Vitamin B12 Industrial Production

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Vitamin B_{12}

- Vitamin B12, also known as cyanocobalamin, belongs to the cobalamin family of compounds, which are composed of a corrinoid ring and an upper and lower ligand.
- The upper ligand can be adenosine, methyl, hydroxy, or a cyano group.
- Vitamin B_{12} (cyanocobalamin) is a water soluble vitamin with complex structure. The empirical formula of cyanocobalamin is $C_{63}H_{90}N_{14}O_{14}PCo$.
- The disease, pernicious anemia, characterized by low levels of hemoglobin, decreased number of erythrocytes and neurological manifestations, has been known for several decades.
- It was in 1926 some workers reported the liver extracts could cure pernicious anemia.
- The active principle was later identified as vitamin B_{12}, a water soluble B-complex vitamin.
- Vitamin B_{12} is exclusively synthesized in nature by microorganisms.
- Vitamin B12 is synthesized by prokaryotes and inhibits the development of pernicious anemia in animals.

Commercial Production of Vitamin B_{12}

- Vitamin B_{12} was first obtained as a byproduct of *Streptomyces* fermentation in the production of certain antibiotics (streptomycin, chloramphenicol, or neomycin).
  - But the yield was very low.
- Later, high-yielding strains were developed. It is estimated that the world's annual production of vitamin B_{12} is around 15,000 kg.
- High concentrations of vitamin B_{12} are detected in sewage-sludge solids.
- This is produced by microorganisms.
- Recovery of vitamin B_{12} from sewage-sludge was carried out in some parts of United States.
- Unlike most other vitamins, the chemical synthesis of vitamin B_{12} is not practicable, since about 20 complicated reaction steps need to be carried out.
- Fermentation of vitamin B_{12} is the only choice.

Microorganisms and Yields of Vitamin B_{12}

- The natural process of vitB12 synthesis by approximately 30 enzyme mediated steps.
- Several microorganisms can be employed for the production of vitamin B_{12}, with varying yields.
- Glucose is the most commonly used carbon source.
- Some examples of microbes and their corresponding yields are given in Table 1.
- The most commonly used microorganisms are —
  - *Propionibacterium freudenreichii,*
  - *Pseudomonas denitrificans,*
  - *Bacillus megaterium*
  - *Streptomyces olivaceus*

Microorganism	*Yield (mg/l)*
Bacillus megaterium	0.51
Streptomyces olivaceus	3.31
Butyribacterium rettgeri	5.0
Micromonospora sp	11.5
Propionibacterium freudenreichii	19.0
Propionibacterium shermanii	35.0
Pseudomonas denitrificans	60.0
Hybrid strain	
Rhodopseudomonas protamicus	135.0

Production of Vitamin B_{12} Using *Propionibacterium* sp.

- *Propionibacterium freudenreichii* and *P. shermanii*, and their mutant strains are commonly used for vitamin B_{12} production.
- The process is carried out by adding cobalt in two phases.

Anaerobic phase:

- This is a preliminary phase that may take 2-4 days.
- In this phase 5'-deoxyadenosylcobinamide is predominantly produced.

Aerobic phase:

- In this phase, 5, 6-dimethyl- Benz imidazole is produced from riboflavin which gets incorporated to finally form coenzyme of vitamin namely 5'-deoxyadenosylcobalamin.
- In recent years, some fermentation technologists have successfully clubbed both an anaerobic and aerobic phases to carry out the operation continuously in two reaction tanks.
- The bulk production of vit B_{12} is mostly done by submerged bacterial fermentation with beet molasses medium supplemented with cobalt chloride.
- The specific details of the process are kept as a guarded secret by the companies.

Recovery of vitamin B_{12}

- The cobalamins produced by fermentation are mostly bound to the cells.
- They can be solubilized by heat treatment at 80-120°C for about 30 minutes at pH 6.5-8.5.
- The solids and mycelium are filtered or centrifuged and the fermentation broth collected.
- The cobalamins can be converted to more stable cyanocobalamins.
- This vitamin B_{12} is around 80% purity and can be directly used as a feed additive.
- However, for medical use (particularly for treatment of pernicious anemia), vitamin B_{12} should be further purified (95-98% purity).

Production of Vitamin B_{12} using *Pseudomonas* sp.

- *Pseudomonas denitrificans* is also used for large scale production of vitamin B_{12} in a cost-effective manner.
- Starting with a low yield (0.6 mg/l) two decades ago, several improvements have been made in the strains of *P. denitrificans* for a tremendous improvement in the yield (60 mg/l).
- Addition of cobalt and 5, 6-dimethyl Benz imidazole to the medium is essential.
- The yield of vitamin B_{12} increases when the medium is supplemented with betaine (usual source being sugar beet molasses).

Carbon Sources for Vitamin B_{12} Production

- Glucose is the most commonly used carbon source for large scale manufacture of vitamin B_{12}.
- Other carbon sources like alcohols (methanol, ethanol, isopropanol) and hydrocarbons (alkanes, decane, hexadecane) with varying yields can also be used.
- A yield of 42 mg/l of vitamin B_{12} was reported using methanol as the carbon source by the microorganism *Methanosarcina barkeri*, in fed- batch culture system.