FACULTY OF ENGINEERING & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

VITAMIN B12

- A **vitamin** is an organic compound and a vital nutrient that an organism requires in limited amounts.

- They are of great value in the growth and metabolism of the living cells.

- Vitamins are obtained with food, but a few are obtained by other means ; humans can produce some vitamins from precursors they consume while certain microorganism produce vitamins too.

- Thirteen vitamins are universally recognized at present, vitamins are classified by their biological and chemical activity.

- Vitamins can be classified as "**Fat soluble vitamins**" and "**Water soluble vitamins**"

FAT SOLUBLE VITAMINS

- **Vitamin A(Retinol)**
- **Vitamin D**

1. Vitamin D2 (Egrocalciferol)
2. Vitamin D3 (Cholecalciferol)

- **Vitamin E (Tocopherol)**
- **Vitamin K (Phylloquinone)**

WATER SOLUBLE VITAMINS

- **Vitamin B Complex**

1. Vitamin B1 (Thiamine)
2. Vitamin B2 (Riboflavin)
3. Vitamin B3 (Niacin)
4. Vitamin B5 (Pantothenic acid)
5. Vitamin B6 (Pyridoxine)
6. Vitamin B7 (Biotin)
7. Vitamin B9 (Folic Acid)
8. Vitamin B12 (Cobalamin)

- **Vitamin C (Ascorbic acid)**

VITAMIN B12

- **Vitamin B_{12}**, also called **Cobalamin**, is a water-soluble vitamin that has a key role in the normal functioning of the brain and nervous system, and the formation of red blood cells.

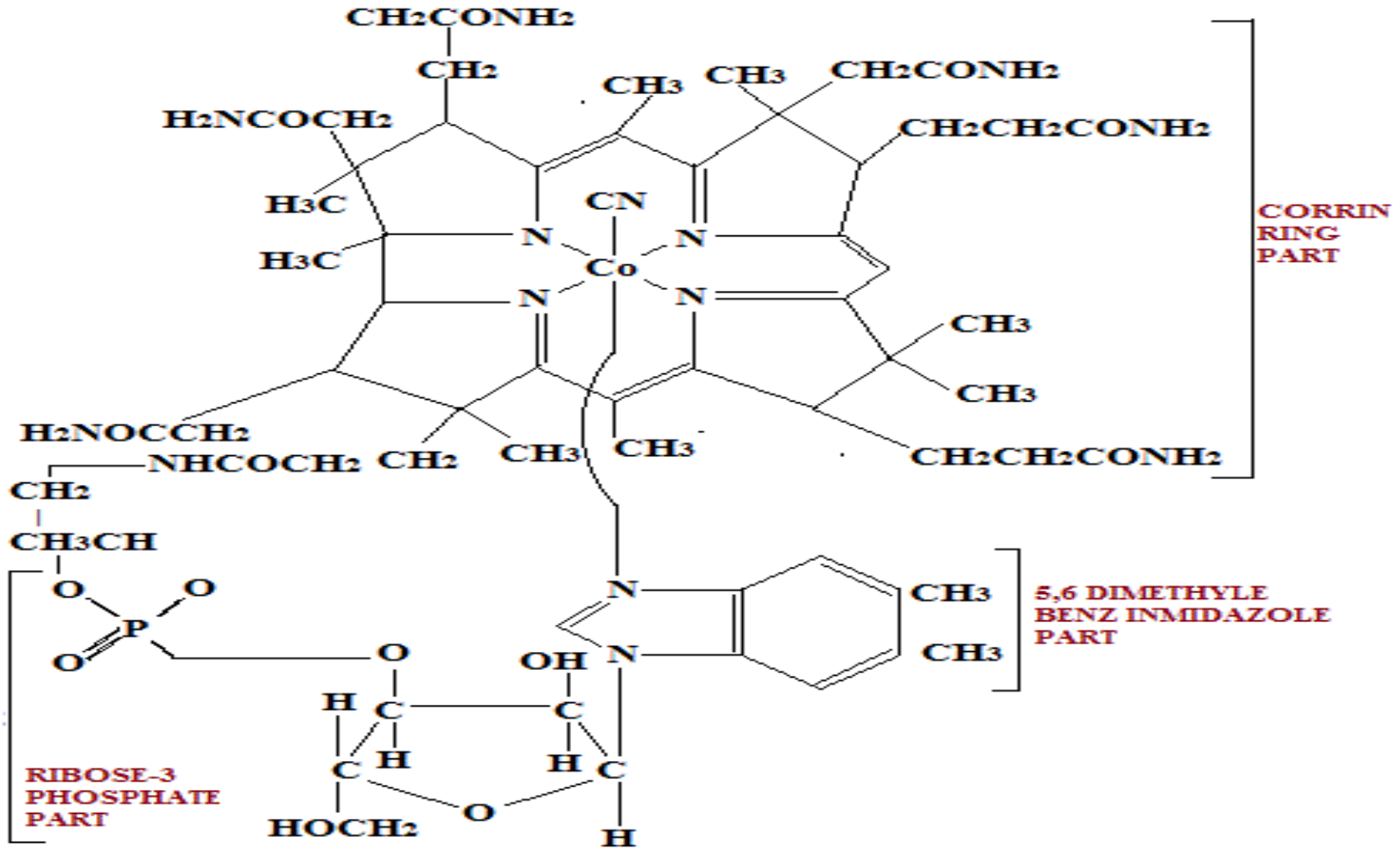
- It is involved in the metabolism of every cell of the human body, especially affecting DNA synthesis, fatty acid and amino acid metabolism.

- It is synthesized only by microorganisms and not by animals (including humans) and plants.

- People with B12 deficiency may eventually develop **Pernicious anemia.**

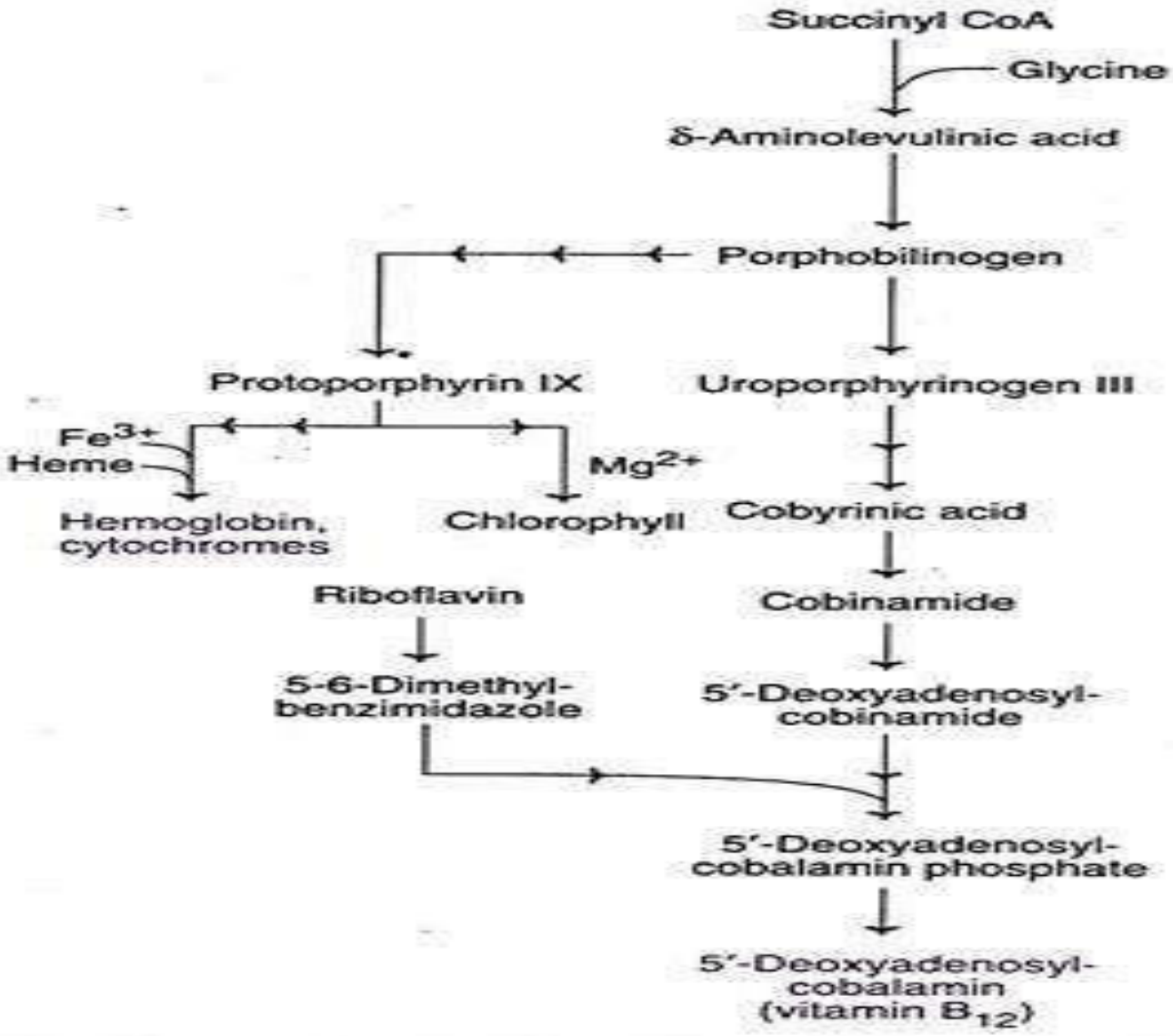
- It is the largest and most structurally complicated vitamin and can be produced industrially only through bacterial fermentation-synthesis.

STRUCTURE



VITAMIN B12
(CYANOCOBALAMIN)

BIOSYNTHESIS



PRODUCTION OF VITAMIN B12

- Cyanocobalamin, is the industrially produced stable Cobalamin form which is not found in nature.

- Vitamin B12 is entirely produced on a commercial basis by the fermentation. It is usually manufactured by submerged culture process. Such a fermentation process is completed in 3-5 days

- Most of the B12 fermentation processes use glucose as a carbon source.

- The microorganisms that maybe employed in the industrial production process are :

i. *Streptomyces griseus*
ii. *Streptomyces olivaceus*
iii. *Bacillus megaterium*
iv. *Bacillus coagulans*
v. *Pseudomonas denitrificans*
vi. *Propionibacterium freudenreichii*
vii. *Propinibacteriun shermanii*

STEP INVOLVED IN PRODUCTION PROCESS

Step 1	• Formulation of the medium
Step 2	• Sterilization of the medium
Step 3	• Making starter culture
Step 4	• Anaerobic fermentation
Step 5	• Aerobic fermentation
Step 6	• Recovery

A. Streptomyces olivaceus

- Production by *Streptomyces olivaceus* yields about 3.3mg / L of vitamin B12.

Process :

A. Preparation Of Inoculum :

Pure slant culture of *S. olivaceus* is inoculated in 100-250ml of inoculum medium, contained in Erlenmeyer flask.

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Seeded flask is incubated on platform of a mechanical shaker to aerate the system.

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This flask culture is then subsequently used to inoculate larger inoculum tanks.

(2 or 3 successive transfers are made to obtain required amount of inoculum cultures.)

- **Media used in preparation of inoculum is Bennett's agar.**

Component	Amount (g/L)
Yeast extract	1.0
Beef extract	1.0
N-Z-Amine A (Enzymatic hydrolase of casein)	2.0
Glucose	10.0
Agar	15.0
D/W	1000 mL
pH	7.3

B. Production Medium :

▪Consist of carbohydrate, proteinaceous material, and source of cobalt and other salts.

Component	Amount (%)
Distiller's Solubles	4.0
Dextrose	0.5 - 1
CaCO3	0.5
COCL2.6H2O	1.5 – 10 ppm
pH	7

- It is necessary to add cobalt to the medium for maximum yield of cobalamin.
- Cyanide is added for conversion of other cobalamins to vitamin B12.

C. Sterilization of the medium :

- Sterilization can be done batchwise of continuously.
  - Batch – medium heated at 250°F for 1 hour.
  - Continuous – 330°F for 13 min by mixing with live steam.

D. Temperature , pH , Aeration and Agitation :

- Temperature : A temperature of 80°F in production tank is satisfactory during fermentation.

- pH : At starting of process pH falls due to rapid consumption of sugar, then rises after 2 to 4 due to lysis of mycelium. pH 5 is maintained with H_2SO_4 and reducing agent Na_2SO_4.

- Aeration and Agitation : Optimum rate of aeration is 0.5 volume air/volume medium/min.

E. Antifoam agent , Prevention of contamination :

▪Antifoam agent : Defoaming agents like soya bean oil , corn oil, lard oil and silicones can be used.

▪Prevention of contamination : Essential to maintain sterility, contamination results in reduced yields, equipments must be sterile and all transfers are carried out under aseptic conditions.

F. Recovery :

▪During fermentation, most of cobalamin is associated with the mycelium; boiling mixture at pH 5 liberates the cobalamin quantitatively from mycelium.

▪Broth containing cobalamin is subjected to further process to obtain crystalline B12.

Filtration of broth to remove mycelium.

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Filtered broth is treated with cyanide to bring conversion of cobalamin to cyanocobalamin.

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Adsorption of cyanocobalamin from the solution is done by passing it through adsorbing agents packed in a column.

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Cyanocobalamin is then eluted from the adsorbent by the use of an aqueous solution of organic bases or solutions of Na-Cyanide and Na- thiocyanate.

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Extraction is carried out by countercurrent distribution between cresol, amylphenol, or benzyl alcohol and water ora single extraction into an organic solvent (e.g. Phenol) is carried out.

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Chromatography on alumina and final crystallization completes the process.

B. Propionibacterium fredenreichii

- Production by *Propionibacterium freudenreichii* yields about 20mg/L of vitamin B12.

A. Production media : glucose , corn- steep , betaine , & cobalt.

- Betaine -0.5 %

- Cobalt – 5µg./ml (excess cause reduced cobalamin formation)

B. pH -7.5

C. Temperature – 30°C

D. Fermentation : It involves to cycles; anaerobic fermentation cycle of 70 hours and Aerobic fermentation cycle of 50 hours.

- **Anaerobic fermentation** :

- Formation of cobinamide occurs.

- The pH falls from 7.5 to 6.5 and then rises upto 8.5.

- Necessary to add 0.1% of 5, 6 – dimethyl benziminazole to the production medium.

- **Aerobic fermentation :**

- Nucleotide formation takes place.

- This nucleotide then links with cobinamide to give cobalamin

Species	Medium	Aeration	Temp. (°C)	Time (hour)	Yield (mg/L)
B.megaterium	Molasses , mineral salts, cobalt	Aerobic	30	18	0.45
P. shermanii	Glucose , corn- steep, ammonia , cobalt pH 7.0	Anaerobic (3 days), aerobic (4 days)	30	150	23
B. coagulants	Citric acid , triethanolamine , corn – steep , cobalt.	Aerobic	55	18	6.0