Microbial Production of Vitamins

Vitamins are organic compounds that perform specific biological functions for normal maintenance and optimal growth of an organism. These vitamins cannot be synthesized by the higher organisms, including man, and therefore they have to be supplied in small amounts in the diet.

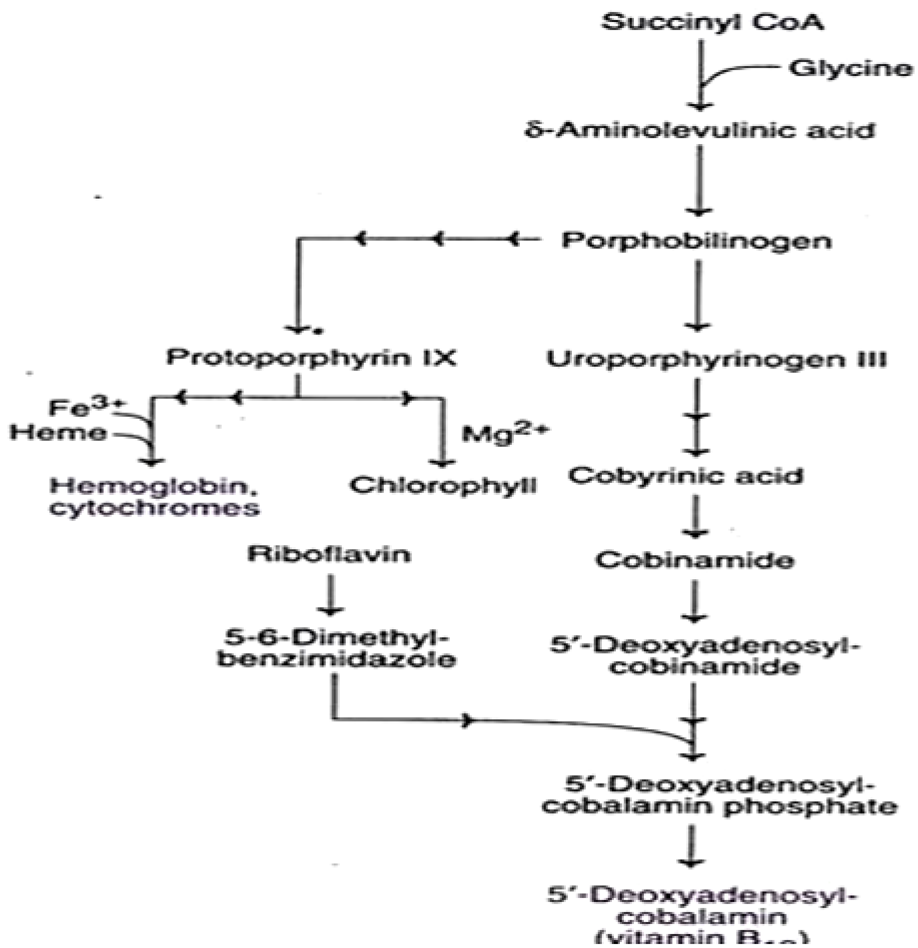
Microorganisms are capable of synthesizing the vitamins. In fact, the bacteria in the gut of humans can produce some of the vitamins, which if appropriately absorbed can partially meet the body's requirements. It is an accepted fact that after administration of strong antibiotics to humans (which kill bacteria in gut), additional consumption of vitamins is recommended.

Microorganisms can be successfully used for the commercial production of many of the vitamins e.g. thiamine, riboflavin, pyridoxine, folic acid, pantothenic acid, biotin, vitamin B_{12}, ascorbic acid, P-carotene (pro-vitamin A), ergosterol (pro-vitamin D). However, from economic point of view, it is feasible to produce vitamin B_{12}, riboflavin, ascorbic acid and p-carotene by microorganisms. For the production of ascorbic acid (vitamin C), the reader must.

1. **Vitamin B_{12}:** 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.

   Occurrence: Vitamin B_{12} is present in animal tissue at a very low concentration (e.g. 1 ppm in the liver). It occurs mostly in the coenzyme forms- methylcobalamin and deoxyadenosylcobalamin. Isolation of vitamin $B_{]2}$ from animal tissues is very expensive and tedious.

   Biosynthesis: Vitamin B_{12} is exclusively synthesized in nature by microorganisms. An outline of the pathway is depicted in Fig. 27.1. The biosynthesis of B_{12} is comparable with that of chlorophyll and hemoglobin. Many of the reactions in the synthesis of vitamin B_{12} are not yet fully understood.



An outline of the biosymthesis of vitamin B_{12}

Commercial Production of Vitamin B_{12}: Vitamin B_{12} is commercially produced by fermentation. It 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. And at present, vitamin B_{12} is entirely produced by fermentation. 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.

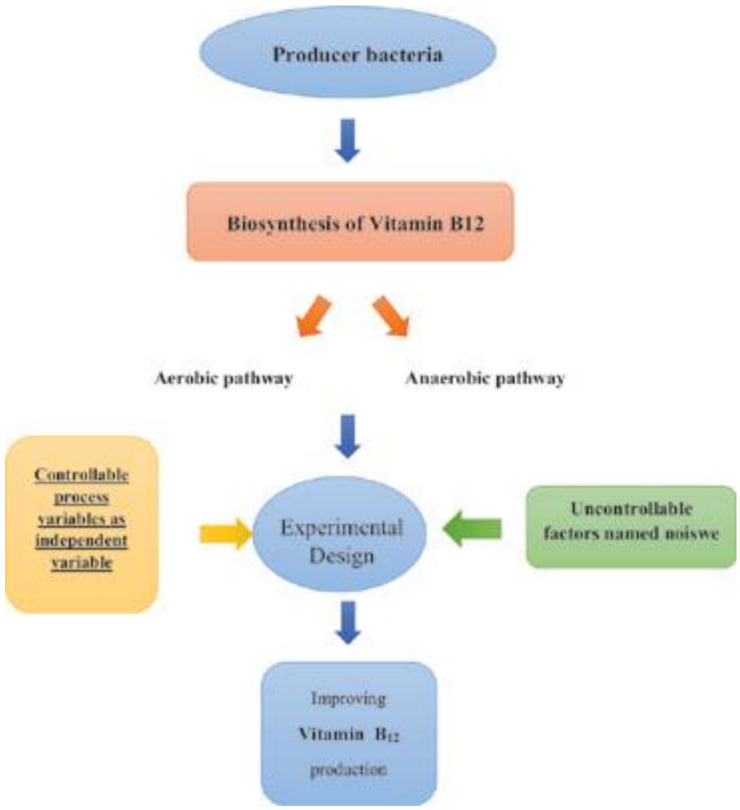
Microorganisms and Yields of Vitamin B_{12}- Several microorganisms can be employed for the production of vitamin B_{12}, with varying yields. Glucose is the most commonly used carbon source. Some examples of microbes and their corresponding yields are given in Table 27.1. The most commonly used microorganisms are — Propionibacterium freudenreichii, Pseudomonas denitrificans, Bacillus megaterium and Streptomyces olivaceus.

Microorganism with corresponding yield of vitamin B^{12}

No.	Microorganism	Yield (mg/l)
1	*Bacillus megaterium*	0.51
2	*Streptomyces olivaceus*	3.31
3	*Butyribacterium rettigeri*	5.0
4	*Micromonospora*	11.5
5	*Propinobacterium fruedenreichii*	19.0
6	*Propinobacterium sharmanii*	35.0
7	*Pseudomonas dendrificans*	60.0
8	*Rhodopseudomonas protamicus*	135.0

Genetically engineered strains for vitamin B_{12} production- By employing modern techniques of genetic engineering, vitamin B_{12} production can be enhanced. A protoplast fusion technique between Protaminobacter rubber and Rhodopseudomonas spheroides resulted in a hybrid strain called Rhodopseudomonas protamicus. This new strain can produce as high as 135 mg/l of vitamin B_{12} utilizing carbon source.

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 the anaerobic 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 B-p namely 5′-deoxyadenosylcobalamin. The bulk production of vitamin 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.

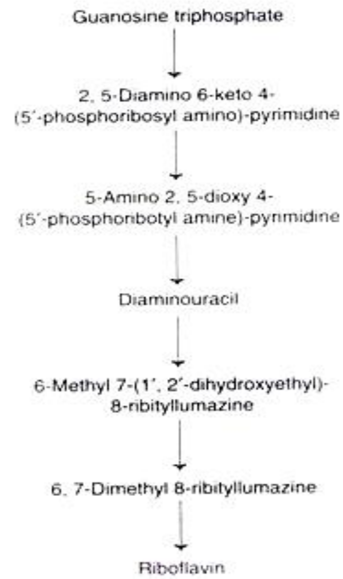
denitrificans for a tremendous improvement in the yield (60 mg/l). Addition of cobalt and 5, 6-dimethyl Benz imidazole to the medium is essential. The yield of vitamin B_{12} increases when the medium is supplemented with betaine (usual source being sugar beet molasses).

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.

2. **Riboflavin-** Riboflavin (vitamin B_{12}) is a water soluble vitamin, essential for growth and reproduction in man and animals. Deficiency of riboflavin in rats causes growth retardation, dermatitis and eye lesions. In humans, vitamin B_2 deficiency results in cheilosis (fissures at the corner of mouth), glossitis (purplish tongue) and dermatitis. Riboflavin exerts its biochemical functions through the coenzymes namely flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN).

   Occurrence- Riboflavin occurs in milk and milk products, meat, eggs, liver and kidney. While in milk and eggs, it is present in free form, in other foods it is found in the form of flavoproteins (i.e. coenzymes of riboflavin bound to proteins).

   Biosynthesis- The biosynthetic pathway of riboflavin, elucidated for the microorganisms Ashbya gossypii and Eremothecium ashbyii. The overproduction of riboflavin in these organisms takes place mainly due to the constitutive nature of the riboflavin synthesizing enzymes. Iron which inhibits the production of vitamin B_{12} in Clostridia and yeasts, has no effect on A. gossypii and E. ashbyii.



Biosynthesis of riboflavin

Commercial Production of Riboflavin: There are three processes employed for the large scale production of riboflavin. The worldwide requirement of riboflavin is estimated to be around 2,500 tones per year.

1. Biotransformation: About 50% of the world's requirement of riboflavin is produced by biotransformation, followed by chemical synthesis. For this purpose, glucose is first converted to D-ribose by mutant strains of *Bacillus pumilus*. The D-ribose so produced is converted to riboflavin by chemical reactions.

2. Chemical synthesis: Approximately 20% of the world's riboflavin is produced by direct chemical synthesis.

3. Fermentation: At least one third of world's riboflavin requirements are met by direct fermentation processes.

Microorganisms and yields of riboflavin:

A. Several microorganisms (bacteria, yeasts and fungi) can be employed for the production of riboflavin. In the acetone-butanol fermentation, employing the organisms Clostridium aceto-butylicum and Clostridium butylicum, riboflavin is formed as a byproduct.

B. Commercial production of riboflavin is predominantly carried out by direct fermentation using the ascomycetes. The different organisms used and the corresponding yields of riboflavin are given in Table 27.2. The two plant pathogens namely Ashbya gossypii and Eremothecium ashbyii are most commonly employed due to high yield. Among these two organisms, A. gossypii is preferred as it is more stable with a high producing capacity of riboflavin.

Microorganism with corresponding yield of vitamin riboflabin

No.	Microorganism	Yield (mg/l)
1	*Clostridium acetobutylicum*	0.097
2	*Clostridium abutylicum*	0.120
3	*Mycobacterium smegmatis*	O,60
4	*Mycocandida rivoflavina*	0.200
5	*Candida flareri*	0.575
6	*Eremothecium ashbyii*	2.500
7	*Ashbya gossypii*	7.500

C. **Genetically engineered strains for riboflavin production-** High yielding strains of Ashbya gossypii have been developed by genetic manipulations. Such strains can yield as high as 15 g/l riboflavin.

D. **Production process of riboflavin-** Industrial production of riboflavin is mostly carried out with the organism, Ashbya gossypii by using simple sugars such as glucose and corn steep liquor. Glucose can be replaced by sucrose or maltose for the supply of carbon source.

E. In recent years, lipids such as corn oil, when added to the medium for energy purpose, have a profound influence on riboflavin production. Further, supplementation of the medium with yeast extract, peptones, glycine, inositol, purines (not pyrimidine’s) also increase the yield of riboflavin.

F. It is essential to carefully sterilize the medium for good yield of riboflavin. The initial pH of the culture medium is adjusted to around 6-7.5. The fermentation is conducted at temperature 26-28°C with an aeration rate 0.3 vvm. The process is carried out for about 5-7 days by submerged aerated fermentation.

G. Riboflavin fermentation by Eremothecium ashbyii is comparable to that described above for Ashbya gossypii. Candida sp can also produce riboflavin, but this fermentation process is extremely sensitive to the presence of iron. Consequently, iron or steel equipment cannot be used. Such equipment have to be lined with plastic material.

Fermentation through phases-Some studies have been carried out to understand the process of fermentation of riboflavin particularly by ascomycetes. It is now accepted that the fermentation occurs through three phases.

Phase I- This phase is characterized by rapid growth of the organism utilizing glucose. As pyruvic acid accumulates, pH becomes acidic. The growth of the organism stops as glucose gets exhausted. In phase I, there is no production of riboflavin.

Phase II- Sporulation occurs in this phase, and pyruvate concentration decreases. Simultaneously, there is an accumulation of ammonia (due to enhanced deaminase activity) which makes the medium alkaline. Phase II is characterized by a maximal production of riboflavin. But this is mostly in the form of FAD and a small portion of it as FMN.

Phase III- In this last phase, cells get disrupted by a process of autolysis. This allows release of FAD, FMN and free riboflavin into the medium.

Recovery- Riboflavin is found in fermentation broth and in a bound form to the cells. The latter can be released by heat treatment i.e. 120°C for about 1 hour. The cells can be discarded after filtration or centrifugation. The filtrate can be further purified and dried, as per the requirements.

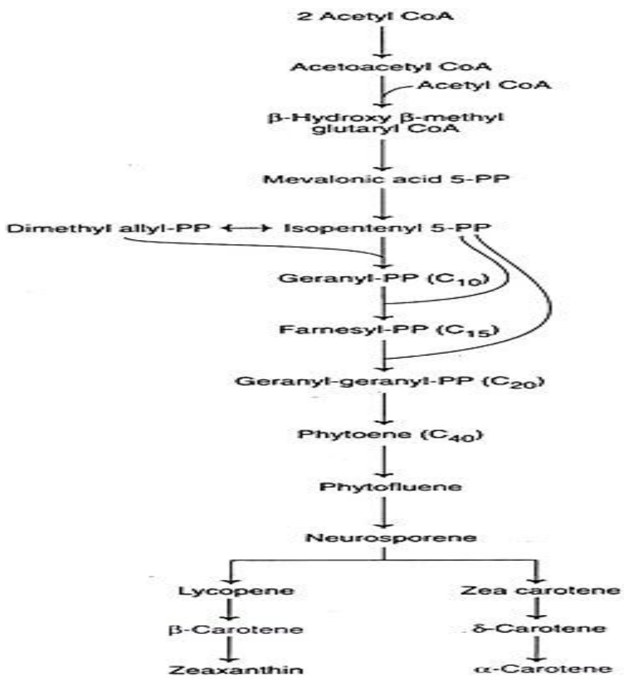
Other carbon sources for riboflavin production- Besides sugars, other carbon sources have also been used for riboflavin production. A pure grade of riboflavin can be prepared by using Saccharomyces sp, utilizing acetate as sole carbon source. Methanol-utilizing organism Hansenula polymorpha was found to produce riboflavin. The other carbon sources used with limited success for riboflavin production are aliphatic hydrocarbons (organism Pichia guilliermoudii) and n-hexadecane (organisms — Pichia miso).

3. **β– Carotene:** β- Carotene is the pro-vitamin A. When ingested, it gets converted to vitamin A in the intestine. Vitamin A is a fat soluble vitamin required for vision, proper growth and reproduction. The deficiency of vitamin A causes night blindness, changes in the skin and mucosal membranes.

   Occurrence- β- Carotene is found in many animal and plant tissues. However, it originates exclusively from plants or microorganisms. Yellow and dark green vegetables and fruits are rich in β-carotene e.g. carrots, spinach, amaranthus, mango, papaya.

Biosynthesis:

The pathway for the biosynthesis of β-carotene and some other important carotenoids, elucidated in plants and fungi, is shown in Fig. 27.3.



Biosynthesis of carotene

Commercial Production of β-Carotene:

β- Carotene can be produced by microbial fermentation. However, for economic reasons, direct chemical synthesis of vitamin A is preferred rather than using its pro-vitamin (β-carotene).

Microorganisms:

The organisms Blakeslea trispora, Phycomyces blakesleeanus and Choanephora cucurbitarum are most frequently used for the production of β– carotene. Among these, Blakeslea trispora is preferred due to high yield. In the Table 27.3, some important carotenoids, the organisms and the production yields are given.

Microbial production of important carotenoids

No.	Carotenoid	Microorganism	Yield (g/l)
1	***B- Carotene***	*Blakeslea trispora (Mixed culture of + and – Sexual forms*	3.0
2	**Lycopene**	*Blakeslea trispora*	0.4
3	**Lycopene**	*Streptomyces chrestomyceticus*	0.5
4	**Zaexanthin**	*Flavobacterium sp.*	0.4

Production process of β-carotene- As already stated, the industrial production of β-carotene is mostly carried out by Blakeslea trispora. The fermentation medium contains corn starch, soybean meal, β-ionone, antioxidants etc. Addition of antioxidants improves the stability of β-carotene within the cells. The fermentation is carried out by submerged process.

The fermentation is usually started by mixing the cultures of both sexual forms, (+) and (-) strains of B. trispora. The yield of β-carotene is significantly higher with mixed cultures, compared to + or – strains (Fig. 27.4).

This is due to the fact that β- carotene production predominantly occurs during the process of zygospore formation. It may be stated here that the use of mixed strains does not improve the yield for other microorganisms (as observed in case of Blakeslea trispora).

4. **Gibberellins — Plant Growth Stimulants-** Gibberellins are plant hormones that stimulate plant growth. They promote growth by cell enlargement and cell division. The observable effects of gibberellins include stimulus to seed germination, flowering and lengthening of stems.

   Microbial Production of Gibberellins- So far only one microorganism, the fungus namely Gibberella fujikuroi has been found to produce gibberellins. This is actually a pathogenic fungus of rice seedlings. Gibberellin production can be carried out by using a glucose-salt medium at pH 7.5 and temperature 25°C for 2-3 days. The fermentation process is conducted in aerated submerged process. After the growth of the fungus is maximum, the production of gibberellins commences.