Reference

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Fermentation

- Biochemist
  - The generation of energy by catabolism of organic compounds
- Indusrial microbiologist
  - Any process for the production of product by the mass culture of a microorganism

The range of Fermentation Process

- Microbial cells (or Biomass)
  - Baker's yeast, SCP(Single Cell Protein)
- Microbial enzyme
  - Protease, amylase, cellulase
- Microbial metabolites
  - Primary metabolites (primary products of metabolism)
  - Secondary metabolites (secondary products of metabolism)
- Transformation process
  - Steroids, antibiotics

Microbial Metabolites

- **Primary metabolites**
  (primary products of metabolism)
  - During the log phase of growth the products produced are essential to the growth of the cells.
  - Ex) Amino acids, Nucleotides, Proteins, Nucleic acids, Lipids, Alcohol, Carbohydrates, Organic acids.
- **Secondary metabolites**
  (Secondary products of metabolism)
  - During the stationary phase some microbial cultures synthesize compounds which are not produced during the trophophase* and do not appear to have any obvious function in cell metabolism.(idiophase*)
  - Ex) Alkaloids, Perfumes, Flavours, Interferons, Monoclonal antibodies.

The Component Parts of Fermentation Process (6 basic components)

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

Various types of fermentation

- **Batch**
  - Standard type of cultivation
  - Initial, limited amount of nutrient
  - Process control for physical parameters possible
- **Continuous**
  - Proper control of reaction
  - Excellent tool for kinetics and regulatory studies
  - Higher costs for experiments
  - Problem of aseptic condition
  - Need for highly trained operator
  - Ex) Few cases of application in industrial scale
    Production of SCP, Waste water treatment.

- **"Chemostat" culture**
  - Perfectly mixed suspension of biomass which medium fed at a constant rate and the culture is harvested at the same rate so that the culture volume remains constant. because the growth rate of the culture is controlled by chemical environment(the availability of one limiting component in the medium)
- **Fed-batch**
  - Simple method for control of regulatory effects
  - Ex) production of baker's yeast, penicillin
- **Repeated fed-batch culture**
  - If a portion of the culture is withdrawn at intervals
  - Volume variation

Microbial Culture

- **Open system**
  - All the materials which compose the system may enter and leave it (Continuous culture - input of nutrient medium, output of biomass and other products)
- **Closed system**
  - Some essential part of the system cannot both enter and leave it(Batch culture - an initial limited amount of nutrient medium)

Nutrients for Growth

1) **Sources of the ‘major’ elements C, H, O and N**
2) **Sources of the ‘minor’ elements P, K, S and Mg**
3) **Vitamins and hormones**
4) **Sources of the ‘trace’ elements Fe, Zn and Si**

Difference of Medium between in a Small and Large Scale

- On a small scale (laboratory scale)
  - Relatively simple to devise a medium containing pure compounds
- On a large scale

  (pilot scale, industrial scale)
  - To avoid expensive medium

Cheap nutrients

Cheap Nutrients (Some Criteria)

1) Maximum yield of product (biomass per gram of substrate used)
2) Maximum concentration of product or biomass
3) Maximum rate of product formation
4) Minimum yield of undesired products
5) Cheap and consistent quality and available all the time
6) Minimal problems with aeration and agitation, extraction, purification, and waste treatment

Carbon & Nitrogen Sources

- **Carbon sources**
  - Cane molasses, beet molasses, cereal grains, starch, glucose, sucrose, lactose
- **Nitrogen sources**
  - Ammonium salts, urea, nitrates, corn steep liquor

Importance of Media

- **Lab. medium is not ideal in a large fermenter (gas transfer pattern)**
- **Media with a high viscosity**
  - a high power input for effective stirring
- **Media**
  - affect pH, foam formation, morphological form of the organism

Medium formulation

- **Essential stage in the design of successful laboratory experiments, pilot scale development and manufacturing processes.**

- **Constituents of a medium**
  - must satisfy the element requirements for cell mass and metabolite production
  - must be an adequate supply of energy for biosynthesis and cell maintenance

Stoichiometric Equation for Cell Growth and Product Formation

Carbon and Energy source + Nitrogen source + Other Requirements

$\longrightarrow$ Cell biomass + Product + CO_2 + H_2O + Heat

Need to be expressed in quantitative terms (for economical design of media)

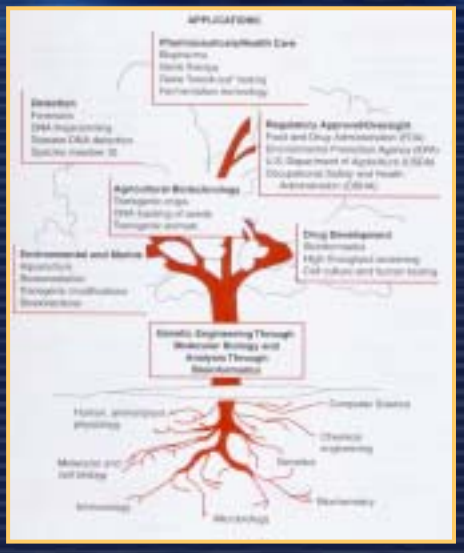
Possible to calculate the minimal quantities of nutrients which will be needed to produce a specific amount of biomass

Carbon Requirements

- Cellular yield coefficient (Y)

$$= \frac{\text{Quantity of cell dry matter produced}}{\text{Quantity of carbon substrate utilised}}$$

The Biotechnology Tree : Different Disciplines Contribute to Biotechnology



Some Commercially Produced Antibiotics

Antibiotic	Producing Microorganism	Class
Produced by fungi		
Cephalosporin	*Cephalosporium acremonium*	Broad-spectrum
Griseofulvin	*Penicillium griseofulvum*	Fungi
Penicillin	*Penicillium chrysogenum*	Gram-positive bacteria
Produced by Gram-positive, Spore-Forming Bacteria		
Bacitracin	*Bacillus subtilis*	Gram-positive bacteria
Polymyxin B	*Bacillus polymyxa*	Gram-negative bacteria
Produced by Gram-Positive Bacterium, Actinomycete		
Amphotericin B	*Streptomyces nodosus*	Fungi
Chloramphenicol	*Streptomyces venezuelae* (*now chemical synthesis*)	Broad-spectrum
Cycloheximide	*Streptomyces griseus*	Pathogenic yeasts
Cycloserine	*Streptomyces orchidaceus*	Broad-spectrum
Erythromycin	*Streptomyces erythreus*	Mostly gram-positive bacteria
Kanamycin	*Streptomyces kanomyceticus*	Gram-positive bacteria
Lincomycin	*Streptomyces lincolnensis*	Gram-positive bacteria
Neomycin	*Streptomyces fradiae*	Broad-spectrum
Nystatin	*Streptomyces noursei*	Fungi
Streptomycin	*Streptomyces griseus*	Gram-negative bacteria (*Mycobacterium tuberculosis*)
Tetracycline	*Streptomyces rimosus*	Broad-spectrum

Produced Amino Acids and Their Uses

Amino Acid	Use
Alanine	Added to fruit juice to improve taste
Aspartate	Added to fruit juice to improve taste
Cysteine	Added to bread and fruit juice to enhance flavor
Glutamate (MSG)	Added to many foods to enhance flavor
Glycine	Enhances flavor of sweetened foods
Histidine + tryptophan	Prevents rancidity in various foods
Lysine	Used in Japan to make bread a more complete protein
Methionine	Makes soybean products a more complete protein

Commercially Important Enzymes Produced by Microorganisms

Enzyme	Activity	Producing Microorganism	Use
Cellulase	Hydrolyzes cellulose	Trichoderma konigi	Digestive aid
Collagenase	Hydrolyzes collagen	Clostridium histolyticum	Promotes wound/burn healing
Diastase	Hydrolyzes starch	Aspergillus oryzae	Digestive aid
Glucose isomerase	Converts glucose to fructose	Streptomyces phaeochromogenes	Converts glucose from hydrolyzed cornstarch to a sweetener
Invertase	Hydrolyzes sucrose	Saccharomyces cerevisiae	Candy manufacture
Lipase	Hydrolyzes lipids	Rhizopus spp.	Digestive aid
Pectinase	Hydrolyzes pectin	Sclerotina libertina	Clarifies fruit juice
protease	Hydrolyzes protein	Bacillus subtilis	Used in detergents

Top Ten Selling Biotechnology Drugs

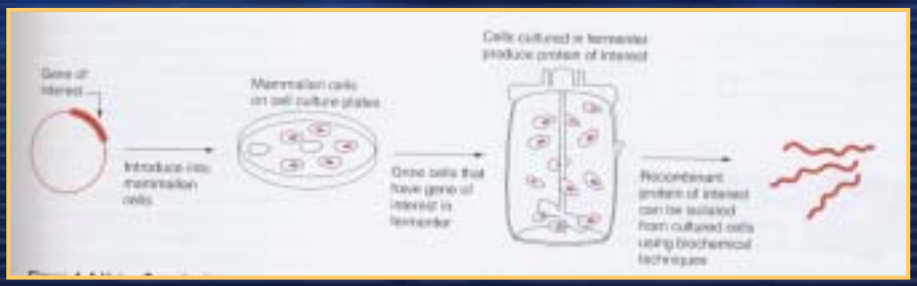
Drug	Developer	Function (treatment of human disease conditions)
Betaseron	Chiron/Berlex	Multiple sclerosis
Cerezyme	Genzyme	Gaucher's disease
Engerix B	Genentech	Hepatitis B vaccine
Epivir	Glaxo Wellcome	Anti-HIV
Epogen	Amgen	Red blood cell enhancement
Genotropin	Genentech	Growth failure
Humulin	Genentech	Diabetes
Intron	Biogen	Cancer and viral infections
Neupogen	Amgen	Neutropenia reduction
Procrit	Amgen	Platelet enhancement

From Ernst & Young LLP, Biotechnology Industry Report (2003)

Examples of Proteins Manufactured from Cloned Genes

Product	Application
Blood factor VIII (clotting factor)	Used to treat hemophilia
Epidermal growth factor	Used to stimulate antibody production in patients with immune system disorders
Growth hormone	Used to correct pituitary deficiencies and short stature in humans; other forms used in cows to increase milk production
Insulin	Used to treat diabetes mellitus
Interferons	Used to treat cancer and viral infections
Interleukins	Used to treat cancer and stimulate antibody production
Monoclonal antibodies	Used to diagnose and treat a variety of diseases including cancers
Tissue plasminogen activator	Used to treat heart attacks and stroke

Using Genetically Modified Cultured Cells to Make a Protein of Interest



Genes of interest can be introduced into bacterial or mammalian cells. Such cells can be grown using cell culture techniques. Recombinant proteins isolated from these cells are used in hundreds of different biotechnology applications. In this example, mammalian cells are shown, but this process is also commonly carried out using bacteria.