WHITE BIOTECHNOLOGY

- Also known as industrial biotechnology applies to industrial processes.
  - Designing of an organism to use to produce a useful chemical.
  - Use of enzymes as industrial catalysts to produce valuable chemicals or destroy hazardous/ polluting chemicals.
- Started in California in 1970s.

IMPORTANCE OF WHITE BIOTECHNOLOGY

- White biotechnology tends to consume less in resources than traditional processes used to produce industrial goods.
- The modern use and application of biotechnology for the sustainable processing and production of chemicals.

IMPORTANCE OF WHITE BIOTECHNOLOGY

- The use of biological methods to optimize industrial processes..
- Research of new enzymes (proteins that remove oily and protein-based stains)
- Applied by the manufacturers of laundry detergents.

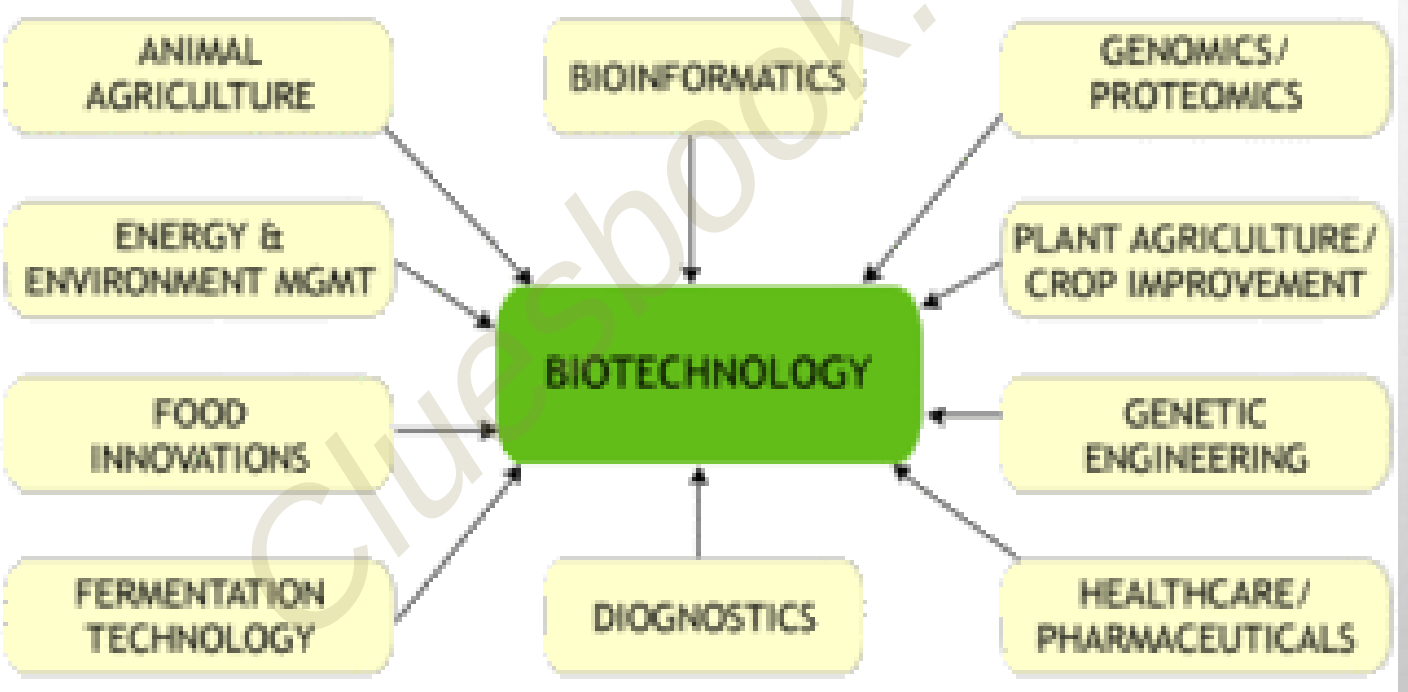
IMPORTANCE OF WHITE BIOTECHNOLOGY

- Biotechnology is used to produces many things like:
  - Pulp & paper
  - Cotton
  - Leather
  - Biogas from organic wastes

IMPORTANCE OF WHITE BIOTECHNOLOGY

- Modern uses of white biotechnology includes:
  - Phyto-remidiation by using transgenic plants
  - Biofuel production by using plants

IMPORTANCE OF WHITE BIOTECHNOLOGY



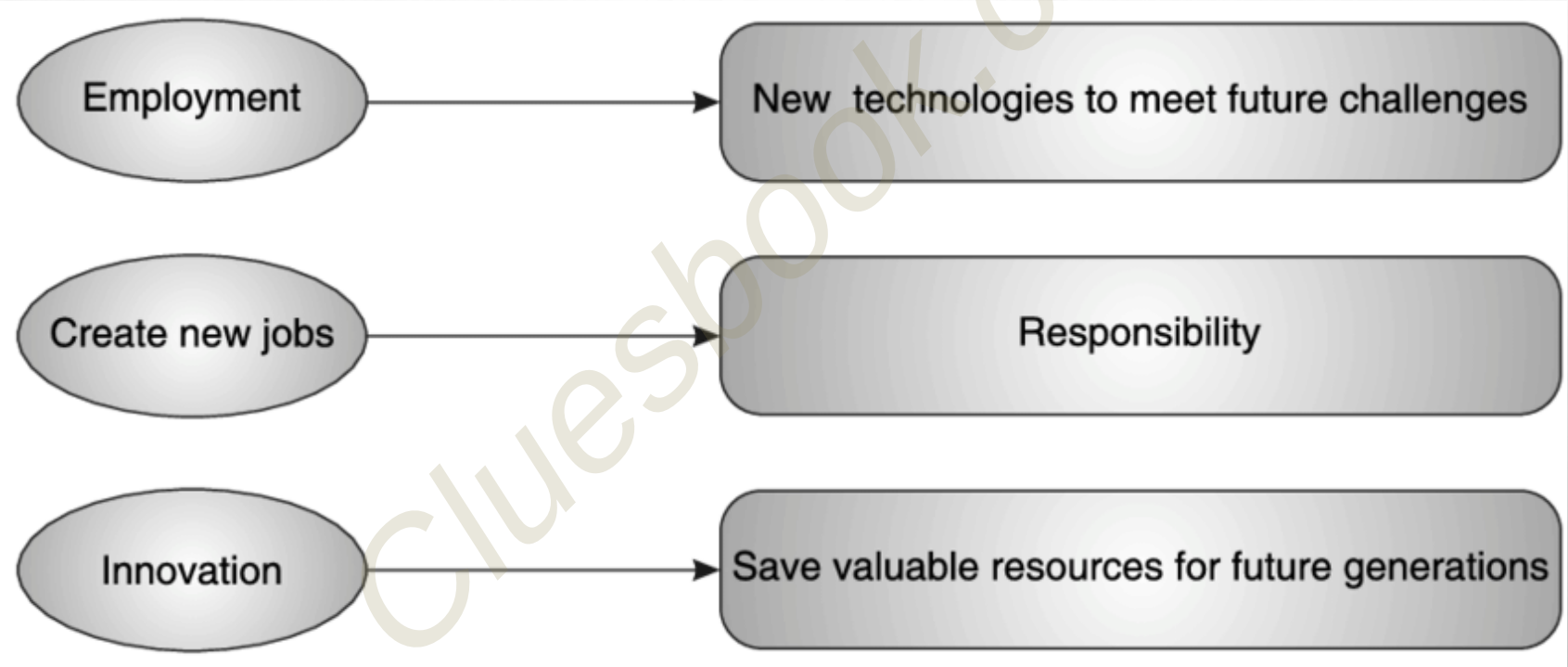
IMPACT OF INDUSTRIAL BIOTECHNOLOGY

- Industrial biotechnology has positive impact on the:
  - Environment
  - Economy
  - Society

IMPACT OF INDUSTRIAL BIOTECHNOLOGY

- The Environment benefits from the industrial biotechnology because:
  - Very little wastes are released in the environment.
  - Excessive materials are used in the further processing.
  - Significant reduction in carbon dioxide emission.
  - Less energy consumption

IMPACT OF INDUSTRIAL BIOTECHNOLOGY



IMPACT OF INDUSTRIAL BIOTECHNOLOGY

- Biotechnology is also benefited for the overall economy:

  - Due to less energy consumption

  - More efficient processes

  - Fermentation and enzymatic processes are commonly used in the fine chemicals sector, to produce for example vitamins, pharmaceutical intermediates and flavors.

IMPACT OF INDUSTRIAL BIOTECHNOLOGY

- They are also making their first inroads into larger volume segments such as polymers, bulk chemicals and bio-fuels, and many other industrial sectors.

IMPACT OF INDUSTRIAL BIOTECHNOLOGY

- Regarding all these environmental and economical impacts the industrial biotechnology is most favorable for the sustainability of the society.

BIOTECHNOLOGICAL PRODUCTS

- Products which are produced by recombinant DNA technology(produced by biotechnology)
- Examples:
  - Antibiotics
  - Vaccines
  - GMO's
  - Transgenic plants
  - Baverages

BIOTECHNOLOGICAL PRODUCTS

- The following are the important products of industrial biotech:
  - Recovery of metals
    - Desulphoration of the coal by Thiobacillus spp.
  - Metabolic production
    - Antibiotics, enzymes, alcohols, organic acids, vitamins

BIOTECHNOLOGICAL PRODUCTS

- Biocatalysts

  - Yeast, bacteria & enzymes

- Biopolymers

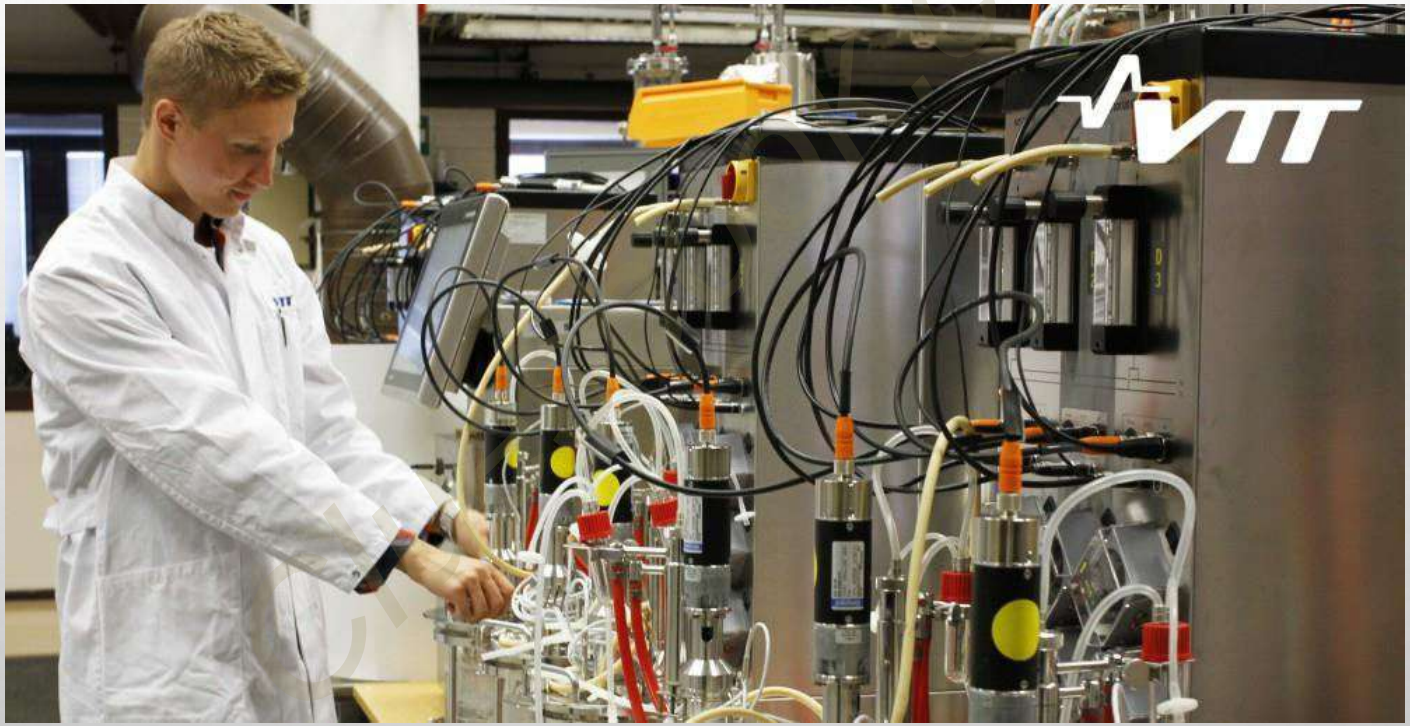
  - Polyactic acid and Poly 3-hydoxy butyrate

BIOTECHNOLOGICAL PRODUCTS

BIOTECHNOLOGICAL PRODUCTS

- Fermentation processes are used to produce a number of important chemicals at very-high volumes, including vitamin C.
- Looking to the future, biotechnology will allow the production of new polymers with improved functionality, not possible by conventional processes.

BIOTECHNOLOGICAL PRODUCTS



VISION 2025

- The development and use of industrial biotechnology is essential to the future competitiveness
- By the year 2025, Biotechnological processes will be used to produce chemicals and materials which are hard or impossible to produce conventionally, or to make existing products in a more efficient way.

ULTERIOR TACTICS

- Increasingly eco-efficient use of renewable resources as industrial raw materials
- Rural bio-refineries will replace port-based oil refineries
- Manufacture products in an economically and environmentally sustainable way

ULTERIOR TACTICS

- Sustainable cooperation and support between the research community, industry, agriculture and civil society
- Green biotechnology will make a substantial contribution to the efficient production of biomass raw materials

ULTERIOR TACTICS

STRATEGIC RESEARCH AGENDA

- Modern white biotechnology is a relatively new discipline, with major areas of knowledge still to be explored.
- It is by nature a multidisciplinary area, comprising biology, microbiology, biochemistry, molecular biotechnology, chemistry, engineering etc.

STRATEGIC RESEARCH AGENDA

STRATEGIC RESEARCH AGENDA

- The industrial biotechnology section of the sustainable chemistry technology platform (suschem) has developed a strategic research agenda, for the development of the white biotechnology.

STRATEGIC RESEARCH AGENDA

- The suschem technology platform will also cooperate on industrial biotechnology issues with other technology platforms such as: the bio-fuels, plants for the future, innovative and sustainable use of forest resources, food for life, textile and clothing, and future manufacturing technologies.

STRATEGIC RESEARCH AGENDA

TECHNOLOGICAL BUILDING BLOCKS

- The development and production of novel, innovative products and processes in a cost- and eco-efficient manner, increasingly using renewable raw materials are the keys to fulfill for the future plans of the industrial biotechnology.

TECHNOLOGICAL BUILDING BLOCKS

- To achieve these, seven major areas of research and technology were identified cooperatively by the stakeholders:

  - Novel enzymes and micro-organisms
  - Microbial genomics and bio-informatics
  - Metabolic engineering and modelling

TECHNOLOGICAL BUILDING BLOCKS

- Biocatalytic process design
- Fermentation science and engineering
- Innovative downstream processing
- Biocatalyst function and optimisation

TECHNOLOGICAL BUILDING BLOCKS

- When the program is implemented, a number of key horizontal issues also need to be addressed, such as:

  - technology transfer
  - overcoming bottlenecks
  - contribution to standards
  - impact assessment
  - issues of perception, awareness and education.

MAJOR CHALLENGES

- Despite the boom in the biotech industry, there are some grave challenges faced by this domain.
  - High levels of risk
  - GM food adoption
  - Societal concerns
  - Environmental harm

MAJOR CHALLENGES

- Production/laboratory safety
- Bioterrorism
- Loss of patents, bringing competition from biosimilars
- High R&D costs
- Low R&D productivity
- A strong requirement of implementing M&A to strengthen the pipeline

BIOMASS AREA

- The development of a closed loop fermentation cycle (where the "bio-waste" of one process can be recycled as input for another process), e.G. Sugar beet pulp as an untapped biomass feedstock for future use.
- Conducting life cycle assessment and economic and environmental impact analysis (ecoefficiency studies) to identify optimal biomass feedstocks.

BIO-PRODUCTS AREA

- The development of new (bio)products with higher performance in existing applications.
- The development of novel processes, bioreactors, and bioreactor operating strategies together with novel downstream processes.

BIO-FUELS AREA

- Utilisation of waste fats and side streams of the edible oil processing industry as raw material for bio-diesel.
- Analysis of the potential to produce bio-diesel economically with biotechnological methods based on methanol or bio-ethanol.
- Identification, evaluation and production of other potential liquid fuels.

MAJOR CHALLENGES

- The methodologies used in the biotech industry have vast room for improvement considering that biotech is in its early stages.

NOVEL ENZYMES AND MICRO-ORGANISMS

- Production of enzymes and micro-organisms which will provide new products and improved processes.
- 70% of the world's enzymes had already produced.
- But the need of new enzymes for the new and innovative products still exists.

INCREASE IN PRODUCTIVITY

- For this purpose the new enzymes and the microorganisms are needed.
- These enzymes and the microorganisms should have the following characteristics:

  - cope with extreme environments like high temperature and high pressure.

  - either in freshwater or marine environment.

INCREASE IN PRODUCTIVITY

- For this purpose two techniques are used nowaday:
  - Meta-genomics
  - High throughput screening

INCREASE IN PRODUCTIVITY

Metagenomics:

- Metagenomics allows the sequencing and genetic characterisation of the full range of species present in a sample from a particular environment at once.

INCREASE IN PRODUCTIVITY

High throughput screening:

- Automated high throughput screening technologies can then help researchers find candidate genes which have both the desired function and are likely to be expressed when transferred to a target micro-organism.

INCREASE IN PRODUCTIVITY

Conclusion:

- Expansion of the range of biological processes:

  - new and improved microbes and enzymes for industrial use.

- New functionality and properties of enzymes via the development and implementation of new tools and technologies.

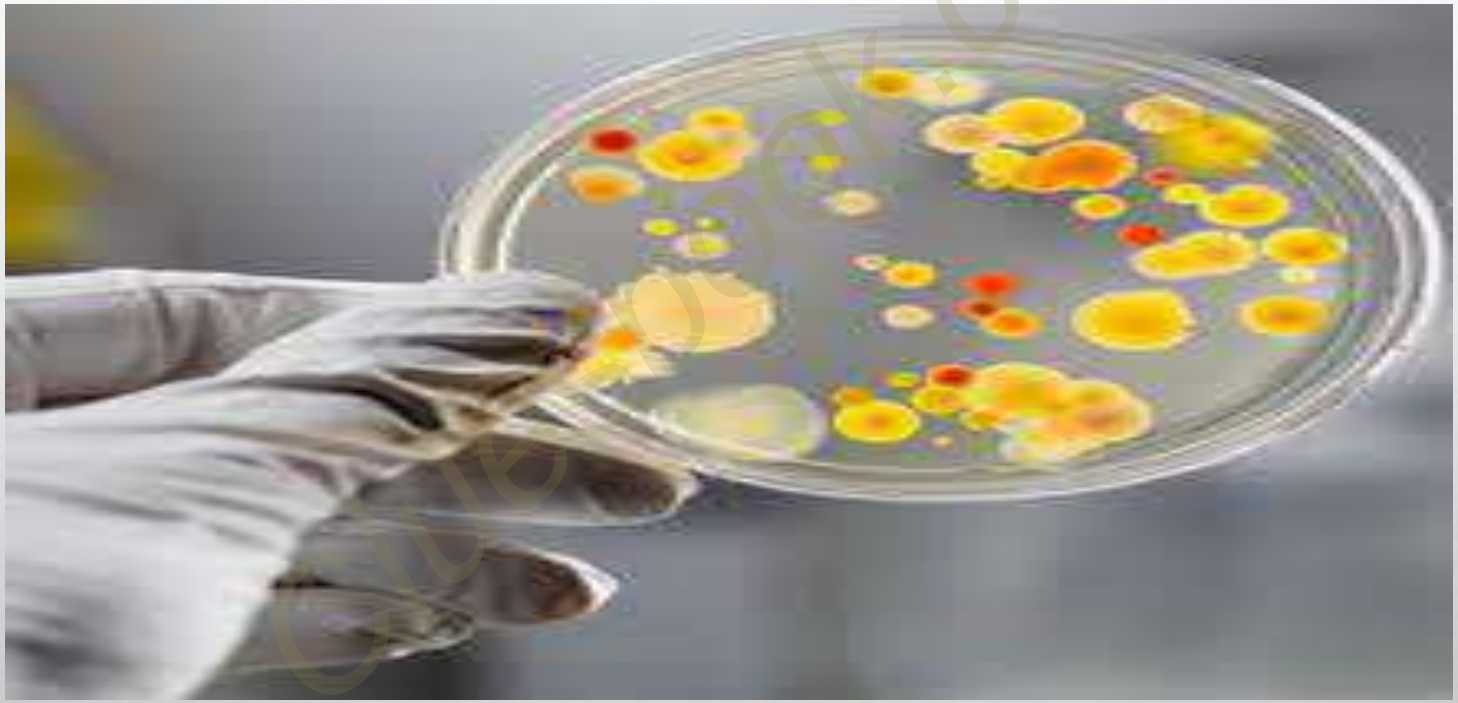
INCREASE IN PRODUCTIVITY

- New products or intermediates from bio-transformations.
- New technologies to make more organisms suitable for metabolic engineering.

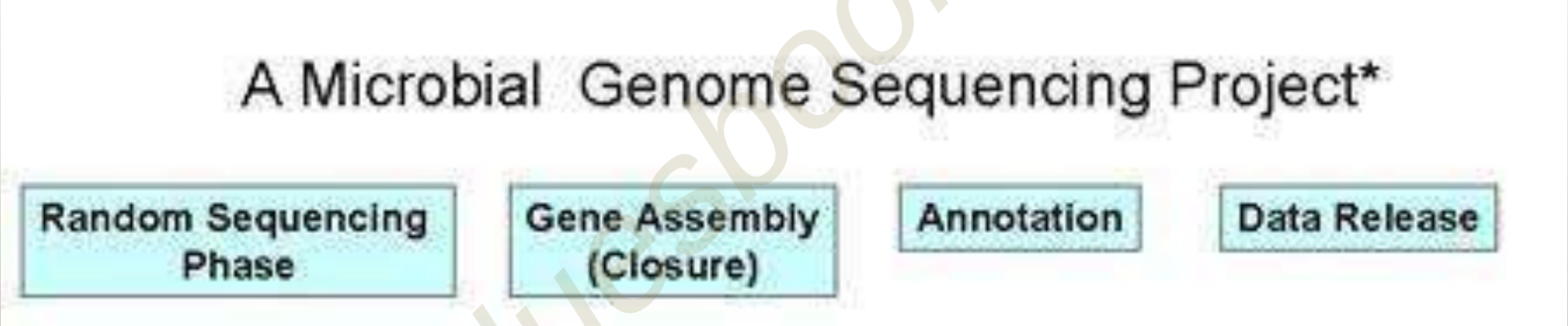
MICROBIAL GENOMICS AND BIO-INFORMATICS

- Genome of microorganism can help us to understand better about their activities.
- With good genome mapping, the identification of desirable metabolic pathways and their adaptation into manufacturing processes will be accelerated.

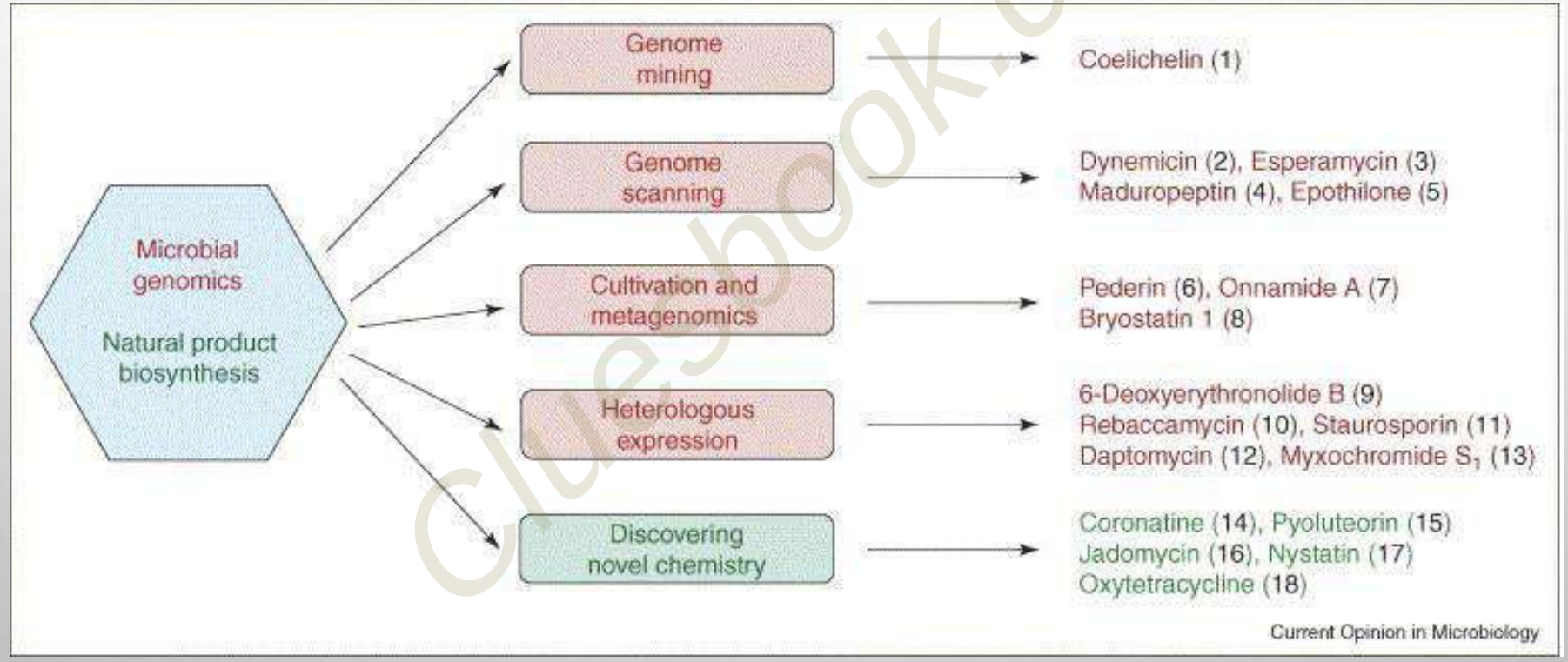
MICROBIAL GENOMICS AND BIO-INFORMATICS



MICROBIAL GENOMICS AND BIO-INFORMATICS



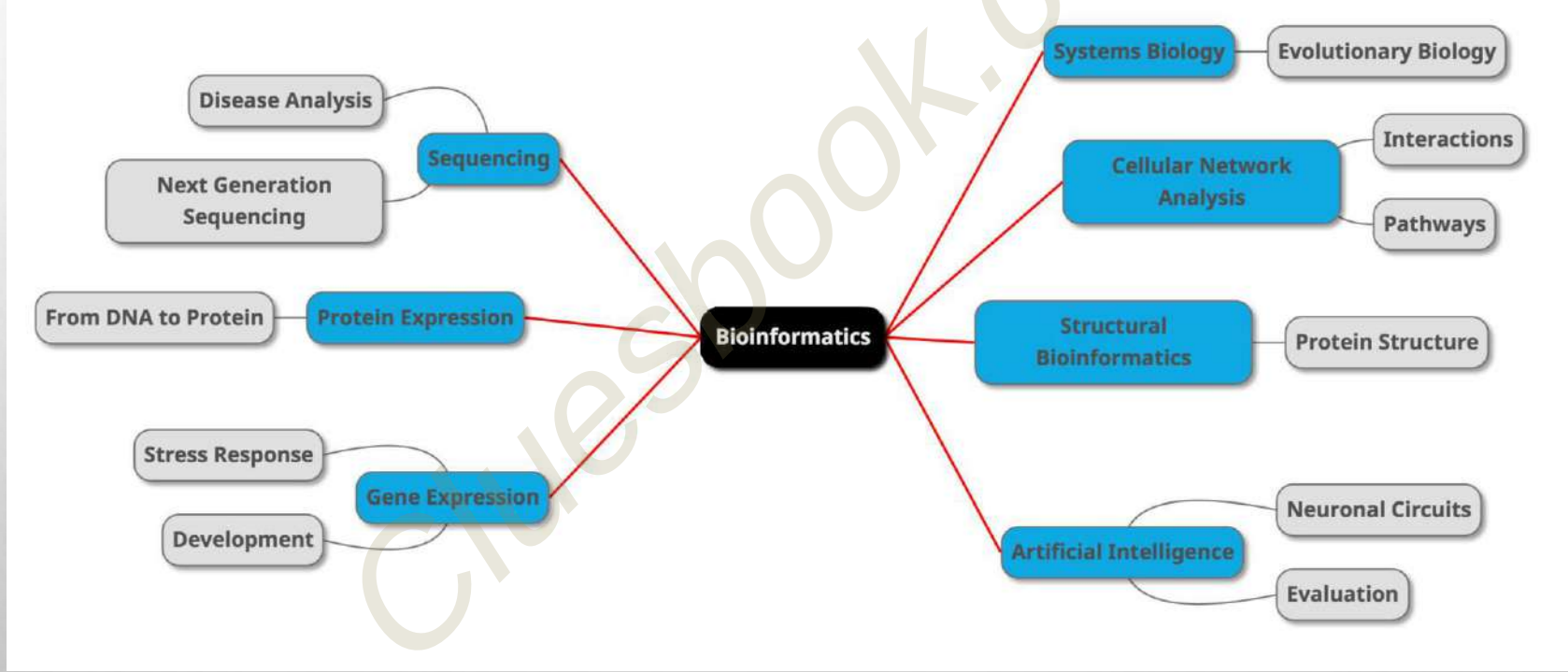
MICROBIAL GENOMICS AND BIO-INFORMATICS



MICROBIAL GENOMICS AND BIO-INFORMATICS

- Bioinformatics is the use of computing technologies to manage and analyse biological data. The huge amounts of genomic data produced through DNA sequencing can often be very confusing and difficult to analyse.
- Using bioinformatics techniques, we can map the positions of genes, elicit their functions and infer evolutionary relationships.

MICROBIAL GENOMICS AND BIO-INFORMATICS



METABOLIC ENGINEERING

- **Metabolic engineering** is the practice of optimizing genetic and regulatory processes within cells to increase the cells' production of a certain substance.
-

METABOLIC ENGINEERING

- Some of the common strategies used for metabolic engineering are:

(1) over-expressing the gene encoding the rate-limiting enzyme of the biosynthetic pathway

(2) blocking the competing metabolic pathways

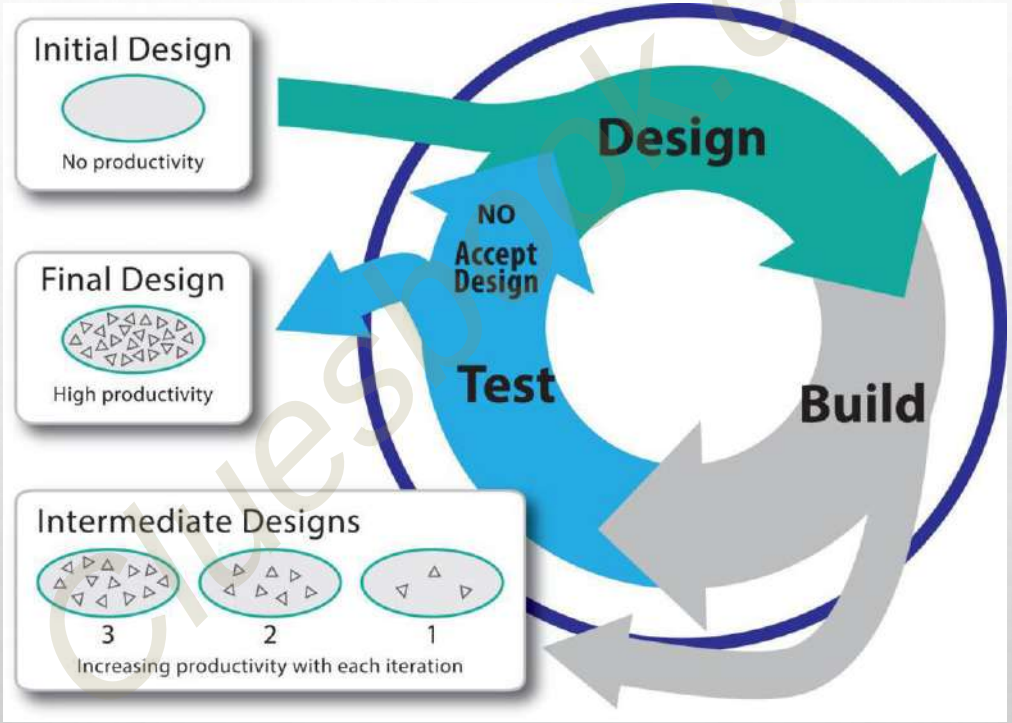
(3) heterologous gene expression

(4) enzyme engineering.

METABOLIC ENGINEERING

- Metabolic engineering typically uses recombinant DNA technology to develop micro-organisms giving improved product yields or even having totally new pathways. Such "**designer organisms**" can be seen as **cell factories** and form the cornerstone of industrial biotechnology.

METABOLIC ENGINEERING



METABOLIC ENGINEERING

- **Goals:**
- Mathematical modelling of microbial metabolism, directed towards both steady state and dynamic models.
- Design of new pathways and synthetic micro-organisms focusing on the synthesis of molecules and products new to nature.

BIOCATALYTIC PROCESS DESIGN

BIOCATALYTIC PROCESS DESIGN

- The use of biological processes in industrial environments, requires particular attention to biocatalytic process design.
- Engineering knowledge base for biotechnology is a prerequisite for its successful introduction in industry.

BIOCATALYTIC PROCESS DESIGN

- Optimal biocatalytic process design will increase the efficiency of production processes.

Case-based reasoning is the norm, promising process interactions are:

- rarely discovered
- exploited in industrial practice.

BIOCATALYTIC PROCESS DESIGN

- That's why, there is a strong need for systematic design Technology which can devise new high-performance processes quickly and reliably

BIOCATALYTIC PROCESS DESIGN

- **Research:**
- Integration of catalysts into industrial processes, as well as development
- Direct integration of enzyme production and enzymatic transformation including
- Downstream processing of target compounds.
- Development of modular and multiphase bioreactors.
- Reduction of the number of unit operations in biocatalytic processes by using multi-step

THE PRODUCER MICROORGANISM

THE PRODUCER MICROORGANISM

Key factors relating to improve the economic efficiency of the process are:

- The strategy for initially obtaining a suitable industrial microorganism
- Strain improvement to enhance productivity and yield
- Maintenance of strain
- Purity, preparation of a reliable inoculum and the continuing
- Development of selected strains

THE PRODUCER MICROORGANISM

- Some microbial products are primary metabolites,
- Produced during active growth (the **trophophase),**
- include amino acids, organic acids, vitamins
- Industrial solvents such as alcohols and acetone

THE PRODUCER MICROORGANISM

- Many of the most important industrial products are secondary metabolites, which are not essential for growth
- e.g. Alkaloids and antibiotics.

THE PRODUCER MICROORGANISM

- These compounds are produced in the stationary phase of a batchculture, after microbial biomass production has peaked (the **idiophase).**

THE FERMENTATION MEDIUM

THE FERMENTATION MEDIUM

The selection of suitable

Vital aspects of process development to ensure maximization of yield and profit are:

- Cost-effective carbon and energy sources
- Essential nutrients
- Media optimization

THE FERMENTATION MEDIUM

- In many instances, the basis of industrial media are waste products from
- Other industrial processes, notably:
- Sugar processing wastes
- Lignocellulosic wastes
- Cheese whey and corn
- Steep liquor

THE FERMENTATION

THE FERMENTATION

- Industrial microorganisms are normally cultivated under rigorously controlled conditions developed to optimize the growth of the organism
- The synthesis of microbial metabolites is usually tightly regulated by the microbial cell.

THE FERMENTATION

- Fermentations are performed in large fermenters
- With capacities of several thousand litres.

These are may be

- Simple tanks, which may be stirred or unstirred,
- Complex integrated systems involving varying levels of computer control

THE FERMENTATION

The fermenter material should have the following characteristics:

- Usually stainless steel
- Can be repeatedly sterilized
- Will not react adversely with the microorganisms
- Will not react with the target products.

THE FERMENTATION

Major influences on fermentation performance are:

- The mode of fermenter operation (batch, fed-batch or continuous systems)
- The method of its aeration and agitation
- Approach taken to process scale-up

THE FERMENTATION

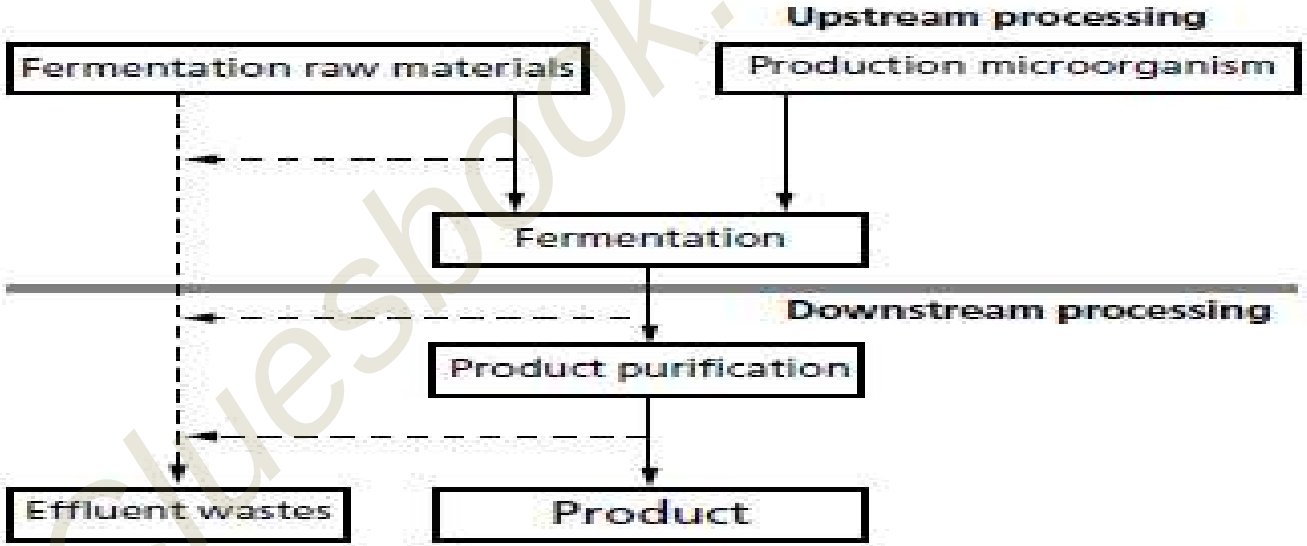


Fig. i Outline of a fermentation process.

THE FERMENTATION

- For some products, especially enzymes, retention of their biological activity is vital.
- There must be safe and inexpensive disposal of all waste products generated during the process.

FERMENTATION PRODUCTS

FERMENTATION PRODUCTS

- Fermentation products can be broadly divided into two categories:
- High volume, low value products

Most food and beverage fermentation products

- Low volume, high value products

Many fine chemicals and pharmaceuticals

FERMENTATION PRODUCTS

Food, beverages, food additives and supplements

- Fermented dairy products, result from the activities of lactic acid bacteria in milk, which modify flavour and texture, and increase long-term product stability
- Yeasts are exploited in the production of alcoholic beverages, notably beer and wine, due to their ability to ferment sugars to ethanol.

FERMENTATION PRODUCTS

- Some microorganisms contain high levels of protein with good nutritional characteristics suitable for both human and animal consumption.
- This so-called ‘single-cell protein’ (scp) can be produced from a wide range of microorganisms cultivated on low-cost carbon sources.

FERMENTATION PRODUCTS

Health-care products:

- Secondary metabolites synthesized by filamentous fungi and bacteria, particularly the actinomycetes.
- Over 4000 antibiotics have now been isolated, but only about 50 are used regularly in antimicrobial chemotherapy.
- The most medically useful antibiotics are the b-lactams, penicillins cephalosporins, and the tetracyclines.

FERMENTATION PRODUCTS

- Important pharmaceutical products derived from microbial fermentation are:
- Alkaloids
- Steroids
- Vaccines.

More recently, therapeutic recombinant human proteins such as insulin, interferons and human growth hormone have been produced by a range of microorganisms

INDUSTRIAL CHEMICALS AND FUELS

INDUSTRIAL CHEMICALS AND FUELS

- chemicals supplied through fermentation include various alcohols, solvents such as acetone, organic acids, polysaccharides, lipids and raw materials for the production of plastics.
- Some of these fermentation products also have applications in food manufacture.

INDUSTRIAL CHEMICALS AND FUELS

- Fossil fuels, especially oil, are likely to become exhausted within the next 50–100 years, resulting in the need to develop alternative sources of energy.
- Biological fuel generation may make an increasing contribution, particularly in the conversion of renewable plant biomass to liquid and gaseous fuels.

INDUSTRIAL CHEMICALS AND FUELS

Plant biomass can be in the form of:

- Cultivated energy crops
- Natural vegetation
- Agricultural wastes
- Industrial wastes
- Domestic organic wastes

INDUSTRIAL CHEMICALS AND FUELS

- Methane and ethanol are the main products, although other potential fuels can be generated using microorganisms, including hydrogen, ethane, propane and butanol

ENVIRONMENTAL ROLES OF MICROORGANISMS

ENVIRONMENTAL ROLES OF MICROORGANISMS

- Microorganisms are particularly important in **wastewater treatment, which utilizes the metabolic activities** of microbial populations capable of degradation.

ENVIRONMENTAL ROLES OF MICROORGANISMS

- The two main objectives are:
- To destroy all pathogenic microbes present in the sewage, particularly the causal organisms of the water-borne diseases cholera, dysentery and typhoid.
- The second objective is to break down the organic matter in waste-water to mostly methane and carbon dioxide, thereby producing a final effluent (outflow) that can be safely discharged into the environment.

ENVIRONMENTAL ROLES OF MICROORGANISMS

- Microbial activities can also be employed in the degradation of man-made xenobiotic compounds
- Environmental biological control is a further area where microorganisms are employed in an effort to reduce our reliance on synthetic chemical pesticides.

ENVIRONMENTAL ROLES OF MICROORGANISMS

- Bacteria, fungi, protozoa and viruses are cultivated to produce biomass or cell products for the control of fungal, insect and nematode pests of agricultural crops, along with some vectors of human and animal diseases.

MICROBIAL CELL STRUCTURE AND FUNCTION

INTRODUCTION

- The cell is the basic unit of all living organisms.
- Some are unicellular some are multicellular.
- All cells are surrounded by a cytoplasmic membrane, primarily composed of lipids and proteins.
- Nucleic acids, the physical carriers of genetic information
- Ribosomes that take part in protein synthesis.

INTRODUCTION

- Cells are divided into two categories:
- **Prokaryotic** (archaeans and eubacteria)
- **Eukaryotic** (cells of fungi, protozoa, algae and other plants, and animals)

INTRODUCTION

- Prokaryotic cells are normally less than 5μm in diameter
- Prokaryotes rarely possess membranebound organelles
- Most prokaryotic cells contain a single chromosome composed of deoxyribonucleic acid (DNA), which is located in a region of the cell referred to as the nucleoid.
- Prokaryotic ribosomes are 70 s.
- Cell division in prokaryotes is normally by simple binary fission.

INTRODUCTION

- Contain a range of membrane-bound organelles, including mitochondria, lysosomes, golgi bodies and an extensive endoplasmic reticulum
- The DNA of eukaryotic cells, in the form of several linear chromosomes, is characteristically complexed with histone proteins and is housed in a double membranebound nucleus.
- Eukaryotic ribosomes, apart from those located within certain organelles, are 80 s.
- Eukaryotic cells divide by a complex process of mitosis and usually have a sexual lifecycle, involving meiosis (reduction division)

PROKARYOTES

PROKARYOTES

- On the basis of the study of phylogenetic (evolutionary) prokaryotes have been separated into two distinct groups
- **Archaea ('ancient' bacteria)**
- **The eubacteria ('true'** bacteria)

PROKARYOTES

- **Archaea:**
- These prokaryotes are quite different from eubacteria and have some features
- Especially aspects of the transcription
- Translation machinery associated with
- Protein synthesis, that are similar to eukaryotic cells.

PROKARYOTES

- Archaeans have relatively small genomes containing **1 *microbial cell structure and function*** less than half the DNA of eubacteria.
- For example,
- The genome of *methanococcus jannaschii has been sequenced* and found to contain 1760 genes composed of 1700 kilobase pairs (kbp).

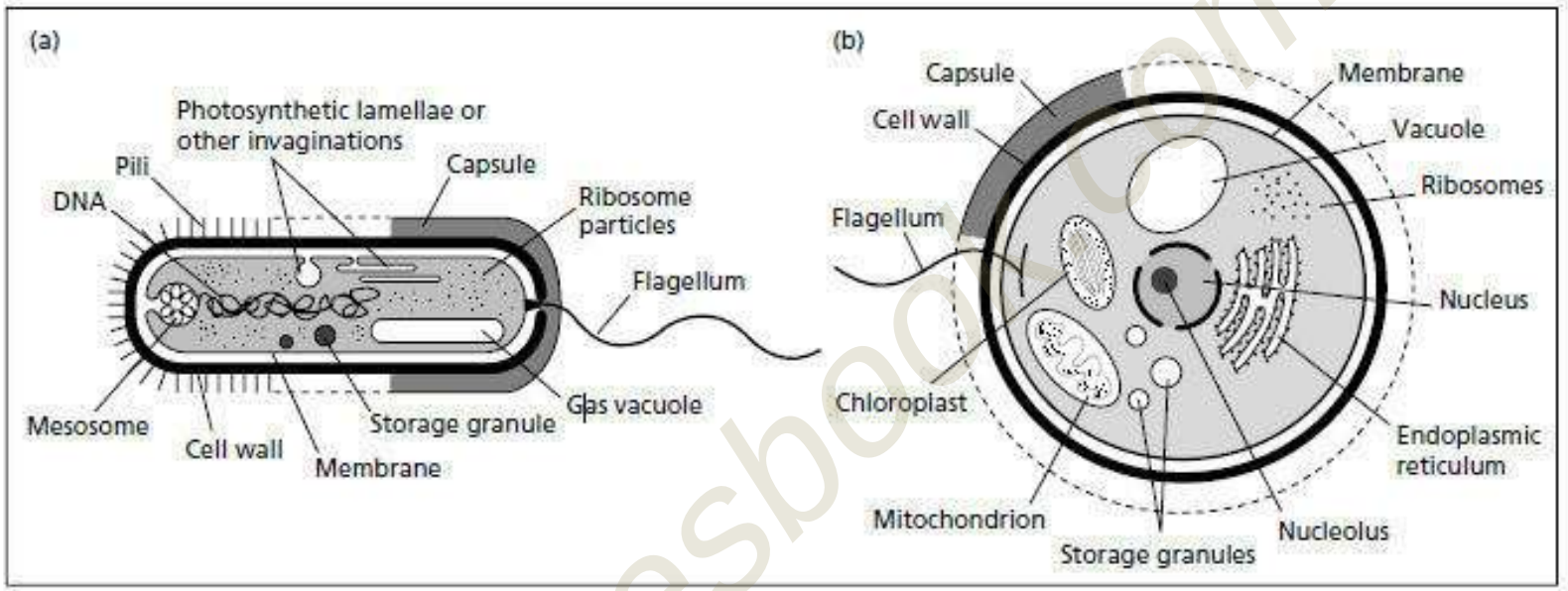


Fig. 1.1 diagrammatic representation of the main structures Of (a) prokaryotic and (b) eukaryotic microbial cells. Not all Structures are always present, including capsules, Chloroplasts, flagellae, pili, storage granules and vacuoles (From dawes & sutherland (1992)).

PROKARYOTES

- The archaea may be divided into three kingdoms.
- **1 euryarchaeota are primarily methanogens, such as**
- *Methanobacterium and methanosarcina, and the*
- Extreme halophiles *halobacterium and halococcus.*

PROKARYOTES

- **2 crenarchaeota are mostly extreme barophiles**
- And thermophiles that include *pyrodictium, pyrolobus,*
- *Sulfolobus and thermoproteus.*
- **3 korarchaeota are hyperthermophiles which, as yet,**
- Have not been isolated as pure cultures.

EUBACTERIA

EUBACTERIA

- **Eubacteria**
- The eubacteria are a very diverse group that may be divided into 12 subgroups.

EUBACTERIA

- **1 the proteobacteria is a major kingdom of gramnegative** bacteria that is divided into five groups, a, b, g, d and e. They include purple photosynthetic bacteria.
- **2 the gram-positive eubacteria are composed of two** major subdivisions:
- (A) the low guanine (G) + cytosine (C) group, (b) the high G + C group

EUBACTERIA

- **3 *cyanobacteria and relatives, which are oxygenic*** Phototrophs, e.G. *Anabaena, nostoc and spirulina.*
- **4 *chlamydia, a group of obligate intracellular*** Parasites.
- **5 *planctomyces and pirella, bacteria lacking*** Peptidoglycan; some with membrane-bound nucleoid.

EUBACTERIA

- **6 *bacteroides and flavobacteria, a subgroup that***
  Contains a mixture of physiological types.
- **7 green sulphur bacteria, such as *chlorobium, an***
  Anaerobic phototroph.
- **8 spirochetes and relatives which are gram-negative**
  Coiled bacteria.

EUBACTERIA

- **9 deinococci, radioresistant micrococci and relatives,** E.G. *Deinococcus radiodurans and thermus aquaticus.*
- **10 green non-sulphur bacteria and anaerobic** Phototrophs.
- **11 *thermotoga and thermosulfobacteria, thermophiles*** From hot springs and marine sediments.
- **12 *aquiflex, a group of obligate chemolithotrophic*** And autotrophic hyperthermophiles.

ESCHERICHIA COLI, A GRAM-NEGATIVE BACTERIUM

ESCHERICHIA COLI, A GRAM-NEGATIVE BACTERIUM

- E. Coli was discovered in 1885 by the German bacteriologist Theodor escherich.
- A major inhabitant of the colon of humans and the lower gut of other warm-blooded animals.
- Some can cause food- and water-borne diseases that produce diarrhea and can be especially problematical for human infants and young animals.

ESCHERICHIA COLI, A GRAM-NEGATIVE BACTERIUM

- A particularly virulent strain is e. Coli 0157:H7.
- Organism's rapid growth rate, with a doubling time as low as 20min, has led to it becoming an important industrial microorganism.
- E. Coli has been used extensively as a model for the study of molecular biology and is often considered to be the ideal host in gene-cloning experiments.

ESCHERICHIA COLI, A GRAM-NEGATIVE BACTERIUM

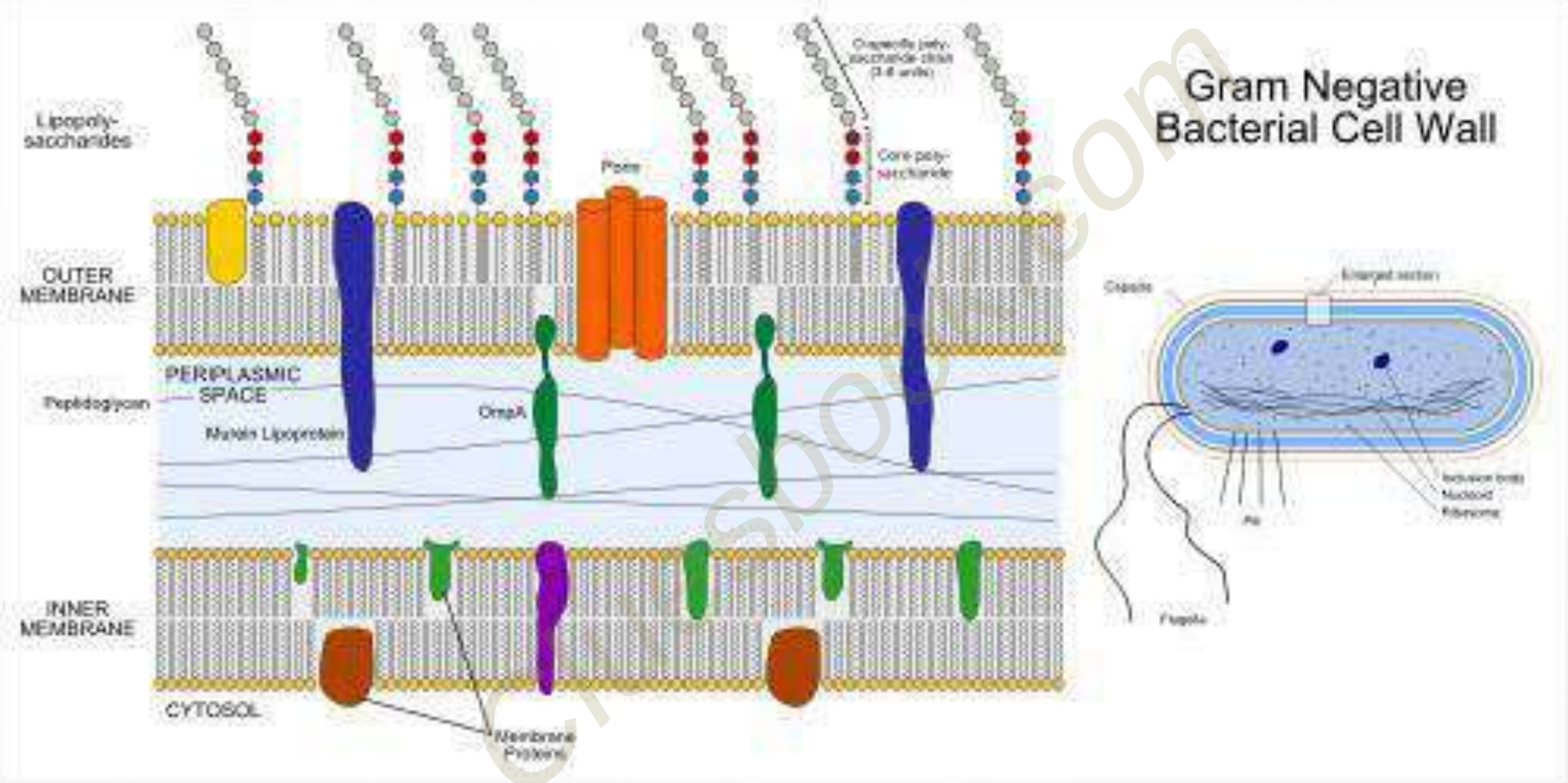
- Extremely useful for the production of heterologous proteins, derived from other organisms.
- E. Coli is a gram-negative facultative anaerobe
- Belonging to the family enterobacteriaceae
- Members are often referred to as enterobacteria or enteric bacteria

ESCHERICHIA COLI, A GRAM-NEGATIVE BACTERIUM

- The cells are short straight rods
- 0.3–1.0mm wide and 1.0–3.0mm long
- Divide by binary fission after elongating to approximately twice their normal length.
- Members of the genus escherichia are oxidase-negative

ESCHERICHIA COLI, A GRAM-NEGATIVE BACTERIUM

- Carry out mixed acid fermentation, producing mainly lactate, acetate, succinate and formate.



OUTER MEMBRANE

OUTER MEMBRANE

- The outer coverings of gram-negative bacterial cells are often referred to as envelopes,
- Composed of two layers
- Layers protect the cell and provide rigidity
- The outermost layer is called the outer membrane

OUTER MEMBRANE

- 7–8nm thick
- Contain lipopolysaccharide and mucopeptide
- This structure does not impede the movement of small molecules, charged or uncharged
- More permeable than the cell/cytoplasmic membrane
- A barrier to hydrophobic molecules and proteins

OUTER MEMBRANE

- Contains porin proteins, composed of three subunits, that form narrow channels of about 1–2nm diameter through which small molecules can pass.
- Non-specific porins allow the passage of molecules up to 600–700da
- Specific porins have binding sites for one or more substances of up to 5000da.

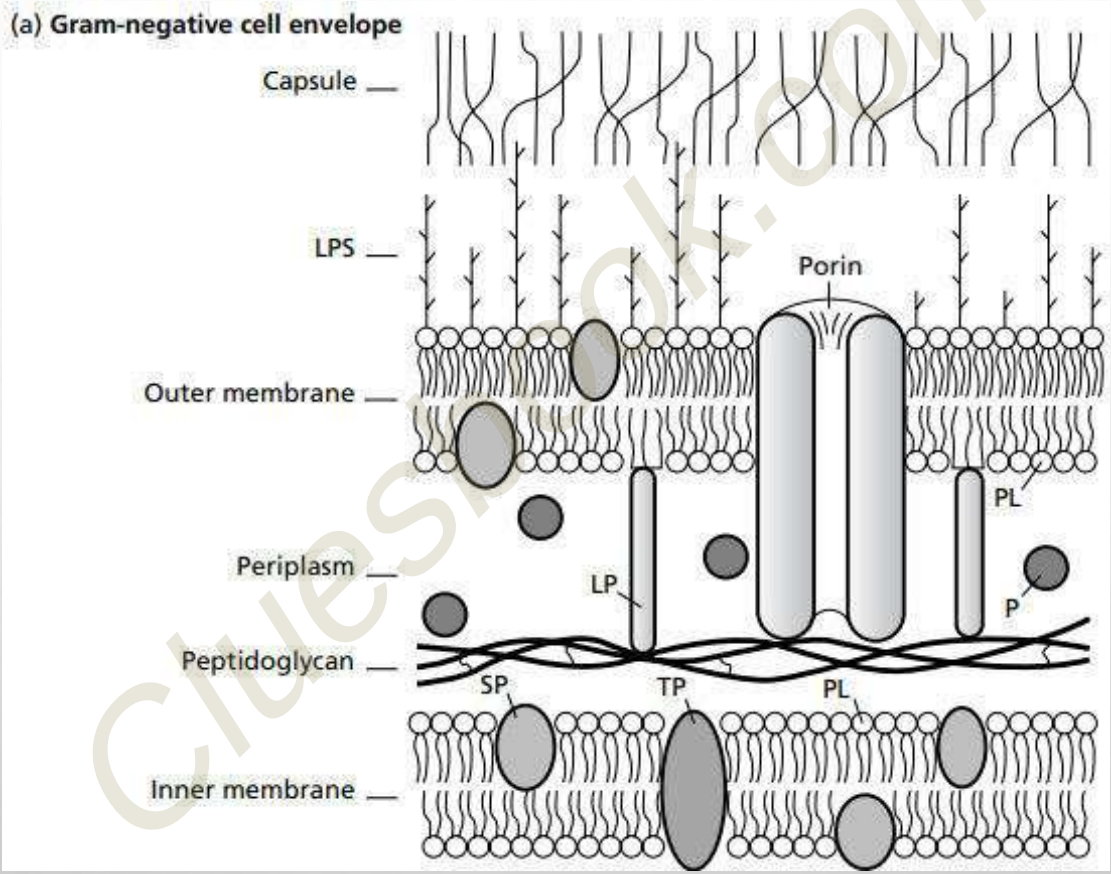
OUTER MEMBRANE

- Most common outer membrane protein is braun's lipoprotein
- Flagellae of motile strains propel the cell through aqueous media.
- Each flagellum is several micrometres long, composed of the protein flagellin
- In addition, fibrils (fimbriae or pili), may be attached to the outer membrane, which are short hair-like projections, 5–7nm in diameter and 400nm long. They enable E. Coli to attach to surfaces, such as intestinal epithelium

OUTER MEMBRANE

- Some strains also possess capsules located outside the outer membrane, which are composed of polysaccharides
- They may provide a barrier to certain molecules, help protect against desiccation, or aid attachment of pathogenic strains to host cell surfaces.

Fig. Diagrammatic representation of the structure of typical (a) gramnegative LPS = lipopolysaccharide; LP = lipoprotein; P = protein; PL = phospholipid; SP = surface protein; TP = transmembrane proteins (from poxton (1993)).



PEPTIDOGLYCAN AND THE PERIPLASMIC SPACE

PEPTIDOGLYCAN AND THE PERIPLASMIC SPACE

- Within the outer membrane of gram-negative bacteria, and covalently attached to it through lipoprotein, is a thin layer of peptidoglycan some 2–3nm thick
- It constitutes only 5–10% of the cell envelope and is composed of one to three layers
- It is a very important structural component
- When the peptiglycan layer is incomplete, bacterial cells may swell and ultimately burst

PEPTIDOGLYCAN AND THE PERIPLASMIC SPACE

- The peptidoglycan extends down into the underlying periplasmic space, which is approximately 12–15nm wide
- Binding proteins initiate transport of specific substances into the cell by taking them to their membrane-bound carriers
- The chemoreceptors are involved in chemotaxis
- Chemotaxis is the movement of a cell towards attractant and away from repellant chemicals.

PEPTIDOGLYCAN AND THE PERIPLASMIC SPACE

- Hydrolytic enzymes, notably alkaline phosphatase, nucleases and proteases
- , Are secreted into the periplasm from the cytoplasm and are retained close to the cell as they cannot normally pass through the outer membrane
- Breakdown of large impermeable nutrients into smaller molecules that can be transported across the cell membrane into the cell
- Some detoxifying and defence enzymes are also located here, e.G. Penicillinase.

CELL (CYTOPLASMIC) MEMBRANE

CELL (CYTOPLASMIC) MEMBRANE

- Below the periplasmic space lies the inner cell (cytoplasmic) membrane that encloses the cytoplasmic matrix
- The membrane is in the form of a lipid bilayer, primarily composed of phosphatidyl ethanolamine
- It is interspersed with both transport proteins, such as lactose permease

CELL (CYTOPLASMIC) MEMBRANE

- It contains pores made up of porins that selectively control the entry of molecules and charged ions into cells
- Respiratory proteins, including cytochromes and other electron transfer proteins, are also located within this membrane

CYTOPLASMIC MATRIX AND CELL CONTENTS

CYTOPLASMIC MATRIX AND CELL CONTENTS

- The cytoplasmic matrix is maintained at ph 7.6–7.8
- It contains metabolic intermediates, and the enzymes and coenzymes required for catabolic and anabolic metabolism
- Machinery for protein synthesis, both transcription and translation, is also located here

CYTOPLASMIC MATRIX AND CELL CONTENTS

- This includes RNA polymerases for transcribing the genetic code of DNA into messenger RNA (mRNA)
- The chromosome resides in the nucleoid region that occupies approximately 10% of the cell's volume
- Plasmids, relatively small circular extrachromosomal DNA molecules, may also be present.

CYTOPLASMIC MATRIX AND CELL CONTENTS

- The polysaccharide glycogen is a main store of carbon and energy, and may be seen as inclusion bodies within the cytoplasmic matrix
- Under certain circumstances osmoprotective betaines (n,n,n-trimethyl glycine) are also accumulated

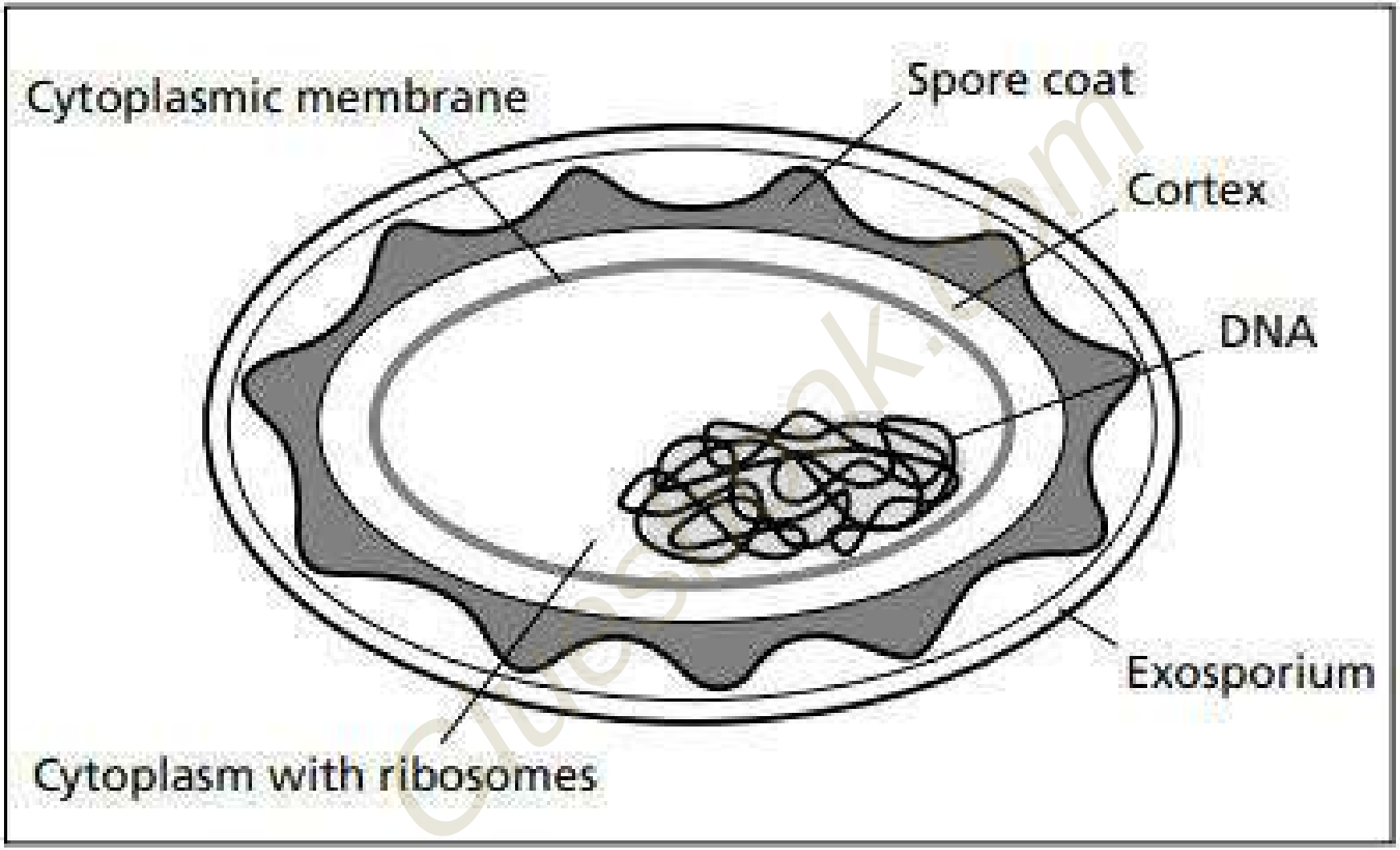
BACILLUS SUBTILIS, A GRAM-POSITIVE BACTERIUM

BACILLUS SUBTILIS, A GRAM-POSITIVE BACTERIUM

- The genus bacillus consists of a large number of diverse, rod-shaped, chemoheterotrophic, grampositive bacteria
- 0.5–2.5mm wide and 1.2–10mm long
- Some species are strictly aerobic, others are facultative anaerobes or microaerophilic, but all are catalase positive

BACILLUS SUBTILIS, A GRAM-POSITIVE BACTERIUM

- Bacillus species also produce oval or cylindrical endospores
- Endospores are resistant to adverse environmental conditions and provide a selective advantage for survival and dissemination



BACILLUS SUBTILIS, A GRAM-POSITIVE BACTERIUM

- B. Anthracis, the cause of anthrax
- B. Subtilis is a common soil microorganism that is often recovered from water, air and decomposing plant residues
- B. Subtilis has used for the production of industrial enzymes, particularly amylases and proteases
- B. Subtilis has also proved very useful for the manufacture of fine chemicals, especially nucleosides, vitamins and amino acids, and some strains are used in crop protection against fungal pathogens

BACILLUS SUBTILIS, A GRAM-POSITIVE BACTERIUM

- The main features of B. Subtilis that distinguish it from E. Coli are the cell wall structure and the ability to produce spores
- They are 20–50nm thick and simply composed of 20–25 layers of peptidoglycan, associated with some lipid, protein and teichoic acid
- Flagellae may be present and flagellated forms are capable of chemotaxis

BACILLUS SUBTILIS, A GRAM-POSITIVE BACTERIUM

- Vegetative cells continue to grow and 12 chapter 1 spore coat cortex DNA exosporium cytoplasm with ribosomes cytoplasmic membrane
- The structure of a typical bacterial endospore. Divide by binary fission until nutrients become limiting
- Unequal cell division produces a smaller forespore cell and a larger mother cell by the formation of an asymmetric septum near one pole of the cell.

FUNGI

FUNGI

- Fungi are a diverse group of eukaryotic microorganisms that occupy a variety of habitats
- Filamentous fungi are non-photosynthetic and have strict chemoheterotrophic absorptive nutrition
- Filamentous fungi originate from either fragments of hyphae or dispersed spores that germinate under suitable environmental conditions

FUNGI

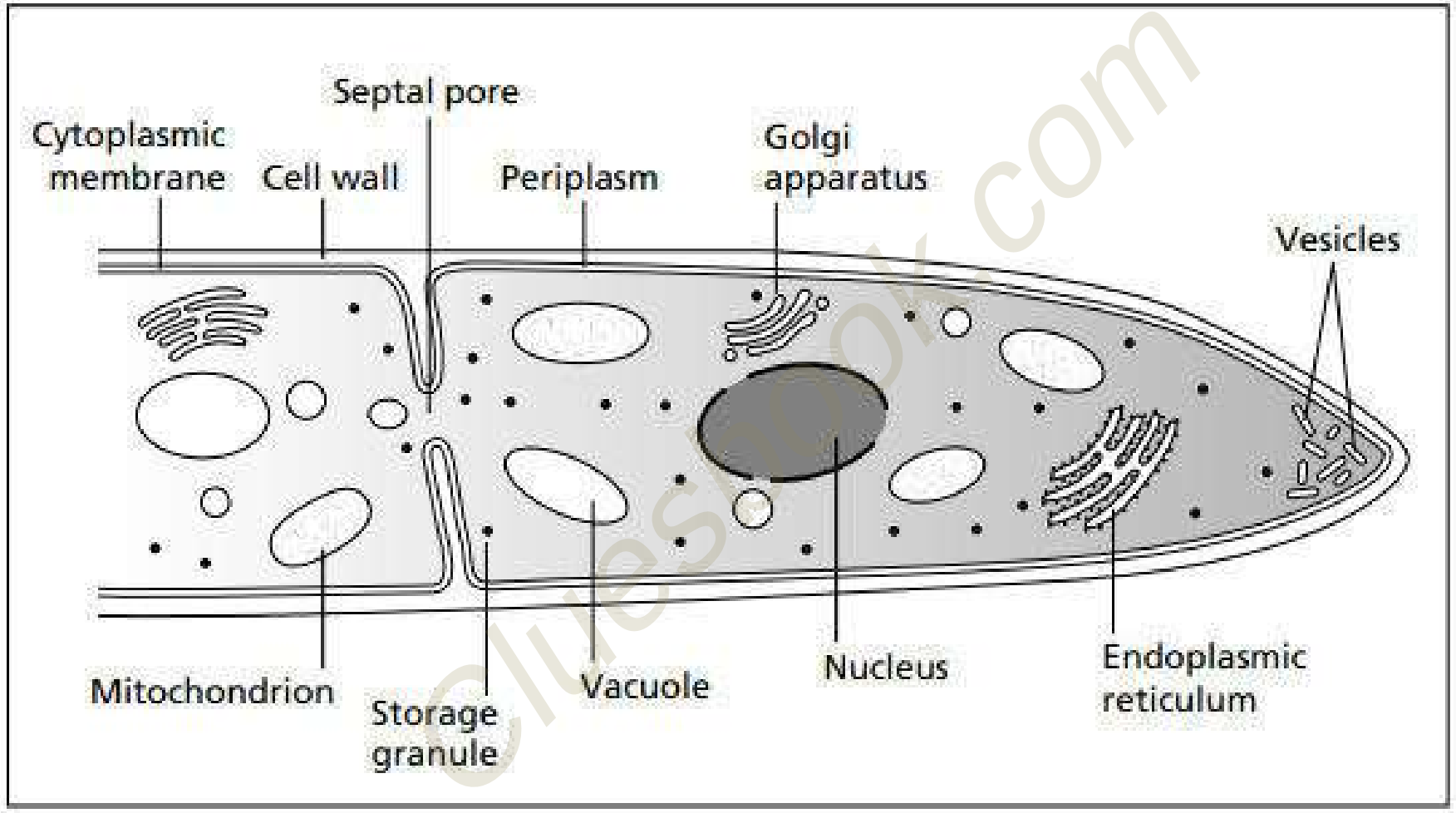
- Most are saprophytic, utilizing dead animal or plant remains.
- Some are facultative parasites of plants or animals
- Several form symbiotic and mutualistic relationships with other organisms, e.G. With an alga to form a lichen (a fungus, the mycobiont, in symbiotic association with an alga, the phycobiont)
- Many secrete a range of hydrolytic enzymes to degrade the complex polymeric molecules encountered into smaller units that can be absorbed

FUNGI

- Hyphae can grow rapidly in length, at rates of up to several micrometres per minute.
- There is generally little increase in girth, which maximizes the surface area for absorption of nutrients
- Individual hyphae are 1–15mm in diameter depending upon the species. Their cell walls are composed of 80–90% polysaccharide, along with some lipid and protein constituents

FUNGI

- Fungal chromosomes and nuclei are relatively small
- Nuclear division is somewhat different from that in most other eukaryotes
- During mitosis, the nuclear envelope remains intact with the spindle located within
- After separation of replicated chromosomes, the nuclear envelope constricts to form two new nuclei, during which the spindle disappears



FUNGI

- The sexual cycle IS syngamy, sexual union of haploid cells of opposite mating strains, + and –, occurs in two stages separated in time: first, plasmogamy, the fusion of cytoplasm, followed some time later by karyogamy, the fusion of nuclei.
- A dikaryon is formed through the pairing of haploid nuclei from each parental strain, but the pairs of nuclei do not fuse immediately. These nuclear pairs within a dikaryon may remain separate and go on dividing synchronously for long periods. Eventually they fuse to form a diploid cell that directly undergoes meiosis, ultimately producing genetically diverse haploid spores

PHYCOMYCETES

PHYCOMYCETES

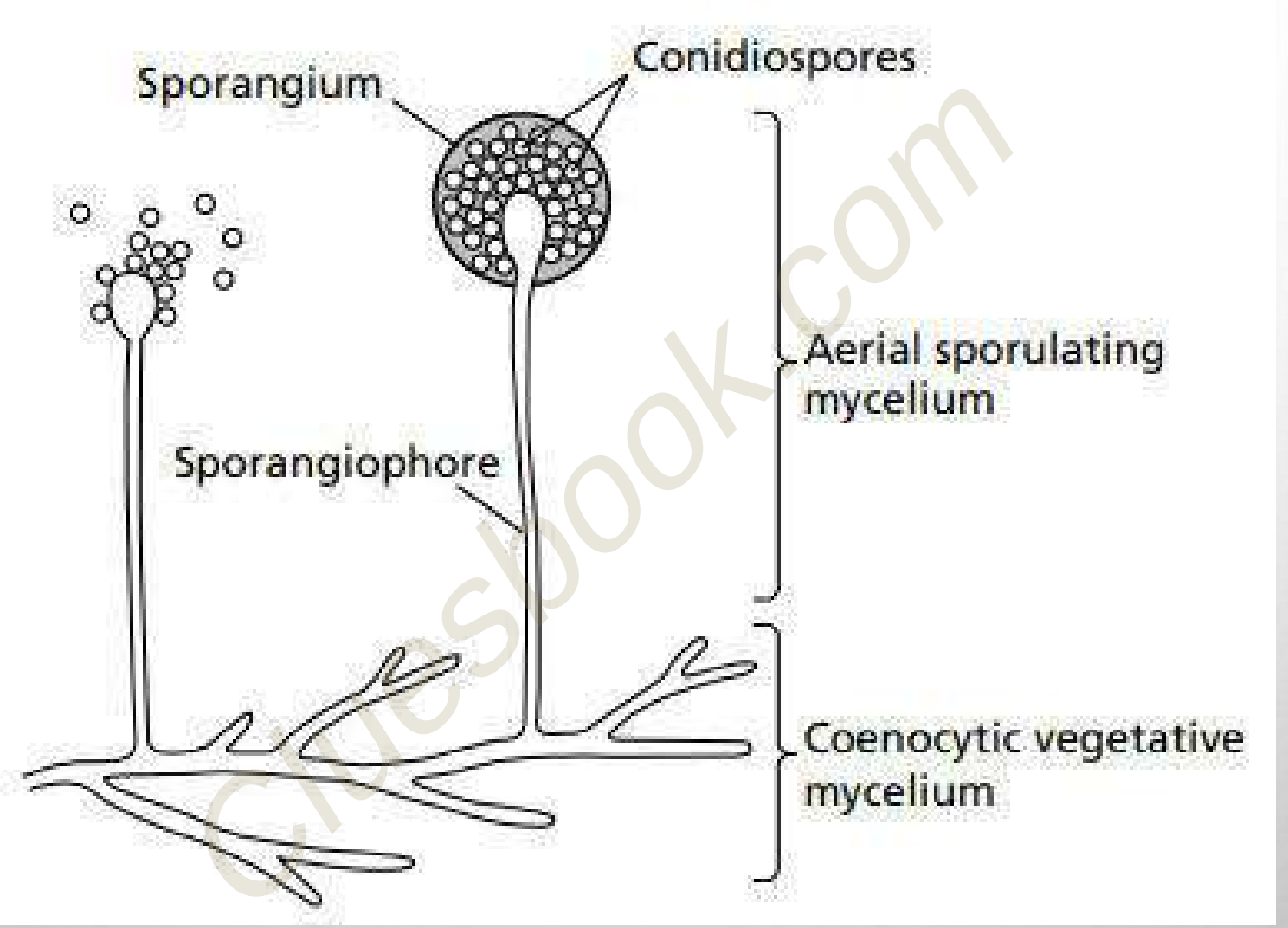
- Phycomycetes are the lower fungi
- These are subdivided into:
- Mastigomycotina (zoosporic, motile spores)
- Zygomycotina (zygosporic).
- They are the simplest fungi
- Their vegetative hyphae are aseptate

PHYCOMYCETES

- Industrially important members of the zygomycotina include mucor, rhizomucor and rhizopus species, which are used in some traditional food fermentations, and whole cell and enzyme bioconversions

PHYCOMYCETES

- Asexual reproduction results in the formation of saclike sporangia at the tips of upright hyphae or sporangiophores
- Hundreds of haploid spores are produced by mitosis within the sporangium, which are then wind dispersed.



PHYCOMYCETES

- In sexual reproduction mycelia of opposite mating types each form gametangia containing several haploid nuclei
- Plasmogamy of + and – gametangia, and pairing of haploid nuclei, results in the formation of a dikaryotic zygosporangium
- This structure is metabolically inactive and resistant to desiccation.
- When conditions become favourable, karyogamy occurs between paired nuclei and the resulting diploid nuclei undergo meiosis to produce haploid spores.

ASCOMYCETES OR ASCOMYCOTINA (SAC FUNGI)

ASCOMYCETES OR ASCOMYCOTINA (SAC FUNGI)

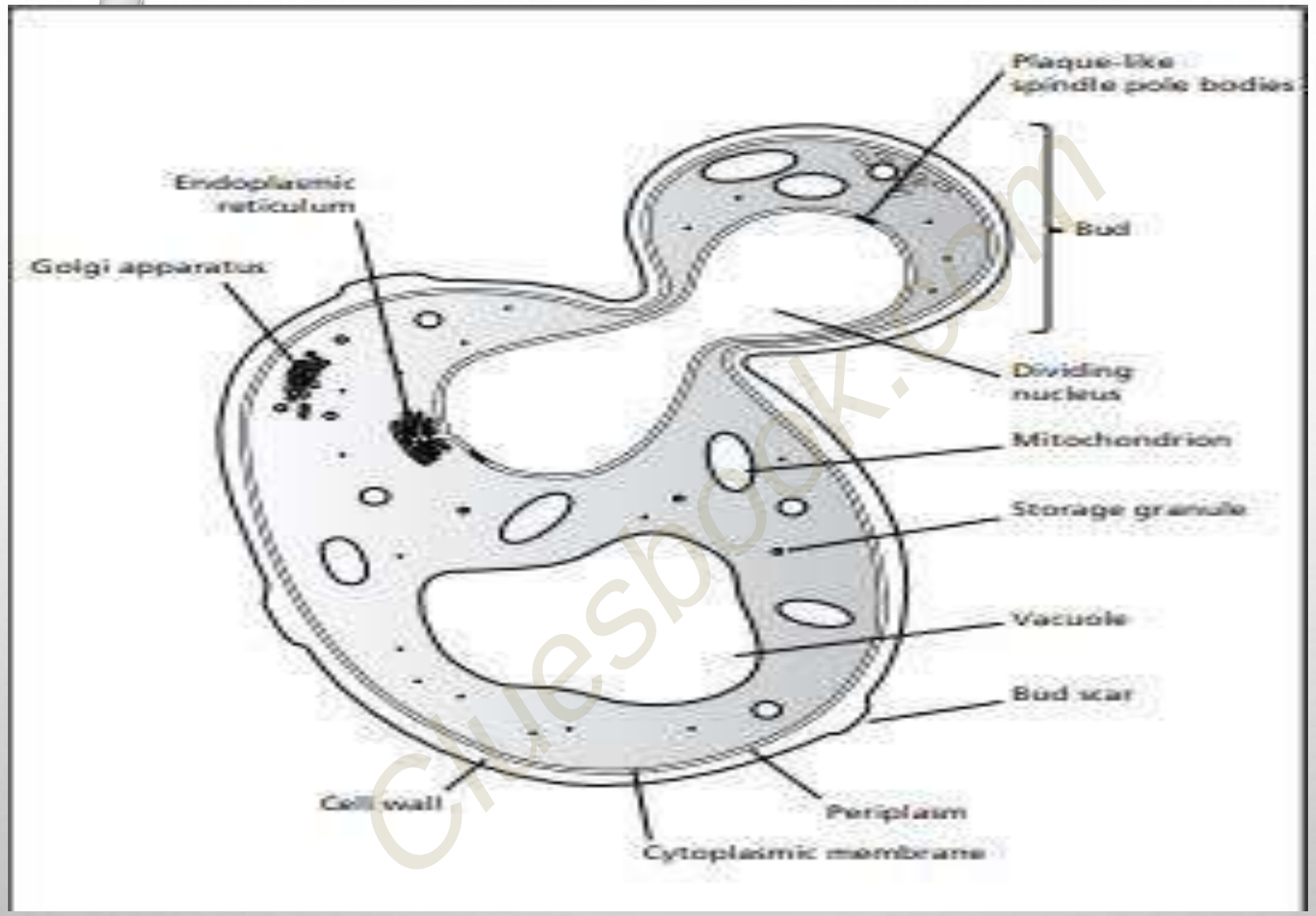
- This is the largest class of fungi and includes the yeasts that are utilized in many industrial fermentation processes
- Neurospora species, claviceps species, and important edible fungi, including morchella species (morels) and tuber species (truffles) are industrially important.
- Their hyphae are septate

ASCOMYCETES OR ASCOMYCOTINA (SAC FUNGI)

- **Asexual reproduction:**
- In asexual reproduction, the tips of specialized hyphae (conidiophores) form chains of haploid conidiospores that are usually wind dispersed.
- **Sexual reproduction:**
- Sexual spores or ascospores are contained within a sac-like ascus, which may be enclosed within a fruiting body or ascocarp.

ASCOMYCETES OR ASCOMYCOTINA (SAC FUNGI)

- The yeasts, such as saccharomyces cerevisiae, produce the equivalent of an ascus during sexual reproduction and most bud during asexual reproduction in a manner similar to the formation of conidiospores



BASIDIOMYCETES OR BASIDIOMYCOTINA (CLUB FUNGI)

BASIDIOMYCETES OR BASIDIOMYCOTINA (CLUB FUNGI)

- Members of this division produce septate hyphae
- Their sexual haploid basidiospores are borne on clubshaped structures called basidia
- , These are contained within large sexual fruiting bodies, the basidiocarps, as in the mushrooms, e.G. Agaricus bisporus (button mushroom) and lycoperdon species (puff balls)

BASIDIOMYCETES OR BASIDIOMYCOTINA (CLUB FUNGI)

- industrially important basidiomycetes are certain wood-rotting fungi involved in biodeterioration and biodegradation processes, e.G. Phanerochaete chrysosporium (white rot)
- Rusts and smuts are important plant pathogens

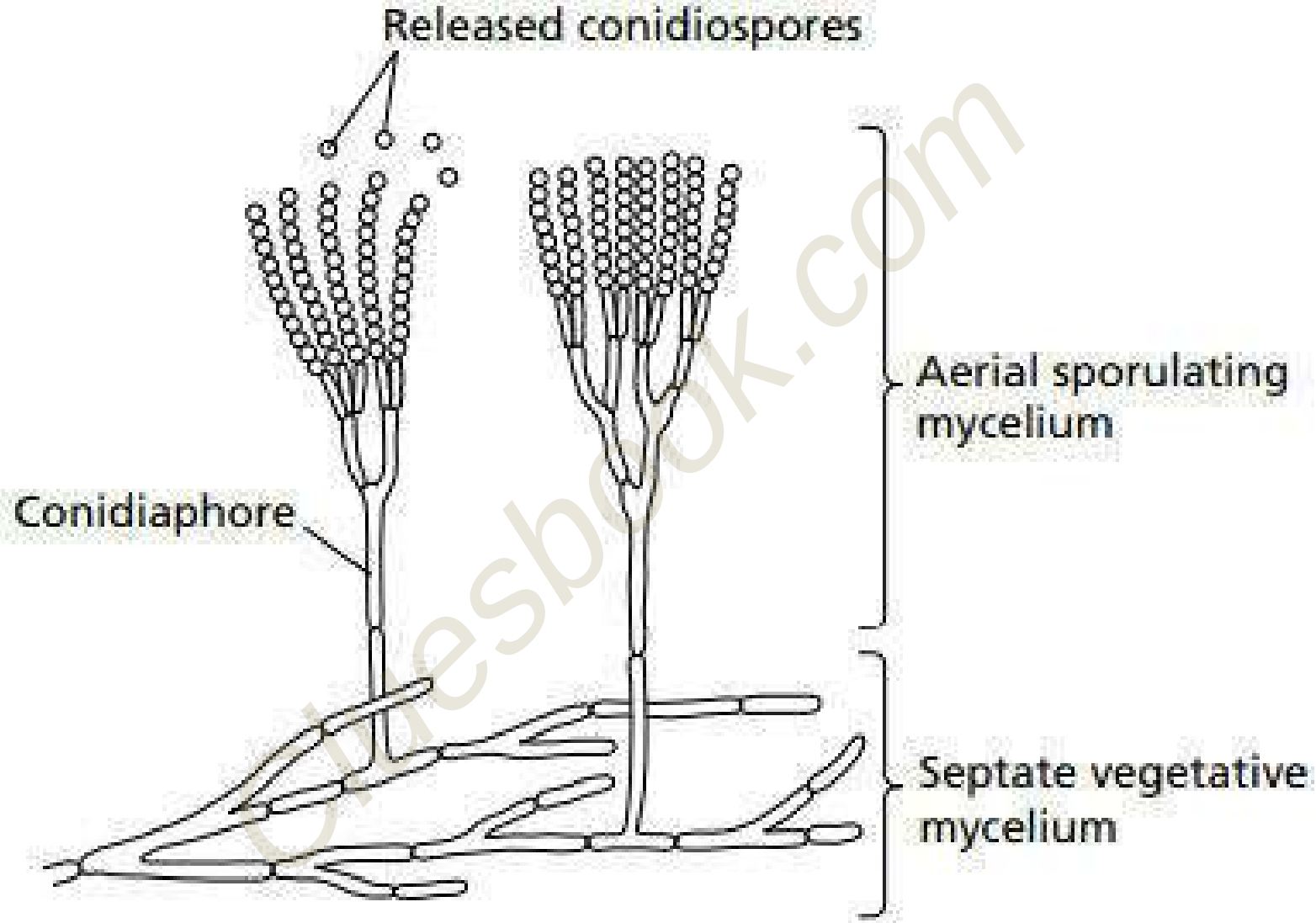
DEUTEROMYCETES OR DEUTEROMYCOTINA ('IMPERFECT' FUNGI)

DEUTEROMYCETES OR DEUTEROMYCOTINA ('IMPERFECT' FUNGI)

- This division contains a diverse group of around 25000 species
- These are grouped together simply because they lack a defined sexual (perfect) stage
- It is assumed that this group may represent the conidial stages of ascomycetes whose ascus stage has not yet been discovered or has been lost in the course of evolution

DEUTEROMYCETES OR DEUTEROMYCOTINA ('IMPERFECT' FUNGI)

- . Parasexuality has been demonstrated in some species, which has proved important for genetic study and strain development
- Many industrially important fungi are classified as deuteromycetes, including species of aspergillus, cephalosporium, fusarium, penicillium and trichoderma.



YEASTS

YEASTS

- The term ‘yeast’ refers to a unicellular form rather than to a phylogenetic classification
- It can be used to describe a unicellular phase of the life-cycles of fungi that may be predominantly filamentous, but exhibit yeast–mould dimorphism
- This term is used more commonly as a generic name for fungi that have only a unicellular phase

YEASTS

- These true yeasts and many others of industrial importance are strains of S. Cerevisiae, a member of the ascomycetes
- Yeasts are heterotrophic
- They are found in a wide range of natural habitats, being particularly common aerial plant organs, especially flowers, and plant debris in the surface layers of soil

YEASTS

- Many yeasts are facultative anaerobes, able to grow in the absence of oxygen
- Strains of S. Cerevisiae are the most well-known and commercially important yeasts, and have long been employed to produce alcoholic beverages and leaven bread
- They are now also used in the production of several other fermentation products, particularly fuel ethanol, single cell protein, enzymes and heterologous proteins, e.G. Human insulin

YEASTS

- Very few yeasts are animal pathogens.
- Cryptococcus neoformans causes a relatively rare form of meningitis, but the best known is candida albicans
- It may commonly infect the skin, and mucosal membranes of the mouth, vagina and alimentary tract
- C. Albicans can become a serious opportunistic pathogen, particularly in individuals whose immune response is weakened. Such infections are difficult to treat, due to the similarity between the host's and the pathogen's metabolism.

CELL GROWTH AND THE YEAST LIFE-CYCLE

CELL GROWTH AND THE YEAST LIFE-CYCLE

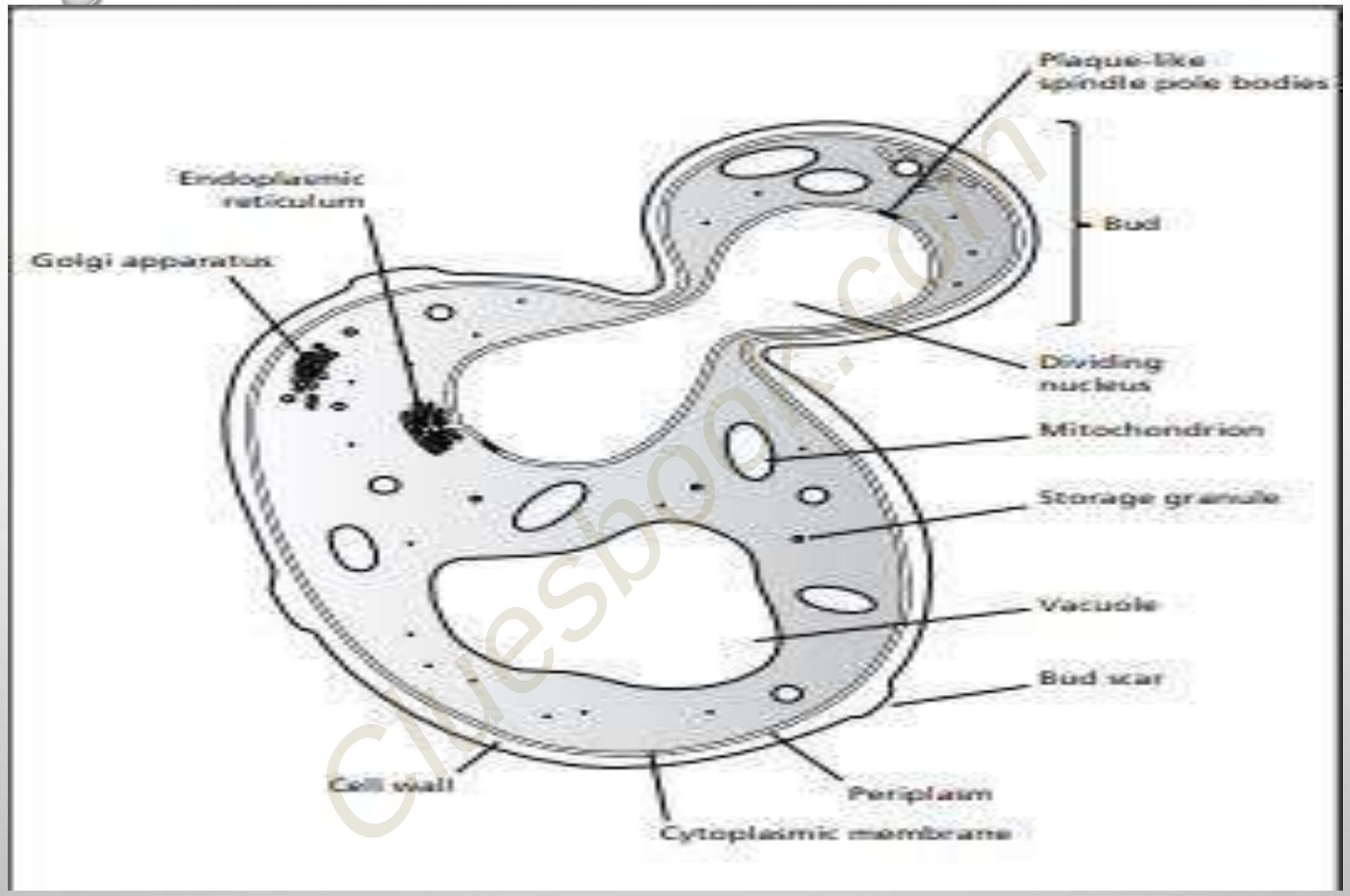
- Members of the genus saccharomyces produce cells that are spherical to ellipsoidal
- Vary in size from approximately 1–7mm wide and 5–10mm long
- The yeast cell is surrounded by a thick wall, withinwhich is the cell membrane, enclosing the cytoplasmic matrix that contains enzymes, storage granules, several different types of organelle and membrane systems

CELL GROWTH AND THE YEAST LIFE-CYCLE

- Their asexual cell division involves budding of a daughter cell from a mother cell and cell growth is largely associated with bud development
- A mature cell initiates a bud at a site where the cell wall has been weakened by lytic enzymes and the bud grows to approximately the same size as the mother cell.
- Wall growth is polarized, mainly by deposition of new cell wall material at its tip, which is associated with microvesicles in the underlying cytoplasmic matrix.

CELL GROWTH AND THE YEAST LIFE-CYCLE

- During this period mitosis occurs.
- The chromosomes replicate, and the mitotic spindle forms and aligns across the junction between the two cells.
- It then elongates and facilitates the formation of two separated sets of chromosomes that become enclosed within separate nuclei, one in each cell.
- The cell wall between the mother cell and bud becomes completed, and the two cells may then separates.



CELL GROWTH AND THE YEAST LIFE-CYCLE

- Sexual reproduction involves haploid cells of two mating types and mating is mediated by the secretion of small peptide pheromones.
- Each mating type responds by halting bud formation, then each cell elongates and differentiates to become a specialized pear-shaped gamete.
- Cells of opposite mating types that are in contact or close proximity undergo plasmogamy and karyogamy to form a diploid cell.
- These diploid forms are stable and can perform repeated cell division.

CELL GROWTH AND THE YEAST LIFE-CYCLE

- However, if the physiological conditions are suitable meiosis occurs.
- This results in sporulation, normally producing four haploid nuclei, which are incorporated into four stress-resistant ascospores, encapsulated within an ascus.

CELL WALL STRUCTURE

CELL WALL STRUCTURE

- Some yeasts develop slimy polysaccharide capsules outside the cell wall that may have a protective function or aid in attachment to surfaces.
- The cell wall itself is approximately 100–200nm thick and comprises 15–25% of the dry weight of a cell.
- Its major components, some 80–90% of wall material, are the polysaccharides glucan, phosphomannan and chitin, along with some proteins

CELL WALL STRUCTURE

- Chitin may form less than 1% of the yeast wall and is primarily found around bud scars
- The number of bud scars on the wall indicates the number of times that a cell has budded and as they never occur twice in the same place, they can be used to estimate the ‘age’ of a cell

CELL WALL STRUCTURE

- Enzymes that have been found here and in the periplasm include those associated with wall biosynthesis and the hydrolysis of substrates unable to cross the cell membrane
- They include glucanase, mannanase, lipase, alkaline phosphatase and invertase, a mannoprotein involved in sucrose utilization
- Flocs are aggregates of cells, not chains of unseparated buds

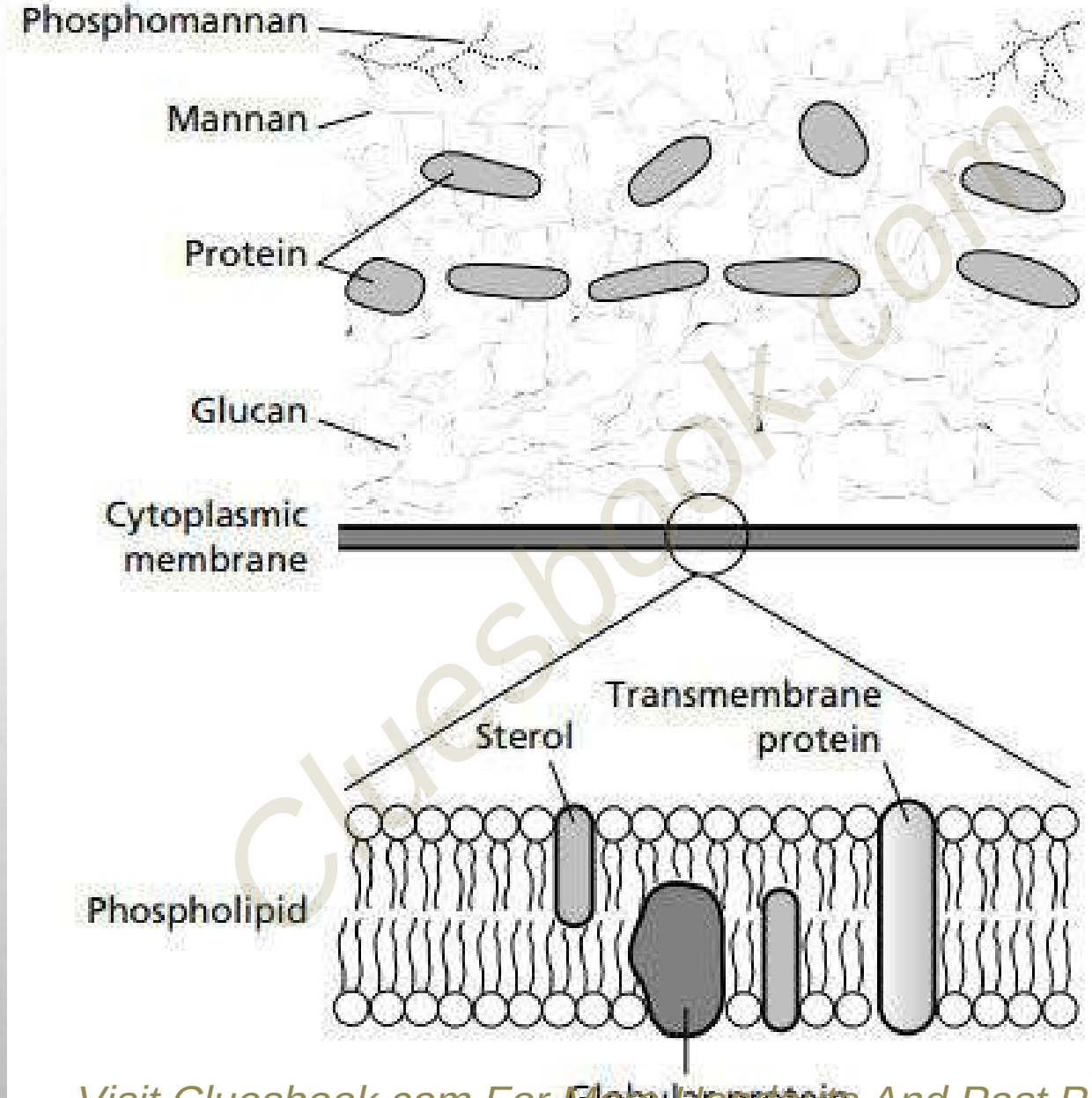
CELL WALL STRUCTURE

- There are two main theories of floc formation.
- The first concerns calcium bridging, where a calcium ion links two cells via negatively charged cell wall components. Support for this theory comes from the observation that flocs are dispersed by the chelating agent ethylenediamine tetraacetic acid (EDTA).
- Secondly, the lectin hypothesis, involving protein–carbohydrate binding, proposes that a surface protein of one cell links to a mannose residue on an adjacent cell. This hypothesis is supported by the fact that sugars, particularly mannose, inhibit floc formation

CELL MEMBRANE STRUCTURE

CELL MEMBRANE STRUCTURE

- Between the cell wall and underlying membrane, there is a periplasmic space of 3.5–4.5nm containing secreted proteins that cannot escape through the wall, including the enzymes
- The cell membrane controls all materials entering and exiting the cell, and is thought to manage cell wall biosynthesis
- Yeast cell membranes, like those of other cells, are primarily composed of a lipid bilayer some 7.5nm thick



CELL MEMBRANE STRUCTURE

- This produces inner and outer hydrophilic regions that sandwich the hydrophobic lipid ‘tails’.
- Unsaturated sterol components include ergosterol and zymosterol, which are not found in prokaryotic cell membranes.
- Membrane lipid composition varies depending upon growth conditions and also affects the cell’s tolerance to ethanol.

CELL MEMBRANE STRUCTURE

- Within and across the lipid bilayer are both structural and functional protein molecules, together with a small portion of carbohydrate.
- Protein components may be involved with solute transport, or are signal transduction components that respond to external stimuli and ultimately initiate an internal response
- There are also wall synthesizing enzymes. For example, chitin synthetase is present in an inactive form, which is activated by proteolytic cleavage when required.

THE NUCLEUS

THE NUCLEUS

- The nucleus is surrounded by a double membrane that has regularly spaced pores.
- This organelle contains the majority (80–85%) of the cellular dna; the remainder is present as circular molecules in the mitochondria or cytoplasm
- Haploid cells of S. Cerevisiae contain approximately 12000kbp of DNA, which is 3–10 times more than a typical bacterium and about 100–150 times less than mammalian cells

THE NUCLEUS

- Nuclear DNA is associated with basic histone proteins and is in the form of 16 linear chromosomes made up of 150–2500kbp.
- Over 6000 genes have been identified, but around 50% have unknown function.
- Associated with the nucleus is a structure referred to as a plaque, which has microtubules that pass into both the nucleus and cytoplasmic matrix.

THE NUCLEUS

- It functions in a similar way to the centrioles of animal cells, forming the spindle upon which the replicated chromosomes separate during mitosis.

THE MITOCHONDRIA

THE MITOCHONDRIA

- Fully developed mitochondria are present only in yeasts that are growing aerobically.
- Under anaerobic conditions they are simple structures, referred to as promitochondria, as anaerobic growth induces their dedifferentiation.
- Fully developed organelles are spherical to rod-shaped, enclosed by a double membrane.

THE MITOCHONDRIA

- The inner membrane possesses proteins involved in electron transport and oxidative phosphorylation, and is folded into the lumen of the organelle to form finger-like structures called cristae. Located within the lumen is a fluid matrix containing most of the enzymes of the tricarboxylic acid (TCA) cycle, ribosomes and 75kbp circular DNA molecules that code for some 10% of mitochondrial proteins.

THE MITOCHONDRIA

- ‘Petite’ yeast mutants lack mitochondria and are therefore incapable of respiration.
- They can grow only fermentatively and on solid media their colonies are very small, compared with wild-type colonies, hence their name.

OTHER ORGANELLES AND CYTOPLASMIC STRUCTURES

OTHER ORGANELLES AND CYTOPLASMIC STRUCTURES

- The cytoplasmic matrix also contains ribosomes, various single membrane-bound organelles and vacuoles, a cytoskeleton composed of microtubules and microfilaments, and an elaborate membrane system, the endoplasmic reticulum
- Endoplasmic reticulum is continuous with the cell membrane and outer membrane of mitochondria.
- At maturity the cell has a large vacuole that arises from the fusion of smaller vacuoles.

OTHER ORGANELLES AND CYTOPLASMIC STRUCTURES

- Peroxisomes contain catalase and various oxidases, and glyoxysomes possess catalase and the enzymes of the glyoxylate cycle.
- The number and size of peroxisomes varies depending upon the environment and available nutrients.
- Cytoplasmic storage granules of yeasts mostly contain lipids or carbohydrates.

OTHER ORGANELLES AND CYTOPLASMIC STRUCTURES

- These carbohydrates may constitute up to 40% of the dry weight of a yeast cell.
- Glycogen is the predominant storage material, but the proportion of trehalose increases under aerobic conditions and under certain stresses.
- It is considered that glycogen has a role during periods of starvation and respiratory adaptation, whereas trehalose may be involved in only the former

OTHER ORGANELLES AND CYTOPLASMIC STRUCTURES

- The cytoplasmic matrix of S. Cerevisiae may also contain 50–60 copies of the extrachromosomal circular 2mm plasmid
- Other extrachromasomal elements have also been found in some strains and include virus-like doublestranded linear RNA molecules.

MICROBIAL NUTRITION

MICROBIAL NUTRITION

- The biosynthesis of cellular components necessary for growth, reproduction and maintenance requires a supply of basic nutrients and an energy source.
- Nutritional classification is established on the basis of specific sources of energy, electrons/hydrogen and carbon used by an organism

MICROBIAL NUTRITION

- Microorganisms have evolved a wide range of mechanisms to gain energy, but are essentially divided into two categories.
- Autotrophs utilize co2 as their sole or primary source of carbon
- Whereas various heterotrophs use a wide range of reduced organic molecules, including hydrocarbons, lipids, organic acids, simple sugars and polysaccharides.

MICROBIAL NUTRITION

- Chemotrophs obtain energy by the oxidation of organic or inorganic compounds
- Phototrophs use energy derived from light.
- Organotrophs oxidize preformed organic molecules, such as sugars, to obtain electrons or hydrogen
- Lithotrophs acquire electrons from reduced inorganic molecules, including hydrogen sulphide and ammonia

Physiological type	Source of		
	Energy	Electrons	Carbon
Chemotroph	Chemical		
Phototroph	Light		
Organotroph		Organic compound	
Lithotroph		Inorganic molecule	
Autotroph			CO_2
Heterotroph			Organic compounds
Chemoorgano (hetero) troph (animals, fungi, protozoa, many bacteria)	Organic compound	Organic compound	Organic compound
Chemolitho (auto) troph (some bacteria)	Inorganic molecule	Inorganic molecule	CO_2
Photolitho (auto) troph (plants, most algae, some bacteria)	Light	Inorganic molecule	CO_2
Photoorgano (hetero) troph (algae, some bacteria)	Light	Organic compound	Organic compound

MICROBIAL NUTRITION

- Microbial cells must obtain a range of chemical elements to fulfil their nutritional requirements.
- Four of these, the macronutrients carbon, hydrogen, oxygen and nitrogen, must be available in gram quantities per litre of growth medium.
- These elements, along with phosphorus and sulphur, are the principal components of major cellular polymers: lipids, nucleic acids, polysaccharides and proteins

Water	70–80%
Protein	15–18%
Polysaccharide	1–3%
Lipid	1–2%
Nucleic acids	3–7%
Inorganic salts	1–2%

Main elemental cell composition = $C_4H_7O_2N$

MACRONUTRIENTS

MACRONUTRIENTS

- Autotrophic fermentations that utilize CO2 are rarely operated on an industrial scale: almost all involve heterotrophic growth.
- In heterotrophic fermentations, carbon sources are required at relatively high media concentrations, often around 10–20g/l or greater, as they provide carbon ‘skeletons’ for biosynthesis and many also serve as an energy source.A

MACRONUTRIENTS

- Hydrogen and oxygen can be obtained from water and organic compounds.
- However, many organisms are also dependent on molecular oxygen as the terminal acceptor in aerobic respiration and for the synthesis of specific compounds, such as unsaturated sterols.

MACRONUTRIENTS

- Microorganisms may contain more than 15% (w/w) nitrogen, mostly within structural and functional proteins, and nucleic acids.
- To fulfil these requirements a nitrogen source is normally supplied in growth media at concentrations of 1–2g/l.
- Ammonium salts are often the preferred nitrogen source, but nitrate, amino acids or nitrogen-rich compounds, such as urea, may sometimes be used.

MACRONUTRIENTS

- In addition, certain specialized nitrogen-fixing bacteria, notably azotobacter and rhizobium species, can utilize molecular nitrogen.

MINOR ELEMENTS

MINOR ELEMENTS

- Phosphorus is generally provided as inorganic phosphate ions, often as a ph buffer.
- This element is essential for the synthesis of nucleic acids, intermediates of carbohydrate metabolism and compounds involved in energy transduction
- E.G. Adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADP).

MINOR ELEMENTS

- Sulphur is required for the production of the sulphurcontaining amino acids cystine, cysteine and methionine, and some vitamins.
- It is often supplied as an inorganic sulphate or sulphide salt at a concentration of 20–30mg/l.
- Other minor elements, mostly calcium, iron, potassium and magnesium, must be provided in relatively small but significant quantities, usually less than 10–20mg/l.

MINOR ELEMENTS

- Iron is essential for several oxidation–reduction enzymes, particularly cytochromes, and potassium is required by enzymes involved in protein synthesis and as counter-ion for the DNA phosphate backbone.
- Magnesium ions are involved in the stabilization of ribosomes, and some are necessary for maintaining cell wall and membrane integrity.
- Sodium and potassium are used in chemiosmotic energy pumps, but the former does not appear to have other specific roles, except in salt ‘loving’ halophilic microorganisms

TRACE ELEMENTS

TRACE ELEMENTS

- Trace elements include cobalt, copper, manganese, molybdenum, nickel, selenium and zinc.
- These elements are usually required at concentrations of 0.1–1mg/L, or less, for a number of specific enzymes.
- Their normal concentrations in water supplies, or as contaminants in other media ingredients, often fulfil this requirement.

TRACE ELEMENTS

- Overall nutrient demands vary for different microorganisms.
- Many bacteria can grow on media merely containing carbon and energy sources, and basic mineral elements.
- Microorganisms that are able to grow on this minimal medium are referred to as prototrophs.
- However, other microorganisms must be given specific compounds in a part or fully constructed form.

TRACE ELEMENTS

- Those that are unable to grow without additional organic substances, such as amino acids or vitamins, are called auxotrophs.
- Culture media for many organotrophic microorganisms often require certain vitamin and growth factor supplements, especially b vitamins (thiamin, b1; riboflavin, b2; pyridoxine, b6; cobalamin, b12; biotin; nicotinic acid; and pantothenic acid).
- Few microorganisms require fat-soluble vitamins (a, d, e and k); and vitamin c, although often improving growth, is not a true microbial growth factor.

NUTRIENT UPTAKE

NUTRIENT UPTAKE

- Nutrients from the environment must be transported across the cell membrane into the cell.
- This is often the rate-limiting step in the conversion of raw materials to products and therefore is of major importance to industrial fermentation processes.
- Uptake of a few nutrients is by passive diffusion, which does not require carriers.

NUTRIENT UPTAKE

- Such nutrients are usually soluble in lipids and can enter hydrophobic membranes, e.G. Glycerol and urea.
- Most solutes must be transported via specific active mechanisms, because membranes are selectively permeable.
- Also, microorganisms usually inhabit natural environments where nutrient concentrations are low. Consequently, it is essential that they can accumulate solutes against concentration gradients, as intracellular concentrations of compounds are often higher than environmental levels.

NUTRIENT UPTAKE

- Most solute uptake involves carrier proteins (permeases) that span the membrane. Their participation in facilitated diffusion requires no direct input of energy. It is driven solely by the concentration gradient across the membrane and is reversible.
- This mechanism, rarely seen in prokaryotes, is used to transport sugars and amino acids, and their selective carriers usually function for a group of related solutes. They greatly increase diffusion rates, at least up to concentrations at which the carrier protein becomes saturated with its nutrient.

NUTRIENT UPTAKE

- Active transport mechanisms allow the accumulation of materials against a concentration gradient, which is important in environments where nutrient levels are low.
- Some systems allow accumulation to 100–1000 times greater than the external concentration.

NUTRIENT UPTAKE

- Proton gradients are involved (see chapter 3, electron transport), the nutrient entering the cell, such as a sugar, amino acid or organic acid molecule, is simultaneously transported with a proton, and is referred to as symport.
- Proton gradients may also be used to establish a sodium ion gradient across the membrane. Here sodium ions leave the cell in exchange for the entry of protons, which is termed antiport.

NUTRIENT UPTAKE

- Some compounds may be modified during uptake. Sugars, for example, can be phosphorylated using phosphoenolpyruvate (PEP) as the phosphate donor. This is referred to as group translocation, which is performed by many prokaryotic cells.

MICROBIAL GROWTH KINETICS

MICROBIAL GROWTH KINETICS

- Microbial growth can be defined as an orderly increase in cellular components, resulting in cell enlargement and eventually leading to cell division.
- This definition is not strictly accurate as it implies that a consequence of growth is always an increase in cell numbers.
- However, under certain conditions growth can occur without cell division,

MICROBIAL GROWTH KINETICS

- In this situation the cell numbers remain constant, but the concentration of biomass continues to increase.
- This is also true for coenocytic organisms, such as some fungi, that are not divided into separate cells.
- Their growth results only in increased size.

MICROBIAL GROWTH KINETICS

- The growth model that will be examined is bacterial binary fission in homogeneous suspension cultures, where cell division produces identical daughter cells.
- Each time a cell divides is called a generation and the time taken for the cell to divide is referred to as the generation time.
- The generation time or doubling time (td) is the time required for a microbial population to double. Theoretically, after one generation, both the microbial cell population and biomass concentration have doubled.

MICROBIAL GROWTH KINETICS

- At any one time there are cells at different stages of their cell cycle. This is termed asynchronous growth.
- Microbial fermentations in liquid media can be carried out under different operating conditions, i.E. Batch growth, fed-batch growth or continuous growth.
- Batch growth involves a closed system where all nutrients are present at the start of the fermentation within a fixed volume. The only further additions may be acids or bases for ph control, or gases (e.G. Aeration, if required).

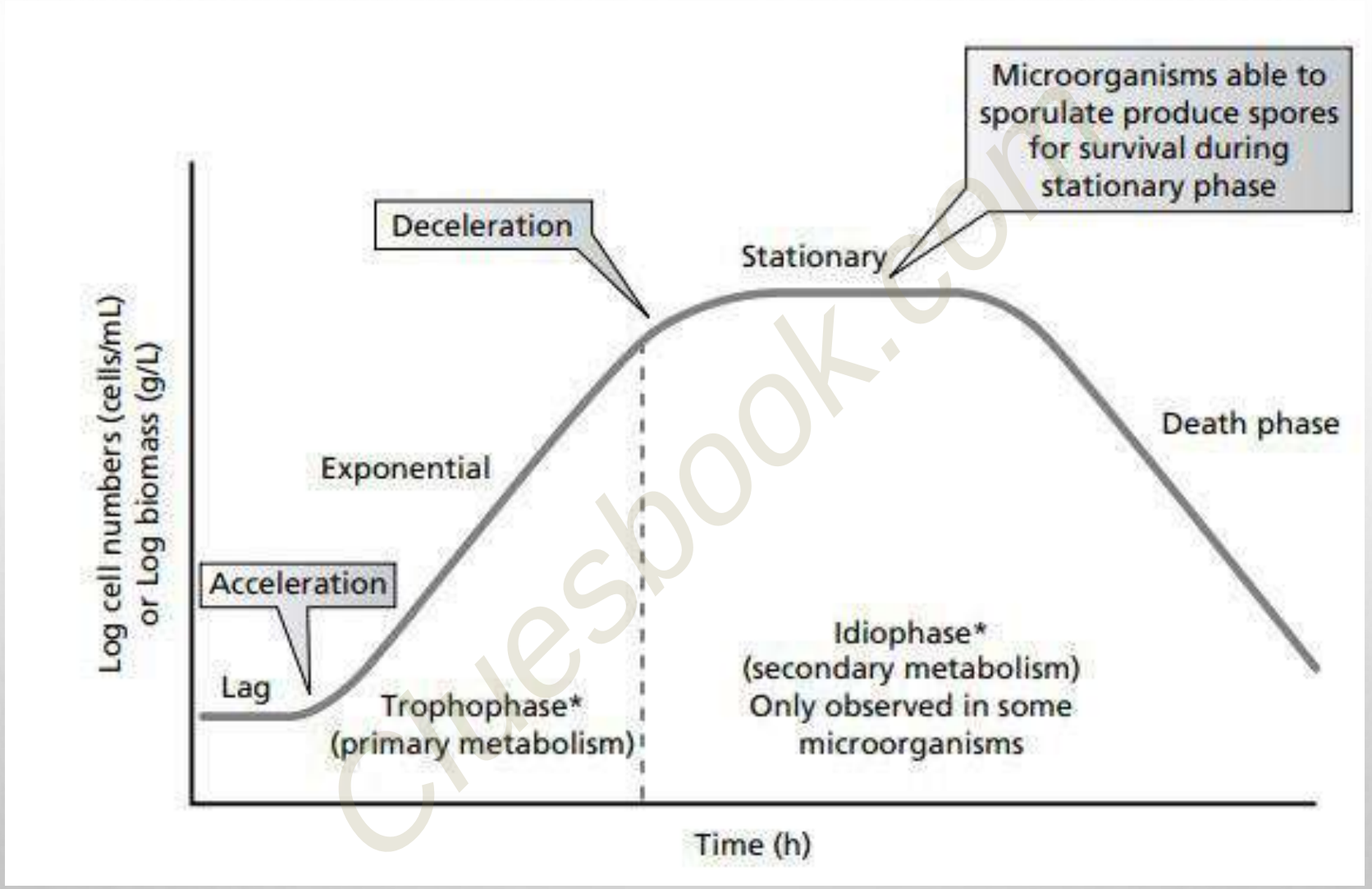
MICROBIAL GROWTH KINETICS

- In fed-batch systems fresh medium or medium components are fed continuously, intermittently or are added as a single supplement and the volume of the batch increases with time.

BATCH GROWTH

BATCH GROWTH

- During batch fermentations the population of microorganisms goes through several distinct growth phases: lag, acceleration, exponential growth, deceleration, stationary and death



BATCH GROWTH

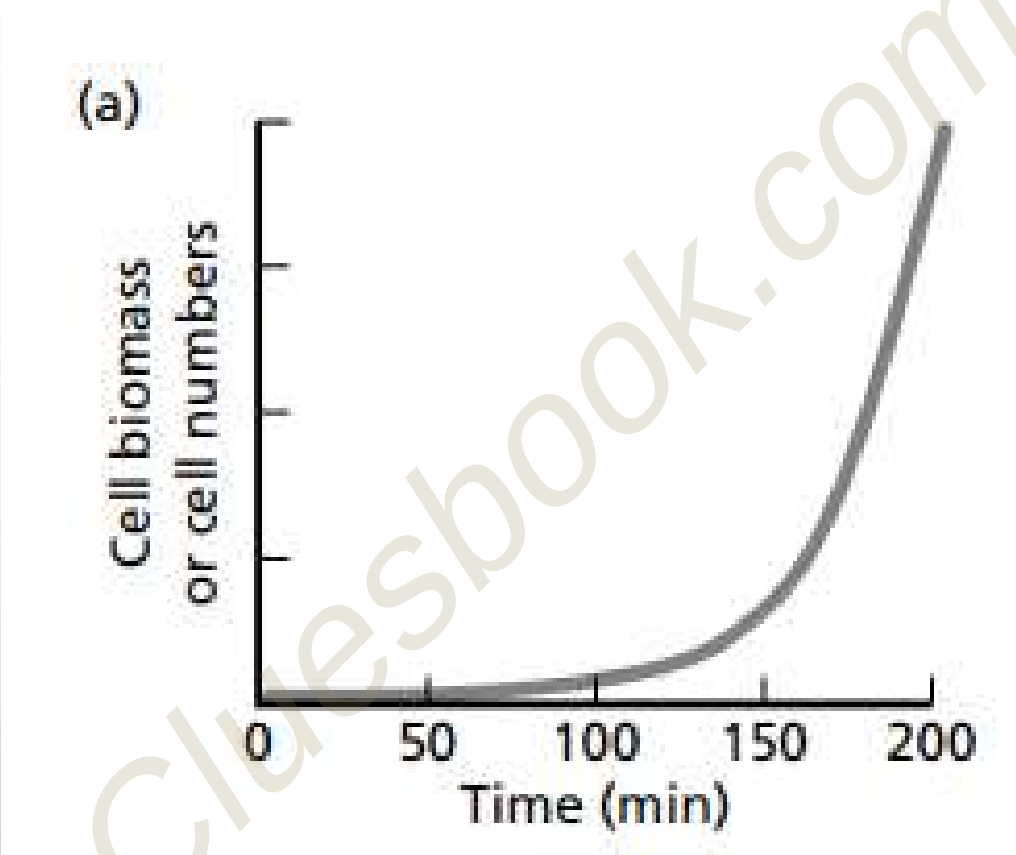
- In the lag phase virtually no growth occurs and the microbial population remains relatively constant.
- Nevertheless, it is a period of intense metabolic activity as the microbial inoculum adapts to the new environment
- It is usually longer if the inoculum was grown up using a carbon source different from that in the fresh medium, because the cells must synthesize enzymes required to catabolize the new substrate.

BATCH GROWTH

- Once the cells have adapted to their new environment they enter the acceleration phase.
- Cell division occurs with increasing frequency until the maximum growth rate (mmax) for the specific conditions of the batch fermentation is reached.
- At this point exponential growth begins and cell numbers/biomass increase at a constant rate. Mathematically, this exponential growth can be described by two methods; one is related to biomass (x) and the other to cell numbers (N).

BATCH GROWTH

- Rate of change of biomass is dx/dt=mx 2.1 where x = concentration of biomass (g/L), m = specific growth rate (per hour) and t = time (h). When a graph is plotted of cell biomass against time, the product is a curve with a constantly increasing slope



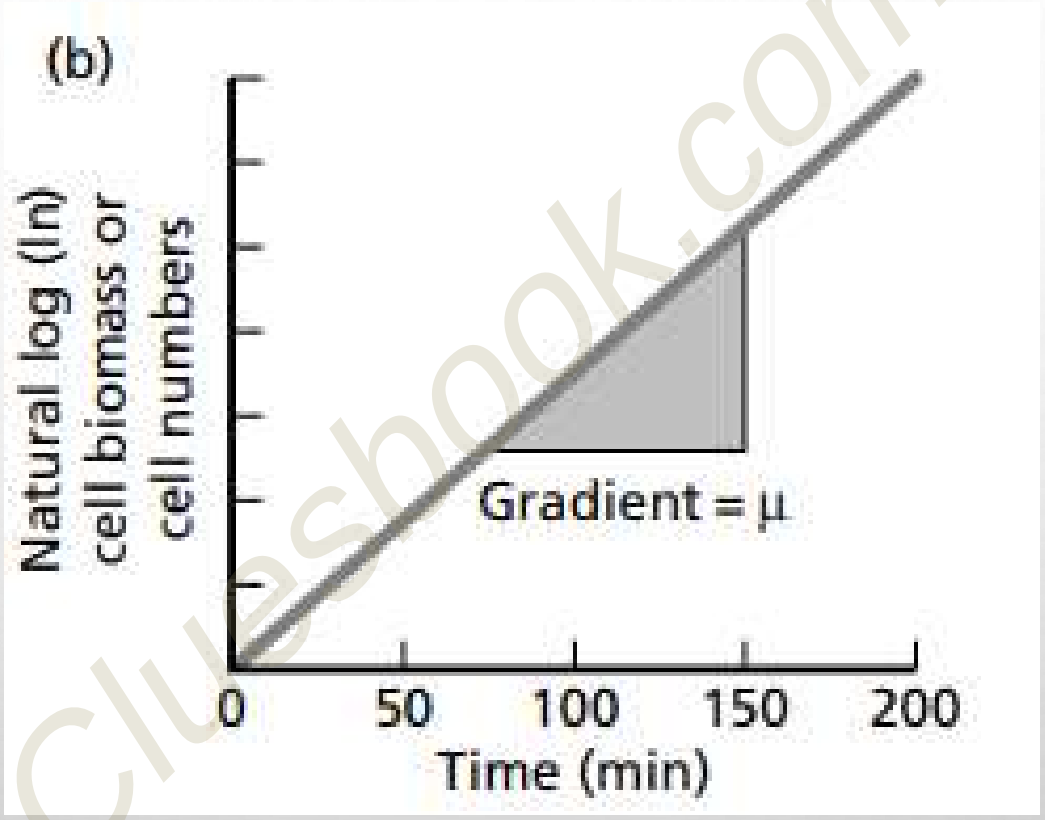
BATCH GROWTH II

BATCH GROWTH II

- Equation 2.1 can also be rearranged to estimate the specific growth rate (m):
- M = 1/x*dx/dt 2.2
- During any period of true exponential growth, equation 2.1 can be integrated to provide the following equation:
- Xt=xoe mt 2.3
- Where xt = biomass concentration after time t, xo = biomass concentration at the start exponential growth, and e = base of the natural logarithm.

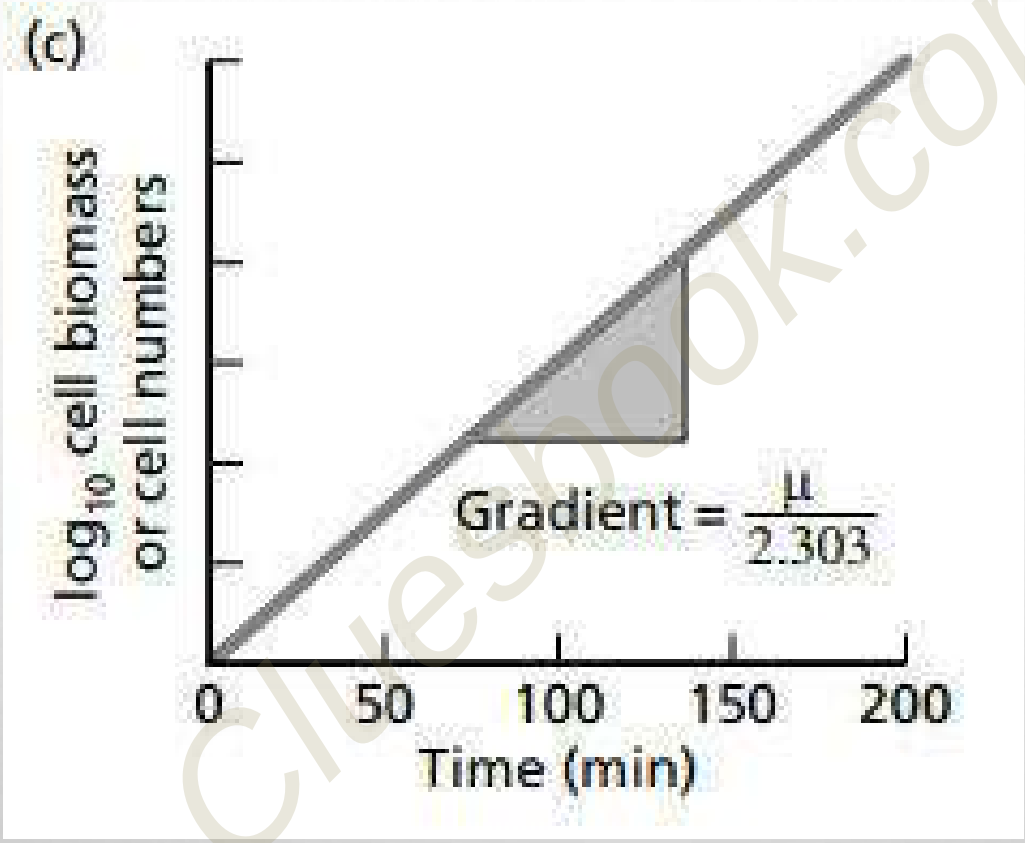
BATCH GROWTH II

- And e = base of the natural logarithm. Taking natural logarithms, loge (ln), gives
- Ln xt= ln xo + mt 2.4
- This equation is of the form y = c (intercept on y axis) + mx where m = gradient (min equation 2.4), which is the general equation for a straight-line graph. For cells in exponential phase, a plot of natural log of biomass concentration against time, a semilog plot, should yield a straight line with the slope (gradient)



BATCH GROWTH II

- $M = (\ln x_t - \ln x_o)/t$ 2.5
- (Note: when plotting log10 values instead of the natural log, the gradient of the semilog plot is equal to m/2.303



BATCH GROWTH III

BATCH GROWTH III

- A second approach is to examine growth in relation to cell number, where the number of cells at the start of exponential growth is no. If we consider a hypothetical case where the microbial cell population at this point of exponential growth is one (no =1), we can describe binary fission as follows:

No. of divisions	0	1	2	3	n
No. of cells	1	2	4	8	2^n
Mathematically	N_o	N_o2	N_o2*2	N_o2*2*2	–
	–	N_o2^1	N_o2^2	N_o2^3	N_o2^n

Consequently, after a period of exponential growth, time (t), the number of cells (N_t) is given by

$$N_t = N_o 2^n \qquad 2.6$$

where n = the number of divisions, N_o = initial cell number. Taking natural logarithms gives

$$\ln N_t = \ln N_o + n \ln 2 \qquad 2.7$$

Therefore, the number of divisions (n) that have taken place is given by

$$n = \frac{\ln N_t - \ln N_o}{\ln 2} \qquad 2.8$$

The number of divisions per unit time during this period of exponential growth is determined by dividing by the time period (t):

$$\frac{n}{t} = \frac{\ln N_t - \ln N_o}{t \ln 2} \qquad 2.9$$

where n/t = **division rate constant** (average number of generations per hour).

Often, we are not really interested in the number of divisions per hour, unit time, but rather in the **mean generation time** or **doubling time** (t_d), that is, the time required to undergo a single generation that doubles the population. Thus,

$$t_d = \frac{t}{n} = \frac{t \ln 2}{\ln N_t - \ln N_o} \qquad 2.10$$

BATCH GROWTH III

- If we consider a situation where at time zero, the cell biomass is xo, then after a fixed period of time (t) of exponential growth, equivalent to one doubling time (td), the microbial biomass will double to 2xo, i.E. Xt=2xo, when t=td. Substituting these parameters into equation 2.3 gives

$$2x_o = x_o e^{\mu t_d} \qquad 2.11$$

Taking natural logarithms produces

$$\ln 2x_o = \ln x_o + \mu t_d \qquad 2.12$$

or

$$\mu t_d = \ln 2 \qquad 2.13$$

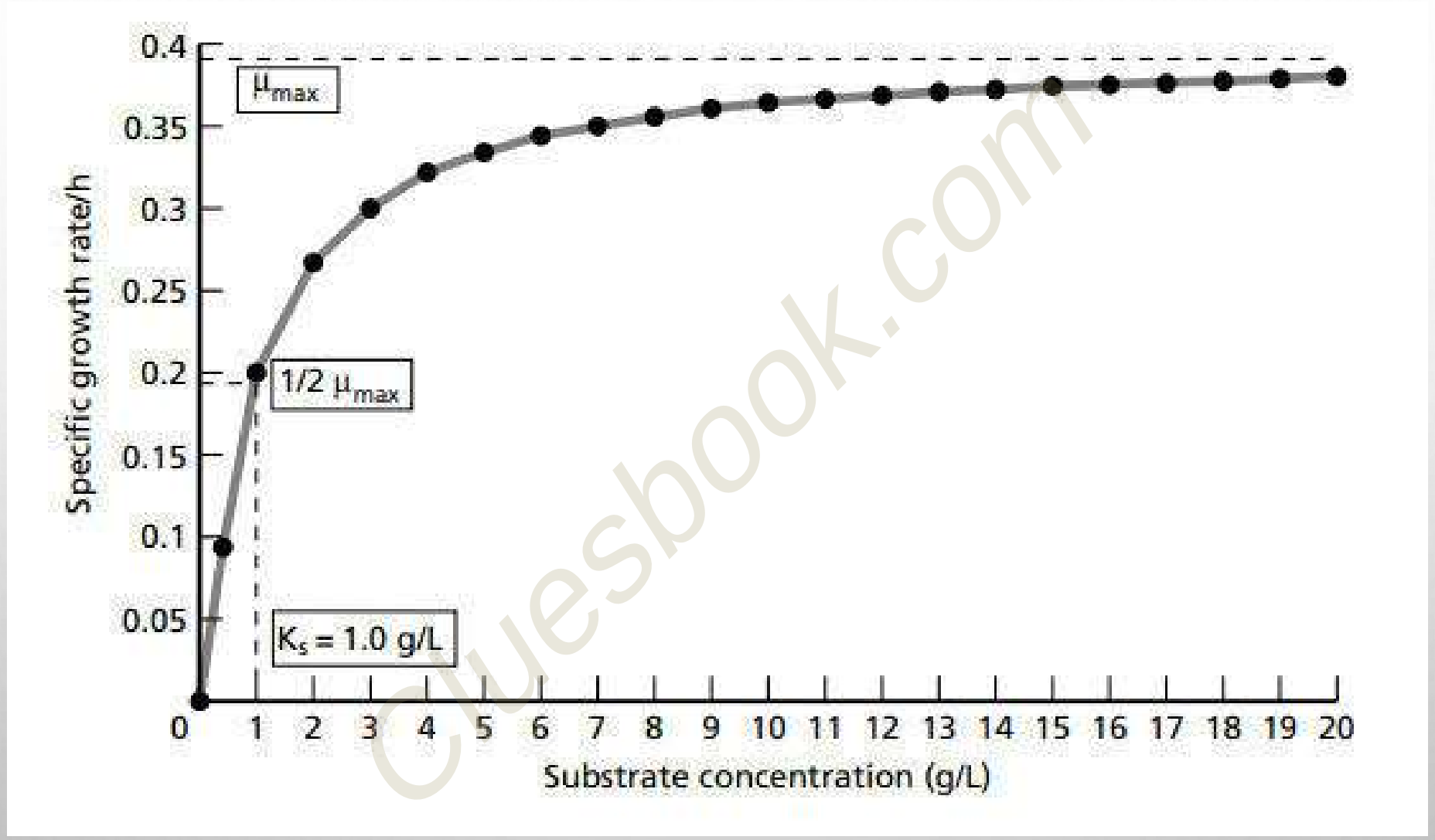
Therefore, in this case

$$t_d = \frac{0.693}{\mu} \qquad 2.14$$

BATCH GROWTH IV

BATCH GROWTH IV

- Monod showed that growth rate is an approximate hyperbolic function of the concentration of the growth-limiting nutrient(s)



BATCH GROWTH IV

- This impact of essential nutrient depletion on growth can be described mathematically by the monod equation, in a form similar to that used in biochemistry, where michaelis–menten kinetics define the rate of an enzyme-catalysed reaction in relation to its substrate concentration:

$$\mu = \frac{\mu_{max} S}{K_s + S} \qquad 2.15$$

BATCH GROWTH IV

- The specific growth rate of the microorganism continues decelerating until all of the available limiting substrate is metabolized.
- Growth is no longer sustainable and the cells enter the stationary phase.
- At this point, the overall growth rate has declined to zero and there is no net change in cell numbers/biomass (rate of cell division equals rate of cell death).

BATCH GROWTH IV

- The duration of the stationary phase varies depending on the microorganisms involved and the environmental conditions.
- For cells unable to survive by forming spores, this is followed by an exponential death phase when the cells die at a constant rate and often undergo lysis.

APPLICATION OF BATCH FERMENTATIONS

APPLICATION OF BATCH FERMENTATIONS

- During batch fermentations certain environmental conditions continually change, particularly nutrient and product concentrations, as does the specific growth rate, because the cells must pass through the sequence of growth phases.
- Consequently, the system never achieves steady-state conditions.

APPLICATION OF BATCH FERMENTATIONS

- A further disadvantage is that several distinct practical stages are associated with the operation of a batch fermentation:
- 1 charging of the fermenter with fresh medium
- 2 sterilization of the fermenter and medium
- 3 inoculation of the fermenter
- 4 production of microbial products

APPLICATION OF BATCH FERMENTATIONS

- 5 harvesting of biomass and spent fermentation broth
- 6 cleaning of the vessel.

OPTIMIZATION OF BATCH FERMENTATIONS

OPTIMIZATION OF BATCH FERMENTATIONS

- This has major economic implications for industrial processes.
- For a considerable period of time, the fermentation vessel is not producing microbial products, but is being cleaned, filled, sterilized, etc.
- The non-productive period is referred to as the down-time of the fermenter.

OPTIMIZATION OF BATCH FERMENTATIONS

- In the case of biomass production, the yield coefficient relates to the quantity of biomass produced per gram of substrate utilized. Therefore, the higher the yield coefficient, the greater the percentage of the original substrate converted into microbial biomass.
- Determination of yield coefficients is vitally important because the cost of the fermentation medium, particularly the carbon source, can be a significant proportion of the overall production cost.

OPTIMIZATION OF BATCH FERMENTATIONS

- By performing a range of experiments under different operating conditions, varying medium constituents and component concentrations, ph, temperature, etc., Optimum growth/production conditions can be established. It is also important to determine the maximum specific growth rate (mmax) of the production organism.

CONTINUOUS GROWTH KINETICS

CONTINUOUS GROWTH KINETICS

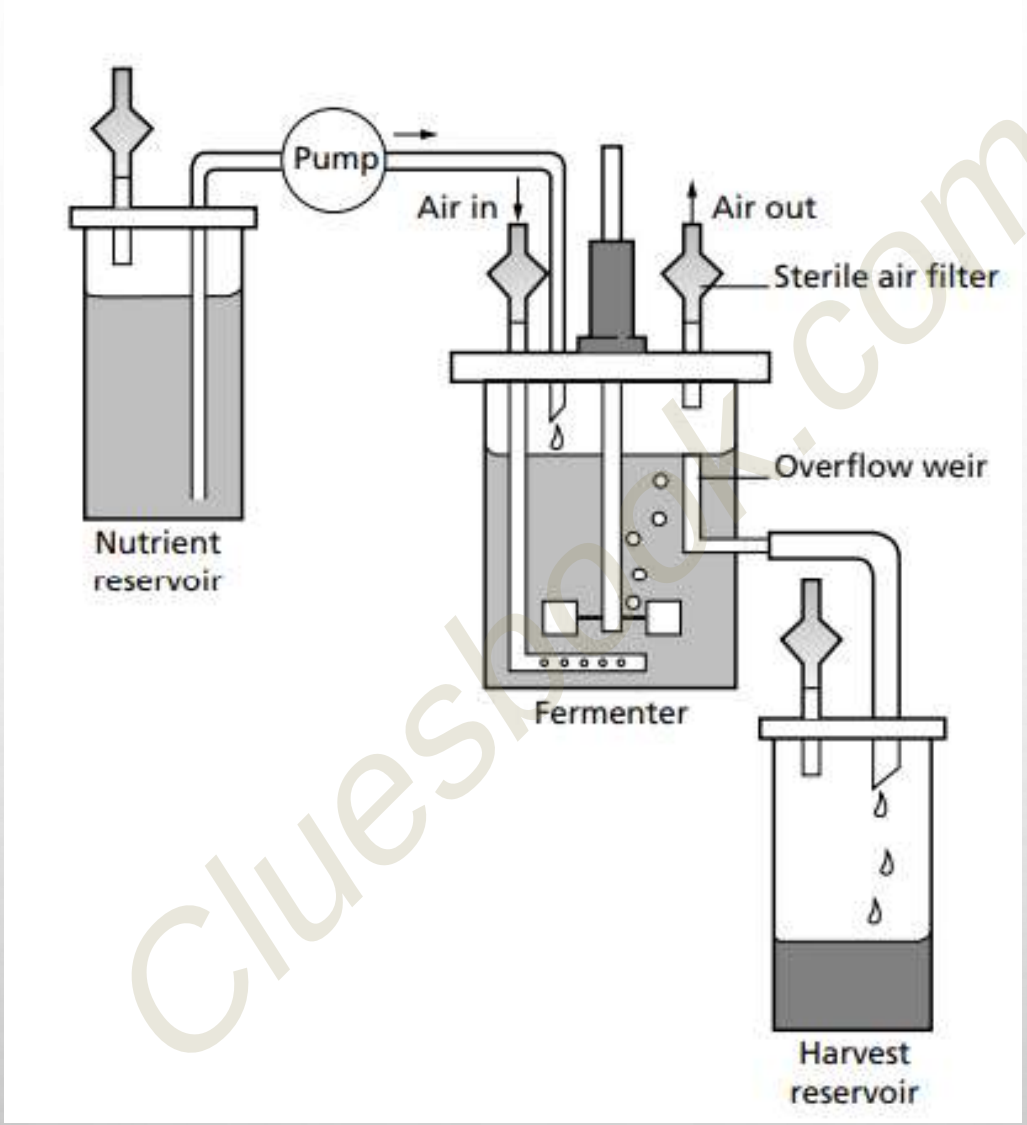
- Continuous culture fermentations have many applications for both laboratory research and industrial-scale processes. Studies can be performed on all aspects of cell growth, physiology and biochemistry.
- They are useful for ecological studies and as a genetics tool for the examination of mutation rates, mutagenic effects, etc.
- Application in industrial fermentations overcomes many limitations of batch processes.

CONTINUOUS GROWTH KINETICS

- Initially, continuous fermentations start as batch cultures, but exponential growth can then be extended indefinitely, in theory, through the continuous addition of fresh fermentation medium.
- The reactor is continuously stirred and a constant volume is maintained by incorporating an overflow weir or other levelling device
- Fresh medium is continuously added and displaces an equal volume of spent fermentation broth and cells at the same rate as fresh medium is introduced.

CONTINUOUS GROWTH KINETICS

- Steadystate conditions prevail, where the rate of microbial cell growth equals the rate at which the cells are displaced from the vessel.



CONTINUOUS GROWTH KINETICS

- As with batch fermentations, the specific rate at which the microorganism grows in continuous culture is controlled by the availability of the rate-limiting nutrient.
- Therefore, the rate of addition of fresh medium controls the rate at which the microorganisms grow.
- However, the actual rate of growth depends not only on the volumetric flow rate of the medium into the reactor, but also on the dilution rate (d). This equals the number of reactor volumes passing through the reactor per unit time and is expressed in units of reciprocal time, per hour.

CONTINUOUS GROWTH KINETICS II

CONTINUOUS GROWTH KINETICS II

$$D = \frac{F}{V} \qquad 2.19$$

CONTINUOUS GROWTH KINETICS II

- Where D = dilution rate (per hour), F = flow (L/h) and V = reactor volume (L).
- The term D is the reciprocal of the mean residence time or hydraulic retention time, as used in waste-water treatment.
- Addition of fresh medium into the reactor can be controlled at a fixed value, therefore the rate of addition of the rate-limiting nutrient is constant.
- Within certain limits, the growth rate and the rate of loss of cells from the fermenter will be determined by the rate of medium input.
- Therefore, under steadystate conditions the net biomass balance can be described as

$$\frac{dx}{dt} = \begin{matrix}\text{rate of growth} \\ \text{in reactor}\end{matrix} - \begin{matrix}\text{rate of loss from} \\ \text{reactor (wash-out)}\end{matrix} \qquad 2.20$$

or

$$\frac{dx}{dt} = \mu x - Dx \qquad 2.21$$

Under steady-state conditions the rate of growth = rate of loss, hence dx/dt=0 and therefore

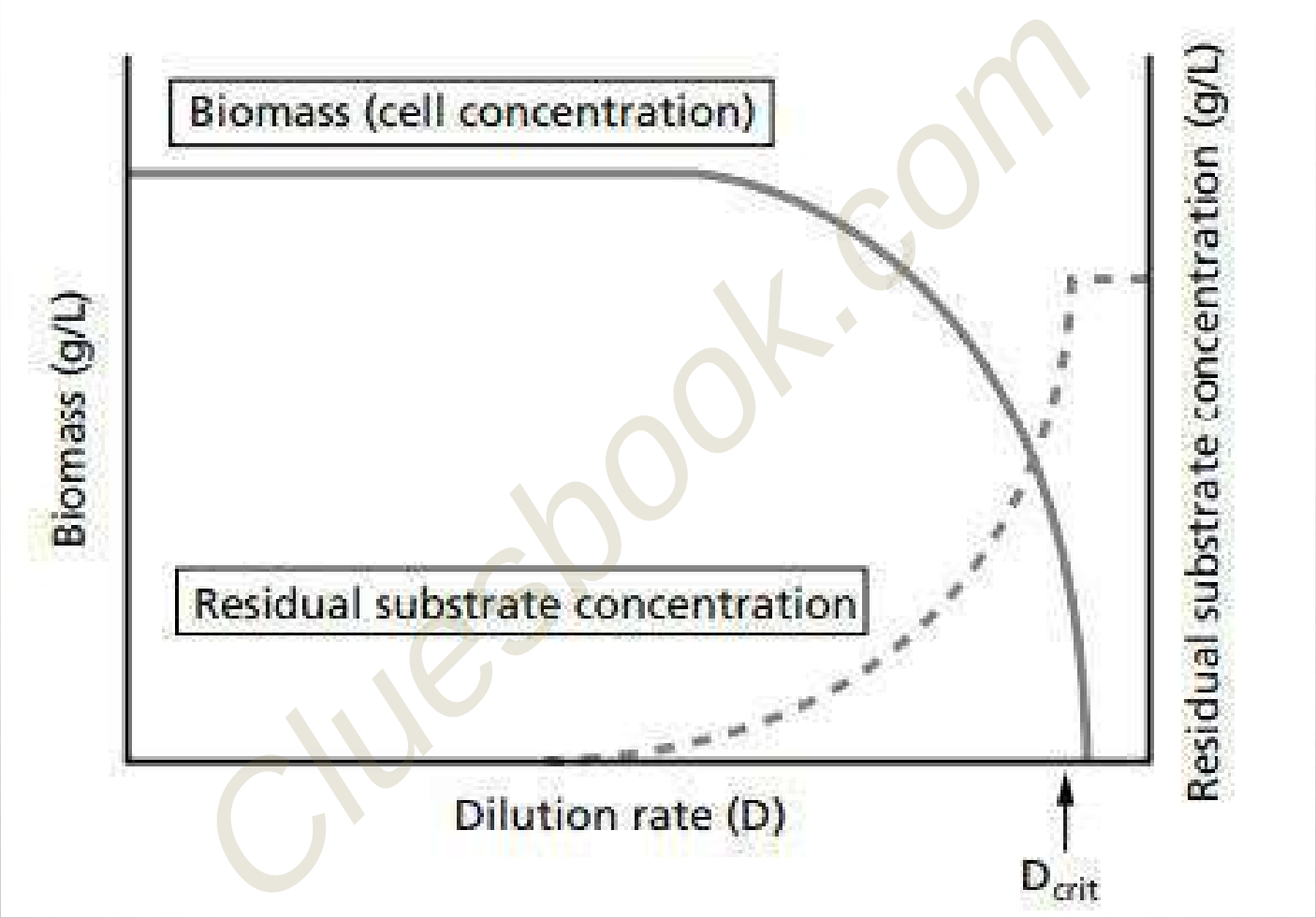
$$\mu x = Dx \qquad 2.22$$

and

$$\mu = D \qquad 2.23$$

CONTINUOUS GROWTH KINETICS II

- Consequently, at fixed flow rates and dilution rates under constant physical and chemical operating conditions, i.E. Under steady-state conditions, the specific growth rate of the microorganism is dependent on the operating dilution rate, up to a maximum value equal to m-max.



CONTINUOUS GROWTH KINETICS III

CONTINUOUS GROWTH KINETICS III

- If the dilution rate is increased above mmax, complete wash-out of the cells occurs, as the cells have insufficient time to ‘double’ before being washed out of the reactor via the overflow.
- The point at which this is just avoided is referred to as the critical dilution rate (dcrit).
- For any given dilution rate, under steady-state conditions, the residual substrate concentration in the reactor can be predicted by substituting d for m in the monod equation

$$D = \frac{\mu_{max} S_r}{K_s + S_r} \qquad 2.24$$

where S_r is the steady-state residual substrate concentration in the reactor at a fixed dilution rate. Rearrangment gives

$$D(K_s + S_r) = \mu_{max} S_r \quad \text{or} \quad DK_s + DS_r = \mu_{max} S_r$$

dividing by S_r then gives

$$\frac{DK_s}{S_r} + D = \mu_{max}$$

$$S_r = \frac{DK_s}{\mu_{max} - D} \qquad 2.25$$

CONTINUOUS GROWTH KINETICS III

- Consequently, the residual substrate concentration in the reactor is controlled by the dilution rate.
- Any alteration to this dilution rate results in a change in the growth rate of the cells that will be dependent on substrate availability at the new dilution rate

CONTINUOUS GROWTH KINETICS III

- Thus, growth is controlled by the availability of a rate-limiting nutrient.
- This system, where the concentration of the rate-limiting nutrient entering the system is fixed, is often described as a chemostat, as opposed to operation as a turbidostat, where nutrients in the medium are not limiting.
- In this case turbidity or absorbance of the culture is monitored and maintained at a constant value by regulating the dilution rate, i.E. Cell concentration is held constant

CONTINUOUS GROWTH KINETICS IV

CONTINUOUS GROWTH KINETICS IV

- At low dilution rates with fixed substrate concentrations, the residual substrate concentration will be low
- However, as D approaches mmax the residual substrate concentration increases along with the growth rate of the microorganism.
- Beyond dcrit, input substrate concentration will equal output concentration, as all the cells have been lost from the system.
- Thus, this continuous reactor can be described as a self-regulating nutrient-limited chemostat.

CONTINUOUS GROWTH KINETICS IV

- The concentration of biomass or microbial metabolite in a continuous fermenter under steady-state conditions can be related to the yield coefficient, as described in the batch fermentation section.
- Inserting the equation for residual substrate (equation 2.25) into the biomass or metabolic product yield coefficient equation (equation 2.16) gives, in this case for steady-state biomass ( ),

CONTINUOUS GROWTH KINETICS IV

$$\bar{x} = Y_{x/S}\left(S_R - \frac{DK_s}{\mu_{max} - D}\right) \qquad 2.26$$

where S_R is the substrate concentration of inflowing medium or

$$\bar{x} = Y_{x/S}(S_R - S_r) \qquad 2.27$$

CONTINUOUS GROWTH KINETICS IV

- Therefore, the biomass concentration under steadystate conditions is controlled by the substrate feed concentration and the operating dilution rate.
- Under non-inhibitory conditions, where there is no substrate or product inhibition, the higher the feed concentration, the greater the biomass concentration and residual substrate concentration remains constant.

MONITORING MICROBIAL GROWTH IN CULTURE

MONITORING MICROBIAL GROWTH IN CULTURE

- During a fermentation, methods are required for the routine determination of the microbial population, cell number and/or biomass, in order to monitor its progress.
- Numerous direct and indirect methods are available for this purpose. Direct procedures involve dry weight determination, cell counting by microscopy and plate counting methods.

MONITORING MICROBIAL GROWTH IN CULTURE

- Indirect methods include turbidimetry, spectrophotometry, estimation of cell components (protein, dna, rna or atp), and online monitoring of carbon dioxide production or oxygen utilization.
- The method adopted in any given situation depends upon the fermentation and any specific requirements. Several factors must be considered, such as the degree of accuracy and sensitivity needed, and the duration of the analysis.

MONITORING MICROBIAL GROWTH IN CULTURE

- Estimation of unicellular organisms, provided that they are not prone to flocculation, is relatively straightforward, but filamentous organisms, fungi and actinomycetes present additional problems.
- Also, culture media vary in viscosity, colour and the quantity of particulate solids, all of which may influence the choice of monitoring method.

MONITORING MICROBIAL GROWTH IN CULTURE

- The speed of analysis may be critical, as an instant indication of biomass or cell concentration is often required.
- However, faster methods are frequently less reliable, and longer procedures are generally more accurate and reproducible.

ELECTRONIC CELL COUNTERS

ELECTRONIC CELL COUNTERS

- Electronic cell counting equipment is also rapid, but most methods are more suitable for larger unicellular microbes, such as yeasts, protozoa and some algae, and less useful for estimating bacteria.

ELECTRONIC CELL COUNTERS

- The coulter counter, for example, is used to count and size particles, and is based on the measurement of changes in electrical resistance produced by non-conductive particles suspended in an electrolyte.
- This method involves drawing a suspension of cells through a small aperture across which an electrical current is maintained.

ELECTRONIC CELL COUNTERS

- As a cell passes through the aperture it displaces its own volume of electrolyte and changes the electrical resistance. These changes are detected and converted to a countable pulse.
- However, it essentially counts particles, and is consequently prone to errors due to cells clumping and the presence of particulate debris.

PLATE COUNTING TECHNIQUES

PLATE COUNTING TECHNIQUES

- Plate counting methods detect viable cells, i.E. Those able to form colonies on an appropriate solid nutrient medium.
- The two methods routinely used are:

i. Spread plating

ii. Pour plating

PLATE COUNTING TECHNIQUES

- In spread plating, samples, usually 0.1ml, are spread on the surface of a suitable agar-based nutrient medium using a sterile spreading device, e.G.
- A bent glass rod. The plates are then inverted and incubated at the optimum growth temperature.
- All resultant colonies should be well separated and easily counted.
- This also enables the isolation of pure cultures where required.

PLATE COUNTING TECHNIQUES

- Pour plates result in the development of microbial colonies throughout the agar. Those organisms with lower oxygen tolerance grow within the agar.
- Colonies of aerobic organisms often have variable sizes, as those nearer the surface have a better oxygen supply.
- Consequently, it is often harder to see similarity in colonial morphology between colonies on the surface and those within the agar.

PLATE COUNTING TECHNIQUES

- Care has to be taken to ensure that the molten agar is not too hot, otherwise some microbial cells may be injured and slow to form visible colonies or even killed.
- For statistical reliability, results are recorded only for plates containing 30–300 colonies.
- Calculation of the cell concentration in the original sample is then carried out, taking into account the dilution and volume plated.
- Both methods measure colony-forming units (cfu).

TURBIDIMETRIC AND SPECTROPHOTOMETRIC TECHNIQUES

TURBIDIMETRIC AND SPECTROPHOTOMETRIC TECHNIQUES

- These methods provide a simple, rapid and convenient means of total biomass estimation.
- They are usually performed at a specific optimum wavelength for each microorganism.
- Turbidimetric methods measure the light scattered by a suspension of cells, which is proportional to the cell concentration.

TURBIDIMETRIC AND SPECTROPHOTOMETRIC TECHNIQUES

- Alternatively, spectroscopy may be employed, using absorbance or transmittance of a cell suspension.
- Some modern fermentation monitoring systems now employ methods based on near-infrared spectroscopy.

TURBIDIMETRIC AND SPECTROPHOTOMETRIC TECHNIQUES

- Turbidimetric and spectrophotometric methods require the construction of appropriate calibration curves, prepared using standard cell suspensions containing known concentrations of cells.
- Also, care must be taken when interpreting the results if the fermentation broth contains particulate matter or is highly coloured.

DRY WEIGHT ESTIMATION

DRY WEIGHT ESTIMATION

- This method determines the weight of total cells, both living and dead, in liquid culture samples.
- It involves separating the biomass from a known volume of a homogeneous cell suspension.
- This is usually achieved by filtration under vacuum, through a preweighed membrane filter with a pore size of 0.2mm or 0.45mm.

DRY WEIGHT ESTIMATION

- The filter with collected cells is ‘washed’ with water to remove any residual growth medium and dried to a constant weight in an oven at 105∞C.
- Results are normally expressed as milligrams of cells per millilitre of culture.

DRY WEIGHT ESTIMATION

- Obviously, any other suspended non-cellular materials above the size of the filter pores is also collected and can lead to errors.
- Further limitations are the time needed to obtain the results and the relatively large volume of sample required to obtain sufficient biomass for accurate weighing, as an individual bacterium weighs only about 10–12 g.

ATP BIOLUMINOMETRY

ATP BIOLUMINOMETRY

- The amount of ATP in a sample can be quantified using ATP bioluminometry.
- This technique utilizes an enzyme–substrate complex, luciferase– luciferin, obtained from the firefly, photinus pyralis, which generates a photon of light for each molecule of ATP.

ATP BIOLUMINOMETRY

- When an aliquot of luciferase–luciferin is added to ATP extracted from a sample of cell suspension, the light generated can be detected in a bioluminometer.
- The resulting signal is amplified and then expressed as a digital or analogue data output.

ATP BIOLUMINOMETRY

$$ATP + O_2 + \text{luciferin} \xrightarrow{\textit{luciferase}} \text{oxyluciferin} + AMP + CO_2 + PP_i + \text{photon of light (562 nm)}$$

This procedure is very rapid and sensitive. Under optimum conditions as little as 10 femtomoles (1 femtomole = 10^{-15} mol) of ATP can be detected, which is approximately equivalent to 1 yeast cell or about 10 bacteria.

ATP BIOLUMINOMETRY

- ATP bioluminometry is most suitable for direct measurement of samples that are not coloured, as quenching of light may be a problem.
- However, as the method is very sensitive, samples may require considerable dilution, which often overcomes any colour quenching problem

ON-LINE ESTIMATION

ON-LINE ESTIMATION

- On-line monitoring of fermenters can provide real-time estimation of biomass, and minimizes the requirement for repeated sampling and off-line analysis.
- Monitoring systems may involve optical density or capacitancebased probes.
- The microorganisms involved in most fermentation processes will either have a requirement for oxygen and/or will produce carbon dioxide.

ON-LINE ESTIMATION

- In such cases it is theoretically possible to establish a mathematical relationship between factors, such as:
- Carbon dioxide evolution
- Oxygen utilization
- The biomass concentration within the bioreactor.

ON-LINE ESTIMATION

- Estimation of biomass concentrations and even product formation can be made by measuring these parameters on-line using carbon dioxide or oxygen detectors and biosensors attached to a computer.
- This can give an accurate estimate of the biomass concentration, provided that the mathematical algorithms developed are reliable.

EFFECTS OF ENVIRONMENTAL CONDITIONS ON MICROBIAL GROWTH

EFFECTS OF ENVIRONMENTAL CONDITIONS ON MICROBIAL GROWTH

- Growth and development of microorganisms are greatly affected by the chemical and physical conditions of their environment.
- Nevertheless, microorganisms have evolved to occupy niches throughout the range of environments on earth, some of which are very hostile.
- Microorganisms from some of these extreme environments have useful properties that can be exploited.

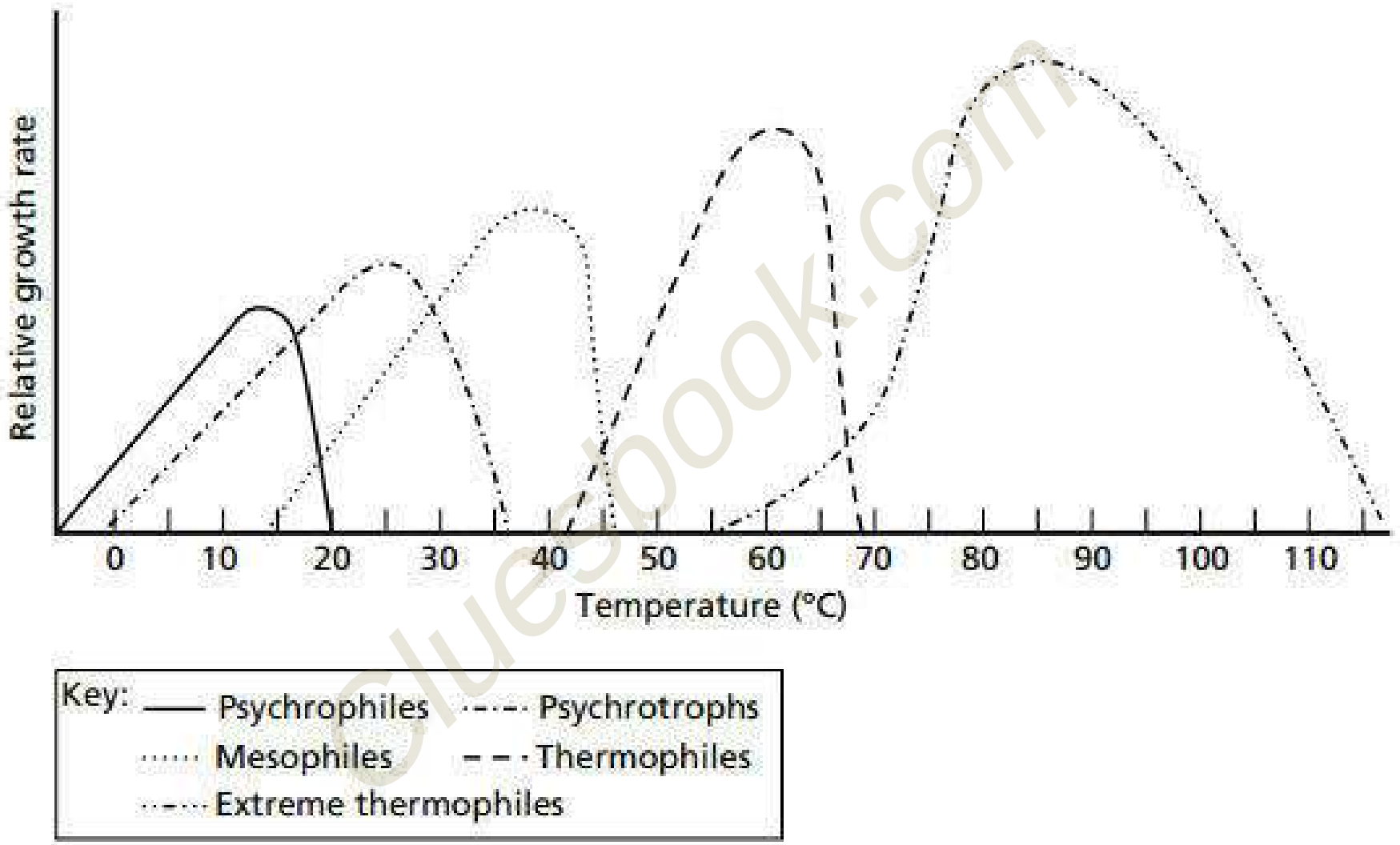
EFFECTS OF TEMPERATURE ON GROWTH

EFFECTS OF TEMPERATURE ON GROWTH

- All organisms have an optimum temperature for growth.
- A typical microorganism, referred to as stenothermal, can grow over a temperature range of approximately 30∞c,
- Eurythermal organisms grow over even wider ranges.
- As for temperature optima, mesophiles have optimum growth temperatures in the range 20–45∞c and a minimum around 15–20∞c
-

EFFECTS OF TEMPERATURE ON GROWTH

- Psychrophiles usually have their optimum temperatures below 15∞C and these organisms are often killed by exposure to room temperature.
- They are able to function at low temperature because their membranes contain a high proportion of unsaturated fatty acids.



EFFECTS OF TEMPERATURE ON GROWTH

- Organisms with optima above 50∞C are termed thermophiles.
- Several algal, protozoal and fungal species have temperature maxima up to 55∞c.
- However, it is only certain prokaryotes that are truly thermophilic, as no eukaryotic microbes grow at such high temperatures.
- Some extreme (hyper-) thermophiles can grow at 100∞c or higher. For example, pyrolobus fumarii has an optimum of 106∞C and continues growing up to 113∞C.

EFFECTS OF TEMPERATURE ON GROWTH

- All of these are archaeans, which are mainly anaerobic sulphate reducers or have other metabolism with lower requirements for thermolabile cofactors such as NADH and NADPH.

Table 2.3 Examples of thermophilic prokaryotes and their temperature optima

	Optimum temperature (°C)
Archaeans	
Methanobacterium thermoautotrophicum (methanogen)	65
Methanococcus jannaschii (methanogen)	85
Pyrococcus furiosis (accumulates H_2)	100
Pyrolobus fumarii (obligate H_2 chemolithotroph)	106
Sulfolobus acidocaldarius (acidophilic sulphur oxidizer)	75
Eubacteria	
Bacillus stearothermophilus	60–65
Clostridium thermocellum (cellulolytic anaerobe)	60
Synechococcus lividans (cyanobacterium)	67
Thermoanaerobacter ethanolicus (ethanol producer)	70

EFFECTS OF TEMPERATURE ON GROWTH

- Microbial spores are particularly heat resistant
- These dormant structures have thick spore coats and low water activity
- Bacterial endospores contain high levels of calcium dipicolinate, which may constitute up to 15% of their dry weight.
- Its role is thought to be in stabilizing nucleic acids and proteins

EFFECTS OF PH ON GROWTH

EFFECTS OF PH ON GROWTH

- As with temperature, every microorganism has a ph optimum and a ph range over which it grows.
- Generally, fungi tend to grow at lower phs (ph 4–6) than most bacteria.
- True acidophiles have ph optima between 1 and 5.5, and often have mechanisms for the exclusion of protons in order to maintain their internal ph at a higher level.

EFFECTS OF PH ON GROWTH

- The majority of microorganisms are neutrophiles, growing between ph 5 and 9, as most natural environments fall within this range.
- Alkalophiles, such as species of bacillus and micrococcus, have ph optima between 8.5 and 11.5, but take up protons to maintain their internal ph at a lower value

EFFECTS OF SOLUTES AND WATER ACTIVITY ON GROWTH

EFFECTS OF SOLUTES AND WATER ACTIVITY ON GROWTH

- Most microorganisms contain approximately 70–80% water and require a certain amount of free water for the performance of specific metabolic activities.
- In dry and hypertonic environments water uptake and retention is problematical.
- Hypertonic environments may contain considerable amounts of water, but it is not necessarily available.
- In fact, the high external levels of solutes, such as salt or sugars, causes water to pass out of most cells and growth is halted.

EFFECTS OF SOLUTES AND WATER ACTIVITY ON GROWTH

- Actual water availability to a microorganism is indicated by the term water activity (aw).
- This is the ratio of the vapour pressure of water in the solution surrounding the microorganism, psoln, to the vapour pressure of pure water:

EFFECTS OF SOLUTES AND WATER ACTIVITY ON GROWTH

$$A_w = \frac{P_{soln}}{P_{water}}$$

i.e. pure water has an A_w of 1.

EFFECTS OF SOLUTES AND WATER ACTIVITY ON GROWTH

- Microbial species vary in their tolerance to dry conditions and environments of high osmotic strength.
- Most bacteria, with a few exceptions, cannot grow below an aw of 0.9
- Many fungi that cause biodeterioration of stored grain (aw = 0.7), including aspergillus restrictus, are xerotolerant and can grow at low moisture levels.
- Truly xerophilic filamentous fungi and osmophilic yeasts, e.G. Zygosaccharomyces rouxii, have evolved to inhabit environments where the aw can be as low as 0.6.

EFFECTS OF SOLUTES AND WATER ACTIVITY ON GROWTH

- Osmophilic, xerophilic and halophilic organisms accumulate compensating solutes, which balance the osmotic strength of the external solute.
- Examples include polyols; arabitol is accumulated by several yeasts and filamentous fungi, and high levels of glycerol are found in the halophilic alga dunaliella salina.

EFFECTS OF OXYGEN ON GROWTH

EFFECTS OF OXYGEN ON GROWTH

- Microorganisms are classified into five main groups on the basis of their requirements for oxygen.
- **1 obligate aerobes** grow only in the presence of oxygen, as it is required as the terminal electron acceptor for electron transport in aerobic respiration
- **2 facultative anaerobes** function with or without oxygen, but grow more efficiently when oxygen is available.

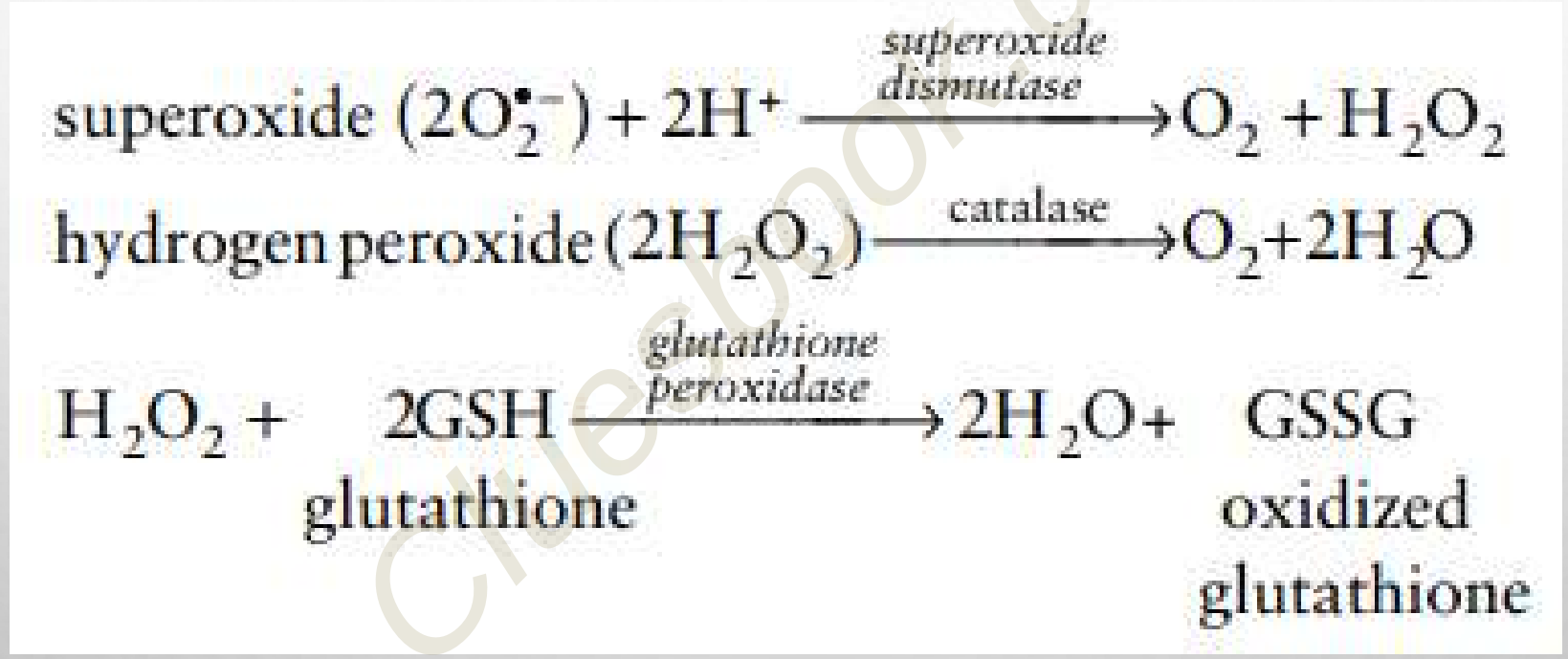
EFFECTS OF OXYGEN ON GROWTH

- **3 microaerophiles** require some oxygen for the biosynthesis of certain compounds, but cannot grow at normal atmospheric oxygen concentrations of 21% (v/v). They must have lower oxygen levels of 2– 10% (v/v).
- **4 aerotolerant anaerobes** essentially ignore oxygen and grow equally well in its presence or absence.
- **5 obligate anaerobes** cannot tolerate oxygen; exposure to it results in their death.

EFFECTS OF OXYGEN ON GROWTH

- On exposure to oxygen, most organisms interact with it to produce highly reactive toxic products.
- These reduction products of atmospheric oxygen include superoxide (O2 •–), hydrogen peroxide (H2O2) and hydroxyl radicals (OH•). unless detoxified, these products react destructively with any organic molecules that they encounter, including lipids, proteins and nucleic acids.
- Three groups of enzymes catalyse free radicals to help to render them harmless: superoxide dismutase, catalase and peroxidases:

EFFECTS OF OXYGEN ON GROWTH



EFFECTS OF OXYGEN ON GROWTH

- In order to grow obligate anaerobes in the laboratory, procedures to exclude oxygen from the culture must be used and all manipulations must be performed in an anaerobic atmosphere.
- Suitable procedures include:
- 1 the use of media containing reducing agents, such as thioglycollate, which chemically combine with oxygen
- 2 physical removal of oxygen from a growth chamber, by pumping out the air with a vacuum pump, and then flushing the vessel with nitrogen gas ± carbon dioxide

EFFECTS OF RADIATION ON GROWTH

EFFECTS OF RADIATION ON GROWTH

- Visible and ultraviolet (UV) light are parts of the electromagnetic spectrum, which extends from strong radiation (g-rays) to very weak radiation (heat and radio waves).
- Even visible light, particularly the more energetic violet and blue regions, can be damaging.
- It may generate singlet oxygen, a very powerful oxidizing agent, which can cause damage to cellular components and may kill some microorganisms.

EFFECTS OF RADIATION ON GROWTH

- UV light, which is most harmful at 260nm, causes specific damage to DNA, notably the formation of thymine dimers.
- Ionizing radiation, such as g-rays emitted from the excited nucleus of 60co, generates free radicals, OH• and H• , and hydrated electrons.
- Resulting damage includes breakage of hydrogen bonds in functional molecules, oxidation of many chemical groups and breakage of DNA strands.

EFFECTS OF RADIATION ON GROWTH

- Many air-borne bacteria are afforded some protection by pigments that absorb radiation, and in other microorganisms resistance involves effective repair mechanisms, especially for damaged DNA.

EFFECTS OF RADIATION ON GROWTH

- Some microorganisms, including E. Coli, are also less susceptible to radiation damage in anaerobic environments than when respiring aerobically, and may be partially protected by reducing agents and sulphydryl compounds.

EFFECTS OF HYDROSTATIC PRESSURE ON GROWTH

EFFECTS OF HYDROSTATIC PRESSURE ON GROWTH

- In nature, many microorganisms are never subjected to pressures in excess of 1atm (101.3kpa or 1.013bar) and higher pressures generally inhibit microbial growth.
- For example, in cider production, yeast performance is impaired where fermenter depths are greater than 14.5m, which produce hydrostatic pressures above 1.5atm.

EFFECTS OF HYDROSTATIC PRESSURE ON GROWTH

- It is difficult to determine which process or function is most damaged and the order of inhibition.
- Interference with both protein synthesis and energy transfer from catabolic to biosynthetic systems is often observed, as is the inability of microorganisms to adapt to new substrates.

EFFECTS OF HYDROSTATIC PRESSURE ON GROWTH

- Many marine organisms must be barotolerant, as they live at depths in oceans where they may be subjected to pressures of up to several hundred atmospheres, and only fail to grow at above 200–600atm.
- Some may even grow better under these conditions and are truly barophilic, capable of living under 700– 1000atm.

THE CONTROL (INHIBITION) OF MICROBIAL GROWTH

THE CONTROL (INHIBITION) OF MICROBIAL GROWTH

- The control or prevention of microbial growth is necessary in many practical situations, particularly in:
- Health care
- Food processing and preparation
- Preservation of materials

THE CONTROL (INHIBITION) OF MICROBIAL GROWTH

- Control may be achieved using physical or chemical agents that either kill microorganisms or inhibit their further growth.
- Agents which kill cells are called '-cidal' agents, whereas '-static' agents inhibit the growth of cells without killing them.
- Sterilization procedures completely destroy or eliminate all viable organisms, including spores, and may be performed using heat, radiation and chemicals, or by the physical removal of cells.

THE CONTROL (INHIBITION) OF MICROBIAL GROWTH

- The population decreases exponentially, by a constant fraction at constant intervals, and several factors influence the effectiveness of any antimicrobial treatment. These include:
- **1 population size**: the larger the microbial population, the longer the time required to kill all the microorganisms present
- **2 population composition**: different microorganisms vary in their sensitivity to a specific lethal agent, and its effectiveness may be influenced by their age, morphology and physiological condition

THE CONTROL (INHIBITION) OF MICROBIAL GROWTH

- **4 period of exposure to the lethal agent:** the longer the exposure, the greater the number of organisms killed
- **5 temperature**: normally a higher temperature increases the effectiveness of the agent

CONTROL OF MICROBIAL GROWTH BY PHYSICAL AGENTS

CONTROL OF MICROBIAL GROWTH BY PHYSICAL AGENTS

- Heat:
- Heat is the most important and widely used means of sterilization and may be achieved through:
- **1** incineration, where burning at 500∞C physically destroys the organisms and is particularly useful for some solid wastes
- **2** moist heat, which is suitable for sterilizing most items, except heat-labile substances that would be denatured or destroyed.

CONTROL OF MICROBIAL GROWTH BY PHYSICAL AGENTS

- It is carried out using steam under pressure to achieve 121∞C for 15min, and is extensively used in fermentation processes for the sterilization of vessels, connecting pipe work and culture media
- **3** dry heat is less efficient than moist heat, requiring higher temperatures and much longer exposure times. It is performed in hot air ovens at 160∞C for 2h, and can be used for glassware, metal objects and moisture-sensitive materials such as powders and oils.

Thermal death time (TDT) is the shortest time required to kill all microorganisms in a sample at a specific temperature and under defined conditions

Decimal reduction time (*D*-value) is the time required to kill 90% of the microorganisms in a sample at a specific temperature. This term is used extensively in the food industry

***Z*-value** is the rise in temperature required to reduce *D* to $^{1}/_{10}$ of its previous value

***F*-value** is the time in minutes at a specific temperature (usually 250°F or 121.1°C) necessary to kill a population of cells or spores

Del factor (∇) = ln (N_o/N_t), where N_o is the number of organisms at the start of sterilization and N_t is the number remaining after time *t*. Therefore, the Del factor is a measure of the fractional reduction in viable organism count produced by a certain heat and time regime. The larger the Del factor, the greater the sterilization regime required. This term is mostly used in the fermentation industry

CONTROL OF MICROBIAL GROWTH BY PHYSICAL AGENTS

- Heat sterilization is commonly employed in canning and bottling, and ultra-high temperature treatments (UHT) are used in some sterile packaging procedures. Pasteurization is a milder heat treatment, used to reduce the number of microorganisms in products or foods that are heat-sensitive and unable to withstand prolonged exposure to high temperatures.

CONTROL OF MICROBIAL GROWTH BY PHYSICAL AGENTS

- Low temperature
- These treatments involve refrigeration or freezing. Organisms are not usually killed, but the majority do not grow or grow very slowly at temperatures below 5∞C. Perishable foods are stored at low temperatures to slow the rate of microbial growth and consequent spoilage. Often, it is psychrotrophs, rather than true psychrophiles, that are the cause of food spoilage in refrigerated foods.

CONTROL OF MICROBIAL GROWTH BY PHYSICAL AGENTS

- LOW WATER ACTIVITY this is used extensively to preserve foods, especially fruits, grains and some meat products. Methods involve removal of water from the product by heating or freeze-drying; alternatively, water activity may be reduced by the addition of solutes, usually salt or sugar.

IRRADIATION

IRRADIATION

- Microwave, UV, X and g radiation can be used to destroy microorganisms.
- Uv radiation is effective, but its use is limited to surface sterilization because it does not penetrate glass, dirt films, water and other substances.
- Ionizing radiation treatment mostly involves x-rays or g-rays.
- This is particularly effective due to its ability to penetrate materials.

IRRADIATION

- Irradiation is used commercially to sterilize items such as petri dishes, and in some countries, spices, fruits and vegetables are irradiated to increase their storage life up to 500% by destroying spoilage microorganisms.
- In some foods the levels of vitamin c and e may be reduced by 5–10% and up to 25%, respectively
- Such treatments are not suitable for all products; for example, when beer and some shampoos are irradiated, hydrogen sulphide may be generated.

IRRADIATION

- Irradiation of food has not been accepted worldwide. Currently, about 40 countries have passed legislation allowing its use for specified materials.
- In the USA, use of these techniques has been extended to the treatment of beef, lamb and pork products following approval by the US food and drug administration (FDA).

HIGH-INTENSITY PULSED ELECTRIC FIELD TREATMENT

HIGH-INTENSITY PULSED ELECTRIC FIELD TREATMENT

- This is another non-thermal treatment and involves exposing the material to an electric field of 15–20kv/cm for just a few milliseconds or less.
- It is considered that highintensity pulsed electric field treatments could, in the future, have several applications, including food preservation.

HIGH-INTENSITY PULSED ELECTRIC FIELD TREATMENT

Sterile filtration

- Sterile filtration involves the physical removal of all cells from a liquid or gas. It is especially useful for sterilizing heat labile solutions of antibiotics and other drugs, amino acids, sugars, vitamins, animal cell culture media and some beverages.

HIGH-INTENSITY PULSED ELECTRIC FIELD TREATMENT

- There are three main types of sterile filter:
- **1 depth filters** are thick fibrous or granular filters that remove microorganisms by physical screening, entrapment and adsorption
- **2 membrane filters** are thin filters with defined pore sizes, usually of 0.2 or 0.45mm, through which the microorganisms cannot pass
- **3 high-efficiency** particulate air (HEPA) filters are used in laminar flow biological safety cabinets and containment rooms to sterilize the air circulating in the enclosure.

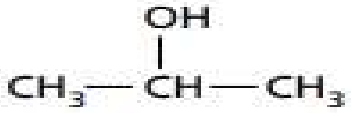
CONTROL OF MICROBIAL GROWTH BY CHEMICAL AGENTS

CONTROL OF MICROBIAL GROWTH BY CHEMICAL AGENTS

- Chemical antimicrobial agents can be used to kill microorganisms or inhibit their growth and may be of natural or synthetic origin. They include chemical preservatives, disinfectants, antiseptics, and drugs used in antimicrobial chemotherapy.
- Some phenolic compounds, for example, are used as disinfectants at high concentration, but at low concentrations they are suitable for use as antiseptics

Alcohols

Ethanol



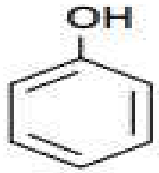
Isopropanol

Aldehydes

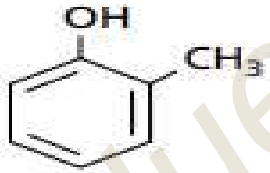
Formaldehyde

Glutaraldehyde

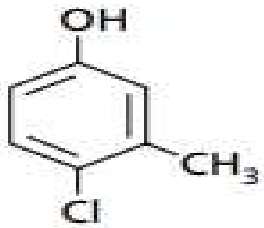
Phenolics



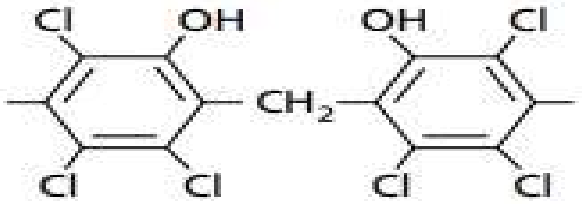
Phenol



Ortho-cresol



Chlorocresol



Hexachlorophene
(di-(3,5,6-trichloro-2-hydroxyphenyl) methane)

Quaternary ammonium compounds

CONTROL OF MICROBIAL GROWTH BY CHEMICAL AGENTS

- Disinfectants are agents that kill microorganisms, but not necessarily their spores.
- They are not safe enough for direct application to living tissues and are used on inanimate objects such as tables, floors, utensils, etc.
- Chlorine, hypochlorites, chlorine compounds and phenols are extensively used as disinfectants.

CONTROL OF MICROBIAL GROWTH BY CHEMICAL AGENTS

- Disinfectants and antiseptics are distinguished on the basis of whether they are safe for application to mucous membranes.
- Often, safety depends on the concentration of the compound.
- Some phenolic compounds, for example, are used as disinfectants at high concentration, but at low concentrations they are suitable for use as antiseptics

ANTIMICROBIAL PRESERVATIVES FOR FOOD AND RELATED PRODUCTS

ANTIMICROBIAL PRESERVATIVES FOR FOOD AND RELATED PRODUCTS

- Antimicrobial preservatives for food and related products:
  These compounds are mostly static agents used to inhibit the growth of microorganisms in food and those pharmaceutical or cosmetic products that may be ingested.

Preservative	Effective concentration	Uses
Acetic acid	0.4% (w/v)	Used in baked goods
Benzoic acid (E210), benzoates and parabens*	0.1% (w/v) 0.01–0.05% (w/v)*	Antimicrobial agents in margarine, cider, soft drinks, cosmetics, etc.
Lactic acid	0.3% (w/v)	Antimicrobial agent in cheeses, buttermilk, yoghurt and pickled foods
Nisin (E234)	50–500 IU/g effective against Gram (+) bacteria; particularly useful against spore formers	Used in processed cheese, other dairy produce and canned products at acid pH
Propionic acid (E280) and propionates	0.3% (w/v)	Antifungal agent in breads, cake, cheeses, dried fruits
Sodium nitrite (E250)	0.02% (w/v) more effective at pH 5.0–5.5	Antibacterial agent in cured meats and fish. Retards growth of *Clostridium botulinum*. Problems due to formation of nitrosamines on cooking
Sorbic acid (E200) and sorbates	0.2% (w/v)	Antifungal agent in cheeses, syrups, cakes, fruit juices, etc.
Sulphur dioxide (E220), sulphites	0.02–0.03% (w/v) active form is undissociated sulphurous acid	Antimicrobial agent in dried fruits, grapes, molasses and beverages. Inhibits fungi and bacteria. Used extensively in the past, now reductions in use due to induction of asthmatic attacks in sensitive individuals

ANTIMICROBIAL PRESERVATIVES FOR FOOD AND RELATED PRODUCTS

- Very few microbial products, apart from organic acids, are used as food preservatives.
- Normally, antibiotic agents are not acceptable in human food due to the potential development of resistant strains, and possible disturbance of the microbial ecology of the gut or allergic reactions.
- Only nisin, and to a lesser extent natamycin, currently have food applications (

ANTIMICROBIAL PRESERVATIVES FOR FOOD AND RELATED PRODUCTS

- In the case of the organic acids and the parabens, it is their undissociated form that exhibits the antimicrobial properties.
- Their pka values (the ph at which there is 50% dissociation of the molecules) lie in the range ph 3–5.
- As the ph falls the concentration of the undissociated form increases, thus elevating the antimicrobial activity.

ANTIMICROBIAL CHEMOTHERAPY

ANTIMICROBIAL CHEMOTHERAPY

- Antimicrobial chemotherapy involves the control or the destruction of disease-causing microorganisms once within the tissues of humans and other animals.
- Probably the most important property of a clinically useful antimicrobial agent is its selective toxicity, i.E. The agent acts to inhibit or kill the pathogenic microorganism, but has little or no toxic effect on the host.

ANTIMICROBIAL CHEMOTHERAPY

- Chemical antimicrobial agents are of synthetic origin, whereas antibiotics are often defined as ‘low molecular weight organic compounds, produced by microorganisms, that kill or inhibit other microorganisms’.
- However, a broader definition of an antibiotic includes natural chemical products from any type of cell that kill or inhibit the growth of other cells.
- Some antibiotics can now be completely chemically synthesized or natural antibiotics may be chemically modified to improve their properties.

ANTIMICROBIAL CHEMOTHERAPY

- Many synthetic chemotherapeutic agents are competitive inhibitors, often referred to as antimetabolites, and may be bacteriostatic or bactericidal.
- Most are ‘growth factor analogues’, which are structurally similar to a microbial growth factor but cannot fulfil its metabolic function.

ANTIMICROBIAL CHEMOTHERAPY

- The sulphonamides were the first compounds found to suppress bacterial infections selectively.
- They inhibit the synthesis of tetrahydrofolic acid (thf), which is essential for one-carbon transfer reactions.
- Folic acid also functions as a coenzyme for the synthesis of purine and pyrimidine bases of nucleic acids.

ANTIMICROBIAL CHEMOTHERAPY

- Quinolones, such as nalidixic acid, are synthetic chemotherapeutic agents that are bacteriocidal, killing mainly gram-negative bacteria.
- They bind to, and inhibit, the DNA gyrase (a topoisomerase).
- This enzyme is essential during DNA replication, as it enables DNA supercoils to be relaxed and re-formed.

ANTIBIOTICS

ANTIBIOTICS

- Antibiotics may have cidal or static effects on a range of microorganisms.
- They are classified according to their range of effectiveness and the general microbial group they act against, i.E. Antibacterial, antifungal or antiprotozoal.
- The range of bacteria or other microorganisms affected by a specific antibiotic is expressed as its spectrum of action.

ANTIBIOTICS

- Antibiotics effective against a wide range of gram-positive and gramnegative bacteria are said to be broad spectrum.
- If they are predominantly effective against gram-positive or gram-negative bacteria, they are narrow spectrum
- Limited spectrum antibiotics are effective against only a single organism or disease.

ANTIBIOTICS

- Antibiotics are low molecular weight compounds that are produced as secondary metabolites by mainly soil microorganisms.
- They are non-protein molecules, although there are some peptide antibiotics.
- Most producer microorganisms form a spore or other dormant cell.

ANTIBIOTICS

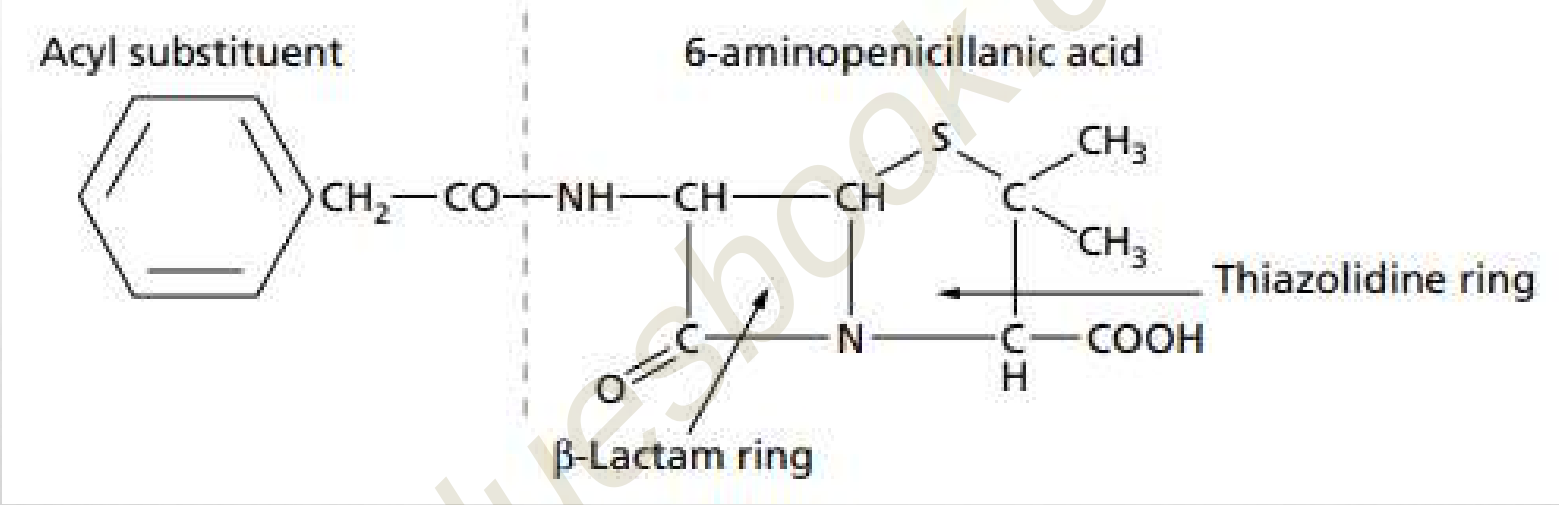
- Among the filamentous fungi, the notable antibiotic producers are penicillium and cephalosporium (acremonium), which are the main source of b-lactam antibiotics, e.G. Penicillin and related compounds.
- Within the bacteria, the filamentous actinomycetes, especially many streptomyces species, produce a variety of antibiotics, including aminoglycosides, macrolides and tetracyclines.

INHIBITION OF CELL WALL SYNTHESIS

INHIBITION OF CELL WALL SYNTHESIS

- Inhibitors of bacterial cell wall/envelope synthesis generally target some step in the formation of peptidoglycan.
- The b-lactam antibiotics, penicillins and cephalosporins, are the best known examples.
- Penicillin was the first antibiotic to be discovered and used therapeutically.
- Natural penicillins, such as penicillin g

INHIBITION OF CELL WALL SYNTHESIS



INHIBITION OF CELL WALL SYNTHESIS

- Penicillins inhibit peptidoglycan synthesis and have no effect on established cell walls.
- Consequently, they are bactericidal to actively growing cells, which through the action of penicillin become sensitive to osmotic stress.
- Cell wall peptidoglycan is made in three stages
- The third step involves carboxypeptidases and transpeptidases in the final cross-linking between peptide side chains, to form a rigid matrix.

INHIBITION OF CELL WALL SYNTHESIS

- Penicillins have a structural resemblance to the end dalanyl-d-alanine residues of the small peptides involved in the peptidoglycan cross-links.
- Thus, penicillins appear to act as substrate analogues.
- They covalently bind to transpeptidase via the b-lactam ring and prevent further functioning of the enzyme.
- As a result, a cell wall containing this loosely formed peptidoglycan is much weaker and the cell undergoes lysis.
- Also, penicillins may induce certain autolytic events.

INHIBITION OF CELL WALL SYNTHESIS

- Bacitracin, a peptide antibiotic produced by bacillus species, also prevents peptidoglycan synthesis
- It acts by inhibiting the release of peptidoglycan subunits from the lipid-carrier molecule that transports them across the cytoplasmic membrane.

CATABOLISM I

Metabolism consists of two processes catabolism and anabolism .

- **the break down of complex molecules into simpler ones with the release of energy is called catabolism.**

•All living cells require continuous supply of energy for growth, movement and maintenance.

•They get this energy from food and transform it by series of enzyme controlled reactions within specific metabolic pathways.

CATABOLISM I

- **This lead to the formation of potential energy in the form of ATP (adenosine 5'-triphosphate), NADH (nicotinamide adenine dinucleotide) and FADH2 (flavin adenin dinucleotide) and heat.**

- Various routes operated by different organisms for catabolism include the following.

➢ **EMBDEN-MEYERHOF-PARNAS (EMP) PATHWAY, is the** most common route and found in all major group of organisms including filamentous fungi, yeasts and many bacteria.

CATABOLISM I

- This pathway can operate under aerobic and anaerobic conditions.
- It consist of 10 enzyme catalysed reactions located within the cytoplasmic matrix.
- Three key regulatory enzymes (hexokinase, phosphofructokinase and pyruvate kinase) act irreversibly.

CATABOLISM I

- All other steps are freely reversible, which is important for the biosynthetic role of the pathway during glucose synthesis.
- Early stages of glucose breakdown consume 2 ATP moleculesin the 3 stage formation of fructose 1,6 biphosphate.

CATABOLISM I

- This ATP molecule then form two different trios (C3) phosphates, glyceraldehyde 3-phosphate (GAP) and dihydroxyacetone phosphate (DAP).
- DAP is directly processed in this pathway and DAP must be isomerized to GAP before it can be used.

CATABOLISM I

- Oxidation of the resultant two GAP molecules to pyruvate generates energy in the form of four ATP molecules .

Net gain is only two ATP, due to its consumption in the earlier reactions.

- GLUCOSE (C6) + 2ADP + 2PI + 2NAD+----------2 PYRUVATE (C3) + 2ATP + 2NADH + 2H+

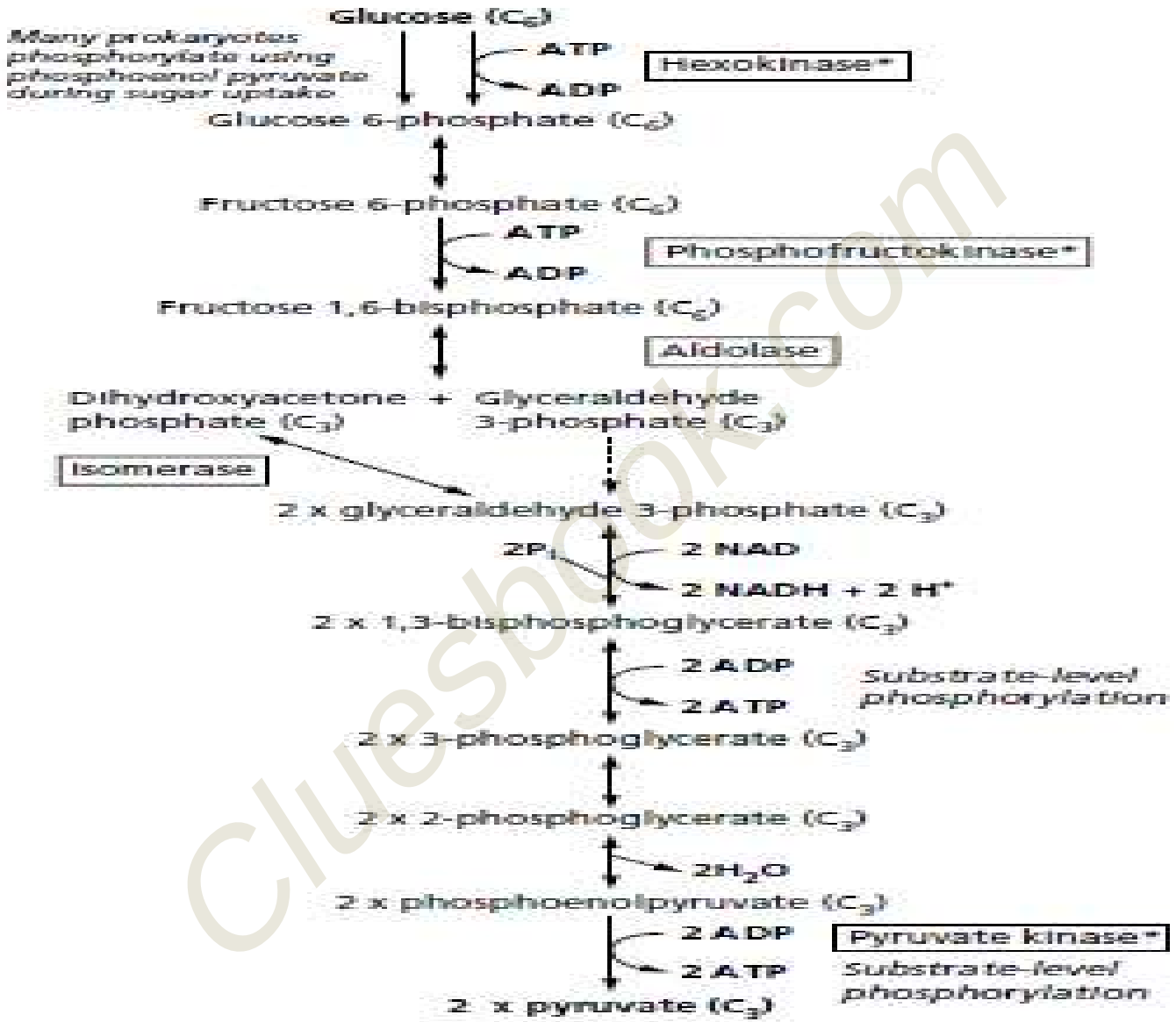


Fig. 3.1 Embden–Meyerhof–Parnas pathway (*irreversible steps).

CATABOLISM II

➢THE PENTOSE PHOSPHATE (PP) PATHWAY OR HEXOSE MONOPHOSPHATE PATHWAY:

- Is found in many bacteria and the majority of eukaryotic organisms this pathway is important in the provision of NADPH, mainly for use in reductive steps in anabolic processes; intermediates for aromatic amino acid synthesis, particularly erythrose-4-phosphate; pentoses,

CATABOLISM II

- Especially ribose for nucleic acid biosynthesis; and other biosynthetic intermediates.
- Pentose sugars such as xylose can also be catabolized via this route.
- This cycle begin with the two-step oxidation of glucose 6-phosphate (g6p) to the pentose (c5) phosphate, ribulose 5-phosphate (rump), via 6-phosphogluconate. This involves the loss of one carbon as CO2 and the formation of two NADPH.

CATABOLISM II

- Following this oxidative phase, rump undergoes rearrangement in a series of two-carbon and three-carbon fragment exchanges, catalysed by the key enzymes transketolase and transaldolase.
- For every three glucose units processed, one GAP, six NADPH and two fructose 6-phosphate (F6P) molecules are generated.

CATABOLISM II

- F6P molecules are converted back to G6P to maintain the operation of the cycle.
- The gap may be oxidized to pyruvate by emp pathway enzymes or it too may be returned to the start of the pathway via conversion of two gap to one g6p.
- 3 glucose 6-phosphate (c6) + 6nadp+ + 3h2o œ 2 fructose 6-phosphate (c6) + glyceraldehyde 3-phosphate (c3) + 3co2 + 6nadph + 6h+

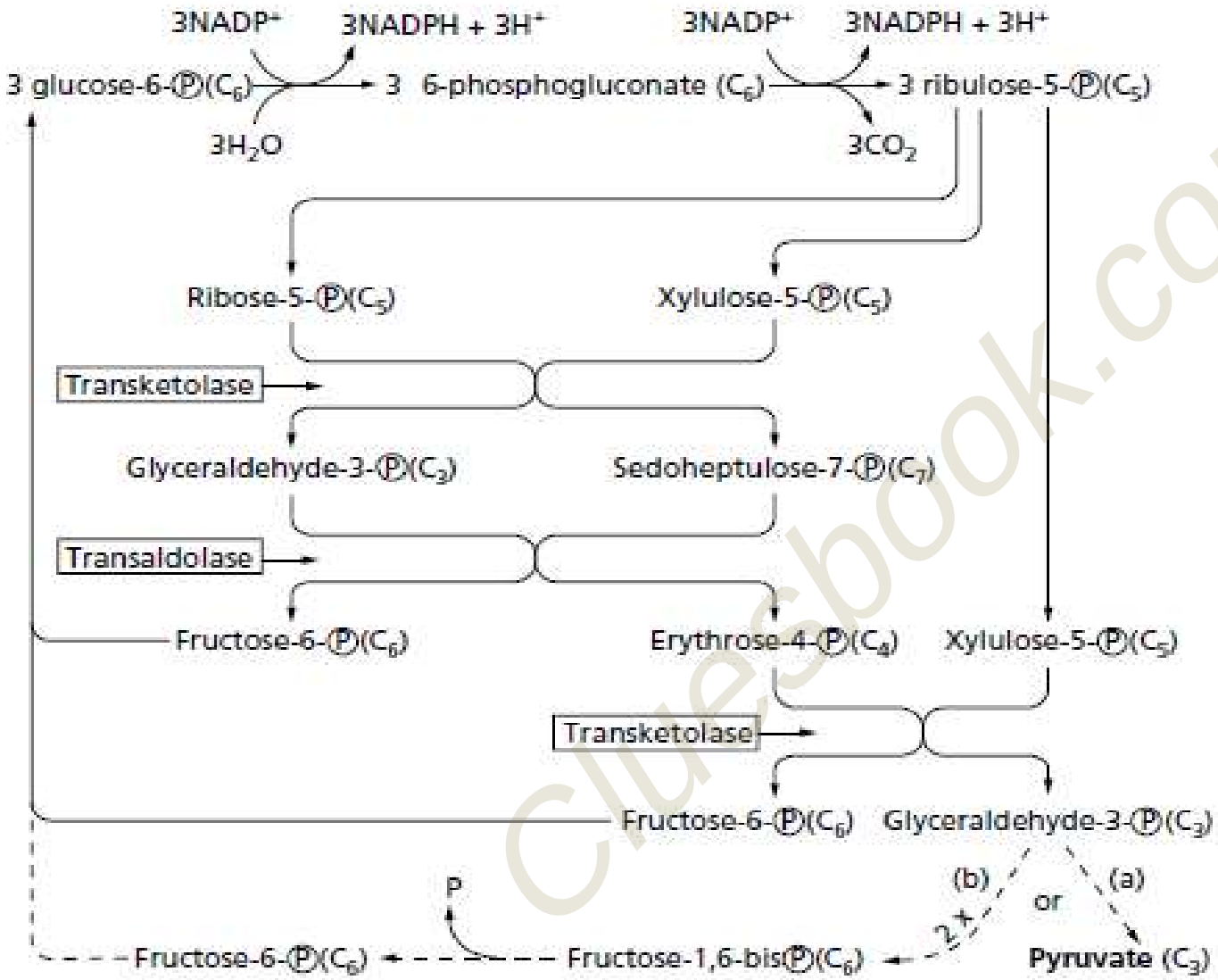


Fig. 3.2 The pentose phosphate pathway.

INDUSTRIAL BIOTECHNOLOGY

- CATABOLISM III

CATABOLISM III

➢ **THE ENTNER-DOUDOROFF (ED) PATHWAY**

- This method is used by a relatively few microorganisms that lack the EMP pathway.
- Most are gram-negative bacteria, including species of *azotobacter, pseudomonas, rhizobium, xanthomonas and zymomonas, but it is rare* in fungi.

CATABOLISM III

- The pathway begins with the formation of 6-phosphogluconate, as in the PP pathway.
- It is then dehydrated, rather than oxidized, to form 2-oxo- 3-deoxy-6-phosphogluconate.

CATABOLISM III

- This six-carbon molecule is cleaved by an aldolase to form two c3 compounds, pyruvate and gap, and the latter may also be converted to pyruvate.
- Overall, from each glucose molecule metabolized, the pathway can generate two pyruvate molecules, one atp, one nadh and one nadph, which is a lower energy yield than for the emp pathway.

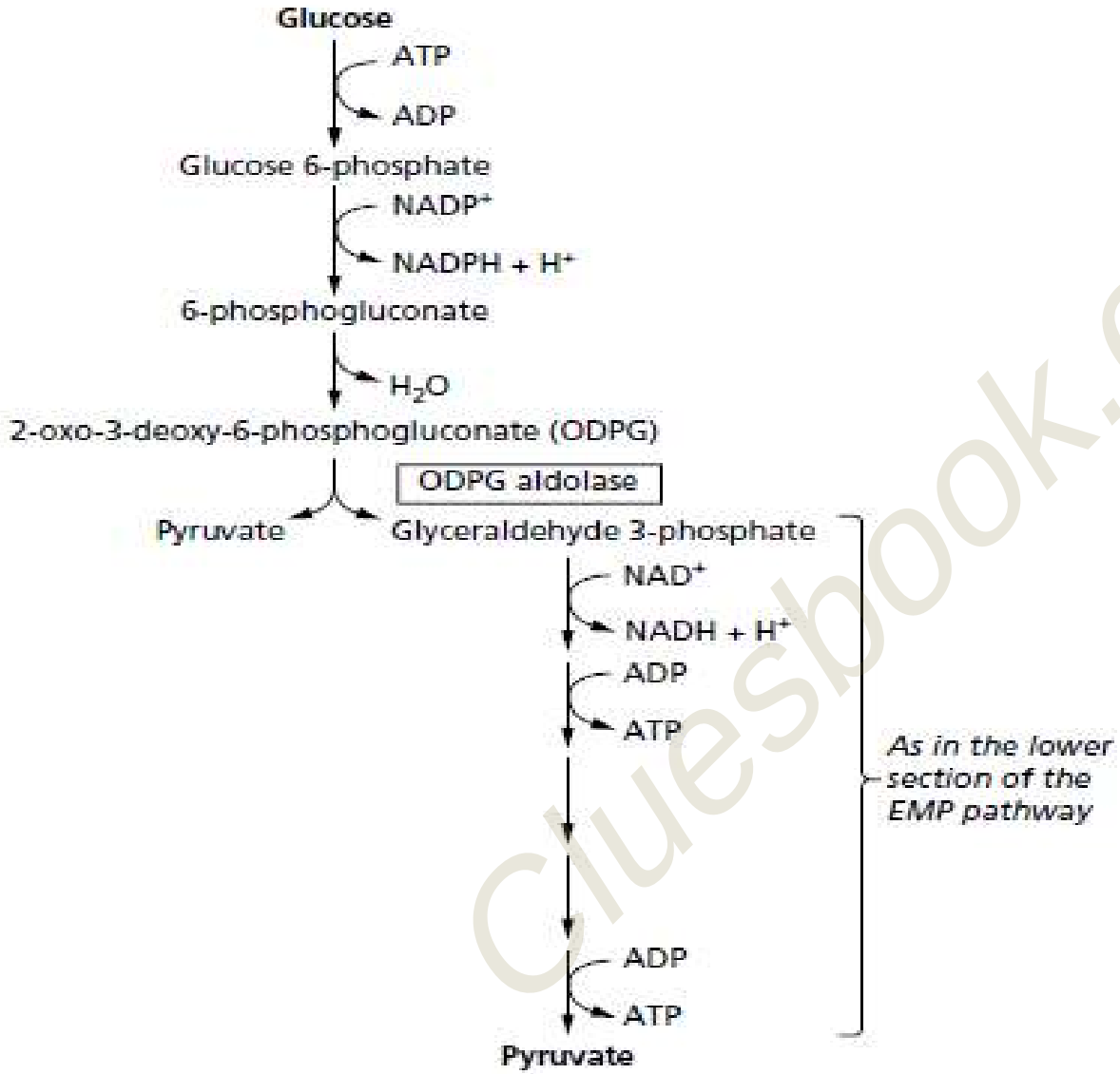


Fig. 3.3 The Entner–Doudoroff pathway.

CATABOLISM IV

- **THE PHOSPHOKETOLASE (PK) PATHWAY OR WARBURG-DICKNES PATHWAY:**

- Is operated by some lactic acid bacteria, notably species of *lactobacillus and leuconostoc.*
- It involves the oxidation and decarboxylation of glucose 6-phosphate to rump, as in the pp pathway.

CATABOLISM IV

- Rump is isomerized to xylulose 5-phosphate (c5) and then cleaved by a phosphoketolase to gap (c3) and acetyl phosphate (c2).
- The former is ultimately converted to lactate and the latter to ethanol.

CATABOLISM IV

- This pathway produces only half the yield of atp compared with the emp pathway.
- However, it does allow pentose formation from hexose sugars for nucleic acid synthesis and the catabolism of pentoses.

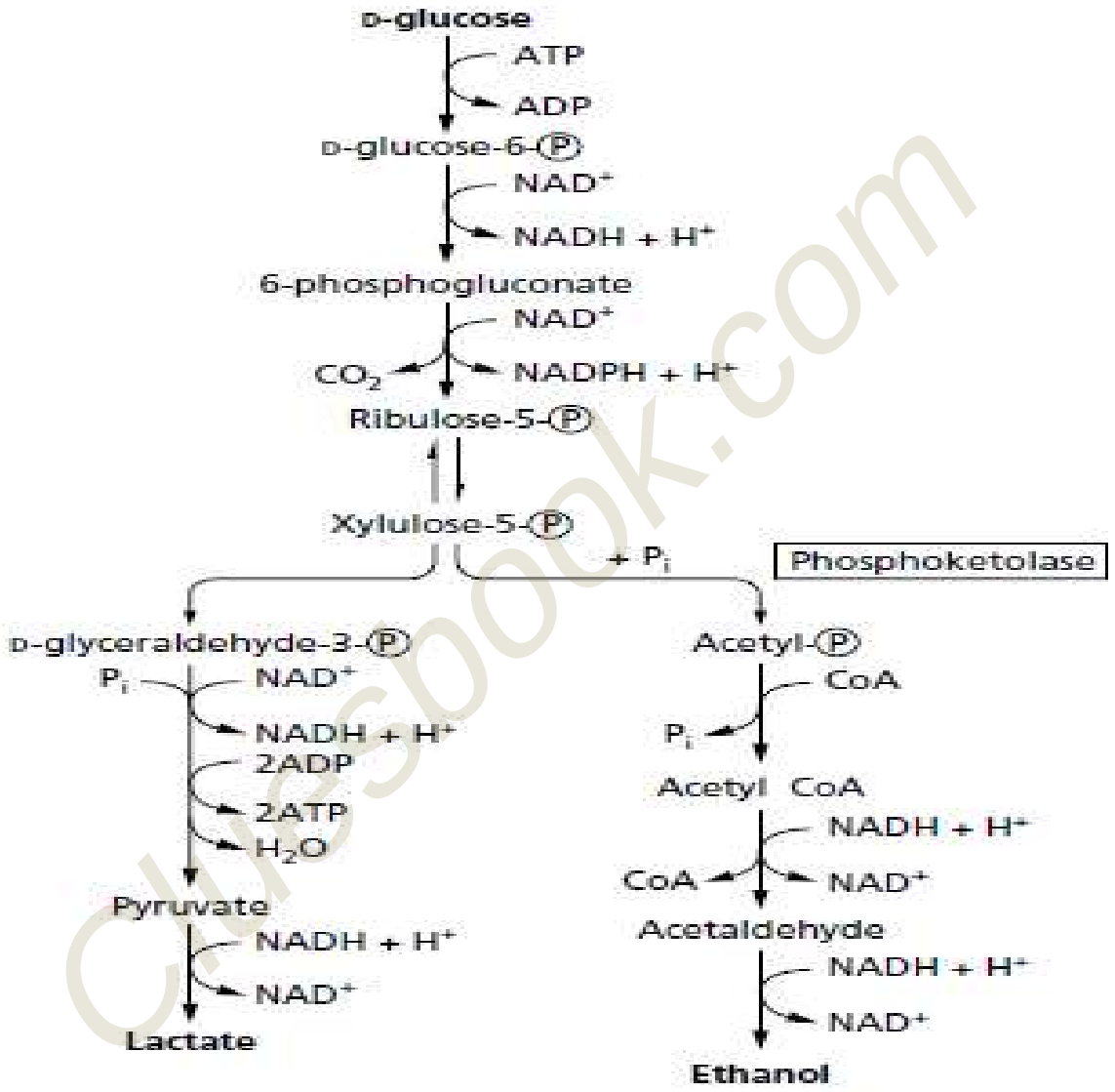


Fig. 3.4 The phosphoketolase pathway.

CATABOLISM V

❖ THE TRICARBOXYLIC ACID CYCLE

- In many bacteria, yeasts, filamentous fungi, algae and protozoa, further catabolism of pyruvate under aerobic conditions involves its direction into the tricarboxylic acid (TCA) cycle.
- Tca cycle enzymes are located within the mitochondrial matrix in eukaryotes, whereas in prokaryotes they are cytoplasmic.
- The step immediately before the cycle involves the oxidative decarboxylation of pyruvate to form the c2 unit acetyl coenzyme a (acetyl coa), catalysed by the multienzyme complex, pyruvate dehydrogenase.

CATABOLISM V

- Acetyl coa can also be formed via breakdown of lipids and some amino acids, and from the oxidation of alkanes, which certain microorganisms can utilize.

PYRUVATE (C3) +NAD+ + COA Æ ACETYL COA (C2) +CO2 + NADH +H+

- The cycle proper commences with the condensation of this two-carbon compound with oxaloacetate (C4) to form citrate (C6).
- During the following eight steps of the complete tca cycle, the two-carbon fragment is oxidized to two co2 molecules and oxaloacetate is regenerated to accept a further two-carbon unit.

CATABOLISM V

- Three reactions within the cycle result in NADH formation and one generates FADH2, and a single ATP molecule is formed indirectly by a substrate-level phosphorylation.
- Net yield is as follows:
- ACETYL COA (C2) + 3NAD+ + FAD + ADP Æ 2CO2 + 3NADH + 3H+ + FADH2 + ATP
- In terms of catabolism, the TCA cycle completes the oxidation of pyruvate to CO2, and reduces electron carriers to produce NADH and FADH2.

CATABOLISM V

- These reduced coenzymes may then be used for further ATP synthesis in respiration (see p. 50), and importantly, are thereby reoxidized to participate in further catabolism.
- Tca cycle is not only a catabolic pathway, but also provides intermediates, c4 and c5 compounds, for the biosynthesis of amino acids, purines and pyrimidines. Under anaerobic conditions it does not function as a cycle.
- This facility to produce biosynthetic intermediates is also present in other microorganisms which lack a complete tca cycle.

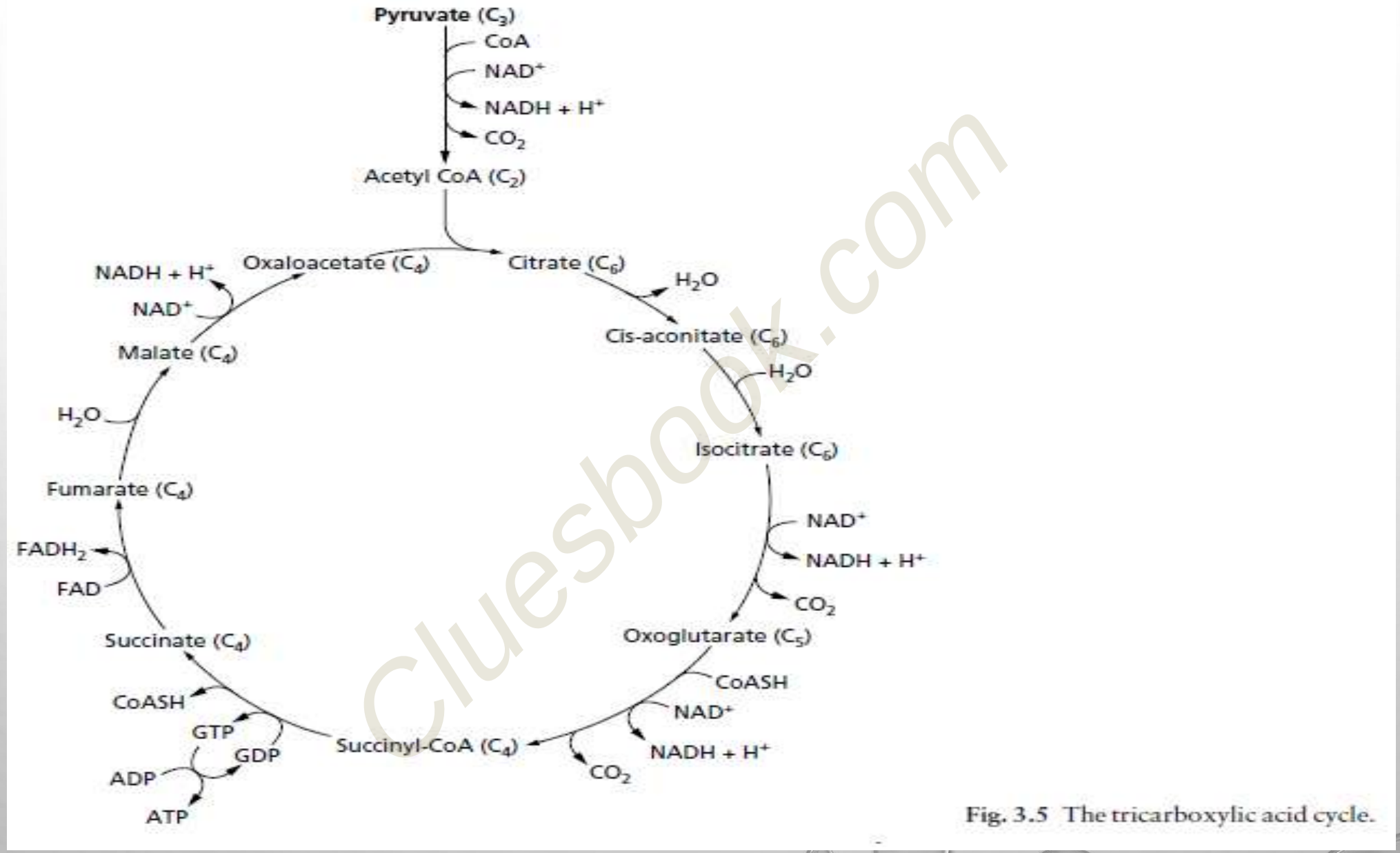


Fig. 3.5 The tricarboxylic acid cycle.

CATABOLISM V

❖ **RESPIRATION**

- Respiration leads to the generation of much more ATP from the oxidation of NADH and FADH2.
- Respiration leads to the generation of much more atp from the oxidation of nadh and fadh2.
- In order to transfer electrons and protons (h+) to
- Oxygen or an alternate final electron acceptor, a special series of intermediate electron carriers, constituting the electron transport system (ETS), is required.
- Eukaryotes have their ETS located in the inner mitochondrial membrane.

CATABOLISM V

- Electrons derived from the oxidation of substrates are passed from NADH or FADH2, through a series of redox or reduction–oxidation reactions, to the terminal acceptor.
- The **chemiosmotic hypothesis** proposed by mitchell (1961). Explains the process as follows:
- As electrons flow through the transport system, protons (H+) are moved from inside to outside the membrane (excretion).
- Because the membrane is essentially impermeable to protons they cannot pass back and a proton gradient is established.

CATABOLISM V

- The ph immediately outside the membrane can reach 5.5, whereas within it approaches 8.5.
- This represents potential energy stored as a proton gradient or proton-motive force, which drives the synthesis of atp via chemiosmotic phosphorylation, mediated by atp synthase (adp + pi œatp).
- A molecule of nadh contains approximately 218 kj of potential energy and it takes approximately 30 kj to generate one atp molecule. Hence, a maximum of seven ATP molecules could, in theory, be generated per NADH if energy conversion were 100% efficient.
- However, these systems are only up to 40% efficient and consequently produce a maximum of only three atp molecules per nadh.

CATABOLISM V

- Some bacteria that are facultative anaerobes or obligate anaerobes perform **anaerobic respiration. This involves** a similar ETS, but has terminal electron acceptors other than oxygen.
- Examples of anaerobic respiration include the following.
- **1 nitrate respiration, which is conducted by facultative** anaerobic bacteria.
- The redox potential of nitrate is +0.42 volts, compared with +0.82 volts for oxygen.
- Denitrifiers include species of *pseudomonas, paracoccus denitrificans* and *thiobacillus denitrificans. Other facultative* anaerobes, including *E*.
- *Coli and relatives, merely reduce* nitrate to nitrite, and the enzyme responsible, nitrate reductase, is repressed in the presence of oxygen.

CATABOLISM V

- **Sulphate respiration is practised by a small group** of heterotrophic bacteria, which are all obligate anaerobes, e.G. *Desulfovibrio species.*
- Product is hydrogen sulphide, formed via several intermediates.
- **3 carbonate respiration is performed by archaeans**
- Such as *methanococcus and methanobacterium. They* are obligate anaerobes that reduce CO2, and sometimes carbon monoxide, to methane.
- These methanogenic bacteria commonly use hydrogen as their energy source and are found in anoxic environments low in nitrate and sulphate, e.G. The gut of some animals, marshes, rice fields and sewage sludge digesters. CO2 + 4H2ÆCH4 + 2H2O

FERMENTATIONS I

- Fermentation is a process by which a substance breaks down into a simpler substance.
- Microorganisms like yeast and bacteria usually play a role in the fermentation process, creating beer, wine, bread, kimchi, yogurt and other foods.
- Fermentations use an organic molecule, pyruvate or a derivative, as this final electron acceptor, thereby regenerating NAD(p)+ and allowing catabolism to continue.

FERMENTATION I

- **Alcoholic fermentation is performed by yeasts and** certain filamentous fungi and bacteria.
- It is a twostep process, where pyruvate from the emp pathway, or via the ed pathway in the case of *zymomonas, is* first decarboxylated to acetaldehyde; NAD+ is then regenerated during reduction of acetaldehyde to ethanol.

FERMENTATION I

- **Lactic acid fermentations are carried out by a number** of bacteria, including *streptococcus, lactobacillus, lactococcus and leuconostoc, along with some fungi*, algae and protozoa.
- There are two forms of this fermentation:
- **1 homolactic fermentation is operated by bacteria such** as *lactobacillus acidophilus and lactobacillus casei*, which reduce virtually all pyruvate generated by glycolysis to lactic acid .
- This also occurs in animal muscle deprived of oxygen.

FERMENTATION I

- **2 heterolactic fermentation generates other products** along with the lactic acid.
- The organisms that perform it include *leuconostoc mesenteroides and lactobacillus brevis, which operate the pk pathway.*
- They form lactate from pyruvate and ethanol from acetyl phosphate, and in certain cases some acetate may be produced.

FERMENTATION I

- **Mixed acid fermentation is carried out by *E. Coli and*** related facultative anaerobes.
- The products include lactate, acetate, small quantities of ethanol and formate.
- Some organisms have the ability to further reduce formate to hydrogen and CO2.

FERMENTATION I

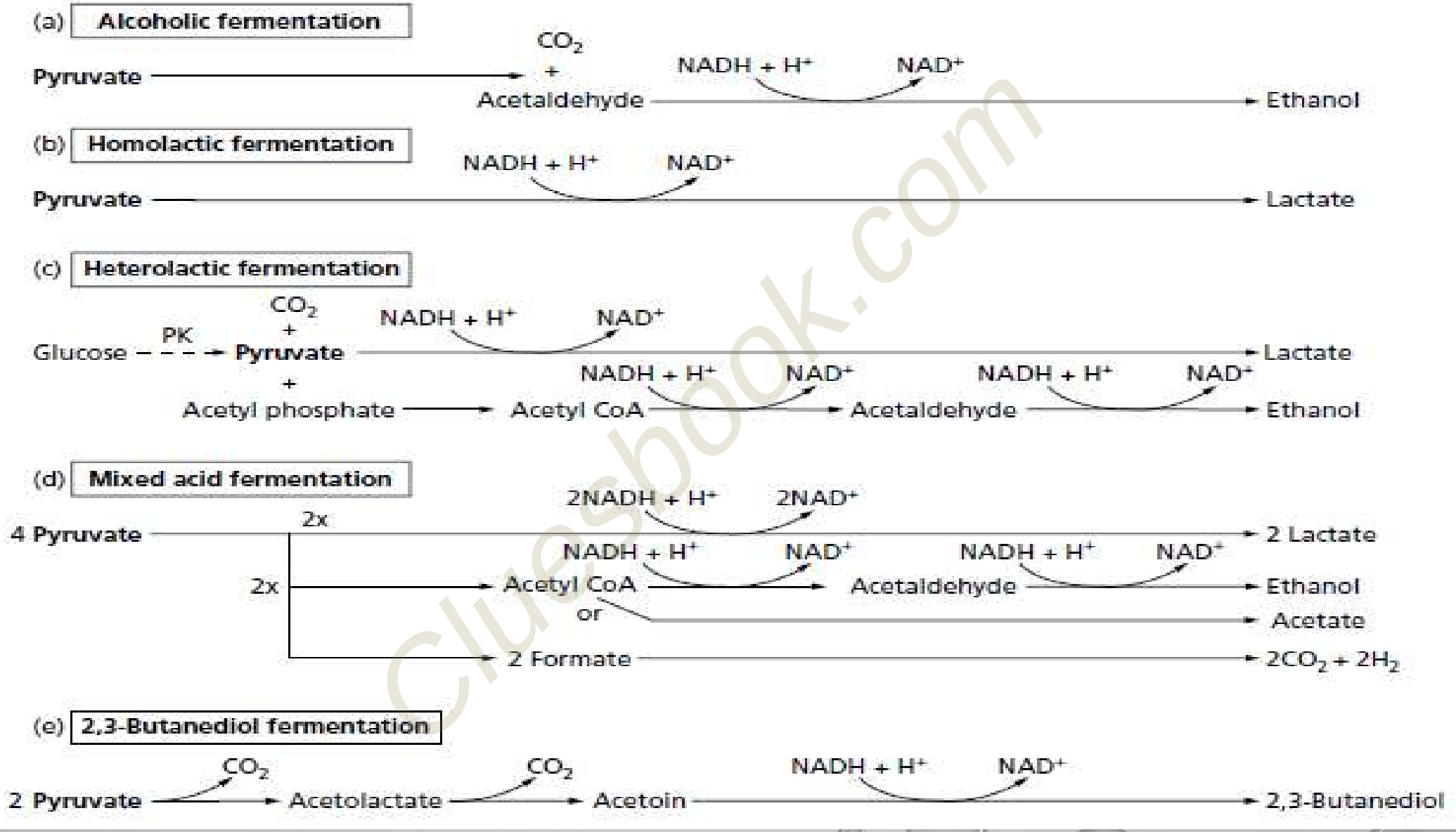
- **2,3-butanediol fermentation is performed by *enterobacter,*** *erwinia, klebsiella and serratia.*
- *It is similar to* the mixed acid fermentation, but generates butanediol, along with ethanol and acids.

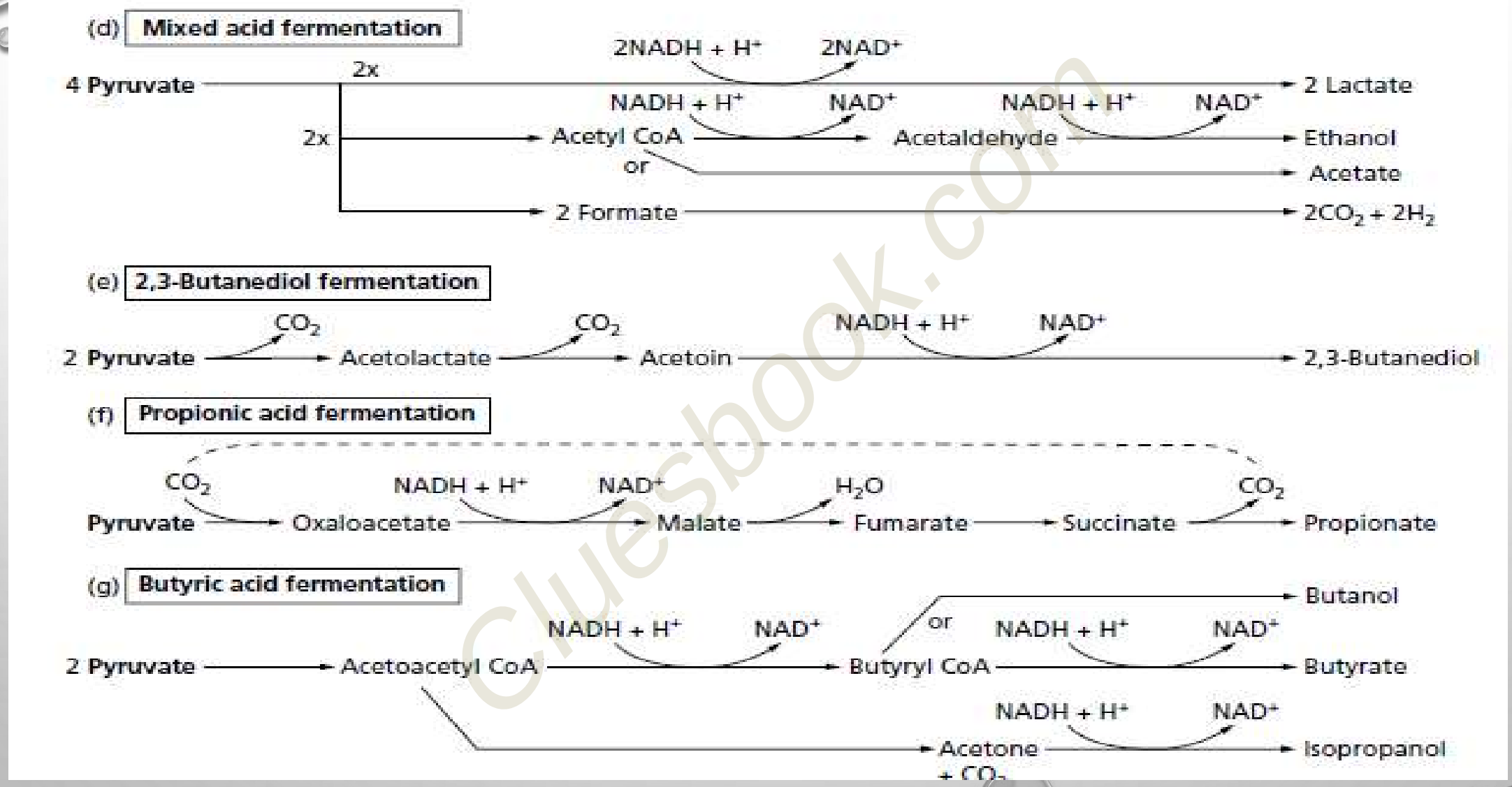
FERMENTATION I

- **Propionic acid fermentation is conducted by several** gut bacteria, such as species *propionibacterium, some* of which are involved in the commercial production of certain swiss-type cheeses and vitamin B12 (cobalamin).
- The propionate is formed from pyruvate via the methylmalonyl coa pathway, where pyruvate is carboxylated to oxaloacetate, and then reduced to propionate via malate, fumarate and succinate.
- This requires the important cofactors, biotin, COA and COB12.

FERMENTATION I

- **BUTYRIC ACID FERMENTATION IS CARRIED OUT BY SPECIES OF** *Clostridium*.
- *These anaerobic spore formers also produce* acetone, butanol, propanol, other alcohols and acids.
- They also ferment amino acids and other nitrogenous compounds, as well as carbohydrates.





FERMENTATION II

➢ CATABOLISM OF LIPIDS AND PROTEINS

- Carbon sources other than carbohydrates must be processed before they can enter catabolism. Lipids such as di- and triglycerides are hydrolysed by lipases to produce free fatty acids and glycerol.
- Fatty acids may then be broken down by the b-oxidation pathway.
- Here fad and nad+ are used to accept electrons, and two-carbon units are successfully removed, in the form of acetyl coa, which can then feed directly into the TCA cycle.

FERMENTATION II

- The glycerol may be phosphorylated to glycerol phosphate, followed by its oxidization to DAP and isomerization to GAP. This C3 unit can then enter the EMP pathway.
- Extracellular proteins are hydrolysed by proteases to produce free amino acids, which can be transported into the cell.
- Amino acid catabolism initially involves removal of their amino group(s).

FERMENTATION II

- This is usually achieved via transamination, where the amino group is donated to a keto host, e.G. Amination of pyruvate to form alanine.
- The keto acid resulting from transamination can then be oxidized within the TCA cycle.
- Excess amino groups may accumulate and are often excreted as ammonium ions, which accounts for the increasing ph of media during the growth of some bacteria.

FERMENTATION II

- **ENERGY STORAGE**
- When excess nutrients are available, microorganisms normally synthesize compounds that can be stored for later use during periods of nutrient shortages.
- **Storage carbohydrates include the polysaccharides, glycogen** and starch, and the disaccharide trehalose, all of which act as both carbon and energy sources.

FERMENTATION II

- Trehalose is produced by filamentous fungi, yeasts and some bacteria, and glycogen is a major storage carbohydrate in many groups of microorganisms.
- Many microorganisms store energy-rich lipids. Poly b-hydroxybutyrate is commonly found in bacteria, but is not produced by eukaryotes.
- Filamentous fungi and yeasts often store neutral lipids (triglycerides) within vacuoles.

FERMENTATION II

- Volutin granules, composed of **polymetaphosphates,** are also stored by some prokaryotes and eukaryotes, which act as both phosphate and energy reserves.
- Sulphur bacteria may accumulate sulphur granules by the oxidation of H2S.
- When no further media supplies of sulphide are available, this sulphur store is oxidized to sulphate, providing reducing equivalents for CO2 fixation or oxidative phosphorylation

ANABOLISM: THE SYNTHESIS OF BIOMOLECULES I

- Anabolism is the *biosynthesis of complex* organic molecules from simpler ones.
- These processes are endergonic, requiring an input of energy to drive them, which mostly comes from the ATP provided by catabolism.
- The anabolic processes often comprise two stages, involving the synthesis of small metabolic intermediates that are then assembled to form polymers.

ANABOLISM: THE SYNTHESIS OF BIOMOLECULES I

- Many catabolic processes not only generate energy, in the form of ATP and reduced coenzymes, for use in biosynthesis, but also provide carbon skeletons that can feed into anabolism.
- Pathways with dual catabolic and anabolic roles are referred to as **amphibolic.**
- **Amphibolic** pathways include the emp pathway and the tca cycle. The former provides pyruvate, hexose phosphates and triose phosphates, whereas the latter supplies oxaloacetate and oxoglutarate.

ANABOLISM: THE SYNTHESIS OF BIOMOLECULES I

- These products can be utilized in the synthesis of cellular components and storage materials.
- Many reactions of amphibolic pathways are freely reversible or have 'bypasses' of irreversible steps to facilitate their dual function.
- The irreversible steps have separate enzymes for the two directions that can provide suitable control points for the independent regulation of catabolism and anabolism.

ANABOLISM: THE SYNTHESIS OF BIOMOLECULES I

- **Anaplerotic (replenishment) reactions**
- As a consequence of removing intermediate compoundsfrom these amphibolic pathways for biosynthesis, their levels may become depleted.
- For example, oxaloacetate is taken from the TCA cycle to furnish the demand for carbon skeletons in amino acid biosynthesis.
- Hence, these intermediates have to be replenished via an alternate route, referred to as an **anaplerotic pathway, in** order to maintain operation of this cycle.

ANABOLISM: THE SYNTHESIS OF BIOMOLECULES I

- One such anaplerotic route is the **glyoxalate cycle.**
- **This** cycle operates in many organisms for the replenishment of oxaloacetate, particularly for gluconeogenesis from lipids and during growth on c2 compounds.
- It involves isocitrate lyase, which cleaves isocitrate to succinate and glyoxalate.
- Acetyl coa is then condensed with glyoxylate to generate malate and coenzyme a (coash) by the action of malate synthase.

ANABOLISM: THE SYNTHESIS OF BIOMOLECULES I

- The malate can then be transformed to oxaloacetate to allow the continued operation of the TCA cycle.
- Gram-negative bacteria such as e. C*oli and other* enteric bacteria can generate oxaloacetate, a fourcarbon compound, by fixing CO2 to the three-carbon compound, phosphoenolpyruvate (PEP), using PEP carboxylase.
- Many gram-positive bacteria and yeasts have a similar carboxylation system, but one which utilizes the biotin-requiring enzyme, pyruvate carboxylase, to add co2 to pyruvate.

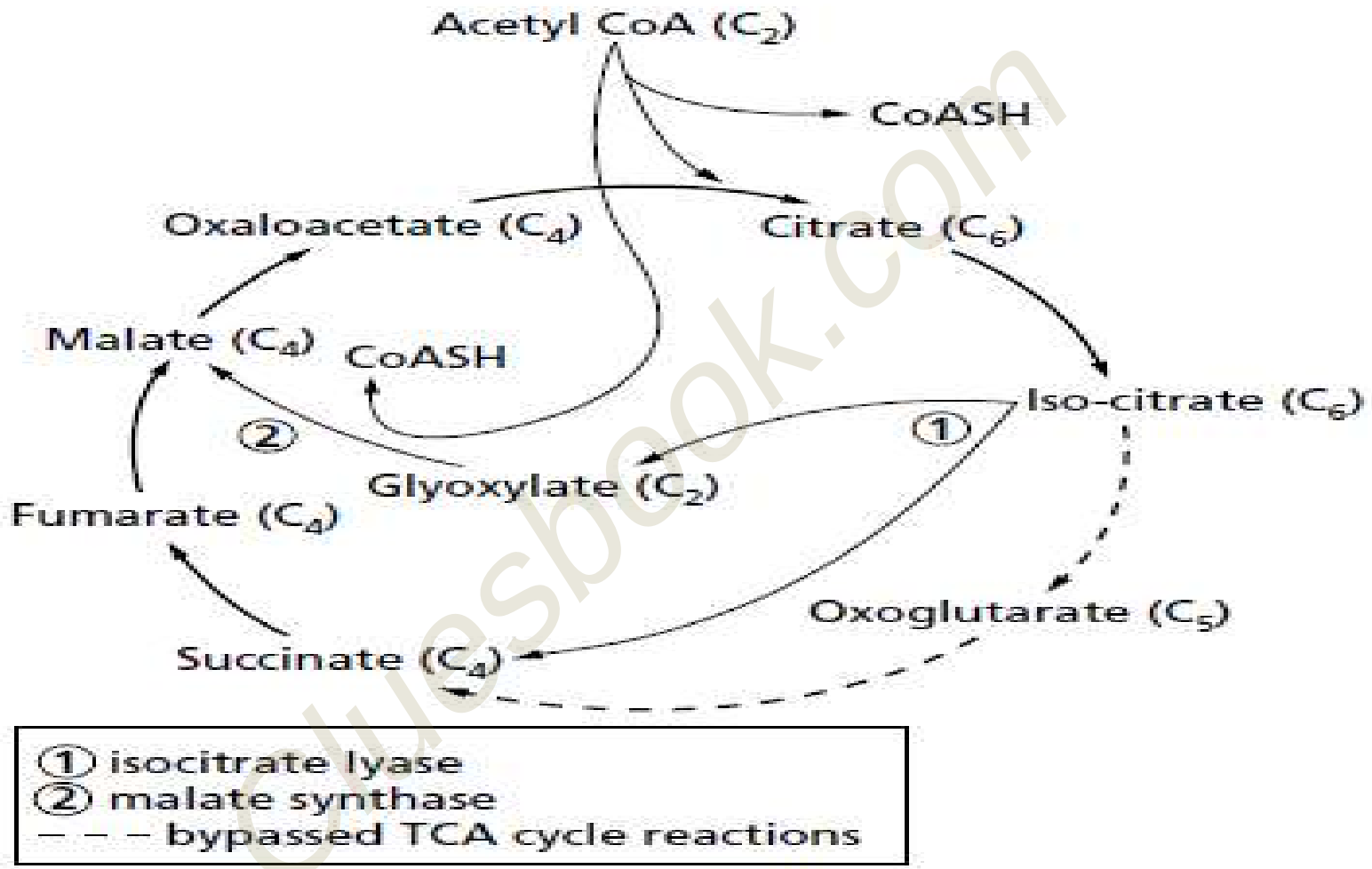
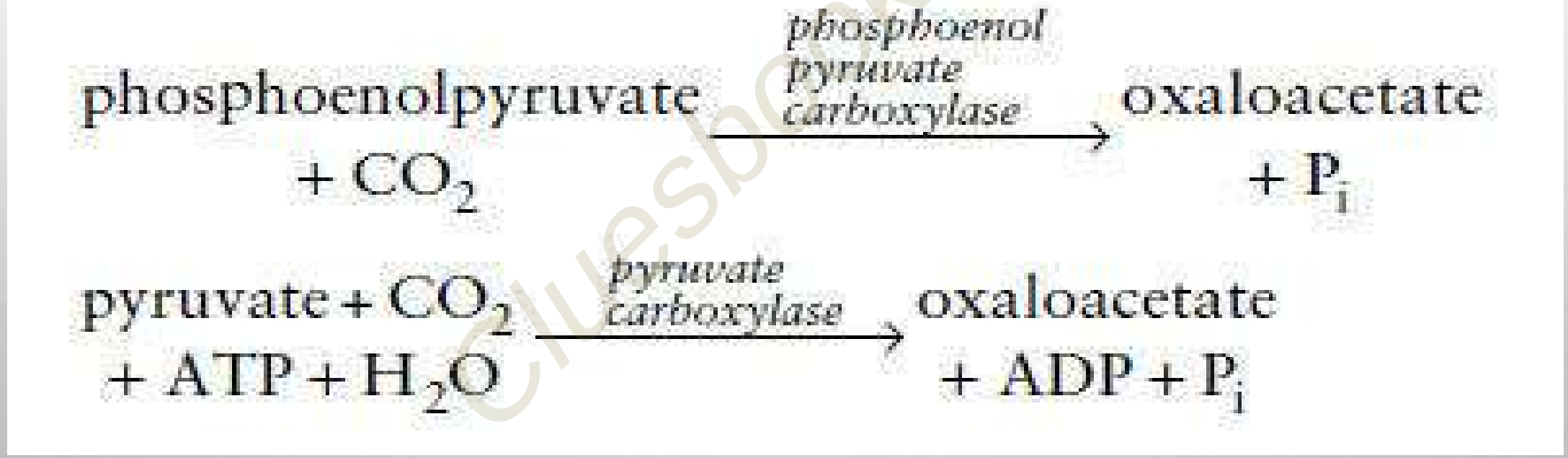


Fig. 3.8 The glyoxylate cycle.

ANABOLISM: THE SYNTHESIS OF BIOMOLECULES I



ANABOLISM: THE SYNTHESIS OF BIOMOLECULES I

- **GLUCONEOGENESIS, ESSENTIALLY THE REVERSAL OF GLYCOLYSIS,**
- Also fulfils a similar anaplerotic role.
- It is particularly important during growth on pyruvate, related c3 compounds and c2 units.
- However, as mentioned earlier, not all steps are reversible and appropriate bypasses are necessary.

ANABOLISM: THE SYNTHESIS OF BIOMOLECULES I

- The reversal of the flow of carbon from pyruvate maintains a supply of hexoses, which would otherwise become depleted.
- These intermediates are mostly required for the synthesis of cell wall components and storage carbohydrates.

ANABOLISM: SYNTHESIS OF BIOMOLECULES II

- **BIOSYNTHESIS OF NUCLEOTIDES AND NUCLEIC ACIDS**
- **Nucleotides are composed of a cyclic nitrogenous** base, a pentose sugar (ribose or deoxyribose) and phosphate.
- The base may be a purine, either adenine or guanine, or one of three pyrimidines, cytosine, thymine or uracil.
- These nucleotides are the building blocks of deoxyribonucleic acid (dna) and ribonucleicacid (rna).
- They are also important constituents of several coenzymes, and have key roles in the activation and transfer of sugars, amino acids and cell wall components.

ANABOLISM: SYNTHESIS OF BIOMOLECULES II

- Most microorganisms can synthesize their own purines and pyrimidines.
- Purine synthesis is complex, the purine skeleton being derived from several components, whereas pyrimidines originate from orotic acid, a product of the condensation of carbamoyl phosphate with aspartic acid.
- Ribose 5-phosphate is generated in the pp pathway and is used here in the activated form of phosphoribosyl pyrophosphate (prpp).

ANABOLISM: SYNTHESIS OF BIOMOLECULES II

- It condenses with the base precursor orotic acid to form pyrimidines.
- In purine biosynthesis, the compounds are built up by several additions of fragments to the prpp molecule. Deoxyribose forms of the nucleosides are then generated by reduction of the sugar moiety of ribonucleosides.
- The nucleosides are finally phosphorylated to form nucleotide triphosphates by successive phosphorylation from atp, in order that they can participate in further metabolic processes.

ANABOLISM: SYNTHESIS OF BIOMOLECULES II

➢ **NUCLEIC ACIDS**

- There are two types of nucleic acid;

1. Dna
2. Rna

- Both are composed of nucleotide building blocks.
- The two nucleic acids differ in the sugar found in their nucleotides, either deoxyribose or ribose.
- Both polymers are made by the formation of covalent bonds between the sugar and phosphate groups of adjacent nucleotides.

ANABOLISM: SYNTHESIS OF BIOMOLECULES II

- Four bases can occur in each nucleic acid.
- Three bases, adenine, guanine and cytosine, are common to both rna and dna.
- But thymine of dna is replaced by uracil in rna.
- The various forms of rna, which play key roles in protein synthesis, include messenger (mrna), ribosomal (rrna) and transfer (trna).

ANABOLISM: SYNTHESIS OF BIOMOLECULES II

- DNA is double stranded, composed of two helical chains each coiled around the same axis.
- Both chains follow right-handed helices, with the two chains running in opposite directions.
- Bases of each strand are on the inside of the helix with the phosphates on the outside.
- A key feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases.

ANABOLISM: SYNTHESIS OF BIOMOLECULES II

- A single base from one chain is hydrogen bonded to a single base from the other chain, so that the two lie side by side.
- One of the pair must be a purine and the other a pyrimidine, and only specific pairs of bases can bond together.
- These base pairs are: adenine (purine) with thymine (pyrimidine) and guanine (purine) with cytosine (pyrimidine).
- The two strands of DNA have a complementary sequence of bases.

ANABOLISM: SYNTHESIS OF BIOMOLECULES II

➢ **BIOSYNTHESIS OF AMINO ACIDS AND PROTEIN**

- Microorganisms are normally capable of synthesizing the 20 amino acids required for protein production.
- The carbon skeletons for the amino acids are derived from intermediary metabolism, and the amino groups are introduced by direct amination, or more usually for most amino acids, via transamination.
- Microorganisms readily assimilate ammonia, but if nitrate, nitrite or more rarely molecular nitrogen are the nitrogen source, they must first be reduced to ammonia.

ANABOLISM: SYNTHESIS OF BIOMOLECULES II

- Primary assimilation of ammonia in many bacteria involves l-glutamate dehydrogenase and l-alanine dehydrogenase, which catalyse the reductive amination without the requirement for ATP.
- When ammonium ion concentrations are low, assimilation of nitrogen may be via glutamine synthetase, which has a higher affinity for ammonium ions but demands expenditure of ATP.
- Glutamate is the predominant component of the free cytoplasmic amino acid pool.

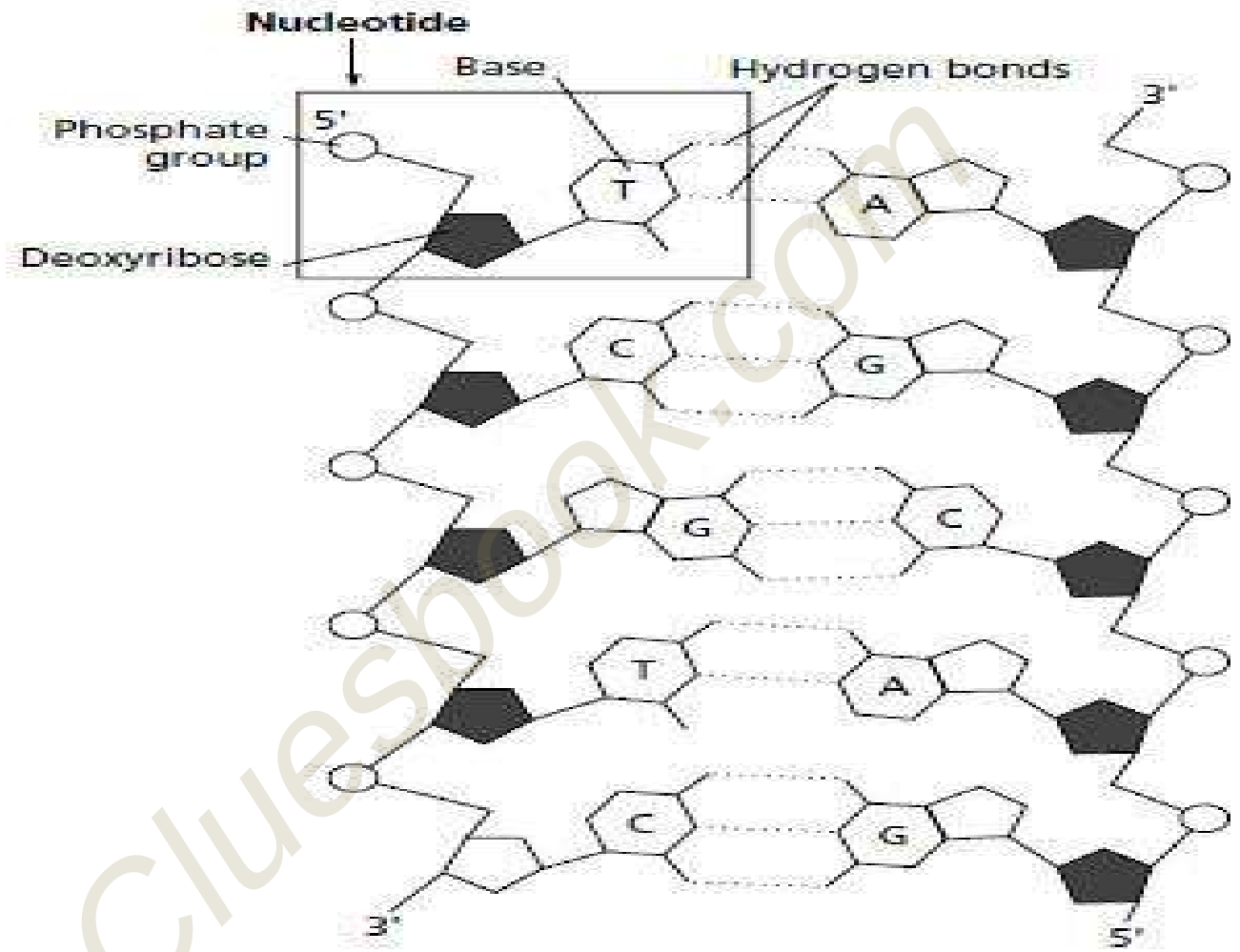


Fig. 3.9 Two-dimensional representation of a section of deoxyribonucleic acid. Bases: adenine (A), thymine (T), cytosine (C) and guanine (G).

ANABOLISM: SYNTHESIS OF BIOMOLECULES II

- Polypeptides consist of a chain of amino acids joined by very stable covalent peptide bonds.
- These bonds form between the amino group of one amino acid and the carboxylic acid group of another.
- The term ‘protein’ normally refers to the final entire functional assembly, which may be composed of one or several polypeptides.
- The order of amino acids within a polypeptide is determined by the base sequence of the DNA that constitutes the structural gene coding for the specific polypeptide.

ANABOLISM: SYNTHESIS OF BIOMOLECULES II

- DNA codes are not read directly, but transcribed into mobile copies of the code, in the form of mrna.
- These act as templates for polypeptide synthesis and are translated into a specific sequence of amino acids.
- Initiation and termination of both transcription and translation are strictly controlled.
- The mrnas are synthesized by RNA polymerases that bind to the promoter site located upstream from the structural gene coding for the polypeptide.
- Base sequences of the template or sense strand of double-stranded DNA, composed of adenine, guanine, cytosine and thymine, are transcribed into single-stranded mrna copies.
- They contain the complementary sequence of bases, except that uracil replaces thymine.

ANABOLISM: THE SYNTHESIS OF BIOMOLECULES III

- The genetic code is composed of a linear series of triplets of bases (codons) that represent the amino acids of proteins, along with start and stop signals for protein synthesis.
- This code is virtually the same for all organisms and is degenerate for most amino acids.
- I.E. There are 20 amino acids and 64 possible triplet combinations from the four bases.
- Consequently, each amino acid is represented by more than one triplet code.

Amino acid groups with common biosynthetic pathways (the compound/skeleton from which they are derived is in parenthesis)

Glutamate family (2-oxoglutarate)	Glutamate, glutamine, proline, arginine
Aspartate family (oxaloacetate)	Aspartate, asparagine, lysine, methionine, threonine, isoleucine
Pyruvate family (pyruvate)	Alanine, leucine, valine
Serine family (3-phosphoglycerate)	Serine, glycine, cysteine
Aromatic amino acids (erythrose 4-phosphate + phosphoenolpyruvate)	Phenylalanine, tryptophan, tyrosine
Histidine (phosphoribosyl pyrophosphate (PRPP) + ATP)	Histidine is the only amino acid member of its biosynthetic route

(a) **Glutamate dehydrogenase (GDH)**

$$\text{Oxoglutarate}^* + \text{NAD(P)H} + \text{H}^+ + \text{NH}_4^+ \rightleftharpoons \text{Glutamate} + \text{NAD(P)}^+ + \text{H}_2\text{O}$$

*5-carbon compound produced in the TCA cycle
(replace with pyruvate for alanine dehydrogenase,
and the product is alanine)
Note: no ATP required

(b) **Glutamine synthetase (GS)**—direct amination

$$\text{Glutamate} + \text{NH}_4 + \text{ATP} \rightleftharpoons \text{Glutamine} + \text{ADP} + \text{P}_i$$

Glutamate synthetase (GOGAT)—a transamination

$$\text{Glutamine} + \text{oxoglutarate} + \text{NAD(P)H} + \text{H}^+ \rightleftharpoons 2\ \text{glutamate} + \text{NAD(P)}^+ + \text{H}_2\text{O}$$

ANABOLISM: THE SYNTHESIS OF BIOMOLECULES III

➢ BIOSYNTHESIS OF MONOSACCHARIDES AND POLYSACCHARIDES

- Carbohydrates can exist either as single units (Monosaccharides) or joined together in molecules ranging from two units (disaccharides) to thousands of units (polysaccharides).
- Monosaccharides containing an aldehyde group are called aldoses, whereas those containing a ketone group are ketoses.
- Monosaccharides are also classified by the number of carbon atoms that they contain, i.E. Trioses, tetraoses, pentoses, hexoses, etc.

ANABOLISM: THE SYNTHESIS OF BIOMOLECULES III

- The monosaccharides are derived from common sugar substrates such as glucose or hydrolysed polysaccharide substrates.
- Within each cell the bulk of the monosaccharides are present in the form of polysaccharides.
- The component sugar units of these polymers are joined by covalent **glycosidic bonds.**
- **They are not assembled from free sugars,** but from sugar nucleotides. Different polysaccharides can be formed by varying the orientation of the glycosidic bond to form a- or b-linkages, or by changing the monomer.

ANABOLISM: THE SYNTHESIS OF BIOMOLECULES III

- Examples of important microbial polysaccharides include the following.
- **1 glycogen and starch, branched-chain homopolymers** of a-linked glucose units that function as carbon and energy reserves. Glycogen, for example, is synthesized through the activities of adp-glucose pyrophosphorylase and the branching enzyme, glycogen synthase.
- **2 cellulose and other cell wall b-glucans, composed of** b-linked glucose units.
- **3 chitin, a polymer of *n-acetylglucosamine*.**
- **4 peptidoglycan, a polymer of *n-acetylglucosamine*** and *n-acetylmuramic acid, cross-linked with peptides.*
- **5 various gums such as dextran (mostly a-1,6 linked** glucose units) and xanthan (essentially a cellulose backbone with a trisaccharide of mannose–glucuronic acid–mannose, linked to alternate glucose units).

ANABOLISM: THE SYNTHESIS OF BIOMOLECULES III

➢ PEPTIDOGLYCAN BIOSYNTHESIS

- Peptidoglycan (PG) consists of a rigid linear polysaccharide backbone of alternating units of *n-acetyl glucosamine* (NAG) and *n-acetyl muramic acid (NAM),* with tetrapeptide side chains whose component amino acid may vary depending upon the bacterium.
- Each tetrapeptide is attached to a NAM residue through a lactate unit.
- The structure is made rigid by crosslinking a proportion of adjacent tetrapeptide side chains either directly or through short peptide bridges to form an overall net-like structure.

ANABOLISM: THE SYNTHESIS OF BIOMOLECULES III

➢ **BIOSYNTHESIS OF FATTY ACIDS AND LIPIDS**

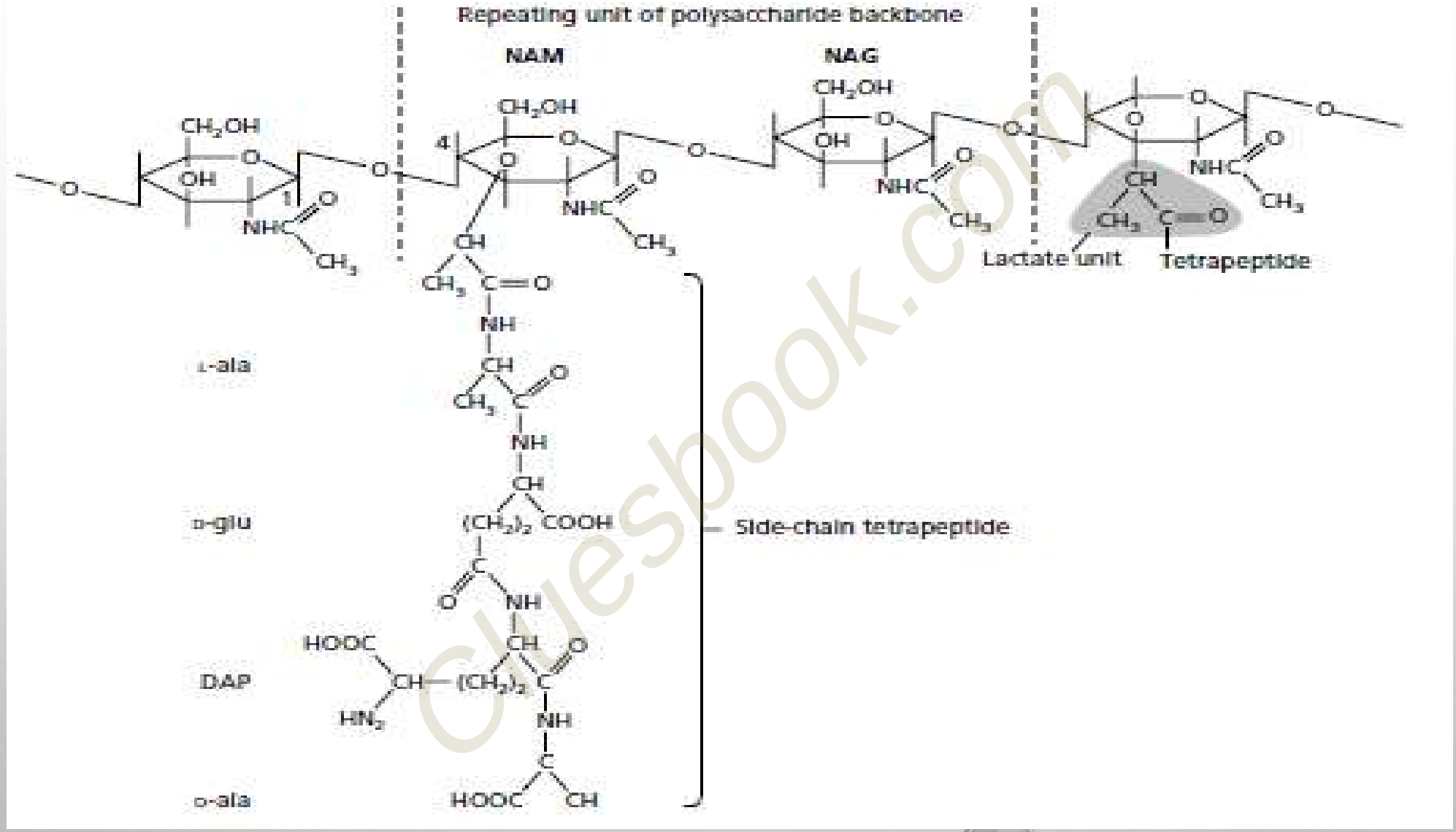
- Lipids are a very diverse family of compounds, grouped together on the basis of their relative insolubility in water and solubility in non-polar solvents.
- They are important components of membranes and may function as energy reserves in some organisms, e.G. Poly bhydroxybutyratem and triglycerides.
- Simple lipids are glycerides, esters of glycerol and fatty acids.
- Each fatty acid consists of a long non-polar chain of 12–24 carbon atoms with a polar carboxylic acid group at one end.
- Most natural fatty acids contain an even number of carbon atoms.

ANABOLISM: THE SYNTHESIS OF BIOMOLECULES III

- They may be saturated, unsaturated or polyunsaturated, containing no double bonds, one double bond or two or more double bonds, respectively.
- Synthesis of saturated fatty acids involves derivatives of coenzyme a and acyl carrier proteins (acp).
- The fatty acid is built up by successive addition of two-carbon units in the form of acetyl units, donated indirectly via acetyl coa (i.E. Following its carboxylation to malonyl coa), which are then reduced by NADPH.

ANABOLISM: THE SYNTHESIS OF BIOMOLECULES III

- Two routes are known for unsaturated fatty acids synthesis, one of which has a requirement for molecular oxygen.
- Monoglycerides, possessing one fatty acid, are rare, but diglycerides are common membrane lipids and triglycerides are often used as energy storage materials by some yeasts.



ANABOLISM: THE SYNTHESIS OF BIOMOLECULES III

- These are highly water-soluble (hydrophilic) at the phosphate end, but very water-insoluble (hydrophobic) at the fatty acid end.
- The phosphate may be further esterified to ethanolamine, inositol or serine. Other important lipids include sterols, such as ergosterol, which are key cell membrane components in fungi, synthesized only under aerobic conditions.
- Glycolipids, including lipopolysaccharides, glycosyldiglycerides and lipoteichoic acid, are also found in many microorganisms.

AUTOTROPHY I

- **Autotrophs** are the organisms that are able to produce Organic compounds from simple inorganic substances such as CO2. They are producers in a food chain, such as plants and algae.
- There are two basic types of autotrophs;

1. **Phototrophs** use energy derived from light and
2. **chemolithotrophs** use inorganic compounds as energy sources.

AUTOTROPHY I

- **Photoautotrophy;**
- **photoautotrophs** are able to absorb light energy and transform it to chemical energy (ATP and reduced coenzyme) via the 'light reactions' of photosynthesis.
- Photosynthesis is initiated by the absorption of quanta of light by specific photosynthetic pigments. There are three main types employed.

AUTOTROPHY I

1. **bacteriochlorophyll is found in the purple sulphur** bacteria, purple non-sulphur bacteria and green bacteria, which includes all photosynthetic eubacteria except the cyanobacteria.
2. **chlorophylls are the photopigments of cyanobacteria,** algae and higher plants.
3. **bacteriorhodopsin, a non-chlorophyll pigment, is** used by archaeans, such as halobacteria

AUTOTROPHY I

➢ **Anoxygenic photosynthetic bacteria;**

- Photosynthetic units in eubacteria, other than cyanobacteria, consist of three components;

1. The lightgathering complex, containing bacteriochlorophyll and accessory pigments;
2. A reaction centre, composed of bacteriochlorophylls and
3. Bacteriopheophytins; and an ETS.

AUTOTROPHY I

- Reduced sulphur compounds, molecular hydrogen or organic compounds are utilized as final electron donors.
- Consequently, oxygen is not generated during this type of photosynthesis.
- When the bacteriochlorophyll within a reaction centre is excited by light, an electron is donated to a bacteriopheophytin.
- It is passed on to a quinone and then, via a series of cytochrome, returning to the oxidized reaction centre bacteriochlorophyll.

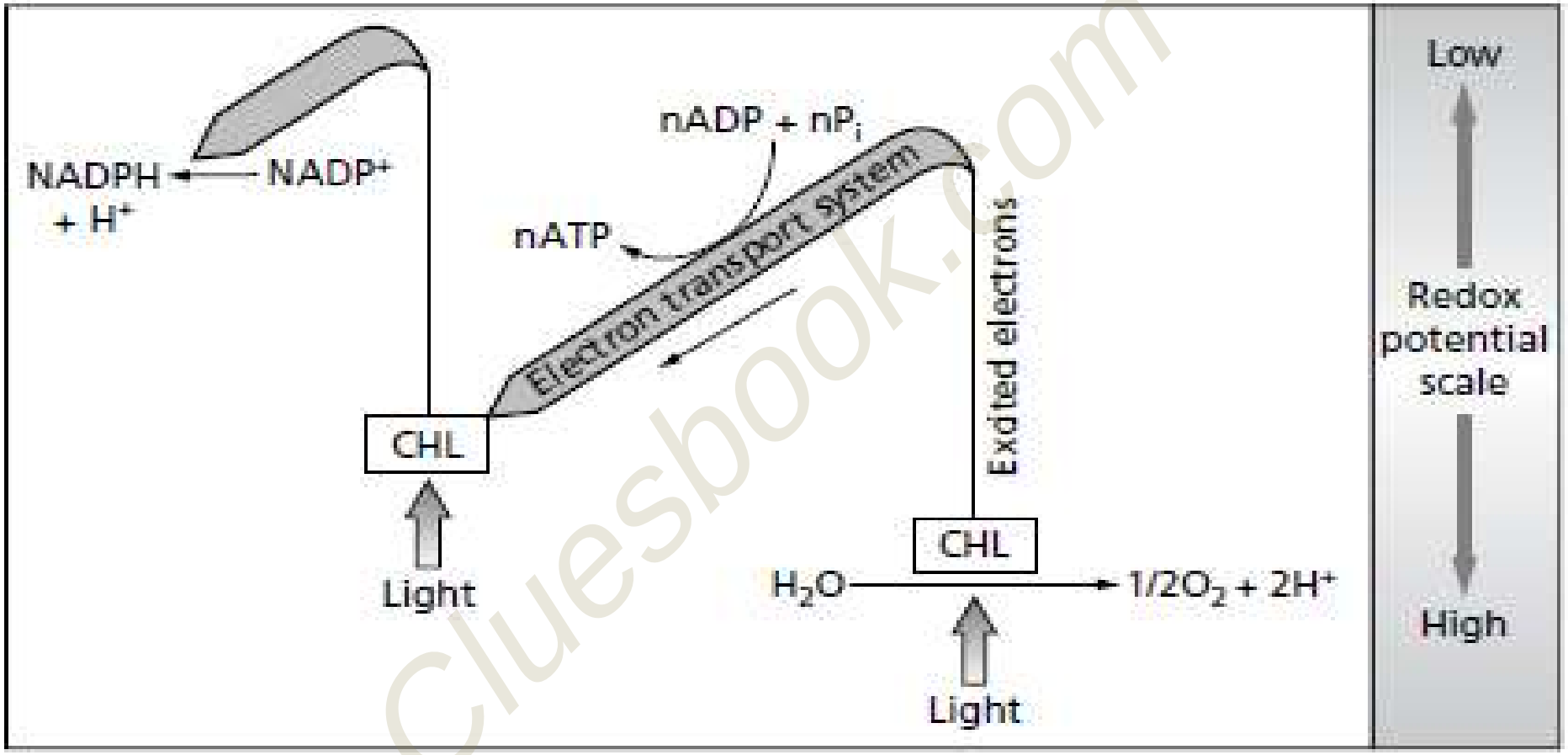
AUTOTROPHY I

- **OXYGENIC PHOTOSYNTHESIS;**

- Cyanobacteria, algae and higher plants have two photosystems, photosystems I and II, linked through a series of electron carriers.
- These systems are responsible for the generation of both atp and NADPH.
- Electrons removed from the reaction centre of photosystem i, when excited by light, are passed to ferredoxin, then directly to NADP+ to form NADPH.

AUTOTROPHY I

- Electrons removed from the reaction centre of photosystem I, when excited by light, are passed to ferredoxin, then directly to NADP+ to form NADPH.
- This leaves photosystem i positively charged and unable to supply any further electrons.
- Electrons are supplied to photosystem i from photosystem ii when it too is energized by light.



AUTOTROPHY I

- They are provided by the photolysis of water and as a result oxygen is released, i.E. This process is oxygenic.
- Photolysis of water:

$$H_2O \text{ (+ light energy)} \text{---------} 2e^- + 2H^+ + 1/2O_2$$

- ATP can also be produced from electrons generated by photosystem I, if they are not used in NADPH production.

AUTOTROPHY I

- The primary electron acceptor of photosystem I can pass electrons through cytochromes and cycles them back to the reaction centre of photosystem I, from where they came.
- During this, ATP is synthesized by cyclic photophosphorylation.

AUTOTROPHY II

- **CHEMOLITHOTROPHIC AUTOTROPHY;**

- Chemolithotrophs use electrons obtained from reduced inorganic compounds as an energy source and inorganic inorganic carbon, usually CO2, as their source of carbon, which is fixed via the calvin cycle.
- They possess electron transport systems, generate a proton-motive force and produce atp by oxidative phosphorylation.

AUTOTROPHY II

- Reducing power, NAD(P)H, is generated using reversed electron transport, as in bacterial photosynthesis. Reversal of electron transport is driven by ATP or the protonmotive force, and electrons are provided by available inorganic donors.
- The ATP and NAD(p)H generated are then used in CO2 fixation.
- Inorganic energy sources utilized include reduced forms of nitrogen, sulphur, iron and hydrogen

AUTOTROPHY II

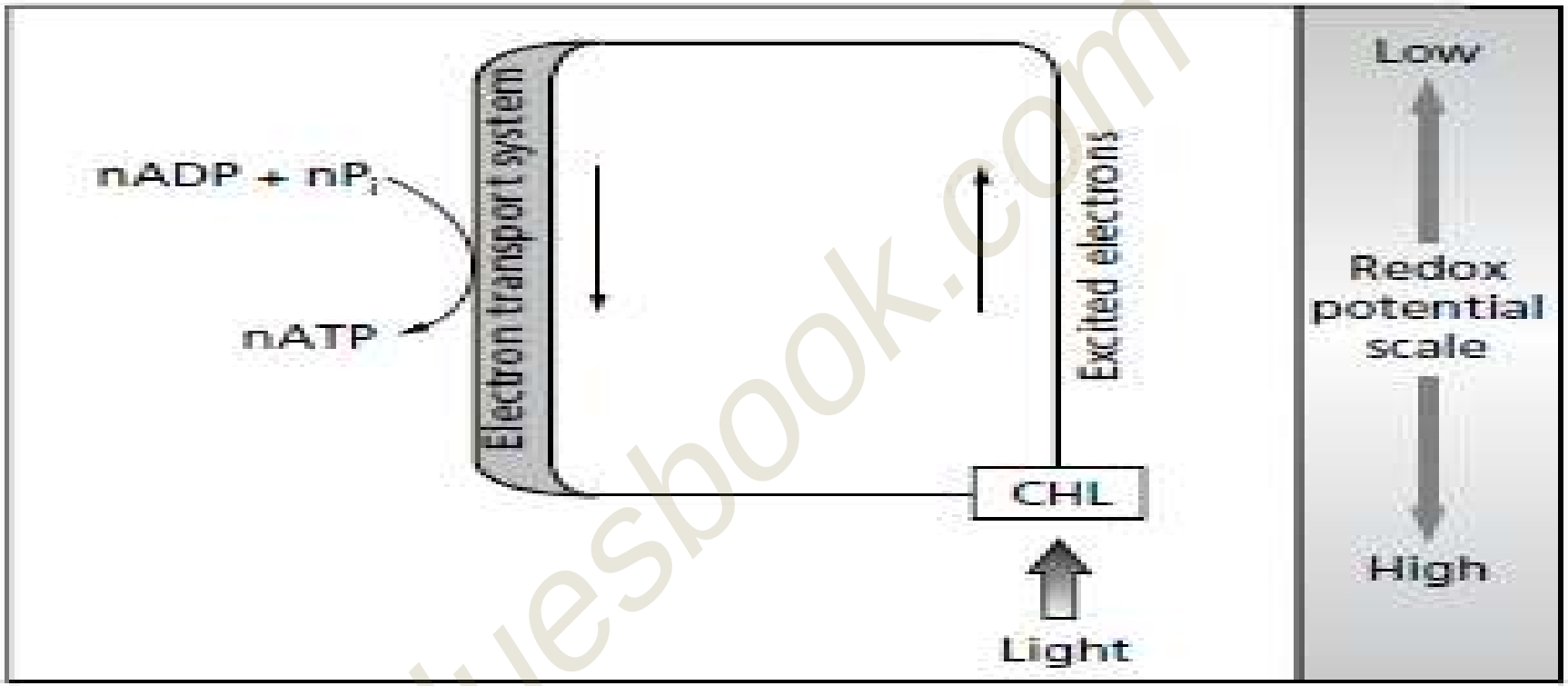
- The ATP requirements for this process are considerable and place an additional burden on an inefficient energy-generating system.
- *Nitrosomonas:*

  2NH4 + + 3O2Æ2NO2 - (nitrite) + 2H2O + 4H+

- *Nitrobacter:*

  2NO2 - +O2Æ2NO3 - (nitrate)

- These organisms are widespread in water and particularly in soil where they have a significant impact on soil fertility.



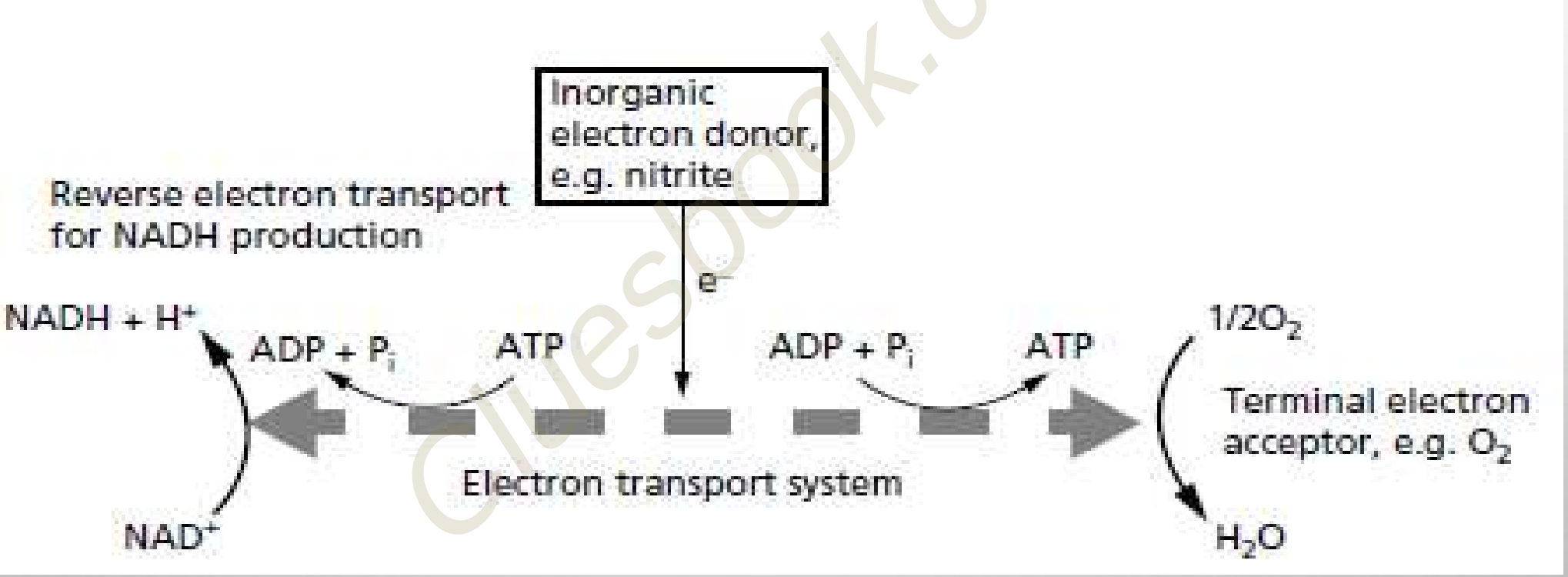
Photosystem I

A simplified scheme for cyclic photophosphorylation.

AUTOTROPHY II

- These organisms are widespread in water and particularly in soil where they have a significant impact on soil fertility.
- Nitrate, the end-product of nitrification, is water-soluble and rapidly leached from soils, whereas ammonia is cationic and adsorbs readily to negatively charged clay minerals.
- Nitrification is therefore an undesirable process in agriculture, especially in areas with high rainfall.

AUTOTROPHY II
REVERSE ELECTRON TRANSPORT



AUTOTROPHY II

- The most common chemoautotrophic energy sources containing reduced forms of sulphur are hydrogen sulphide (H2S), elemental sulphur (S0) and thiosulphate (S2O3 2-).
- Sulphate (so4 2-) is the end-product of sulphur oxidation.

Thiosulphate: S2O3 2- + 2O2 +H2O ----------2(SO4) 2- + 2H+

Free sulphur: 2SO + 2H2O + 3O2 ---------- 2SO4 2- + 2H+

AUTOTROPHY II

➢ **CARBON DIOXIDE FIXATION (ASSIMILATION) VIA THE CALVIN CYCLE;**

- The calvin cycle is essentially divided into three phases:

1. Carboxylation
2. Reduction
3. Regeneration

- Initial carboxylation involves the addition of CO2 to a C5 acceptor molecule, ribulose 1,5-bisphosphate (rubp), by rubp carboxylase. The resulting C6 product is immediately split into two C3 units, forming two molecules of 3-phosphoglycerate (PGA).

AUTOTROPHY II

- In the following reduction phase, a phosphate group (from ATP) and hydrogen (from NADPH) are added to each PGA molecule to produce GAP.
- The cycle must complete three turns, fixing a co2 molecule each time, in order to produce a net gain of one gap (c3), which can then enter anabolic metabolism.
- $3CO_2 + 9ATP + 6NADPH + 6H^+ + 6H_2O$ ---------- GAP + 9ADP + 6Pi + $6NADP^+$

METABOLIC REGULATION I

- Microorganisms naturally have tight control over their metabolism,
- But this often needs to be overcome when they are used in industrial fermentations, e.G. In attempting to over-produce specific metabolites.
- Consequently, the study of metabolic regulation is of special importance in industrial microbiology.

METABOLIC REGULATION I

➢ **MODIFICATION OF ENZYME ACTIVITY**

- This involves the stimulation or inhibition of specific enzymes to rapidly change the flow of carbon within a pathway.
- **Physical separation of enzyme and substrate;** the simplest mechanism for the regulation of enzyme activity merely involves controlling the concentration of available substrate and coenzymes.
- The most obvious mechanism is the control of substrate entry into a cell across the cytoplasmic membrane.

METABOLIC REGULATION I

- In eukaryotic cells, which have numerous membranebound organelles, compartmentalization can be used to control the transport of metabolites and coenzymes between different regions of the cell.
- Compartmentalization also allows the simultaneous, but separate, operation and regulation of similar pathways.
- Prokaryotes lack organelles, but development of concentration gradients of metabolites and coenzymes, between different regions of the cytoplasmic matrix, may play a similar regulatory role.

METABOLIC REGULATION I

➢ **REGULATORY ENZYMES**

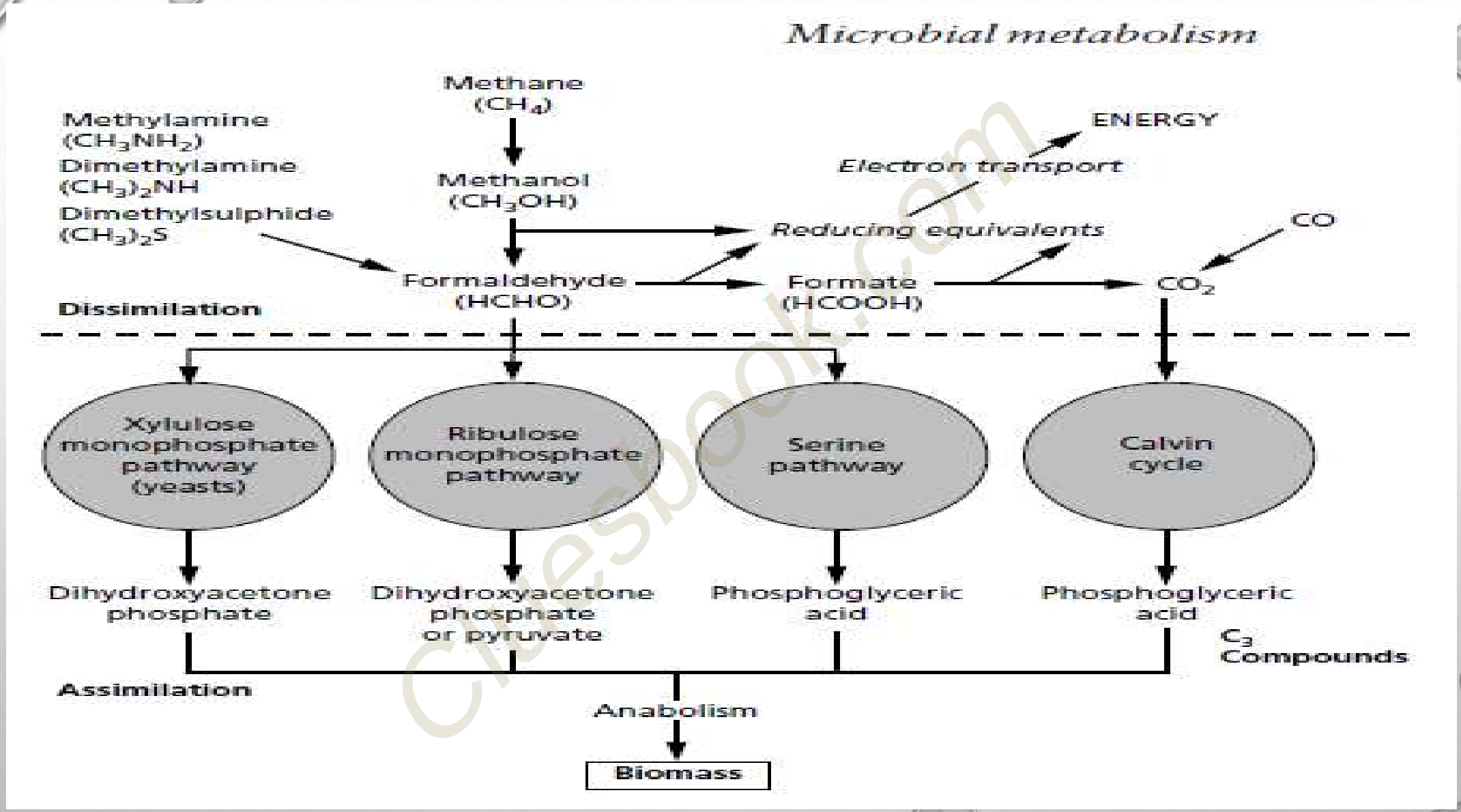
- Many metabolic pathways contain **regulatory enzymes** whose activity can be adjusted to provide a mechanism for fine tuning the flux of carbon through a pathway.
- Some of these enzymes are **allosteric, usually composed** of several subunits, and do not follow michaelis– menten kinetics.

METABOLIC REGULATION I

- Their activity can be altered by a small molecule called an effector or modulator.
- This effector molecule binds reversibly through non-covalent forces to the regulatory site on the enzyme, causing the enzyme to change shape. As a result, the catalytic site is altered.
- Positive effectors bring about an increase in enzyme activity and negative effectors cause enzyme inhibition.

METABOLIC REGULATION I

- Every metabolic pathway has at least one **pacemaker enzyme that catalyses the slowest or rate-limiting reaction.**
- This is usually the first step or first unique step of the pathway and it is often irreversible.
- It controls the overall rate of flux through the pathway and is normally inhibited by the product of the last step in the pathway, which is called feedback inhibition or end-product inhibition.



METABOLIC REGULATION I

- End- products of each branch often inhibit both the enzyme at the start of their respective branch and the pacemaker enzyme at the beginning of the main pathway.
- In some branched pathways, several isoenzymes (isozymes) may control the first step, rather than having a single initial pacemaker enzyme.
- This provides a more subtle means of control for the flow of carbon, as each isozyme can be controlled by a different end-product.

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

- 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

- 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

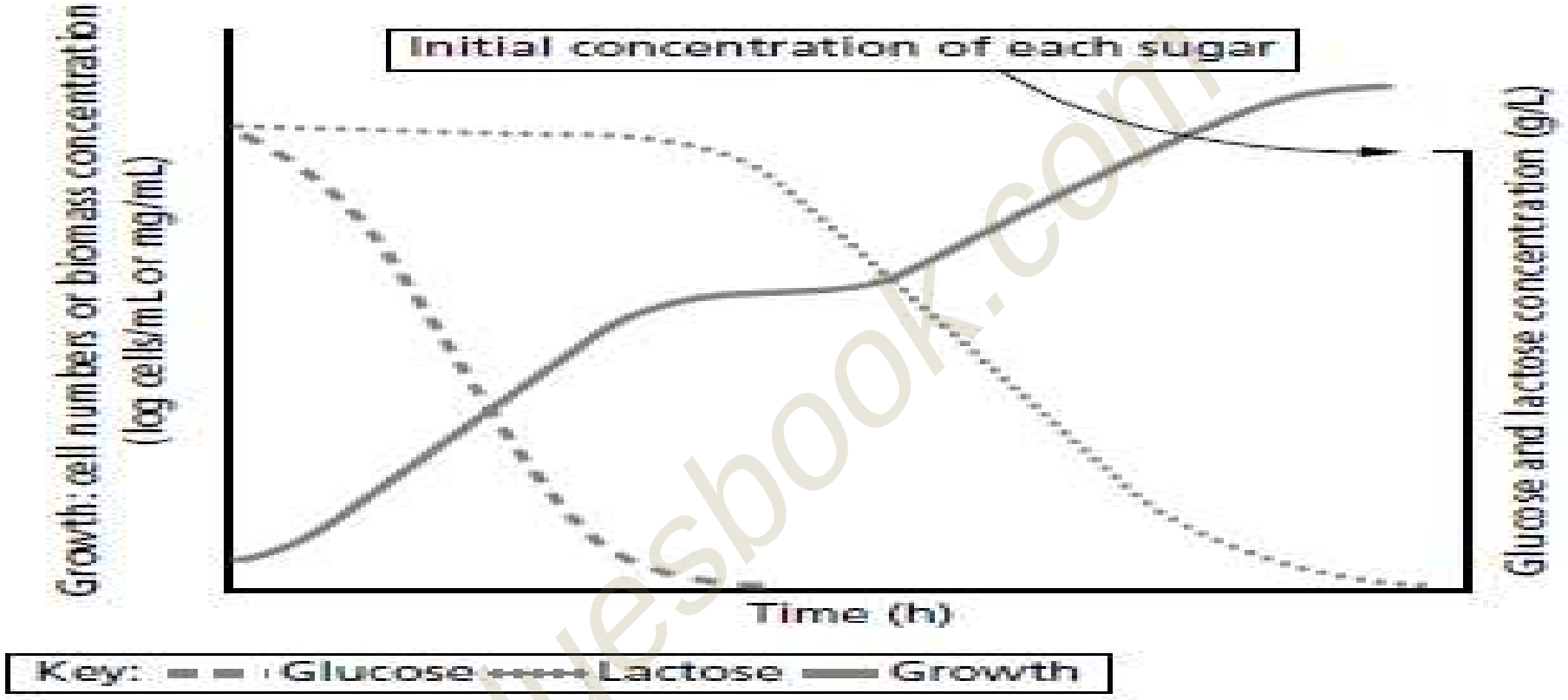
- In some cases, it is due to glucose-induced inactivation of key enzymes; for others, repression results from rapid glucose uptake, which causes the intracellular level of cyclic adenosine monophosphate (camp) to fall.
- This important compound is involved in the positive control of many catabolic enzymes,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

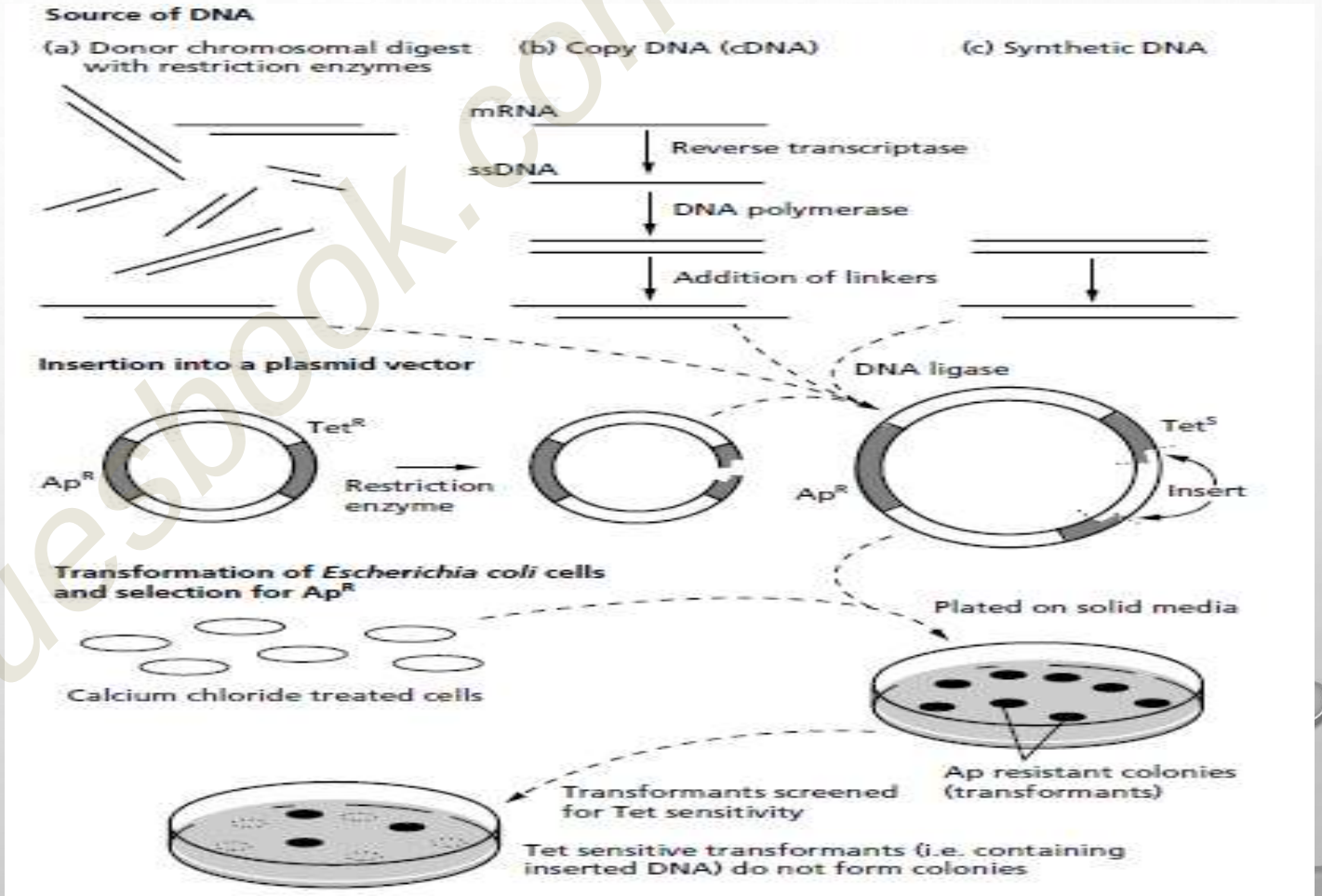
- Here the differences between prokaryotic and eukaryotic gene expression must be taken into account.
- Eukaryotic genes contain non-coding regions, introns, which would obviously cause problems if the gene was directly transferred to a prokaryotic system.
- However, introns are excised during normal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

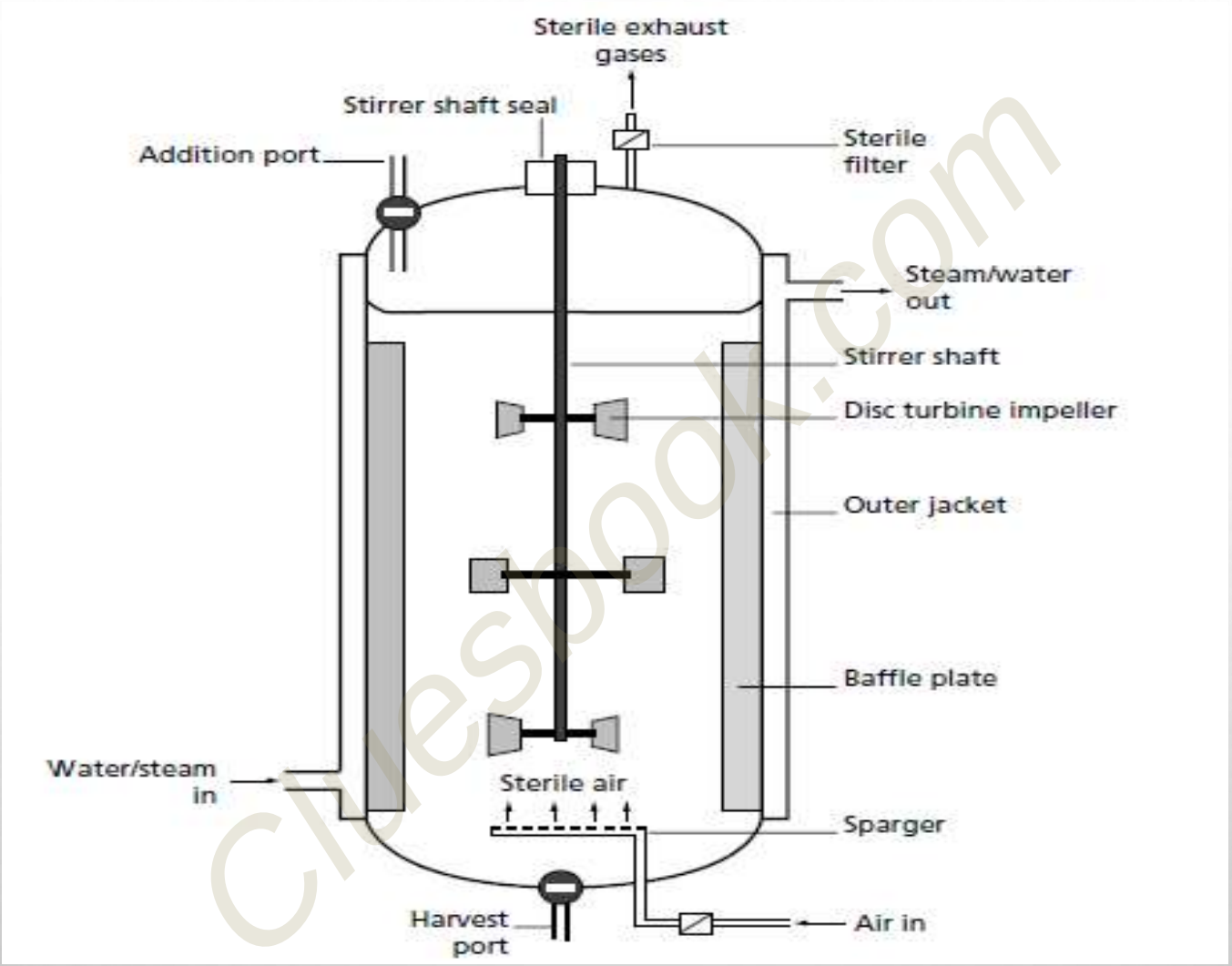


Diagram of a stirred tank reactor.

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,**

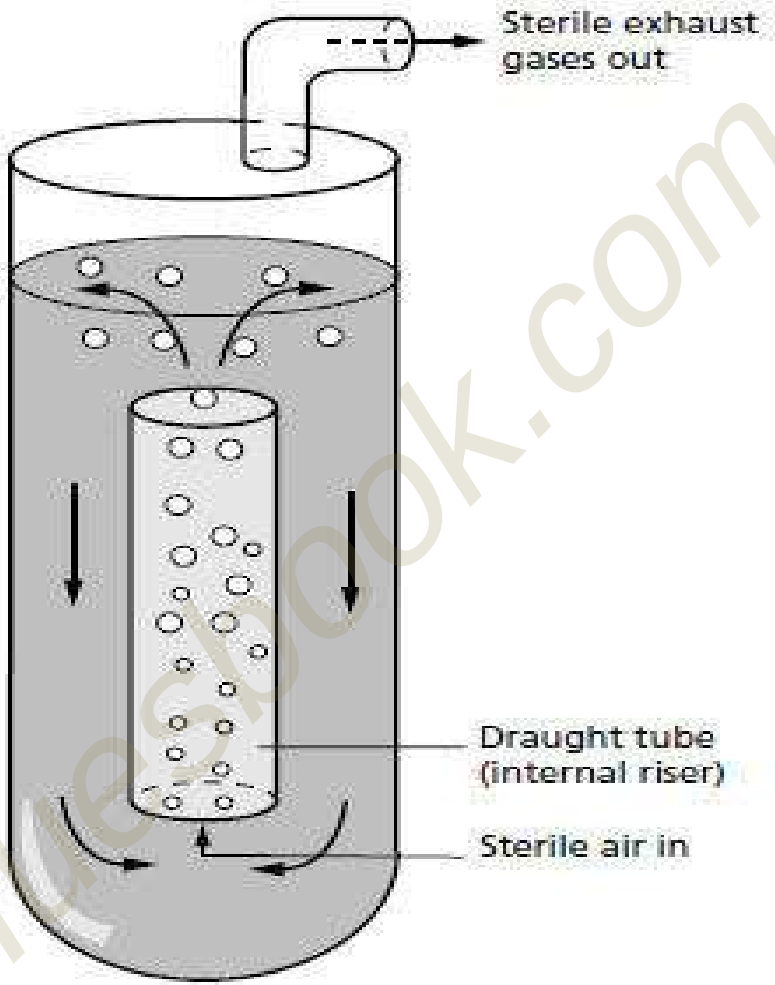
- **Such as airlift fermenters, have no** Moving parts and use the expansion of compressed gas to bring about the mixing These systems have Lower energy requirements and create less shear than STRs.
- Liquid movement is initiated by the injection of Compressed air at the bottom of the internal or external 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

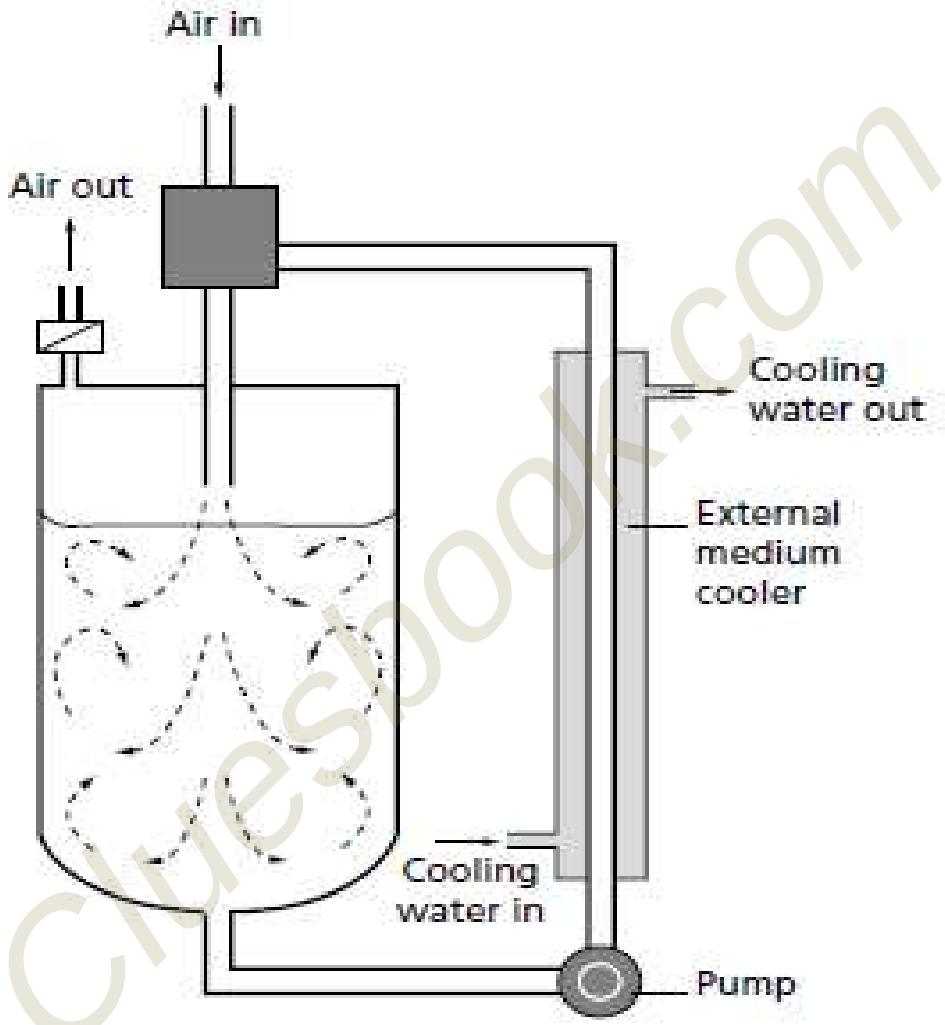
- Even large fermenters do not require internal cooling coils as a jacket can normally provide sufficient heat transfer, due to the rapid movement of fluid within the vessel.



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

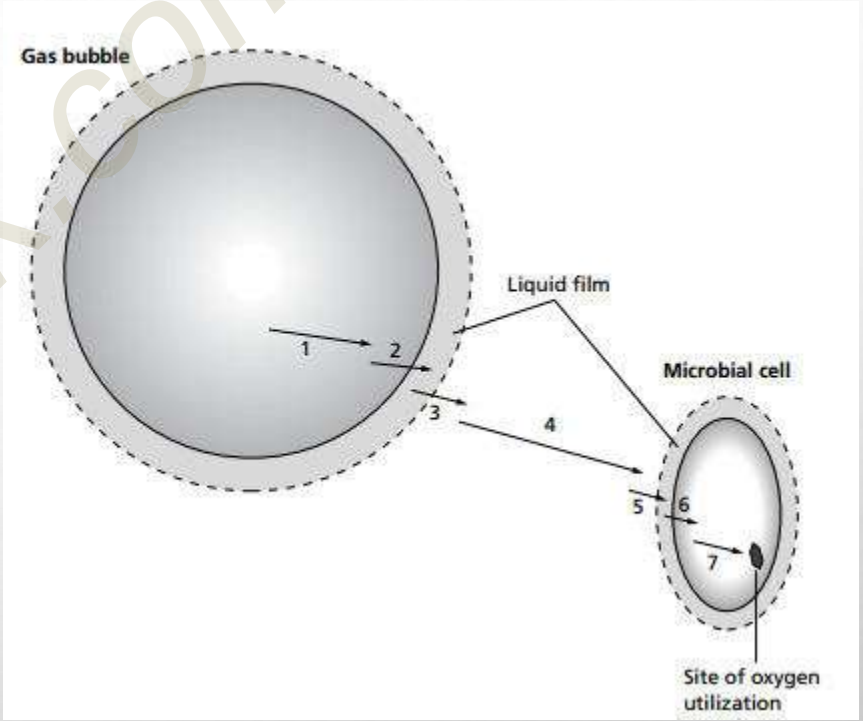
•The two steps associated with an oxygen mass balance are the rate at which oxygen can be delivered to the biological system (oxygen transfer rate, OTR) and the rate at which it is utilized by the microorganisms (critical oxygen demand).

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 $K_l a$ 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.

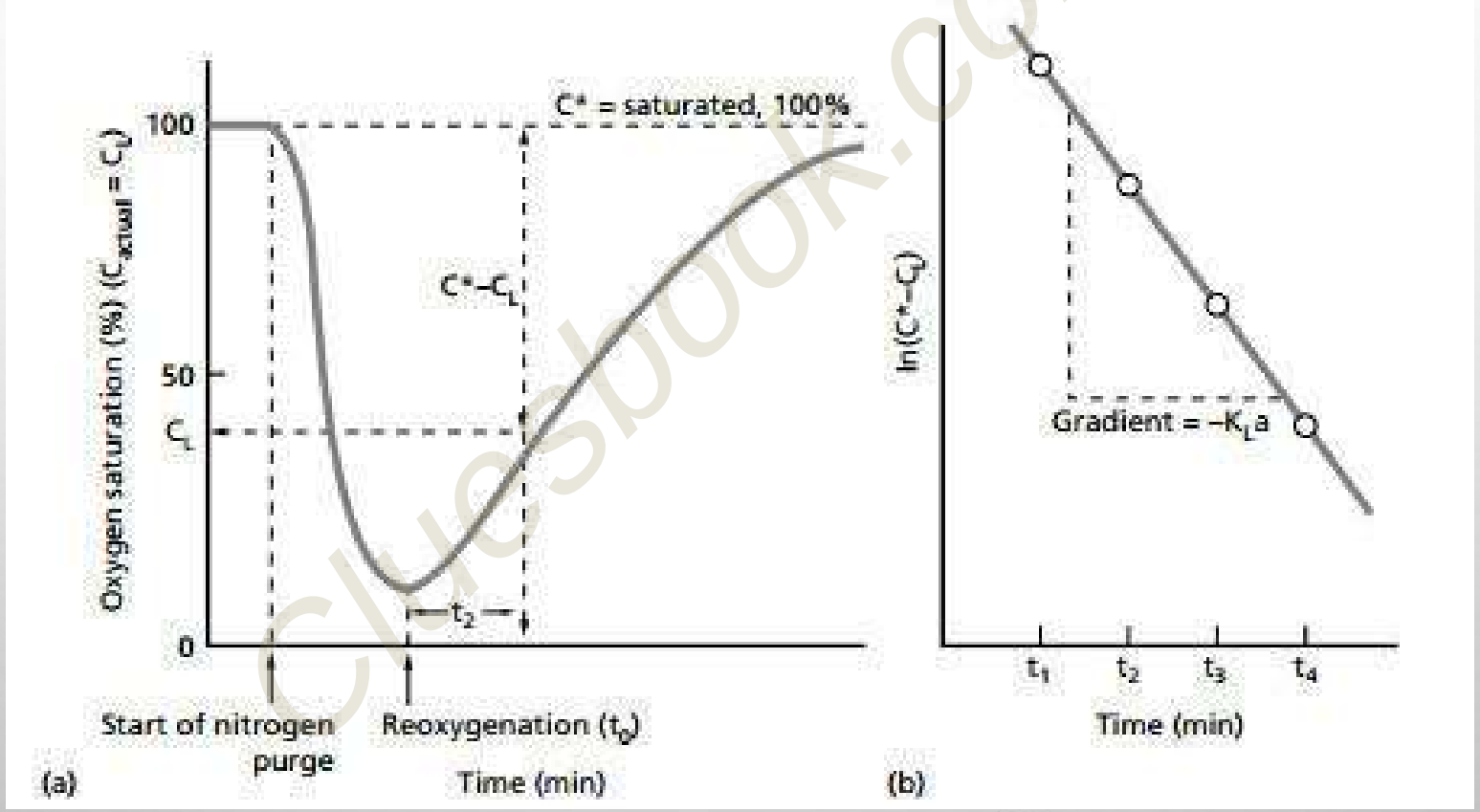
FERMENTER CONTROL AND MONITORING I

- 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

Sensor	Measurement		
	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 O2 and CO2 are determined using O2 and CO2 electrodes.

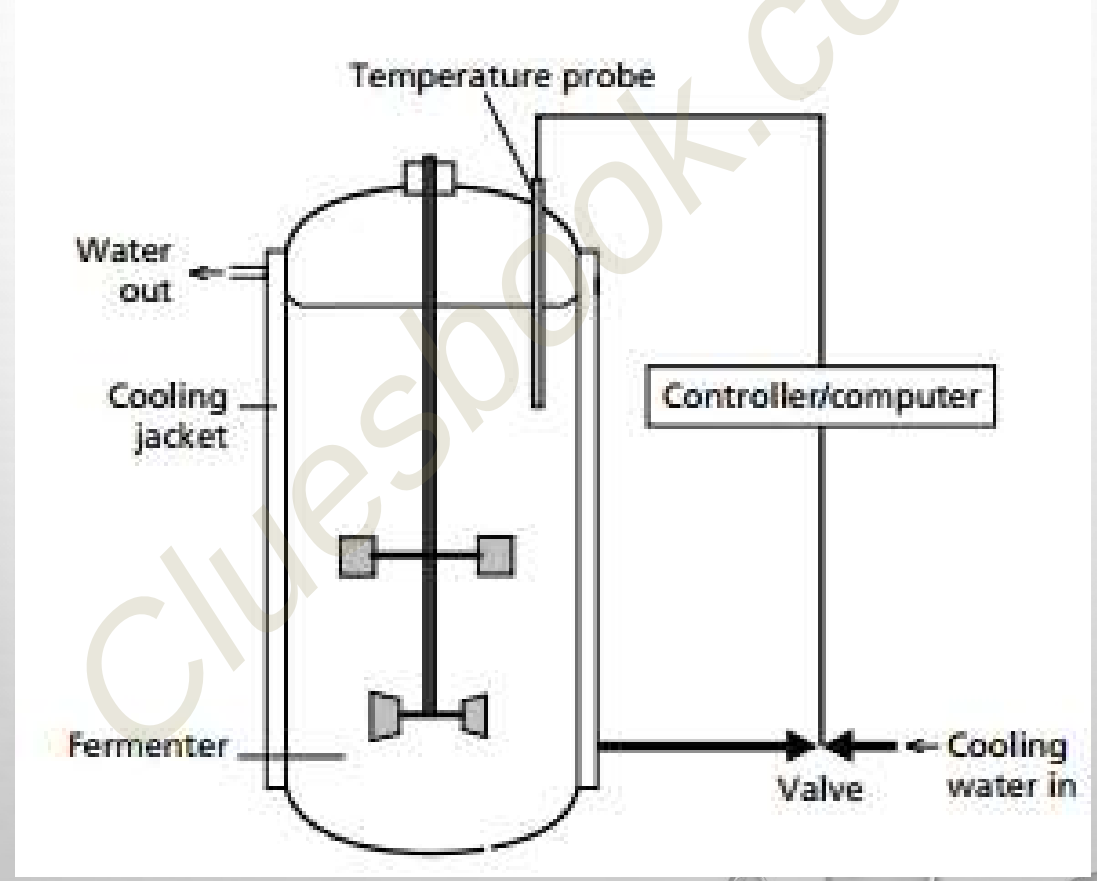
FERMENTER CONTROL AND MONITORING I

•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.

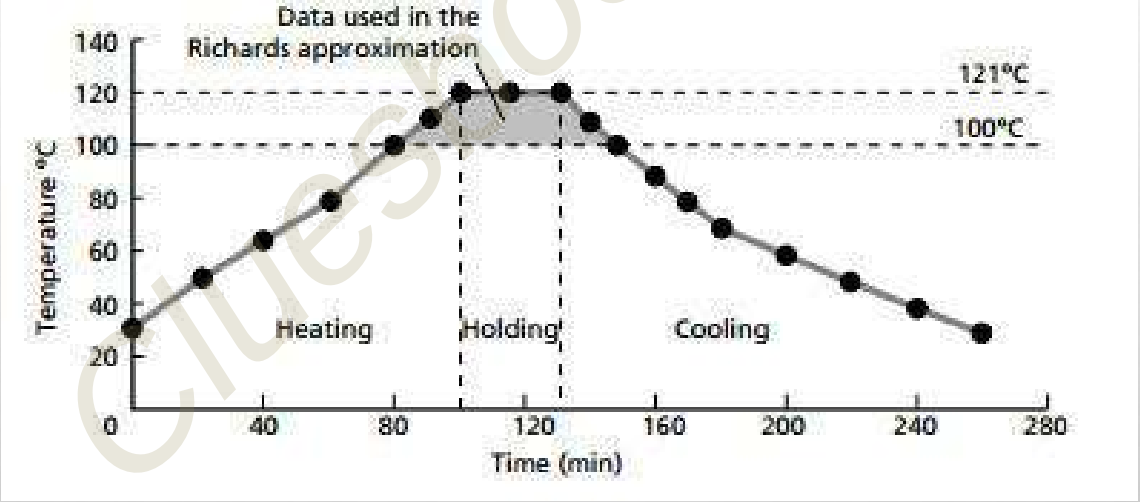
•The substrates used are often cereal grains, bran, legumes and lignocelluloses materials, such as straw, wood chippings, etc.

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.

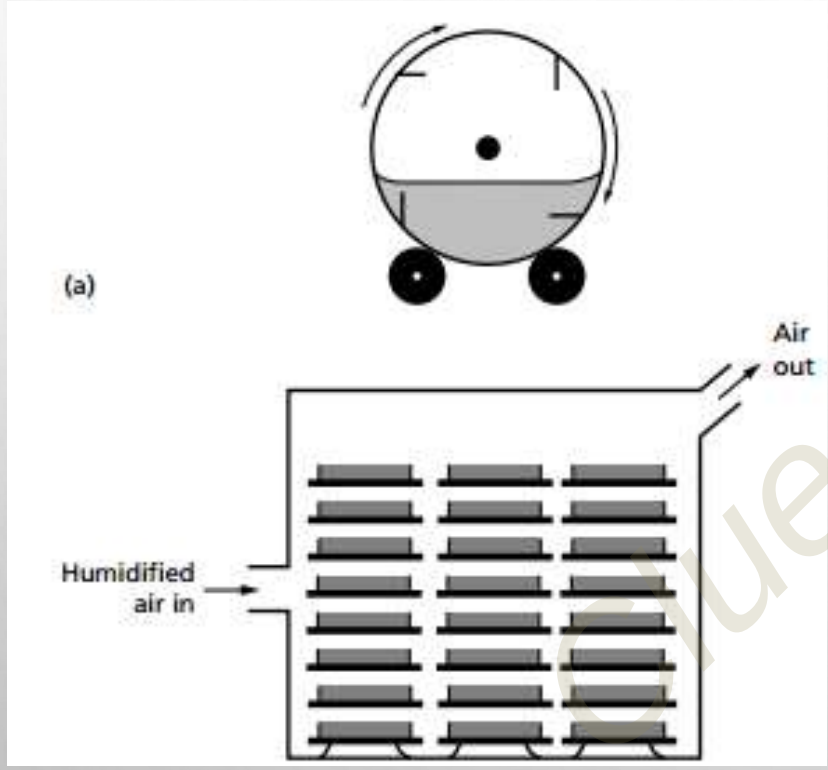
BIOREACTORS USED FOR SOLID-SUBSTRATE FERMENTATION

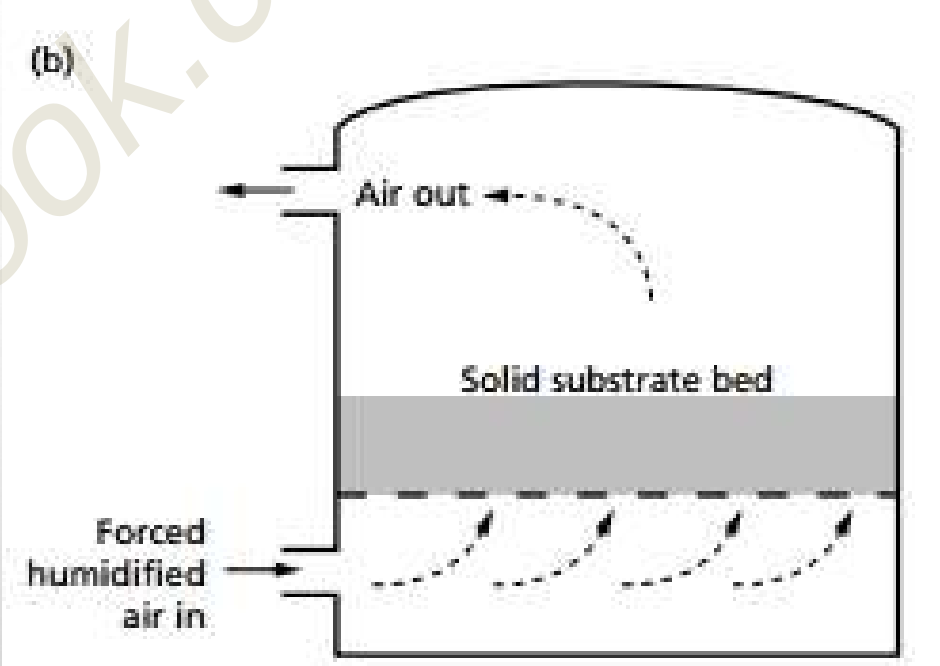
•Some processes do not require bioreactors, they simply involve spreading the substrate onto a suitable floor.

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.

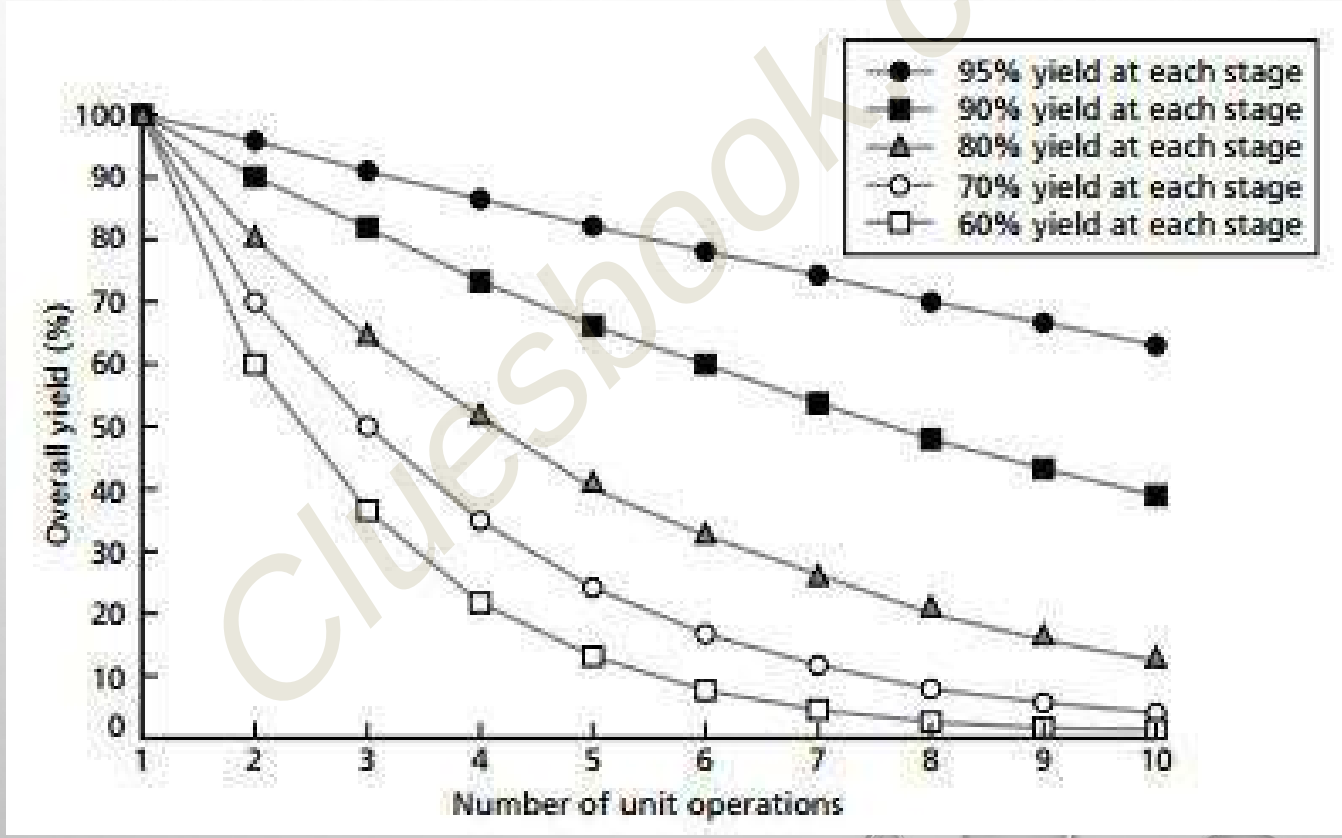
DOWN STREAMING PROCESS II

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

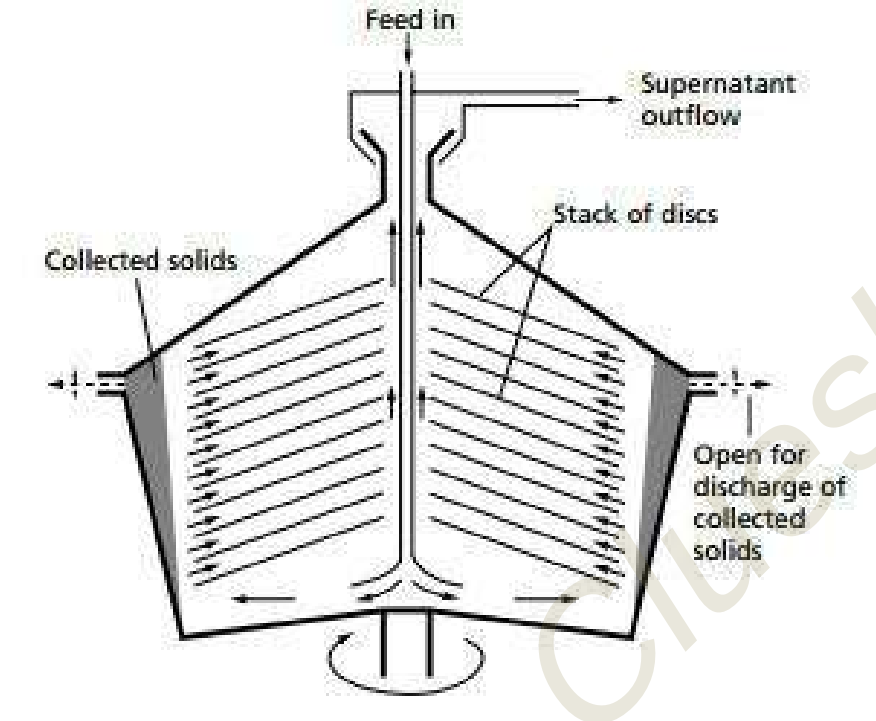
CELL HARVESTING II

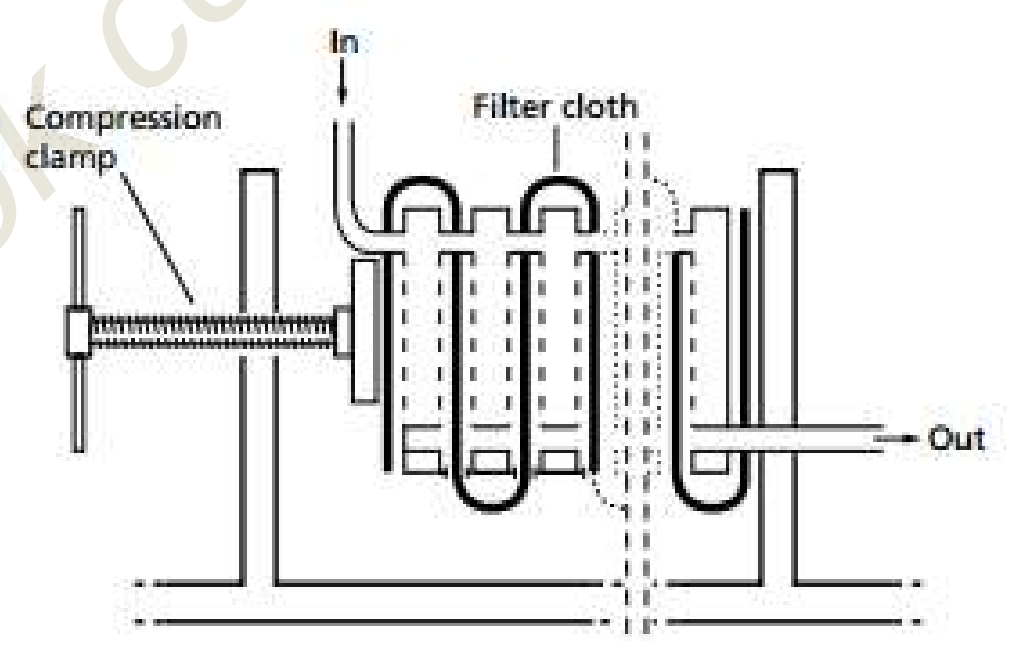
Multichamber bowl centrifugesconsist of a bowl that is divided by vertically mounted interconnecting cylinders and are capable of operating at 5000–10 000g

CELL HARVESTING II

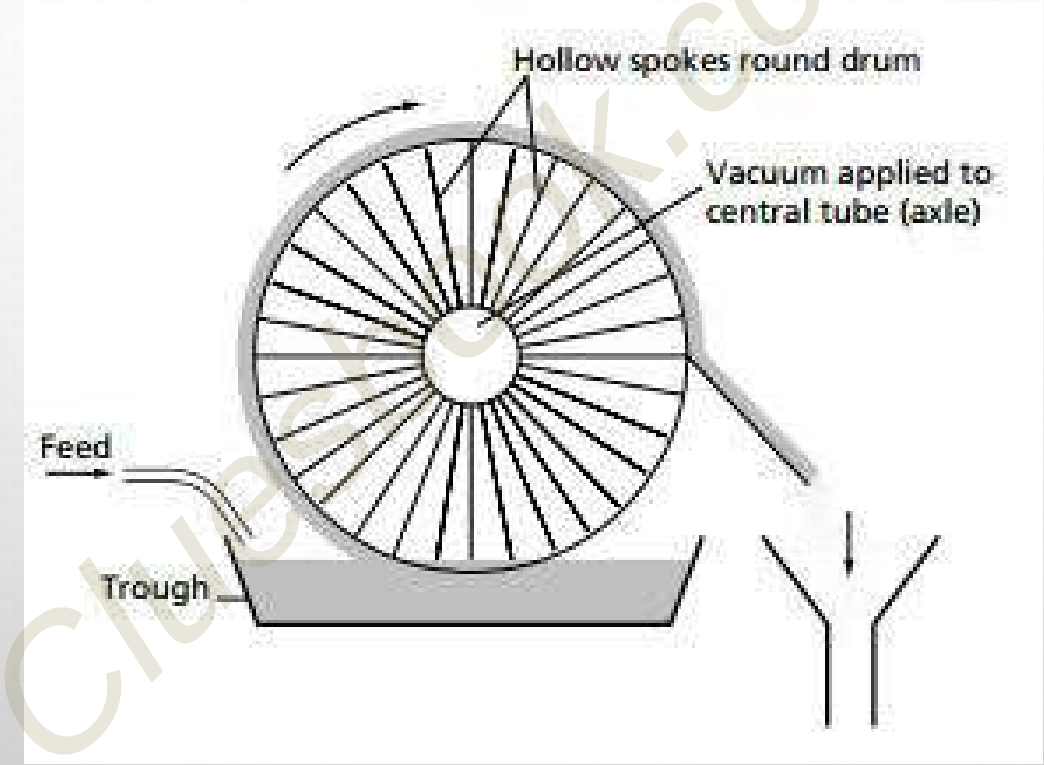
Disc stack centrifugescan operate at 5000–13 000g.
The centrifuge bowl contains a stack of conical discs whose close packing aids separation

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

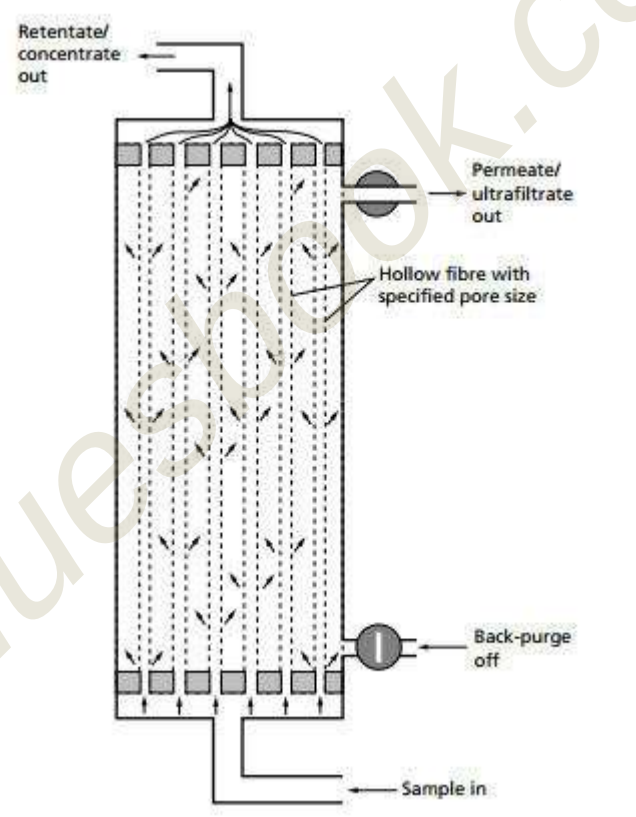
Solids accumulate on the filter medium as liquid filtrate is drawn, under vacuum, through the filter medium into the hollow drum to a receiving vessel.

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

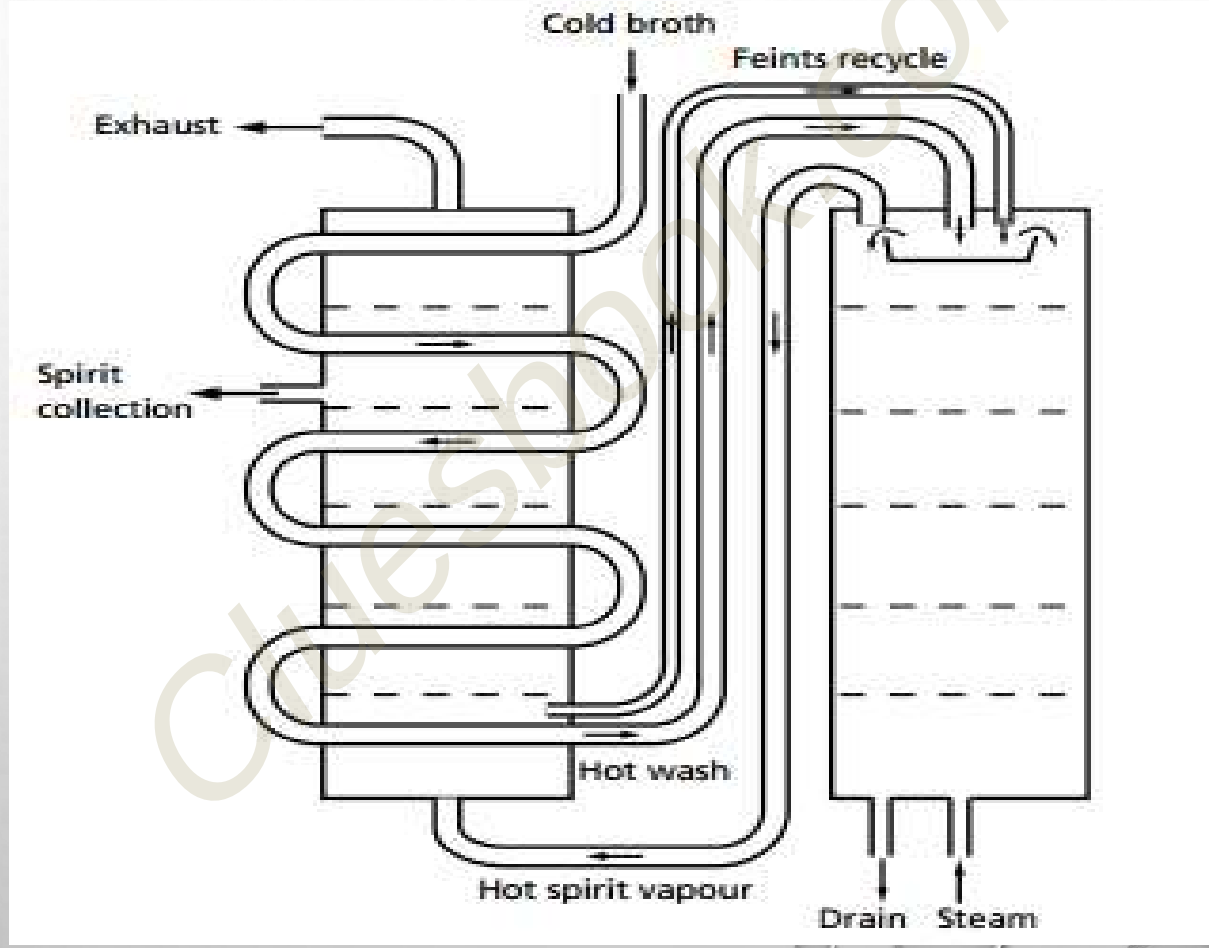
membrane separation techniques are primarily used for the removal of low molecular weight solutes and inorganic ions from a solution

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

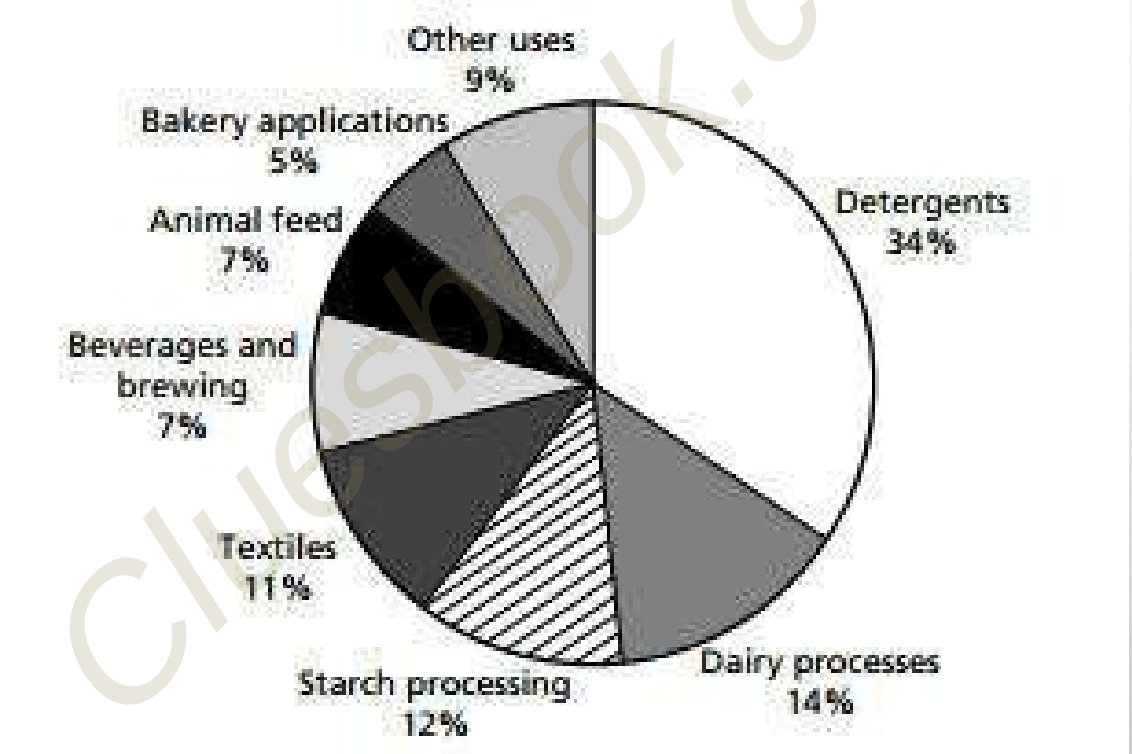
MICROBIAL ENZYMES AND THEIR APPLICATIONS I

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	Bacteriophage T3/T7	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, CO2and H2;
2 butanol–isopropanol (Clostridium butylicum), additional products: butyric acid, acetic acid, CO2 and H2; and
3 butyric acid–acetic acid(Clostridium butyricum), additional products: CO2and H2

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

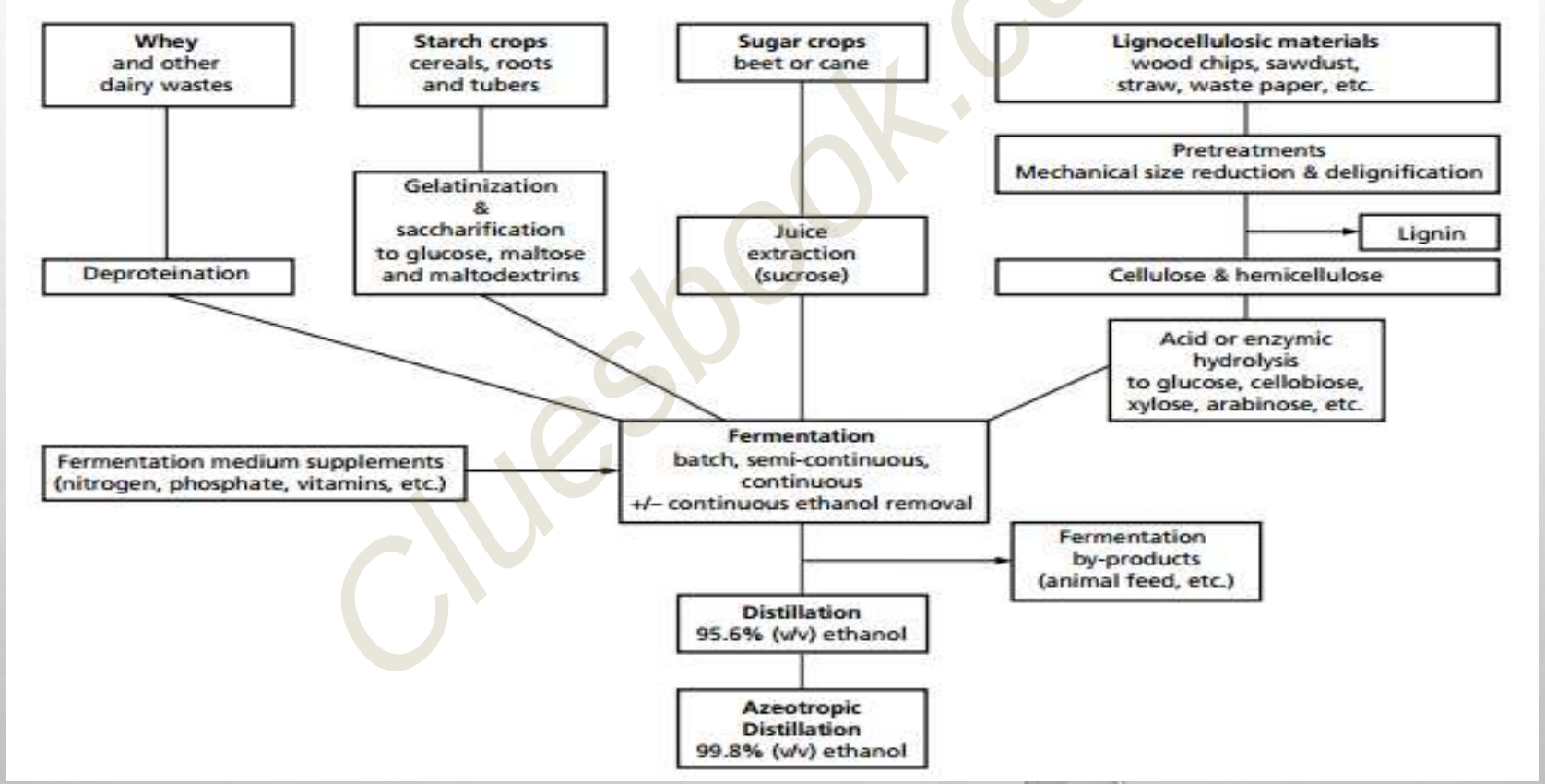
FUELS AND INDUSTRIAL CHEMICALS I

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.

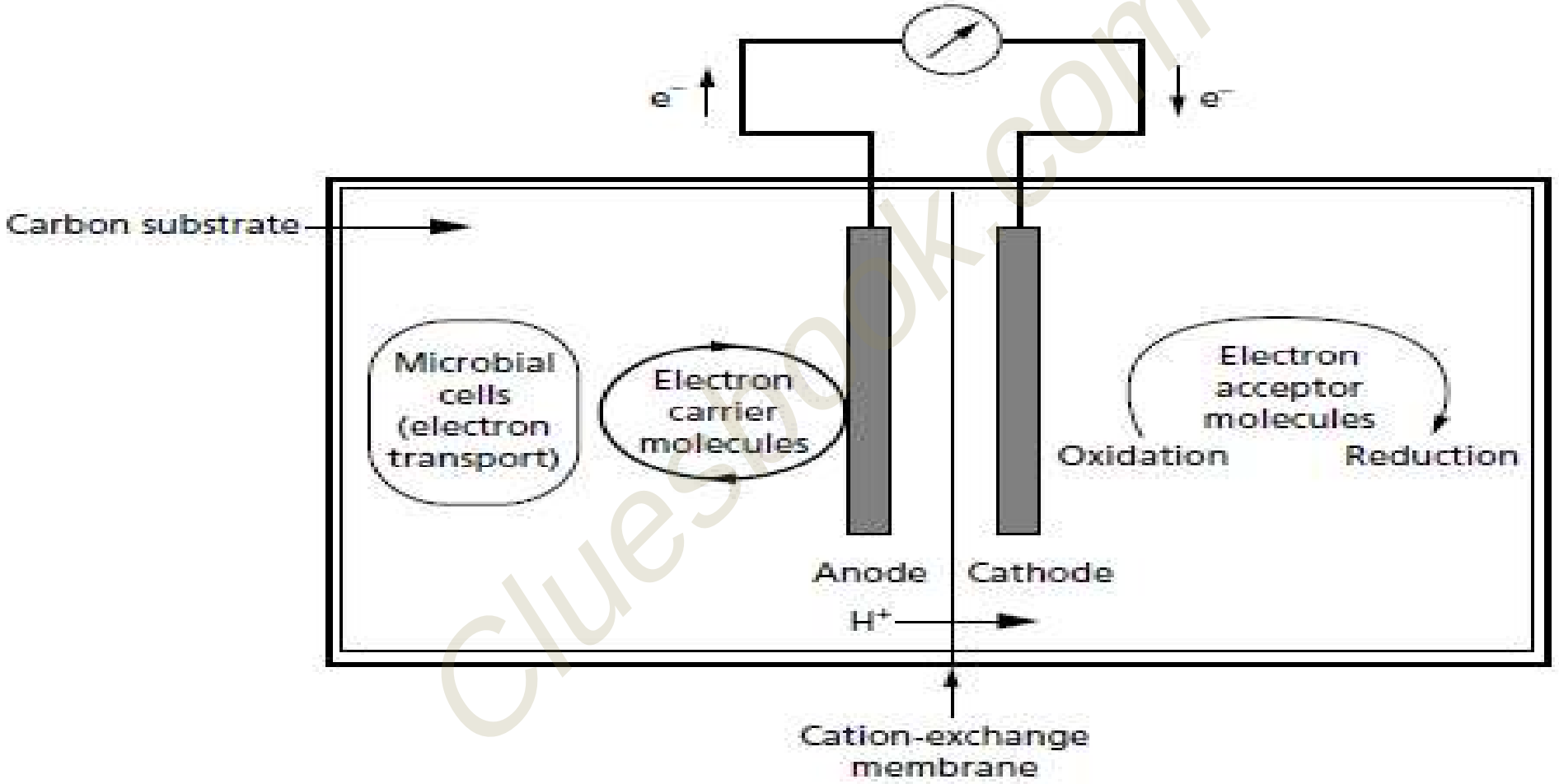
ELECTRICITY

- 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

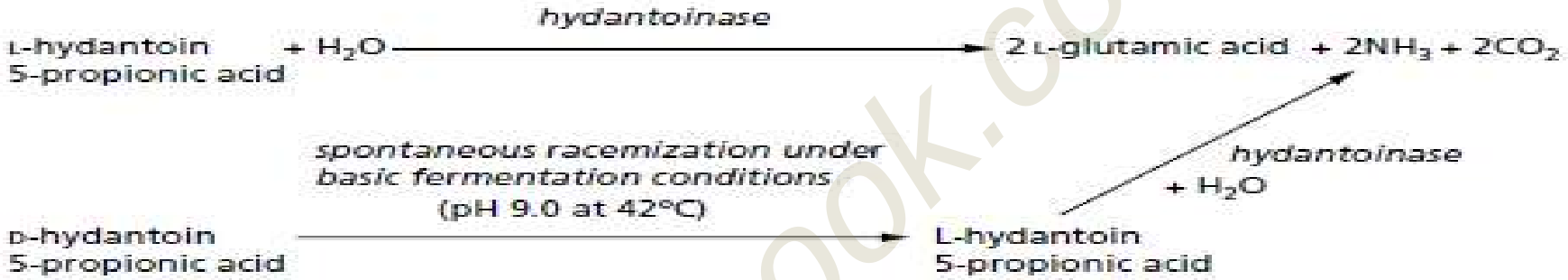
➢ CITRIC ACID

- 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.

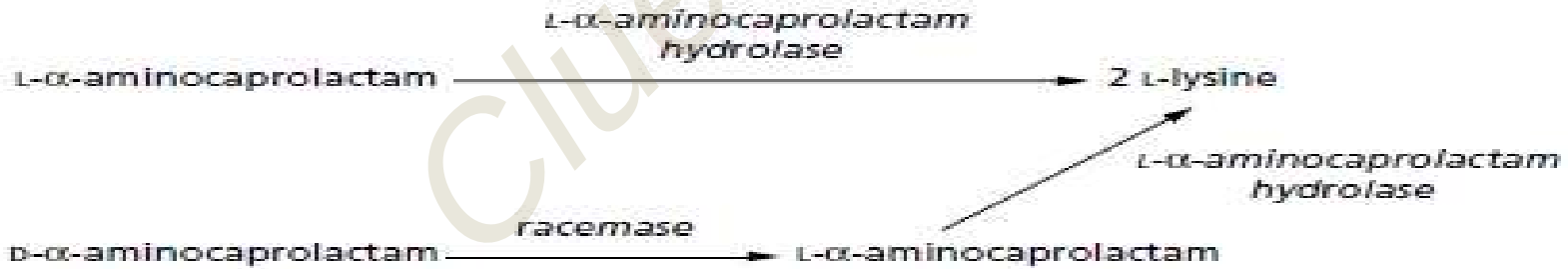
(a) L-Glutamic acid

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

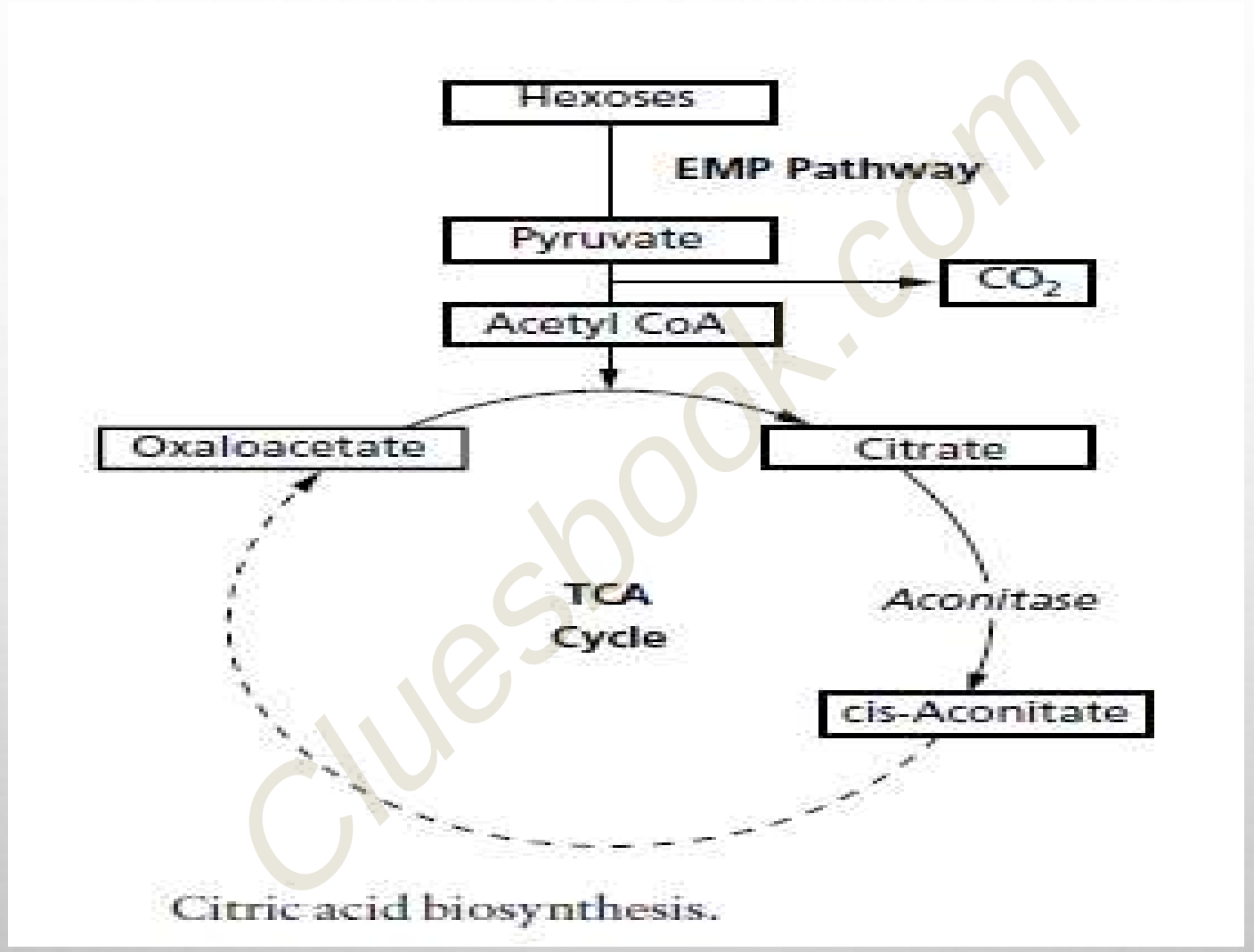
- The metabolic pathways involved in citric acid biosynthesis are the embden–meyerhof–parnas (EMP) pathway and the TCA cycle. *A. Niger also operates* the pentose phosphate pathway, which can compete with glycolysis for carbon units.
- The first stages of citric acid formation involve the breakdown of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

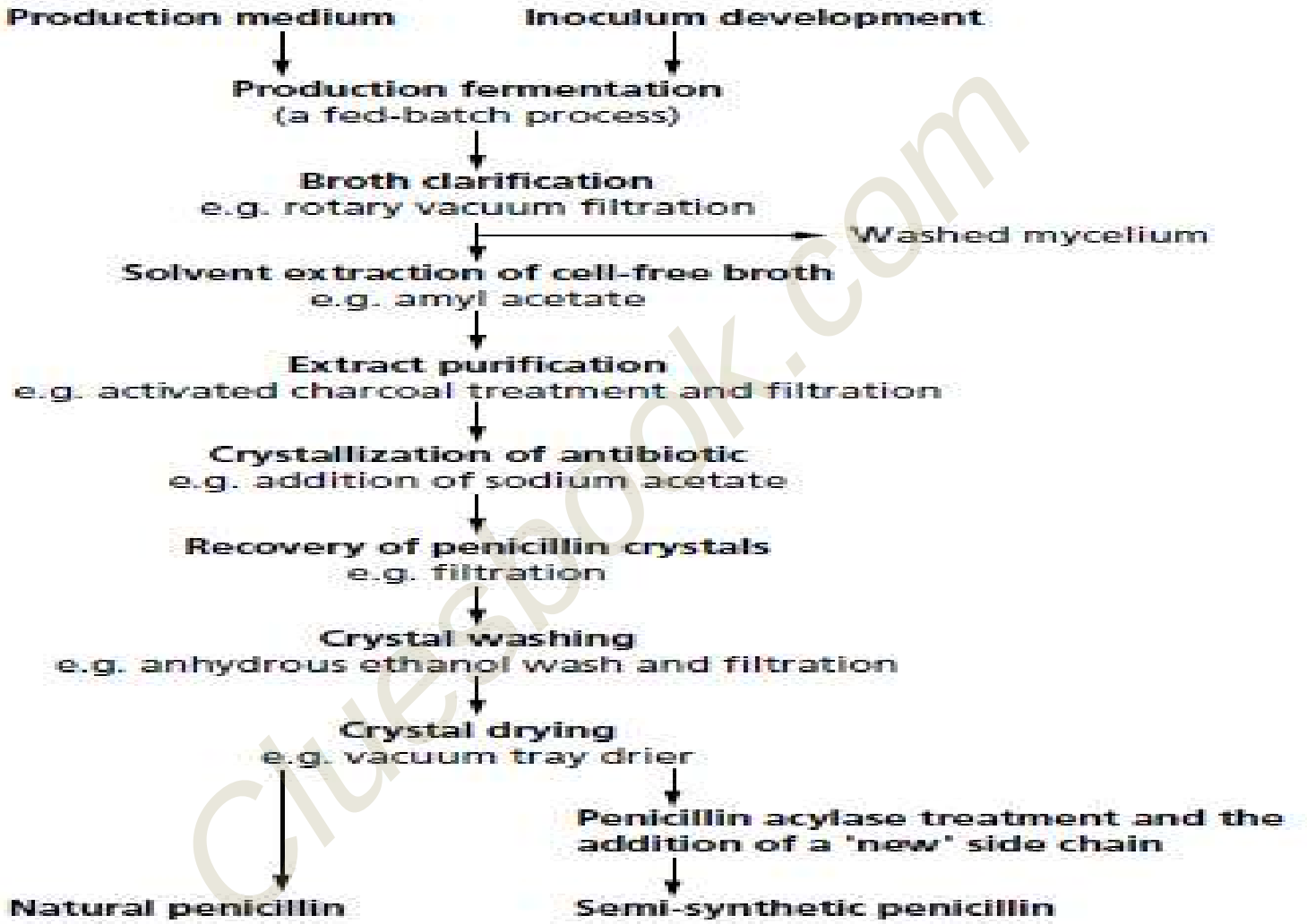
- The basic structure of the penicillins is **6-aminopenicillanic acid (6-APA), composed of a thiazolidine ring** fused with a b**-lactam ring whose 6-amino position** carries a variety of **acyl substituents.**
- **This blactam–** thiazolidine structure**,** synthesized from **l-aaminoadipate, l-cystine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

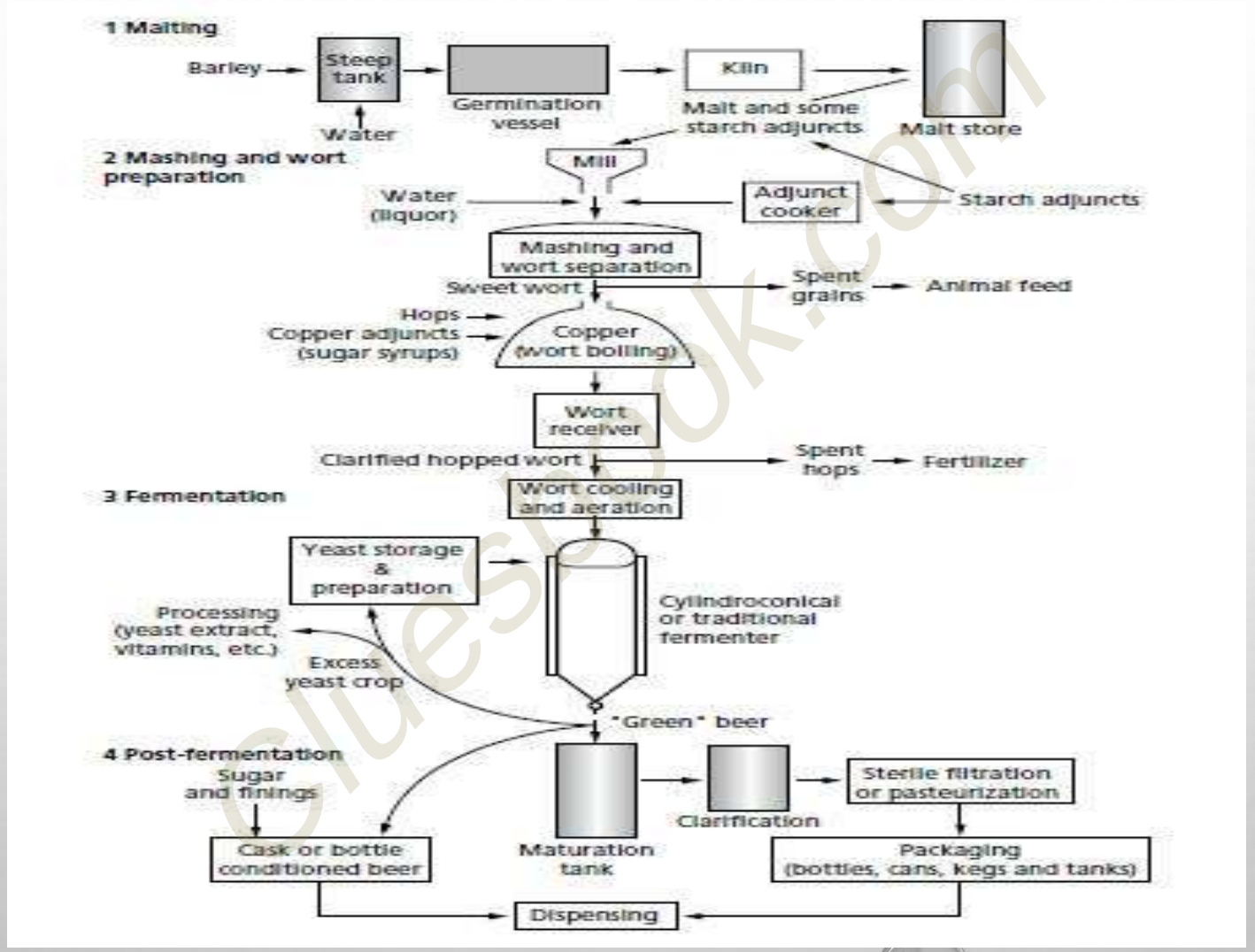
4. Post-fermentation treatments are conducted to mature

- 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.

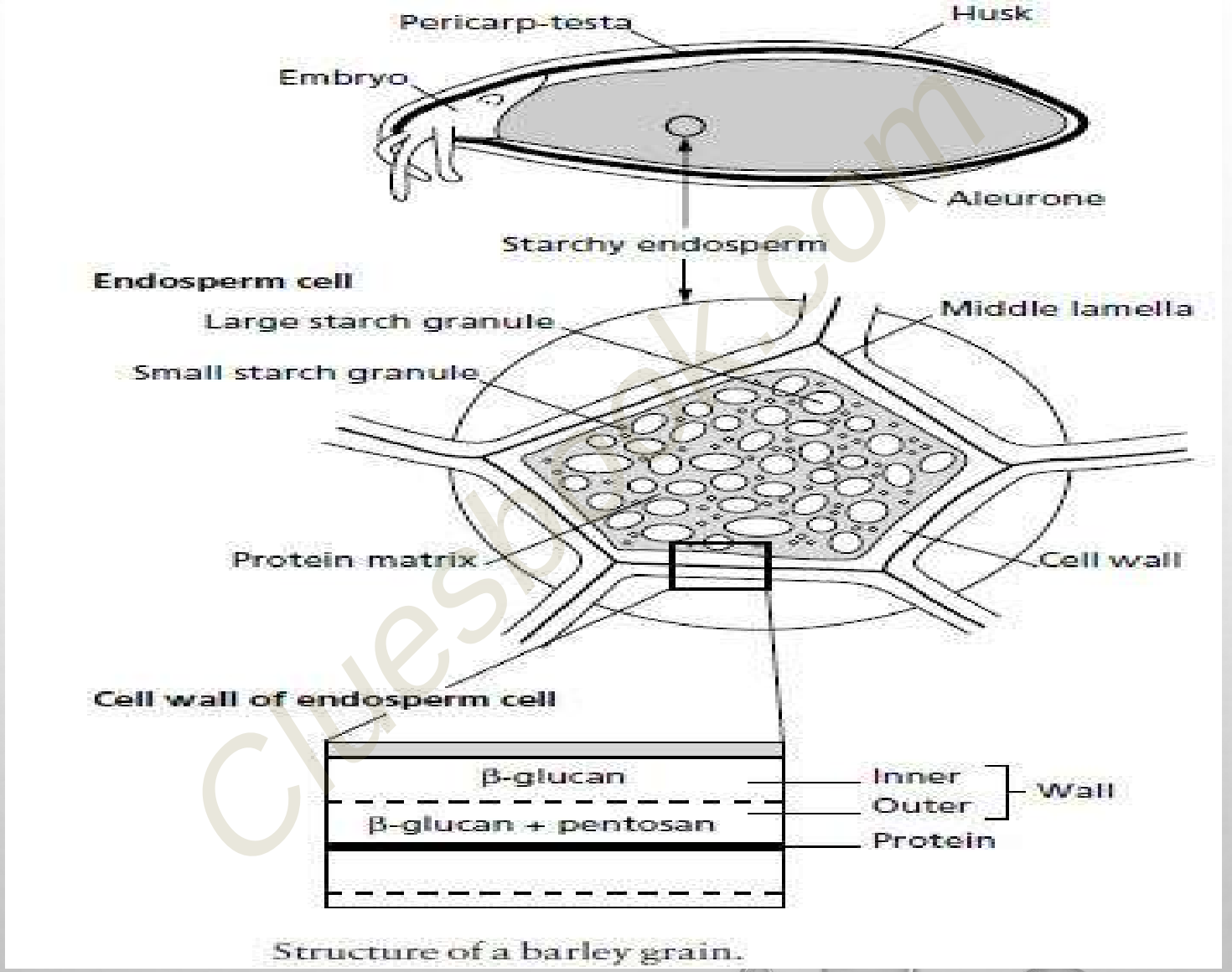


FOOD AND BEVERAGE FERMENTATIONS II

- 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

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

FOOD ADDITIVES AD SUPPLEMENTS I

The term beer is given to non-distilled alcoholic beverages made from partially germinated cereal grains, referred to as malt.

FOOD ADDITIVES AD SUPPLEMENTS I

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

FOOD ADDITIVES AD SUPPLEMENTS I

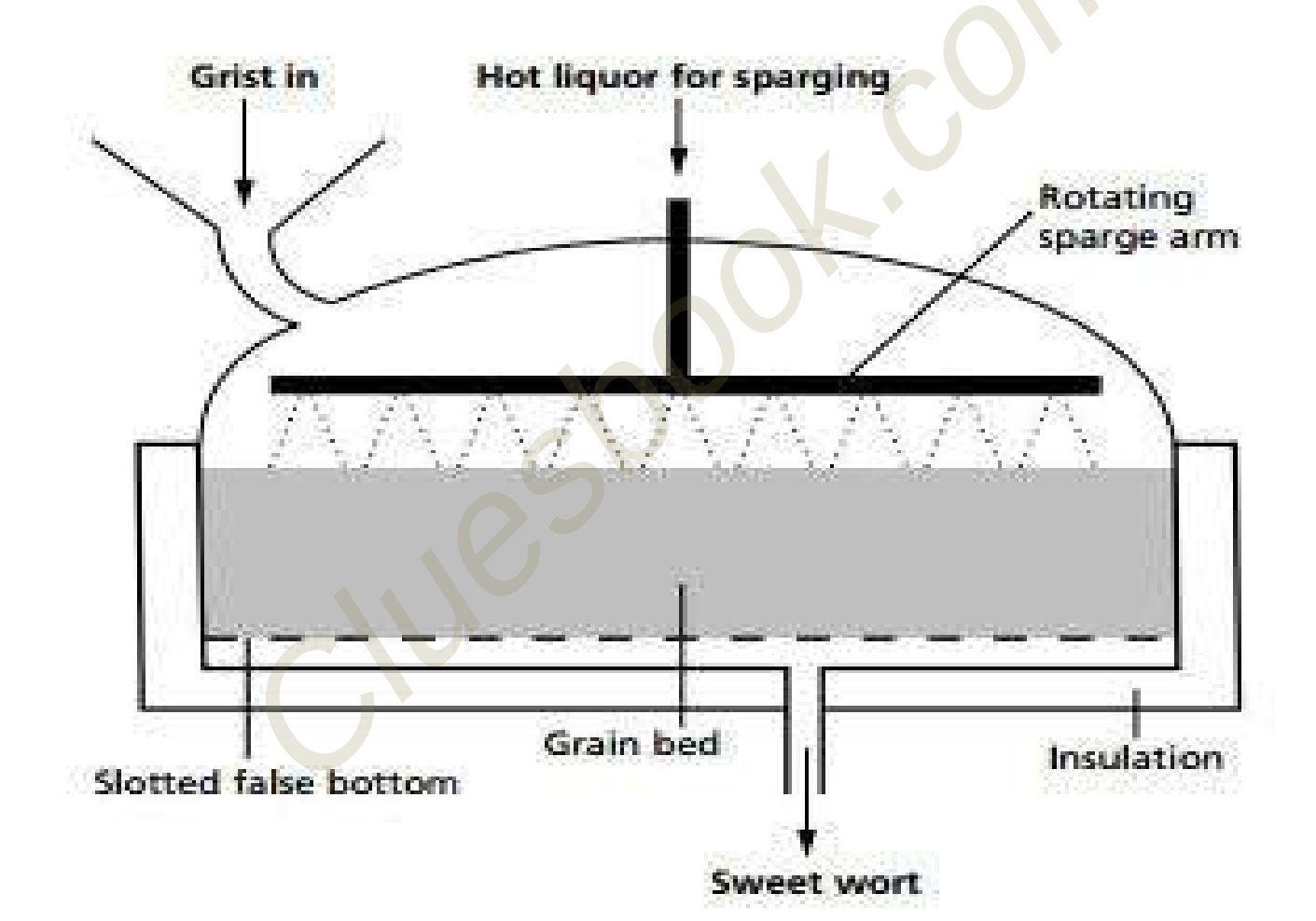
The objectives of wort preparation are to form, and extract into solution, fermentable sugars, amino acids, vitamins, etc., from malt and other solid ingredients.

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.

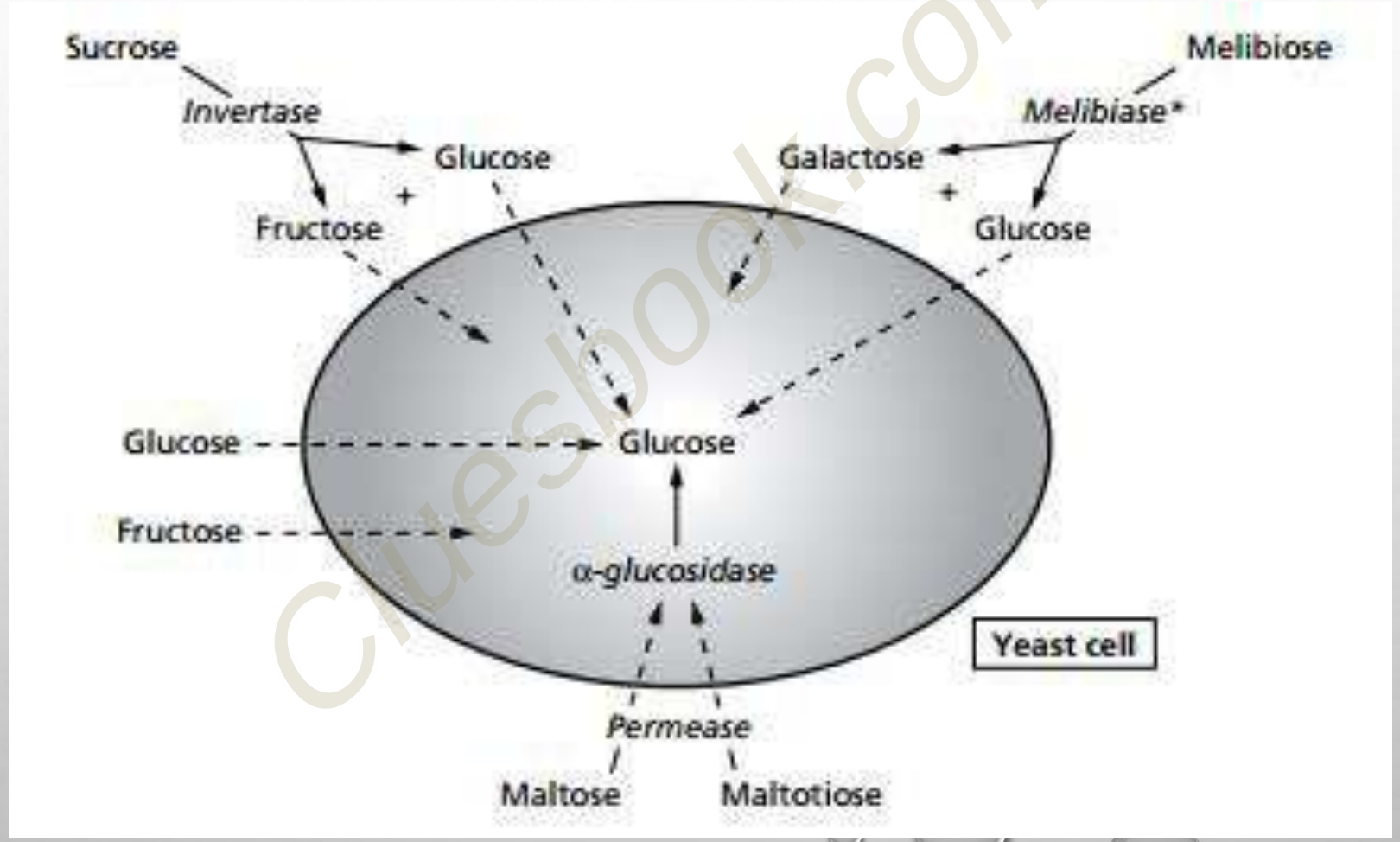
FOOD ADDITIVES AD SUPPLEMENTS I

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