Nutrient Media for Cultivation of Industrial Microorganisms and Production of Microbial Products

1. 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.

Carbon	Nitrogen	Minerals	Water	Growth factors
Provides both energy and carbon units for biosynthesis	Needed for the synthesis of amino acids, lipids, enzyme cofactors and other substances.	Required for the activity in cell.	Water is a raw material of vital importance in industrial microbiology.	Require organic compounds that cannot be synthesized by the organisms

Carbon or energy

C or energy requirements are usually met from carbohydrates (glucose, starch or cellulose & …., etc) . Energy sources may include hydrocarbons, alcohols, or even organic acids. In formulation industrial medium, the carbon content must be adequate for the production of cells.

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. Any nitrogen compound which the organism cannot synthesize must be added.

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.

2.Criteria for Raw Materials in Industrial Media

2.1 Cost of the material

2.2 Ready availability of the raw material

2.3 Transportation costs

2.4 Ease of disposal of wastes resulting from the raw materials

2.5 Uniformity in the quality of the raw material and ease of standardization

2.6 Adequate chemical composition of medium

2.7 Presence of relevant precursors

2.8 Satisfaction of growth and production requirements of the microorganisms

2.1 Cost of the material

- The cheaper the raw materials the more competitive the selling price of the final product.
- Lactose is more suitable than glucose in some processes (e.g. penicillin production) because of the slow rate of its utilization, it is usually replaced by the cheaper glucose.
- The raw materials used in many industrial media are usually waste products from other processes.

2.2 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.

2.3 Transportation costs

- The closer the source of the raw material to the point of use the more suitable it is for use, if all other conditions are satisfactory.

2.4 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.
- Thus, spent grains from breweries can be used as animal feed.
- But in some cases no further use may be found for the waste from an industry.
- Its disposal could be expensive.
- When choosing a raw material therefore the cost, if any, of treating its waste must be considered.

2.5 Uniformity in the quality of the raw material and ease of standardization

- Composition must be reasonably constant in order to ensure uniformity of quality in the final product and the satisfaction of the customer.
- E.g., molasses as waste product of sugar industry.
- Each batch of molasses must be chemically analyzed before being used in a fermentation industry in order to ascertain how much of the various nutrients must be added.
- A raw material with extremes of variability in quality is undesirable as extra costs are needed.

  - Analysis of the raw material,

  - Nutrients may need to be added to attain the usual and expected quality in the medium.

2.6 Adequate chemical composition of medium

- The medium must have adequate amounts of C, N, minerals and vitamins in the appropriate quantities and proportions necessary for the optimum production of the commodity in question.
- The compounds in the medium must utilizable by the organisms.
- Thus most yeasts utilize hexose sugars, whereas only a few will utilize lactose.
- Cellulose is not easily used and is utilized only by a limited number of organisms.
- Some organisms grow better in one or the other substrate.
- Fungi will for instance readily grow in corn steep liquor while actinomycetes will grow more readily on soya bean cake.

2.7 Presence of relevant precursors

- 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.
- Precursors include amino acids & small molecules.
- For penicillin G to be produced the medium must contain a phenyl compound.
- Corn steep liquor contains phenyl precursors.
- Other precursors are cobalt in media for Vitamin B12 production & chlorine for the chlorine containing antibiotics, chlortetracycline, & griseofulvin (Fig. 4.1).

2.8 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.**
- **In the first** phase cell multiplication takes place rapidly, with little or no production of the desired material.
- It is in the second phase that production of the material takes place, usually with no cell multiplication and following the elaboration of new enzymes.
- Often these two phases require different nutrients or different proportions of the same nutrients.
- The medium must be complete and be able to cater for these requirements.
- For example high levels of glucose and phosphate inhibit the onset of the idiophase in the production of a number of secondary metabolites of industrial importance.
- The levels of the components added must be such that they do not adversely affect production.

3. Raw Materials Used in Compounding Industrial Media

The raw materials to be used in compounding industrial media should be cheap, ready availability, constancy of chemical quality, etc.

(a) Corn steep liquor

(b) Pharmamedia

(c) Distillers solubles

(d) Soya bean meal

(e) Molasses

(f) Sulfite liquor

(g) Other Substrates

- Alcohol
- Acetic acid
- Methanol
- Methane
- Fractions of crude petroleum

3.1 Corn steep liquor

- Corn steep liquor is a by-product of starch manufacture from maize.
- Corn steep liquor is highly acidic and must be neutralized (usually with $CaCO_3$)
- The lowered pH inhibits most other organisms, but encourages the development of naturally occurring lactic acid bacteria especially *Lactobacillus spp.*
- Corn steep liquor rich in carbohydrates, nitrogen, vitamins, and minerals.

Approximate composition of corn steep liquor (%)

Lactose	3.0-4.0
Glucose	0.-0.5
Non-reducing carbohydrates (mainly starch)	1.5
Acetic acid	0.05
Glucose lactic acid	0.5
Phenylethylamine	0.05
Amino aids (peptides, mines)	0.5
Total solids	80-90
Total nitrogen	0.15-0.2%

3.2 Pharmamedia

- Pharma media 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
- It also rich in calcium, iron, chloride, phosphorous, and sulfate.

3.3 Distillers solubles

- Distillers solubles is a by-product of the distillation of alcohol from fermented grain.
- It is prepared by filtering away the solids after distilling fermented cereals and concentrating to a syrup which is then drum-dried to give distillers soluble
- It is rich in nitrogen, minerals, and growth factors

3.4 Soya bean meal

- Soya bean meal has about 11% nitrogen, and 30% carbohydrate
- Its nitrogen is more complex than that found in corn steep liquor and is not readily available to most microorganisms, except actinomycetes.

3.5 Molasses

- Molasses is a by-product of the sugar industry.
- 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.
- There are two types of molasses : Beet Molasses and Cane Molasses

3.6 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.
- Sulfite liquor of various compositions are produced, depending on the severity of the treatment and the type of wood.
- Hardwoods not only yield a higher amount of sugar (up to 3% dry weight of liquor) but the sugars are largely pentose, in the form of xylose. Hardwood hydrolyzates also contains a higher amount of acetic acid. Soft woods yield a product with about 75% hexose, mainly mannose.
- Sulfite liquor is used as a medium for the growth of microorganisms after being suitably neutralized with CaCO3 and enriched with ammonium salts or urea, and other nutrients.

4. Growth Factors

- Growth factors are materials which are not synthesized by the organism and therefore must be added to the medium.
- They usually function as cofactors of enzymes and may be vitamins, nucleotides etc.
- Growth factors are required only in small amounts.

Growth factor	*Source*
Vitamin B	Rice polishing, wheat germ, yeasts
Vitamin B_6	Corn steep liquor, yeasts
Vitamin B_2	Cereals, corn steep liquor
Nicotinamide	Liver, penicillin spent liquor
Panthothenic Acid	Corn steep liquor
Vitamin B_{12}	Liver, silage, meat

5. Water

- Water is a raw material of vital importance in industrial microbiology
- It is required as a major component of the fermentation medium, as well as for cooling, and for washing and cleaning.
- It is therefore used in rather large quantities, and measured in thousands of liters a day depending on the industry.
- In some industries such as the beer industry the quality of the product depends to some extent on the water.
- In order to ensure constancy of product quality the water must be regularly analyzed for minerals, color, pH, etc. and adjusted as may be necessary.

6.Sources of Components of Industrial Media

6.1 Carbohydrate Sources

These are all polysaccharides and have to be hydrolyzed to sugar before being used.

(a)Cassava (manioc)

The roots of the cassava-plant *Manihot esculenta* Crantz serve mainly as a source of carbohydrate for human (and sometimes animal) food in many parts of the tropical world. Its great advantage is that it is high yielding, requires little attention when cultivated, and the roots can keep in the ground for many months without deterioration before harvest. The inner fleshy portion is a rich source of starch and has served, after hydrolysis, as a carbon source for single cell protein, ethanol, and even beer. In Brazil it is one of the sources of fermentation alcohol (Chapter 13) which is blended with petrol to form gasohol for driving motor vehicles.

(b) Sweet potato

Sweet potatoes *Ipomca batatas* is a warm-climate crop although it can be grown also in sub tropical regions. There are a large number of cultivars, which vary in the colors of the tuber flesh and of the skin; they also differ in the tuber size, time of maturity, yield, and sweetness. They are widely grown in the world and are found in South America, the USA, Africa and Asia. They are regarded as minor sources of carbohydrates in comparison with maize, wheat, or cassava, but they have the advantage that they do not require much agronomic attention. They have been used as sources of sugar on a semi-commercial basis because the fleshy roots contain saccharolytic enzymes. The syrup made from boiling the tubers has been used as a carbohydrate (sugar) source in compounding industrial media. Butyl alcohol, acetone and ethanol have been produced from such a syrup, and in quantities higher than the amounts produced from maize syrup of the same concentration. Since sweet potatoes are not widely consumed as food, it is possible that it may be profitable to grow them for use, after hydrolysis, in industrial microbiology media as well as for the starch industry. It is reported that a variety has been developed which yields up to 40 tonnes per hectare, a much higher yield than cassava or maize.

(c) Yams

Yams (*Dioscorea spp*) are widely consumed in the tropics. Compared to other tropical roots however, their cultivation is tedious; in any case enough of this tuber is not produced even for human food. It is therefore almost inconceivable to suggest that the crop should be grown solely for use in compounding industrial media. Nevertheless yams have been employed in producing various products such as yam flour and yam flakes. If the production of these materials is carried out on a sufficiently large scale it is to be expected that the waste materials resulting from peeling the yams could yield substantial amounts of materials which on hydrolysis will be available as components of industrial microbiological media.

(d) Cocoyam

Cocoyam is a blanket name for several edible members of the monocotyledonous (single seed-leaf) plant of the family *Araceae* (the aroids), the best known two genera of which are *Colocasia* (tano) and *Xanthosoma* (tannia). They are grown and eaten all over the tropical world. As they are laborious to cultivate, require large quantities of moisture and do not store well they are not the main source of carbohydrates in regions where they are grown. However, this relative unimportance may well be of significance in regions where for reasons of climate they can be suitably cultivated. Cocoyam starch has been found to be of acceptable quality for pharmaceutical purposes. Should it find use in that area, starchy by-products could be hydrolyzed to provide components of industrial microbiological media.

(e) Millets

This is a collective name for several cereals whose seeds are small in comparison with those of maize, sorghum, rice, etc. They are classified as the minor cereals because they generally do not form major components of human food. They are however hardy and will tolerate great drought and heat, grow on poor soil and mature quickly. Attention is being turned to them for this reason in some parts of the world. It is for this reason also that millets could become potential sources of cereal for use in industrial microbiology media.

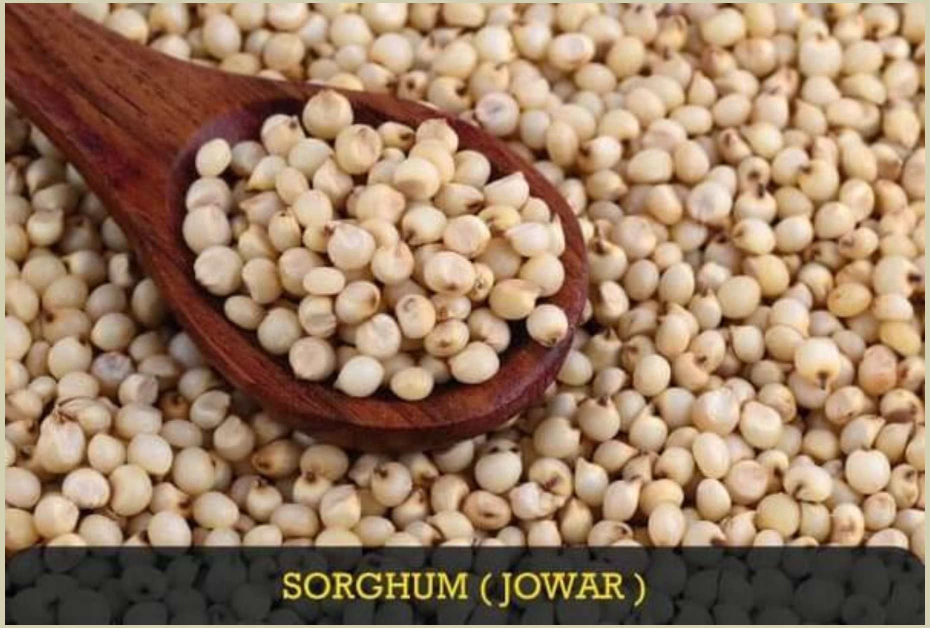
Millets are grown all over the world in the tropical and sub-tropical regions and belong to various genera: *Pennisetum americanum* (pearl or bulrush millet), *Setaria italica* (foxtail millet), *Panicum miliaceum* (yard millet), *Echinochloa frumentacea* (Japanese yard millet) and *Eleusine corcana* (finger millet). Millet starch has been hydrolyzed by malting for alcohol production on an experimental basis as far back as 50 years ago and the available information should be helpful in exploiting these grains for use as industrial media components.

(f) Rice

Rice, *Oryza sativa* is one of the leading food corps of the world being produced in all five continents, but especially in the tropical areas. Although it is high-cost commodity, it has the advantage of ease of mechanization, storability, and the availability of improved seeds through the efforts of the International Rice Research Institute, Philippines and other such bodies. The result is that this food crop is likely in the near future to displace, as a carbohydrate source, such other starch sources as yams, and to a lesser extent cassava in tropical countries. The increase in rice production is expected to become so efficient in many countries that the crop would yield substrates cheap enough for industrial microbiological use. Rice is used as brewing adjuncts and has been malted experimentally for beer brewing.

(g) Sorghum

Sorghum, *Sorghum bicolor,* is the fourth in term of quantity of production of the world's cereals, after wheat, rice, and corn. It is used for the production of special beers in various parts of the world. It has been mechanized and has one of the greatest potential among cereals for use as a source of carbohydrate in industrial media in regions of the world where it thrives. It has been successfully malted and used in an all-sorghum lager beer which compared favorably with barley lager beer



(h) Jerusalem artichoke

Jerusalem artichoke, *Helianthus tuberosus*, is a member of the plant family compositae, where the storage carbohydrate is not starch, but inulin a polymer of fructose into which it can be hydrolyzed. It is a root-crop and grows in temperate, semi-tropical and tropical regions.

6.2 Protein Sources

(a) Peanut (groundnut) meal

Various leguminous seeds may be used as a source for the supply of nitrogen in industrial media. Only peanuts (groundnuts) *Arachis hypogea* will be discussed. The nuts are rich in liquids and proteins. The groundnut cake left after the nuts have been freed of oil is often used as animal feed. But just as is the case with soya bean, oil from peanuts may be used as anti-foam while the press-cake could be used for a source of protein. The nuts and the cake are rich in protein.

(b) Blood meal

Blood consists of about 82% water, 0.1% carbohydrate, 0.6% fat, 16.4% nitrogen, and 0.7% ash. It is a waste product in abattoirs although it is sometimes used as animal feed. Drying is achieved by passing live steam through the blood until the temperature reaches about 100°C. This treatment sterilizes it and also causes it to clot. It is then drained, pressed to remove serum, further dried and ground. The resulting blood-meal is chocolate-colored and contains about 80% protein and small amounts of ash and lipids. Where sufficient blood is available blood meal could form an important source of proteins for industrial media.

(c) Fish Meal

Fish meal is used for feeding farm animals. It is rich in protein (about 65%) and, minerals (about 21% calcium 8%, and phosphorous 3.5%) and may therefore be used for industrial microbiological media production. Fish meal is made by drying fish with steam either aided by vacuum or by simple drying. Alternatively hot air may be passed over the fish placed in revolving drums. It is then ground into a fine powder.

7. Plant Waste Materials in Industrial Microbiology Media: Saccharification of Polysaccharides

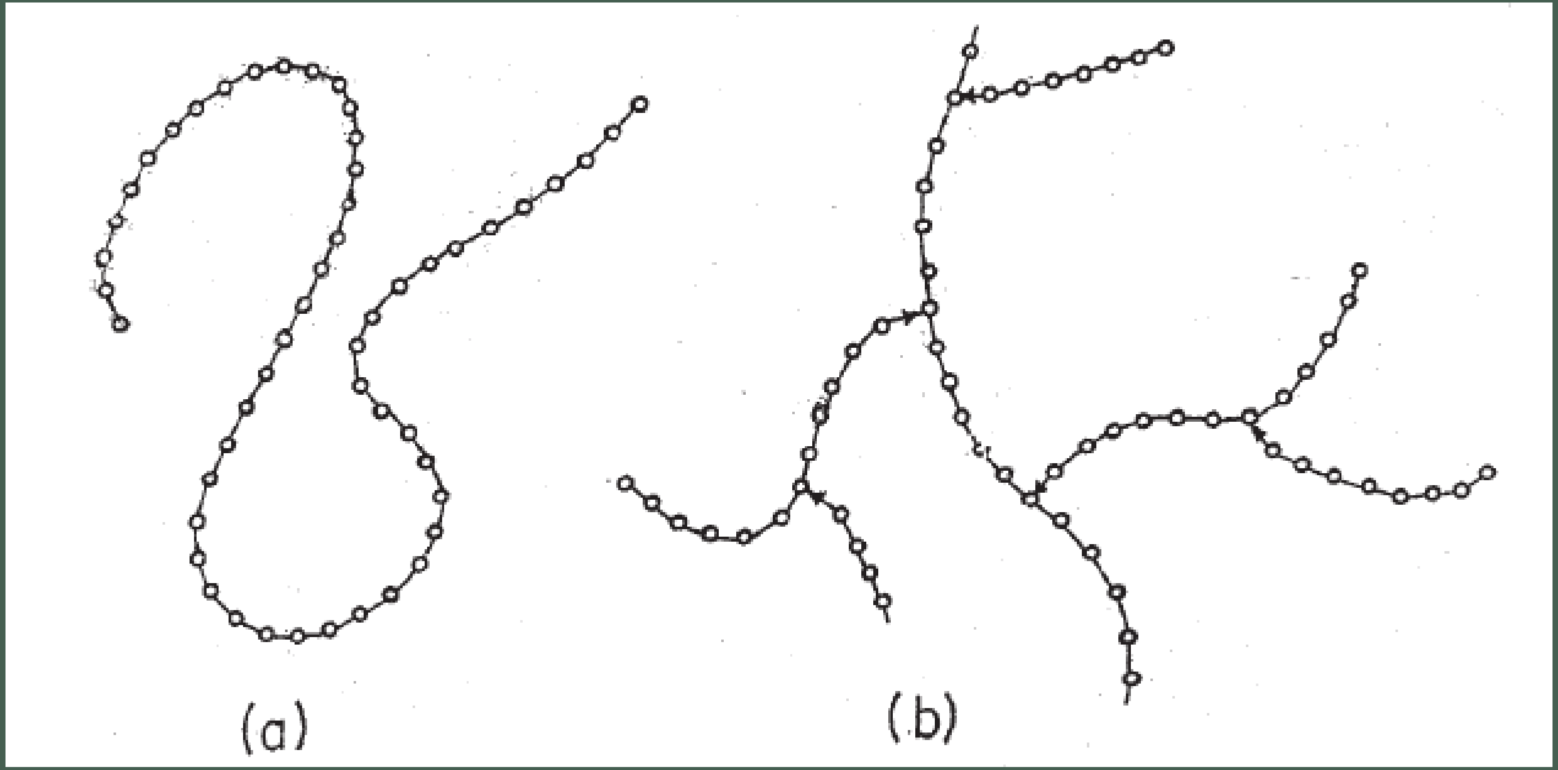
The great recommendation of plant agricultural wastes as sources of industrial microbiological media is that

1. plentiful
2. renewable.

7.1 Starch

- It is a mixture of two polymers of glucose: amylose and amylopectin.
- Amylose is a linear (1-4) – D glucan usually having a degree of polymerization (D.P., i.e. number of glucose molecules) of about 400 and having a few branched residues linked with (1-6) linkages.
- Amylopectin is a branched D glucan with predominantly – D (1-4) linkages and with about 4% of the – D (1-6) type..
- Amylopectin consists of amylose – like chains of D. P. 12 – 50.

Structure of amylose (a) and amylopectin (b)



- Starches differ in their proportion of amylopectin and amylose according to the source.
- The common type of maize, for example, has about 26% of amylose and 74% of amylopectin.
- Others may have 100% amylopectin and still others may have 80 – 85% of amylose.

Some properties of amylose and amylopectin

Property	*Amylose*	*Amylopectin*
Structure	Linear	Branched
Behavior in water	Precipitates	
	Irreversibly	Stable
Degree of polymerization	10^3	10^4-10^5
Average chain length	10^3	20-25
Hydrolysis to maltose (%)		
(a) β - amylase	87	54
(b) β - amylase and		
debranching enzyme	98	79
Iodine Complex max (nm)	650	550

7.1.1 Saccharification of starch

Starch occurs in discrete crystalline granules in plants, and in this form is highly resistant to enzyme action. However when heated to about 55°C – 82°C depending on the type, starch gelatinizes and dissolves in water and becomes subject to attack by various enzymes.

Before saccharification, the starch or ground cereal is mixed with water and heated to gelatinize the starch and expose it to attack by the saccharifying agents. The saccharifying agents used are dilute acids and enzymes from malt or microorganisms.

a. Saccharification of starch with acid

The starch-containing material to be hydrolyzed is ground and mixed with dilute hydrochloric acid, sulfuric acid or even sulfurous acid. When sulfurous acid is used it can be introduced merely by pumping sulfur dioxide into the mash.

The concentrations of the mash and the acid, length of time and temperature of the heating have to be worked out for each starch source. During the hydrolysis the starch is broken down from starch (about 2,000 glucose molecules) through compounds of decreasing numbers of glucose moieties to glucose. The actual composition of the hydrolysate will depend on the factors mentioned above. Starch concentration is particularly important: if it is too high, side reactions may occur leading to a reduction in the yield of sugar. At the end of the reaction the acid is neutralized. If it is desired to ferment the hydrolysate for ethanol, yeast or single cell production, ammonium salts may be used as they can be used by many microorganisms.

b. Use of enzymes

Enzymes hydrolyzing starch used to be called collectively diastase. With increased knowledge about them, they are now called amylases.

Enzymatic hydrolysis has several advantages over the use of acid:

1. Since the pH for enzyme hydrolysis is about neutral, there is no need for special vessels which must stand the high temperature, pressure, and corrosion of acid hydrolysis;
2. Enzymes are more specific and hence there are fewer side reactions leading therefore to higher yields;
3. Acid hydrolysis often yields salts which may have to be removed constantly or periodically thereby increasing cost;
4. It is possible to use higher concentrations of the substrates with enzymes than with acids because of enzyme specificity, and reduced possibility of side reactions.

c. Enzymes involved in the hydrolysis of starch

Several enzymes are important in the hydrolysis of starch. They are divisible into six groups.

1.Enzymes that hydrolyse a – 1, 4 bonds and by-pass a – I, 6 bonding:

- The typical example is a - amylase.
- This enzyme hydrolyses randomly the inner a - (1 4) - D - glucosidic bonds of amylose and amylopectin.
- The cleavage can occur anywhere as long as there are at least six glucose residues on one side and at least three on the other side of the bond to be broken.
- The result is a mixture of branched - limit dextrins (i.e., fragments resistant to hydrolysis and contain the a - D (1-6) linkage derived from amylopectin) and linear glucose residues especially maltohexoses, maltoheptoses and maltotrioses.
- a -Amylases are found in virtually every living cell and the property and substrate pattern of a - amylases vary according to their source.
- Animall a - amylases in saliva and pancreatic juice completely hydrolyze starch to maltose and D-glucose.
- Among microbial a - amylases some can withstand temperatures near 100°C.

2. Enzymes that hydrolyse the a–1, 4 bonding, but cannot by-pass the a– 1,6 bonds: Beta amylase:

- Originally found in plants but has now been isolated from micro-organisms. Beta amylase hydrolyses alternate a– 1,4 bonds sequentially from the non-reducing end to yield maltose
- Beta amylase has different actions on amylose and amylopectin, because it cannot by-pass the a –1:6 – branch points in amylopectin.
- Therefore, while amylose is completely hydrolyzed to maltose, amylopectin is only hydrolyzed to within two or three glucose units of the a– 1.6 - branch point to yield maltose and a 'beta-limit' dextrin which is the parent amylopectin with the ends trimmed off.
- Debranching enzymes are able to open up the a– 1:6 bonds and thus convert beta-limit dextrins to yield a mixture of linear chains of varying lengths; beta amylase then hydrolyzes these linear chains.
- Those chains with an odd number of glucose molecules are hydrolyzed to maltose, and one glucose unit per chain.
- The even numbered residues are completely hydrolyzed to maltose.
- In practice there is a very large population of chains and hence one glucose residue is produced for every two chains present in the original starch.

3. Enzymes that hydrolyze (a —1, 4 and a — 1:6 bonds:

- The typical example of these enzymes is amyloglucosidase or glucoamylase.
- This enzyme hydrolyzes a - D - (1-4) -D – glucosidic bonds from the non-reducing ends to yield D – glucose molecules.
- When the sequential removal of glucose reaches the point of branching in amylopectin, the hydrolysis continues on the (1-6) bonding but more slowly than on the (1-4) bonding.
- Maltose is attacked only very slowly. The end product is glucose.

4. De-branching enzymes:

At least two de-branching enzymes are known: pullulanase and iso-amylase.

Pullulanase: This is a de-branching enzyme which causes the hydrolysis of a — D – (1 6) linkages in amylopectin or in amylopectin previsouly attacked by alphaamylase. It does not attack a - D (1-4) bonds. However, there must be at least two glucose units in the group attached to the rest of the molecules through an a -D- (1-6) bonding.

Iso-amylase: This is also a de-branching enzyme but differs from pullulanase in that three glucose units in the group must be attached to the rest of the molecules through an a - D – (1 6) bonding for it to function.

5. Enzymes that preferentially attack a - 1, 4 linkages:

Examples of this group are glucosidases. The maltodextrins and maltose produced by other enzymes are cleaved to glucose by α - glucosidases. They may however sometime attack unaltered polysaccharides but only very slowly.

6. Enzymes which hydrolyze starch to non-reducing cyclic D-glucose polymers known ascyclodextrins or Schardinger dextrins:

Cyclic sugar residues are produced by *Bacillus macerans*. They are not acted upon by most amylases although enzymes in Takadiastase produced by *Aspergillus oryzae* can degrade the residues.

d. Industrial saccharification of starch by enzymes

- In industry the extent of the conversion of starch to sugar is measured in terms of dextrose equivalent (D.E.).
- This is a measure of the reducing sugar content, determined under defined conditions involving Fehling's solution.
- The D.E is calculated as percentage of the total solids.
- Acid is being replaced more and more by enzymes.
- Sometimes acid is used initially and enzymes employed later.
- Practical upper limit of acid saccharification is 55 D.E.
- Beyond this, breakdown products begin to accumulate.
- Furthermore, with acid hydrolysis reversion reactions occur among the sugar produced.
- These two withdraws are avoided when enzymes are utilized.
- By selecting enzymes specific sugars can be produced.

- Industrialy used enzymes are produced in germinated seeds and by micro-organisms.
- Barley malt is widely used for the saccharification of starch.
- It contains large amounts of various enzymes notably -amylase and -glucosidase which further split saccharides to glucose.
- All the enzymes discussed above are produced by different micro-organisms and many of these enzymes are available commercially.
- The most commonly encountered organisms producing these enzymes are *Bacillus spp, Streptomyeces spp, Aspergillus spp, Penicillium spp, Mucor spp and Rhizopus spp.*

7.2 Cellulose, Hemi-celluloses and Lignin in Plant Materials

7.2.1 Cellulose

- Cellulose is the most abundant organic matter on earth.
- Does not exist pure in nature and even the purest natural form (that found in cotton fibres) contains about 6% of other materials.
- Three major components, cellulose, hemi-cellulose and lignin occur roughly in the ratio of 4:3:3 in wood.

7.2.2 Hemicelluloses

- Group of carbohydrates whose main and common characteristic is that they are soluble in, and hence can be extracted with, dilute alkali.
- They can then be precipitated with acid and ethanol.
- They are very easily hydrolyzed by chemically or biologically.
- The nature of the hemicellulose varies among plants.
- In cotton the hemicelluloses are pectic substances, which are polymers of galactose.
- In wood, they consist of short (DP less than 200) branched heteropolymers of glucose, xylose, galactose, mannose and arabinose as well as uronic acids of glucose and galactose linked by 1 – 3, 1 – 6 and 1 – 4 glycosidic bonding.

7.2.3 Lignin

- Lignin is a complex three-dimensional polymer formed from cyclic alcohols.
- It is important because it protects cellulose from hydrolysis.
- Cellulose is found in plant cell-walls which are held together by a porous material known as middle lamella.
- In wood the middle lamella is heavily impregnated with lignin which is highly resistant and thus protects the cell from attack by enzymes or acid.

7.2.4 Pretreatment of cellulose-containing materials before saccharification

- In order to expose lignocellulosics to attack, a number of physical and chemical methods are in use, or are being studied, for altering the fine structure of cellulose and/or breaking the lignin-carbohydrate complex.

- Chemical methods include the use of swelling agents such a NaOH, some amines, concentrated H_2SO_4 or HCl or proprietary cellulose solvents such as 'cadoxen' (tris thylene-diamine cadmium hydroxide). These agents introduce water between or within the cellulose crystals making subsequent hydrolysis, easier. Steam has also been used as a swelling agent. The lignin may be removed by treatment with dilute H2SO4 at high temperature.

- Physical methods of pretreatment include grinding, irradiation and simply heating the wood.

Table 4.8 Various pretreatment methods used in lignocellulose substrate preparation

Pretreatment type	*Specific method*
Mechanical	Weathering and milling-ball, fitz, hammer, roller
Irradiation	Gamma, electron beam, photooxidation
Thermal	Autohydrolysis, steam explosion, hydrothermolysis, boiling, pyrolysis, moist or dry heat expansion
Alkali	Sodium hydroxide, ammonium hydroxide
Acids	Sulfuric, hydrochloric, nitric, phosphoric, maleic
Oxidizing agents	Peracetic acid, sodium hypochlorite, sodium chlorite, hydrogen peroxide
Solvents Ethanol,	butanol, phenol, ethylamine, acetone, ethylene glycol
Gases	Ammonia, chlorine, nitrous oxide, ozone, sulfur dioxide
Biological	Ligninolytic fungi

7.2.5 Hydrolysis of cellulose

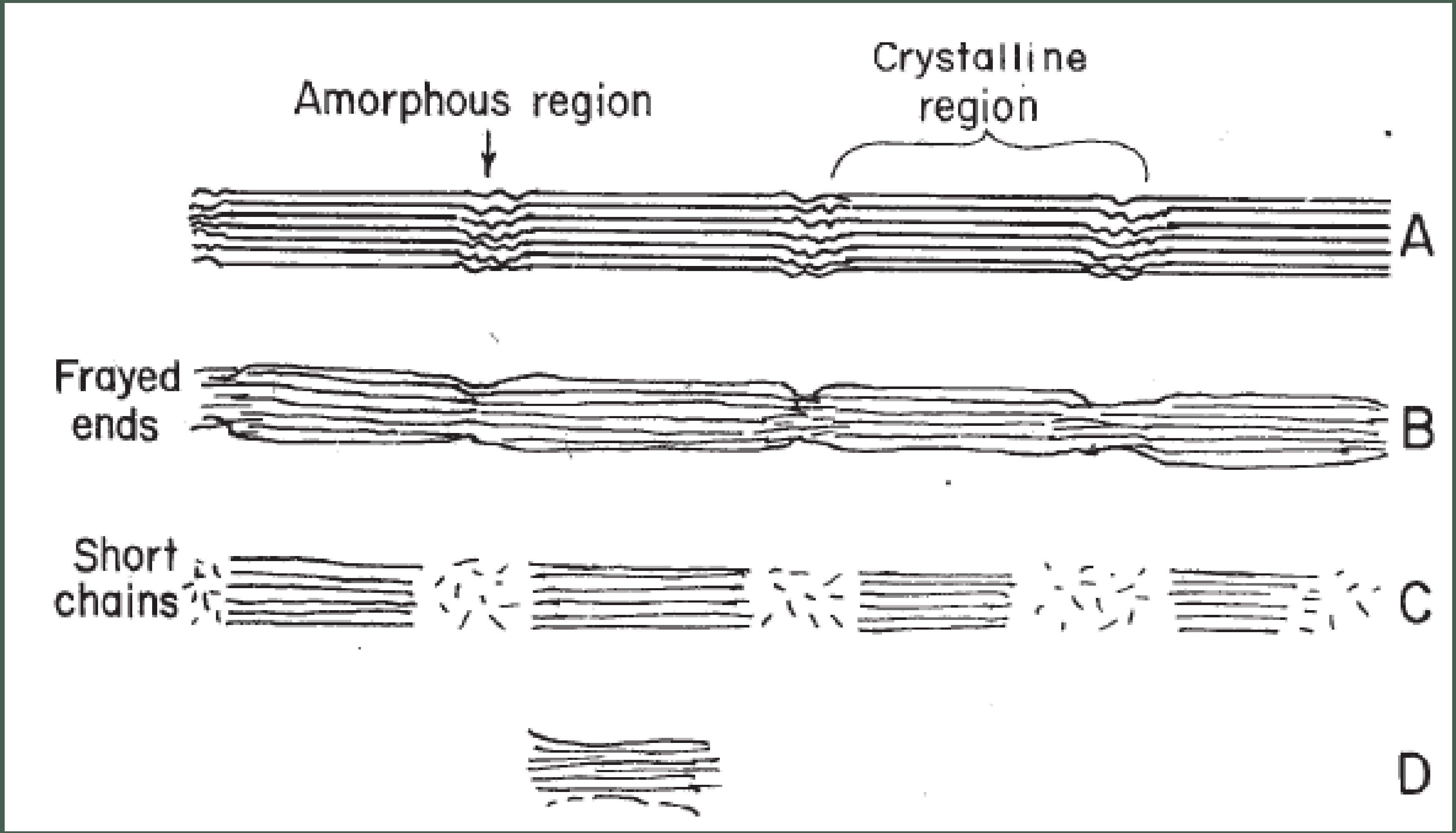
- After pretreatment, wood may be hydrolyzed with dilute HCl, H_2SO_4 or sulfites of Ca, Mg or Na under high temperature and pressure.
- When, however, the aim is to hydrolyze wood to sugars, the treatment is continued for longer than is done for paper manufacture.

- Fungi was the main source of cellulolytic enzymes.
- *Trichoderma viride* and *T. koningii* have been the most efficient cellulase producers.
- *Penicillicum funiculosum* and *Fusarium solani* have also been shown to possess potent cellulases.

- Cellulase has been resolved into at least three components:
  1) C_1 : attacks crystalline cellulose and loosens the cellulose chain
  2) C_x : are β - (1→4) glucanases and hydrolyse soluble derivatives of cellulose or swoollen or partially degraded cellulose. Enzymes attack on the cellulose molecule and may also act by removing successive glucose units from the end of a cellulose molecule.
  3) β-glucosidases : β-glucosidases hydrolyze cellobiose and short-chain oligo-saccharides derived from cellulose to glucose, but do not attack cellulose

Molecular structure of cellulose

Cellulose is a linear polymer of D-glucose linked in the Beta-1, 4 glucosidic bondage



A = Original cellulose fibril

B = Initial attack on amorphous region

C = Residue crystalline region

D = Attack on crystalline region