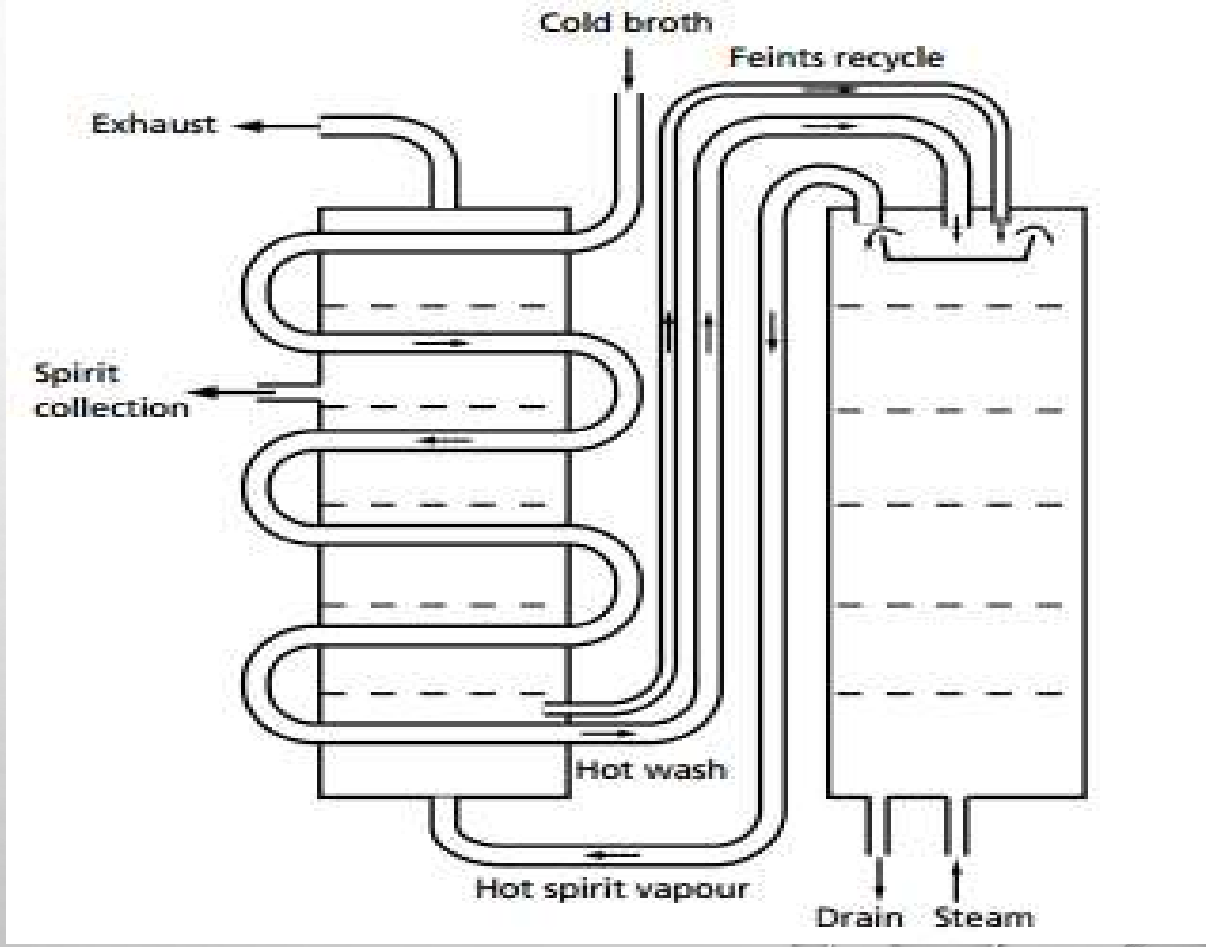
membrane separation techniques are primarily used for the removal of low molecular weight solutes and inorganic ions from a solution

DISTILLATION

Batch distillation in pot stills continues to be used for the production of some whiskies

Some continuous stills may be in the form of four or five separate columns, but the Coffey-type still comprises just two columns, the ‘rectifier’ and ‘analyser’, each containing a stack of 30–32 perforated plates

DISTILLATION



DISTILLATION

Product crystallization may be achieved by evaporation, low-temperature treatment or the addition of a chemical reactive with the solute.

DISTILLATION

Drying involves the transfer of heat to the wet material and removal of the moisture as water vapour. Usually, this must be performed in such a way as to retain the biological activity of the product.

DISTILLATION

Freeze-drying(lyophilization) is often used where the final products are live cells, as in starter culture preparations, or for thermolabile products.

DISTILLATION

Many recombinant proteins are formed as inclusion bodies, which are insoluble inactive aggregates of over-expressed polypeptides.

Once the producer cells are broken open and the large cell debris is removed, the inclusion bodies are easily recovered from the cell-free extracts by low-speed centrifugation at 5000–20 000gfor 15–60 min.

DISTILLATION

Organisms can be modified to suppress the production of byproducts and enzymes that may interfere with DSP operations or degrade the target product.

DISTILLATION

Enzyme cleavage sites include those for end opeptidases, enterokinase and thrombin, whereas chemical sites are those targeted by acid, cyanogen bromide or hydroxylamine.

FINISHING STEPS

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INCLUSION BODIES AND THE ROLE OF GENETIC ENGINEERING IN DOWN STREAM PROCESS

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PRODUCT DEVELOPMENT, REGULATION AND SAFETY

he development of any new fermentation product depends on many factors, including the market, the current level of scientific knowledge and the regulatory environment.

PRODUCT DEVELOPMENT, REGULATION AND SAFETY

In the case of medical products, a new treatment for a previously untreatable disease or a more effective drug can be extremely profitable for a company.

PRODUCT DEVELOPMENT, REGULATION AND SAFETY

he level of scientific knowledge is a further key factor. Some dvances in science result in the rapid development of industrial processes, termed ‘scientific push’.
For example, Fleming’s discovery of penicillin in 1928 ultimately led to the establishment of the antibiotic industry in the 1940s.

PRODUCT DEVELOPMENT, REGULATION AND SAFETY

In drafting a proposal for the production of any new fermentation product, and throughout its development, a great deal of input must come from disciplines other than microbiology.

PRODUCT DEVELOPMENT, REGULATION AND SAFETY

A typical pharmaceutical, for example, currently takes 10–12 years to come to the market and incurs development costs in excess of $200 million

PRODUCT DEVELOPMENT, REGULATION AND SAFETY

Patents can be separated into three distinct types. Product patents(substances, composition of matter and devices) such as bioinsecticides, recombinant proteins, monoclonal antibodies, plasmids, etc.

PRODUCT DEVELOPMENT, REGULATION AND SAFETY

There are concerns over breeding pigs with part human immune systems for use as sources of organs for transplants xenotransplantation), patenting of human DNA sequences and the release of recombinant DNA systems into the environment

PRODUCT QUALITY AND SAFETY

Prior to approval for marketing, any new food or health-care product that is to be used by humans and domestic or farm animals must be thoroughly tested.

PRODUCT QUALITY AND SAFETY

In the case of pharmaceuticals, for example, the process of manufacture must be validated and the product ‘well haracterized’.

PRODUCT QUALITY AND SAFETY

Approval for non-food and non-drug products is rather less costly and the risk assessments will depend on the microorganism involved, the specific product, and the mode of its production and downstream processing.

PRODUCT QUALITY AND SAFETY

A product, such as an inject able drug for human use, would have a somewhat different and more stringent set of quality criteria than silage for feeding to farm animals.

PRODUCT QUALITY AND SAFETY

Validated standard operating procedures (SOPs) are also required. Their validation involves establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product that meets the predetermined specifications and quality attributes.

PRODUCT QUALITY AND SAFETY

Overall, implementation of these practices should result in the controlled production of a safe product with the elimination of significant batch-to-batch variations in quality.

MANUFACTURING AND ENVIRONMENTAL SAFETY

Certain downstream processing unit operations, such as the use of centrifuges for cell harvesting and some mechanical methods of cell disruption, may generate bioaerosols that could otentially affect employees.

MANUFACTURING AND ENVIRONMENTAL SAFETY

Safety within fermentation industries is particularly important where known pathogens and certain GMMs are employed.

MANUFACTURING AND ENVIRONMENTAL SAFETY

Cultivation of microorganisms necessitates the implementation of different levels of barrier/containment systems depending upon the fermentation process and the classification of the producer microorganism

MANUFACTURING AND ENVIRONMENTAL SAFETY

Overall safety is primarily controlled by international and individual government agencies that regulate: foods, beverages and pharmaceuticals; protection of the environment; and safety in the workplace.

MANUFACTURING AND ENVIRONMENTAL SAFETY

In Japan this is largely overseen by the Ministry of Health and Welfare. Within the EU, the European Commission and European Parliament, as well as individual member states, frame new regulations and ensure their implementation.

MANUFACTURING AND ENVIRONMENTAL SAFETY

Before any new industrial process can start operating, most countries now require that an assessment of risk analysisbe carried out.

MANUFACTURING AND ENVIRONMENTAL SAFETY

Risk group 1 microorganisms are the least likely to cause a problem and, for industrial use, simply require good industrial large-scale practices (GILSP). Most industrial microorganisms are in this category.

MANUFACTURING AND ENVIRONMENTAL SAFETY

Inspectors from the regulatory agencies must give permission for a process to start and for its continued operation.

MICROBIAL ENZYMES AND THEIR APPLICATIONS I

Commercial microbial enzymes are increasingly replacing conventional chemical catalysts in many industrial processes.

Certain enzymes are not restricted to aqueous environments and can operate in two-phase water–organic solvent systems and in non-aqueous organic media, particularly hydrophobic solvents.

MICROBIAL ENZYMES AND THEIR APPLICATIONS I

Enzyme classification is based on a system originally established by the Commission on Enzymes of the International Union of Biochemistry (1979). There are six main classes, grouped according to the type of reaction

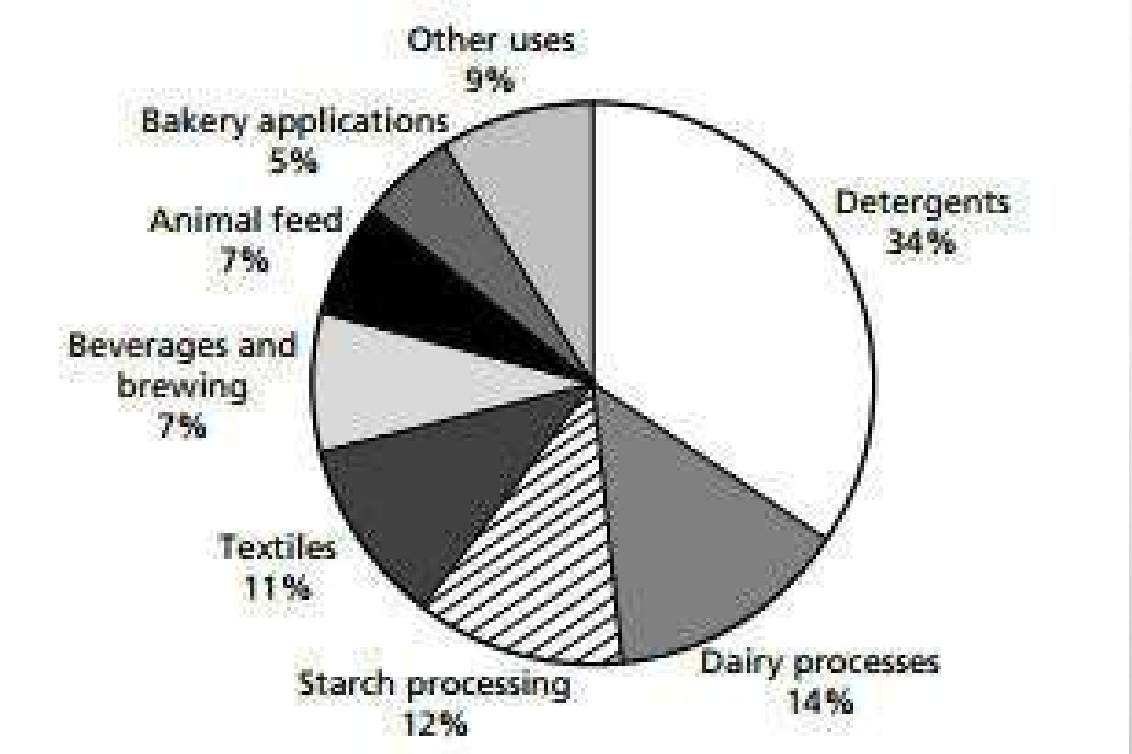
MICROBIAL ENZYMES AND THEIR APPLICATIONS I

Several thousand tonnes of commercial enzymes are currently produced each year, which have a value in excess of US$1500 million.

MICROBIAL ENZYMES AND THEIR APPLICATIONS I

esides their role as aids in traditional processes, ‘bulk’ enzymes are at the centre of many novel processes and biotechnological innovation continues to expand the range of applications.

MICROBIAL ENZYMES AND THEIR APPLICATIONS I



MICROBIAL ENZYMES AND THEIR APPLICATIONS I

There is an increasing demand for fine enzymes used in molecular biology, particularly restriction endonucleases and DNA polymerases,

Enzyme	Source	Use
α-Amylase EC 3.2.1.1	*Aspergillus* & *Bacillus* species	Digestive aids
Alcohol dehydrogenases EC 1.1.1.1 or alcohol oxidases EC 1.1.3.13	*Saccharomyces cerevisiae* *Candida boidinii* & *Pichia pastoris*	Ethanol testing
Asparaginase EC 3.5.1.1	*Escherichia coli*, *Serratia marcescens* & *Erwinia carotovora*	Maintains low asparagine levels in treating cancers (lymphomas and leukaemias) whose cells cannot synthesize the amino acid
Catalase EC 1.11.1.6	*Aspergillus niger*, *Corynebacterium glutamicum* & *Micrococcus lysodeikticus*	Contact lens cleaning systems
Cholesterol esterase EC 3.1.1.13	*Pseudomonas fluorescens*	Monitoring serum cholesterol levels
Creatininase EC 3.5.2.10 (creatinine amidohydrolase)	*Pseudomonas putida* & recombinant *Escherichia coli*	Determination of serum creatinine levels
Glucose oxidase EC 1.1.3.4	*Aspergillus niger*	Analysis of blood glucose levels
β-Lactamase EC 3.5.2.6	*Bacillus cereus* & *Escherichia coli*	Treatment of penicillin allergy
Proteases (various)	Several bacterial & fungal sources	Used as digestive aids, for wound debridement and contact lens cleaning
Rhodanase EC 2.8.1.1 (thiosulphate sulphur transferase)	*Trichoderma* species	Treatment for cyanide poisoning
Streptokinase EC 3.4.22.10 (a cysteine protease)	Various haemolytic streptocci	To break down blood clots
Urease EC 3.5.1.5	*Lactobacillus fermentum*	Removal of urea from blood in renal failure
Uricase EC 1.7.3.3 (urate oxidase)	*Arthrobacter globiformis* & *Candida utilis*	Used in the treatment and diagnosis of gout
Some microbial enzymes used in molecular biology (*DNA directed enzymes)		
DNA ligases (e.g. ATP, EC 6.5.1.1)	*Escherichia coli*	Joining pieces of DNA
DNA polymerase* EC 2.7.7.7	*Thermus aquaticus*	DNA synthesis, e.g. in PCR for forensic analysis
Restriction endonucleases e.g. *Bam* HI and *Eco* RI	*Bacillus amyloliquefaciens* H & *Escherichia coli* RY13	'Cutting' DNA at specific base sequences
RNA polymerase* EC 2.7.7.6	*Salmonella typhimurium*	RNA synthesis

MICROBIAL ENZYMES AND THEIR APPLICATIONS II

Microbial enzymes are predominantly produced by submerged fermentations, although some solid-substrate fermentations are used, particularly for the production of extracellular fungal enzymes

MICROBIAL ENZYMES AND THEIR APPLICATIONS II

The fermenters for bulk enzyme production are up to 100 m3 capacity, but fine enzymes may be produced on smaller scales of a few hundred litres or less.

MICROBIAL ENZYMES AND THEIR APPLICATIONS II

A programme of microorganism screening and selection is necessary, to determine enzyme properties, such as optimum pH and heat resistance, and examination of the ability to secrete the target enzyme

MICROBIAL ENZYMES AND THEIR APPLICATIONS II

The fermentation system and conditions for maximum production of the enzyme per unit of biomass, using inexpensive carbon and nitrogen feed stocks, must then be determined.

Enzyme	EC number	Source	Product/role
Aminoacylase	3.5.1.14	*Aspergillus oryzae*	L-amino acids
Amyloglucosidase (glucoamylase)	3.2.1.3	*Aspergillus niger* *Rhizopus niveus*	glucose production from starch
Glucose isomerase	5.3.1.5	*Actinomyces missouriensis* *Bacillus coagulans*	high fructose corn syrup
Hydantoinase	4.5.2.2	*Flavobacterium ammoniagenes*	D- and L-amino acids
Invertase	3.2.1.26	*Saccharomyces cerevisiae* *Aspergillus niger*	invert sugar (glucose + fructose)
Lactase (β-galactosidase)	3.2.1.23	*Aspergillus oryzae* *Khyveromyces fragilis*	lactose-free milk and whey
Lipase	3.1.1.3	*Rhizopus arrhizus*	cocoa butter substitutes
Naringinase* (hesperidinase)	3.2.1.40 3.2.1.21	*Penicillium decumbens*	debittering of citrus fruit juice
Nitrile hydratase	4.2.1.84	*Rhodococcus rhodochrous*	acrylamide
Penicillin acylase (penicillin amidase)	3.5.2.6	*Escherichia coli* *Bacillus subtilis*	penicillin side chain cleavage
Melibiase (raffinase) (α-galactosidase)	3.2.1.32	*Aspergillus niger* *Saccharomyces cerevisiae*	removal of raffinose from sugar beet extracts
Thermolysin (a zinc protease)	3.4.24.27	*Bacillus thermoproteolyticus*	Aspartame (L-aspartyl-L-phenylalanine methyl ester) a low-calorie sweetener

MICROBIAL ENZYMES AND THEIR APPLICATIONS II

Expression in GRAS-listed organisms provides obvious advantages. Prime candidates for this role are species within three genera of microorganisms, namely Bacillus, Aspergillusand Saccharomyces.

MICROBIAL ENZYMES AND THEIR APPLICATIONS II

A Swiss chemist Jaag, who worked for the detergent company Gebrüder Schnyder, developed a new product containing a bacterial protease, which replaced the animal trypsin that had previously been incorporated into these detergent products.

MICROBIAL ENZYMES AND THEIR APPLICATIONS II

Proteases are not the only enzymes used in detergents; since the late 1980s, amylases and lipases have been available for incorporation, e.g. Lipolase from Novo Nordisk.

STARCH PROCESSING ENZYMES AND RELATED CARBOHYDRATES

Since the 1950s, fungal amylases have been used to manufacture sugar syrups containing specific mixtures of sugars that could not be produced by conventional acid hydrolysis of starch.

STARCH PROCESSING ENZYMES AND RELATED CARBOHYDRATES

A further success has been amyloglucosidase (glucoamylase) from Aspergillus niger and Rhizopus species, which first became available in the early 1960s

STARCH PROCESSING ENZYMES AND RELATED CARBOHYDRATES

Glucose is omerasehas also been a notable success in the starch processing industry.
This enzyme can be obtained from many bacteria, including species of Bacillus and Streptomyces, and is usually immobilized for use in the conversion of glucose to fructose.

STARCH PROCESSING ENZYMES AND RELATED CARBOHYDRATES

Lactase(b-galactosidase) is employed in several industrial processes that require the hydrolysis of lactose from milk, where the disaccharide is present at a concentration of 4.7% (w/v).

STARCH PROCESSING ENZYMES AND RELATED CARBOHYDRATES

east enzymes have been immobilized by incorporation into cellulose acetate fibres and the Aspergillusenzymes have been immobilized on 0.5 mm diameter porous silica for use in packed bed reactors

OTHER BENEFICIAL ENZYMES

Enzymes in cheese production

Rennet preparations from the stomachs of calves, lambs and kids have been used in cheese production for thousands of years.

OTHER BENEFICIAL ENZYMES

Enzymes in plant juice production
Several microbial enzymes are employed in fruit juice processing, but probably the most important are pectinases.

OTHER BENEFICIAL ENZYMES

In wine making, commercial enzymes are used for several purposes, as well as for juice extraction. For red wines, colour extraction from the grape skins during pressing can be promoted by the addition of commercial cellulases, e.g. from the fungus Trichoderma reesei.

OTHER BENEFICIAL ENZYMES

Enzymes in textile manufacture
Enzymes are being increasingly used in textile processing for the finishing of fabrics and garments, especially in desiring, iopolishing and denim washing

OTHER BENEFICIAL ENZYMES

Enzymes in leather manufacture

Proteases and lipases are now extensively used in the processing of hides and skins. These enzymes are easier to use, more pleasant to handle and safer than the harsh chemicals that were previously employed.

OTHER BENEFICIAL ENZYMES

wood pulps

Paper manufacture is a major world industry. In the USA alone over 70 million tonnes of paper and paperboard are manufactured each year, which have a value in excess of US$50 billion.

OTHER BENEFICIAL ENZYMES

Enzymes as catalysts in organic synthesis
There is a rapidly growing market for microbial enzymes used in the synthesis of high-value organic compounds for the chemical, food and pharmaceutical 142 Chapter 9 industries.

OTHER BENEFICIAL ENZYMES

Enzyme-based processes can be used to prepare specific enantiomers and resolve enantiomers from racemic mixtures.

FUELS AND INDUSTRIAL CHEMICALS I

Biological fuel generation is likely to become increasingly important, especially as it can provide both liquid and gaseous fuels.

FUELS AND INDUSTRIAL CHEMICALS I

Numerous other important chemical compounds are now most economically produced by microbial fermentation and biotransformation processes.

FUELS AND INDUSTRIAL CHEMICALS I

Methane is used for both domestic and industrial fuel.
At present, supplies mostly come from gas and oil fields or the gasification of coal.

FUELS AND INDUSTRIAL CHEMICALS I

1 acetone–butanol(Clostridium acetobutylicum), additional products: butyric acid, acetic acid, acetoin, ethanol, $CO_2$and H_2;
2 butanol–isopropanol (Clostridium butylicum), additional products: butyric acid, acetic acid, CO_2 and H_2; and
3 butyric acid–acetic acid(Clostridium butyricum), additional products: $CO_2$and H_2

FUELS AND INDUSTRIAL CHEMICALS I

Besides the existing role as a solvent and chemical feedstock, butanol has several properties that are favourable for motor fuel use, either alone or when blended with gasoline

FUELS AND INDUSTRIAL CHEMICALS I

In the past, the economically viable production of butanol has normally required a fermenter volume of at least 1000 m_3

FUELS AND INDUSTRIAL CHEMICALS I

A proportion of butanol production in China and a few other countries may still be via similar fermentation processes, but the last industrial acetone–butanol– ethanol fermentation operated in the western world closed in the early 1990s.

FUELS AND INDUSTRIAL CHEMICALS I

The traditional batch methods have suffered from several problems, including contamination by lactobacilli, bacteriophage attack, product inhibition, high energy costs for distillation and the fact that a mixture of fermentation products is obtained.

FUELS AND INDUSTRIAL CHEMICALS I

Most regions of the world have traditionally produced alcoholic beverages from locally available substrates

Ethanol is an attractive fuel because it may be used alone or mixed with other liquid fuels, e.g. ‘gasohol’, a blend of 10–22% (v/v) ethanol with gasoline

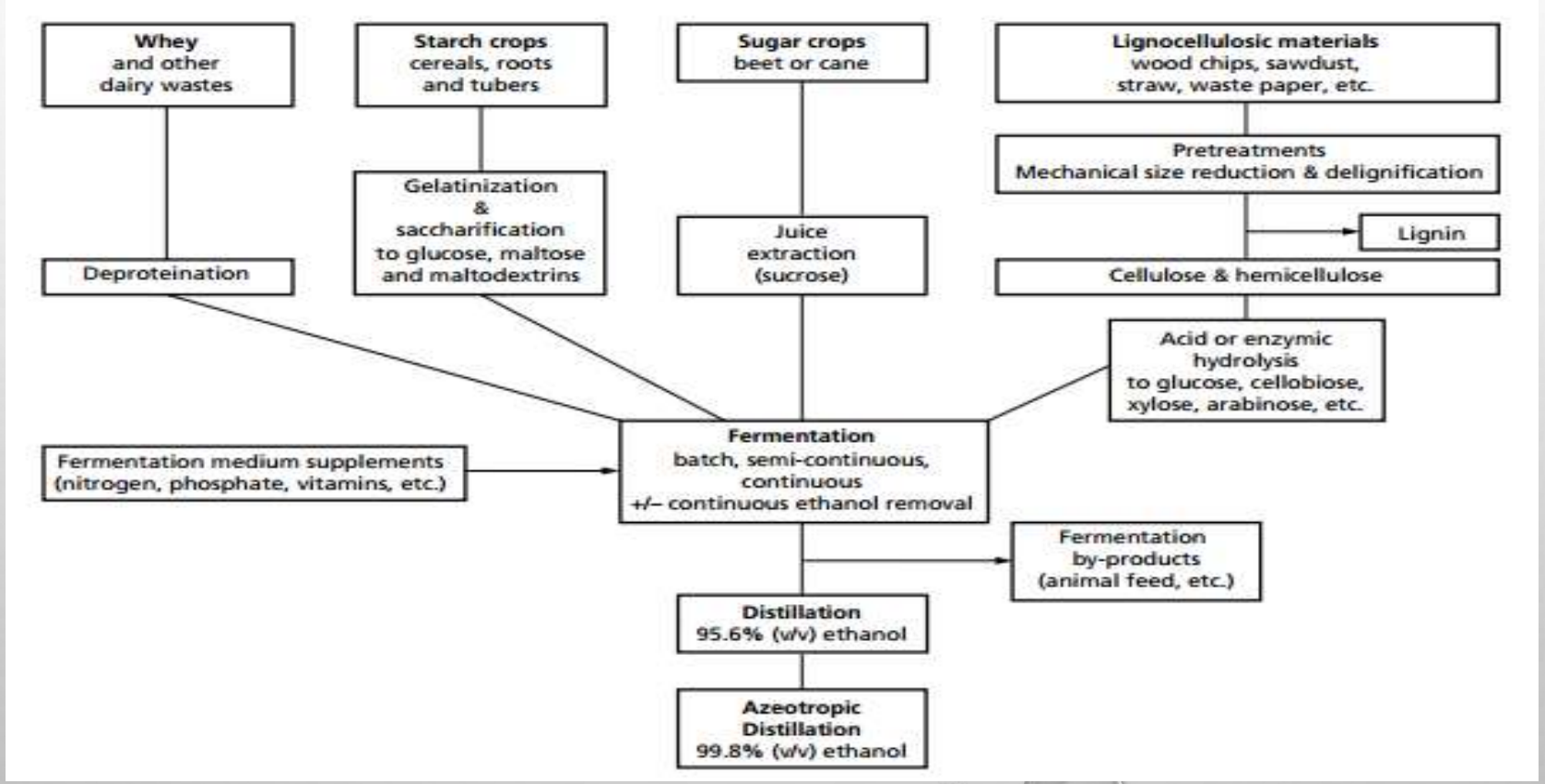
FUELS AND INDUSTRIAL CHEMICALS I

In North America, wet or dry milling processes have been developed for maize processing to separate corn oil from the starch.

FUELS AND INDUSTRIAL CHEMICALS I

Fermentations may be batch or continuous processes, often with some form of cell recycle, which reduces both the fermentation time and the amount of substrate ‘wasted’ in conversion to unwanted biomass.

FUELS AND INDUSTRIAL CHEMICALS I



FUELS AND INDUSTRIAL CHEMICALS I

Unlike energy crops (cereals, sugar cane and beet, etc.), lignocellulosic plant wastes (sawdust, wood chips, straw, bagasse, waste paper, etc.) have no direct food use.

FUELS AND INDUSTRIAL CHEMICALS I

Hydrogen is a very attractive fuel because of its high energy content (118.7 kJ/g), which is about four-fold greater than ethanol and over two-fold higher than methane.

FUELS AND INDUSTRIAL CHEMICALS I

heoretically, 4 mol of hydrogen could be generated from each mole of glucose, which represents only a 33% energy yield. However, most organisms produce much less.

ELECTRICITY

- THE ROLE OF MICROORGANISMS IN ELECTRICITY GENERATION
- MAY INVOLVE MICROBIALLY PRODUCED GASEOUS AND LIQUID
- FUELS, SUCH AS ETHANOL OR METHANE, BEING USED TO DRIVE
- CONVENTIONAL MECHANICAL GENERATORS. ALTERNATIVELY, DIRECT
- GENERATION MAY BE USED, BUT THIS IS STILL IN THE EARLY
- STAGES OF DEVELOPMENT.

ELECTRICITY

- POSSIBLE ROUTES ARE VIA INTACT
- MICROORGANISMS OR MICROBIAL ENZYMES INCORPORATED
- WITHIN FUEL CELLS (FIG. 10.6). ENZYME-BASED SYSTEMS ARE
- PREFERRED, AS ELECTRON TRANSFER BETWEEN WHOLE CELLS AND
- ELECTRODES IS GENERALLY LESS EFFICIENT. IN SOME CASES, IMMOBILIZED
- ENZYMES MAY BE USED.

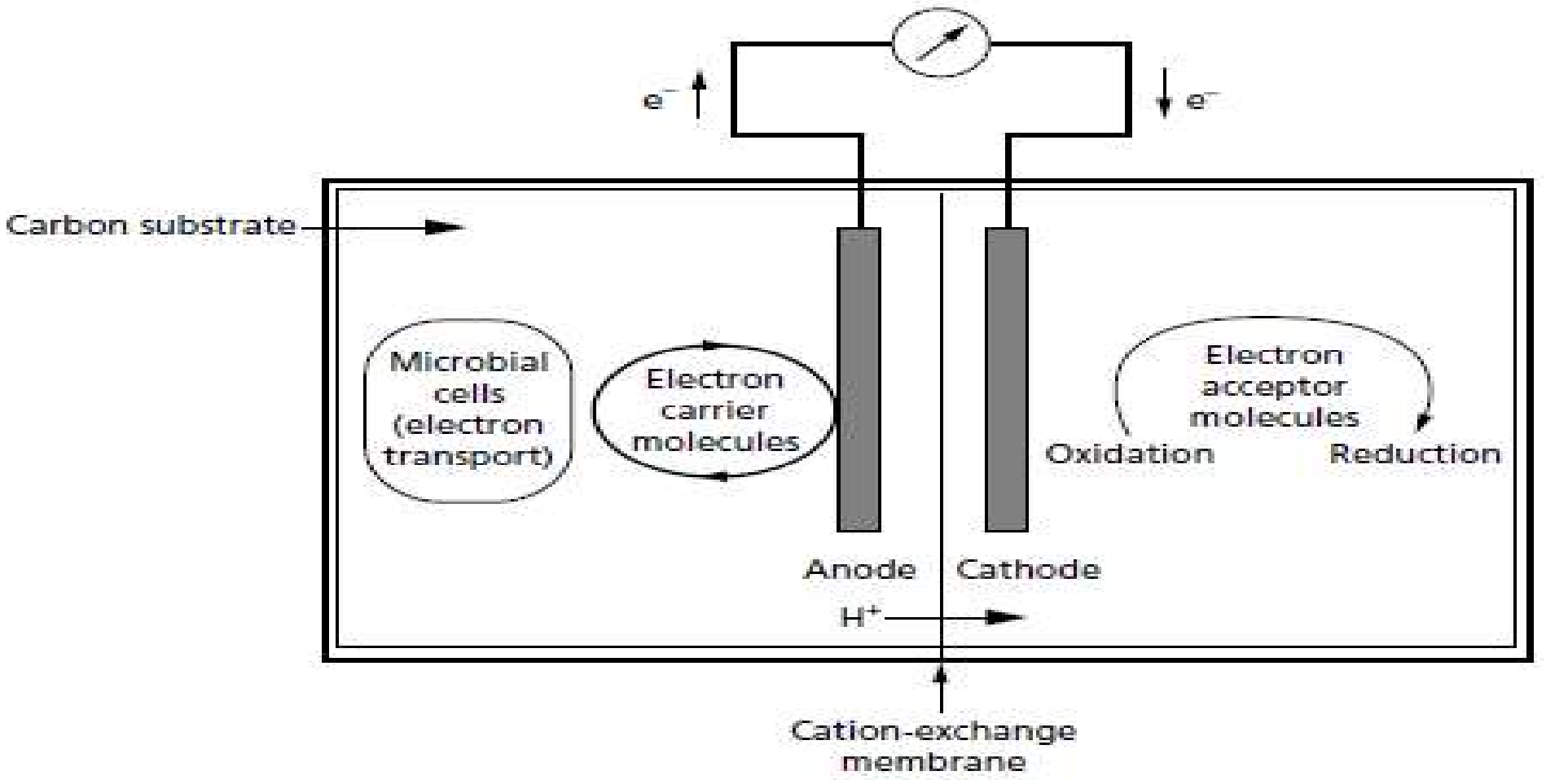
ELECTRICITY

- POSSIBLE CANDIDATES ARE
- MICROBIAL DEHYDROGENASES COUPLED TO ELECTRODE SYSTEMS
- AND CATALYSING THE INTERCONVERSION OF HYDROGEN AND
- ELECTRICITY. ALSO, THERE IS THE POSSIBILITY THAT PHOTOTROPHIC
- MICROORGANISMS, OR THEIR PHOTOACTIVE SYSTEMS,
- COULD DIRECTLY CONVERT SUNLIGHT TO ELECTRICITY.

ELECTRICITY

- FOR
- EXAMPLE, USING ARTIFICIAL MEMBRANES INCORPORATING
- BACTERIORHODOPSIN-BASED SYSTEMS FROM ARCHAEANS, E.G. *HALOBACTERIUM HALOBIUM*. *SUCH SYSTEMS FACILITATE THE*
- LIGHT-DEPENDENT TRANSLOCATION OF PROTONS AND THE RESULTING
- TRANSMEMBRANE ELECTROCHEMICAL GRADIENT CREATED
- COULD BE USED TO GENERATE ELECTRICITY.

A microbial fuel cell.



AMINO ACIDS

- Several amino acids are produced in commercial quantities via direct fermentation processes using overproducing microbial strains, or by microbial biotransformation.
- They are mostly employed as food or animal feed supplements and flavour compounds.
- However, several amino acids also have uses in pharmaceuticals and cosmetics, and in the chemical industry for the manufacture of polymers.

AMINO ACIDS

- **L-GLUTAMIC ACID**

- Of all the amino acid production processes, that of lglutamic acid is probably the most important in terms of quantity.
- Its main use is as the flavour enhancer, monosodium l-glutamate (MSG), which can heighten and intensify the flavour of foods without adding significant flavour of its own.

AMINO ACIDS

- MSG is naturally present in certain foods and was discovered to be the ‘active’ component of a traditional flavour-enhancing seaweed stock used in far eastern foods.
- This compound was first isolated from the seaweed, *laminaria japonica, in* 1908.
- Commercial production in japan followed almost immediately, using extracts of soya protein and wheat gluten.

AMINO ACIDS

- In 1959 the us food and drug administration (FDA) classified MSG as ‘generally regarded as safe’ (GRAS) due to its history of safe use, and the joint food and agriculture organization (FAO)/world health organization (WHO) expert committee on food additives (1970) gave the acceptable daily intake as 0–120 mg/kg body weight.

AMINO ACIDS

- Since the early 1960s, the classical production methods using plant sources have largely been replaced by fermentation processes, which are now responsible for an annual production in excess of 400 000 tonnes.
- The price of MSG in international trade is an average of US$1.20/kg and apart from extensive use in oriental foods, it is added to a wide range of food products, particularly soups, gravies, sauces and snack foods.

AMINO ACIDS

- Glutamic acid-producing microorganisms include species of the closely related genera *arthrobacter, brevibacterium, corynebacterium, microbacterium* and *micrococcus*.
- *These are gram-positive, biotinrequiring,* non-motile bacteria that have intense glutamate dehydrogenase activity.

AMINO ACIDS

- The overall strategy for achieving overproduction of the amino acid involves:

1. **increasing the activity of anabolic enzymes;**
2. **manipulation of regulation to remove feedback** control mechanisms;
3. **blocking pathways that lead to unwanted** byproducts;
4. **blocking pathways that result in degradation of the** target product; and
5. **limiting the ability to process the immediate precursor** of l-glutamic acid, namely oxoglutaric acid, to the next intermediate of the tricarboxylic acid (TCA) cycle, succinyl coenzyme A (coa), i.e. Use of mutants lacking oxoglutaric acid dehydrogenase.

AMINO ACIDS

- During the growth phase these mutants produce essential intermediates from isocitrate via the glyoxylate cycle.
- In addition, as these bacteria do not normally secrete glutamate, a range of treatments are employed to render the cells more permeable and aid release of the amino acid into the medium.

AMINO ACIDS

- These treatments include: biotin limitation, restriction of phospholipid biosynthesis by adding C16–C18 saturated fatty acids during the growth phase, and inclusion of surfactants (e.g. Tween 40) and penicillin in the production media.

ORGANIC ACIDS

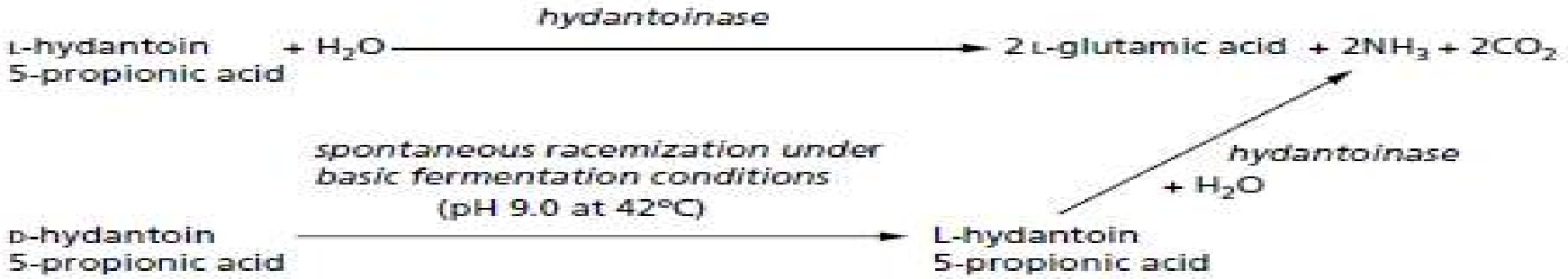
- **CITRIC ACID**

- Citric acid is widely used in the food industry as an acidulant and flavouring agent in beverages, confectionery and other foods, and in leavening systems for baked goods.
- As a food constituent, its use is unrestricted because it has GRAS status.

Production of L-amino acids by biotransformation.

(a) L-Glutamic acid

Starting material: a racemic mixture of D- and L-hydantoin 5-propionic acid



(b) L-Lysine

Starting material: a racemic mixture of D- and L-α-aminocaprolactam



ORGANIC ACIDS

- This organic acid also has many non-food applications.
- They include roles in maintaining metals in solution for electroplating, as a cleaning and ‘pickling’ agent for metals, and as a replacement for polyphosphates in the detergent industry, along with several pharmaceutical uses.
- Until the 1920s citric acid was mainly prepared from lemon juice, but in 1923 pfizer began operating a fermentation- based process in the USA.

ORGANIC ACIDS

- The production organism was the filamentous fungus *aspergillus niger,* an obligate aerobe, which was grown in surface culture on a medium of sucrose and mineral salts.
- Virtually all the worldwide output is now produced by fermentation, which is primarily located in western europe, the USA and china.
- Citric acid has become one of the world's major fermentation products, with an annual production of over 550 000 tonnes and a value approaching US$800 million.

ORGANIC ACIDS

- The demand for citric acid is still increasing, particularly for beverage applications.
- Surface methods are still operated, but since the latem 1940s, submerged fermentations have become the principal mode of production.
- Many microorganisms, including filamentous fungi, yeasts and bacteria, can be used to produce this primary metabolite.

ORGANIC ACIDS

- However, *A. Niger still remains as the predominant industrial producer.*
- Specific strains have been developed for various types of fermentation processes, which are capable of generating high yields of citric acid, often in excess of 70% of the theoretical yield from the carbon source.

ORGANIC ACIDS

- CITRIC ACID BIOSYNTHESIS

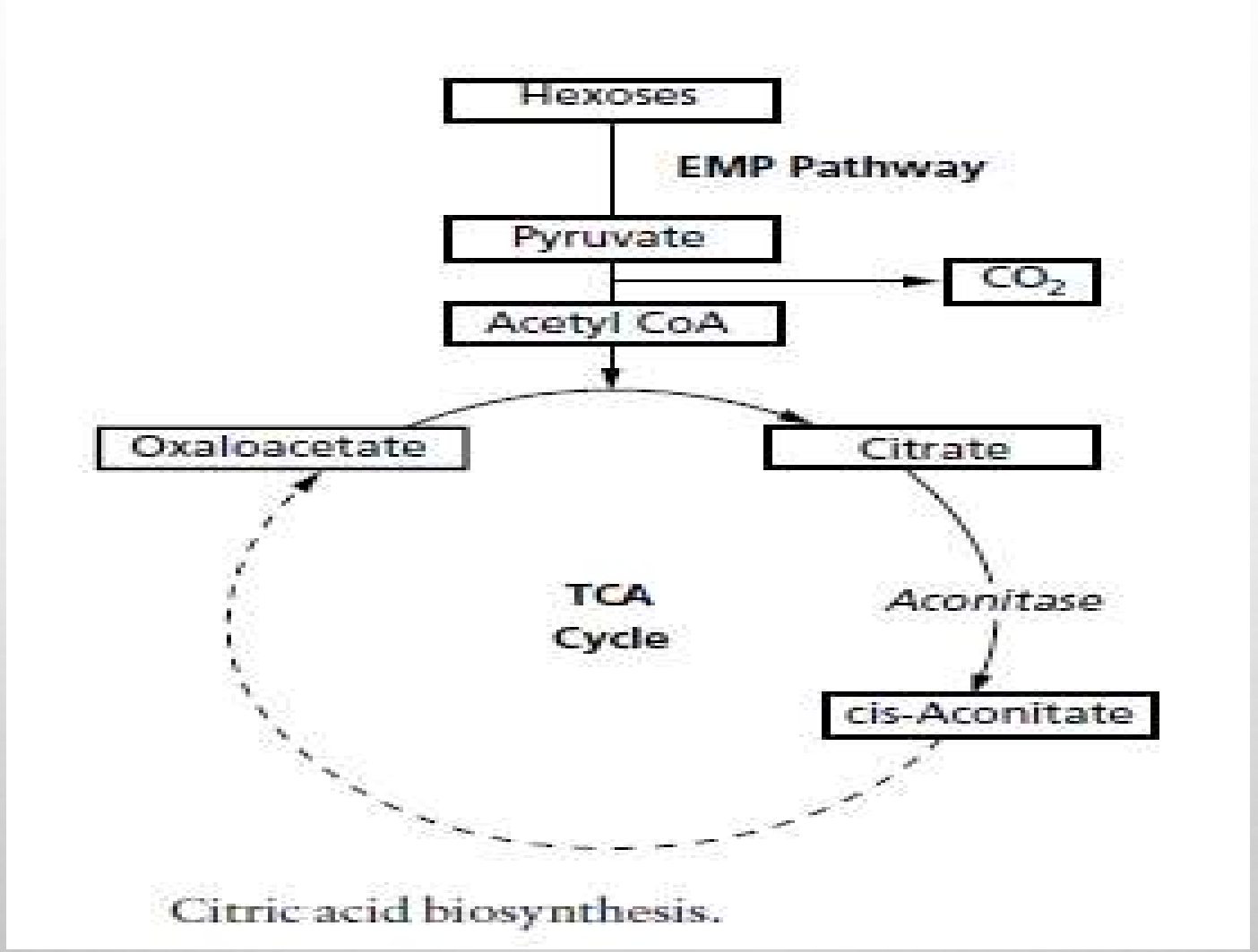
- The metabolic pathways involved in citric acid biosynthesis are the embden–meyerhof–parnas (EMP) pathway and the TCA cycle. *A. Niger also operates* the pentose phosphate pathway, which can compete with glycolysis for carbon units.
- The first stages of citric acid formation involve the breakdown of hexoses to pyruvate in glycolysis, followed by its decarboxylation to produce acetyl COA.

ORGANIC ACIDS

- Very importantly, the CO2 released during this reaction is not lost, but is recycled by pyruvate carboxylase (produced constitutively in *aspergillus) in the* anaplerotic formation of oxaloacetate.
- Normally, oxaloacetate would largely be supplied through the completion of the TCA cycle, allowing recommencement of the cycle by condensing with acetyl coa to form citrate, catalysed by citrate synthase.

ORGANIC ACIDS

- In order to accumulate citrate, its onward metabolism (continuation of the cycle) must be blocked.
- This is achieved by inhibiting aconitase, the enzyme catalysing the next step in the TCA cycle.
- Inhibition is accomplished by removal of iron, an activator of aconitase.
- Consequently, during citrate accumulation, the TCA cycle is largely inoperative beyond citrate formation, hence the importance of the anaplerotic routes of oxaloacetate formation.



Citric acid biosynthesis.

HEALTH-CARE PRODUCTS I

- Antibiotics are probably the most important group of compounds synthesized by industrial microorganisms.
- They are not produced in the greatest quantity, nor are they the most economically valuable.
- Nevertheless, over the last 60 years their influence in improving human health has been immense.

HEALTH-CARE PRODUCTS I

- The other major health-care products derived from microbial fermentations and/or biotransformation are alkaloids, steroids, toxins and vaccines; along with vitamins, certain enzymes, and viable microbial cell preparations used as probiotics.
- In addition, genetic engineering techniques have made it possible for microorganisms to produce a wide variety of mammalian proteins and peptides that have various therapeutic properties.

HEALTH-CARE PRODUCTS I

- Those of considerable medical importance and with established markets include insulin, interferons, human growth hormone and monoclonal antibodies.
- Apart from these therapeutic agents, which cure or reduce the incidence of disease, many diagnostic products are also derived from microorganisms.
- These are extensively used to test for the presence of various health and disease states.

HEALTH-CARE PRODUCTS I

- **ANTIBIOTICS**
- Most antibiotics are secondary metabolites produced by filamentous fungi and bacteria, particularly the actinomycetes.
- Well over 4000 antibiotics have been isolated from various organisms, but only about 50 are used regularly in antimicrobial chemotherapy.

HEALTH-CARE PRODUCTS I

- The best known and probably the most medically important antibiotics are the b**-lactams, penicillins and** cephalosporins; along with **aminoglycosides, such as** streptomycin, and the broad-spectrum **tetracyclines.**
- The remainder fail to fulfil certain important criteria, particularly their lack of selectivity, exhibiting toxicity to humans or animals, or their high production costs.
- Some antibiotics have applications other than in antimicrobial chemotherapy.

HEALTH-CARE PRODUCTS I

- For example, actinomycin and mitomycin, produced by *streptomyces peucetius and S. Caepitosus, respectively, have* roles as antitumour agents;
- and other antibiotics are used for controlling microbial diseases of crop plants, or as tools in biochemistry and molecular biology research.
- Several antibiotics are also added to animal feed as growth promoters.

HEALTH-CARE PRODUCTS I

- However, worries about the development of resistance has meant that some antibiotics, used or intended for human use, may be withdrawn from use in animal feed.
- For example, the EU commission voted to ban the application of bacitracin, spiromycin, tylomycin and virginiamycin as growth promoters after january 1999.

HEALTH-CARE PRODUCTS I

- B**-lactams** over 100 b-lactams, mostly penicillins and cephalosporins, have been approved for human use, and they account for over half of the antibiotics produced worldwide.
- This group is especially useful because of their wide margin of safety.
- They specifically target the synthesis of peptidoglycan, a vital bacterial cell wall component, which is not present in eukaryotic organisms, thus providing a high level of selectivity.
- They primarily inhibit the cross-linking transpeptidation reaction, resulting in the formation of incomplete peptidoglycan, severely weakening the bacterial cell wall structure.

HEALTH-CARE PRODUCTS I

- PENICILLIN

- penicillin was discovered by fleming in 1928 following his famous observation of an inhibitory zone surrounding a fungal contaminant, *penicillium notatum, on a* plate of *staphylococcus aureus.*
- *In the late 1930s florey,* chain and heatley characterized the inhibitory compound responsible, penicillin, and developed a protocol that allowed it to be produced in a pure form.

Examples of important antibiotics

Antibiotic	Producer organism	Activity against	Site or mode of action
β-Lactams			
Natural penicillins	*Penicillium chrysogenum*	Gram-positive bacteria	Wall synthesis
Penicillin G (benzylpenicillin)			
Penicillin V (phenoxymethylpenicillin)			
Semi-synthetic penicillins			
Improved spectrum penicillins		Some Gram-negative rods	
Aminopenicillins			
Ampicillin			
Amoxicillin			
Hetacillin			
Penicillinase-resistant penicillins		Antistaphylococcal	
Methicillin			
Cloxacillin			
Dicloxacillin			
Nafcillin			
Oxacillin			
Extended spectrum penicillins		Antipseudomonal	
Carboxypenicillins			
Carbenicillin			
Ticarcillin			
Ureidopenicillins			
Azlocillin			
Mezlocillin			
Piperacillin			
Cephalosporins	*Cephalosporium acremonium*	Broad spectrum	Wall synthesis
Monobactams	*Chromobacterium violaceum*	Gram-negative bacteria	Wall synthesis

Examples of important antibiotics

Antibiotic	Producer organism	Activity against	Site or mode of action
Polypeptide antibiotics			
Bacitracin	*Bacillus subtilis*	Gram-positive bacteria	Wall synthesis
Polymyxin B	*Paenibacillus polymyxa*	Gram-negative bacteria	Cell membrane
Macrolides			
Erythromycin	*Saccharapolyopora erythraea* (formerly *Streptomyces erythraeus*)	Gram-positive bacteria	Protein synthesis
Tylosin	*Streptomyces fradiae*	Gram-positive bacteria	Protein synthesis
Aminoglycides			
Gentamicin	*Micromonospora purpurea*	Broad spectrum	Protein synthesis
Neomycin	*Streptomyces fradiae*	Broad spectrum	Protein synthesis
Streptomycin	*Streptomyces griseus*	Gram-negative bacteria	Protein synthesis
Tetracyclines	*Streptomyces* species	Broad spectrum	Protein synthesis
Vancomycin	*Streptomyces orientalis*	Gram-positive bacteria	Peptidoglycan
Rifamycins	*Amycolatopsis mediterranei* (formerly *Streptomyces mediterranei*)	Tuberculosis	RNA synthesis
Antifungal antibiotics			
Polyenes			
Amphotericin B	*Streptomyces nodosus*	Fungi	Cell membrane
Nystatin	*Streptomyces noursei*	Fungi	Cell membrane
Griseofulvin	*Penicillium griseofulvum*	Dermatophytic fungi	Microtubules

HEALTH-CARE PRODUCTS II

- Penicillin exhibits the properties of a typical secondary metabolite, being formed at or near the end of exponential growth.
- Its formation depends on medium composition and dramatic overproduction is possible.
- However, *P. Notatum, the organism originally found to* produce the antibiotic, generated little more than 1 mg/L from the surface cultures initially used for penicillin production.

HEALTH-CARE PRODUCTS II

- A 20–25-fold increase in yield was achieved when corn steep liquor was incorporated into the fermentation medium.
- This byproduct of maize processing contains various nitrogen sources, along with growth factors and side-chain precursors, and remains as a major ingredient of most penicillin production media.

HEALTH-CARE PRODUCTS II

- Even greater penicillin yields were obtained from a closely related species, *penicillium chrysogenum, which was* originally isolated from a mouldy cantaloup melon.
- Further increases in yield were achieved when productio went over to submerged fermentation.
- The wartime requirements for penicillin stimulated the rapid development of a large-scale submerged culture system using stirred tank reactors (strs).

HEALTH-CARE PRODUCTS II

- Each vessel was continuously stirred via vertical shaft-driven turbine impellers and incorporated air sparging.
- These technological developments had a major impact on the advancement of the whole field of fermentation technology.
- Since the 1940s, penicillin yield and fermentation productivity has been vastly improved by extensive mutation and selection of producer strains.

HEALTH-CARE PRODUCTS II

- He traditional approach to improving penicillin yields involved random mutation and selection of higher producing strains.
- Resulting mutants were grown in liquid medium and culture filtrates were assayed for penicillin.
- This was slow and painstaking as large numbers of strains had to be tested.
- Nevertheless, such methods were the key to the dramatically increased yields achieved since the discovery of penicillin.

HEALTH-CARE PRODUCTS II

- Penicillin fermentations now produce yields in excess of 50 g/L, a 50 000-fold increase from the levels first produced by fleming's original isolate.
- The contribution of classical methods of strain improvement has so far outweighed all other approaches, including more recently available genetic manipulation techniques.
- The latter have contributed more to our understanding of the complex mechanisms of penicillin biosynthesis, particularly the genetic arrangement of improved strains, the identification of bottlenecks inpenicillin synthesis and the regulation of secondary metabolism in overproducing strains.

HEALTH-CARE PRODUCTS II

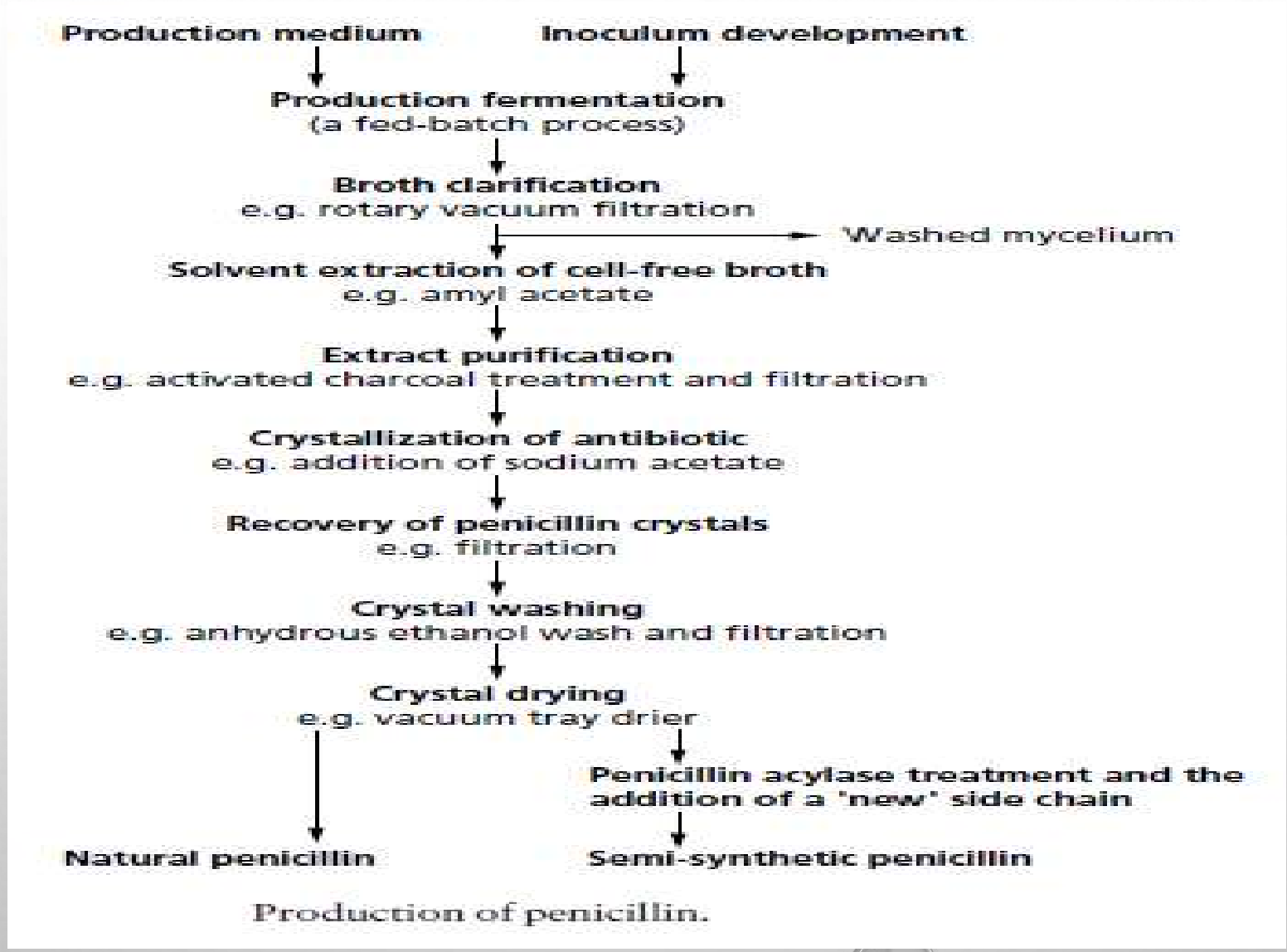
- The basic structure of the penicillins is **6-aminopenicillanic acid (6-APA), composed of a thiazolidine ring** fused with a b**-lactam ring whose 6-amino position** carries a variety of **acyl substituents.**
- **This blactam–** thiazolidine structure**,** synthesized from **l-aaminoadipate, l-cystine and l-valine, is common to** penicillins, cephalosporins and cephamycins.

HEALTH-CARE PRODUCTS II

- In the absence of added side-chain precursors to the fermentation medium of *P. Notatum* or *P. Chrysogenum, a mixture* of natural penicillins is obtained from culture filtrates, notably penicillin G (benzyl penicillin) and the more acid-resistant penicillin V (phenoxymethyl penicillin).
- These penicillins are most active against grampositive bacteria.
- However, an expanded role for the penicillins came from the discovery that different biosynthetic penicillins can be formed by the addition of side-chain precursors to the fermentation medium and that natural penicillins can be modified chemically to produce compounds with improved characteristics.

HEALTH-CARE PRODUCTS II

- *Commercial production of penicillin* penicillin production is usually via a fed-batch process carried out aseptically in stirred tank fermenters of 40000–200000L capacity, although airlift systems are sometimes used.
- The fermentation involves an initial vegetative growth phase followed by the antibiotic production phase.



Production of penicillin.

FOOD AND BEVERAGE FERMENTATIONS I

- Microorganisms have long played a major role in the production of food and beverages.
- Traditional fermented foodstuffs include:

1. **alcoholic beverages, especially beers, wines and distilled** spirits, which are derived from sugars and starches;
2. **dairy products, particularly cheeses, yoghurt, sour** cream and kefir;
3. **fish and meat products, such as fish sauce and fermented** sausages;

FOOD AND BEVERAGE FERMENTATIONS I

4. PLANT PRODUCTS, NOTABLY CEREAL-BASED BREADS AND

- Fermented rice products; along with fermented fruits, vegetables and legumes, including preserved olives and gherkins, sauerkraut, soy sauce, tofu, fermented cassava, cocoa and coffee beans. Many of these products originally evolved as a means of food preservation.

FOOD AND BEVERAGE FERMENTATIONS I

- The stabilizing microbial activity may result in lower water activity, modified ph, generation of inhibitory compounds (alcohol, bacteriocins, etc.
- And removal of nutrients readily utilized by potential spoilage organisms.
- Importantly, besides providing long-term stability, these fermentation processes also generate desirable flavour, aroma and texture.

FOOD AND BEVERAGE FERMENTATIONS I

- The role of microorganisms in this field is now even more diverse.
- Microbial biomass is used as novel food, such as single cell protein products, speciality mushrooms and probiotic preparations;
- numerous microbial fermentation products are also incorporated as food additives and supplements. In addition, microbial enzymes are utilized extensively as food processing aids, and some may be added to animal feed to improve its nutritional value.

FOOD AND BEVERAGE FERMENTATIONS I

➢ ALCOHOLIC BEVERAGES

- Alcoholic beverages have been produced throughout recorded human history.
- They are manufactured worldwide from locally available fermentable materials, which are sugars derived either from fruit juices, plant sap and honey, or from hydrolysed grain and root starch.
- Some alcoholic beverages are drunk fresh, but more commonly they are aged to modify their flavour, whereas others are distilled to increase alcoholic strength.

FOOD AND BEVERAGE FERMENTATIONS I

- Although bacteria such as *zymomonasspecies* may be involved in the production of certain products, yeasts are primarily used, either in single or mixed cultures.
- Their fermentation products are ethanol, a range of desirable organoleptic (flavour and aroma) compounds and co2 (provides carbonation for some products).

FOOD AND BEVERAGE FERMENTATIONS I

- The yeasts involved in these alcoholic fermentations are mostly strains of *saccharomyces cerevisiae, which cannot directly ferment starch.*
- *They* require prior hydrolysis of the polysaccharide to simple sugars and small dextrins (not greater than three glucose units).
- Traditionally, this is achieved by using fungal or plant amylases. These enzymes may be inherent elements of the carbohydrate source or added during processing.

FOOD AND BEVERAGE FERMENTATIONS I

➢ BEER BREWING

- The term beer is given to non-distilled alcoholic beverages made from partially germinated cereal grains, referred to as malt.
- They include ales, lagers and stouts, which normally contain 3–8% (v/v) ethanol.
- Their other main ingredients are hops (giving beer a characteristic flavour and aroma), water and yeast.
- The brewing process is essentially divided into four main stages.

FOOD AND BEVERAGE FERMENTATIONS I

1. **MALTING IS THE PARTIAL GERMINATION OF CEREAL GRAIN FOR** 6–9 days to form malt.

- This is the primary beer ingredient and contains mostly starch, some protein and hydrolytic enzymes.
- Malted barley is predominantly used, but beers are also made with malted wheat, occasionally malted oats and even malted sorghum.

FOOD AND BEVERAGE FERMENTATIONS II

2. **Mashing and wort preparation involves the production** of the aqueous fermentation medium, otherwise known as **wort.**

- **It contains fermentable sugars, amino** acids and other nutrients, and is prepared by solubilizing malt components through the action of endogenous hydrolytic enzymes.
- In most countries, a proportion of adjuncts are now also added, which are unmalted cereal and non-cereal starch sources, and sugar syrups.

FOOD AND BEVERAGE FERMENTATIONS II

3. yeast fermentation is a non-aseptic batch process that uses a starter culture of a selected brewing strain of *S. Cerevisiae*.

- *The inoculated wort undergoes an alcoholic* fermentation to produce ethanol, CO2 and minor metabolites that contribute to flavour and aroma.
- Fermentations usually last for 2–7 days depending upon the type of beer being produced.

Some examples of alcoholic beverages from around the world

Substrate	Non-distilled beverage	Product of alcoholic fermentation distilled to form	Location
Fruit juices and plant saps			
Apples	Cider	Apple brandy, calvados, etc.	N. Europe, N. America
Cacti/succulents	Pulque	Tequila	Mexico, Central America
Grapes	Wine	Grape brandy, cognac, armagnac, etc.	S. Europe, N. & S. America, Australia, New Zealand
Palmyra	Toddy	Arak	India
Pears	Perry	Pear brandy, williams, etc.	N. Europe
Honey	Mead		UK
Sugar cane or molasses	–	Rum	West Indies
Starches			
Barley, plus other cereals*	Beer	Whisk(e)y†	Beer: ales—UK mostly; lager—worldwide
	–		Whisk(e)y: Scotland, Ireland, USA, Canada, Japan
Rice‡	Sake	Shochu	Japan
	Pachwai		India
	Sonti		
Sorghum*‡	Kaffir		Central and South Africa

FOOD AND BEVERAGE FERMENTATIONS II

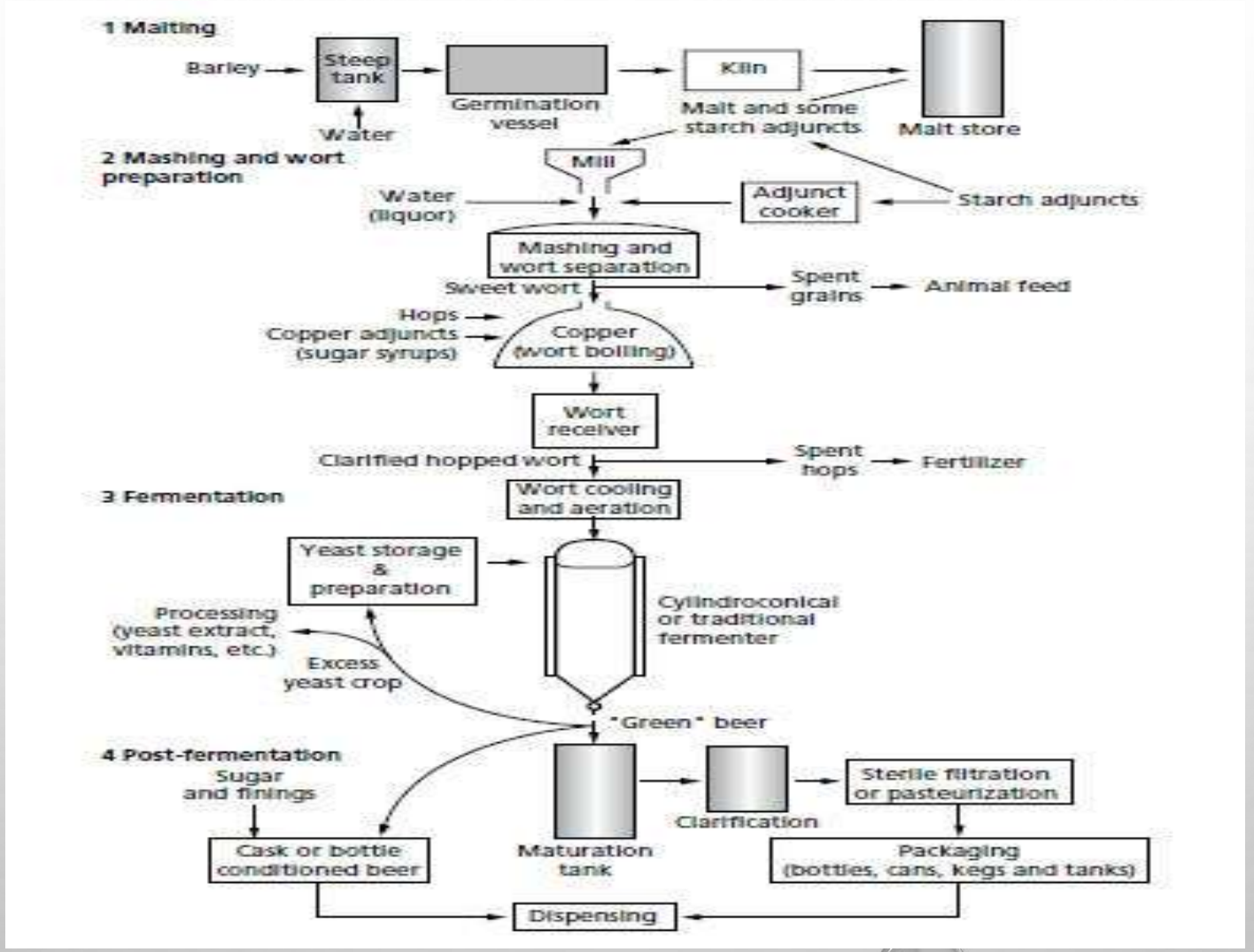
4. Post-fermentation treatments are conducted to mature

- Or condition the new beer to make it ready for consumption, which may take from one to several weeks.
- The raw materials, excluding hops, the preparation of the wort and the yeast fermentation, are essentially the same for the production of both whisky and malt vinegar.
- However, the major objectives are somewhat different. For whisky and vinegar production, the main aim is to maximize alcohol production prior to the respective steps of distillation and acetification.

FOOD AND BEVERAGE FERMENTATIONS II

- MALT AND MALTING malting involves the controlled partial germination of barley grain.
- This modifies the hard vitreous grain into a friable (easily crushed) form containing more readily degradable starch and generates hydrolytic enzymes, especially amylases, b-glucanases and proteases.

The beer brewing process.

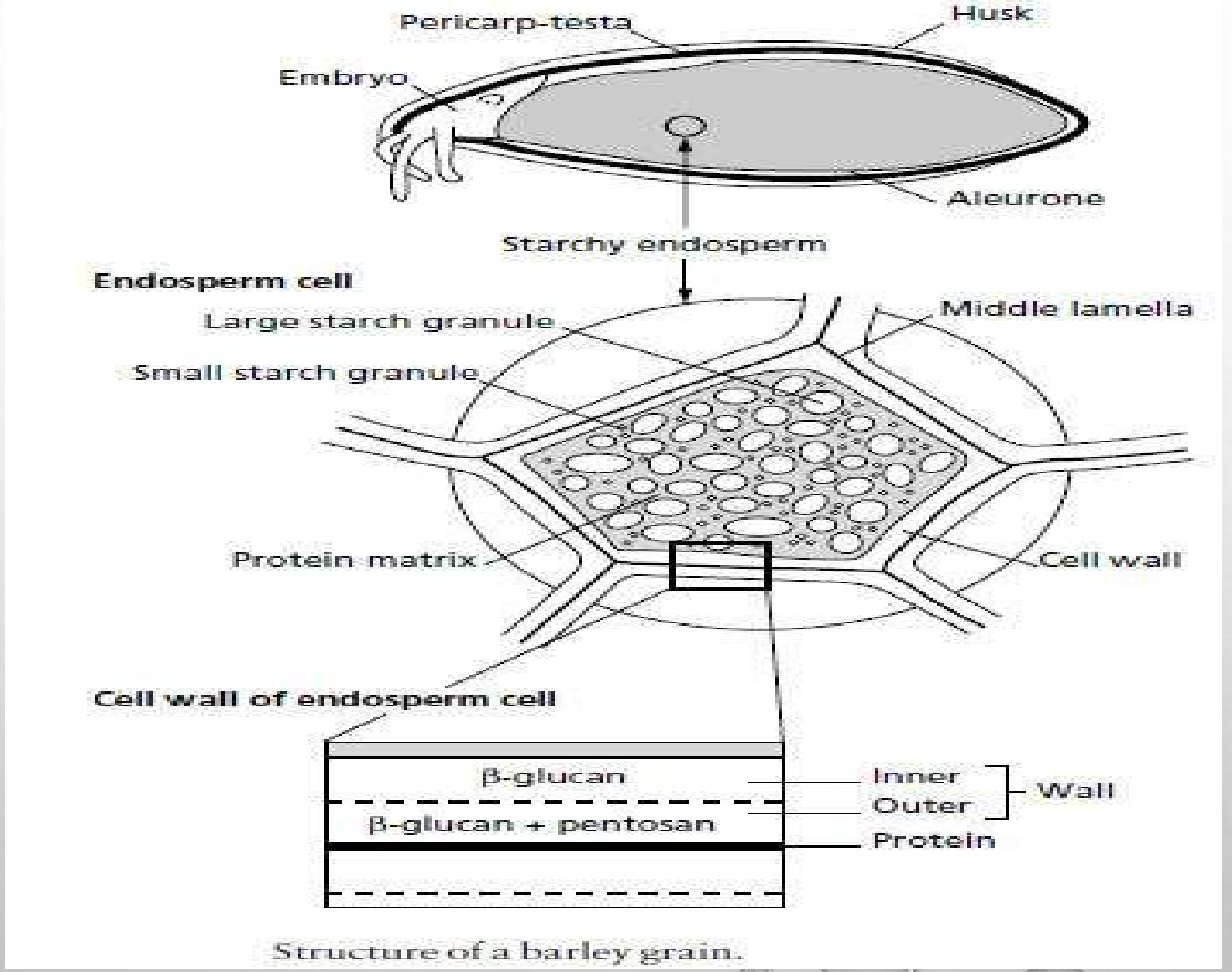


FOOD AND BEVERAGE FERMENTATIONS II

- Barley grains contain approximately 65% starch, located within the endosperm region.
- Endosperm cells are filled with starch granules embedded in a protein matrix, and their walls are composed of a mixed linkage b-glucan (b-1,3; 1,4), hemicellulosic pentosans and protein.
- The starch granules cannot be accessed until the cell walls have been breached and the protein matrix has been at least partially degraded.

FOOD AND BEVERAGE FERMENTATIONS II

- Malting begins by soaking or steeping the barley in water for 2 days at 10–16°C, in order to increase the moisture content to around 45% (w/w).
- Periodically, the water is temporarily drained off and aeration is provided, thus preventing anaerobic conditions that can cause embryo damage.
- The water used for steeping is often reused to save on costs of both water and effluent treatment.



Structure of a barley grain.

FOOD AND BEVERAGE FERMENTATIONS II

- After steeping, the barley is partially germinated for 3–5 days at 16–19°C.
- Traditionally, this simply involved spreading the grain on malting floors to a depth of 10–20cm. However, various mechanized systems are now operated, which have grain beds of about 1m in depth.
- These are aerated with moist cool air and turned mechanically every 8–12 h to aid respiration by the grain and prevent the build-up of heat, otherwise the embryo may become damaged.

FOOD ADDITIVES AD SUPPLEMENTS I

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FOOD ADDITIVES AD SUPPLEMENTS I

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FOOD ADDITIVES AD SUPPLEMENTS I

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FOOD ADDITIVES AD SUPPLEMENTS I

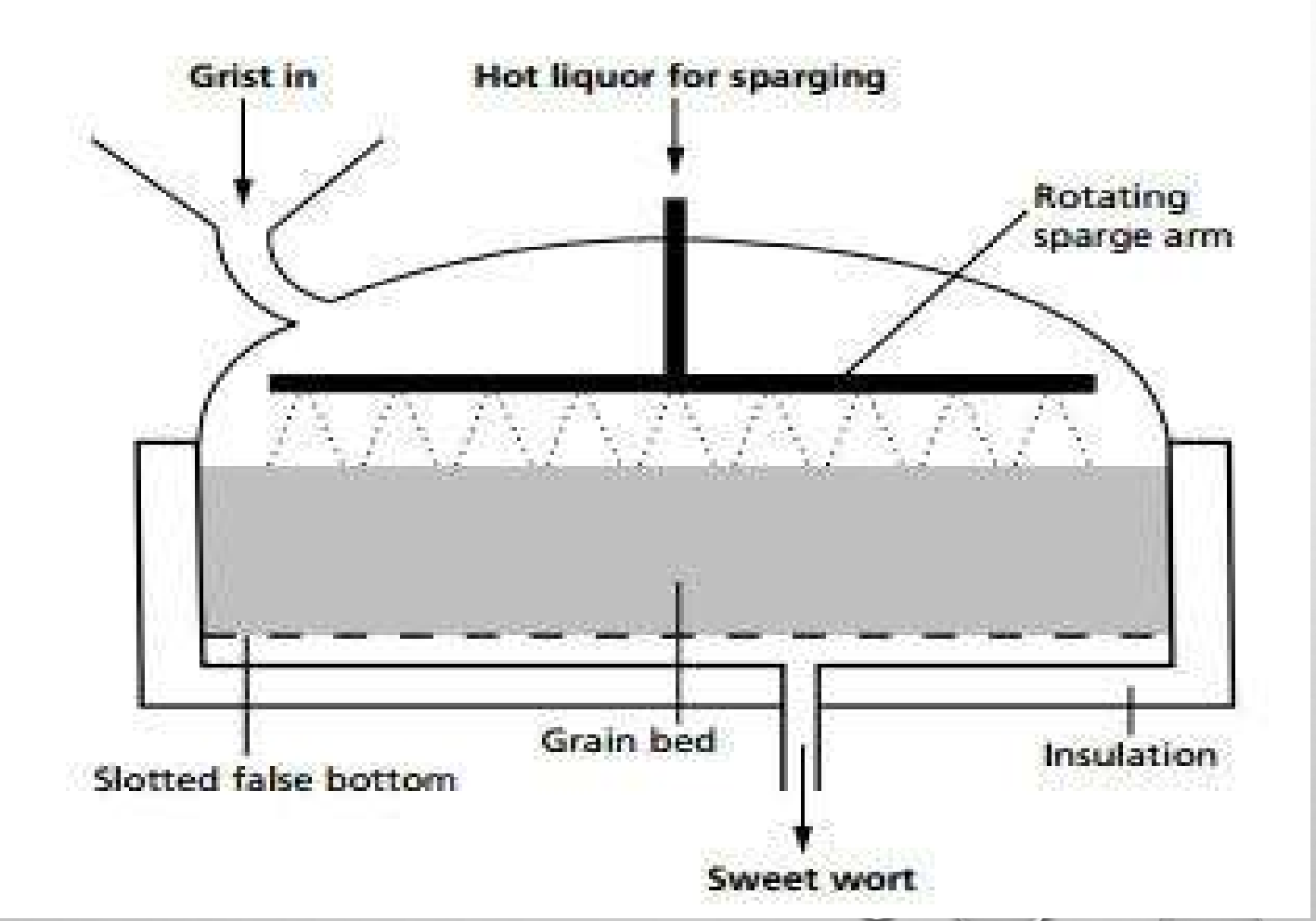
The objectives of wort preparation are to form, and extract into solution, fermentable sugars, amino acids, vitamins, etc., from malt and other solid ingredients.

FOOD ADDITIVES AD SUPPLEMENTS I

Mashing systems

Three main mashing systems are operated. 1 Infusion mashingis the classic British method for ales and stouts, using well-modified malt and relatively simple equipment.

FOOD ADDITIVES AD SUPPLEMENTS I



FOOD ADDITIVES AD SUPPLEMENTS I

The objective in mashing is to convert as much of the malt and adjunct starch as possible to fermentable sugars. Starch is composed of 25% amylose, a linear polymer of a-1,4-linked glucose units, and 75% amylopectin, which is a branched polymer containing both a-1,4 and a-1,6 linkages.

FOOD ADDITIVES AD SUPPLEMENTS I

Sweet wort obtained from the mash is transferred to a ‘copper’ (‘kettle’) for boiling along with dried hops or hop extracts.

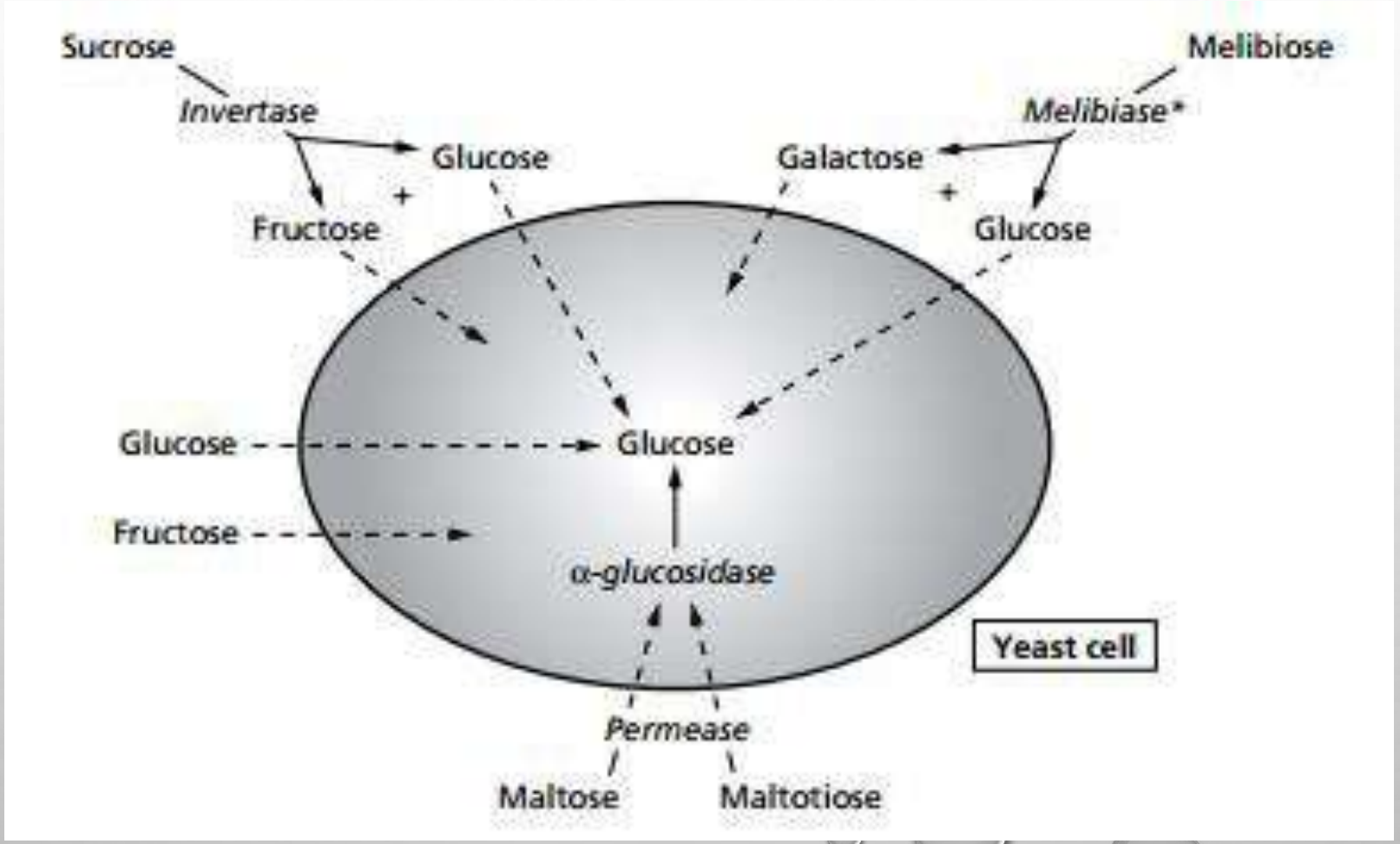
FOOD ADDITIVES AD SUPPLEMENTS I

In beer brewing, both top-fermenting strains and bottom-fermenting strains of S. cerevisiae are employed; the latter were formerly classified as S. carlsbergensisorS. uvarum. Top-fermenting yeasts exhibit flotational-flocculation behaviour and have been primarily used for making ales and stouts.

FOOD ADDITIVES AD SUPPLEMENTS I

Stock cultures of yeasts are maintained at low temperature or stored in freeze-dried form

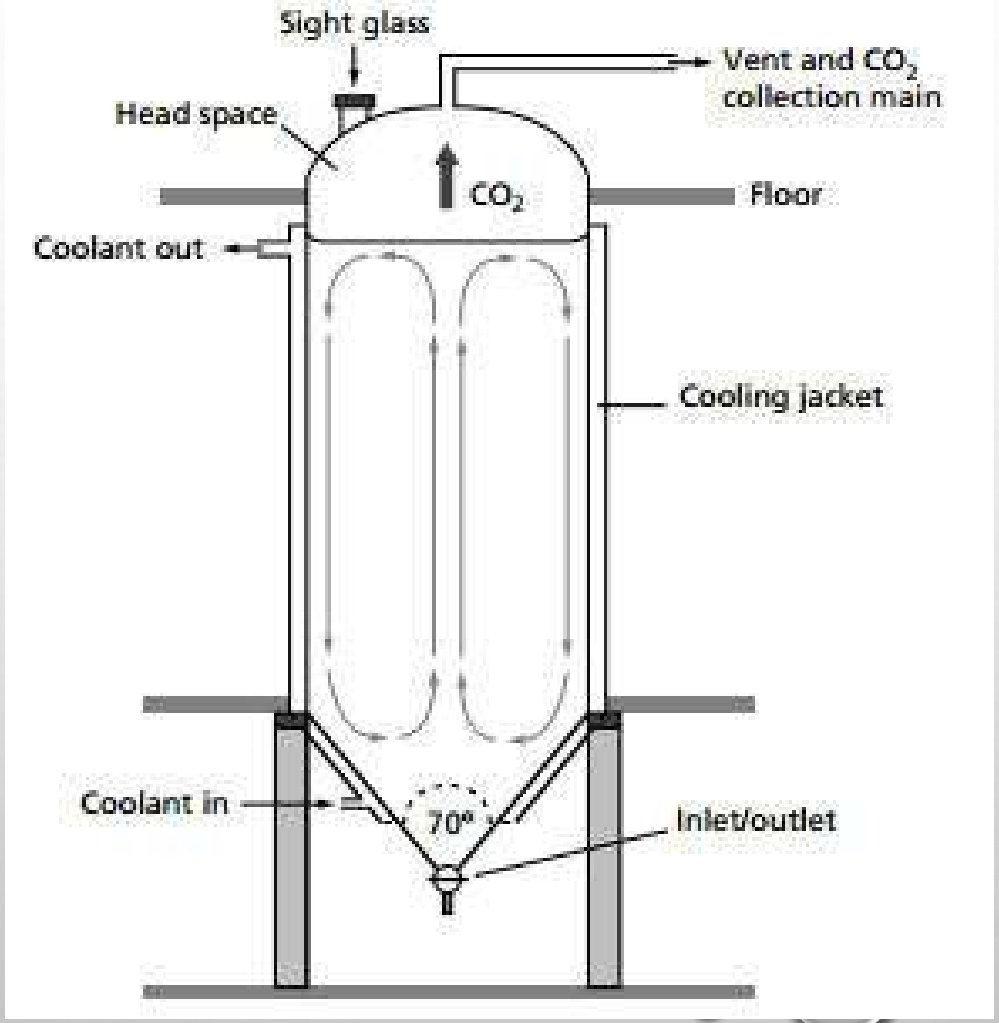
FOOD ADDITIVES AD SUPPLEMENTS I



FOOD ADDITIVES AD SUPPLEMENTS I

The resultant wort contains glucose, fructose, sucrose (only small quantities), maltose, maltotriose, and both linear and branched higher dextrins.

FOOD ADDITIVES AD SUPPLEMENTS I



VU MEDICAL ZONE

ADMIN:

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INDUSTRIAL BIOTECHNOLOGY

BT 604 FINAL TERM PPT

Lecture 91 to 167