International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 3061-3071 www.ijprajournal.com ISSN: 2249-7781

Vitamin B12: A potent biomolecule of microbial origin

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Submitted: 14-06-2023 Accepted: 24-06-2023

ABSTRACT
In this research investigation, we first give a historical overview of vitamin B12 research and provide an overview of the more types of microorganisms that are usually used to produce it industrially. Due to the difficulty of its chemical synthesis, vitamin B12 is a compound that is widely employed in the feed and food, healthcare, and medical industries. It can only be developed by fermentation. We propose optical spectroscopy, which incorporates High-performance liquid chromatography (HPLC), Capillary electrophoresis (CE), and Radioisotope and mass spectrometry as a viable method for understanding the nature of materials method for tracking and identifying vitamin B12. The generation of vitamin B12 from microorganisms must be maximized, requiring novel methods and technology. We present an in-depth discussion of developments in the microbial synthesis of vitamin B12 in this review.

Keywords: VitaminB12, Biosynthesis, Cyanocobalamin Production, Escherichia coli

INTRODUCTION

Vitamin B12, commonly known as cyanocobalamin, is a molecule of the cobalamin family that consists of a corrinoid ring with an upper and lower ligand. The top ligand can be adenosine, methyl, hydroxy, or a combination of these the cyano group [1]. Vitamin B12 is an important nutrient for humans and animals because it functions as a coenzyme in several mitochondrial and cytosolic processes (tricarboxylic acid cycle, one-carbon metabolism including methionine and folate cycles), as well as methylation-mediated pathways. Although crucial for gut microbiota, it also regulates sex steroids (metabolites, DNA, RNA, and proteins) as a result of host-microbe metabolic interactions. E. coli has evolved into a well-studied cell factory for the manufacture of different compounds such as terpenoids, non-natural alcohols, and poly-(lactate-glycolate) [8-10]. In addition to producing ALA through the C4 and C5 pathways, Escherichia coli have been employed as a microbial cell factory E. coli can also synthesize vitamin B12 through the salvage pathway [13, 14]. Vitamin B12 is related to molecules known as "cobalamins" that contain cobalt and have four upper ligands and a common bottom ligand called 5, 6-dimethyl benzimidazole (DMB) in the position. Methylcobalamin (MeCbl), 5'-deoxyadenosylcobalamin (AdoCbl), hydroxocobalamin (OHCbl), and cyanocobalamin (CNCbl) are the four forms of the -position (cyano, hydroxyl, methyl, or 5'-desoxyadenosyl radical), respectively [5]. The same team also established the structure of adenosylcobalamin (AdoCbl), one of the cobalamin's two active forms, in 1957 [5]. Due to his discoveries, Hodgkin received the Chemistry Nobel Prize in 1960. Methylcobalamin (MetCbl), another physiologically active vitamin B12 isoform, was found two years later. AdoCbl and MetCbl were discovered to function as cofactors in several enzymes, and throughout the following years, numerous enzymes dependent on MetCbl and AdoCbl were discovered and characterized. Some of them crystallized, including L-methyl malonyl-CoA mutase from Propionibacterium shermanii and methionine synthase from Escherichia coli [6, 7]. Meanwhile, the human gut bacteria Ruminococcus bromine, Clostridium spiroforme, and others have been demonstrated to require vitamin B12 for growth (auxotrophy), which has been experimentally confirmed and predicted by bioinformatics. Serratia fonticola, Serratia marcescens, Shigella flexneri, dysentery-causing Shigella sonnei, Equine enterococci, Escherichia coli Lactobacillus delbrueckii, Lactobacillus sakei Bacteroides ovatus, Bacteroides caccae, and Bacteroides the taiotaomicron [12].

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VITAMINB12 STRUCTURE

According to Figure 1, the corrin ring is made up of four pyrrole units (C4H5N), two of which are coupled directly, two of which are connected by a C-H link on one side, and two of which lack the methine bridge that connects the A and D subunits in the original structure. More recognized porphyrins, like hemoglobin.

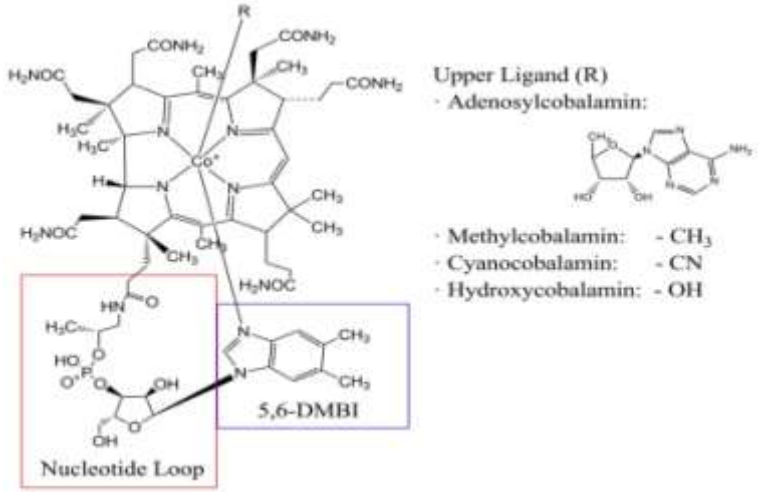

Figure 1. Shows the structure that all cobalamin isoforms share schematically. Also displayed are the primary groups that often serve as the top ligand. 5, 6-DMBI is the chemical name for benzimidazole.

The center Co ion is connected to two further ligands in addition to the four N atoms of the pyrrole units. The base 5, 6-dimethyl benzimidazole (DMBI), which is connected to the central Co ion through the N7-atom in a -axial configuration, is the lower ligand. DMBI is connected to one of the corrin ring's side chains; its phosphate group connects to an aminopropanol group that is connected to the propionic acid side chain of the pyrrole unit D [18].

Six ligands are connected to a central cobalt atom in the vitamin, which is unique in that four of them have been structurally reduced to create a corrin ring. The former together encircles it with direct nitrogen connections. An -axial 5, 6-dimethyl benzimidazole (DMB) ligand is located directly below the central cobalt and binds to the corrin ring through a phosphoribosyl moiety, giving the vitamin exceptional selectivity for intrinsic factor (IF) binding in the lower gastrointestinal tract [19].

Cobalamin biosynthetic pathway

1] De novo pathway

Depending on the timing of cobalt insertion and the conditions present, cobalamin can be synthesized de novo in prokaryotes via two different pathways. Need for molecular oxygen. The aerobic route includes these [19]. ALA is the first committed precursor in the route that produces tetrapyrroles. Either the C4 pathway or the C5 pathway can synthesize ALA. The enzyme ALA synthase catalyzes the production of ALA from glycine and succinyl-CoA in the C4 pathway. Through three enzymatic processes, ALA is produced from glutamate in the C5 pathway [20]. Precorrin-2 is where the anaerobic and aerobic routes split off, and coby (II) runic acid a, c-diamide is where they came together. During the de novo manufacture of cobalamin, eight peripheral methylation processes take place in the same temporal order and spatial arrangements in the anaerobic and aerobic pathways, respectively. There is a lot of sequence similarity among the methyltransferase enzymes engaged in these processes [23]. Adenosylation of cobyrinic acid a, c-diamide results in adenosyl cobyrinic acid a, c-diamide. Adenosylation of cob (I) syringic acid a, c-diamide by the enzyme additional corrinoids with amidated carboxyl groups at least in the a and c locations. To produce adenosyl cobyric acid, adenosyl cobyrinic acid is subjected to four stepwise amidation reactions at carboxyl groups at positions b, d, e, and g. It has become possible to connect (R)-1-amino-2-propanol or (R) - 1-amino-2-propanol phosphate at the f location of the molecule using two different techniques. Adenosyl cobyric acid's carboxyl group is involved in both aerobic and anaerobic processes. Before the binding of the lower axial ligand to the corrinoid ring in the anaerobic pathway, the linker between the two is the phosphorylated Ring of corrinoid.

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2] Salvage pathway

For bacteria and archaea, the salvage route offers an energy-efficient means of obtaining cobalamin. Exogenous corrinoids are carried into the cell by gram-negative bacteria using an ATP-binding cassette (ABC). BtuC, BtuD, and BtuF are components of the membrane permease, ATPase, and periplasmic-binding protein, respectively, transport system. Corrinoid is transported via BtuB, a TonB-dependent transporter in the outer membrane, to the periplasmic corrinoid binding protein BtuF. The latter then supplies corrinoid to the inner membrane's BtuCD complex [30]. In the salvage process, two further reactions add lower axial ligands to AdoCbi-GDP to create AdoCbl, much like in the de novo pathway. ABC transporters are also utilized by Archaea for corrinoid absorption. In a strain of Halo bacterium sp., archaeal orthologs of the bacterial BtuC, BtuD, and BtuF have been discovered NRC-1 [31]. After passing across the membrane, ATP: co (I) crinoid adenosyltransferases (ACATs) adenylate nicotinamide. There are three ACAT families: CobA, EutT, and PduO [32].

Cell Factories for Cobalamin Production

1] Escherichia coli

Due to its ability to grow for 24 h (as opposed to 180 h for the complete fermentation in P. denitrificans) and its ability to synthesize ALA, which plays a crucial role as a precursor in the formation of tetra pyrrolidine, salvager E. coli is another promising host for the production of vitamin B12 [24,104].

Later, 28 genes from six designed modules derived from Rhodopseudomonas palustris, B. melitensis, S. meliloti, S. Typhimurium, and R. capsulatus were expressed in E. coli strains. Recombinant CobU, CobT, CobS, and CobC, as well as the natural E. coli adenosyltransferase BtuR, work together to concurrently synthesize de novo vitamin B12 and build the nucleotide loop via the salvage pathway [105].

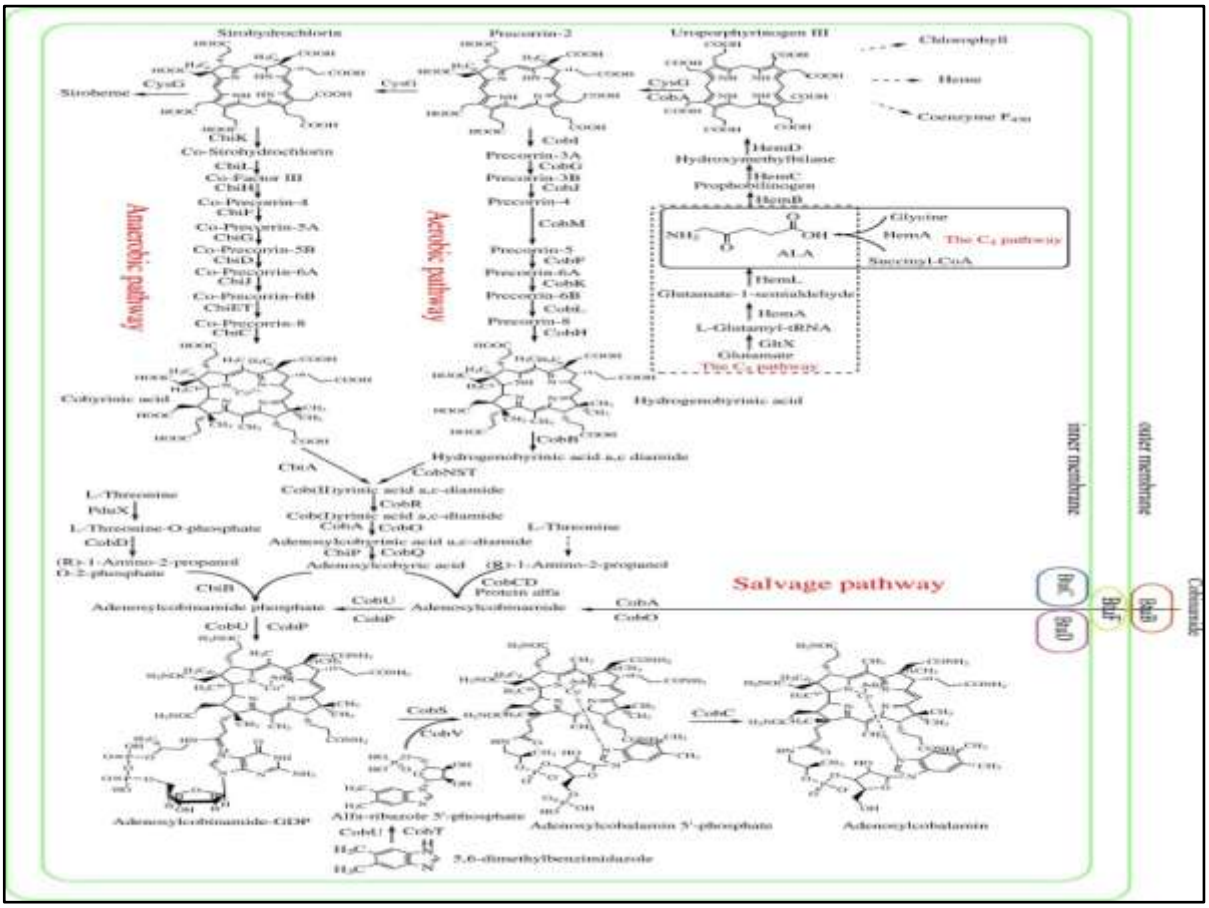

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Figure 2. The processes involved in tetrapyrrole molecules' production. The C4 or C5 pathway can both yield ALA. Adenosylcobalamin may be made by de novo or salvage procedures. S. Typhimurium or P. denitrificans, which utilizes either the aerobic pathway or the anaerobic pathway, respectively, is the source of the enzymes displayed in the adenosylcobalamin biosynthesis pathway.

2] Propionibacteria

The Propionibacterium species are frequently utilized in the industrial production of propionic acid, lactic acid, and vitamin B12 as well as dairy products (cheese) [25]. Studies on media optimization for Propionibacterium species revealed that specific carbon sources, the addition of amino acids, minerals, precursors, vitamins, and vitamers (chemical vitamins), and aeration of the mixture were all beneficial Production of Cbl is impacted by blue light, co-fermentation, and co-cultivation [95].

3] Pseudomonades

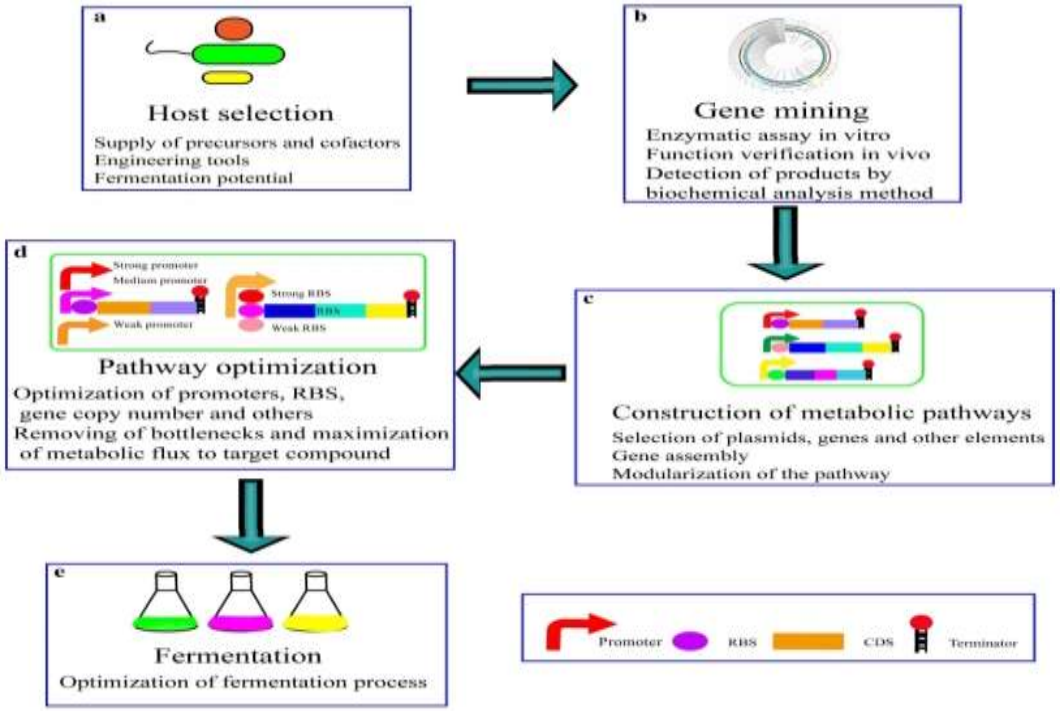
It is included that Pseudomonas spp may produce a variety of chemical compounds for commercial use, including vitamin B12. Pseudomonas spp can use a variety of environmental carbon and nitrogen sources; hence efforts to increase vitamin B12 production in their various species were made under constrained parameters [54].

4] Bacillus megaterium

It was successfully discovered that the B. megaterium DSM319 strain can grow on cheap carbon sources, such as raw glycerol from the manufacturing of biodiesel, making it an excellent production host. Aside from that for the parent strain, the cobalt bioavailability allowed for a rise in B12 production from 2 to 13 g/L, and bypassing the natural B12 synthesis regulating system by cloning resulted in even greater yields, over 220 g/L [54,102].

Biosynthetic pathway for the vitamin B12 production

Synthetic biology is a powerful technology that may be used to recreate genetic or biochemical networks to manufacture substances in a heterologous host. The development of a biosynthetic route for vitamin B12 in a heterologous host includes picking an appropriate host, constructing the biosynthetic route with useful components, and pathway fine-tuning.



When choosing the right host, a few things should be taken into consideration.

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Figure 3:(1) Precursors should be able to be supplied by the host (e.g., for the synthesis of the desired chemical, ALA), and cofactors (such as S-adenosylmethionine) are required. For instance, substantial ALA synthesis has been observed for the heterologous C4 route in E. coli [13]. (2) There must be enough genetic engineering tools, including chromosomal gene expression vectors, transformation methods, and Systems that knock out or integrate to control the host [53]. A heterologous biosynthetic pathway's design. (A) The capacity of the precursor and cofactor supply is taken into consideration when choosing a host for the heterologous biosynthetic pathway. Tools for genetic engineering and the capacity for large-scale fermentation using inexpensive and easily accessible carbon sources. (B) Enzyme activity is initially confirmed in vitro and then in vivo. Products of intracellular reactions or in vitro assays can be found by spectroscopic analysis, mass spectrometry, or microbiological tests. (C) Heterogeneous genes and other functional components are put together on plasmids using methods for gene assembly like DNA assembler, SLIC, CPEC, Gibson, Golden Gate, LCR, or integrated into the genome. The metabolic pathway is broken down into distinct modules to make construction less complicated. These modules are constructed after being sequentially confirmed in a heterologous host. (D) Based on the quantification of metabolites, metabolic bottlenecks should be eliminated and compound maximization should be the objective. Promoter, RBS, and gene copy numbers are developed and put into practice at the transcriptional or translational levels to optimize gene expression in the metabolic process.

Table 1. Strains for the fermentation of vitamin B12.

Strain and Pathway	Strategy and Tactic	Precursors	Product Yield Statistics	Reference
P. denitrificans SC510, salvage/aerobic	Optimization of promoters, RBSs, and terminators; random mutagenesis using UV light and chemicals (ethyleneimine and nitrosomethyluretane); overexpression of the cobF-cobM gene cluster; and cobA and cobE gene overexpression.	betaine, sucrose, and DMB	214 mg/L	[101]
S. meliloti MC5-2, aerobic/salvage	Random mutagenesis based on atmospheric and room-temperature plasma (ARTP); overexpression of hemE; deletion of cobI, and usage of a riboswitch based on butB element from Salmonella Typhimurium in front of a gaff reporter gene driven by the constitutive promoter PmelA	sucrose, DMB	156 mg/L	[23]
Pseudomonas sp. PCSIR-B-99, aerobic	Optimization of the fermentation process	methanol, DMB	3500 µg/L	[89]
S. olivaceus NRRL B-1125, aerobic	Optimization of the fermentation process	glucose, DMB	1–3.3 µg/mL	[90]
P. Sherman, anaerobic	Overexpression of biosynthetic genes	glucose, DMB	206 mg/L	[54]

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P. freudenreichii CICC 10019	Optimization of fermentation process	glucose, corn extract, DMB	58.8 mg/L	[95]
B. megaterium, anaerobic	Overexpression of hemACDBL, sirA, cbiXJCDETLFGA, cysGA, cbiY, but, glmS, metH, rtpR with the xylose inducible promoter; antisense RNA for hemE, hemZ, sirB. Bypassing of the B12 riboswitch	glucose; ALA; DMB	0.220 mg/L	[102]
B. megaterium, wild strain, anaerobic	Optimization of the fermentation process	glucose; ALA; DMB	204.46 µg/L	[103]
E. coli, salvage	The 22 native cob genes located in six operons from P. denitrificans ATCC 13867 were PCR-amplified and cloned in three compatible plasmids under the strong inducible T7 promoter	ALA	0.65 µg/g	[104]
E. coli, aero-BIC/anaerobic/salvage	Optimization of fermentation process; expression was conducted by assembling six modules comprising 28 genes from R. capsulatus, B. melitensis, S. meliloti, S. typhimurium, and R. palustris	ALA; glycine, succinic acid, betaine	307 µg/g	[105]

Table 2.

Strain/Pathway	Strategy/Tactic	Main Precursors	Product Yield	Reference
P. freudenreichii IFO12426	Optimization of fermentation process, overexpression of hem genes from R. sphaeroides	glucose; ALA	1.46 mg/L	[94]
Mesophilic methane bacteria from digested sludge	Optimization of fermentation process: enriching trace metal salts by an electrolysis process	H_2/CO_2 medium (biogas or coal gas)	185 mg/L	[106]
S. meliloti CGMCC 9638 aerobic/salvage	Optimization of the fermentation process (9–12 days)	sucrose, glycine betaine, corn liquor, DMB	at least 50 mg/L (Up to 180 mg/L)	[107]
(Food-grade) P. freudenreichii	Aqueous fermentation of cereal-based matrices	Cobalt, nicotinamide, riboflavin, and malted barley flour	712 µg/kg	[96]

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Hazardous freudenreichii	suppression of propionic acid feedback	cobalt, glucose, DMB, and corn steep liquor	59.5 mg/L (0.59 mg/L/h)	[97]
DSM 20271 P. freudenreichii	5.0 pH-controlled co-fermentation with L. brevis ATCC 14869	Water and wheat bran dough (15:85)	332 ± 44 ng/g dry weigth (3 days)	[100]

ESTIMATION OF VITAMINB12

1] High-performance liquid chromatography (HPLC)

By passing a mixture of the sample substance and one or more liquid solvents via a microporous column, HPLC is a chemical process that separates various chemicals present in a liquid sample (50). HPLC was originally used to isolate vitamin B12 in 1997 (51) and since then, other research has focused on simultaneously identifying multiple B-vitamin family members by HPLC (52–54).

2] Capillary electrophoresis (CE)

This method involves mixing the sample under study into a capillary of liquid electrolyte, then applying an electric field across the capillary to separate the ions in the sample (55, 56). The various cobalamin derivatives separate as they move through the capillary and are discovered at its end by the sample's ultraviolet light (266 nm) absorption, a procedure that takes 25 min and yields the recognizable chemicals in the sample (57).

3] Radioisotope and mass spectrometry

Detection based on radioactivity measurements is made possible by the use of a radioactive isotope of vitamin B12. The initial studies involve administering a radioactive vitamin B12 isotope to participants, typically in the form of (^{57}Co) vitamin B12 (58, 59). More recently, mass spectrometry (MS) and a version of vitamin B12 containing 14C have been utilized to gauge human absorption of the vitamin (68). Even though this restriction relates to identifying the altered form of cobalamin that necessitates an involved and expensive synthesis requires a sophisticated experimental setup, rather than the vitamin B12 found in nature) that culture broth is subjected to.

Vitamin B12 Downstream Processing and Post-Modification Strategies

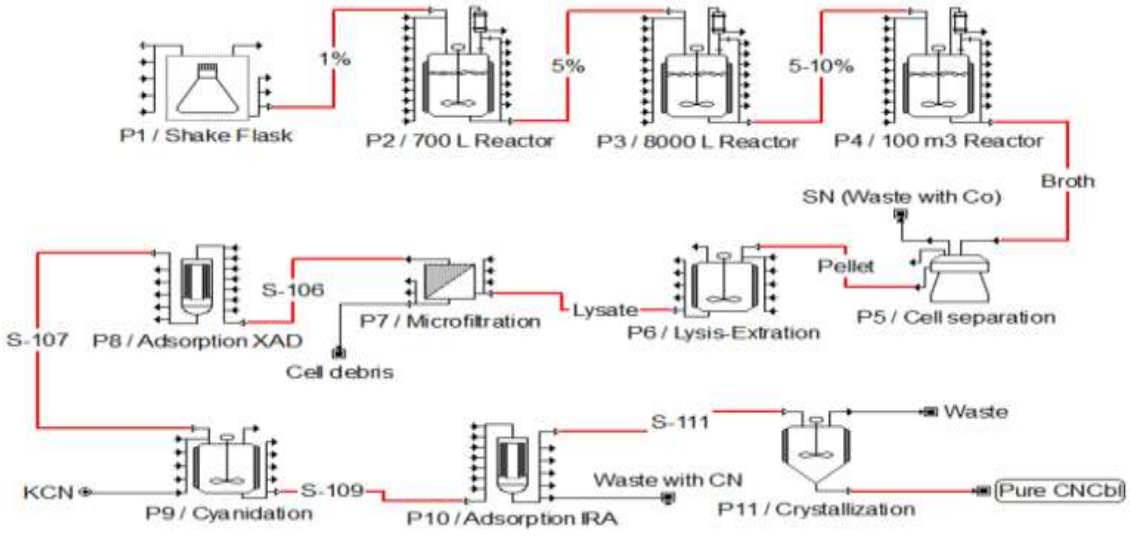

Figure 4: Cyanocobalamin can be obtained via a traditional bioprocess. The primary CNCbl stream has a red highlight. To make the depiction simpler, intermediate storage vessels have been left out. Processes 1 through Process 11 are denoted by P1 and P11, accordingly. SuperPro Designer® V9 Academic was used to depict the bioprocess. Intelligen, Inc. (Scotch Plains, New Jersey, USA), Site Edition.

To the best of the authors' knowledge, the industrial-scale recovery of vitamin B12 has followed the same well-described procedure for decades (see Figure 4 for a typical bioprocess scheme). Briefly, the yield and viability of the

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entire process are affected by the separation and purification phases (such as extraction, filtering, and adsorption In any case, all species of corrinoids are removed by heating for 10–30 minutes at a temperature of 80–120 °C and a pH of 6.5–8.5. After the initial filtration and adsorption processes, or during the extraction process, cyanidation might be done [74, 88]. In both instances, several corrinoids are when potassium cyanide or thiocyanate are added, and CNCbl is created. Sodium nitrite and heat are frequently used in this procedure [80].

The CNCbl solution is then cleared using one or more adsorption (XAD resin) and filtration (micro- and/or nano-) techniques. The vitamin solution is frequently treated with zinc chloride and copper chloride if the generated Cbl is intended for animal feed. To create the finished product, precipitated with organic solvents such as acetone [89]. To increase its bioavailability, vitamin B12 may go through several post-modifications before being utilized as a food supplement or oral medicine. Protecting these substances can be particularly interesting when there is an intrinsic non-function extremely low Cbl bioavailability factor [90, 91].

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