Environmental Microbiology
Course 10

Microbial Transformations

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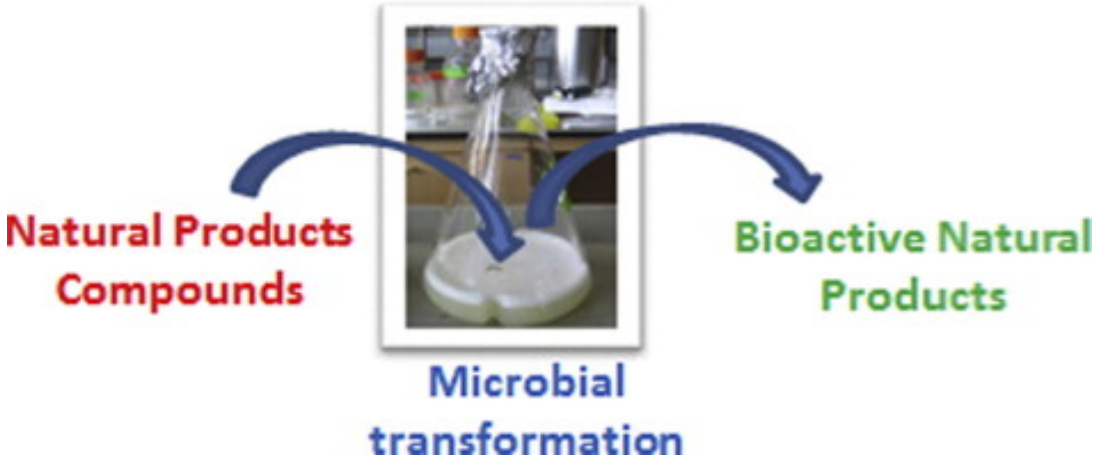
Biotransformation

- Biotransformation is a biological process whereby an organic compound is converted into structurally related products.
- These involves simple, chemically defined reactions catalyzed by enzymes present in the cell.
- Cells (i.e., microbial, plants and animal) provide the enzymes to catalyze the transformation reactions.

Microbial transformation

- Biotransformation can be clarified as the specific modification of a definite compound to a distinct product with structural similarity, by the use of biological catalysts including microorganisms like fungi and bacteria



Comparison of microbial transformation with others

Microbial cells are preferred more as compared to animal cells or plant cells due to the following reasons:

Surface-volume ratio: The microorganisms have high surface-volume ratio as compared to the plant or animal cell culture.

Growth Rate: The microorganisms have high growth rate as compared to the plant or animal cell culture thus the transformation using the cell culture is a less time consuming.

Comparison of microbial transformation with others

Sterility: Sterility is the major factor that should be taken care of. In case of plant or animal cell culture it is difficult to maintain sterility as compared to the transformation using microorganism.

Metabolism Rate: The microorganisms possess high rate of metabolism for the efficient transformation of the substrate added as compared to the plant or animal culture.

Sources of Biocatalysts and techniques for biotransformation

- A wide variety of biological catalysts can be used for biotransformation reactions.
- Includes:

→ Growing cells,

→ Resting cells,

→ Killed cells,

→ Immobilized cells,

→ Cell-free extract,

→ Enzymes and

→ Immobilized enzymes.

INDUSTRIAL PRODUCTS AND THE MICROORGANISMS THAT MAKE THEM

- Properties of a useful industrial microbe include
  - Produces spores or can be easily inoculated
  - Grows rapidly on a large scale in inexpensive medium
  - Produces desired product quickly
  - Should not be pathogenic
  - Allow genetic manipulation

Major products of industrial microbiology

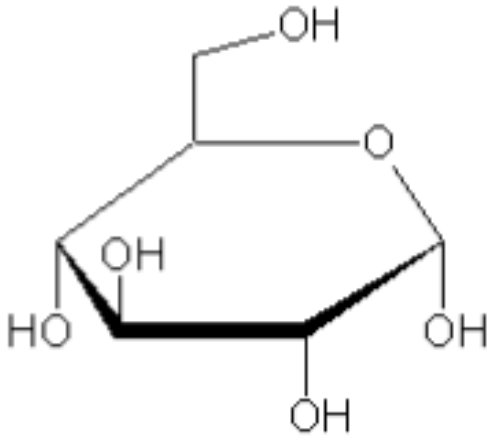
Product	*Example*
Antibiotics	Penicillin, tetracycline
Enzymes	Glucose isomerase, laundry proteases and lipases
Food additives	Vitamins, amino acids
Chemicals	Biofuels (alcohol and biodiesel), citric acid
Alcoholic beverages	Beer, wine, distilled spirits

Important Applications

- Production of primary metabolites (Acids & Alcohol)
- Secondary metabolites (Antibiotic)
- Production of whole microbial cells (Food, Vaccine)
- Biotransformation reactions (Enzymes, steroids)
- Exploitation of metabolism (Leaching and wastes treatment)
- Recombinant proteins (Therapeutic proteins, gene delivery vectors, etc.)

Metabolites

- ✓Intermediates and products of Metabolism;
- ✓Metabolites have various functions, including fuel, structure, signaling, stimulatory and inhibitory effects on enzymes, catalytic activity of their own, defense, and interactions with other organisms; and,
- ✓These are categorized as Primary & Secondary Metabolites.



a-D-Glucopyranose

Primary Metabolites

- ✓Intermediates and products of Primary Metabolism;
- ✓Directly involved in normal growth, development, and reproduction; and,
- ✓Absence of primary metabolites can result in immediate death.

Primary Metabolism

- ✓Glycolysis;
- ✓Photosynthesis;
- ✓Citric Acid Cycle;
- ✓Pentose Phosphate Pathway;
- ✓β-oxidation of fatty acids' and,
- ✓Synthesis of proteins, enzymes and coenzymes.

Secondary Metabolites

- ✓Organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism;
- ✓Chemical compounds that are found only in specific organisms or groups of organisms as an expression of their individuality;
- ✓Used as defense, attractants, and coloring agents;
- ✓Provide most of the pharmacologically active natural products; and,
- ✓Absence of secondary metabolites can result to long-term impairment of the organism's survivability, fecundity, or aesthetics, or perhaps in no significant change at all.

Secondary metabolites

- Secondary metabolites have no function in the growth of the producing cultures (although, in nature, they are essential for the survival of the producing organism), functioning as:

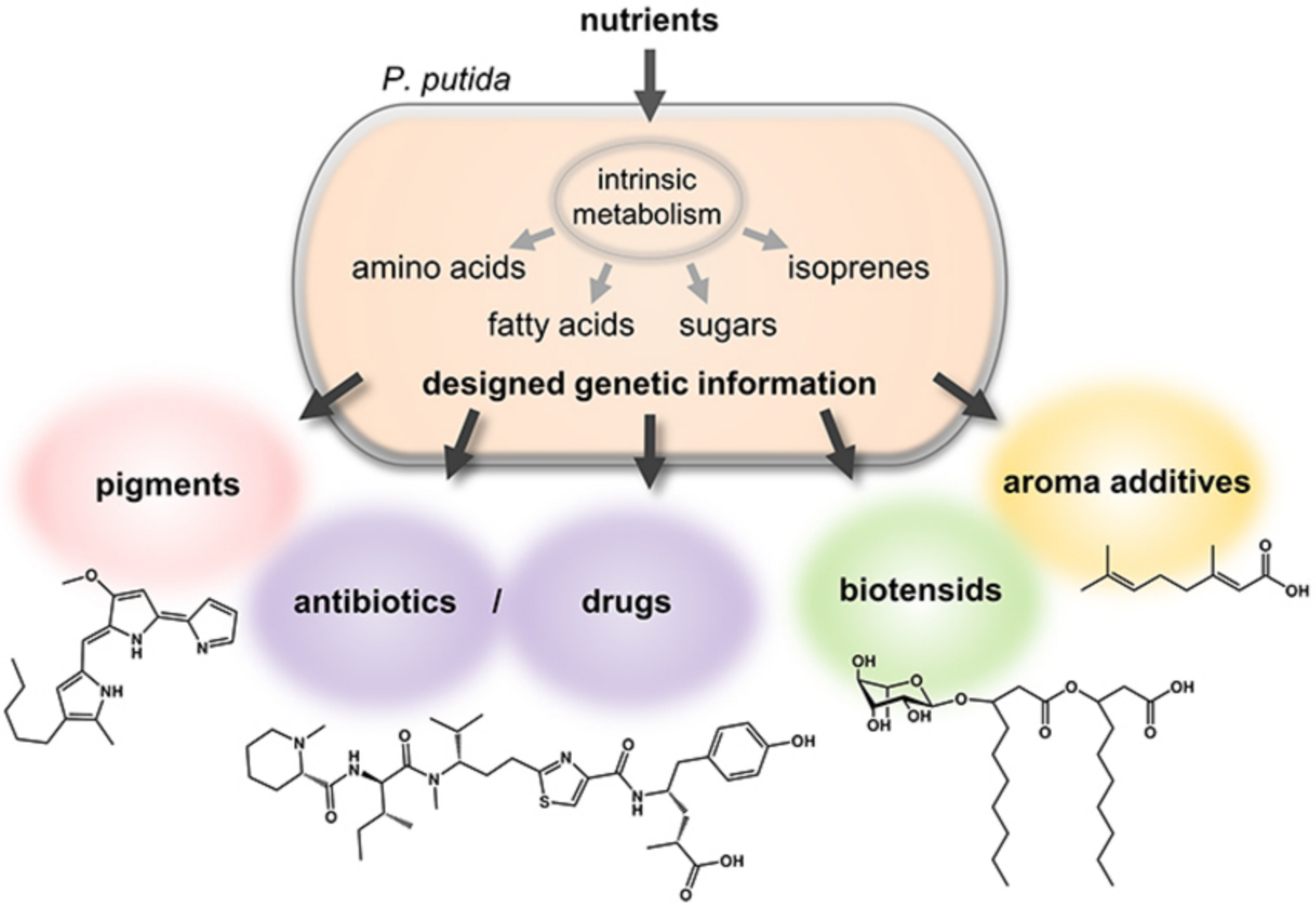
  (1) sex hormones;

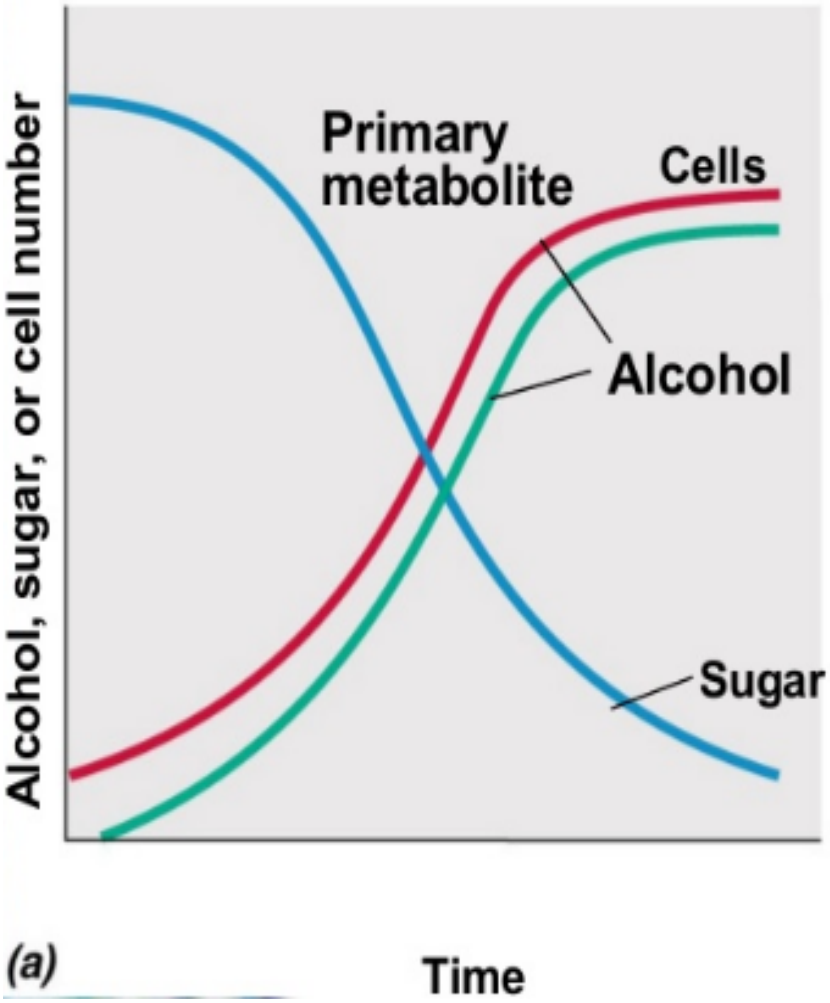
  (2) Antibiotics

  (3) ionophores;

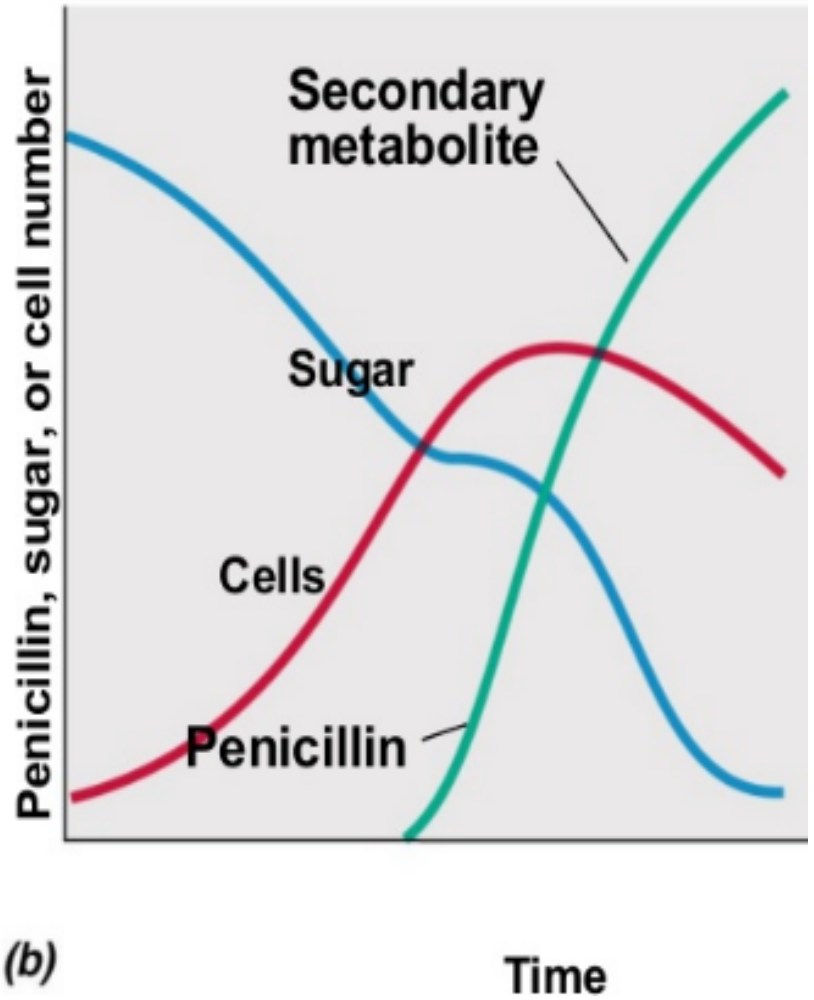
  (4) competitive weapons against other bacteria, fungi, amoebae, insects and plants;

  (5) agents of symbiosis etc.
- Microbially produced secondary metabolites are extremely important for health and nutrition.
  - Antibiotics
  - Other medicinals
  - Toxins
  - Biopesticides
  - Animal and plant growth factors


nutrients
P. putida
intrinsic metabolism
amino acids
isoprenes
fatty acids
sugars
designed genetic information
pigments
antibiotics / drugs
biotensids
aroma additives


Alcohol, sugar, or cell number
Primary metabolite
Cells
Alcohol
Sugar
Time

(a)


Penicillin, sugar, or cell number
Secondary metabolite
Sugar
Cells
Penicillin
Time

(b)

Antibiotics

- The best-known group of the secondary metabolites are the antibiotics.
- Their targets include
- DNA replication (Actinomycin)
- Transcription (Rifamycin)
- Translation (Chloramphenicol, tetracycline, erythromycin and streptomycin)
- Cell wall synthesis (cycloserine, bacitracin, penicillin, cephalosporin and vancomycin)

Antibiotic-producing microorganisms

- Penicillium and Cephalosporium

Beta-lactam antibiotics: *penicillin and cephalosporin*

- Actinomycetes, Streptomyces species

Tetracyclines *Aminoglycosides*

Macrolides *Chloramphenicol*

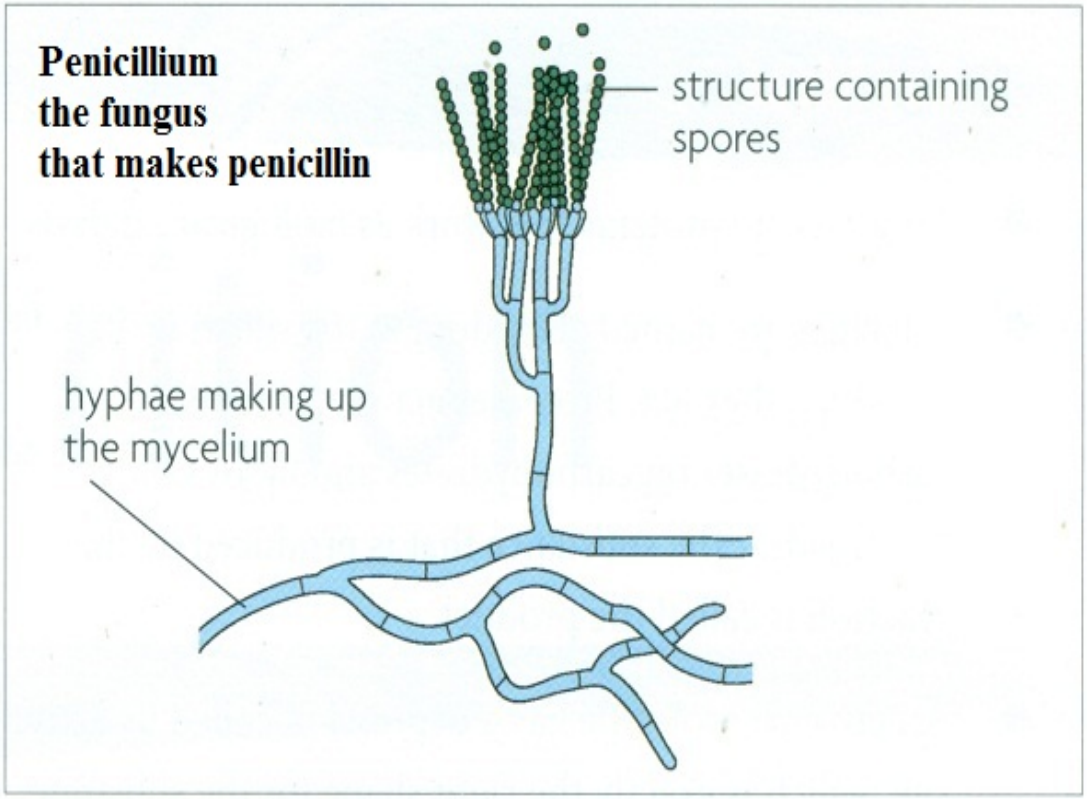
- Bacillus species

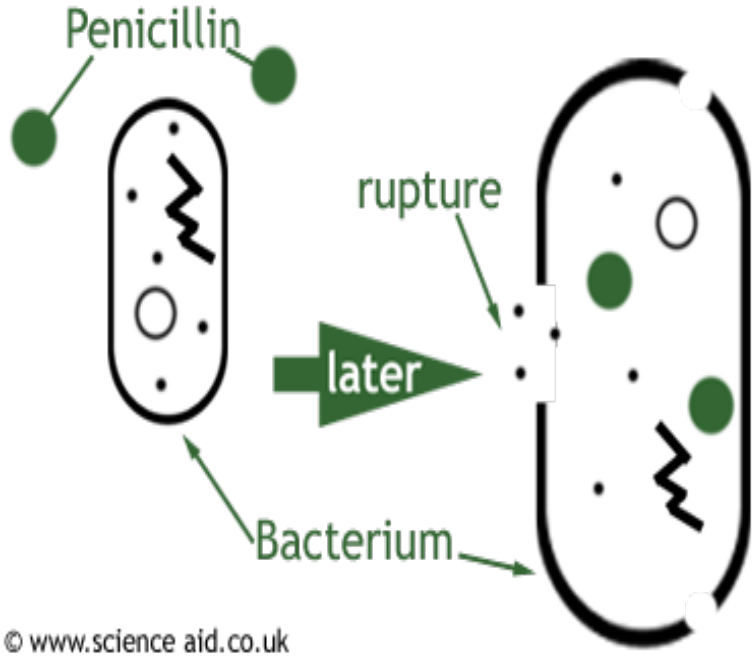
Polypeptide antibiotics*: polymyxin and bacitracin*

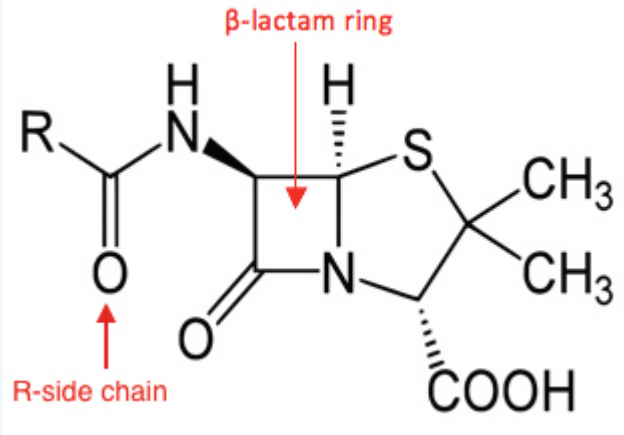
Industrial Production of Penicillins and Tetracyclines

- Penicillins are *β-lactam antibiotics*
  - *Natural* and *biosynthetic penicillins*
  - *Semisynthetic penicillins*
    - *Broad spectrum* of activity
- Penicillin production is typical of a secondary metabolite
  - Production only begins after near-exhaustion of carbon source
  - High levels of glucose repress penicillin production

Penicillium (Fungi)







• The industrial production of antibiotics begins with screening for antibiotic producers (**Figure 30.7**).

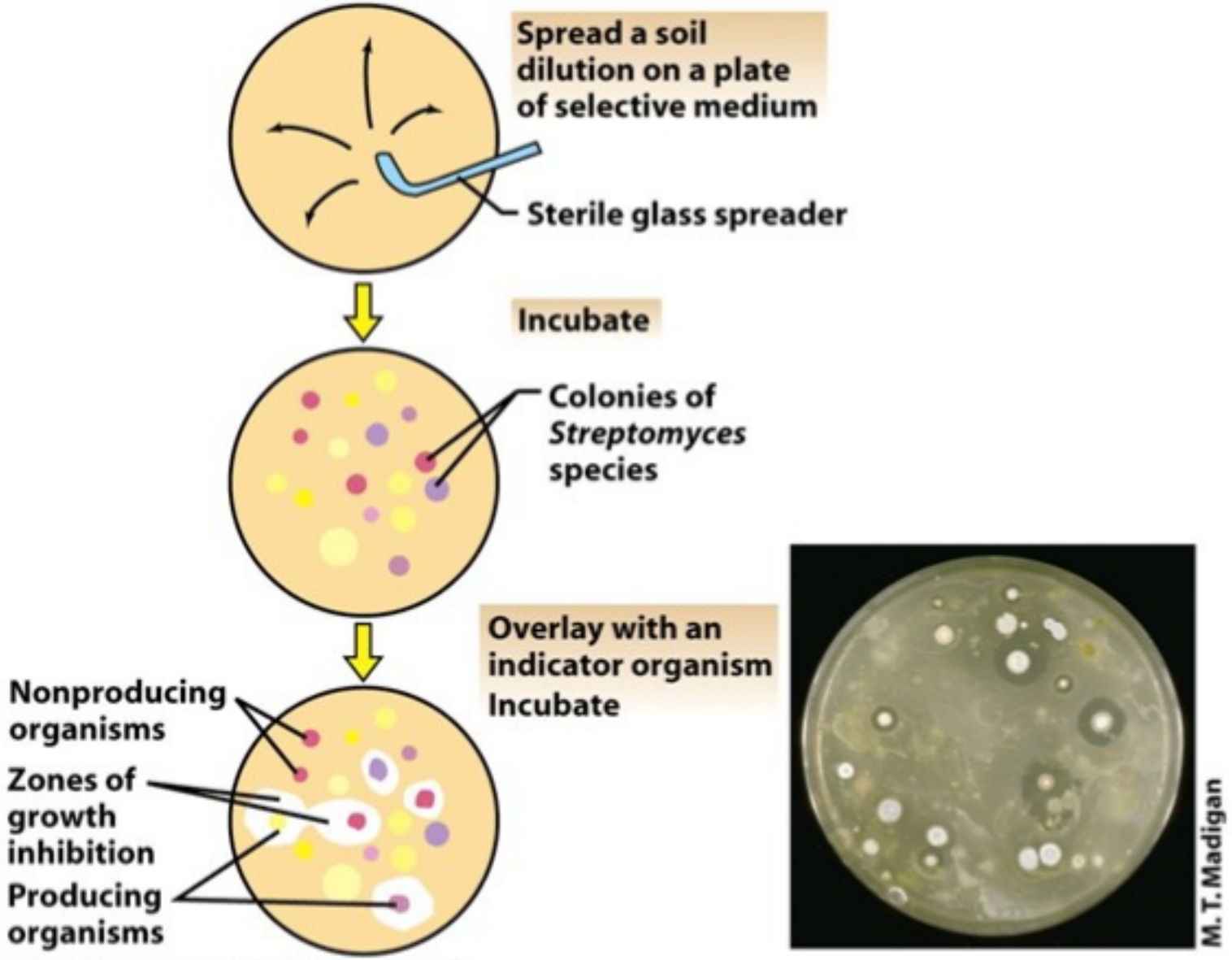


Figure 30-7a Brock Biology of Microorganisms 11/e

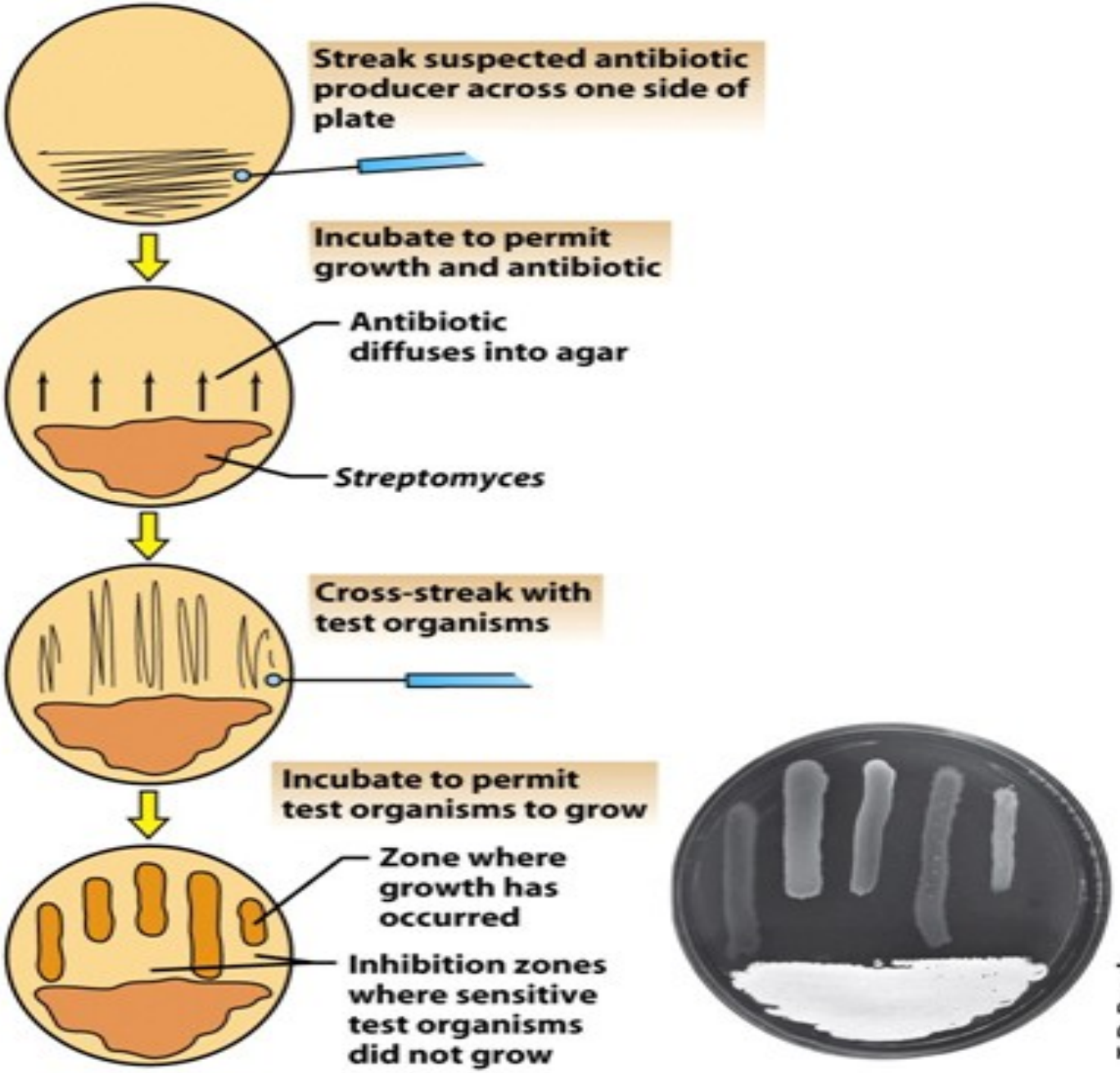


Figure 30-7b Brock Biology of Microorganisms 11/e

Biosynthesis of Penicillin

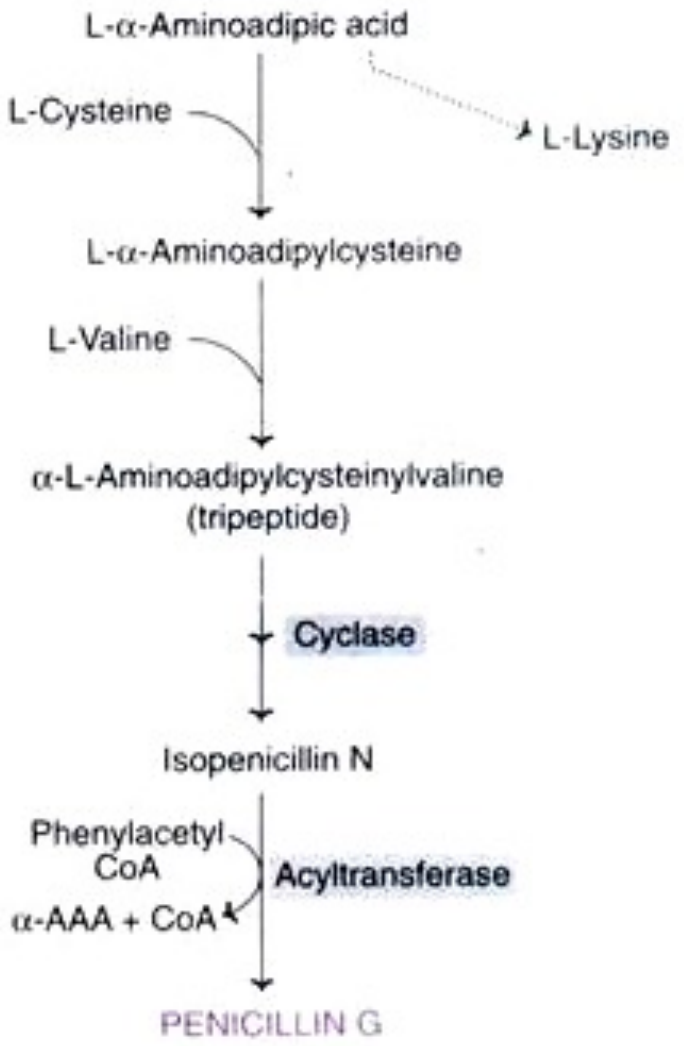


***Fig. 25.2** : Biosynthesis of penicillin by* Penicillium chrysogenum *(α-AAA — α-Amino adipic acid; CoA–Coenzyme A.*

- L-α-Aminoadipic acid combines with L-cysteine, and then with L-valine to form a tripeptide namely α-L-aminoadipylcysteinylvaline.
- This compound forms isopenicillin which reacts with phenyl acetyl CoA (catalysed by the enzyme acyltransferase) to produce penicillin G (benzyl penicillin).
- In this reaction, aminoadipic acid gets exchanged with phenylacetic acid

Biosynthesis of Penicillin

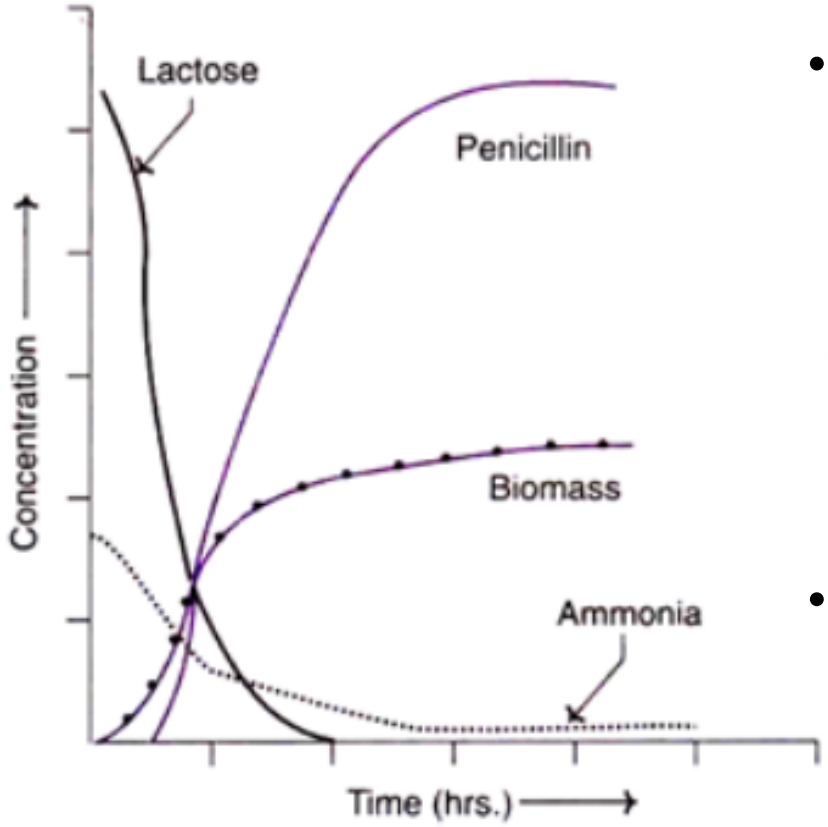


Fig. 25.4 : Penicillin production in relation to substrates utilization and biomass formation.

- Penicillin biosynthesis is inhibited by glucose through catabolite repression. For this reason, penicillin was produced by a slowly degraded sugar like lactose. The concentrations of phosphate and ammonia also influence penicillin synthesis.
- Sometimes, ammonium sulfate is added for the supply of nitrogen. Phenylacetic acid (or phenoxyacetic acid) which serves as a precursor for penicillin biosynthesis is continuously fed.

Biosynthesis of Penicillin

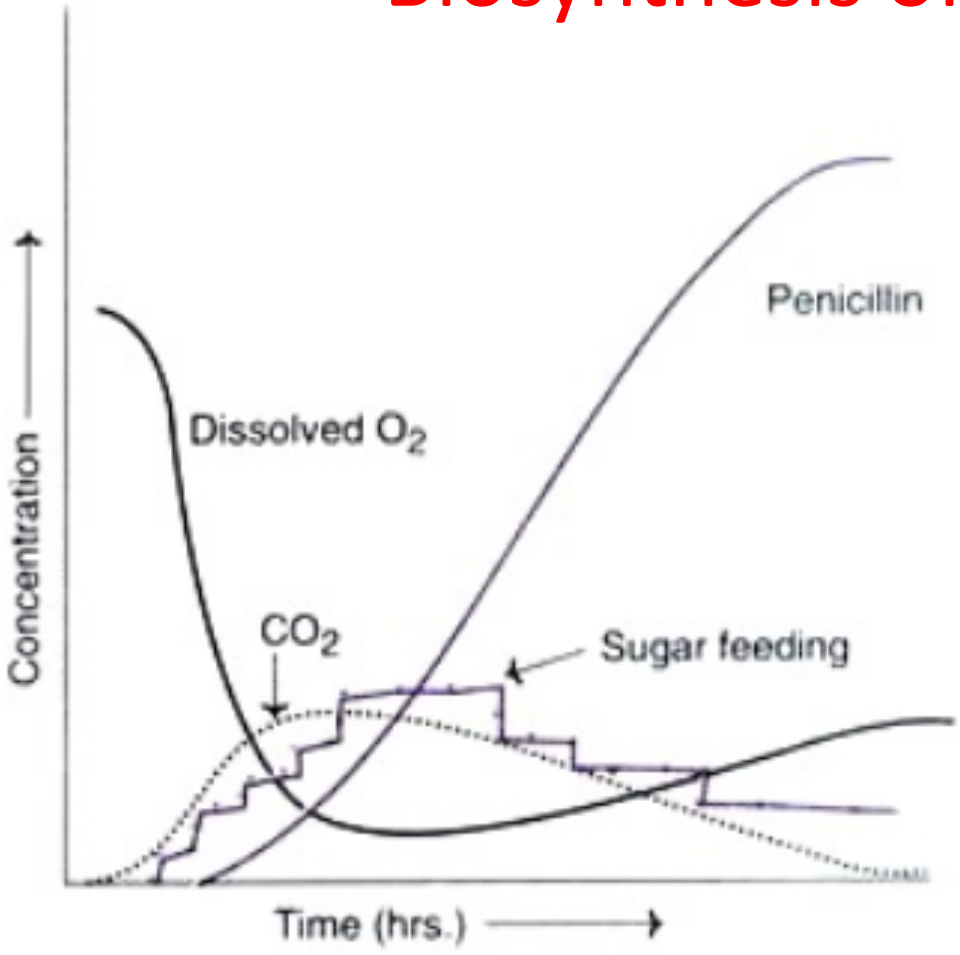
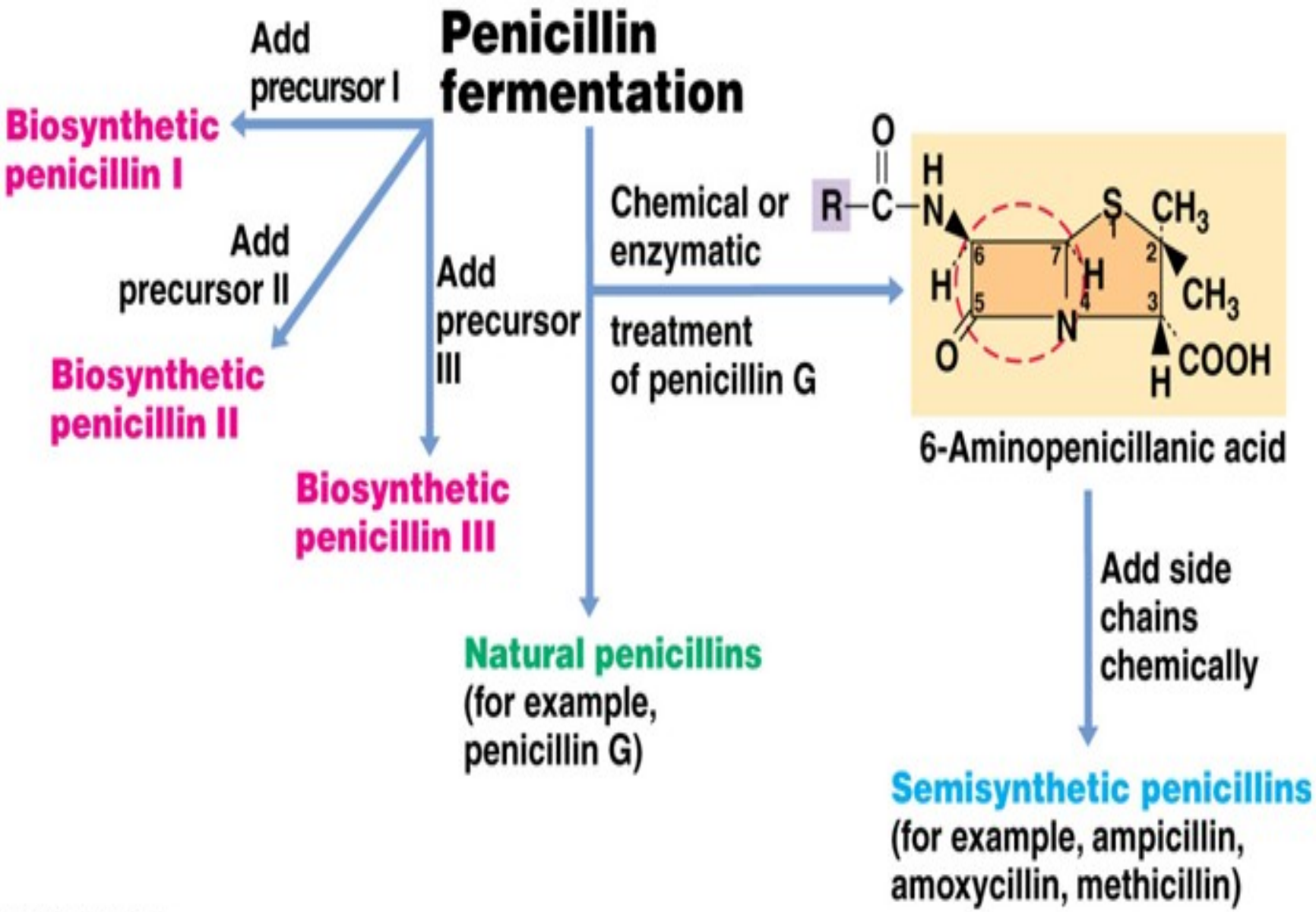


***Fig. 25.5:** Penicillin production in relation to continuous feeding of sugar, O_2 utilization, and CO_2 formation.*

- Penicillin production is an aerobic process and therefore, a continuous supply of O_2 to the growing culture is very essential.
- Further, continuous feeding of sugar is advantageous for a good yield of penicillin.
- Thus, by adding lactose and acetic acid, the yield can be increased by about 25%

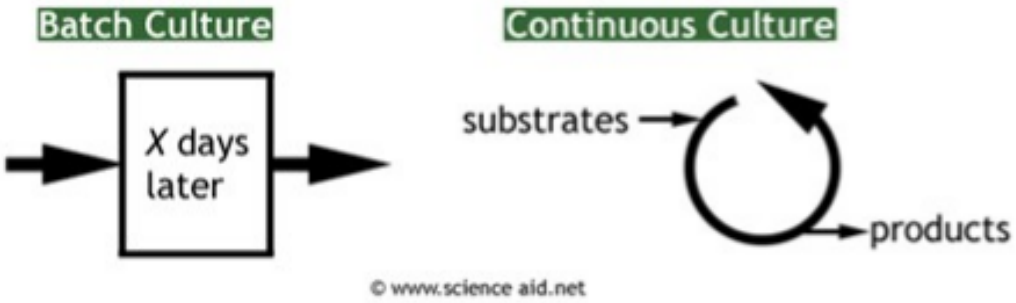
• If the penicillin fermentation is carried out without addition of side-chain precursors, the **natural penicillins** are produced. The fermentation can be more directed by adding to the broth a side-chain precursor so that only one desired penicillin is produced.



Fermentation

- Fermentation could be:

- Batch mode
- Fed batch mode (continuous)



Batch fermentation

- Most fermentations are batch processes
- Nutrients and the inoculum are added to the sterile fermenter and left to get on with it!
- Anti-foaming agent may be added.
- Once the desired amount of product is present in the fermenter the contents are drained off and the product is extracted.
- After emptying, the tank is cleaned & prepared for a new batch.

Continuous fermentation

- Some products are made by a continuous culture system.
- Sterile medium is added to the fermentation with a balancing withdrawal of broth for product extraction.

INDUSTRIAL PRODUCTION OF ANTIBIOTIC- PENICILLIN

- The industrial production of penicillin was broadly classified in to two processes namely,

- Upstream processing
- Downstream processing

UPSTREAM PROCESSING

- Upstream processing encompasses any technology that leads to the synthesis of a product. Upstream includes the exploration, development and production.

DOWNSTREAM PROCESSING

- The extraction and purification of a biotechnological product from fermentation is referred to as downstream processing.

UPSTREAM PROCESSING
INOCULUM PREPARATION

- The medium is designed to provide the organism with all the nutrients that it requires.
- Inoculation method- submerged technique
- Spores -major source of inoculum

RAW MATERIALS

- **CARBON SOURCES:**
  Lactose acts as a very satisfactory carbon compound, provided that is used in a concentration of 6%. Others such as glucose & sucrose may be used.

NITROGEN SOURCES:

- Corn steep liquor (CSL)
- Ammonium sulphate and ammonium acetate can be used as nitrogenous sources.

MINERAL SOURCES:
Elements namely potassium, phosphorus, magnesium, sulphur, zinc and copper are essential for penicillin production. Some of these are applied by corn steep liquor.

- **Calcium** can be added in the form of chalk to counter the natural acidity of CSL
- **PAA** - precursor

FERMENTATION PROCESS

- The medium is inoculated with a suspension of conidia of Penicillium chrysogenum.
- The medium is constantly aerated and agitated, and the mould grows throughout as pellets.
- After about seven days, growth is complete, the pH rises to 8.0 or above, and penicillin production ceases

STAGES IN DOWNSTREAM PROCESSING

Removal of cells

- The first step in product recovery is the separation of whole cells and other insoluble ingredients from the culture broth by technique such as filtration and centrifugation.

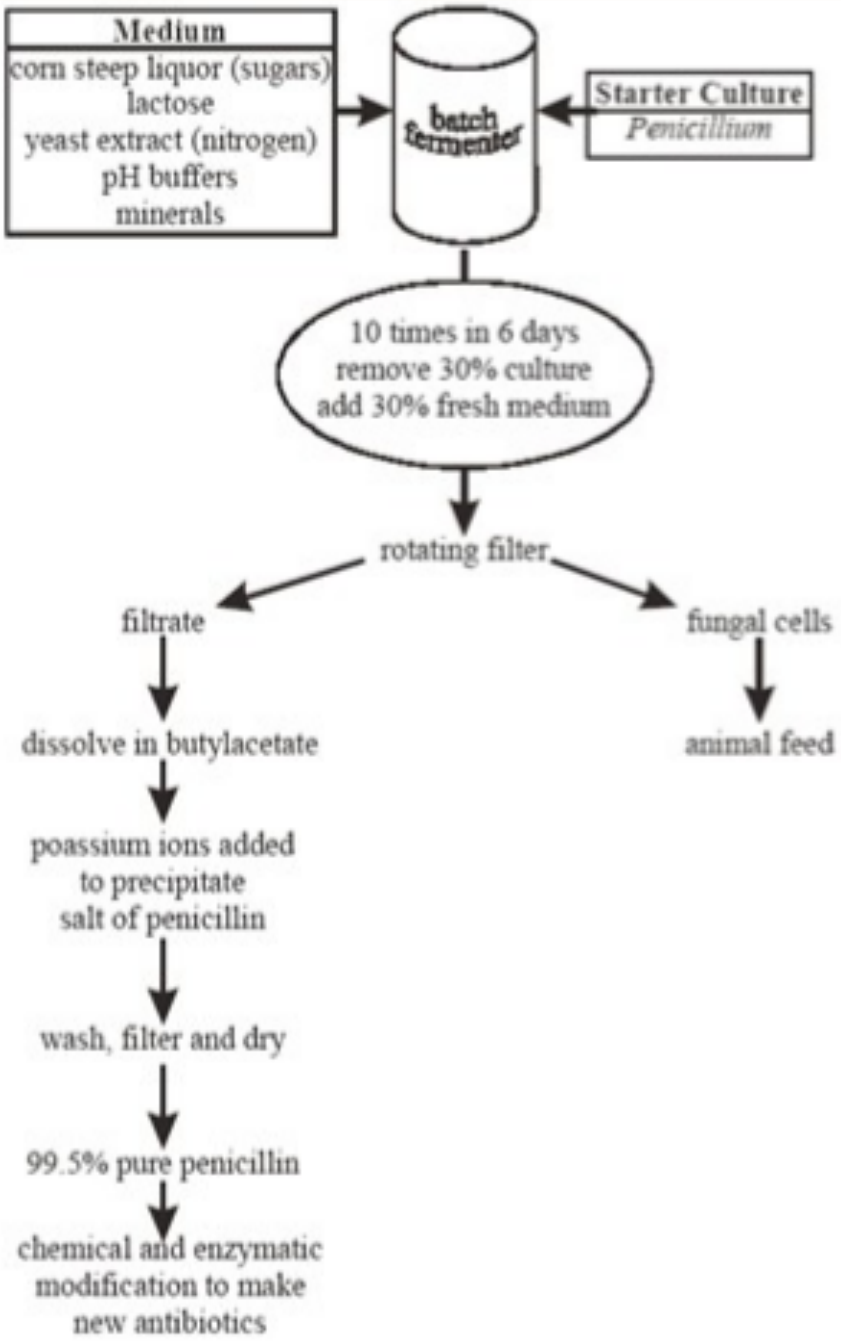
ISOLATION OF BENZYL PENICILLIN

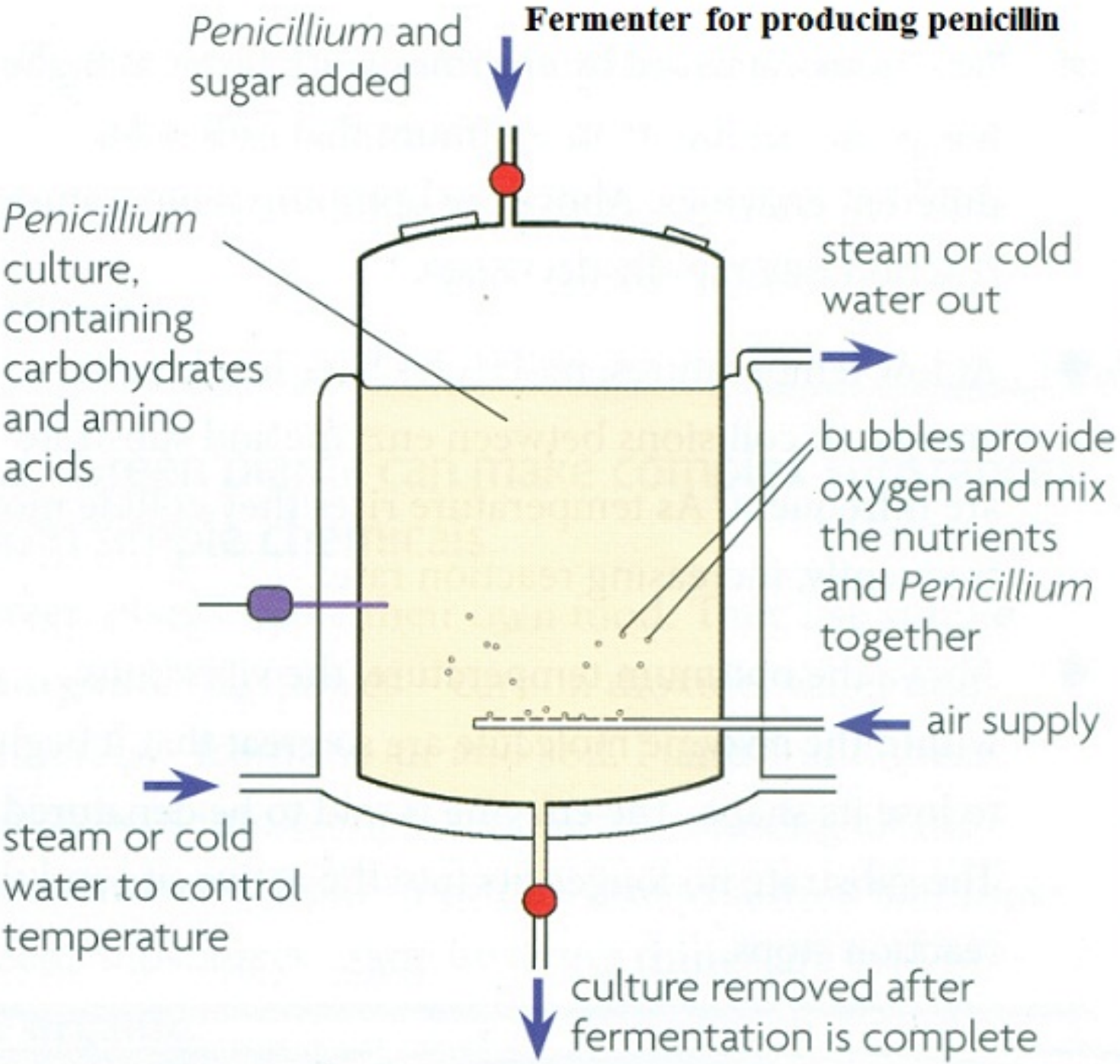
- The PH is adjusted to 2-2.5 with the help of phosphoric or sulphuric acids.
- In aqueous solution at low PH values there is a partition coefficient in favor of certain organic solvents such as butyl acetate.
- This step has to be carried out quickly for penicillin is very unstable at low PH values.
- Antibiotic is then extracted back into an aqueous buffer at a PH of 7.5, the partition coefficient now being strongly in favor of the aqueous phase. The resulting aqueous solution is again acidified & re-extracted with an organic solvent.
- These shifts between the water and solvent help in the purification of penicillin.

- The treatment of the crude penicillin extract varies according to the objective, but involves the formation of an appropriate penicillin salt.
- The solvent extract recovered in the previous stage is carefully extracted back with aqueous sodium hydroxide.
- This is followed by charcoal treatment to eliminate pyrogens and by sterilization.
- Pure metal salts of penicillin can be safely sterilized by dry heat, if desired. Thereafter, the aqueous solution of penicillin is subjected to crystallization.

The main stages of Penicillin production are:

1. A medium of corn steep liquor (a by product of starch manufacture), yeast extract and others substrates added to the fermenter.
2. After 40 hours, Penicillin begins to be secreted by the fungus
3. The mould mycellium (cell matter) is filtered from the harvested product.
4. Penicillin is extracted in the organic solvent: butylacetate, in which it dissolves.
5. Potassium salts are added and a penicillin precipitate is formed, this is washed and dried.


Medium
corn steep liquor (sugars)
lactose
yeast extract (nitrogen)
pH buffers
minerals
batch fermenter
Starter Culture
Penicillium
10 times in 6 days
remove 30% culture
add 30% fresh medium
rotating filter
filtrate
fungal cells
dissolve in butylacetate
animal feed
poassium ions added
to precipitate
salt of penicillin
wash, filter and dry
99.5% pure penicillin
chemical and enzymatic
modification to make
new antibiotics


Fermenter for producing penicillin
Penicillium and sugar added
Penicillium culture, containing carbohydrates and amino acids
steam or cold water out
bubbles provide oxygen and mix the nutrients and *Penicillium* together
air supply
steam or cold water to control temperature
culture removed after fermentation is complete

- Precursors improve the yield or quality of products that are incorporated without any major change in the product. E.g., phenyl acetic acid for penicillin, cobalt for vitamin B 12.

- Inducers: The majority of the enzymes used in industrial fermentation are inducible and are synthesized in response of inducers: e.g. starch for amylases, maltose for pollulanase, pectin for pectinase.

- Chelators: Chelators are the chemicals used to avoid the precipitation of metal ions. Chelators like EDTA, citric acid, polyphosphates are used in low concentrations.

- Antifoaming agents(alcohol anf fatty acids)

- Phosphates as buffers

- Sometimes growth factors and calcium carbonate

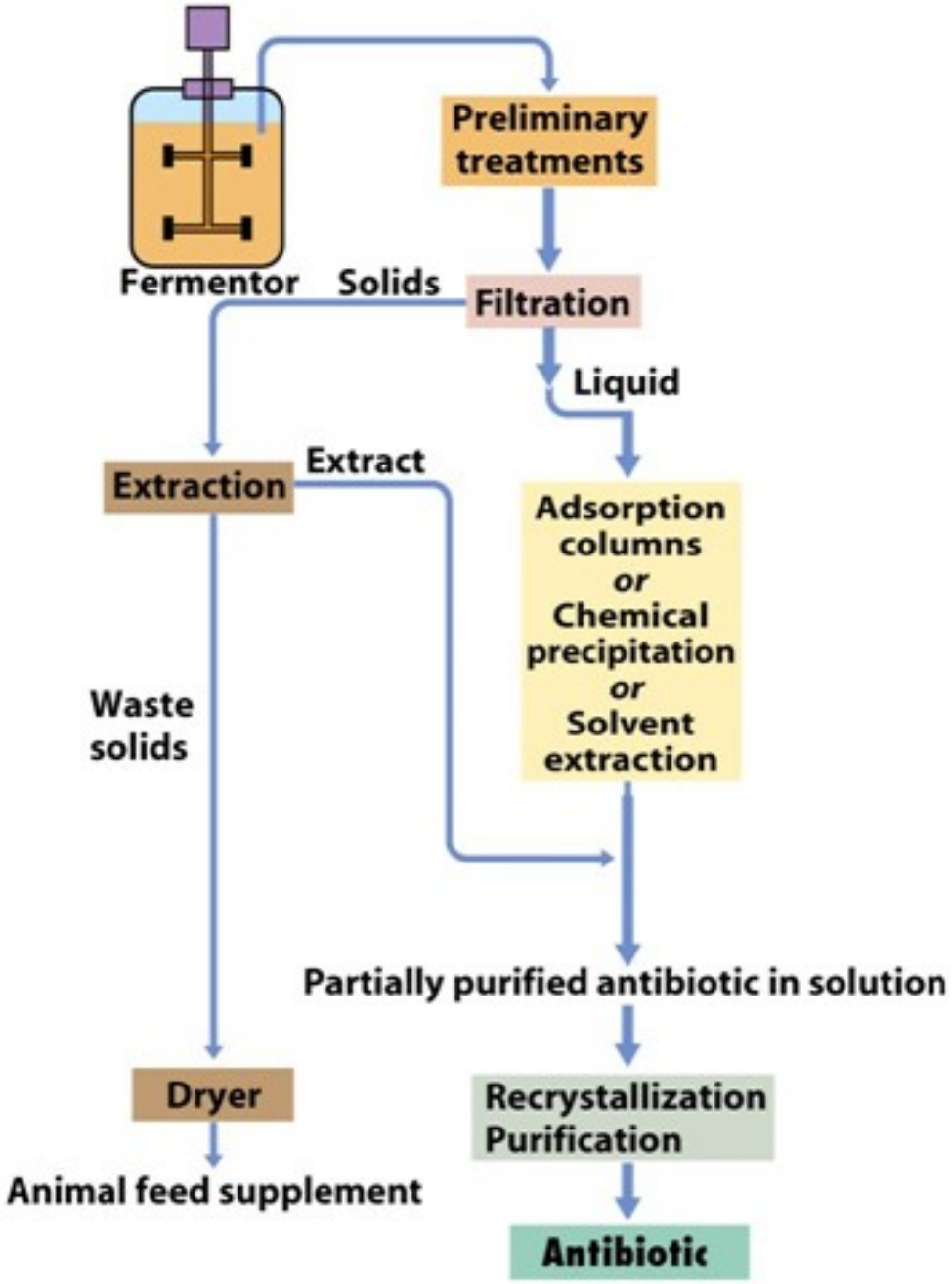


Figure 30-8a Brock Biology of Microorganisms 11/e

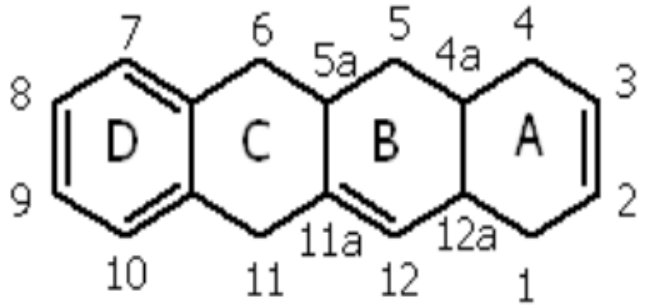
Non β-lactam antibiotics

Tetracyclines

Obtained from :

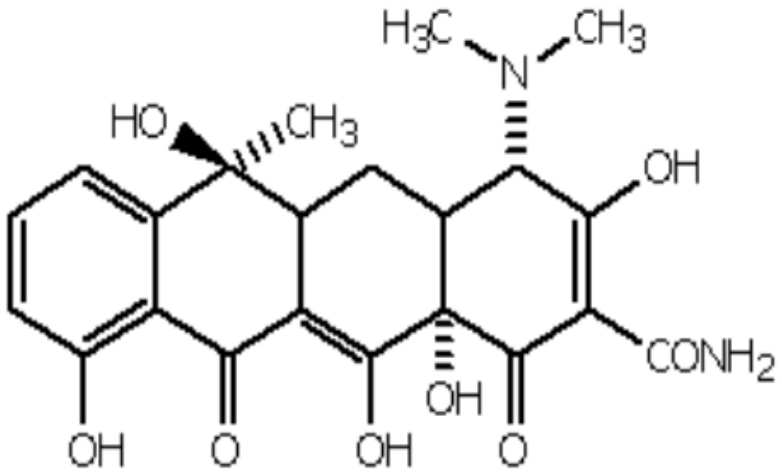
(1) Fermentation procedures from *Streptomyces* species.

(2) Chemical transformation of natural products.

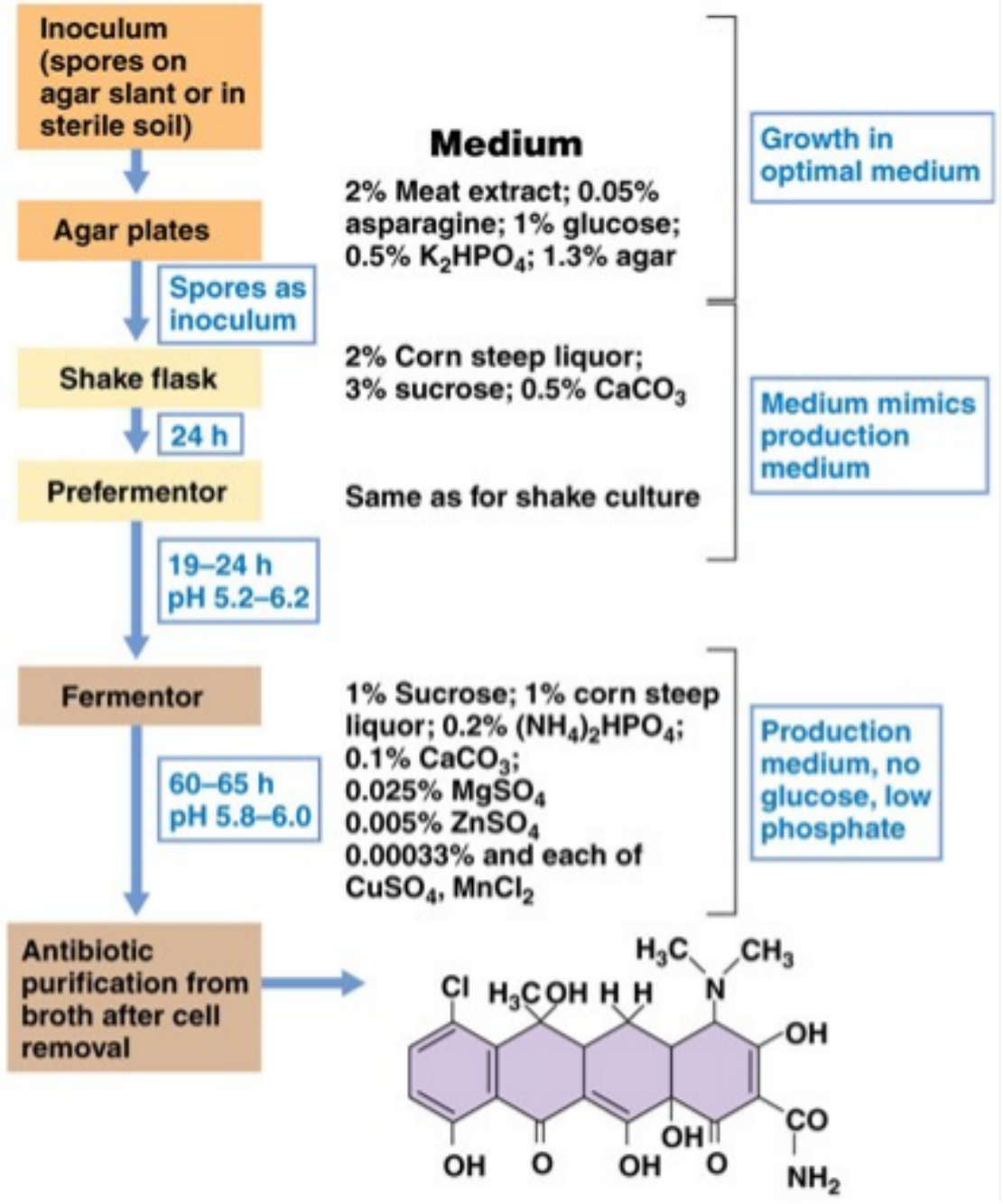


Octahydronaphthacene

[5 double bonds]



Tetracycline Production



Vitamins and Amino Acids

- Vitamins produced microbially include vitamin B_{12} and riboflavin

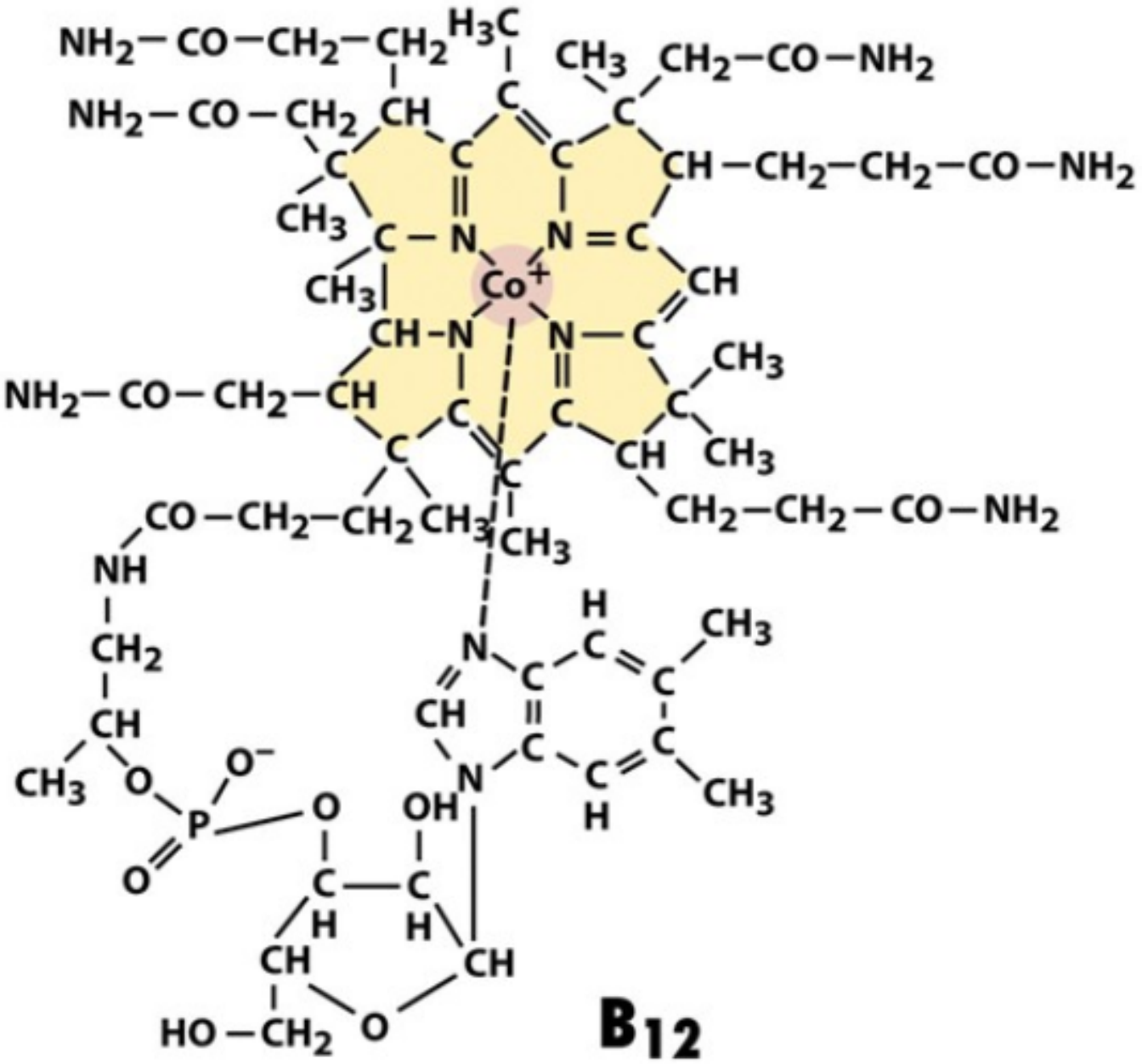


Figure 30-12a Brock Biology of Microorganisms 11/e

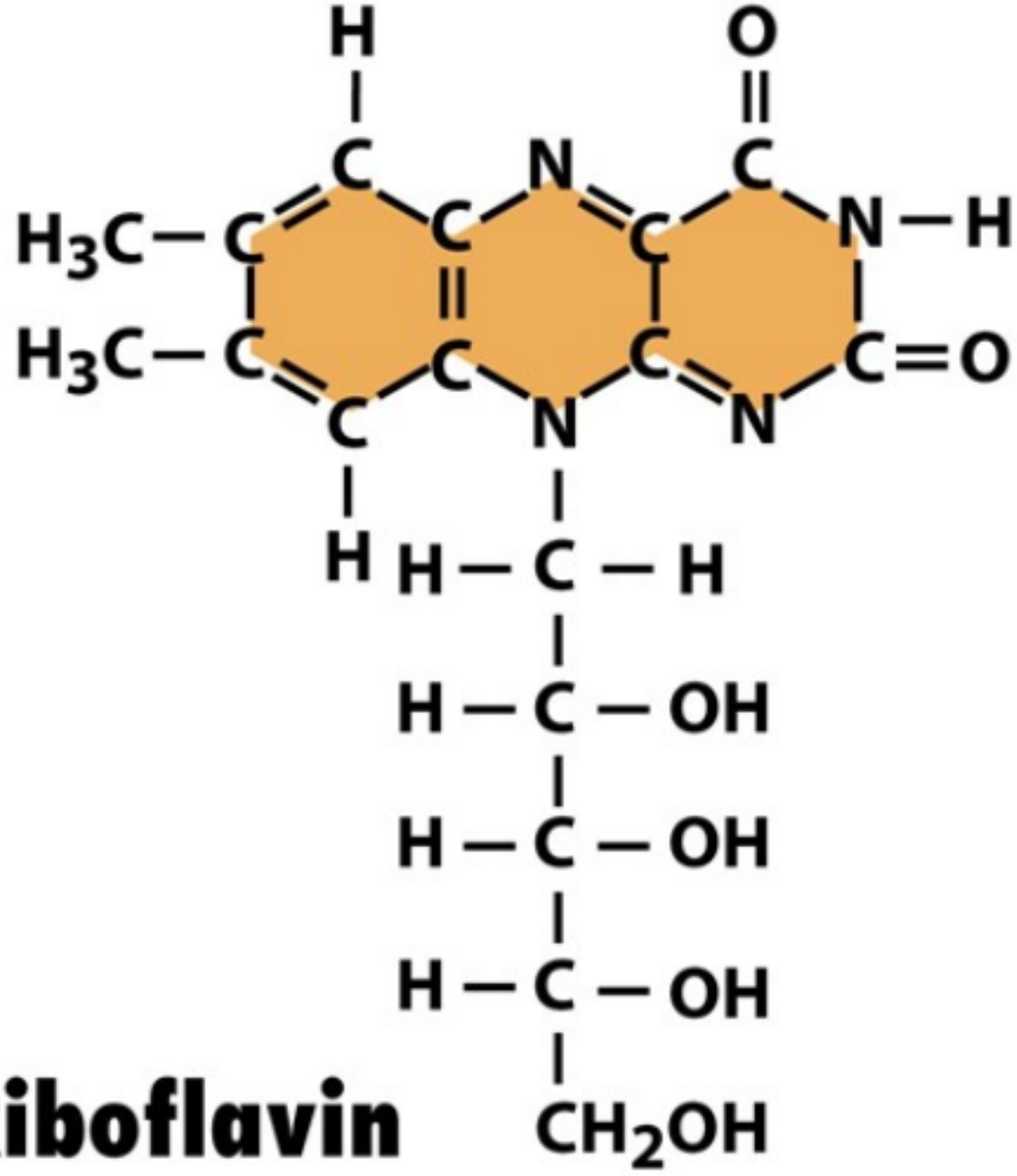


Figure 30-12b Brock Biology of Microorganisms 11/e

- The most important amino acids produced commercially are glutamic acid, aspartic acid, phenylalanine, and lysine

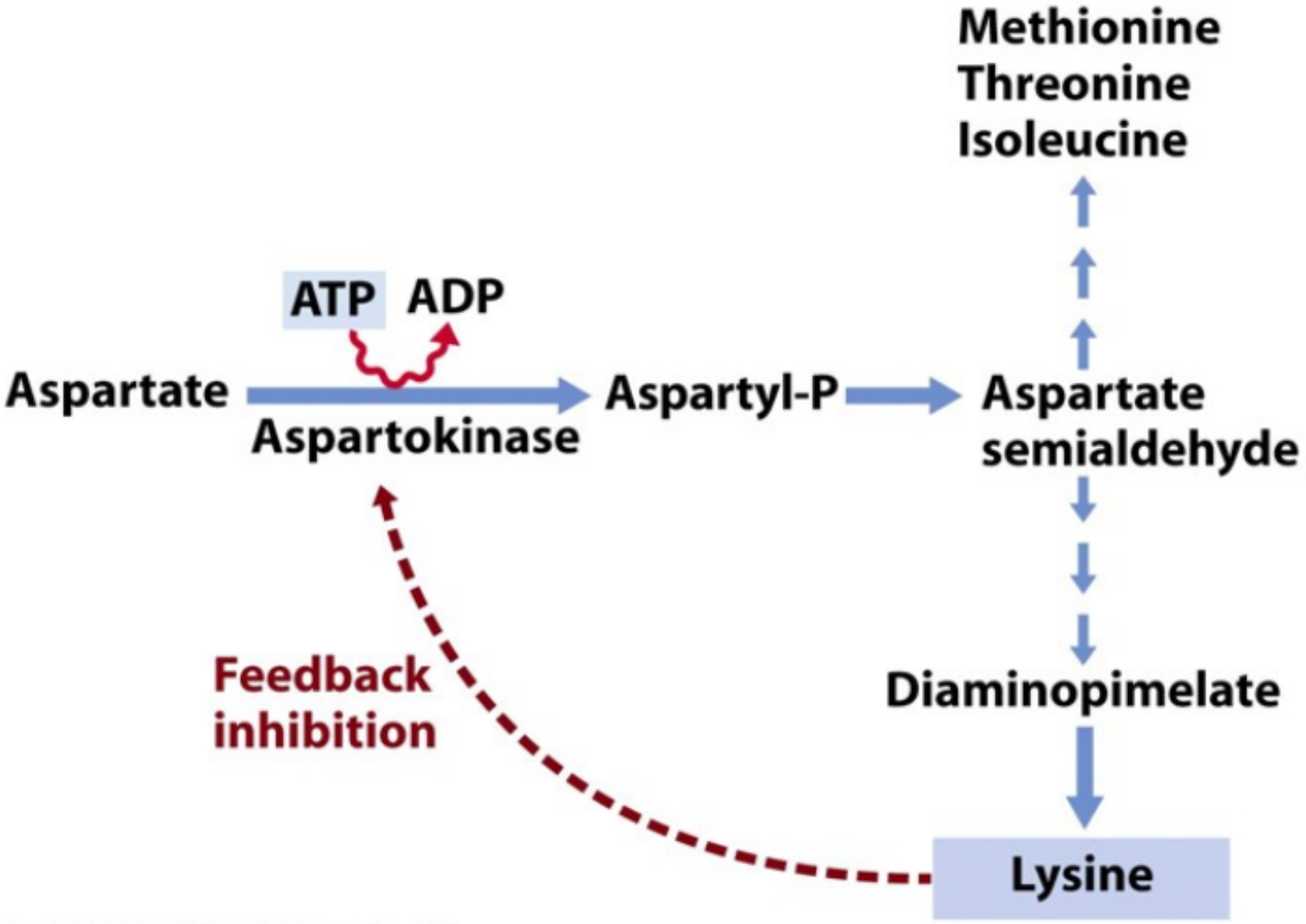


Figure 30-13a Brock Biology of Microorganisms 11/e

Table 30.3 Amino acids used in the food industry[a]

Amino acid[b]	Annual production worldwide (metric tons)	Uses	Purpose
L-Glutamate (monosodium glutamate, MSG)	370,000	Various foods	Flavor enhancer; meat tenderizer
L-Aspartate and alanine	5,000	Fruit juices	"Round off" taste
Glycine	6,000	Sweetened foods	Improves flavor; starting point for organic syntheses
L-Cysteine	700	Bread Fruit juices	Improves quality Antioxidant
L-Tryptophan + L-Histidine	400	Various foods, dried milk	Antioxidant, prevent rancidity; nutritive additives
Aspartame (made from L- phenylalanine + L-aspartic acid)	7,000	Soft drinks, chewing gum, many other "sugar-free" products	Low-calorie sweetener
L-Lysine	70,000	Bread (Japan), feed additives	Nutritive additive
DL-Methionine	70,000	Soy products, feed additives	Nutritive additive

[a] Data from Glazer, A. N., and H. Mikaido. 1995. *Microbial Biotechnology*, W. H. Freeman, New York.
[b] The structures of these amino acids are shown in Figure 3.12.

Table 30-3 Brock Biology of Microorganisms 11/e

Table 30.4 Microbial enzymes and their applications

Enzyme	Source	Application	Industry
Amylase (starch-digesting)	Fungi	Bread	Baking
	Bacteria	Starch coatings	Paper
	Fungi	Syrup and glucose manufacture	Food
	Bacteria	Cold-swelling laundry starch	Starch
	Fungi	Digestive aid	Pharmaceutical
	Bacteria	Removal of coatings (desizing)	Textile
	Bacteria	Removal of stains; detergents	Laundry
Protease (protein-digesting)	Fungi	Bread	Baking
	Bacteria	Spot removal	Dry cleaning
	Bacteria	Meat tenderizing	Meat
	Bacteria	Wound cleansing	Medicine
	Bacteria	Desizing	Textile
	Bacteria	Household detergent	Laundry
Invertase (sucrose-digesting)	Yeast	Soft-center candies	Candy
Glucose oxidase	Fungi	Glucose removal, oxygen removal	Food
		Test paper for diabetes	Pharmaceutical
Glucose isomerase	*Bacteria*	High-fructose corn syrup	Soft drink
Pectinase	Fungi	Pressing, clarification	Wine, fruit juice
Rennin	Fungi	Coagulation of milk	Cheese
Cellulase	*Bacteria*	Fabric softening, brightening; detergent	Laundry
Lipase	Fungi	Breaks down fat	Dairy, laundry
Lactase	Fungi	Breaks down lactose to glucose and galactose	Dairy, health foods
DNA polymerase	*Bacteria*	DNA replication in polymerase chain	Biological research;
	Archaea	reaction (PCR) technique (Section 7.9)	forensics

Table 30-4 Brock Biology of Microorganisms 11/e

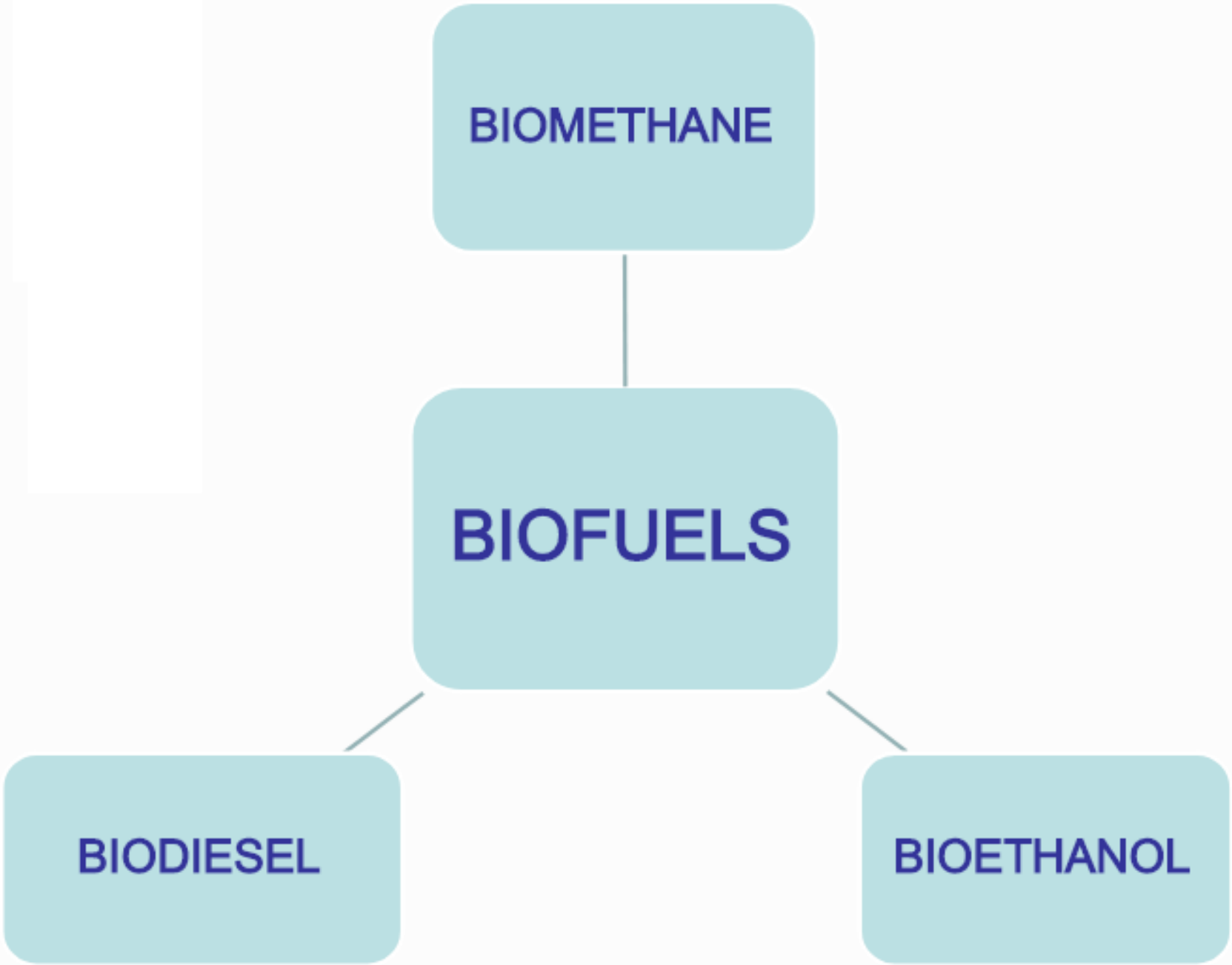
Bioenergy

INTRODUCTION:

Biofuel is a fuel that uses biomass from living organisms which may be plant, animal microorganisms etc.

It uses sunlight as a renewable source.

In short, a hope to meet the global energy demands.


BIOMETHANE
BIOFUELS
BIODIESEL
BIOETHANOL

BIOETHANOL:

Ethanol derived from agricultural sources such as sugar cane, corn, potato etc., produced by sugar or cellulosic fermentation is called bioethanol.

Distinct features:

- It is renewable
- Posses a large variety of substrate
- Biodegradable
- Less toxic

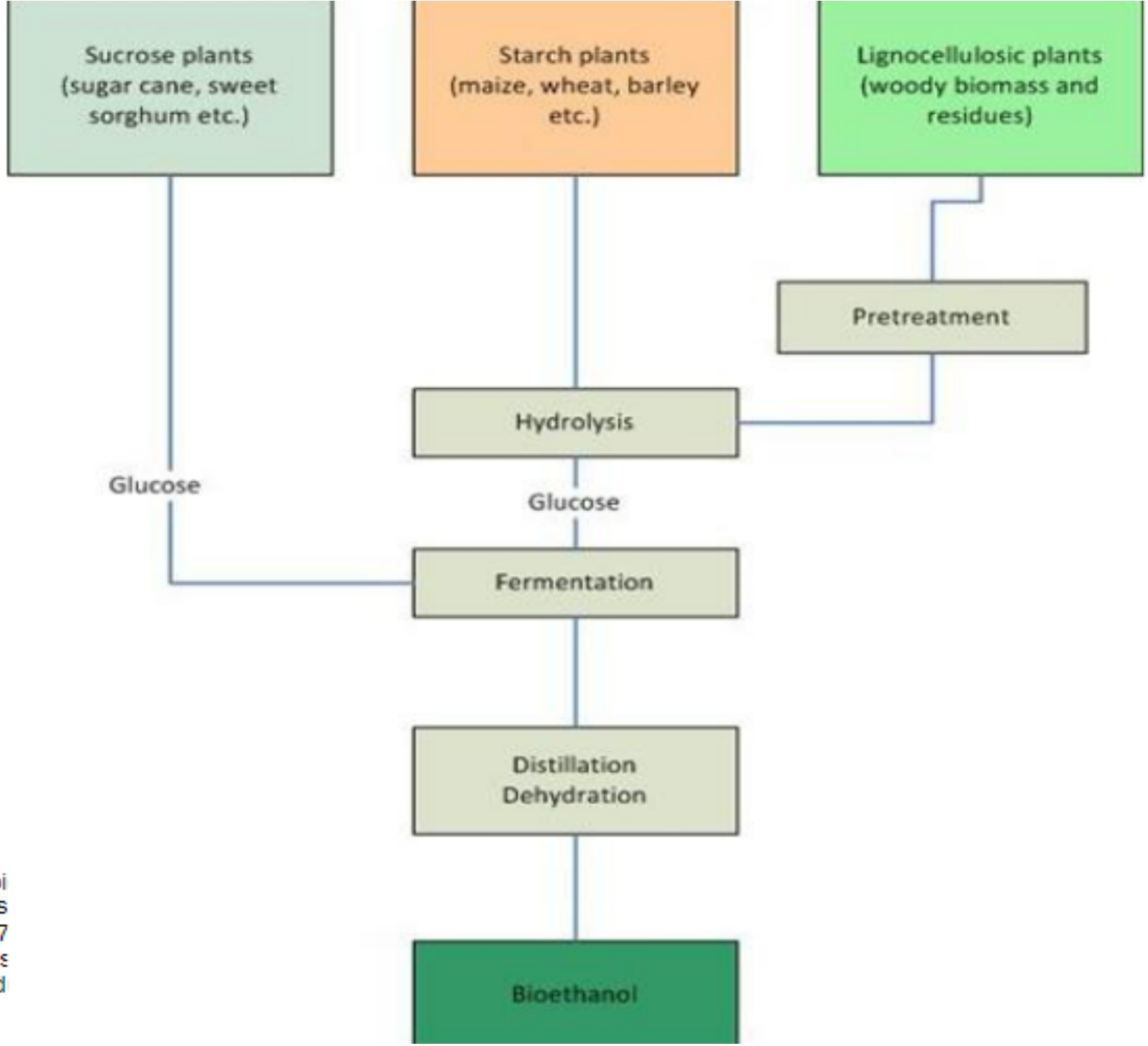
WHAT ARE THE RAW MATERIALS FOR BIOETHANOL?

There are in general three groups of raw material:

1). Sugar : Beet, Sugar Cane, Sweet Sorghum and Fruits.
2). Starchy Material such as corn, wheat, rice, potatoes, cassava, sweet potatoes etc.
3). Cellulose materials like wood, used paper, crop residues etc.

STEPS FOR ETHANOL PRODUCTION

- Fermentation Process
- Distillation Process
- Dehydration Process



http://www.bi
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bioaithanolhs
ethanol-prod
biomass-p1

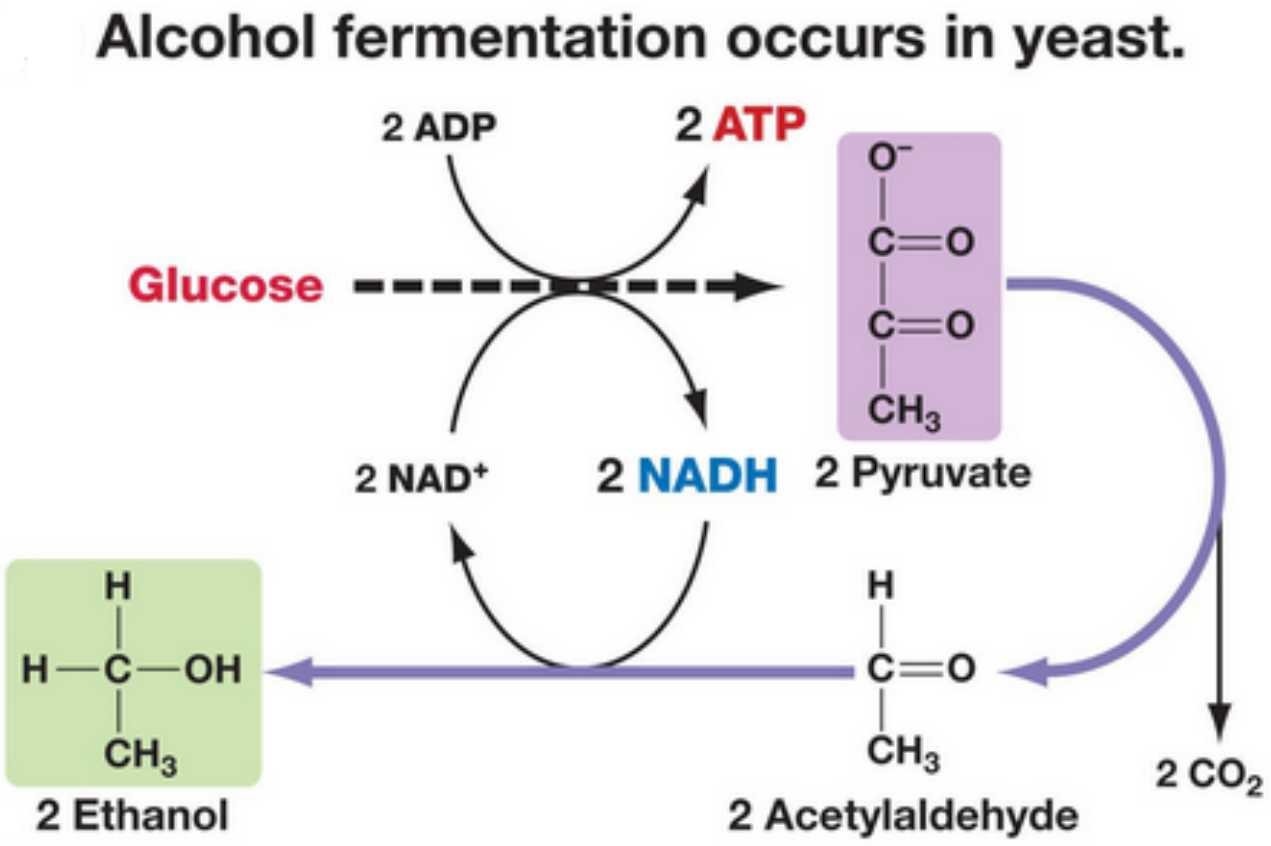
BIOETHANOL PRODUCTION

Ethyl alcohol production is based on two major procedures :

(1). **Fermentation** . *Cereal grains (corn, wheat, barley, sorghum, or rye);* sugarcane (molasses); sugar beets; fruit product wastes; other starch crops (potatoes or rice); sulfite liquors (paper pulping); and such high cellulose-containing materials as wood, crop residues, and cultivated fiber crops.

(2). **Chemical synthesis** . *Petroleum and natural gas; coal; oil shales; and* tar sands.
Synthetic alcohol is not purer or better quality than fermentation alcohol for industrial use.

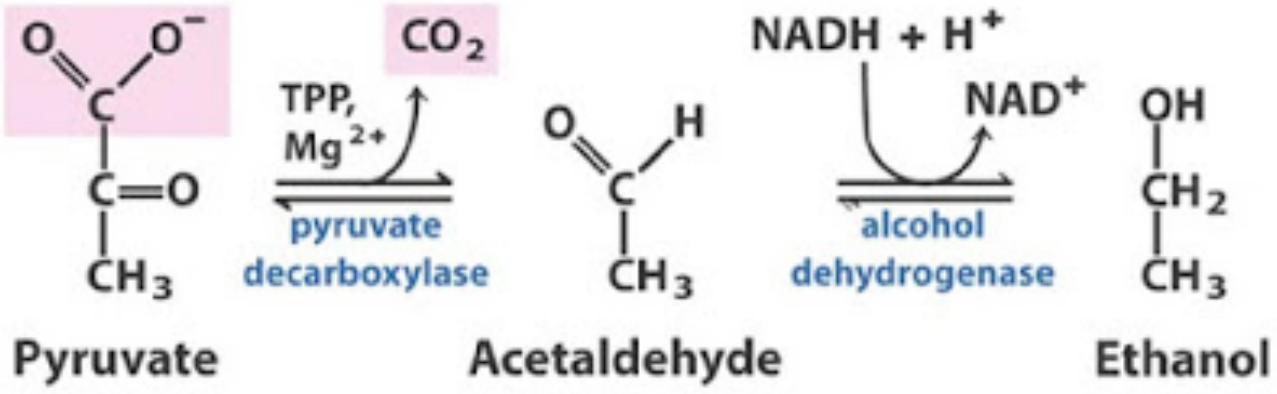
Ethanol pathway



Ethanol pathway: conversion of **pyruvate** to **ethanol** in alcoholic fermentation, e.g. by yeast

- **Pyruvate** is decarboxylated to **acetaldehyde/ethanal** (CH_3CHO).

  - This accepts hydrogen from reduced NAD and is reduced to **ethanol** (C_2H_5OH), releasing NAD.

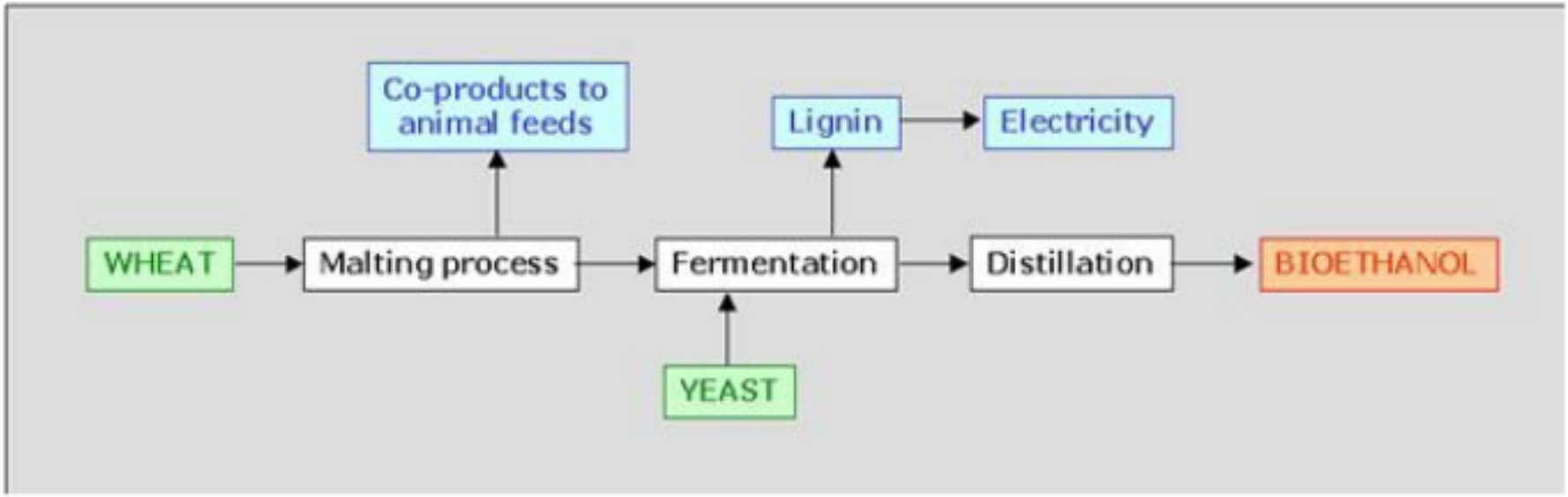


MICROORGANISMS for BIOETHANOL PRODUCTION

Bacteria	*Zymomonas mobilis* *Bacillus stearothermophilus* *Escherichia coli* *Klebsiella oxytoca*
Yeast	*Saccharomyces cerevisiae* *Pachysolen tannophilus* *Candida shehatae* *Pichia stipitis*

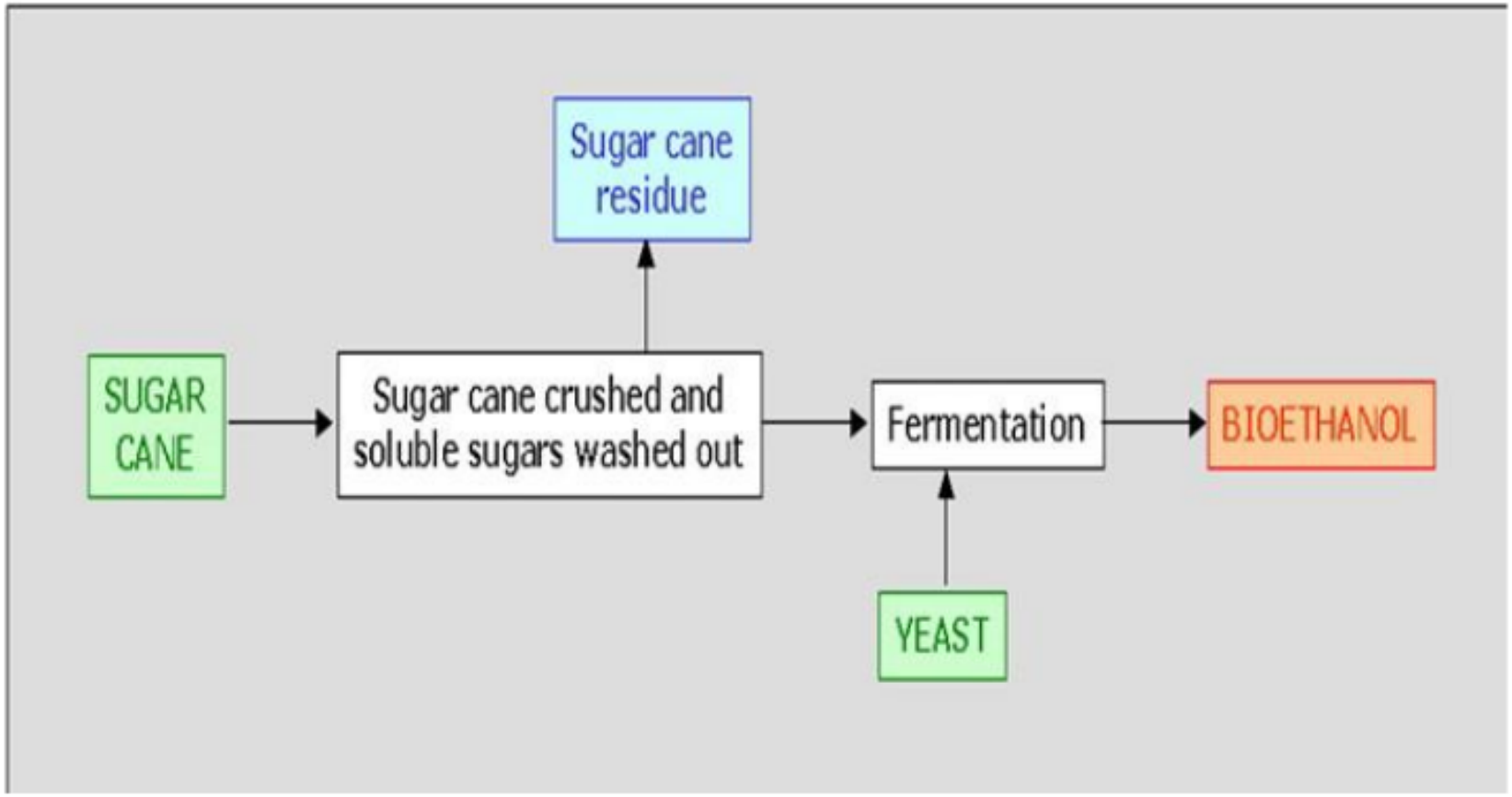
Conversion of Starch to sugar and then sugar to ethanol

Wheat



- Ethanol is produced at 10-15% concentration and the solution is distilled to produce ethanol at higher concentrations

Sugar cane


Sugar cane residue
SUGAR CANE
Sugar cane crushed and soluble sugars washed out
Fermentation
BIOETHANOL
YEAST

Saccharomyces cerevisiae **Yeast** :

Invertase converts sucrose into glucose and fructose.

Zymase , another enzyme present in yeast converts glucose and fructose into ethanol and carbon dioxide.

The carbon dioxide formed is allowed to escape, but air is not allowed to enter. In presence of air ethanol formed would be oxidised to acetic acid.

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow[\text{in yeast}]{\text{Invertase}} \underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{fructose}}{C_6H_{12}O_6}$$

$$\underset{\text{glucose or fructose}}{C_6H_{12}O_6} \xrightarrow[\text{in yeast}]{\text{Zymase}} \underset{\text{ethanol}}{2C_2H_5OH} + \underset{\text{carbon dioxide}}{2CO_2}$$

The fermentation is complete in 3 days.

The carbon dioxide obtained as byproduct is recovered and can be sold.

Distillation

- The fermented liquor contains 9-10% of ethanol and is called **wash or wort.** It is distilled to remove water and other impurities.
- The steam condenses and the alcohol vapors escaping near the top are condensed in the condenser.
- The distillate contains about **90% alcohol** and the **residue** left in the still is used as **cattle feed** .

Lignocellulosic Biomass Composition

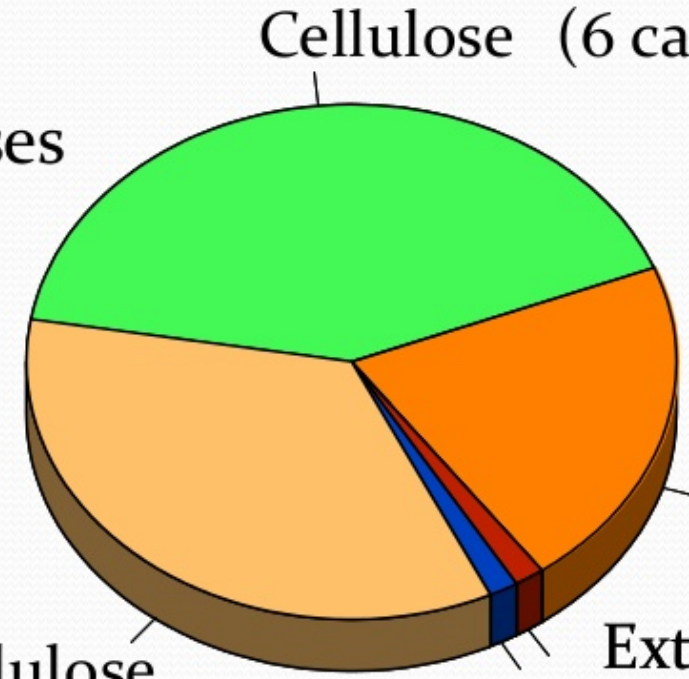
Basically, the lignocellulosic biomass comprises of cellulose, hemicellulose and lignin.

Cellulose is a linear, crystalline homopolymer with a repeating unit of glucose strung together beta-glucosidic linkages. The structure is rigid and harsh treatment is required to break it down. Hemicellulose consists of short, linear and highly branched chains of sugars.

Hemicellulose is a hetero-polymer of D-xylose, D-glucose, D-galactose, D-mannose and L-arabinose.

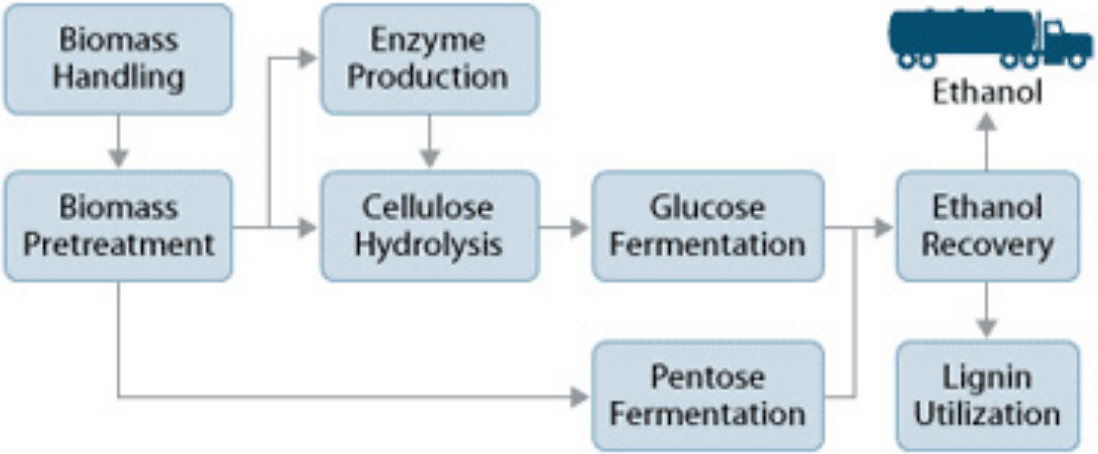
Composition of Lignocellulose

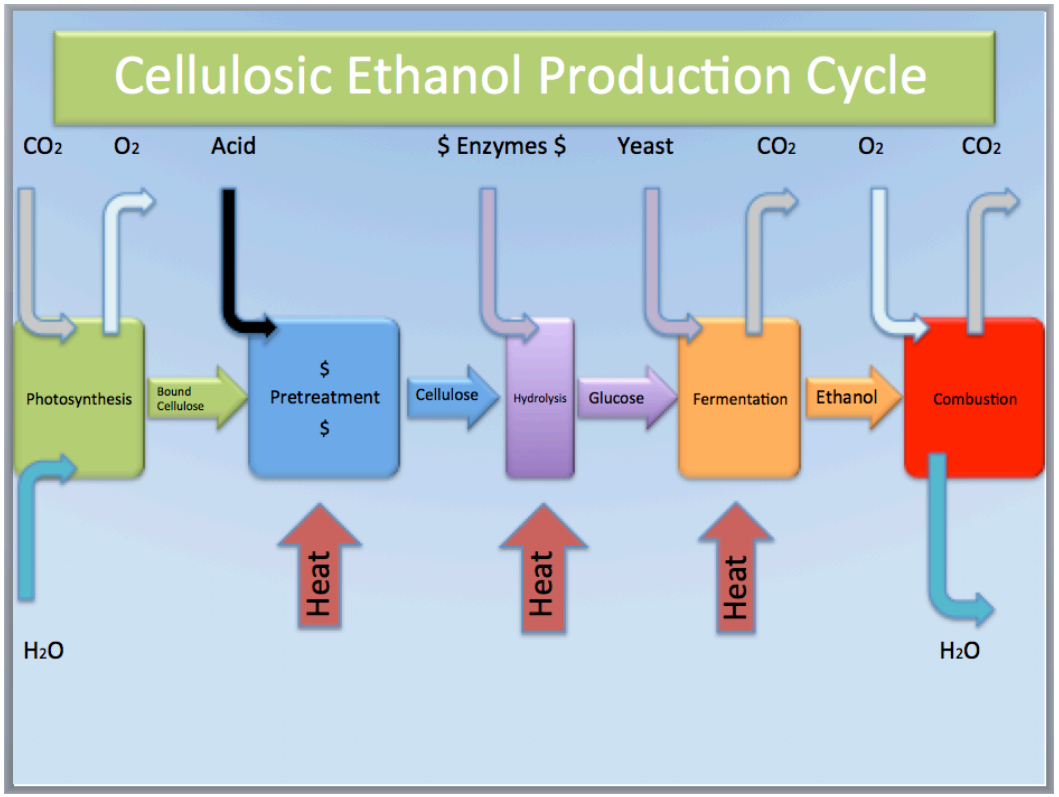
- Cellulose
- Hemicelluloses
- Lignin

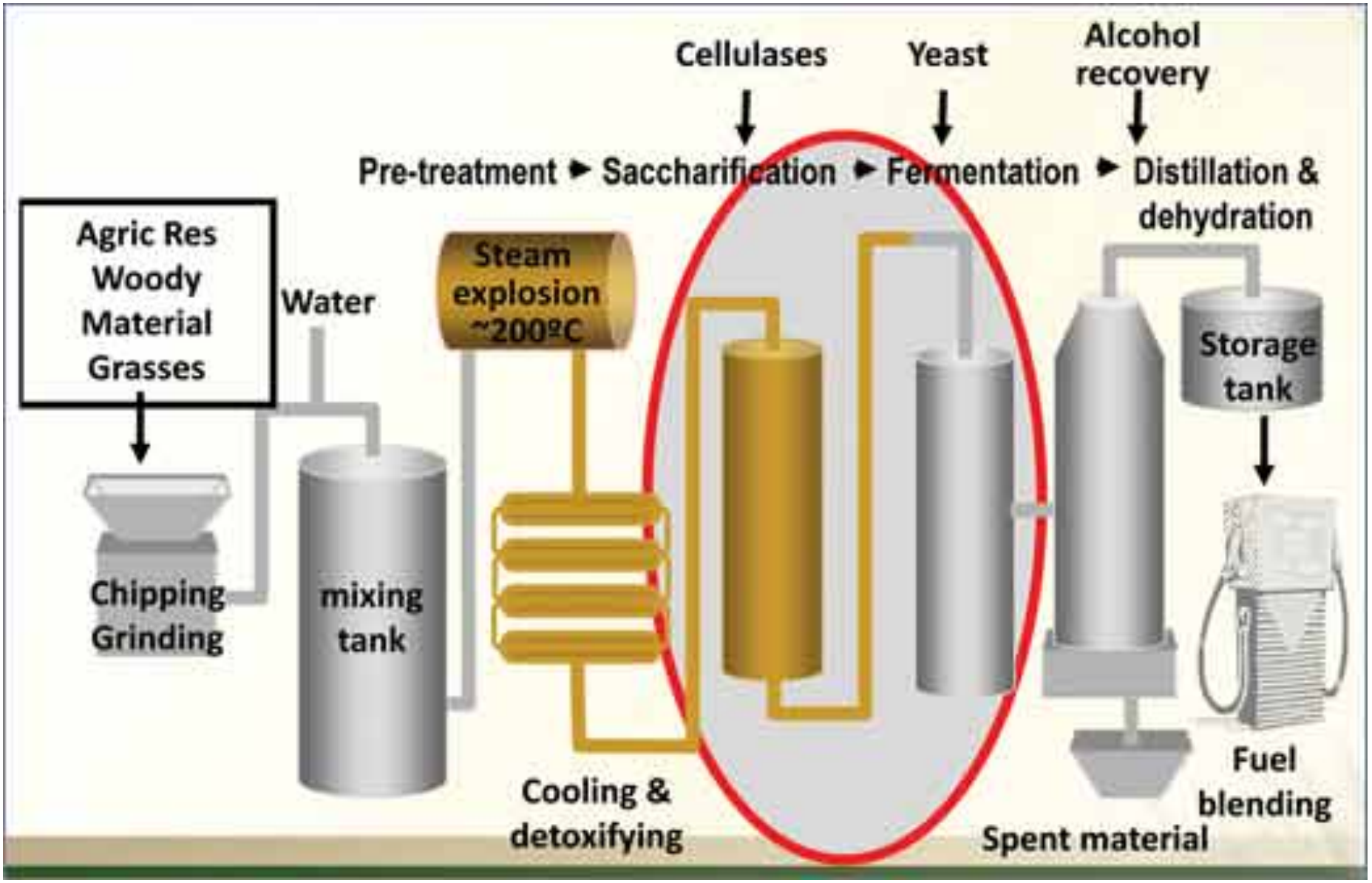


Chapple, 2006; Ladisch, 1979, 2006

Schematic of a Biochemical Cellulosic Ethanol Production Process

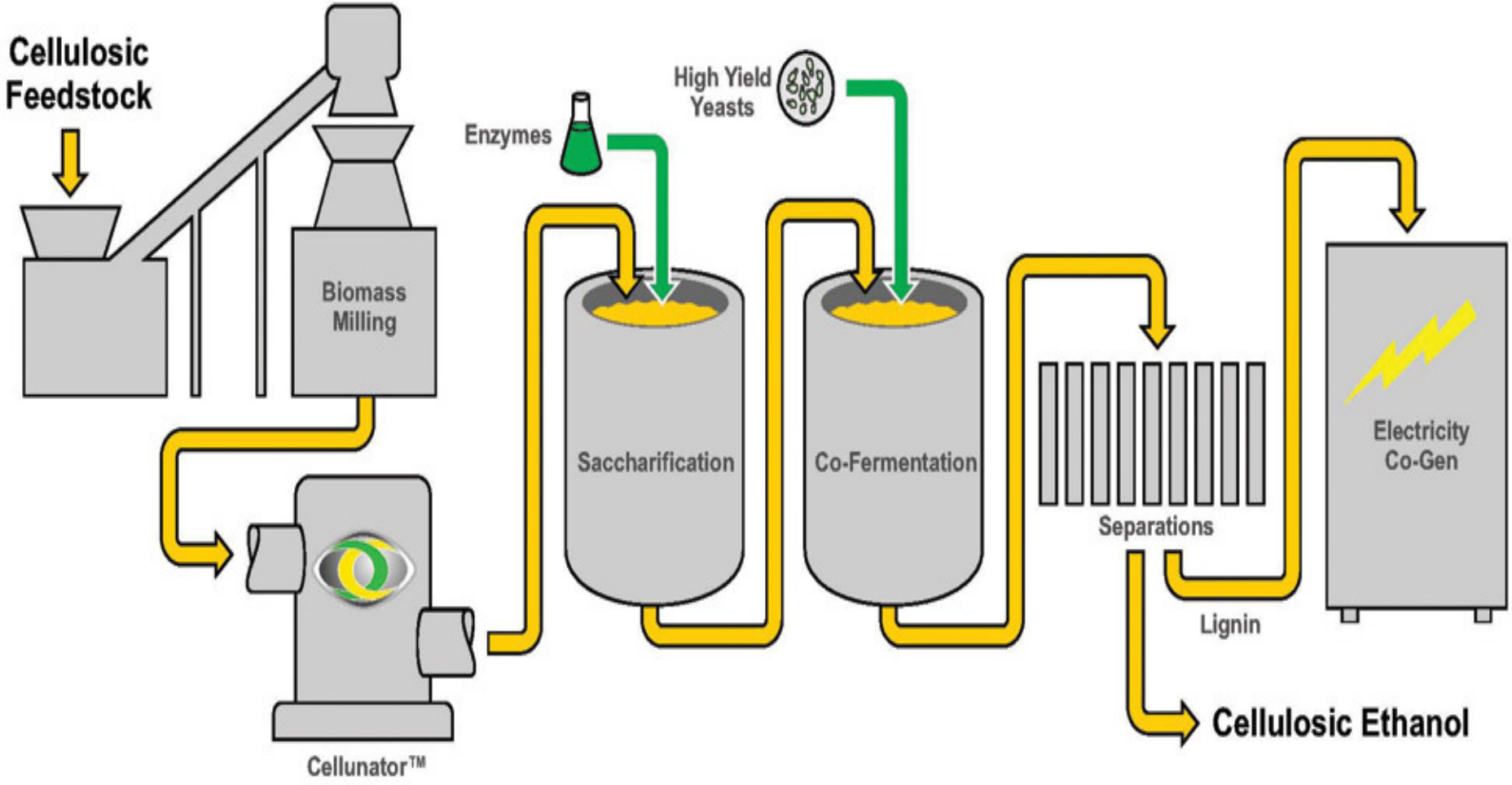





Cellulases
Yeast
Alcohol recovery
Pre-treatment
Saccharification
Fermentation
Distillation & dehydration
Agric Res Woody Material Grasses
Water
Steam explosion ~200ºC
Storage tank
Chipping Grinding
mixing tank
Cooling & detoxifying
Spent material
Fuel blending

Steps of conversion of cellulose and hemicellulose to Bioethanol

1. Pretreatment
2. Hydrolysis
3. Fermentation
4. Distillation of the product mixture to separate ethanol


Cellulosic Feedstock
Biomass Milling
Cellunator™
Enzymes
High Yield Yeasts
Saccharification
Co-Fermentation
Separations
Lignin
Electricity Co-Gen
Cellulosic Ethanol

Pretreatment

- break down the shield formed by lignin and hemicellulose
- Open the fiber structure
- reduce the degree of polymerization of cellulose.

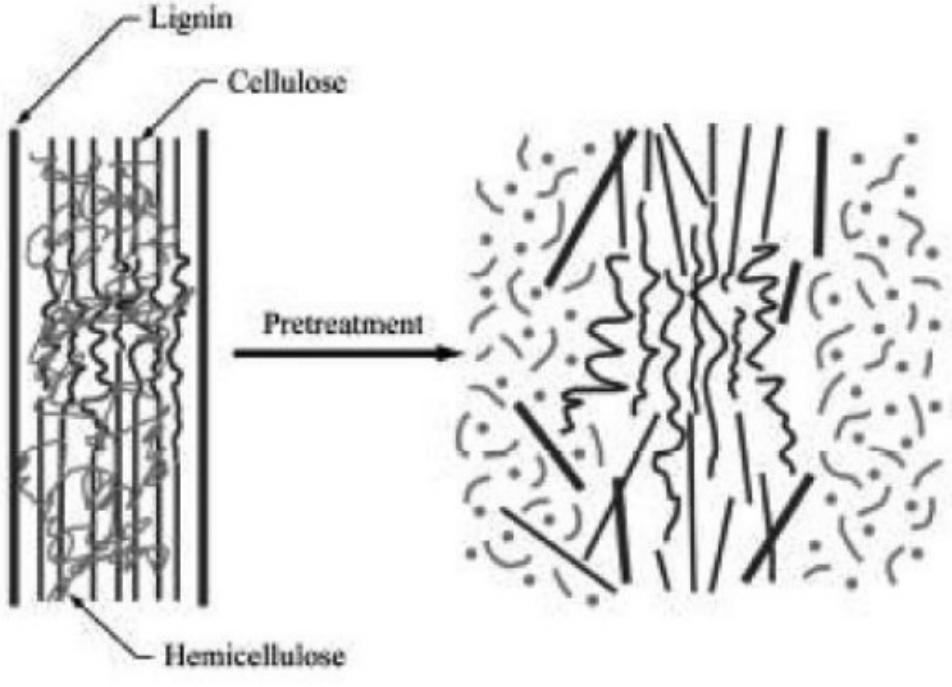
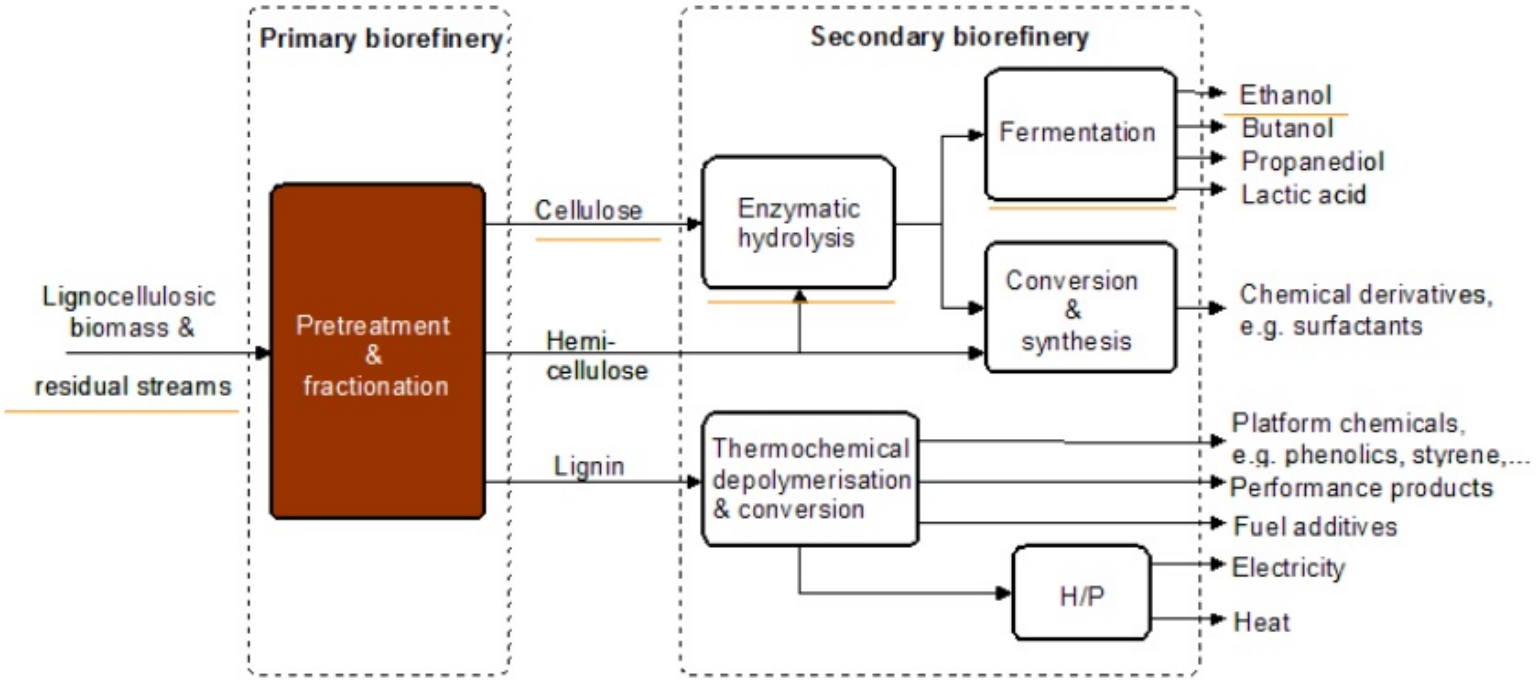


Figure 1 Schematic of pretreatment effect on lignocellulosic biomass

Source: Overview of biomass pretreatment for cellulosic ethanol production; 2009

Potential Lignocellulose Biorefinery



A possible biorefinery including biomass fractionation.

1) Pretreatment

The solubilization and separation of one or more of the four major components of biomass – hemicellulose, cellulose, lignin, and extractives – to make the remaining solid biomass more accessible to further chemical or biological treatment.

2) Hydrolysis

The breaking down of the glycosidic bonds in cellulose and hemicellulose

* **acid hydrolysis**

  Sugars made after acid hydrolysis get converted into furfural in the acidic medium which can act as fermentation inhibitors.

  - *Reaction should be rapid*
  - *Sugars should be rapidly removed*

* **enzymatic hydrolysis**

Enzyme hydrolysis

- Bacteria and fungi are used as sources of cellulases, hemicellulases that could be used for the hydrolysis of pretreated lignocelulosics.

- There are two technological developments.
  - Enzymatic conversion
  - Direct microbial conversion (DMC)

Direct Microbial Conversion (DMC)

- A single microorganism does both hydrolysis and fermentation (cellulose → sugar → bioethanol)

- ✓ Advantage
  - ✓ Cellulose enzyme production or purchase is a significant cost in enzymatic hydrolysis under development → with DMC, a dedicated step for production of cellulase enzyme is not necessary.

- ✓ Disadvantage
  - ✓ Currently available microbes cannot do both processes at the required efficiencies

Applications of Enzymatic Hydrolysis

(a). Simultaneous Saccharification and Fermentation (SSF)

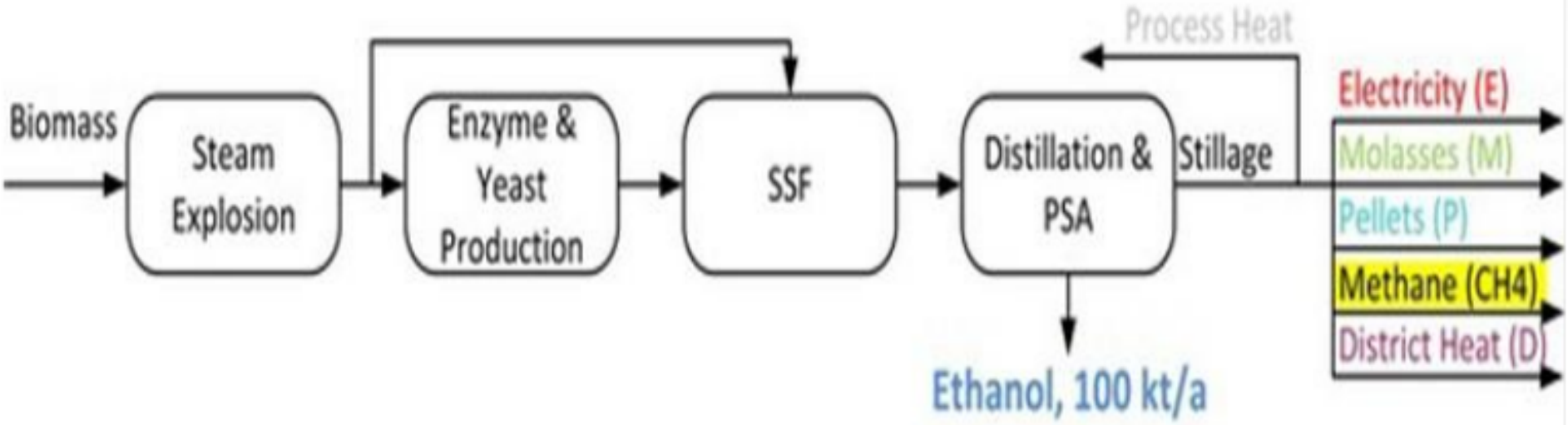
- Cellulase enzymes & fermenting microbes are added to one vessel (hydrolysis and fermentation happen in one reaction vessel).

Advantage :

Minimize accumulation of sugars in the fermenter. ➔ As a result, inhibition of the enzyme β-glucosidase (acts upon β-1,4 bonds linking two glucose or glucose-substituted molecules (i.e., the disaccharide cellobiose) by its product sugars is reduced, and higher hydrolysis rates and yields are possible than for straight saccharification

Disadvantage :

Cellulase enzymes and the fermentation enzymes have to operate under the same conditions - decreases the sugar and ethanol yields.


Process Heat
Biomass
Steam Explosion
Enzyme & Yeast Production
SSF
Distillation & PSA
Stillage
Electricity (E)
Molasses (M)
Pellets (P)
Methane (CH4)
District Heat (D)
Ethanol, 100 kt/a

(b) Sequential Hydrolysis and Fermentation (SHF)

- Hydrolysis and fermentation are done in separate reaction chambers.

✓ Advantage→

  ✓Enables optimization of conditions for the enzymes.

✓ Disadvantage→

  ✓Operational and maintenance costs are high.

3). Fermentation

Fermentation of both C_5 and C_6 sugars

Problem

The ability to ferment pentoses along with hexoses is not widespread among microorganisms.

Solution

Develop genetically modified microorganisms using recombinant DNA technology which can ferment both forms of sugars.

e.g. *Zymomonas mobilis*

4) Distillation

This is done to separate ethanol from other products.