Microbial production of Vitamins (Vitamin B_{12})

One of the most interesting and fascinating molecules in the world of science and medicine is vitamin B_{12} (cobalamin), which was originally discovered as the anti-pernicious anemia factor in the early 1920s, when two American physicians, Minot and Murphy, demonstrated it to cure pernicious anemia, a disorder first described in 1835, with a diet that included raw liver. In humans, the vitamin is required in trace amounts (approximately 1 mg/day) to assist the action of only two enzymes, methionine synthase and (R)-methylmalonyl-CoA mutase; yet commercially more than 10 t of B_{12} are produced each year from a number of bacterial species. The term vitamin B_{12} is widely used to describe compounds of the cobalamin group. Natural forms are adenosylcobalamin, methylcobalamin and hydroxocobala-min. Cyanocobalamin, by definition vitamin B_{12}, is the industrially produced stable cobalamin form which is not found in nature.

Vitamin B_{12} is obtained exclusively by fermentation process. It is produced by a number of pharmaceutical companies to meet annual demands worldwide. Merck began production of vitamin B_{12} by *Pseudomonas denitrificans* in 1952 and have improved the efficiency of culture more than 30-fold relative to the performance of the original soil isolates by genetic manipulations and microbial screening. At first, vitamin B_{12} for human therapy and as a food or feed supplement was obtained as a byproduct of *Streptomyces* antibiotic (neomycin, chlortetracycline) fermentation. Good strains were also isolated from manure and sewage sludge. Mutagenic treatments have resulted in improved activity, but in all cases cobalt ions and 5,6-dimethylbenzimidazole (5,6-DMBI) have to be added in addition to the precursors such as glycine, threonine, and aminopropanol. During the past two to three decades, several microorganisms have been employed for the efficient production of vitamin B_{12}. The list of various microorganisms producing vitamin B_{12} and the respective yields are shown in Table 1.

Table1. Species of microbial producers and microbiological processes recommended for the production of vitamin B_{12}

Species of microorganism	Main component of culture medium	Conditions of fermentation	Vitamin B_{12} production/(mg/L)
Propionibacterium freudenreichii	Glucose	Anaerobiosis, 5,6-dimethyl benzimidazole	206.0
Rhodopseudomonas protamicus	Glucose	5,6-dimethyl benzimidazole	135.0
Propionibacterium shermanii	Glucose	5,6-dimethyl benzimidazole	60.0
Pseudomonas denitrificans	Sucrose	Aerobiosis, betaine	60.0
Nocardia rugosa	Glucose	Aerobiosis	18.0
Rhizobium cobalaminogenum	Sucrose	Aerobiosis	16.5
Micromonospora sp.	Glucose	5,6-dimethyl benzimidazole	11.5
Streptomyces olivaceus	Glucose	5,6-dimethyl benzimidazole	6.0
Nocardia gardneri	Hexadecane	Aerobiosis	4.5
Butyribacterium methylotrophicum	Methanol	Anaerobiosis	3.6
Pseudomonas sp.	Methanol	5,6-dimethyl benzimidazole	3.2
Arthrobacter hyalinus	Isopropanol	5,6-dimethyl benzimidazole	1.1

Vitamin B_{12} biosynthesis is restricted to microorganisms. Most of the steps in the biosynthesis of vitamin B_{12} have been characterized in *Pseudomonas denitrificans, Salmonella typhimurium* and *Propionibacterium freudenreichii.* Some authors have reported about the requirement of more than 30 genes for the entire *de novo* biosynthesis of cobalamin, which amounts to about 1 % of a typical bacterial genome. Two different biosynthetic routes for vitamin B_{12} exist in nature:

• aerobic, or more precisely an oxygen-dependent pathway that is found in organisms like *P. denitrificans,* and

• anaerobic, oxygen-independent pathway investigated in organisms like *P. shermanii, Salmonella typhimurium* and *Bacillus megaterium*.

The major problem in vitamin B_{12} production using *Propionibacterium* is the growth inhibition of the cell due to the accumulation of inhibitory metabolites such as propionic acid and acetic acid.

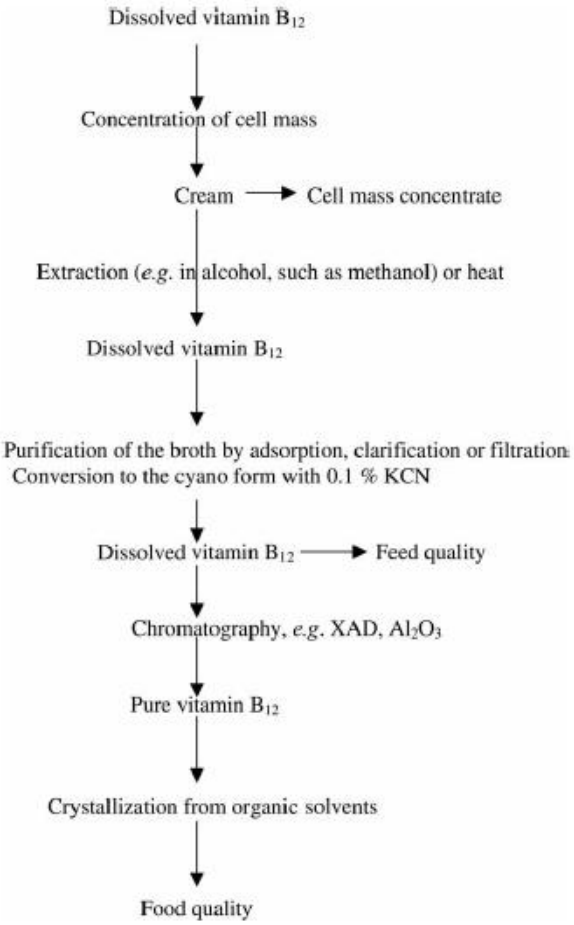

Fig. 1. Downstream processing of vitamin B_{12}

Recovery of vitamin B_{12}

The study of separation and purification processes of the fermentation products is most important for their commercial success. Recovery and purification of high value bioproducts from their crude sources involve various steps (extraction, membrane filtration, and sorption), which lead to low overall yields. Conventional chromatographic and chemical processes are capital and energy consuming due to a number of post- and pretreatment steps involved in a processing scheme. A rapid and selective mode of recovery of the target molecule from the crude feedstock can prove highly advantageous in improving the product yields and thus reducing the overall cost of downstream processing. Adsorptive separations are often used in the downstream processing using various interactions, *e.g.* ionic, hydrophobic, affinity, *etc*. for the recovery of biomolecules, namely antibiotics and vitamins.

The steps in the downstream processing for the recovery of vitamin B_{12} are summarized in Fig. 1. The biomass is separated by centrifugation to obtain a cell mass concentrate, which is then dried. Alternatively, the entire contents of the fermentor can be concentrated or spray dried. Cell lysis by heating the centrifuged cell mass in an aqueous solution, or by other methods to get corrinoids, can be used. Corrinoids are converted to vitamin B_{12} or cyanocobalamin by the addition of potassium cyanide, usually in the presence of sodium nitrite and heat. The vitamin solution is clarified subsequently by filtration, treatment with zinc chloride, and then precipitated out by the addition of tannic acid or cresol to give the product of 80 % purity, which is suitable for use as animal food additive. For greater purity, which is required for pharmaceutical use, the clarified solution is extracted with organic solvents, such as carbon tetrachloride, and then with water and butanol, followed again by organic solvents. In addition, adsorption processes such as on ion exchangers, aluminium oxide, or activated carbon can be used. Pure vitamin B_{12} can be obtained by crystallization after the addition of organic solvents, such as phenol and water.