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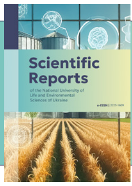

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# *Chlorella vulgaris* microalgae as a potential source of vitamins $B_{12}$ and $D_2$

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**Abstract.** The research relevance is determined by the need to summarise current knowledge on the mechanisms of vitamin $B_{12}$ and $D_2$ accumulation in *Chlorella vulgaris* and the factors determining their biochemical variability in microalgae cultures. The study aimed to systematise literature data for 2020-2025 and identify key factors regulating sterol metabolism and vitamin profile in *Chlorella vulgaris*. The study was based on targeted information search, content analysis, critical comparison, and qualitative synthesis of previous scientific works. Based on the analysis of sources, the study demonstrated that the type of nutrient medium determined the level of micronutrients in the biomass of *Chlorella vulgaris*. Mineral media provided stable growth but did not contain cobalamin (vitamin $B_{12}$), while organic substrates – liver extract, whey, permeates, and anaerobic digestates demonstrated a higher potential for cell enrichment due to natural coenzymes, proteins and trace elements. Cobalt ions ($Co^{2+}$) were shown to promote greater accumulation of cobalamin in biomass, while magnesium ($Mg^{2+}$), zinc ($Zn^{2+}$), iron ($Fe^{2+}/Fe^{3+}$) and copper ($Cu^{2+}$) were substantial cofactors for enzymes and transport systems that influenced sterogenesis. Regarding vitamin $D_2$ (ergocalciferol), the literature confirmed the decisive role of ultraviolet radiation in the B range (UV-B), which initiated the photoconversion of ergosterol. The intensity and spectral composition of light determined the rate of sterol accumulation, while salinity, nitrogen or phosphorus deficiency, and other stress factors contributed to increased lipidation and changes in the sterol pool. The study determined that in the stationary phase of the culture, lipid and sterol fractions accumulated more

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intensively than during the period of active cell division. A generalised analysis of sources showed that the most effective enrichment of *Chlorella vulgaris* with vitamins $B_{12}$ and $D_2$ is achieved by combining organic nutrient media, an optimised lighting spectrum with a UV-B component, and a controlled mineral composition of the medium

**Keywords:** : cobalamin; ergocalciferol; organic extracts; light spectrum; ergosterol; metal ions; stress factors

## Introduction

Vitamin $B_{12}$ ensures the synthesis of deoxyribonucleic acid (DNA), normal functioning of the nervous system and blood formation processes, while vitamin $D_2$ participates in the regulation of calcium metabolism, maintains bone tissue health and performs an immunomodulatory function. Available epidemiological data indicate the prevalence of deficiencies in these micronutrients among various population groups, creating a need for affordable and plant-based sources of vitamins (Peshuk *et al.*, 2024). Regarding vitamin $D_2$, a key limitation is that its formation in microalgae depends on the availability of sterol precursors and photochemical conditions, which vary significantly in natural environments. Thus, the determination of the potential of *Chlorella* as a source of both vitamins is a relevant scientific task that combines the issues of food security and optimisation of biotechnological production.

To outline the nutritional potential of microalgae, O.V. Kozhemiaka & L.V. Peshuk (2023) described *Chlorella* as a valuable component of health and functional products. The study emphasised its pronounced biological activity and potential for the creation of food supplements. To summarise biotechnological approaches to the intensive cultivation of *Chlorella*, V. Grishko *et al.* (2024) systematised cultivation regimes, performance parameters and areas of practical application. Technological factors that determine the quality of biomass in industrial and laboratory conditions were outlined. To further analyse the vitamin composition of microalgae, J.A. Coronado-Reyes & J.C. González-Hernández (2023) summarised the sources of micronutrients in different types of algae. The study highlighted the key features of vitamin profile formation and the importance of microalgae as alternative nutrient resources. In the direction of studying the interaction of microalgae with microbial communities, M. Ribeiro *et al.* (2023) investigated lactobacilli from *Chlorella* photobioreactors as potential producers of vitamin $B_{12}$. The study established the ability of certain strains to synthesise cobalamin and outlined the prospects for their use in food technology.

Emphasising bioactive properties, R.A. Hamouda *et al.* (2022) evaluated the antioxidant and antitumour effects of *Chlorella vulgaris* extracts. The study demonstrated significant activity of the biomass in a series of biochemical tests, confirming its functional potential. In the context of the need to control nutrient stability, M. Islam (2024) analysed the consistency of vitamin $B_{12}$ content in commercial microalgae powders. Differences between product batches were recorded, and their possible impact on the nutritional value of the products was assessed. Within the framework of analysing the interaction of microalgae with soil microbial systems, C. Liu *et al.* (2025) showed that a synthetic microalgal community stimulated pepper growth on mineral substrates. The study also demonstrated an enrichment of root endophytic bacteria and fungi, which accompanied an increase in plant biomass. In the context of studying bioremediation processes, D. Zhao *et al.* (2025) established the effectiveness of a consortium of microalgae and bacteria in removing $Cu^{2+}$ ions from water systems. In addition, the physiological reactions of the co-culture were described, and an Artificial Neural Network (ANN) model for predicting detoxification efficiency was constructed.

Concerning the role of mineral nutrition, M. Peng *et al*. (2025) determined changes in the growth of *Corethron sp*. under the influence of nitrogen, phosphorus, iron, and silicon. A simultaneous increase in lipid content and transformation of the fatty acid profile depending on the combinations of nutrients was demonstrated. Emphasising light regulation of metabolism, S. Maulana *et al*. (2025) evaluated the responses of *Navicula sp*. to red and blue light. The results demonstrated changes in lipid, protein, carbohydrate, and pigment composition depending on the spectral mode. Within the framework of bioinformatic generalisation, A. Voshall *et al*. (2021) compared sterol pathways in green algae. Key enzymatic steps separating ergosterol and phytosterol biosynthesis pathways were identified. In the spectrum of functional applications, A.R. Mendes *et al*. (2024) systematised data on the chemical components of *Chlorella vulgaris*. A wide range of bioactive effects were noted, determining its potential for food, medical and feed technologies. Therefore, a comprehensive analysis of the presented research suggests that varying the composition of the nutrient medium (organic extracts, metal ions, fixed C/N ratio) with controlled lighting parameters (including ultraviolet light) may be an effective strategy for increasing the nutritional value of *Chlorella vulgaris* biomass.

The study aimed to analyse current scientific approaches to enriching *Chlorella vulgaris* biomass with vitamins $B_{12}$ and $D_2$ based on the determination of various factors (lighting, nutrient medium composition) that influence the synthesis and accumulation of these metabolites. The objectives of the study were to determine the conditions that influence the accumulation of vitamins $B_{12}$ and $D_2$ in *Chlorella vulgaris*, to characterise changes in the sterol profile under different light and nutrient regimes, and to evaluate the role of organic substrates in modifying the biochemical parameters of the culture.

## Materials and Methods

The study was conducted in the format of a narrative literature review, within which a systematic review and qualitative analysis of publications devoted to the mechanisms of vitamin $B_{12}$ accumulation, sterol metabolism regulation, and vitamin $D_2$ biosynthesis in *Chlorella vulgaris* was conducted. The initial stage of the information search covered 412 publications found using complex key queries in the Scopus, Web of Science, PubMed, SpringerLink, Wiley Online Library, ScienceDirect, MDPI, and Google Scholar databases. After filtering by publication year (2020-2025), completeness of experimental data, availability of quantitative indicators, and relevance to the areas of photovitaminogenesis, sterogenesis, and *Chlorella cultivation* conditions, 136 sources were retained. At the content filtering stage, works that duplicated results, contained contradictory or unclear data, did not have a quantitative part, were predominantly descriptive, and all Russian-language publications were removed; a total of 87 materials were rejected. The final review corpus consisted of 21 full-text sources that met the thematic, methodological, and quality criteria.

Search queries were formed using combined keywords covering various aspects of the topic: *Chlorella vulgaris*, ergosterol, vitamin $D_2$, vitamin $B_{12}$ accumulation, UV-induced conversion, light spectra, salinity stress, metal cofactors, nitrogen limitation, lipid accumulation, photobioreactor optimisation, and microalgae sterols.

The selected materials were summarised through a structural analysis of the experimental conditions, which included lighting parameters (intensity, spectral composition, photoperiod), temperature regimes, pH of the environment, salinity, concentrations of macro- and microelements, addition of organic substrates (liver extracts, whey, permeate, amino acid additives), ultraviolet (UV) irradiation regimes – Ultraviolet A (UVA, long-wave UV radiation), Ultraviolet B (UVB, medium-wave UV radiation) and Ultraviolet C (UVC, short-wave UV radiation), as well as stress factors, in particular the presence of heavy metals, oxidative stress, salinity of the environment, nitrogen or phosphorus deficiency and their ratio. The analysis covered various cultivation systems: fermenters, stirred photobioreactors, tubular systems, thin-film installations and biofilm structures.

The results of different authors were compared using a set of integrative parameters, which formed a consistent model of factors that regulate the biochemical composition of *Chlorella vulgaris*. These parameters included: (1) vitamin $B_{12}$ content depending on the presence of microbial consortia, sources of organic nitrogen, and cultivation conditions; (2) ergosterol profile at different light intensities, growth phases, and ion composition of the medium; (3) vitamin $D_2$ content after photochemical conversion of ergosterol under the action of different ranges of UV irradiation; (4) lipid metabolism, in particular changes in triglycerides, polyunsaturated fatty acids and membrane lipids; (5) the influence of mineral factors $Mg^{2+}$, $Zn^{2+}$, $Fe^{2+}$/$Fe^{3+}$, $Cu^{2+}$ as cofactors of sterogenesis enzymes; (6) the influence of salinity, which has been considered a trigger for lipid and sterol accumulation; (7) spectral dependence of growth and biochemical composition, especially with regard to blue and red light, which modulate lipid and pigment synthesis in different ways; (8) the effect of organic stimulants, such as liver extracts containing a complex of vitamins (in particular $B_{12}$) and microelements capable of increasing biomass yield with enriched elemental composition. Generalisation was achieved by comparing data between studies and identifying common patterns, such as the relationship between the content of cobalamin and ergosterol in microalgae biomass and the light spectrum, the influence of metal ions on the synthesis and accumulation of corresponding metabolites, the role of UV in the formation of $D_2$, the influence of growth phases on lipid dynamics and the transition of metabolism to a stress regime.

## Results

### Vitamin $D_2$: Biosynthesis, influence of factors, peculiarities of microalgae cultivation

The biosynthesis of vitamin $D_2$ in microalgae is noteworthy due to the specific mechanisms of ergocalciferol formation. A. Voshall *et al.* (2021) showed that in *Chlorella*, vitamin $D_2$ is formed from its own sterols, in contrast to vitamin $B_{12}$, which microalgae do not synthesise. The study determined that the process involves two key steps: first, sterogenesis, during which ergosterol is formed via the mevalonate pathway (cycloartenol→lanosterol→ergosterol); second, photochemical conversion, in which ergosterol is converted to pre-$D_2$ under the action of UV light, which is then isomerised to ergocalciferol. Researchers emphasise that it is the photochemical conversion of sterols that is the critical phase determining the final yield of vitamin $D_2$ in green algae. A simplified diagram of the biosynthesis (according to the Kyoto Encyclopedia of Genes and Genomes (KEGG)) of vitamin $D_2$ in *Chlorella vulgaris* is shown in Figure 1. Under the influence of ultraviolet light, ergosterol is photochemically converted to previtamin $D_2$, which is further isomerised to ergocalciferol (vitamin $D_2$). The biosynthesis of ergosterol in microalgae is influenced by the growth phase, light intensity and spectrum, salt and metal content in the environment, temperature, pH, and subsequent UV irradiation, which converts ergosterol into vitamin $D_2$. In this context, C. Carlberg (2022) considered the photoproduction of vitamin D in a broader evolutionary framework, emphasising the role of UV-dependent sterol conversions as an adaptive mechanism that explains the variability of metabolic responses in different groups of photosynthetic organisms. C. Der *et al.* (2024) emphasised the multifunctionality of sterols in cellular signalling processes and showed that their synthesis is significantly modified under the influence of environmental stress factors, which is consistent with data on the sensitivity of microalgae to light and chemical cultivation parameters. O.H. Abdelkarim *et al.* (2025) emphasised the species diversity of microalgae as sources of sterols and demonstrated that the level of ergosterol formation is determined by a combination of lighting, medium composition and physiological state of cultures, confirming the key role of environmental conditions in the regulation of sterol metabolism. The study demonstrated that the ability of microalgae to produce sterols significantly depends on their species and cultivation conditions, including light regime, which shapes their different potential for vitamin $D_2$ photoproduction. M.F. Leung & P.C.K. Cheung (2021) summarised experimental data on UV-induced vitamin $D_2$ synthesis in fungi and provided quantitatively confirmed examples of a sharp increase in its content after irradiation, emphasising the universality of the photochemical mechanism among different eukaryotic groups.

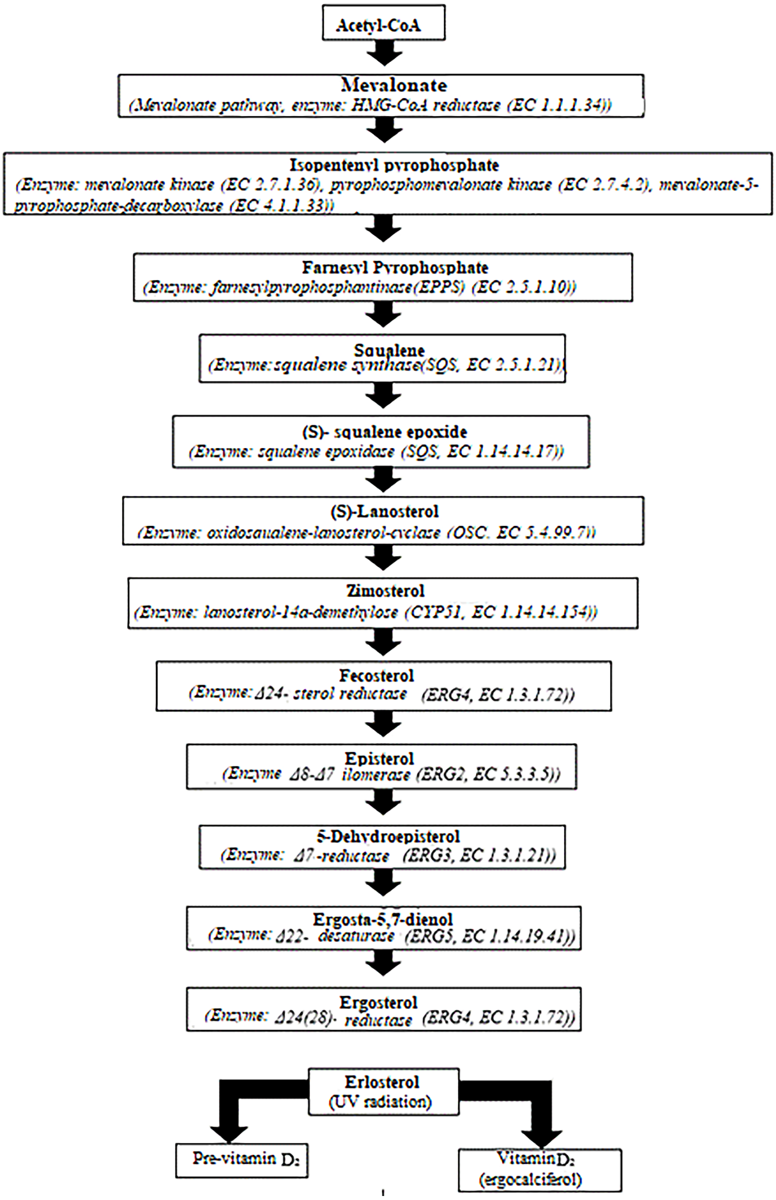


***Figure 1.*** *Ergocalciferol biosynthesis by Chlorella microalgae*

**Source:** compiled by the authors based on M. Durdakova *et al.* (2024), Kyoto Encyclopedia of Genes and Genomes (n.d.)

T. Zhang & J. Long (2025) determined that the intensity and spectral composition of light directly determine the level of lipids and sterols in green microalgae. The researchers showed that increased light intensity stimulates lipidation in *Chlorella vulgaris*, increasing the proportion of lipids from approximately 19.5% to 25.6% of dry weight (+31%), while in *Chlorella sorokiniana*, blue light provides an accumulation of about 34% lipids within 10 days of cultivation. The study emphasised that varying light intensity and cultivation parameters accelerate the formation of lipid and sterol fractions, confirming the light dependence of the anabolic pathways of green algae. A.N.M. Azhan *et al.* (2025) showed that the spectral quality of light, in particular blue radiation, modulates the synthesis of fatty acids and chlorophyll, creating favourable conditions for increased lipid formation in *Chlorella sorokiniana*, which is consistent with the results regarding the potential for sterol accumulation and photobiological regulation of lipid metabolism. Trace elements, particularly divalent metals, acted as cofactors in the enzymatic reactions of the sterol pathway, as magnesium ($Mg^{2+}$) ensured stable enzyme function and supported biomass growth, which indirectly enhanced ergosterol accumulation, and the results of Y. Feng *et al.* (2021) showed that varying concentrations of available trace elements, including $Mg^{2+}$, directly affected the intensity of lipid metabolism, highlighting their regulatory function in sterogenesis.

Zinc ($Zn^{2+}$) is a cofactor of numerous dehydrogenases and other enzymes involved in sterol biosynthesis; it participates in the sequential conversion of squalene to lanosterol and further to ergosterol, i.e., it directly affects the key stages of this metabolic pathway (Kyoto Encyclopedia of Genes and Genomes, n.d.). Iron ($Fe^{2+}/Fe^{3+}$) is substantial in the functioning of enzymes that ensure cellular respiration and energy metabolism, creating conditions for the stable course of sterol pathways. Copper ($Cu^{2+}$) in low concentrations can activate several oxidases and maintaining the overall metabolic tone of the cell, which also creates the conditions for enhanced ergosterol synthesis. This is indicated by the results of A.L. Souza & G.J. Patti (2021), who demonstrated that changes in the availability of transition metals restructure the cell's metabolome and affect the activity of metal-dependent enzymes. This shift in metabolic flows enhances reactions associated with lipid and sterol synthesis, thus consistent with data on the importance of trace elements in the regulation of sterologenesis. For example, in yeast, where the mechanisms have been studied in detail, the addition of zinc increased the carbon flux towards ergosterol by +28.6%. The presence of $Zn^{2+}$ and $Cu^{2+}$ enhanced sterol synthesis even in the presence of inhibitors, while Fe deficiency reduced sterol levels (Souza & Patti, 2021). A similar mechanism of action is expected in microalgae. At the same time, it should be emphasised that an excess of any of these metals has a toxic effect on microalgae cells, reduces their viability and can inhibit enzyme activity; therefore, the selection of trace element concentrations is critical for optimising cultivation conditions and increasing ergosterol yield (Feng *et al.*, 2021).

G. Salbitani & S. Carfagna (2021) showed that forms of nitrogen can significantly affect structural and energy metabolism in microalgae: ammonium, as a more "desert" substrate for the cell, accelerates nitrogen assimilation and affects the balance between protein and lipid synthesis. The study also demonstrated that under conditions of variable nitrogen availability, cells switch to increased accumulation of energy and structural lipids, which is consistent with a possible increase in sterologenesis. Such metabolic changes confirm that nitrogen compounds are one of the key regulators that determine the level of sterol formation in microalgae. The increase in ergosterol content under conditions of moderate salinity is also confirmed by data from H.E.A. Ali *et al.* (2021), determining that the combination of light and salt stress in *Chlorella vulgaris* cultures activated lipid synthesis, enhanced the accumulation of sterol fractions, and was accompanied by an increase in the level of secondary metabolites, including carotenoids and antioxidants. Thus, moderate salt stress acts as an effective trigger for increased accumulation of ergosterol in microalgae. The optimal parameters for *Chlorella* growth, a temperature of 25-30°C and a pH of ≈7, are critical for the activity of sterol pathway enzymes, which is consistent with the findings of A.E. Kholif & O.A. Olafadehan (2021), demonstrating that *Chlorella vulgaris* was highly sensitive to temperature and pH shifts, which directly affected its chemical composition and metabolic processes. The study noted that changes in the physicochemical parameters of the environment can significantly affect the synthesis of lipids, proteins, and secondary metabolites, which indirectly indicates the potential variability of the sterol profile. A generalised description of the influence of certain factors on sterol biosynthesis in microalgae is given in Table 1.

***Table 1.*** *The influence of certain factors on sterol biosynthesis in Chlorella microalgae*

| Factor | Condition | Effect |
|---|---|---|
| Lighting intensity | Increase in sensitivity | Increase in lipid content in *C. vulgaris* cells from ~19.5% to ~25.6% (≈+31%). |
| Visible light spectrum | Blue light (450-470 nm) | On the 10th day of cultivation, *C. sorokiniana* cells showed an increase in lipid content of ≈34.06% compared to other spectra. |
| $Zn^{2+}$ ions | Addition of $Zn^{2+}$ ions to the culture medium | The addition of $Zn^{2+}$ ions to the yeast culture medium led to a 28.6% increase in ergosterol content. A similar effect is also expected for *Chlorella* microalgae. |
| Growth phase | Phase 2 (stationary) | The ergosterol content in the stationary phase of *C. sorokiniana* cultivation increased by 22.8% compared to the active growth phase. |

**Source:** compiled by the authors based on A.E. Kholif & O.A. Olafadehan (2021), A.L. Souza & G.J. Patti (2021), H.E.A. Ali *et al.* (2021), T. Zhang & J. Long (2025), A.N.M. Azhan *et al.* (2025)

Therefore, to increase ergosterol biosynthesis and vitamin $D_2$ content in *Chlorella* biomass, it is advisable to cultivate cells in a stationary growth phase using stress factors (salinity), provide lighting with blue spectrum elements, maintain optimal temperature and pH, and add microdoses of Zn and Fe.

**Vitamin $B_{12}$: Accumulation mechanism, influence of factors, peculiarities of microalgae cultivation**

The *Chlorella* is considered a potential source of vitamin $B_{12}$, but the presence of this micronutrient depends on the activity of bacterial symbionts and the form of the vitamin that accumulates, while S.P. van den Oever & H. Mayer (2022) demonstrated that microalgae-based products contained predominantly non-bioavailable forms of cobalamin rather than its inactive analogues, which are unable to perform physiological functions in the human body. The study also noted that the formation of the active form of $B_{12}$ depends on the presence of specific bacterial producers, and their absence in artificial cultivation conditions explains the minimal level of this metabolite in *Chlorella*. This approach demonstrates that the microbial environment with which algae interact is central to $B_{12}$ accumulation, rather than the algae themselves. A comparative characterisation of vitamin $B_{12}$ content (true and substitutes) in different types of microalgae is presented in Table 2.

Following Table 2, *Chlorella* grown in natural conditions contains significant amounts of true vitamin $B_{12}$ (~100 mcg/100 g) and a few of its analogues (<10 mcg/100 g). It has a positive effect on $B_{12}$ levels in the body. Artificially cultivated *Chlorella*, especially under sterile conditions, contains no or very little vitamin $B_{12}$, so it is not an effective source of it. Other microalgae, such as *Spirulina* and *AFA*, are high in $B_{12}$ analogues, which may even impair the absorption of this vitamin.

***Table 2.*** *Comparative table of $B_{12}$ content in microalgae*

| Microalgae genus | True $B_{12}$ (μg/100 g) | Analogue of $B_{12}$ (mcg/100 g) | The effect of $B_{12}$ contained in microalgae on $B_{12}$ status in the human body |
|---|---|---|---|
| *Spirulina* | ~35 | ~120 | negative |
| *AFA (Aphanizomenon flos-aquae)* | ~30 | ~570 | negative |
| *Nori* (red algae) | varies | varies | not determined |
| *Chlorella* (natural habitat) | ~100 | <10 | positive |
| *Chlorella* (artificially cultivated, fermented) | – | – | neutral |

**Source:** compiled by the authors based on Vitamin $B_{12}$ in algae: Spirulina, Chlorella and nori (n.d)

In contrast to bacteria, microalgae, in particular *Chlorella vulgaris*, are not capable of autonomous synthesis of cobalamin, since their genome lacks the genes for biosynthesis of this coenzyme (Peshuk *et al*., 2024). However, they can accumulate and store $B_{12}$ that enters the environment from exogenous sources. In natural ecosystems, Chlorella obtained vitamin $B_{12}$ through symbiosis with producer bacteria, which secreted biologically active forms of cobalamin (methylcobalamin and adenosylcobalamin) into the environment, which were absorbed by algae cells through specialised transport systems. M. Peng *et al*. (2025) demonstrated that the availability of macro- and microelements significantly modified these metabolic processes, affecting the overall efficiency of vitamin absorption. This effect is explained by the synergy of metabolisms: bacteria provide cobalamin, and microalgae create conditions for their growth by releasing oxygen and organic substrates. Thus, the study proved that co-cultivation of *Chlorella* with bacteria significantly increases the content of biologically active $B_{12}$ in biomass. The summary by B. Nowruzi *et al.* (2022) demonstrates that the interaction between algae and bacteria is multi-level: bacteria not only supply vitamin $B_{12}$, but also regulate the signalling pathways and metabolic activity of algae. The study noted that the exchange of nutrients, the release of phytohormones, and the stabilisation of the microenvironment create conditions in which both groups of organisms function more efficiently. This interdependence accounts for a higher level of cobalamin in co-cultures compared to microalgae monocultures (Fig. 2).

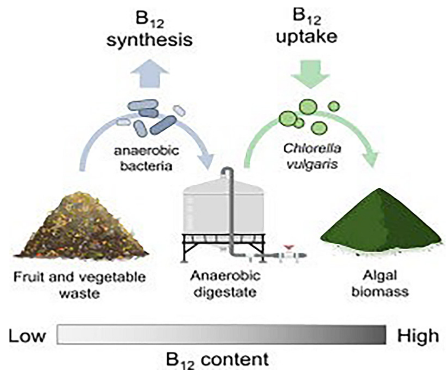


***Figure 2.*** *Simplified scheme of $B_{12}$ accumulation in microalgae biomass*

**Source:** compiled by the authors based on K.P. Papadopoulos *et al.* (2023)

In addition to symbiotic relationships with producer microorganisms, a significant factor is the presence of metal ions in the environment, which can affect the level of $B_{12}$ biosynthesis by bacteria, the bioavailability of the vitamin, and the efficiency of its accumulation by *Chlorella* cells. Cobalt ($Co^{2+}$) is the central atom in the structure of cobalamin. The addition of cobalt ions to the environment stimulates both the synthesis of $B_{12}$ by bacteria (precoryne formation) and the accumulation of the vitamin in microalgae (when co-cultured with producer bacteria) (Kyoto Encyclopedia of Genes and Genomes, n.d.). Some experiments have shown that optimal concentrations (0.01-0.1 mg/L) significantly increase the content of active forms of vitamin $B_{12}$ methylcobalamin, 5'-deoxyadenosylcobalamin andhydroxycobalamin in biomass (an increase of 7-12% compared to the control). X. Ji *et al.* (2021) further emphasised the significance of cobalamin in "algae-bacteria" systems: the authors showed that the presence of an external source of vitamin $B_{12}$ alters the expression of proteins associated with nutrient uptake and intercellular interaction. Under the influence of cobalamin, the consortium demonstrated increased metabolic activity, which was reflected in better nitrogen and phosphorus uptake and more active algae growth. This effect indicates that even minor changes in coenzyme availability can significantly affect the functioning of mixed cultures and the accumulation of $B_{12}$ in algal biomass.

Zinc ($Zn^{2+}$), magnesium ($Mg^{2+}$) and iron ($Fe^{2+}$/$Fe^{3+}$) acted as cofactors for enzymes involved in vitamin $B_{12}$ metabolism and transport, while iron additionally influenced the efficiency of electron transport during photosynthesis, which enhanced cobalamin uptake by cells, and L.M. González-González & L.E. De-Bashan (2021) showed that changing the mineral composition of the environment in mixed "microalgae-bacteria" systems directed metabolic flows towards more intensive formation of secondary metabolites, emphasising the significance of micronutrient interactions. The results demonstrated that a balanced supply of metal ions not only maintains enzymatic activity but also enhances cooperation between bacterial cobalamin producers and algal cells, contributing to more efficient accumulation of vitamin $B_{12}$ in biomass.

Excessive concentrations of heavy metals ($Cu^{2+}$, $Ni^{2+}$, $Pb^{2+}$) inhibited *Chlorella* growth and reduced vitamin $B_{12}$ absorption efficiency due to oxidative stress, while controlled addition of micronutrients, especially cobalt, could serve as a tool to increase cobalamin content in microalgae biomass. In this context, R.P. Singh *et al.* (2023) demonstrated that different types of abiotic stress, particularly metal-induced and osmotic stress, significantly modified the metabolic activity of microalgae, enhancing the accumulation of lipids and secondary metabolites at moderate metal concentrations, but causing metabolic disorders and loss of biosynthetic potential at toxic levels. $B_{12}$ transporters, homologous to bacterial carrier proteins (BtuB, BtuFCD), function in the cell membranes of microalgae. They ensure the active transport of cobalamin across the membrane, after which the vitamin is deposited in the cytoplasm in the form of methyl- and adenosylcobalamin. The presence of these systems explains the ability of *Chlorella* to rapidly absorb exogenous $B_{12}$ within a few hours after its addition to the medium (Fig. 3).

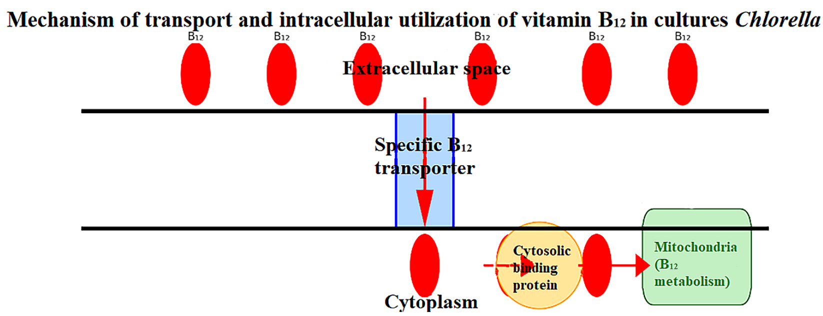


***Figure 3.*** *Mechanism of $B_{12}$ transport into Chlorella cells*
**Source:** compiled by the authors based on C. Nef et al. (2022), N. Zhao et al. (2024)

It is worth noting that in most eukaryotic cells (including algae), vitamin $B_{12}$ metabolism is not uniform and occurs in several parts of the cell. In microalgae, particularly *Chlorella*, the situation is more complex. Studies show that $B_{12}$-dependent methionine synthase (METH) may be present in the cytoplasm and chloroplasts. At the same time, no evidence of $B_{12}$-dependent methylmalonyl-CoA mutase, characteristic of animals, was found in many green algae. This means that *Chlorella* most likely uses $B_{12}$ mainly in the cytoplasm and chloroplasts, rather than in the mitochondria (Nef *et al*., 2022). The *Chlorella vulgaris* can be cultivated on different types of nutrient media: mineral (BG-11, Chu-10, WC, LC Oligo), organic (whey, food processing waste, meat extracts) and mixed (combinations of mineral and organic components). The choice of medium determines the growth rate, chemical composition of cells and accumulation of vitamin $B_{12}$. Classic mineral media provide macro- and microelements (nitrogen, phosphorus, magnesium, potassium, iron, and microelements), but do not contain vitamin $B_{12}$. For species that depend on cobalamin, it must be added separately, for example, in the form of an f/2 vitamin solution. This is critical if the culture is maintained under axenic conditions, i.e., it is a pure culture without any accompanying microorganisms, in particular $B_{12}$-producing bacteria. In such cases, microalgae cannot obtain the vitamin from an external source other than artificially added (Singh *et al*., 2023).

Different media have different effects on growth and metabolism. For *Chlorella vulgaris*, the study demonstrated that LC Oligo and Chu-10 media provide higher biomass productivity and alter the carbohydrate-to-lipid ratio compared to WC, where growth is less intense. Thus, in semi-continuous cultivation, the specific growth rate is 0.84 $d^{-1}$ in LC Oligo medium, 0.79 $d^{-1}$ in Chu-10, and only 0.62 $d^{-1}$ in WC, i.e., biomass productivity in LC Oligo is almost 35% higher than in WC. The BG-11 medium is one of the most common for phototrophic microalgae cultivation due to its optimal content of macroelements and microelements, which ensures stable growth and high biomass concentration (Ampofo & Abbey, 2022). In contrast, Tris-Acetate-Phosphate (TAP) medium contains organic carbon in the form of acetate, which ensures growth of *Chlorella* in a mixotrophic mode, combining photosynthesis with the use of an external source of organic matter. This often leads to increased growth rates and changes in biomass composition, including greater lipid accumulation, although TAP contains virtually no vitamin $B_{12}$ (Singh *et al*., 2023).

The ability of *Chlorella* to assimilate ammonium, nitrate and urea is well known; ammonium is often the most energy-efficient source of N when pH is controlled, while urea or nitrate is better for some strains. The correct selection of N form increases growth rate and protein content. Some studies indicate that urea can maintain higher productivity and a more stable pH than nitrate, which indirectly improves the physiological condition of cells (Papadopoulos *et al*., 2023). The transition to organic or waste-based substrates (whey, permeates, anaerobic digestates, pig manure permeates, etc.) often increases growth (mixo-/heterotrophy) and paves the way for the intake of exogenous $B_{12}$ through associated microbiota or vitamin content in the streams (Ali *et al*., 2021). For *Chlorella*, the study demonstrated that whey and permeates can increase biomass yield relative to controls in several modes, provided that trace elements are corrected. Thus, when 10% whey was added to the mineral nutrient medium in fed-batch mode, biomass growth increased to 17.2 ± 1.3 g/L, although the lipid fraction decreased to 20.5 ± 0.3%, which still exceeds the control values (mineral medium). In mixed media, "pig manure permeate + whey" (instead of BG-11) achieves comparable or higher productivity due to the high content of N, P and organic carbon; this also significantly reduces the cost of media (González-González & De-Bashan, 2021).

Anaerobic digestates are another effective source of nutrients and $B_{12}$. Studies have shown that even when vegetable and fruit waste is used as a substrate for fermentation, a significant amount of cobalamin is produced, which can be effectively accumulated by *Chlorella vulgaris*. With this approach, the vitamin $B_{12}$ content in the biomass reaches 10.6 μg/g dry weight, which significantly exceeds the level in standard mineral media (Feng *et al*., 2021). Additionally, the concentration of $B_{12}$ in the digestate reached 13.8 μg/L at the time of inoculation, and on day 13, *Chlorella* absorbed approximately 26% of the vitamin present in the medium. The study also noted that ammonia toxicity caused by high pH is a major growth limiter and can be overcome by adjusting the pH to increase productivity (Papadopoulos *et al.*, 2023).

The use of beef liver extract is notable. This product is one of the richest natural sources of vitamin $B_{12}$ (up to 60-80 μg/100 g). The use of liver extract as a component of the medium provides not only a supply of cobalamin, but also proteins, peptides and trace elements that further stimulate growth. Patent developments since the 1980s have noted that the combination of liver extract with optimal spectral lighting promotes the intensive accumulation of $B_{12}$ in *Chlorella* cells (Der *et al.*, 2024). Since *Chlorella vulgaris* is unable to synthesise cobalamin, the most common approach to its enrichment is to add organic substrates containing this vitamin to the culture medium. Studies from the last decade (2015-2025) show that organic extracts (meat, milk, food waste) can significantly increase $B_{12}$ levels in biomass while stimulating cell growth (Salbitani & Carfagna, 2021). Animal liver, particularly beef liver, is one of the most concentrated natural sources of vitamin $B_{12}$, with a content of 60-80 μg/100 g of product (Durdakova *et al.*, 2024). Therefore, liver extracts are most often used in biotechnological experiments. Their advantage lies not only in their high cobalamin content, but also in the presence of proteins, peptides, iron and trace elements, which further stimulate the growth of *Chlorella vulgaris* (Zhang & Long, 2025).

As early as 1980-1990, a method for using liver extract to enrich *Chlorella* with vitamin $B_{12}$ was patented in Japan (Kholif & Olafadehan, 2021). The patent documents emphasised that the technological approach of combining such an extract with controlled spectral lighting (blue range) provided a high level of vitamin accumulation. Modern studies have confirmed that the introduction of liver extract into the medium can significantly increase the content of biologically active $B_{12}$ in *Chlorella vulgaris* biomass, as well as increase the dry matter yield compared to mineral media (Souza & Patti, 2021). Therefore, the liver is a particularly promising substrate for microalgae cultivation. In addition to liver, milk whey, soy protein hydrolysates, food waste extracts, and anaerobic digestates are used as nutrient media (Mendes *et al.*, 2024). They may also contain traces of cobalamin synthesised by microbial communities and serve as sources of readily available nitrogen and organic carbon. However, these sources are inferior to liver in terms of $B_{12}$ content.

Thus, the use of organic extracts, in particular liver extracts, for the cultivation of *Chlorella vulgaris* has a dual effect: enrichment of biomass with biologically active vitamin $B_{12}$, which is substantial for the creation of functional foods (especially for vegans and vegetarians); rational use of food and meat industry waste, which is in line with modern concepts of bioeconomics. Liver extract is one of the richest organic sources of vitamin $B_{12}$; therefore, its use in the nutrient medium ensured a higher content of cobalamin in the biomass of *Chlorella vulgaris* compared to other organic and mineral substrates. Light is one of the key regulatory factors of *Chlorella vulgaris* growth and metabolism. The intensity, duration and spectral composition of light determine photosynthetic efficiency, pigment content and the rate of metabolite biosynthesis, including vitamin $B_{12}$. In general, increasing light intensity stimulates the rate of photosynthesis and biomass accumulation up to a certain limit, after which photosaturation and photodestructive processes occur. The optimal light intensity for *Chlorella vulgaris* varies depending on the spectrum, cultivation conditions and type of medium (Table 3).

***Table 3.*** *Light intensity ranges and their effect on Chlorella growth*

| Illuminance range, $\mu mol \cdot m^{-2} \cdot s^{-1}$ | Biological effect |
|---|---|
| ~80 | Beginning of noticeable growth |
| 80-150 | Efficient area: stimulation of growth and biomass accumulation |
| >150 | Possible photo suppression (especially >200) |

**Source:** compiled by the authors based on A.E.P. Cunha *et al.* (2023); B.M. de Brito *et al.* (2023)

Continuous lighting (24/0) stimulates more intensive growth but is sometimes accompanied by a decrease in photosynthetic efficiency. An alternative is to use 16/8 or 12/12 (light/dark) modes, which mimic natural daily cycles and can promote more stable metabolism (Bialevich *et al.*, 2022). Wavelength (spectral composition) is a critical parameter for metabolism. Blue light (450-470 nm) induces intense absorption by chlorophyll *a* and promotes increased photosynthetic activity. Studies show that under blue light, *Chlorella* accumulates more protein and vitamin $B_{12}$ (Voshall *et al.*, 2021). Red light (650-680 nm) activates photosystem II and stimulates carbohydrate biosynthesis, increasing biomass yield (Liu *et al.*, 2025). Green light (520-550 nm) penetrates the photobioreactor, improving cell illumination in dense cultures. It is less effective for pigment biosynthesis but can stabilise the photosynthetic process in mixed lighting (Gao *et al.*, 2023). A combination of blue and red light is often found to be most effective for the growth of *Chlorella vulgaris*, as it provides a balance between photosynthetic productivity and metabolite biosynthesis. Patent and experimental studies have shown that the potential technology of adding a source of $B_{12}$ (e.g., liver extract) in combination with blue lighting can increase the accumulation of cobalamin in *Chlorella* cells (Ashaari *et al.*, 2024). This confirms the substantial role of spectral conditions in regulating vitamin biosynthesis and transport. A general description of the factors and their influence on the accumulation of vitamin $B_{12}$ by *Chlorella* microalgae is provided in Table 4.

***Table 4.*** *The influence of certain factors on the accumulation of $B_{12}$ in Chlorella microalgae*

| Factor | Conditions/examples | Effect on $B^{2+}$ |
|---|---|---|
| Symbiosis with bacteria | Co-cultivation with *Propionibacterium freudenreichii* | Increase in $B_{12}$ content in biomass several times over (exact figures were not provided by S. Maulana *et al.* (2025)) |
| Metal ions | Addition of $Co^{2+}$ (0.01-0.1 mg/l) | 7-12% increase in $B_{12}$ in biomass compared to control (Ji *et al.*, 2021) |
| | $Zn^{2+}$, $Mg^{2+}$, $Fe^{2+}$/Fe | Indirect stimulation of $B_{12}$ (enzyme cofactors) absorption (González-González & De-Bashan, 2021) |
| | Excess of $Cu^{2+}$, $Ni^{2+}$, $Pb^{2+}$ | Decreased *Chlorella* growth and reduced $B_{12}$ accumulation (Singh *et al.*, 2023) |
| Environment type | BG-11, Chu-10, WC, LC Oligo | Mineral media do not contain $B_{12}$; therefore, the vitamin must be added externally (Ricky *et al.*, 2022) |
| | TAP (mixotrophy, acetate) | Higher height and lipids, but low $B_{12}$ levels (Ricky *et al.*, 2022) |
| | Organic/waste substrates (whey, permeates) | Increased biomass yield, exogenous $B_{12}$ intake via associated microbiota (Kashyap *et al.*, 2025) |
| Anaerobic digestates | Substrate – vegetable and fruit waste | $B_{12}$ content in biomass up to 10.6 μg/g dry weight; in the medium – 13.8 μg/l; absorption ~26% in 13 days (Kolesovs & Semjonovs, 2023) |
| Organic extracts | Beef liver extract (60-80 mcg/100 g $B_{12}$) | Significant increase in $B_{12}$ and biomass; the patent describes the method but does not provide numerical data (Ashaari *et al.*, 2024) |
| Light (intensity and spectrum) | 80-150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ | Optimal intensity for growth; above – photostress (Cunha *et al.*, 2023) |
| | 12/12 mode (light/dark) | Stable metabolism, improved balance (Bialevich *et al.*, 2022) |
| | Blue light (450-470 nm) | Increased protein and $B_{12}$ content (Kamolrat *et al.*, 2023) |
| | Red light (650-680 nm) | Increased protein and carbohydrate content (Gao *et al.*, 2023) |
| | Mixed blue + red | Most effective for growth and $B_{12}$ (Gao *et al.*, 2023) |

**Source:** compiled by the authors

Thus, the accumulation of vitamin $B_{12}$ in *Chlorella vulgaris* largely depends on external factors. The most effective strategies are the use of organic extracts, anaerobic digestates and co-cultivation with cobalamin-producing bacteria, while optimal lighting conditions and the presence of metal ions act as additional regulators of this process.

### Potential biotechnological applications of *Chlorella* microalgae

Enriched with vitamin $B_{12}$, *Chlorella vulgaris* biomass has a wide range of potential applications in the food, feed, and pharmaceutical industries, as well as in the bioeconomy. In the food industry, *Chlorella vulgaris* is already used as a "superfood" containing high levels of protein, essential amino acids, fatty acids, pigments and trace elements. Biomass enriched with vitamin $B_{12}$ can be used as a basis for functional products such as drinks, protein bars or dietary supplements, especially for people on vegan diets who lack natural sources of cobalamin (Peshuk *et al*., 2024). In addition, *Chlorella* can be a source of ergocalciferol (vitamin $D_2$), which is formed from ergosterol, a natural cell sterol, under the influence of UV radiation. The additional $D_2$ content in the biomass enhances the functional value of the products by supporting normal calcium metabolism, bone health and immune function (Hamouda *et al*., 2022). In animal husbandry, the use of microalgae not only provides animals with an additional source of nutrients, but also improves their growth, immunity and reproductive functions. Enriched with $B_{12}$ and $D_2$, *Chlorella* helps to increase the content of cobalamin and ergocalciferol in meat, milk and eggs, reduces the need for synthetic premixes, and is also a valuable component in fish farming and aquaculture, where microalgae are a natural part of the diet (Abdelkarim *et al*., 2025).

In pharmaceuticals and medicine, *Chlorella vulgaris*, which contains biologically active forms of vitamin $B_{12}$ and ergocalciferol, can be used to prevent and treat deficiencies of these vitamins, which are a global problem and are associated with anaemia, bone metabolism disorders, cognitive dysfunction and an increased risk of cardiovascular disease. Enriched microalgae biomass is considered a basis for the creation of functional preparations capable of supporting the nervous system, blood formation and bone health. In addition, *Chlorella* exhibits antioxidant, immunomodulatory, and detoxification properties, which expand its therapeutic potential (Selvaraj, 2024). Equally relevant is the use of microalgae in ecology and economics. The use of by-products from the food and meat industries, in particular liver extracts, as components of nutrient media for the cultivation of *Chlorella vulgaris*, combines the utilisation of organic waste with the production of biomass enriched with vitamin $B_{12}$ (Zhang & Long, 2025). This approach reduces production costs and environmental impact while providing high-quality raw materials for food, pharmaceutical and feed purposes, which correlate with the principles of sustainable development.

## Discussion

The results showed that vitamin $D_2$ formation in *Chlorella* is a multi-component process in which ergosterol synthesis and its subsequent photochemical transformations under the action of ultraviolet light were central. An increase in light intensity and a change in the spectrum were accompanied by an increase in the lipid pool by about one-third, which simultaneously increased the potential for sterol accumulation and created favourable conditions for the formation of $D_2$. Salt stress also had a significant effect on this process: the addition of 300 ppm NaCl in combination with the late growth phase resulted in a 28-43% increase in ergosterol, reflecting the adaptive restructuring of cells under osmotic stress. The action of microelements was also substantial: changes in the concentrations of $Mg^{2+}$, $Zn^{2+}$ and $Fe^{2+}/Fe^{3+}$ modified the activity of enzymes involved in the sterol pathway, promoting the formation of ergosterol. Maintaining optimal temperature conditions (25-30°C) and neutral pH further stabilised sterol metabolism and maintained its intensity.

Among the studies investigating the ability of microalgae to accumulate cobalamin under various conditions, the most notable were those by B. Xu *et al.* (2021) and A. Tzachor *et al.* (2024). B. Xu *et al.* determined that *Chlorella* can absorb vitamin $B_{12}$ more efficiently in mixed cultures: the combination of microalgae with fungi and the presence of organic substrate and available cobalt ions in the environment significantly enhanced the accumulation of active forms of cobalamin in cells. The study also emphasised that the concentration of trace elements and the type of environment determined whether microalgae could absorb $B_{12}$ in a biologically available form. A study by A. Tzachor *et al.* demonstrated that light regimes have a significant effect on the accumulation of active vitamin $B_{12}$. The researchers showed that the combination of light intensity, spectrum, and culture growth stage determined how effectively cells were able to accumulate $B_{12}$. Compared to these studies, the present study covered a wider range of conditions that influenced the enrichment of *Chlorella* biomass with active forms of cobalamin: cobalt availability, type of medium, interaction with microorganisms, growth phase, salinity level, light intensity, and the presence of moderate stress factors. This approach provided a more comprehensive overview of the parameters that ensure the maximum content of bioavailable $B_{12}$ in microalgae biomass.

Within the scope of studies devoted to biologically active components of microalgae, L. Klamann *et al.* (2023) and A.W. Almutairi *et al.* (2021) examined factors influencing the metabolism and accumulation of key compounds. The study by L. Klamann *et al.* analysed the conditions under which phototrophic and mixotrophic cultures of different species of Lemnaceae can accumulate bioavailable vitamin $B_{12}$. The study demonstrated that cobalamin levels increased sharply in the presence of available micronutrients, organic carbon, and stable interaction with producer bacteria. Although the model objects differed from *Chlorella*, the study outlined the main conditions necessary for the accumulation of active forms of $B_{12}$. This idea is consistent with the results of analysis by A.W. Almutairi *et al.* on the lipid reactions of *Chlorella vulgaris* at elevated salinity. The results confirmed that stressful conditions can activate metabolic pathways, but the authors did not analyse the absorption or accumulation of cobalamin. The present study complements these findings by incorporating the conditions that determine the uptake and accumulation of $B_{12}$, the role of trace elements, light, bacterial symbionts, and environmental characteristics.

In the field of research on alternative sources of nutrients and conditions for the accumulation of vitamin $B_{12}$ by microalgae, the results of S. Sultana *et al.* (2023) and F. Bunbury *et al.* (2022) were substantial. S. Sultana *et al.* demonstrated that not all marine bacteria that produce cobalamin release it into the environment in quantities sufficient for assimilation by microalgae. The study emphasised that the availability of active forms of $B_{12}$ significantly depends on the bacterial strain and environmental characteristics, which explains the limited accumulation of this vitamin in microalgae under artificial cultivation conditions. The results of F. Bunbury *et al.* supplemented this observation: the study found that effective $B_{12}$ uptake by microalgae is only possible with close and prolonged contact with producing bacteria. The study demonstrated that the stability of the interaction and the availability of metabolically active forms of cobalamin determine the actual level of its accumulation in cells. Compared to these sources, this study covered a wider range of factors, as it included an assessment not only of the bacterial supply of $B_{12}$, but also of the role of trace elements, salt and light triggers, and environmental conditions that affect the further accumulation of the vitamin – blocks that were not considered in the aforementioned studies.

Within the scope of studies that intersected with the topic of the biochemical quality of microalgae, the most relevant in terms of content were the studies by E. Barber-Lluch *et al.* (2021) and C.Y. Tong *et al.* (2025). E. Barber-Lluch *et al.* showed significant variability in vitamin $B_{12}$ concentrations in the coastal waters of the north-western Pyrenean shelf, which was determined by hydrological conditions and the activity of microbial communities. The study emphasised that the unstable availability of cobalamin in the natural environment directly affects the ability of microalgae to accumulate biologically active forms of $B_{12}$. C.Y. Tong *et al.* demonstrated that co-cultivation of *Chlorella sorokiniana* with bacteria that overproduce vitamins resulted in synergistic biomass growth, increased lipid content, and more efficient uptake of $B_{12}$ into cells. The study determined that bacterial producers can compensate for cobalamin deficiency in the environment and create conditions under which microalgae accumulate significantly more vitamin than under autotrophic cultivation. Compared to these studies, the study provided a broader analytical context, as it additionally covered the influence of light regimes, salinity, metal cofactors, nitrogen sources, and growth stages on the accumulation of $B_{12}$ and sterols. Aspects such as regulated availability of micronutrients, UV-dependent sterol transformations, and stress factors were not considered in the studies by E. Barber-Lluch *et al.* and C.Y. Tong *et al.*, which renders the presented review more comprehensive.

The analytical field of research was expanded by A. Ahirwar *et al.* (2024) and S. Vogeler *et al.* (2021). A. Ahirwar *et al.* considered fermentation approaches to increasing the nutritional value of algal biomass but did not analyse the accumulation of vitamin $B_{12}$, the role of metals, the conditions for sterol synthesis, or the photochemical formation of $D_2$. S. Vogeler *et al.* studied the distribution of $B_{12}$ in the tissues of bivalve molluscs to evaluate natural sources of micronutrients, but the mechanisms of $B_{12}$ accumulation by microalgae, the effect of trace elements, salinity, and light regimes were not evaluated. Compared to these studies, the presented research provided a broader picture of the processes, as it combined the mechanisms of $B_{12}$ and $D_2$ synthesis, the sterol pathway, UV conversion of ergosterol, the influence of $Mg^{2+}$, $Zn^{2+}$, $Fe^{2+}/Fe^{3+}$, and the addition of organic substrates. This demonstrated the factors regulating the biochemical composition of *Chlorella*, which previous studies had not covered.

Within the scope of studies on light regimes, stress factors and metabolic changes in microalgae, the most relevant were those by B.A. Pessi & O. Bernard (2024) and K. Gopalakrishnan *et al.* (2024). B.A. Pessi & O. Bernard modelled the dynamics of metabolic flows in the "microalgae-bacteria" symbiosis, identifying the conditions that stimulated lipid synthesis under the action of regulators, in particular biotin, but the work did not cover the sterol pathway and the photochemical formation of vitamin $D_2$. K. Gopalakrishnan *et al.* analysed the co-cultivation of microalgae and bacteria in wastewater to increase biomass productivity, but the influence of salinity, the role of trace elements and the accumulation of ergosterol were not considered. In contrast to these approaches, the presented study exceeded the scope of assessment of growth parameters and covered the full range of biochemical processes: ergosterol synthesis, its modulation by metals, the response of cells to different light spectra and salinity, as well as the photoconversion of ergosterol to vitamin $D_2$. This breadth of coverage formed a holistic view of the mechanisms of sterol and calciferol accumulation, which was not presented in the aforementioned studies. A summary of the results showed that this work formed a comprehensive multifactorial model of the conditions that determined the accumulation of cobalamin by microalgae, from interaction with bacterial producers to the role of organic substrates, trace elements, lighting and stress factors, offering a much broader and deeper analytical approach than previous studies.

## Conclusions

A summary of the literature data shows that *Chlorella vulgaris* biomass is a promising source of functional nutrients, in particular vitamins $B_{12}$ and $D_2$. Due to the lack of endogenous synthesis systems, the accumulation of cobalamin in microalgae cells depends on its external supply; therefore, the type of nutrient medium and the availability of exogenous sources of vitamin $B_{12}$ are substantial. Organic media (liver extract, whey, food processing waste) provide a significantly higher $B_{12}$ content in microalgae biomass compared to classic mineral media (BG-11, Chu-10, WC). Light conditions are an additional regulator: blue light stimulates photosynthetic activity and transport systems, promoting better vitamin absorption. A substantial factor is the presence of metal ions in the medium, in particular cobalt, which is part of cobalamin, and other trace elements that indirectly affect the overall metabolism of *Chlorella* microalgae and the accumulation of vitamin $B_{12}$.

Vitamin $D_2$ synthesis in *Chlorella vulgaris* is based on the photochemical conversion of ergosterol under the action of UV radiation, which confirms the analogy with the mechanisms in fungi. The yield of ergocalciferol depends on light parameters, nitrogen nutrition, the C:N ratio, and the state of the photosynthetic apparatus. Metal ions (Mg, Fe, Zn) are essential cofactors for sterol metabolism, while excess Cu or Zn can induce oxidative stress and alter the sterol balance. Stress factors are an additional regulator of productivity. Increased salinity stimulates metabolic restructuring and promotes lipid accumulation, which can also affect the sterol pool associated with $D_2$ synthesis. At the same time, the growth prophase (logarithmic stage) is critical for the active synthesis of biomass and metabolites, while in the stationary growth stage, the accumulation of lipids and certain vitamins is higher, which must be considered in the optimisation of cultivation conditions.

Thus, effective enrichment of *Chlorella vulgaris* biomass with vitamins $B_{12}$ and $D_2$ is possible when several factors are combined: the use of organic media containing exogenous cobalamin; an optimal light spectrum (blue and UV-B ranges); controlled mineralisation of the medium, incorporating metal ions; and the application of stress conditions that stimulate the accumulation of lipids and sterol precursors. This creates the possibility of simultaneously obtaining biomass enriched not only with $B_{12}$, but also with $D_2$, which increases its functional value for food, pharmaceutical and nutraceutical products. Further research should address optimisation of the cultivation conditions of *Chlorella vulgaris* to increase the production of vitamins $B_{12}$ and $D_2$, improving light and mineral regimes, and clarifying the key biochemical mechanisms of their accumulation.

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None.

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# Мікроводорості *Chlorella vulgaris* як перспективне джерело вітамінів $B_{12}$ та $D_2$

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**Анотація.** Актуальність даного дослідження обумовлена необхідністю узагальнити сучасні знання щодо механізмів накопичення вітамінів $B_{12}$ і $D_2$ у *Chlorella vulgaris* та факторів, що визначають їхню біохімічну варіативність у культурах мікроводоростей. Метою роботи було систематизувати дані літератури за 2020-2025 роки та визначити ключові чинники, що регулюють стерольний метаболізм і вітамінний профіль *Chlorella vulgaris*. Дослідження базувалося на методах цільового інформаційного пошуку, контент-аналізу, критичного порівняння та якісного синтезу результатів попередніх наукових робіт. На основі аналізу джерел було показано, що тип живильного середовища визначав рівень мікронутрієнтів у біомасі *Chlorella vulgaris*. Мінеральні середовища забезпечували стабільний ріст, але не містили кобаламіну (вітаміну $B_{12}$), тоді як органічні субстрати – печінковий екстракт, молочна сироватка, пермеати та анаеробні дигестати – демонстрували вищий потенціал збагачення клітин завдяки природним коферментам, білкам і мікроелементам. Показано, що іони кобальту ($Co^{2+}$) сприяли більшому накопиченню кобаламіну в біомасі, а магній ($Mg^{2+}$), цинк ($Zn^{2+}$), залізо ($Fe^{2+}/Fe^{3+}$) і мідь ($Cu^{2+}$) виступали важливими кофакторами ферментів та транспортних систем, які впливали на стерогенез. Щодо вітаміну $D_2$ (ергокальциферолу), літературні дані підтвердили вирішальну роль ультрафіолетового випромінювання діапазону В (UV-B), яке ініціювало фотоконверсію ергостеролу. Інтенсивність та спектральний склад освітлення визначали швидкість накопичення стеролів, тоді як солоність, дефіцит азоту чи фосфору та інші стресові чинники сприяли посиленню ліпідогенезу й змінам стерольного пулу. Встановлено, що у стаціонарній фазі культури ліпідні та стерольні фракції накопичувалися інтенсивніше, ніж у період активного поділу клітин. Узагальнений аналіз джерел показав, що найбільш ефективне збагачення *Chlorella vulgaris* вітамінами $B_{12}$ і $D_2$ досягається за умови поєднання органічних живильних середовищ, оптимізованого спектра освітлення з UV-B компонентом та контрольованого мінерального складу середовища

**Ключові слова:** кобаламін; ергокальциферол; органічні екстракти; спектр світла; ергостерол; іони металів; стресові фактори