THE BASIC NUTRIENT REQUIREMENTS OF INDUSTRIAL MEDIA

All microbiological media, whether for industrial or for laboratory purposes must satisfy the needs of the organism in terms of carbon, nitrogen, minerals, growth factors, and water. In addition they must not contain materials which are inhibitory to growth.Ideally it would be essential to perform a complete analysis of the organism to be grown in order to decide how much of the various elements should be added to the medium.However, approximate figures for the three major groups of heterotrophic organisms usually grown on an industrial scale are available and may be used in such calculations

Carbon or energy requirements are usually met from carbohydrates, notably (in laboratory experiments) from glucose. It must be borne in mind that more complex carbohydrates such as starch or cellulose may be utilized by some organisms.

Nitrogen is found in proteins including enzymes as well as in nucleic acids hence it is a key element in the cell. Most cells would use ammonia or other nitrogen salts.

Minerals form component portions of some enzymes in the cell and must be present in the medium. The major mineral elements needed include P, S, Mg and Fe. Trace elements required include manganese, boron, zinc, copper and molybdenum.

Growth factors include vitamins, amino acids and nucleotides and must be added to the medium if the organism cannot manufacture them.

Under laboratory conditions, it is possible to meet the organism's requirement by the use of purified chemicals since microbial growth is generally usually limited to a few liters. However, on an industrial scale, the volume of the fermentation could be in the order of thousands of liters. Therefore, pure chemicals are not usually used because of their high expense, unless the cost of the finished material justifies their use. Pure chemicals are however used when industrial media are being developed at the laboratory level.

CRITERIA FOR THE CHOICE OF RAW MATERIALS USED IN INDUSTRIAL MEDIA

In deciding the raw materials to be used in the production of given products using
designated microorganism(s) the following factors should be taken into account.

(a) Cost of the material

The cheaper the raw materials the more competitive the selling price of the final product will be.

(b) Ready availability of the raw material

The raw material must be readily available in order not to halt production. If it is seasonal or imported, then it must be possible to store it for a reasonable period. Many industrial establishments keep large stocks of their raw materials for this purpose.

(c) Transportation costs

Proximity of the user-industry to the site of production of the raw materials is a factor of great importance, because the cost of the raw materials and of the finished material and hence its competitiveness on the market can all be affected by the transportation costs.

(d) Ease of disposal of wastes resulting from the raw materials

The disposal of industrial waste is rigidly controlled in many countries. Waste materials often find use as raw materials for other industries.

(e) Uniformity in the quality of the raw material and ease of standardization

The quality of the raw material in terms of its composition must be reasonably constant in order to ensure uniformity of quality in the final product and the satisfaction of the customer and his/her expectations.

(f) Adequate chemical composition of medium

As has been discussed already, the medium must have adequate amounts of carbon, nitrogen, minerals and vitamins in the appropriate quantities and proportions necessary for the optimum production of the commodity in question.

(g) Presence of relevant precursors

The raw material must contain the precursors necessary for the synthesis of the finished product. Precursors often stimulate production of secondary metabolites either by increasing the amount of a limiting metabolite, by inducing a biosynthetic enzyme or both.

(h) Satisfaction of growth and production requirements of the microorganisms

Many industrial organisms have two phases of growth in batch cultivation: the phase of growth, or the **trophophase**, and the phase of production, or the **idiophase**

SOME RAW MATERIALS USED IN COMPOUNDING INDUSTRIAL MEDIA

The raw materials to be discussed are used because of the properties mentioned above: cheapness, ready availability, constancy of chemical quality, etc.

(a) Corn steep liquor

This is a by-product of starch manufacture from maize. As a nutrient for most industrial organisms corn steep liquor is considered adequate, being rich in carbohydrates, nitrogen, vitamins, and minerals.

(b) Pharmamedia

Also known as proflo, this is a yellow fine powder made from cotton-seed embryo. It is used in the manufacture of tetracycline and some semi-synthetic penicillins. It is rich in protein, (56% w/v) and contains 24% carbohydrate, 5% oil, and 4% ash, the last of which is rich in calcium, iron, chloride, phosphorous, and sulfate.

(c) Distillers solubles

This is a by-product of the distillation of alcohol from fermented grain. It is rich
in nitrogen, minerals, and growth factors .

(d) Soya bean meal

Soya beans (soja) (*Glycine max*), is an annual legume which is widely cultivated
throughout the world .The seeds are heated before being extracted for oil that is used for food, as an antifoam in industrial fermentations, or used for the manufacture of margarine.

(e) Molasses

Molasses is a source of sugar, and is used in many fermentation industries including the production of potable and industrial alcohol, acetone, citric acid, glycerol, and yeasts. It is a by-product of the sugar industry. There are two types of molasses depending on whether the sugar is produced from the tropical crop, sugar cane or beet.
Cane and beet molasses differ slightly in composition (Table 4.4). Beet molasses is alkaline while cane molasses is acid.
High test' molasses (also known as inverted molasses) is a brown thick syrup liquid used in the distilling industry and containing about 75% total sugars (sucrose and reducing sugars) and about 18% moisture. Strictly speaking, it is not molasses at all but invert sugar, (i.e reducing sugars resulting from sucrose hydrolysis). It is produced by the hydrolysis of the concentrated juice with acid. In the so-called Cuban method, invertase is used for the hydrolysis. Sometimes 'A' sugar may be inverted and mixed with 'A'molasses

Table 4.4 Average composition of beet and cane molasses

	Beet Molasses % (W/W)	*Cane Molasses* % (W/W)
Water	16.5	20.0
Sugars:	53.0	64.0
Sucrose	51.0	32.0
Fructose	1.0	15.0
Glucose	-	14.0
Raffinose	1.0	-
Non-sugar (nitrogeneous Materials, acids, gums, etc.)	19.0	10.0
Ash	11.5	8.0

(f) Sulfite liquor

Sulfite liquor (also called waste sulfite liquor, sulfite waste liquor or spent sulfite liquor) is the aqueous effluent resulting from the sulfite process for manufacturing cellulose or pulp from wood. Depending on the type, most woods contain about 50% cellulose, about 25% lignins and about 25% of hemicelluloses.

(g) Other Substrates

Other substrates used as raw materials in fermentations are alcohol, acetic acid,
methanol, methane, and fractions of crude petroleum.

THE NATURE OF METABOLIC PATHWAYS

In order to be able to manipulate microorganisms to produce maximally materials of economic importance to humans, but at minimal costs, it is important that the physiology of the organisms be understood as much as is possible.

The series of chemical reactions involved in converting a chemical (or a metabolite) in the organism into a final product is known as a metabolic pathway. When the reactions lead to the formation of a more complex substance, that particular form of metabolism is known as *anabolism* and the pathway an anabolic pathway.

When the series of reactions lead to less complex compounds the metabolism is described as *catabolism.* The compounds involved in a metabolic pathway are called *intermediates* and the final product is known as the end-product (see Fig. 5.1).

Catabolic reactions have been mostly studied with glucose. Four pathways of glucose breakdown to pyruvic acid (or glycolysis) are currently recognized. They will be discussed later. Catabolic reactions often furnish energy in the form of ATP and other high energy compounds, which are used for biosynthetic reactions. A second function of catabolic reactions is to provide the carbon skeleton for biosynthesis.

Anabolic reactions lead to the formation of larger molecules some of which are constituents of the cell.

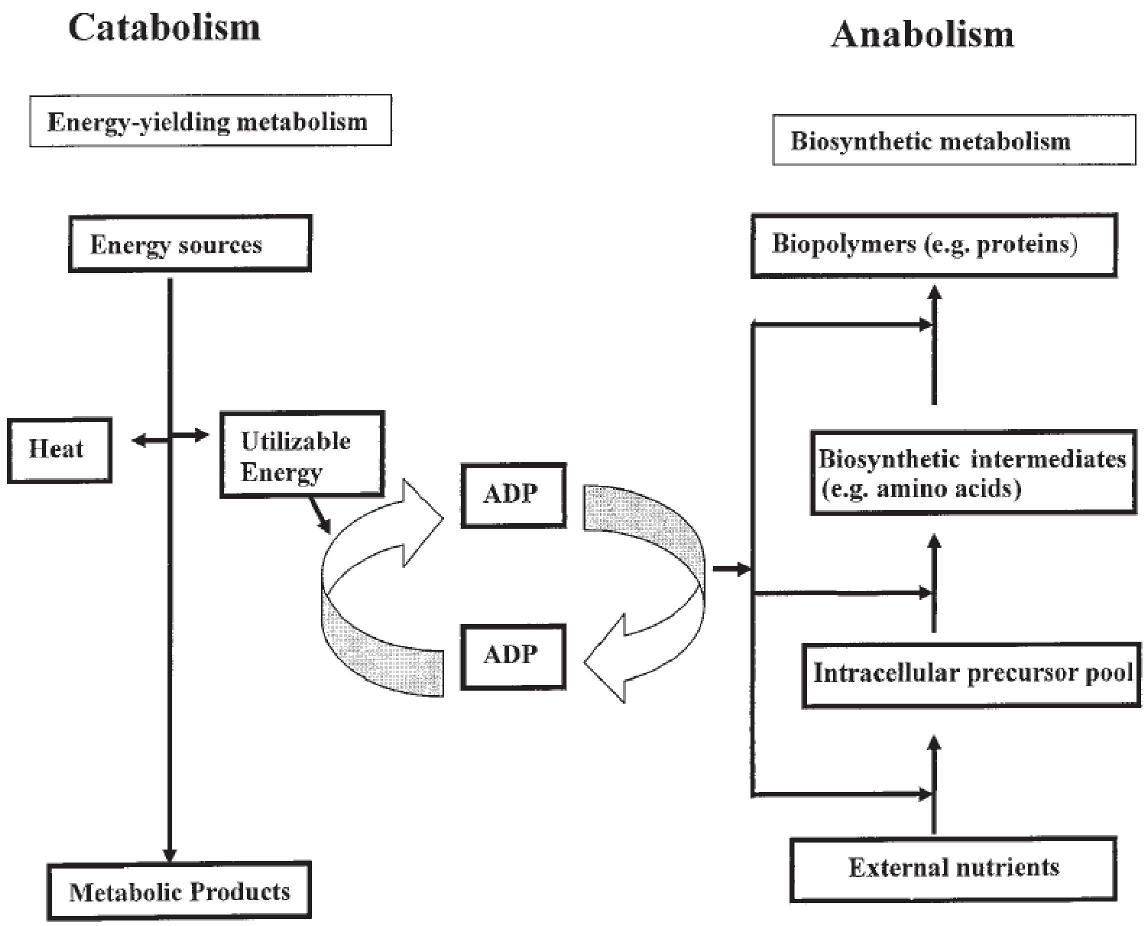

Fig. 5.1 Metabolism: Relationship between Anabolism and Catabolism in a Cell

INDUSTRIAL MICROBIOLOGICAL PRODUCTS AS PRIMARY AND SECONDARY METABOLITES

Products of industrial microorganisms may be divided into two broad groups, those which result from primary metabolism and others which derive from secondary metabolism. The line between the two is not always clear cut, but the distinction is useful in discussing industrial products.

1. Products of Primary Metabolism

Primary metabolism is the inter-related group of reactions within a microorganism
which are associated with growth and the maintenance of life. Primary metabolism is essentially the same in all living things and is concerned with the release of energy, and the synthesis of important macromolecules such as proteins, nucleic acids and other cell constituents. When primary metabolism is stopped the organism dies.
Products of primary metabolism are associated with growth and their maximum
production occurs in the logarithmic phase of growth in a batch culture. Primary
catabolic products include ethanol, lactic acid, and butanol while anabolic products
include amino-acids, enzymes and nucleic acids. Single-cell proteins and yeasts would also be regarded as primary products (Table 5.1)

Table 5.1 Some industrial products resulting from primary metabolism

Anabolic Products	*Catabolic Products*
1. Enzymes	1. Ethanol and ethanol-containing products, e.g. wines
2. Amino acids	2. Butanol
3. Vitamins	3. Acetone
4. Polysaccharides	4. Lactic acid
5. Yeast cells	5. Acetic acid (vinegar)
6. Single cell protein	
7. Nucleic acids	
8. Citric acid	

2. Products of Secondary Metabolism

In contrast to primary metabolism which is associated with the growth of the cell and the continued existence of the organism, secondary metabolism, which was first observed in higher plants, has the following characteristics (i) Secondary metabolism has no *apparent* function in the organism. The organism continues to exist if secondary metabolism isblocked by a suitable biochemical means. On the other hand it would die if primary metabolism were stopped.

(ii) Secondary metabolites are produced in response to a restriction in nutrients. They are therefore produced after the growth phase, at the end of the logarithmic phase of growth and in the stationary phase (in a batch culture). They can
be more precisely controlled in a continuous culture.

(iii) Secondary metabolism appears to be restricted to some species of plants and microorganisms (and in a few cases to animals). The products of secondary metabolism also appear to be characteristic of the species

(iv) Secondary metabolites usually have 'bizarre' and unusual chemical structures and several closely related metabolites may be produced by the same organism in wild-type strains. This latter observation indicates the existence of a variety of alternate and closely-related pathways.

(v) The ability to produce a particular secondary metabolite, especially in industrially important strains is easily lost. This phenomenon is known as strain degeneration.