Recent Advances in Microbial Production of Vitamin B12: A Review of Optimization Strategies and High-Yielding Strains

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ABSTRACT:

Vitamin B12 is a crucial water-soluble vitamin involved in key metabolic processes, including serving as a cofactor for enzymes in DNA synthesis, promoting red blood cell formation, and supporting healthy nerve tissue and brain function. While plants cannot synthesize vitamin B12, it can be found in certain plants and mushrooms through microbial interaction. Additionally, vitamin B12 is abundant in animal tissues, making the meat and milk of ruminant's valuable dietary sources. Due to its essential role in human health and the growing demand, there has been significant interest in industrial-scale production of vitamin B12 using microbes. Commonly utilized microorganisms include *Sinorhizobium meliloti*, *Propionibacterium shermanii*, and *Pseudomonas denitrificans*. The production process often employs inexpensive, carbon-rich substrates derived from agro-industrial waste, providing a cost-effective solution for microbial cultivation. Both solid-state and submerged fermentation methods are optimized for vitamin B12 yield, with specific culture conditions tailored to different microbial strains. This review offers a comprehensive overview of vitamin B12, highlighting its natural sources, cost-effective substrates for microbial production, and the optimized culture conditions necessary for efficient purification and extraction. Future prospects in this field include enhancing production yields and exploring novel microbial strains and substrates to meet the increasing global demand for vitamin B12.

Keywords: Cobalamin production, Sources, Optimized conditions, Heterologous pathway development.

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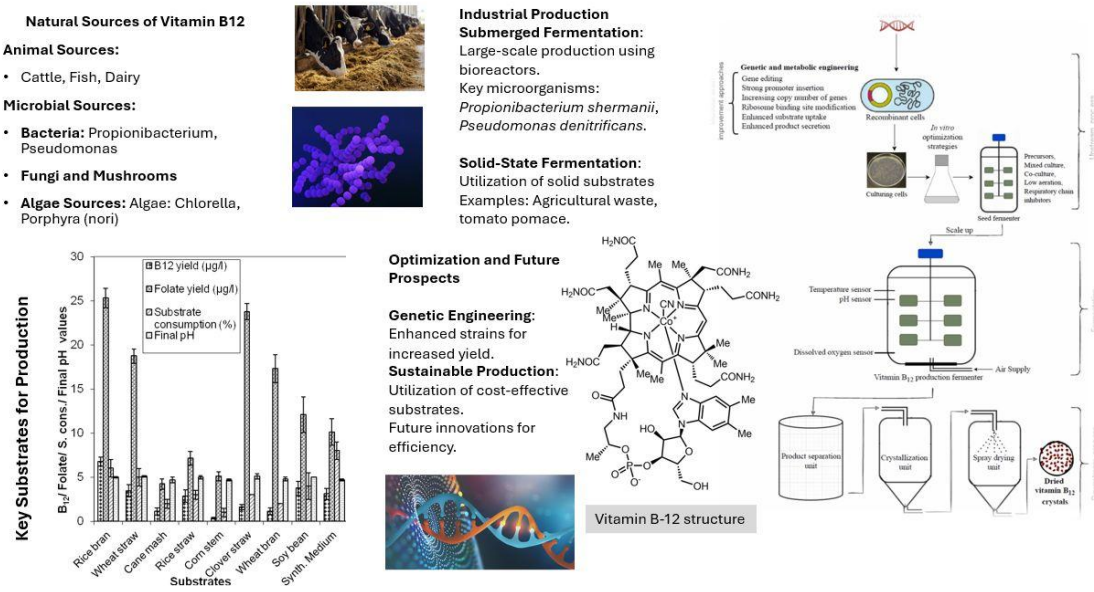

Graphical Abstract

Chapter 1: Introduction

Vitamin B12, defined as the anti-pernicious anemia factor, plays a crucial role in animal and human metabolism. Studies by two American physicians, Minot and Murphy, in the early 1920s, showed that a specific type of anemia known as pernicious anemia could be cured using liver extract or whole liver. The main component present in the liver, vitamin B12, has the potential to cure pernicious anemia. After ten years, in 1942, vitamin B12 was discovered using a specific technique known as X-ray crystallography. Additionally, its unique ligand, known as the corrin ligand, was revealed. Anaerobic microorganisms produce B12 coenzymes, which play a crucial role as cofactors in various important enzymatic reactions during their metabolism. Naturally, several types of microorganisms can act as sources of B12 derivatives and depend on these derivatives for their production, including cobalt and corrinoids.

Among vitamins, vitamin B12 has the most complex chemical structure. In humans, it acts as a cofactor for the enzymes methionine synthase (EC 2.1.1.13) and (R)-methylmalonyl-CoA mutase (EC 5.4.99.2). These enzymes are involved in the breakdown of important components, including fatty acids and amino acids. The methionine synthase enzyme also has an indirect association with DNA synthesis. Due to vitamin B12's major role in metabolism, its deficiency can lead to serious problems, including dementia, psychological issues, neurological problems,

megaloblastic anemia, and changes in mood and behavior. Prolonged vitamin B12 deficiency can adversely affect the heart, neurons and spinal cord. Humans obtain cobalamin through their diet as they cannot synthesize it. Three main reasons for cobalamin deficiency, reduced consumption of food of animal origin, infections caused by microorganisms in the gastrointestinal tract, such as Helicobacter pyloriand reduced absorption of micronutrients required by the body. Cobalamin demand has increased significantly in recent years across multiple industries, including food, beverages, dietary supplements and nutraceuticals due to rising health consciousness and the growing popularity of alternative diets, like vegetarian diets. Due to dietary restrictions, vegans and vegetarians are at increased risk of vitamin B12 deficiency (Kumar et al., 2023; Calvillo et al., 2022; Goryacheva and Kalinia, 2024).

Vitamin B12 production can be achieved at an industrial scale through microbial fermentation using different microbes, including *Sinorhizobium meliloti*, *Propionibacterium shermanii*, and *Pseudomonas denitrificans*. However, there are certain limitations to using these strains, including long fermentation cycles and the absence of methods for genetic engineering of these strains. Traditionally, scientists have used methods such as optimizing culture conditions and random mutagenesis using different chemicals or radiation, with very limited research on genetic engineering. Escherichia coli has been used as a suitable microorganism for the production of multiple vitamins, non-natural alcohols, minerals, terpenoids, poly-(lactate-co-glycolate), and many enzymes. Now, genetic engineers are focusing on E. coli as a microbial factory for the significant production of vitamin B12. Furthermore, novel techniques in metabolic engineering and synthetic biology have the significantly increased production of many of these compounds (Lee et al., 2008; Jarboe et al., 2010; Tripathi et al., 2024).

Sources:

Various archaea and bacteria can synthesize vitamin B12, but it has not been synthesized in plants. The synthesized vitamin B12 in microbes can be transferred and made available in many plants and mushrooms through microbial interaction. It is also present in animal tissues. Animal products are the traditional source of vitamin B12. For humans, the milk and meat of ruminants are vital sources of vitamin B12. Humans obtain vitamin B12 from cattle and sheep. In ruminant's stomach, certain types of symbiotic microbes are present that are involved in the formation of vitamin B12, which acts as a specific nutrient for them. Specific types of phytoplankton in the aquatic environment obtain vitamin B12 through symbiotic relationships

with different types of bacteria, which are then consumed by bivalves and fish. Edible mushrooms and various plants contain very low levels of vitamin B12, mainly due to bacteria in the soil and on other surfaces. In most cases, humans obtain vitamin B12 from ruminants, shellfish, and other fish (Abdel et al., 2024; Calinoiu et al., 2024).

Moreover, it has been reported that milk can also serve as a source of vitamin B12. Plasma tests related to B12 composition show higher levels in fish, meat, and milk, particularly in Western countries. It has also been reported that milk can increase serum B12 levels. Different types of meat, including lamb, mutton, veal, and beef, are obtained from ruminant's muscles. Other sources of meat, include older animals such as pigs and chickens. Bovine milk and common milk products, including fermented milk such as yogurt and cheese, are easily available and can be good sources of B12. Herbivores like cattle and sheep eat grasses that are free from B12. The ruminant's stomach is composed of four chambers, which include different types of beneficial microorganisms, some of which are involved in the formation of B12. After B12 is formed in the stomach, it is absorbed in the intestine and can be transferred to the blood or stored in muscles and the liver. Cobalt is a very important component of the diet of ruminants, involved in the synthesis of vitamin B12 and its deficiency can also cause vitamin B12 deficiency. Currently, many methods are being used to increase the B12 content in milk and meat. Omnivores, including pigs and chickens, consume both plants and animals and can be valuable sources of B12. However, the B12 content in meat from chickens and pigs is lower compared to that from ruminants. The production of meat in poultry can be induced using lactic acid bacteria. In the context of human consumption, chicken eggs do not significantly change serum B12 levels (Parisis et al., 2024; Wang et al., 2022; Sultana et al., 2023).

The dried fruiting bodies of certain mushrooms, such as oysters, parasol, black morels, and porcini mushrooms, contain trace amounts of B12 (about <0.1 μg/100 g dry weight). However, slightly higher levels of vitamin B12 have been found in the fruiting bodies of black trumpet (*Craterellus cornucopioides*) and golden chanterelle (*Cantharellus cibarius*) (1.09–2.65 μg/100 g dry weight). Significant variations in B12 content have been observed in dried and commercially available shiitake mushrooms (*Lentinula edodes*), with an average value of about 5.6 μg/100 g dry weight. It is believed that the vitamin B12 found in shiitake mushroom fruiting bodies does not come from a de novo biosynthetic pathway but from other sources, such as bacteria that synthesize vitamin B12 or those present in bed logs. Similarly, white button

mushrooms (Agaricus bisporus) have about 0.2 μg B12 per 100 g of dry weight, with the peel portion containing the highest content. Vitamin B12 has also been found in compost at similar levels. Such studies suggest that Agaricus bisporus can absorb B12 from compost or from bacteria capable of synthesizing B12. Truffles (Tuber species), which mostly grow underground, form an intimate mycorrhizal relationship with the roots of particular host trees. Truffle fruiting bodies have a higher B12 content (approximately 11.5 μg/100 g dry weight) compared to other foodstuffs. However, there is no information about the physiological function of B12 in these mushrooms.

Porphyra sp., a type of red algae, is a marketable marine culture known for its use as seafood. Various species of Porphyra contain significant quantities of B12 (approximately 77.6 μg/100 g dry weight) and are mostly used as dried nori sheet products. Chinese dried nori (zicai), New Zealand dried nori (karengo), Korean dried nori (kim), and Welsh dried nori (laverbread) are reported to contain approximately 60.2, 28.5, 66.8, or 2.8 μg B12, respectively, per 100 g of weight. Genomic analysis of Porphyra umbilicalis has provided evolutionary insights and suggested the physiological function of B12 in red algae. Studies of natural plants with high B12 content show that nori is currently the most appropriate source of vitamin B12 for vegans. In rats depleted of B12, B12 is significantly absorbed from dried nori and remains functional (Calinoiu et al., 2024).

One of the microalgae, Chlorella, is produced in large quantities and widely sold as a nutraceutical crop and supplementary food in China, Taiwan, the United States, Indonesia, and Japan. Chlorella vulgaris biomass contains sustainable nutrients, including proteins, carbohydrates, lipids, minerals, and vitamins. The biologically active form of vitamin B12 (methylcobalamin), which is rare among foods, has been identified in the biomass of C. vulgaris. Green algae of the Chlorella species, used as supplements to human food, contain bioactive B12. Recent analyses of dried Chlorella health supplements have shown B12 content varying from <0.1 μg to about 415 μg in large vessels of culture (closed cultivation conditions) and in other types of Chlorella grown in open cultivation tanks. Chlorella pyrenoidosa showed much higher B12 content in open culture than Chlorella vulgaris. It has been reported that Chlorella cells have an exogenous uptake system for B12, meaning that the B12 compounds derived from Chlorella cells are mainly from B12-synthesizing bacteria present under culture conditions or organic B12 ingredients or crystalline B12.

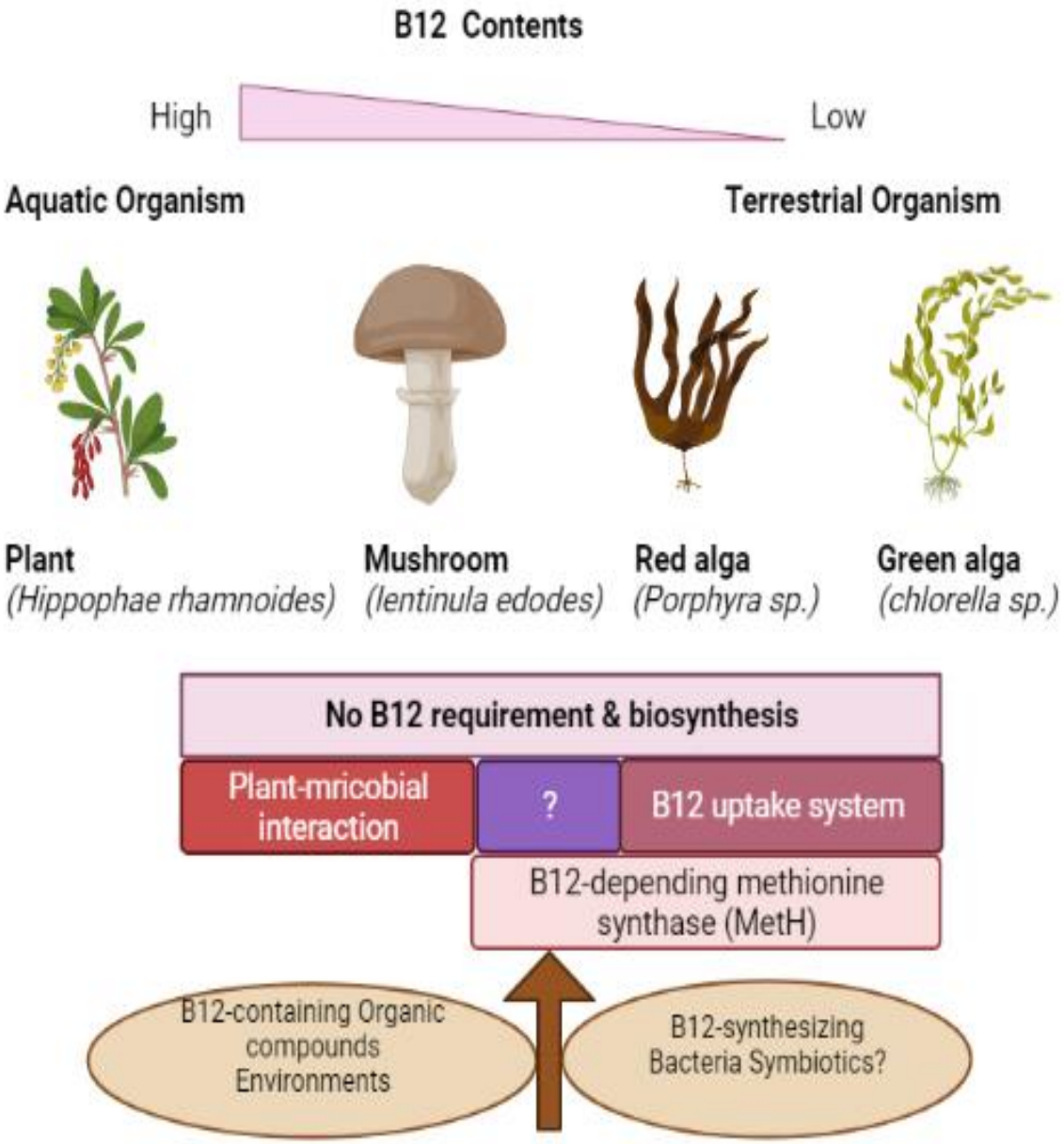

Figure 1. Microbial interactions in edible plants, algae, and mushrooms and Sources of Vitamin B12 sources (Watanabe and Bito, 2018)

Vitamin B12 is produced on a large industrial scale through microbial fermentation, mainly using *Sinorhizobium meliloti, Propionibacterium shermanii,* or *Pseudomonas denitrificans* (Martens et al., 2002). These strains, however have limitations, such as an inadequate genetic engineering system, complex and expensive media requirements, and long

fermentation cycles. Most research by the production companies has so far concentrated on classical strategies, such as optimization of fermentation processes and random mutagenesis, and the very limited role of metabolic engineering. In recent eras, scientists have emphasized *Escherichia coli* as a microbial factory for the production of vitamin B12. It is a well-studied cell and can broadly be used for the production of various chemical substances such as poly- (lactate-glycolate), non-natural alcohols, and terpenoids (Martin et al., 2003; Choi et al., 2016; Tripathi et al., 2024).

Chapter 2: Development of a heterologous biological pathway for the production of vitamin B12

The construction of biosynthetic heterologous pathways in model organisms not only involves altering indigenous microbial hosts but also identifying novel microbial hosts to produce cobalamin. Synthetic biology is a useful tool for reconstructing a heterologous host's pathways or genetic networks to produce compounds.

(Fig. 2) illustrates the development of the vitamin B12 biosynthesis pathway in a heterologous host, including the selection of a suitable host and the construction of the biosynthetic pathway with functional components. When selecting the ideal host, several points should be considered: (1) The host should be able to supply the desired chemical cofactors (e.g., S-adenosyl methionine) and precursors (ALA) for production. For example, in E. coli, ALA production is high with a heterologous C4 pathway, avoiding the need for exogenous ALA addition (Zhang et al., 2013). (2) Several genetic engineering approaches, including transformation protocols, expression vectors, and chromosome/gene knockout/integration systems, can be used to manipulate the host (Lee et al., 2009). (3) The host should be able to utilize readily available and inexpensive carbon sources, such as xylose, glucose, and arabinose, in industrial fermentation. Multiple candidate genes from several native producers of vitamin B12 can be expressed in the host if it is appropriately selected. The application of in vitro enzymatic analysis is an effective method for identifying optimal enzymes. Since the intermediate pathway for synthetic vitamin B12 is often absent, desired chemicals must be isolated or enzymatically prepared to create substrates for in vitro enzymatic analysis. Spectroscopic analysis, mass spectrometry, and microbiological testing can then be used to detect the products of an in vitro trial (Parisis et al., 2024; Calinoiu et al., 2024).

Sometimes, the production of heterologous enzymes does not work, and new enzymes from different sources must be screened. All forms of indigenous regulation, such as riboswitches, should be removed for heterologous expression studies. The valuable genes for cobalamin production can be expressed polycistronically or monocistronically on plasmids, after adding elements responsible for transcription and translation, including ribosome binding sequences, promoters, and terminators. Several tools in synthetic biology facilitate the construction of heterologous pathways. Various methods, such as Gibson Assembly, SLIC, Golden Gate Cloning, CPEC, LCR, and DNA Assembly, are capable of assembling heterologous genes (Kok et al., 2014).

Creating a metabolic pathway can be challenging when transferring excessively heterogeneous genes to a heterologous host. The metabolic pathway can be divided into several modules, which can be assembled after validating their sequence and function. The final structure can then be transferred to the selected host for heterologous expression, enabling the host to synthesize cobalamin. The engineered strains are then cultivated under optimal conditions to assess their capacity for vitamin B12 production.

(Fig. 2) outlines the design for developing a biosynthetic heterologous pathway: (a) Genetic engineering tools and low-cost, readily available carbon sources are selected to supply precursors and cofactors required for the heterologous biosynthesis pathway in the host. (b) Enzyme activity is checked both in vitro and in vivo. Multiple assays, including mass spectrometry, microbiological assays, and spectroscopic analyses, can be conducted to determine in vitro tests or intracellular reaction products. (c) In plasmids, methods such as LCR, CPEC, Gibson, SLIC, DNA assembler, and Golden Gate are used to assemble integrated heterologous genes and other functional elements. The pathway is divided into separate modules to simplify metabolic pathway construction. These modules are sequentially checked and then assembled in a heterologous host. (d) To maximize the target compound, metabolic flux, and bottlenecks should be addressed based on metabolite quantification. Promoters, RBS, and gene copy numbers are designed and implemented at the transcriptional or translational levels to optimize gene expression in the metabolic pathway. (e) The properties of engineered strains are verified through fermentation. Various substrates and conditions (e.g., ALA, cobalt ions, betaine, and DMB) can be optimized to increase yield and productivity, including adjusting factors like dissolved oxygen concentration, pH, and temperature.

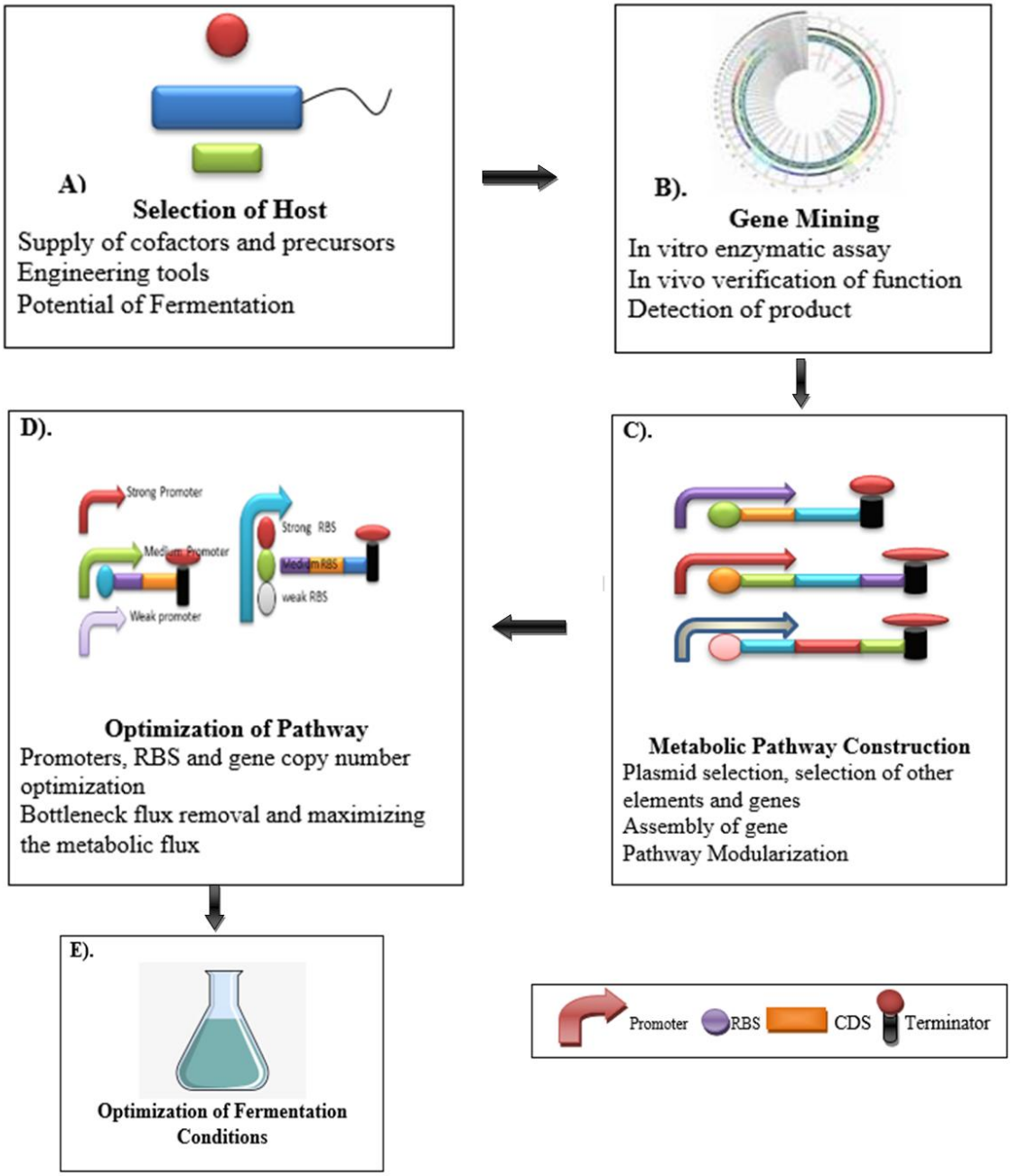

Figure 2: The design of a biosynthetic heterologous pathway modified from (Fang et al., 2017)

Substrate for Production of Vitamin B12

It is possible to reduce costs by using relatively cheaper raw materials, including agricultural, industrial, and food waste, such as frying oils, molasses, and high-starch waste. In the future, these residues can be used as substrates for large-scale production of biomaterials, such as vitamins. Vitamin B12 can be produced from various carbon sources, such as tomato pomace, sucrose, household sugar, molasses, glycerol, whey, and kefir, using *Propionibacteria*. Molasses has proven to be one of the cheap and effective substrates for producing various compounds. Another inexpensive substrate could be waste frying oil (WFO). The biosynthesis of vitamin B12 using sunflower oil (WFO) produced by frying shows promising results. Large volumes of frying oils are employed at domestic and industrial levels in food production. Frying oil in use typically contains over 30% polar compounds, which can pose serious environmental hazards if disposed of in aquatic environments without effective treatment. A hydrophobic layer can form on the aqueous phase of different effluent streams, partly preventing air penetration by grey ingredients of vegetable oil waste. Such conditions are not conducive for living organisms, so this waste should be used reasonably, such as for vitamin B12 production (Hajfarajollah et al., 2015; Calinoiu et al., 2024).

Using agroindustrial waste as a substrate in the production of vitamin B12 with microbes is another area of interest. These substrates are available in large quantities, have low value and cost, but are rich in nutrients for microbial growth (carbohydrates, proteins, and minerals). Liquid acid soy protein (LAPRS), obtained as a byproduct from the soybean-isolated protein (SIP) process, is a particularly significant residue. For example, a single industrial plant generates about 50,000 m^3 of LAPRS each month to produce SIP. LAPRS mainly consists of carbon and protein and has a very high biochemical oxygen demand (>20,000 mg O_2 L^{-1}). This means it cannot be disposed of directly into the environment, requiring costly and undesirable effluent treatment. However, LAPRS has been reported as an efficient growth medium for producing probiotic bacteria and their main product, lactic acid, and has also been recently used in vitamin B12 production (Assis et al., 2020).

The tomato (*Lycopersicon lycopersicum*) is native to the Andes in South America, a mountainous region where it has been used for food long before Europeans arrived. During the 16th century, it gradually spread and is now one of the most popular garden plants. Tomatoes are warm-season plants and require outdoor conditions, as most varieties do not fruit below 14°C or

above 29°C. Direct sunlight is desirable for at least six hours a day. In Jordan, extensive areas are dedicated to cultivating fresh salad ingredients, and about 100,000 tons of pizza pastes and similar retail products are processed each year. The annual waste product of this industry is a lignocellulosic material that is mostly dumped in landfills. Small quantities of this waste are dried and fed to animals, but economic pressures favor exploiting it more profitably. One possible approach involves hydrolyzing these saccharides into food-grade products or animal feed. Using tomato pomace as a synthetic substrate for B-group vitamins, such as vitamin B12 (cyanocobalamin), is highly recommended (Haddadin et al., 2001).

Refined sucrose served as the exclusive carbon source, resulting in high costs for the entire industrial fermentation medium (Hugenschmidt et al., 2010). Alternatively, a low-cost fermentation medium using beet molasses as the main substrate was developed, significantly reducing fermentation costs due to the use of beet molasses (a sugar byproduct) containing favorable ingredients for vitamin B12 production, such as sucrose, glutamate, and betaine (Xia et al., 2015).

Vitamin B12 Production by Fermentation

For several centuries, solid-state fermentation (SSF) and submerged fermentation (SMF) have been used to produce well-controlled biological agents using moist solid raw materials, such as cornstalks, cotton stalks, and sugarcane bagasse, as the main technology in agricultural waste. This is an alternative way to grow fluid nutrient medium microorganisms (Atta et al., 2008).

Submerged Fermentation:

Free-flowing substrates like molasses and broth are used in submerged fermentation. The fermentation broth is supplemented with bioactive chemicals. The substrate is used up quickly, so replacements are needed regularly. Microorganisms thrive best with this fermentation technique like bacteria that require high humidity. Additionally, this process is advantageous because it facilitates the easy cleaning of products. Submerged fermentation is particularly used for producing the "secondary metabolites" needed in fluid form (Dasari et al., 2019). (Table 1 shows several microbial species that produce substantial amounts of vitamin B12 using submerged fermentation).

Solid-State Fermentation:

Solid-state fermentation uses solid waste, such as paper, bagasse, or bran, as the substrate. The key advantage of using these substrates is that they are nutrient-rich waste materials, providing a

rich source of carbon and other organic compounds and being recyclable. The substrate is used slowly and consistently during fermentation, allowing long-term use (Haddadin et al., 2001).

Table 1 represents different substrates used, mode of fermentation, and yield of Vitamin B12 by using different substrates

Sources	Substrate	Fermentation mode	Vitamin yielding unit	References
Propionibacterium freudenreichii	liquid acid protein residue of soybean	Submerged fermentation	(0.6 mg/g cells)	(Assis et al., 2020)
Propionibacterium shermanii	Tomato pomace	Submerged fermentation	11.1 mg l^{-1}	(Haddadin et al., 2001)
Pseudomonas denitrificans	maltose syrup and corn steep liquor	Submerged fermentation	198.27 ± 4.60 mg/l	(Xia et al., 2015)
*Propionibacterium freudenreichii sp No.*PTCC 1674	waste frying sunflower oil	Submerged fermentation	2.60 mg/L	(Hajfarajollah et al., 2015)
Chlorella vulgaris	fermented milk	Submerged fermentation	154.9 ± 1.14 µg / 100 g	(Jalilian et al., 2019)
Bacillus firmus AZ-78B	Agriculture waste + basal media	Solid substrate fermentation	37.7µg/ml	(Atta et al., 2008)
Streptomyces halstedii, AZ- 8A	Agriculture waste + basal media	Solid substrate fermentation	37.7µg/ml	(Atta et al., 2008)
Pseudomonas denitrificans	Molasses	Submerged fermentation	181.75mgL–1	(Li et al., 2013)

Factors Affecting Vitamin B12 Production

There are following factors that may affect the production of vitamin B12.

pH:

The pH has a profound impact on microbial growth and product formation as it has a major effect on ion stability, nutrients, substrate solubilization, and use by microorganisms (Bertoldo and Antranikian, 2002). In most studies, the optimum pH is (6.5 to 7) and can be

adjusted with various buffers to produce vitamin B12 (Haddadin et al., 2001; Jalilian et al., 2019).

Temperature:

Temperature significantly affects vitamin B12 production as it influences the growth conditions of different microbes. The optimal temperature range for producing certain bioactive compounds, including vitamins, generally falls between 30 to 35°C, according to several studies (Hajfarajollah et al., 2015; Jalilian et al., 2019; Atta et al., 2008).

Carbon Source:

Vitamin B12 can be synthesized from various carbon sources, including tomato pomace, sucrose, household sugar, molasses, glycerol, whey, and kefir. Molasses, in particular, is one of the most cost-effective substrates for producing a range of compounds (Hajfarajollah et al., 2015). These substrates, along with agricultural waste and byproducts from the sugar industry, can serve as carbon sources, yielding valuable quantities of vitamin B12. Production of vitamin B12 using maltose as a carbon source was lower compared to sucrose. Although the differences were not statistically significant, sucrose was identified as the optimal carbon source for *P. denitrificans* fermentation for vitamin B12 production (Li et al., 2013).

Minerals:

Adequate amounts of cobalt, copper, iron, manganese, molybdenum, and zinc are typically present in water supplies and other media components. For instance, steep maize contains a variety of minerals that usually meet the minor and mineral requirements. Cobalt, in particular, plays a crucial role in the production of vitamin B12, as it significantly increases the concentration of this vitamin (Assis et al., 2020).

Inoculum Size:

Inoculum size can also affect vitamin B12 production. A study by (Li et al., 2013) used an inoculum size of 4% (v/v). Most studies suggest that using 10% of the total volume as inoculum yields effective results (Assis et al., 2020).

Table 2. Culture conditions for the production of vitamin B12 from different microbial strains

Sr.No	Sources	Optimum Ph	Optimum Temperature (°C)	References
1.	*Propionibacterium freudenreichii*	6.5	30	(Assis et al., 2020)
2.	*Propionibacterium shermanii*	6.8	30	(Haddadin et al., 2001)
3.	*Pseudomonas denitrificans*	6.7	30	(Xia et al., 2015)
4.	*Propionibacterium freudenreichii sp No.*PTCC 1674	7	30	(Hajfarajollah et al., 2015)
5.	*Chlorella vulgaris*	6.8	26 ± 2	(Jalilian et al., 2019)
6.	*Bacillus firmus* AZ-78B	5.5	35	(Atta et al., 2008)
7.	*Streptomyces halstedii,* AZ- 8A	5.5	37	(Atta et al., 2008)
8.	*Pseudomonas denitrificans*	7.2	32	(Li et al., 2013)

Agitation:

Proper mixing of the media is essential to ensure that nutrients are evenly distributed to all microbes. The agitation rate depends on the type of substrate being used. A common agitation

rate is 200 rpm, although this can vary based on specific requirements, as it helps facilitate the supply of nutrients to the microbes (Li et al., 2013).

Incubation Period:

Different microbes require varying incubation periods to achieve maximum growth. For instance, *Pseudomonas denitrificans* typically requires about 6-7 days to produce a substantial amount of vitamin B12 (Li et al., 2013).

Extraction and Purification:

To extract vitamin B12, the freeze-dried biomass of *C. vulgaris* is suspended in a specific amount of deionized water. A green extraction process involves autoclaving the suspension at 121°C for 10 minutes. After autoclaving, the suspension is cooled and centrifuged at 6000 rpm for 10 minutes, and the vitamin B12 is found in the supernatant. The pH of the supernatant is adjusted to 6.0 for purification.

The purification process involves two stages. First, the samples are passed through Amberlite XAD-2 and a Sep-Pak cartridge. A prepared column of Amberlite XAD-2, filled to a height of 15–16 cm with a methanol slurry, is used. After most of the methanol is drained, the column is equilibrated with deionized water. Vitamin B12 extracts are slowly loaded onto the column, drained over approximately 3 hours for increased purification efficiency, and eluted with 80% methanol.

For further purification, the samples are passed through a Sep-Pak cartridge, which is washed with 75% ethanol before use and equilibrated with deionized water. Cleaned samples are loaded onto the cartridge with 25% ethanol. The purified vitamin B12 is then concentrated under reduced pressure. The concentration and purity of the vitamin B12 are confirmed using HPLC and biological methods (Jalilian et al., 2019).

CONCLUSION:

The discussion demonstrates that optimized culture conditions and cost-effective substrates, such as agricultural waste, molasses, waste frying oil, and tomato pomace, can be effectively utilized for the microbial fermentation of Vitamin B12. By leveraging these low-cost and readily available resources, substantial quantities of Vitamin B12 can be produced, making the process economically viable for large-scale industrial production. The significance of this lies not only in meeting the high market demand for Vitamin B12 but also in harnessing its numerous

medicinal properties. This approach offers a sustainable and efficient pathway to address global needs for this essential nutrient.

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