Vitamins :

Riboflavin :

Vitamins are organic compounds required in small amounts in the diets of animals in order to maintain the normal physiological function of the body. Though vitamins are required in small amounts, the animals are unable to synthesis them. The dietary deficiency of vitamins or its malabsorption results in diseases with characteristic symptoms. The vitamins are the constituent of the coenzymes which are essentially required by the enzymes to catalyze various biochemical reactions. The role of vitamin A in the vision of animals has been clearly demonstrated, vitamin D increases the permeability of the intestinal mucosal cells to calcium ion helping in its absorption. Riboflavin is a precursor of coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) which are hydrogen carriers in biological redox reactions catalyzed by various dehydrogenases. Cyanocobalamin assists the action of methionine synthase and (R)-methylmalonyl-CoA mutase in humans. The vitamins are also essential for proper growth of microorganisms in fermentation processes.

Animal and plant tissues were mainly used for extracting vitamins in the beginning, although dried baker’s yeast preparation was also employed as a rich source of B vitamins. Microorganisms have been known to produce vitamins during normal metabolism and are being used for commercial production of various vitamins such as thiamine, riboflavin, folic acid, pyridoxine, cobalamin, biotin, folic acid, L-ascorbic acid, β-carotene, ergosterol and pantothenic acid. Direct fermentation methods are employed for the production of some vitamins, whereas biotransformations or combined chemical and microbiological processes are used for the production of certain other vitamins. In this section microbial production of riboflavin and cyanocobalamin will be discussed.(p.36).

Biosynthesis of Riboflavin :

Riboflavin biosynthesis has been studied in both gram-positive and gram-negative bacteria but it has been worked out in more detail in *B. subtilis* (Perkins and Pero, 2002) and *Escherichia coli* (Bacher *et al.* 1996). It has been well established that the synthesis of this vitamin involves seven enzymes encodes by *rib* operon. Various enzymes and steps involved in the biosynthesis of riboflavin are shown in Fig. 17. The synthesis of one molecule of riboflavin requires one molecule of GTP and two molecule of ribulose-5-phospate. In order to perform its metabolic function, riboflavin is biochemically transformed to the coenzymes FMN and FAD by an essential bifunctional flavokinase/FAD synthetase.

Production Process:

Ashbya gossypii NRRL Y-1056 is being currently used for the production of riboflavin. Studies focused on strain improvement and optimization of fermentation process parameters have enhanced the yield to 10-15 g/L. Media containing 2.25% corn steep liquor, 3.5% commercial peptone, 4.5% soy bean oil, supplemented with glycine, distiller's solubles or yeast extract are used for the production of riboflavin. The fermentation is initiated by using small inoculum (0.75-2.0%) of 24-48 h old actively growing culture and the medium pH is maintained around 6.5-7.0. The fermentation is allowed to proceed for 7 days with an aeration of 0.3 vvm at 34-37 ºC.

The fermentation is performed in two stages, in the first stage the organism is allowed to grow and increase its biomass and in second stage riboflavin production is stimulated by restricting the growth or by feeding glucose and inositol along with micronutrients such as iron to obtain high yield of riboflavin. After completion of fermentation, the pH of the fermentation broth is adjusted to 4.5. For feed grade product, the broth is concentrated to about 30% solids and dried on double-drum driers and to obtain crystalline product, the broth is heated for 1 h at 121 ºC to solublize the riboflavin and also release the bound vitamin from the cells. Insoluble matter is removed by centrifugation and the riboflavin so recovered is converted to less soluble form either chemical or by microbiological methods .

Yeasts (*Candida flaeri*, *C. famata*, etc.) and bacteria are also reported to produce riboflavin. Genetically engineered *Bacillus subtilis* and *Corynebacterium ammoniagenes* overexpressing genes of the enzymes involved in riboflavin biosynthesis produces 4.5 g/L and 15.3 g/L of riboflavin respectively in the fermentation broth. Some yeasts utilize other carbon sources e.g., *Pichia guilliermondii* (C_{10}-C_{18} aliphatic hydrocarbon), *P. miso* (n-hexadecane, corn steep liquor, urea), *Hansenula polymorpha* (methanol), *Saccharomyces* (acetate) and have exhibited a fairly high potential for the production of this vitamin.