UNIT – 3 ANTIBIOTIC PRODUCTION

Antibiotics are chemicals produced by microorganisms and which in low concentrations are capable of inhibiting the growth of, or killing, other microorganisms.

Antibiotics may be wholly produced by fermentation. Nowadays, however, they are increasingly produced by semi-synthetic processes, in which a product obtained by fermentation is modified by the chemical introduction of side chains. Some wholly chemically synthesized compounds are also used for the chemotherapy of infectious diseases e.g. sulfonamides and quinolones. Some antibiotics e.g. chloramphenicol were originally produced by fermentation, but are now more cheaply produced by chemical means.

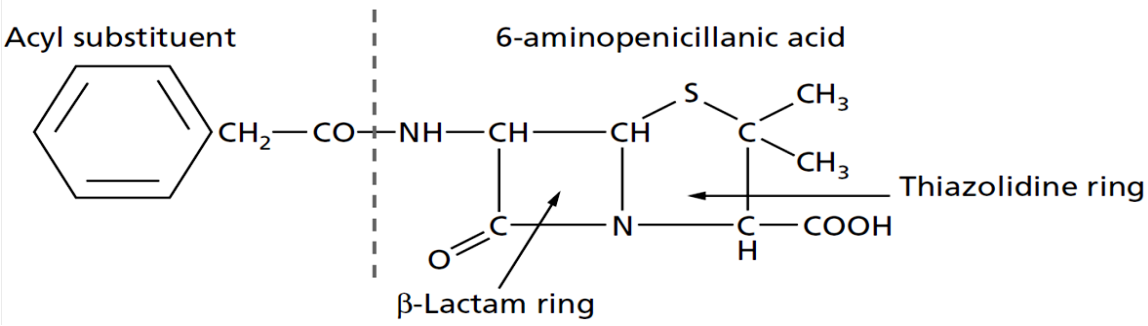
Thousands of antibiotics are known; and every year dozens are discovered. However, only a small proportion of known antibiotics is used clinically, because the rest are too toxic.

Penicillin Production

Penicillin was discovered by Alexander 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. The discovery of penicillin and its later characterization and purification ultimately led to major advancements in both medicine and fermentation technology. The speed of these developments was greatly influenced by the urgent need to supply penicillin during World War 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 1mg/L from the surface cultures initially used for penicillin production. A 20–25-fold increase in yield was achieved when corn steep liquor was incorporated into the fermentation medium.

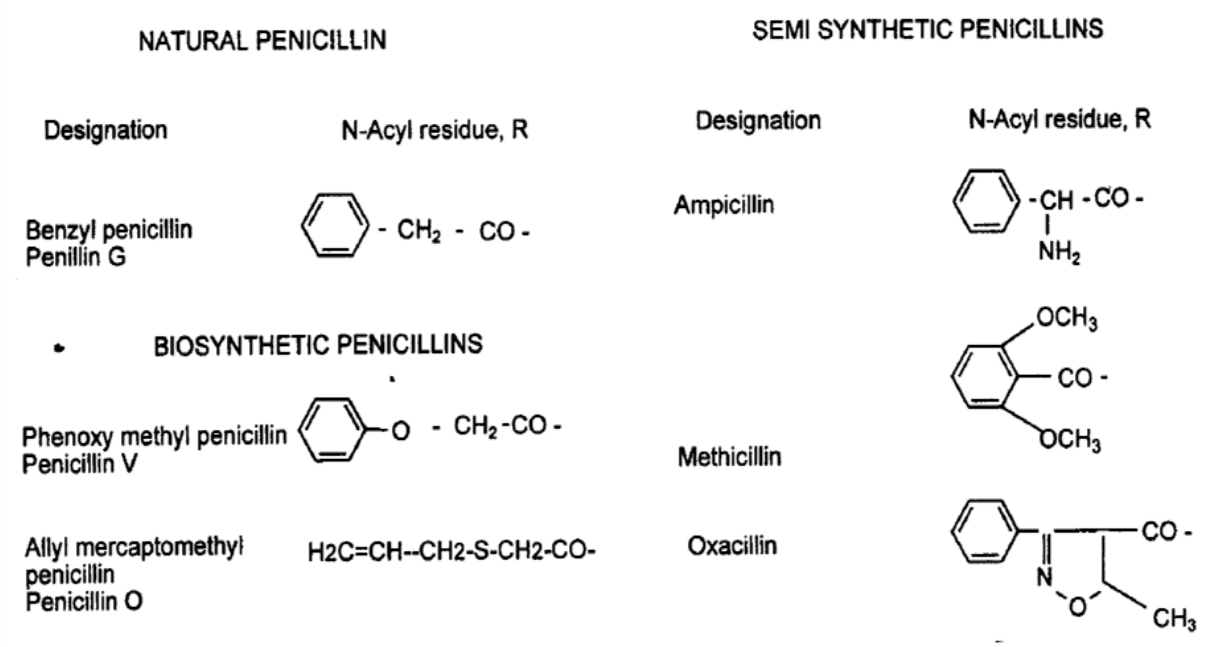
Structure of penicillin

The basic structure of the penicillins is **6-aminopenicillanic acid (6-APA)**, composed of a **thiazolidine ring** fused with a β-**lactam ring** whose 6-amino position carries a variety of **acyl** substituents.



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 Gram positive 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.

Most penicillins are now semisynthetic (see below), produced by the chemical modification of natural penicillins, obtained by fermentation using strains of *P. chrysogenum.* Modification is achieved by removing their natural acyl group, leaving 6-APA, to which other acyl groups can be added to confer new properties. These semisynthetic penicillins, such as methicillin, carbenicillin and ampicillin , exhibit various improvements, including resistance to stomach acids to allow oral administration, a degree of resistance to penicillinase and an extended range of activity against some Gram-negative bacteria.



The penicillin nucleus, to which the 6-amino-penicillanic acid (6-APA) side-chain is attached, is believed to be synthesized from the amino acids L-cysteine and valine, as shown in the following diagram:

L-Cysteine ---------- L-Valine

↓

L-Cysteinyl-L-Valine

↓ PAA

Benzyl Penicillin

Strain of organism used in penicillin fermentation

In the early days of penicillin production, when the surface culture method was used, a variant of the original culture of *Penicillium notatum* discovered by Sir Alexander Fleming was employed. When however the production shifted to submerged cultivation, a strain of ***Penicillium chrysogenum*** designated NRRL 1951 (after Northern Regional Research Laboratory of the United States Department of Agriculture) discovered in 1943 was introduced. In submerged culture it gave a penicillin yield of up to 250 Oxford Units.

(1 Oxford Unit = 0.5988 µg of sodium benzyl penicillin) which was two to three times more than given by *Penicillium notatum.*

1 Oxford unit = 0.6 µg of penicillin
1 mg of penicillin = 1666 units

A '**super strain**' was produced from a variant of NRRL 1951 and designated X 1612. By ultraviolet irradiation of X-1612, a strain resulted and was named WISQ 176 after the University of Wisconsin where much of the stain development work was done. On further ultra violet irradiation of WISQ 176, BL3-D10 was produced, which produced only 75% as much penicillin as WISQ 176, but whose product lacked the yellow pigment the removal of which had been difficult. Present-day penicillin producing *P. chrysogenum* strains are far more highly productive than their parents. They were produced through natural selection, and mutation using ultra violet irradiation, x-irradiation or nitrogen mustard treatment.

Inoculum preparation

The inoculum is usually built up from lyophilized spores or a frozen culture and developed through vessels of increasing size to a final 5-10% of the fermentation tank. Inoculum development is usually initiated by adding lyophilized spores to a small fermentor. Fungal mycelium may then be grown up through one or two further stages until there is sufficient to inoculate the production fermentor.

Many sporulation media have been developed to obtain large numbers of spores.

- **Moyer and Cog-hill sporulation medium**:

Component	**g/ l ltr.**
Glycerol	7.5
Cane molasses	7.5
Corn-steep liquor	2.5
Peptone	5.00
NaCl	4.00
$MgSO_4.7H_2O$	0.05
KH_2PO_4	0.06
Fe-tartarate	0.005
$CuSO_4.5H_2O$	0.004
Distilled water to make	1.0 litre

Adequate oxygen is supplied in the form of sterile air. In addition, the temperature (24°C) is controlled. Contamination in the inoculum tanks is tested both by microscopic observations and by subcultures to broth medium. The volume of culture increases approximately ten-fold with each successive stage in the progression. The mold, *P. chrysogenum,* grows in a filamentous form.

Inoculation Methods*:* Any one of the following inoculation methods may be used to inoculate the fermentation medium in the submerged culture production of penicillin:
(i) Dry spores may be used to seed the fermentation medium.
(ii) Inoculation of the fermentation medium may be accomplished by a suspension of non-germinated mold spores. A non-toxic wetting agent (e.g. 1 :10,000 sodium lauryl sulfonate) in sterile water is used for preparing a more uniform suspension than can be obtained with sterile, water alone. Such suspension may be applied by means of a suitable technique (e.g. spray guns, pipettes, and other means). This is followed by agitation and aeration of the fermentation medium for the equal distribution of the spore suspension.
(iii) The fermentation medium may be seeded by pellet inocula which are obtained by the germination of spores, with the formation of mycelial growth, under submerged conditions.

Fermentation medium / Raw material

Raw Materials: Three points should be kept in mind in choosing raw materials for the manufacture of penicillin:
(i) An abundant growth of mycelium, (ii) Maximum accumulation of penicillin, and (iii) Ease of extraction and purification of the antibiotic.

Carbon Sources: The medium for penicillin production now usually has as carbohydrate source glucose, beet molasses or lactose. Lactose acts as a very satisfactory carbon compound, provided that it is used in a concentration of 6 per cent (Moyer and Coghill, 1946). Other carbohydrates, for example, glucose and sucrose, may be used as satisfactory substitutes for lactose, if slow feeding rates are used. Glucose suppresses secondary metabolism and excess of it therefore limits penicillin production.

Nitrogen Sources: Corn-steep liquor supplies cheap and readily available nitrogen. Cotton seed, peanut, linseed or soybean meals have been used as alternate nitrogen sources. Ammonium sulfate, ammonium acetate, ammonium lactate, or other materials can be used as nitrogenous compounds. Nitrogen is often supplied as ammonia gas.

Mineral Sources: Elements, namely potassium, phosphorous, magnesium, sulphur, zinc, and copper are essential for penicillin production. Sulfur compounds are sometimes added for additional yields since penicillin contains sulfur. Some of these are supplied by corn-steep liquor. Potassium and phosphorus are customarily supplied as potassium dihydrogen phosphate, whereas magnesium and sulphur, as magnesium sulphate ($MgSO_4$. $7H_2O$). Iron and copper, if required in the medium, are usually supplied as sulphates. Corn-steep liquor seems to contain sufficient amounts of potassium dihydrogen phosphate and magnesium sulphate ($MgSO_4$. $7H_2O$). However, small amounts of these salts may be incorporated in the fermentation medium.

pH: The pH is maintained at between 6.8 and 7.4 by the automatic addition of H_2SO_4 or NaOH as necessary.

Buffers: Calcium carbonate or phosphates may be added as a buffer.

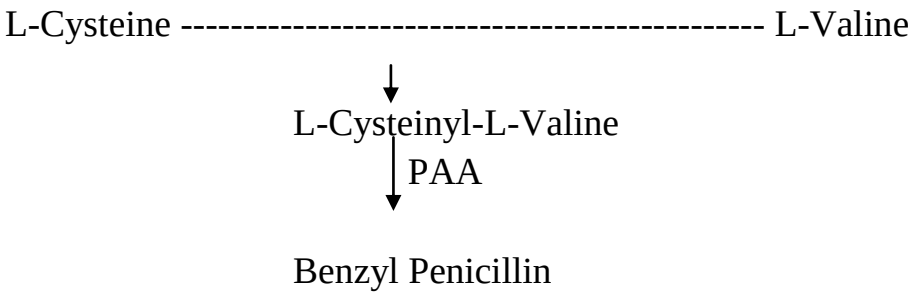
Corn Steep Liquor: It stimulates production of penicillin due to presence of arginine, histidine, and glutamic acid, and possibly to phenyl-acetic acid derivatives.

Precursors: There are many related penicillins (e.g. penicillin G, penicillin V, penicillin X, etc.). The most important naturally occurring penicillins are penicillin G (benzyl-penicillin) and penicillin V (phenoxymethyl-penicillin). The formation of desirable penicillin can be stimulated by the addition of phenylacetic acid derivatives. For example, the mold, *Penicillium chrysogenum,* synthesizes large quantities of penicillin G, if phenyl- acetic acid (C_6H_5 . CH_2. COOH) is present in the fermentation medium. Phenylacetic acid (PAA) supplies the side chain of penicillin G. For this purpose, Phenyl acetic acid or phenoxyacetic acid is added in the medium.

Fortunately, corn-steep liquor, employed in the formulation of the fermentation medium, is a source of phenylacetic acid derivatives. High levels

of PAA are, however, toxic to the mold, and so all of it cannot be added at a time.

The penicillin nucleus, to which the 6-amino-penicillanic acid (6-APA) side-chain is attached, is believed to be synthesized from the amino acids L-cysteine and valine, as shown in the following diagram:



Jackson's typical medium

Component	**%**
Corn-steep liquor solids	3.5
Lactose	3.5
Glucose	1.0
Calcium Carbonate	1.0
Potassium dihydrogen phosphate (KH_2PO_4)	0.4
Edible Oil	0.25
Penicillin precursor (PAA) Phenyl Acetic Acid	0.1

Fermentation conditions during production of penicillin

Penicillin production is usually via a fed-batch process carried out aseptically in stirred tank fermenters of 40000–200000 L capacity, although airlift systems are sometimes used. The fermentation involves an initial vegetative growth phase followed by the antibiotic production phase. Throughout the process, the oxygen level is very important and must be maintained at 25–60 mmol /L /h. These processes are maintained at 25–27°C and pH 6.5–7.7, the specific conditions depending upon the *P. chrysogenum* strain used.

Initially, there is a vegetative growth phase devoted to the development of biomass, which doubles every 6 h. This high growth rate is maintained for the first 2 days. To ensure an optimum yield of penicillin in the following production phase, the mycelium must develop as loose pellets, rather than compact forms. During the following production phase, the carbon source is fed at a low rate and penicillin production increases. This continues for a further 6–8 days, provided that appropriate substrate feeds are maintained.

Extraction and purification of penicillin after fermentation

The fermentation broth contains a large number of other materials and the method used for the separation of penicillin from them is based on the solubility, adsorption and ionic properties of penicillin. Since penicillins are monobasic carboxylic acids they are easily separated by solvent extraction as described below.

a) **Cooling and Acidification:** At the end of the fermentation the broth is transferred to a settling tank. Penicillin is highly reactive and is easily destroyed by alkali conditions (pH 7.5-8.0) or by enzymes. It is therefore cooled rapidly to 5-10°C. A reduction of the pH to 6 with phosphoric or sulphuric acids.

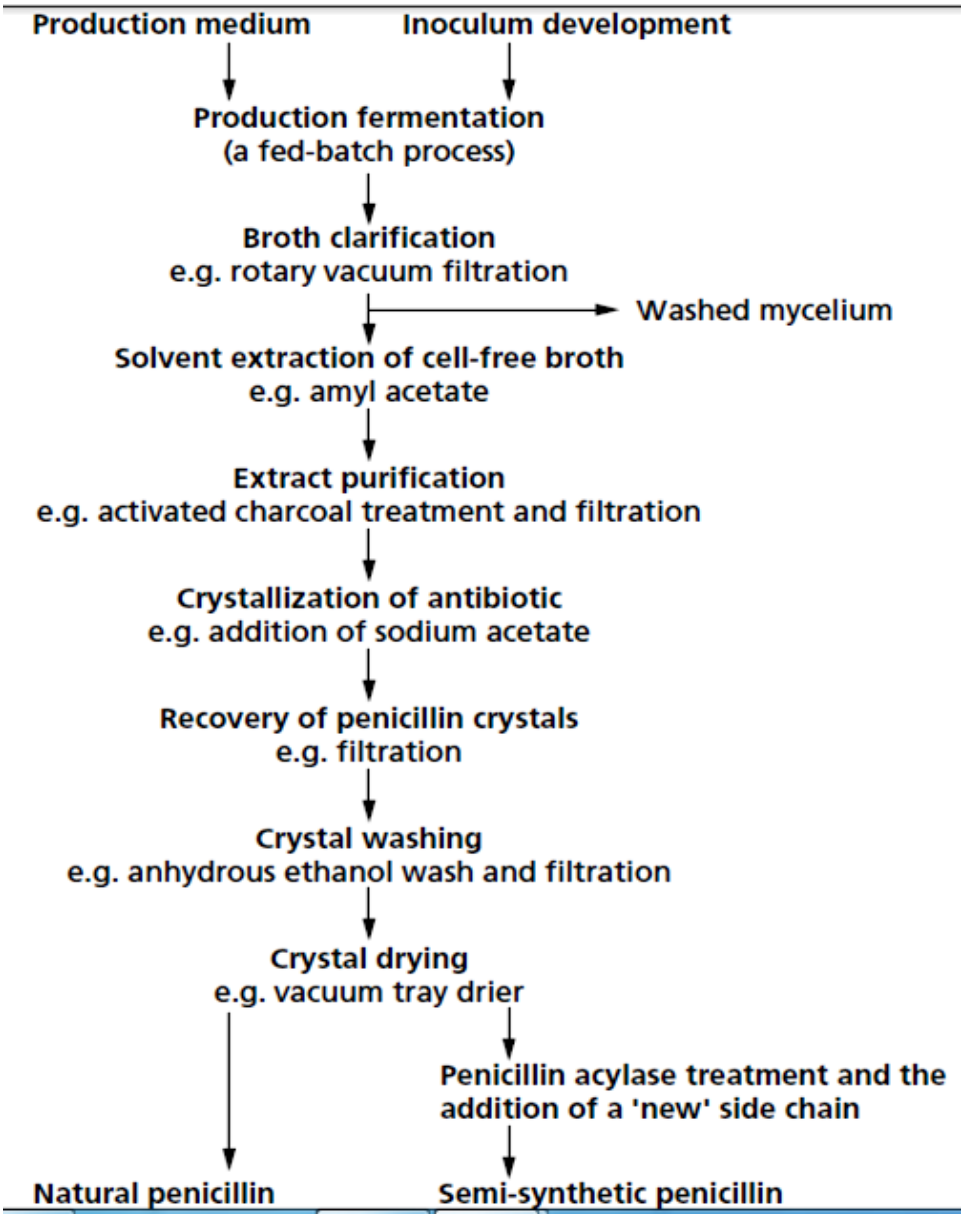
b) Removal of mycelium: The first step is to remove the mycelium by filtration. A rotary vacuum filter is employed for the filtration of fermented production medium. This stage is carried out under aseptic conditions to avoid contamination of the filtrate with penicillinase producing micro-organisms which may cause serious or total loss of an antibiotic.

c) Countercurrent solvent extraction of penicillin: Antibiotic recovery is often by solvent extraction of the cell-free medium, which gives yields of up to 90%. This involves reducing the pH of the filtered medium to 2.0–2.5 by addition of sulphuric or phosphoric acid, followed by a rapid two-stage continuous countercurrent extraction at 0–3°C using amyl acetate, butyl acetate or methyl isobutyl ketone. The low temperature is necessary to reduce damage to penicillin due to the low pH. The antibiotic is then extracted back into an aqueous buffer at pH 7-7-5, the partition co-efficient now being strongly in favour of the aqueous phase. The resulting aqueous solution is again acidified

and re-extracted with an organic solvent. These shifts between the water and the solvent help in the purification of the penicillin.

d) Removal of pigment: Any pigments and trace impurities are removed by treating with activated charcoal.

f) Salts of Penicillin: The penicillin is then retrieved from the solvent by addition of sodium or potassium acetate. This reduces the solubility of the penicillin and it precipitates as a sodium or potassium salt. Resultant penicillin crystals are separated by rotary vacuum filtration. Penicillin crystals are mixed with a volatile solvent, usually anhydrous ethanol, butanol or isopropanol, to remove further impurities. The crystals are collected by filtration and air dried. Metal salts of penicillin can be safely sterilized by dry heat, if desired. At this stage the penicillin is 99.5% pure. This product may be further processed to form a pharmaceutical grade product or is used in the production of semisynthetic penicillins.



Production of semisynthetic penicillins and cephalosporins

As mentioned previously, the objective in semisynthetic penicillin production is to generate compounds with improved properties, e.g. acid stability, resistance to enzymic degradation, broader spectrum of activity, etc. It involves removal of the side chain of the base penicillin to form 6-aminopenicillanic acid (6-APA). This is achieved by passage through a column of immobilized **penicillin acylase**, usually obtained from *Escherichia coli*, at neutral pH.

Penicillin G, for example, is converted to 6-APA and phenylacetic acid. The 6-APA is then chemically acylated with an appropriate side chain to produce a semisynthetic penicillin.

Acylation of the 6-APA is accomplished by a chemical reaction with a suitably activated derivative of a carboxylic acid (most frequently an acid chloride or a mixed anhydride). The coupling reaction can be carried out in organic solvents under anhydrous conditions or in aqueous solution. Organic amines (e.g. triethyl amine) are added to the anhydrous reactions. Alternatively, the aqueous reactions are frequently carried out in acetone-water mixtures in the presence of sodium bicarbonate. The formed semisynthetic penicillins are usually isolated by distributing the product as its free acid into a water-immiscible organic solvent and the salts and other impurities into water.

Yields of cephalosporins from direct fermentations are much lower than those for penicillins. Consequently, as 6-APA can also serve as a precursor of cephalosporins, it is often used as the starting material for their semisynthetic production. A base natural penicillin is converted to 6-APA, as described above, followed by its conversion to the preferred precursor, 7-amino deacetoxycephalosporic acid (7-ADCA), by ring expansion. A suitable side chain can then be readily attached.

==========

PRODUCTION OF VITAMINS

INTRODUCTION

Vitamins are very important compounds in the diet because they are of great value in the growth and metabolism of the living cell. All the vitamins may be synthesized by prototrophic microrganisms. These compounds are constituted from simple ingredients supplied by the growth medium. But, they are produced in the amounts enough to fulfill the organism's needs. In other words, they do not accumulate in the growth medium. There are certain micro-organisms which excrete vitamins greatly in excess of their own metabolic needs if grown under highly specified and artificial conditions. Thus, such micro-organisms are of industrial interest.

VITAMIN B_{12}

Vitamin B_{12} is an important dietary component for normal growth in human beings and domesticated animals. Its daily requirement for human beings is 0.001 mg/day.

The most-commonly used man-made forms are cyancobalamin, ethylcobalamin, and hydroxocobalamin. Vitamin B12 has a number of additional functions. It is needed for:

- production of elements of DNA
- production of red blood cells
- regeneration of bone marrow and the lining of the gastrointestinal and respiratory tracts
- maintaining the health of the nervous system and spinal cord
- prevention of megaloblastic anemia

Symptoms of vitamin B12 deficiency include:

- shakiness
- muscle weakness
- stiff, spastic muscles
- fatigue (Low energy)
- incontinence (lack of control)
- low blood pressure
- mood disturbances

Symptoms of a vitamin B12 deficiency can include:

- Constantly feeling tired or chronic fatigue
- Muscle aches and weakness
- Joint pain
- Difficulty breathing or shortness of breath
- Feeling dizzy
- Poor memory
- Inability to concentrate well
- Mood changes, like increased depression and anxiety
- Having abnormal heart problems, such as palpitations
- Poor dental health, including bleeding gums and mouth sores
- Digestive problems like nausea, diarrhea or cramping
- A poor appetite
- A more serious deficiency can also cause a form of anemia called pernicious anemia, a serious condition that can cause memory loss, confusion and even long-term dementia
- The most serious condition associated with vitamin B12 deficiency is megaloblastic anemia. This is a chronic blood disorder in which the bone marrow produces overly large, immature blood cells. As a result, the body doesn't have enough healthy red blood cells to carry oxygen around the body.

Recommended Daily Amount of Vitamin B12

According to the NIH, the Recommended Dietary Allowance (RDA) for vitamin B12 is:

- Infants 0–6 months: 0.4 micrograms
- Infants 7–12 months: 0.5 micrograms
- Toddlers 1–3 years: 0.9 micrograms
- Children 4–8 years: 1.2 micrograms
- Children 9–13 years: 1.8 micrograms
- Adult men and women over age 14: 2.4 micrograms
- Women who are pregnant: 2.6 micrograms
- Women who are breastfeeding: 2.8micrograms

Top 9 Vitamin B12 Benefits

1. Helps Maintain Energy Levels

Vitamin B12 benefits your metabolism because it's needed to convert carbohydrates into useable glucose in the body. Glucose from carbohydrate foods is used as a form of energy, so this is the reason why people with vitamin B12 deficiencies often experience **fatigue**. Vitamin B12 is also needed for neurotransmitter signaling that helps your muscles contract and gives you energy to go about your day without feeling tired and run down.

2. Prevents Memory Loss and Lowers Risk of Neurodegenerative Disease

A vitamin B12 deficiency may cause various neurologic and psychiatric disturbances. Because of its role in nerve health and neurotransmitter signaling, vitamin B12 benefits cognitive function and is used to lower the risk of neurodegenerative diseases, including **Alzheimer's disease** (memory loss,) and dementia (gradual decrease in the ability to think and remember).

3. Boosts Mood and Helps the Nervous System to Properly Function

One of the most researched vitamin B12 benefits is its ability to help in healthy regulation of the nervous system, including reducing such mood disorders as depression and **anxiety**. Vitamin B12, along with folate, is needed as a major determinant of one-carbon metabolism, which produces the compound called SAM (*S-adenosyl methionine)*. SAM is crucial for neurological function, **dealing with stress** and mood regulation.

Vitamin B12 is needed for concentration and cognitive processes, such as learning, so a vitamin B12 deficiency can result in difficulty focusing and an increased risk for attention disorders.

4. Plays a Role in Maintaining Heart Health

Vitamin B12 benefits cardiovascular health in several ways, which is important considering the fact that heart disease is currently the number one cause of death worldwide. Vitamin B12 helps to reduce elevated homocysteine levels, which is now considered a major risk factor for heart disease.

Homocysteine is an amino acid and its levels in the blood are influenced by blood levels of B-complex vitamins, including vitamin B12.

Vitamin B12 helps to protect against heart disease like a heart attack or stroke by lowering high homocysteine levels in the blood. There is also some evidence that B12 can help control **high cholesterol** and high blood pressure levels. B vitamins are also able to control atherosclerotic diseases, in which someone experiences a dangerous build-up of plaque in the arteries.

5. Needed for Healthy Skin and Hair

Vitamin B12 is essential for healthy skin, hair and nails because it plays a major part in cell reproduction. Vitamin B12 benefits skin health by reducing redness, dryness, inflammation and acne blemishes — and can be applied to the skin for **psoriasis** and **eczema**. It can also reduce hair breakage and help nails to become stronger.

6. Aids in Digestion

Due to its role in helping with digestive enzyme production, vitamin B12 is needed to support a healthy metabolism and the breakdown of foods within the stomach. One of the ways that vitamin B12 benefits digestion? It helps foster healthy bacteria within the gut environment. The elimination of harmful bacteria in the digestive tract — and simultaneously the presence of beneficial bacteria — is what prevents digestive disorders like **inflammatory bowel disease** (IBS) or Candida.

7. Needed for a Healthy Pregnancy

Vitamin B12 is needed to create nucleic acid, or DNA — the basic genetic material that's used to create the entire body. Therefore, vitamin B12 is not only a key nutrient for growth and development, but a vital component of a healthy pregnancy. Vitamin B12 also interacts with folate in the body, so it may help lower the risk of birth defects, such as neural tube defects.

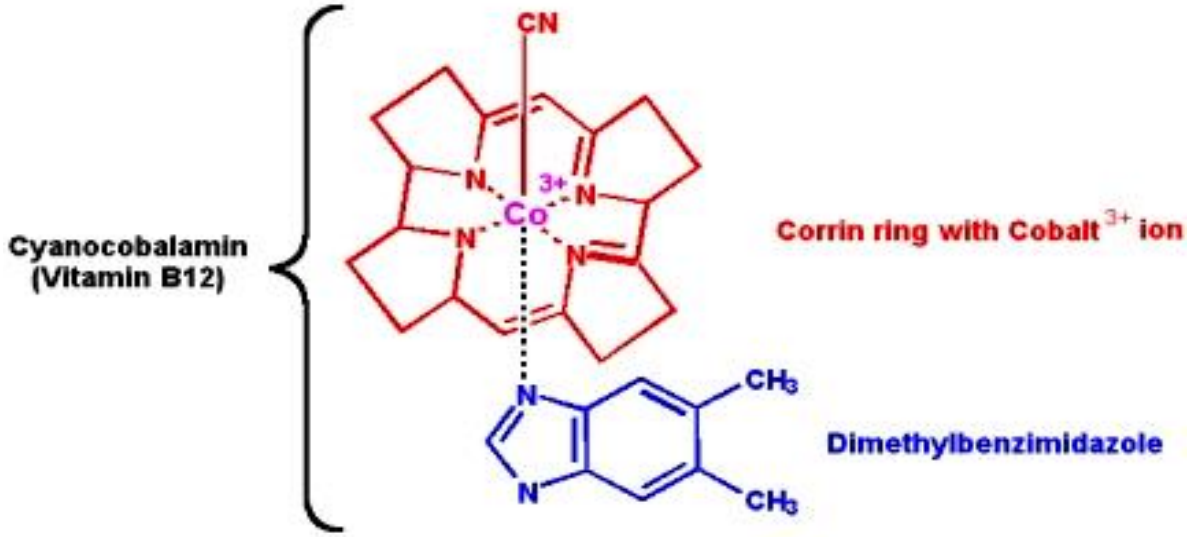
8. May Help Prevent Cancer

Vitamin B12 supplementation is now being studied as a way to help lower the risk of certain kinds of cancers, especially when taken with folate. (15) Some preliminary research shows that vitamin B12 benefits the immune system enough to potentially help prevent **cancer**, including cervical, prostate and colon cancers.

9. Helps Produce Red Blood Cells and Prevent Anemia

Vitamin B12 is needed to help produce a healthy level of red blood cells. It helps prevent a type of anemia called megaloblastic anemia, which results in symptoms like **chronic fatigue** and weakness.

Looking to the chemistry of vitamin B_{12}, it is a complex chemical compound whose structural formula is as shown in Figure.



It is cyanocobalamin, consisting of a molecule of cobinamide linked to a nucleotide. The cobina mide molecule has a central atom of cobalt linked to a cyanide group, surrounded by four reduced pyrrole rings joined to form a macro-ring. Moreover, a number of carbon atoms carry methyl or other substituent groups. The nucleotide is atypical in having 5, 6-dimethyl benzimidazole as its base, instead of a purine or pyrimidine base.

Vitamin B_{12} is entirely produced on a commercial basis by the fermentation. Generally, it is manufactured by primary fermentation. In some instances, it is obtained from broths used primarily for the production of antibiotics. This is largely a matter of economics, since maximum production of the antibiotic and maximum production of cobalamin generally do not take place under the same fermentation conditions. Its production has become one of the important fermentation industries in the United States.

Micro-organisms that may be employed in the industrial production of vitamin B_{12} are:

Streptomyces griseus, S. olivaceus, Bacillus megaterium, B. coagulans, Pseudomonas denitrificans, Propionibacterium freudenreichii, P. shermanii, and a mixed fermentation of a *Proteus* spp. and *Pseudomonas spp.*

Vitamin B_{12} production using *Streptomyces olivaceus* NRRL B-1125

Preparation of inoculum:

In the building up of the inoculum, pure agar slant culture of *Streptomyces olivaceus* NRRL B-1125 is inoculated and grown in 100 to 250 ml of inoculum medium contained in Erlenmeyer flasks.

Composition of Bennett's agar used in the preparation of inoculum.

Component	***Amount* (g./Litre)**
Yeast extract	1.0
Beef extract	1.0
N-Z-Amine A (Enzymatic hydrolysate of casein)	2.0
Glucose	10.0
Distilled water	1000 ml
(pH is adjusted to 7.3 with NaOH)	

These seeded flasks are to be kept on the platform of mechanical shaker during incubation in order to aerate the medium. Subsequently, the flask cultures are used to inoculate larger amounts of inoculum media contained in inoculum tanks in series. Usually, two or three such successive transfers are made to obtain the required amount of inoculum culture. It has been shown that inoculation of the production tank with about 5% of the volume of production medium is satisfactory.

Production medium:

Production media used in this fermentation, usually, consist of carbohydrate, proteinaceous material, and a source of cobalt and other salts. A typical production medium cmpositon is as follows

Component	***Amount* (per cent)**
Distillers' soluble	4.0
Dextrose	0.5 to 1
$CaCO_3$	0.5
$COC1_2.6H_2O$	0.5 ppm
(pH adjusted to about 7 with NaOH)	

Most forms of distillers' solubles, soya bean meal, yeast, casein, etc. are found satisfactory under suitable conditions. It is necessary to add cobalt to the medium for the maximum yields of cobalamin. It is to be noted that, cobalt has nothing to do with the growth of the *S. olivaceus.* In some cases, it is essential to add cyanide to the medium to aid the conversion of other cobalamins to vitamin B_{12}.

Sterilization of the medium may be practiced batch wise or continuously. In batch wise sterilization, the medium contained in the production tank is heated at 250°F for 1 hour. In the latter method, the production tank is charged (e.g. at 330°F for 13 minutes) by mixing it directly with live steam During sterilization, steam is blown into openings and all transfer lines are kept filled with live steam when not in use to ensure sterility.

Temperature:

A temperature of 80°F in the production tanks is satisfactory during fermentation.

pH:

There are few tenths of a unit fall in pH and a rapid consumption of sugar in the first 24 hours o the fermentation. After 2 to 4 days, lysis of the mycelium begins resulting in the rise of pH. The stabilization of the mash is practiced by reducing

the pH to about 5 with sulphuric acid and adding small amounts of a reducing agent (e.g. sodium sulphite).

***Aeration and agitation**:*

A proper rate of aeration and a correct speed of agitation are essential because the rate of the growth of the streptomycete strain depends on the rate of aeration and agitation. Aeration rates higher than optimum cause excessive foaming. The optimum rate of aeration is about 0-5 volume air/volume medium/minute. The sterilization of air is, usually, practiced by passing it through columns filled with activated charcoal.

***Antifoam agents**:*

With this fermentation, foam formation is a serious problem, particularly at the beginning and at the end of the fermentation. There are many antifoam agents that may be used to suppress the foam formation. Important defoaming agents are: soya bean oil, corn oil, lard oil and silicones. A defoaming agent in its sterile form is added to the medium during foaming according to the requirement. In some instances, an antifoam agent is added at the time of the production medium make up.

***Prevention of contamination**:* It is very essential to maintain sterility until the fermentation ends, because contamination invariably results in very low yields. Therefore, all equipments must be sterile. In addition, transfers are carried out under aseptic conditions.

Yields: The yields of cobalamin are usually in the range of 1 to 2 mg per litre in the fermented broth.

Recovery:

During the major part of the fermentation period, most of the cobalamin is associated with the mycelium. But, a considerable portion of the cobalamin is in the solution at the end of the fermentation period. Heating the mixture to boiling at pH 5 or below liberates the cobalamin quantitatively from the

mycelium. Broth containing cobalamin is subjected to further work-up depending on the type of the product to be produced.

In order to obtain crystalline vitamin B_{12}, the very first operation is of filtration to remove mycelium. Then the filtered broth is treated with cyanide to bring about the conversion of cobalamin to cyanocobalamin. Alternatively, this conversion may also be done after some concentration has been secured while fermentation is in progress. The adsorption of the cyanocobalamin from the solution is practiced by passing it through an adsorbing agent packed in columns. Several adsorbents are available for this purpose; activated charcoal, bentonite, fuller's earth, and ion-exchange resins.

Finally, elution of cyanocobalamin from adsorbent is accomplished by the use of an aqueous solution of materials ranging from organic bases to hydrochloric acid. Several materials have been reported for this purpose: water, water-acetone, and solutions of sodium cyanide or sodium thiocyanate.

Thereafter, some type of extraction is usually carried out. It may be countercurrent distribution between cresol, amylphenol, or benzyl alcohol and water or a single extraction into an organic solvent, (e.g. phenol). Precipitation as a copper or zinc cyanide-cyanocobalamin complex has been reported.

Another purification step applicable to aqueous concentrates consists in dissolving a zinc salt in the slight acid solution and then raising the pH to bring about precipitation of zinc hydroxide, which eliminates many impurities.

Chromatography on alumina and final crystallization from methanol-acetone, ethanol-acetone, or acetone-water, usually complete the process.

In order to obtain a concentrate of vitamin B_{12} to use as feed-supplement, the final fermented broth is evaporated to dryness. These final broths containing about 3% solids are first subjected to evaporation *in vacuo* to solids content of 15 to 20%. Then the syrups are drum-dried or spray-dried. The concentrates obtained in this way by cobalt-supplemented fermentation may contain 10 to 30 mg/lb of cobalamin.

==========

Amino acid fermentation : L-lysine (direct method)

Introduction:

Amino acids have the general formula

From amino acids proteins are made. 20 amino acids are found in proteins, of these eight are essential and must be supplied in the food, since animals and human beings cannot synthesize them. All the amino acids, except glycine have two optically active isomers, the D – or the L form.
Natural proteins are usually made up of L- (or the so-called natural amino acids.)

USES OF AMINO ACIDS

Amino acids find use in a large number of activities, including human and animal nutrition, medicine, cosmetics, and in the synthesis of chemicals.

- ***Use in human and animal nutritional supplementation*:**
- ***Flavor and taste enhancement in foods*:** Amino acids are important in deciding the taste of meats and such foods. L-Glutamic acid is utilized in the manufacture of monosodium L-glutamate which, because of its meatlike flavor, is used as a flavoring agent. L-Lysine is an essential amino acid, but it often is present in only small amounts in foods prepared from cereal grains. To remedy this situation, L-lysine often is used to fortify such foods in order to make them nutritionally more equivalent to meat proteins. Amino acids influence the taste of foods. Some are very sweet; for example glycine is as sweet as sugar and is sometimes used in soft drinks and soups.
- ***Medical uses*:** The greatest application of amino acids in medicine is in transfusion; which is administered when the oral consumption of proteinaceous food is not possible such as after an operation. Various amino acids are used for ammonia detoxification in blood in liver diseases, in the treatment of heart failure, in cases of peptic ulcer and male sterility. Methyldopa (L-methy1-3, 4 dihydroxy-phenylalanin) is widely used as an antihypertensive with relatively few side effects. Dopa is used in treating

Parkinson's disease. A derivative of serine, cycloserine is an antibiotic produced by a streptomycete; it is used for the treatment of tuberculosis.

- ***Use as an industrial synthetic raw materials*:**
- ***Surface-active agents:*** Sodium lauryl sarcosinate is used in toothpaste and shampoo because it has a bacteriocidal as well as foaming action. These derivatives are also used as fungicides and pesticides.
- ***Production of polymers from amino acids*:** Polymers derived from amino acids are used in making synthetic leather, fire-resistant fabrics and anti-static materials.
- ***Use as cosmetics:*** Amino acids exhibit a buffering action that help maintain normal skin function by regulating pH and a protective action against bacteria. Detergents (surface action agents) derived from amino acids are less irritating than soaps because the pH of 5.5-6.0 is closer to that of the skin, whereas soap is slightly alkaline. The addition of different amino acids to shampoo is practiced to achieve different ends: anti-dandruff shampoos contain cysteine; thioglycolic acid is employed as a reducing agent for the cold waving of hair.

METHODS FOR THE MANUFACTURE OF AMINO ACIDS

(i) ***Protein hydrolysis*:** Protein hydrolysis was the original method of amino manufacture. Hair, keratin, blood meal and feathers are hydrolyzed using acid and the amino acid extracted.

(ii) ***Chemical synthesis*:** few amino acids are prepared by chemical synthesis. Amino acids produced by chemical synthesis are glycine and methionine;

(iii) ***Microbiological methods*:** Microbiological methods are of three types:

1. Semi-fermentation;
2. Use of microbial enzymes or immobilized cells;
3. Direct fermentation.

AUXOTROPHS / AUXOTROPHIC MUTANTS

An auxotrophic mutant is a cell which, through mutation, has lost the ability to produce one or more enzymes of a biosynthetic pathway and, because of this metabolic block, it requires that a specific metabolite or metabolites just beyond the block in the metabolic pathway be supplied in its growth medium.

Mutation and selection programs are commonly employed for increasing the yields of fermentation products by industrially important microorganisms. While such programs often provide strains with increased yield capacity, in most instances it is not apparent as to just which biosynthetic pathways of the organism have been altered. This picture, however, is clearer with auxotrophic mutants and, in some instances; the auxotrophic mutations allow specific control over microbial metabolic pathways such that high yields of desired fermentation products is required.

Thus, because of the metabolic block, cell is not able to synthesize the specific metabolite (or metabolites) required for its growth. An auxotrophic mutant can be of considerable value as a fermentation organism, because the intermediate compound or compounds of the biosynthetic sequence just preceding the metabolic block may accumulate in quantity in the culture broth due to the lack of an enzyme for bringing about further chemical conversion.

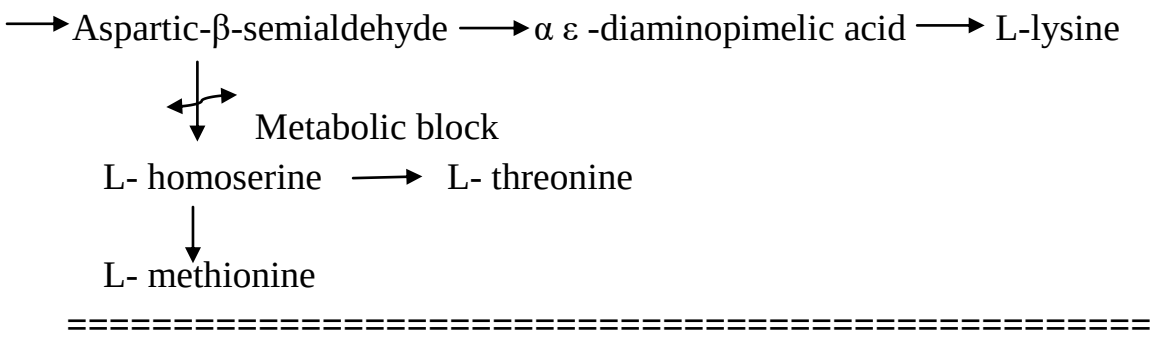
Auxotrophs have been used for industrial fermentations, but to date their application has been limited almost exclusively to amino acid fermentations. Of the various amino acids, there is real commercial demand only for L-glutamic acid and L-lysine, and fermentation processes utilizing auxotrophs (or organisms resembling auxotrophs) are employed for the production of these two amino acids.

A) DIRECT FERMENTATION of L-Lysine

The strain of *Micrococcus gluamicus* utilized in commercial glutamic acid production was mutated to obtain an auxotroph capable of producing L-lysine by direct fermentation. This auxotroph requires L-homoserine or a mixture of L-threonine and L-methionine for growth, and produces greater than 20 g per liter of L-lysine in the fermentation broth. In addition, the L-lysine is not destroyed by the organism during the fermentation, because it also lacks the ability to produce L-lysine decarboxylase.

The fermentation utilizes a glucose medium, high aeration, 30^0 C temp, 6 to 8 pH, urea and 2 days incubation period. The level of L-homoserine (or L-threonine plus L-methionine) provided initially in the medium must be optimum. Too small an amount impedes growth and, hence, L-lysine production, and an excess also prevents L-lysine production. A branching point in the biosynthetic pathway occurs at aspartic-β-semialdehyde. One branch without a mutational block leads in the direction of α ε -diaminopimelic acid

and L-lysine. The other route, however, has a mutational block between aspartic-β-semialdehyde and L-homoserine. Since a further branching of the sequence occurs just beyond L-homoserine, it is obvious that either L-homoserine or L-threonine plus L-methionine must be supplied for growth of the organism.



==========