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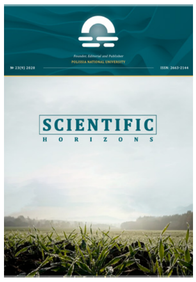

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Production of *Bacillus subtillis* protein mass on the microbial mass of *Methylococcus capsulatus*

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Abstract. The growing demand for food of animal origin has contributed to the annual increase in the agricultural population of productive animals in the world, which in turn has led to a significant shortage of feeds and an increase in their cost. Therefore, the purpose of this research was to explore the qualitative composition of feed products obtained by culturing methanotrophic microorganisms *Methylococcus capsulatus* with probiotic bacteria *Bacillus subtillis*. Arbitrage methods for determining the quality of feeds, which are regulated by regulatory documents (ISO), were used in the research. The results of the research demonstrate that the protein product obtained by growing *Methylococcus capsulatus* culture has a high content of "crude" protein at the level of 60.9%, which corresponds to high-protein feeds of animal origin by its technical characteristics. The addition of probiotic microorganisms *Bacillus subtillis* to the medium for cultivation significantly improved the qualitative parameters of the synthesised protein by increasing by 1.5% the amount of essential amino acids, increasing by 3.8% fatty acids and increasing the concentration of calcium and phosphorus. The increase in the concentration of lysine, isoleucine, valine and asparagic acid, due to the co-cultivation of two microorganisms allowed increasing their nutritional value for cattle since these amino acids are the most demanded in ruminant diets when using corn silage and other plant feeds. The addition of *Bacillus subtillis* culture allowed reducing the content of "crude" fibre and improving its biological characteristics by changing the microbiological composition of the obtained product. In the future, the biotechnological method of obtaining feed protein for animals will reduce the dependence of animal breeding on the cultivation of fodder crops and weather conditions

Keywords: methanotrophs; microbial culture; probiotic culture; co-cultivation; feed product; amino acid composition

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INTRODUCTION

The growth of food consumption is closely connected with the increase in the world population, as a result of which agriculture has developed intensively in recent years. The demand for meat and dairy products is growing most intensively. High demand for food of animal origin contributes to the increase in the number of livestock and their productivity. S.A. Terry *et al.* (2020) indicate that cattle, compared to other domestic animals, have the lowest efficiency of meat production, despite the highest demand for this particular type of product. Therefore, one of the urgent directions of future livestock production will be the intensification of beef cattle breeding to meet the growing demand of the population. This shortly, is possible only through extensive growth of the population of animals of meat breeds. However, according to S.R. Fariña and P. Chilibroste (2019), in most developed countries such population growth will be artificially limited through existing environmental regulations and limited land resources. However, the main constraint is feed scarcity.

According to M.R.C. Cordeiro *et al.* (2022), increased livestock production will increase global demand for feed from 11 to 17% for food crops and from 6 to 15% for feed crops. Therefore, rapid and uncontrolled growth in animal numbers will lead to a situation where feed security becomes critical to meet future global meat demand and any increase in livestock production will only occur if sufficient feed is available. The Food and Agriculture Organisation (FAO) research (2023) indicates that an increase in the share of feed crops such as maize and soya in the world crop mix should be expected shortly. Only for the grain group FAO (2023) forecasts indicate an annual growth of 0.3-0.5% per year in the required volumes of feed grains for animal feed, which in physical equivalent becomes 783-1000 million tonnes per year. It will cause an increase in the area of arable land used for fodder production and, accordingly, a decrease in the area of land for food crops. And considering the annual increase in the number of animals, the situation will only worsen. Even countries with large areas of land suitable for agricultural production are experiencing shortages of animal feed.

Kazakhstan is no exception: the availability of roughage in 2023 is only 80%, succulent fodder 60% and concentrated fodder 50% of the need (The Ministry of Agriculture…, 2023). To ensure intensive development of livestock production, new approaches to protein feed production will have to be found and utilised more efficiently. Such a source, according to A.U. Fabiszewska *et al.* (2019) a biotechnological method of processing industrial and food wastes using microorganisms can become such a source. This method will allow accumulating of protein compounds due to the growth of microbial biomass. The most promising area is using methane-oxidising microorganisms as protein producers, which will allow, in addition to solving the problem of the lack of feed resources, to solve environmental problems associated with the emission of greenhouse gases by animals.

The intensification of production and the growth of the animal population have become one of the reasons for the increase in greenhouse gas emissions, which are leading to an ecological disaster on the planet. According to H. Guo *et al.* (2022) global greenhouse gas emissions from livestock production have increased by 51%, and methane accounts for 50% of total emissions. Ruminants are the largest producer of greenhouse gases, accounting for about 72% of the livestock sector, of which one-third is the highly hazardous methane gas (Gerber *et al.*, 2013).

Therefore, the purpose of this work was to develop an industrial biotechnological method that allows using live microorganisms with methane as a nutrient medium to produce protein mass suitable as feed in livestock production.

LITERATURE REVIEW

Biotechnological approaches have been used in forage production for a long time. They have been applied according to R. Kapoor *et al.* (2018) and M.A. Moselhy *et al.* (2022) in the preparation of green forages and their conservation for year-round use as mono-feed. R.R. Singhania *et al.* (2017) and S. Bala *et al.* (2023) proposed biotechnological approaches for utilisation of animal farm wastes, while R.P. Singh *et al.* (2022) and K.C.P. Cruz *et al.* (2022) conducted studies for microbiological synthesis of farm animal feed additives with therapeutic and preventive effects. However, research on using microbial synthesis products for animal nutrition using animal wastes themselves as nutrient media has become particularly relevant. The works of K. Rasool *et al.* (2023) and also A.E. Graham and R. Ledesma-Amaro (2023) indicate that microbially synthesized protein can be used without restrictions in animal feeding, and the amino acid composition of such products is more complete in comparison with plant proteins. B. Bajić *et al.* (2023) indicate that considering the composition and characteristics, and the huge amount of waste in livestock production, this approach to feed protein production has great potential for industrial production.

Environmental conditions have a significant influence on the yield of microbial protein during cultivation. S. Ismail (2022) identified the influence of temperature, pH level, and nitrogen and carbon sources on the yield of protein and nucleic acids in the cultivation of *Cupriavidus necator* bacteria. Using methane as a carbohydrate and energy source for biotechnological processes is described in M.L.K. Khider *et al.* (2021). Methanotrophs, unlike industrial microorganisms, do not compete for food, which is quite suitable for growing animal feed biomass. Similar conclusions were reached by P. Tsapekos *et al.* (2018), who demonstrated that the resulting microbial biomass is suitable for use as an animal feed additive. A. Kuźniar *et al.* (2019) found that methanotrophic biomass contains high concentrations of K, Mg and Fe in addition to protein compounds, and it can be considered as a feed additive for enriching diets with protein, macronutrients and some trace elements.

Using microbial protein in diets of different types of productive animals can be traced in scientific works published recently, indicating the relevance of such research worldwide. The work of S. Polyorach *et al.* (2023) found no adverse effect of adding microbiological protein to the diets of dairy cows on their productivity compared to traditionally used vegetable proteins. Therewith, the researchers observed an increase in the level of diet digestibility ($p>0.05$) with the addition of biotechnological products. Similar results were obtained by E. Direkvandi *et al.* (2020) in research on sheep. The addition of microbial biomass to the diet had no effect on feed intake and live weight of lambs, but the positive result was a reduction in the methanogen population in the rumen and a reduction in greenhouse gas emissions. In addition to ruminants, studies have demonstrated positive results when feeding poultry E.G. Olson *et al.* (2022), hydrobionts-American eel W. Lu *et al.* (2023), and black sea bream B. Xu *et al.* (2021).

As reported by B. Valverde-Pérez *et al.* (2020), to date, the industrial production of protein compounds from methane is limited due to the explosive nature of the media in fermenters during production and needs further improvements in technology.

MATERIALS AND METHODS

Studies were conducted using the culture of methanotrophic bacteria *Methylococcus capsulatus*. For the cultivation of microorganisms, a bioreactor with a volume of 40 litres (working volume 25 litres), equipped with a stirrer with a speed of 3500 thousand revolutions per minute and having an operating pressure of up to 5 atmospheres was used. Methanotrophic microorganisms were cultured on depleted mineral nutrient media, which reduces the risk of contamination by pathogenic and opportunistic microorganisms. Bacteria *Methylococcus capsulatus* were grown on nutrient medium prepared according to the following recipe: KCl – 0.125 g/l, $MgSO_4 * 7H_2O$ – 0. 125 g/l, $FeSO_4 * 7H_2O$ – 10.75 mg/l, $CuSO_4 * 5H_2O$ – 10 mg/l; $MnSO_4 * 5H_2O$ – 9.5 mg/l, H_3BO_3 – 6.25 mg/l, $ZnSO_4 * 7H_2O$ – 1.5 mg/l, $Na_2MoO_4 * 2H_2O$ – 0.25 mg/l, $CoCl_2 * 6H_2O$ – 0.25 mg/l, H_3PO_4 (70%) – 0.35 mg/l. The PH value of this medium was maintained with 10% aqueous ammonia solution. Microbial biomass accumulation was conducted at 42°C and pH 5.8 for 48 hours.

To reduce the concentration of carbon dioxide in the fermenter, which in high concentration inhibits the growth of methanotrophic bacteria, a culture of *Chlorella vulgaris* was used. The gas phase separated in the degassing chamber was passed through the bioreactor with growing Chlorella before being returned to the bioreactor to reduce the carbon dioxide concentration. To improve the quality of protein products obtained by microbiological synthesis, after the end of *Methylococcus capsulatus* cultivation, *Bacillus subtillis* mother liquor was added to the fermenter in the amount of 5% by volume and cultivation was continued for another day at 38-40°C.

After completion of the cultivation process, a medium sample was taken from the fermenter. The resulting cell biomass was precipitated by centrifugation (4000 rpm, 15 min), and the cell precipitate was further dried at constant temperature. All subsequent results were obtained by dry matter examination. The obtained product was subjected to laboratory research for the purpose of determining a number of physicochemical parameters and nutritional value of the synthesised microbiological biomass. The content of:

- "crude" protein by Kjeldahl method (VELP Scientifica equipment set) (ISO 20588:2019, 2019);
- "crude" fat by Soxhlet method (ISO 1099:2010, 2010);
- "crude" fibre by extraction method (ISO 6865:2000, 2000);
- "crude" ash by ashing in a muffle furnace (ISO 5984:2022, 2022);
- amino acid content was determined using an Agilent 1260 Infinity II amino acid analyser (ISO 13903:2005, 2005);
- calcium – by titrimetric method (ISO 6490-1:1985, 1985);
- phosphorus – by spectrometric method (ISO 6491:1998, 1998).

The obtained research results were subjected to mathematical processing and grouping using Excel software product included in the Microsoft 365 core application package.

RESULTS

The main area of research, the results of which are presented in this research, was to obtain alternative sources of protein feeds for livestock farming, using wastes obtained as a result of animal housing or products of their vital activity. Therefore, it was decided to use methane as a source of nutrients for microbiological synthesis, based on its availability, cost and the need to utilise it as an associated gas resulting from the maintenance of ruminants, which in turn will reduce the impact of greenhouse gases on the environmental situation. A culture of the methanotrophic bacteria *Methylococcus capsulatus*, which is capable of consuming 85 to 90% of the methane dissolved in special nutrient media, was used for methane utilisation. An additional positive factor that contributed to the choice of these microorganisms as an object of research was their relatively high incubation temperature (from +38 to +48°C), which reduces the risk of contamination of the obtained biomass with pathogenic and opportunistic microorganisms. Microorganisms causing fodder and other diseases in cattle have a lower cultivation temperature. Such characteristics of the selected microbiological culture guarantee the absence of infectious disease development in animals after using microbiological protein in diets.

As a gas nutrient mixture for the cultivation of methanotrophic bacteria *Methylococcus capsulatus*, the most favourable for their growth mixture of methane and oxygen with an oxygen content of 15% was used. Experimentally selected cultivation conditions based on the partial pressure of gases and their solubility, and mineral nutrient medium allowed obtaining microbial biomass of *Methylococcus capsulatus* with a concentration of 7-8 g/l of culture medium.

Although the results of microbial biomass growth of *Methylococcus capsulatus* under experimental conditions were at a high level, the selection of possible symbiotic microorganisms was performed to increase the productivity and growth rate of methanotrophic bacteria. The reasons for this approach were the findings of S. Golaghaiee *et al.* (2017), who managed to increase the yield of "crude" protein by co-culturing methanotrophic bacteria with microscopic fungi, and their report on the improvement of quality parameters of the resulting protein synthesis for animals. The culture of microorganisms that allowed increasing the efficiency of protein raw material synthesis were opportunistic fungi of the *Candida* genus. Considering that according to the conditions of the research programme, it is not recommended to use pathogenic or disease-causing microorganisms in the process of scientific work, therefore, using these symbionts or fungi with similar properties was not conducted. In this regard, it was decided to increase the quantity and quality of synthesised protein from one of the probiotic cultures that can develop in thermophilic conditions – *Bacillus subtilis* strain, which processes methanotrophs into protein raw material with positive feed characteristics. The absence of information about diseases caused by microorganisms of *Bacillus subtilis* strains or products of their vital activity allows considering them as safe organisms, which, moreover, have been identified as promising producers of probiotic compounds. This strain is actively cultured on metabolites of *Methylococcus capsulatus* and their microbial biomass, biotransforming them into its cell mass and contributing to the improvement of the quantity and quality of microbial protein. The properties of protein biomass obtained by biotechnological cultivation of *Bacillus subtilis* are well-explored, they have no contraindications for use as animal feed additives and additionally have probiotic properties.

For the purpose of increasing the quantity and quality of protein biomass, after the end of *Methylococcus capsulatus* cultivation time, *Bacillus subtillis* mother liquor was added to the fermenter in the amount of 5% of the volume and continued cultivation for a day at 38-40°C. The results of chemical composition of protein raw materials obtained under different variants of methanotrophs cultivation are presented in Table 1.

Table 1. *Chemical composition of microbiological biomass obtained as a result of cultivation of Methylococcus capsulatus and co-cultivation of Methylococcus capsulatus + Bacillus subtillis, %*

Name of indicator	*Methylococcus capsulatus*	*Methylococcus capsulatus + Bacillus subtillis*
Crude protein	60.89	55.67
Total moisture	7.24	13.02
Crude fat	0.59	4.36
Crude fiber	1.98	1.25
Crude ash	7.97	9.56
Nitrogen-free extractive substances (calculation method)	23.31	17.38
Calcium	0.6	1.04
Phosphorus	0.06	0.39

Source: *compiled by the authors*

The "crude" protein yield of *Methylococcus capsulatus* monoculture was 5.2% higher than that of co-cultivation with the probiotic bacteria *Bacillus subtillis*. However, despite the insignificant decrease in protein content, the quality of the obtained feed product was significantly higher when the two microorganisms were co-cultured. It was accompanied by a decrease in the amount of nucleic acids in the protein fraction by 14.4%, and an increase in the amount of "crude" fat and ash in the dry matter of microbial biomass, due to an increase in the concentration of macronutrients – calcium and phosphorus. It, in turn, allowed reducing the cost of purchasing mineral feed for animals when balancing rations.

In addition, when *Methylococcus capsulatus was co-cultured with Bacillus subtillis,* a decrease in the proportion of nitrogen-free extractives (NFE) was observed, which was associated with the replacement of

Gram-negative methanotrophic microorganisms, whose cell wall is covered with a protective layer of polysaccharides, by more digestible Gram-positive *Bacillus subtillis*. This assumption is confirmed by the decrease in the amount of "crude" fibre in the bacteriological mass, which indicates a change in the species composition of the microbial product and a decrease in the amount of structural (cellular) carbohydrates. In addition to the replacement of part of cells in the dry product of cultivation of one species of microorganisms for another, a significant increase in the quality of protein associated with the enzymatic activity of symbiotic bacteria, whose enzymes contribute to the destruction of the cell wall of *Methylococcus capsulatus*. Therefore, even against the background of a lower level of "crude" protein in the microbiological product during the joint incubation of methanotrophs and symbiotic bacteria, the protein quality appeared to be more nutritious and suitable for animal feeding. To explore protein quality in more detail under different cultivation options for methanotrophs, amino acid analysis of proteins was performed and the results are presented in Table 2.

Table 2. *Amino acid profile of "crude" protein obtained as a result of different variants of cultivation of methanotrophic bacteria Methylococcus capsulatus, %*

Name of indicator	*Methylococcus capsulatus*		*Methylococcus capsulatus + Bacillus subtillis*	
	Content in the product	Protein content	Content in the product	Protein content
Substituted amino acids				
Asparagic acid (Asp)	6.32	10.36	6.67	11.97
Threonine (Thr)	3.32	5.44	2.62	4.7
Serine (Ser)	1.9	3.11	1.71	3.07
Glutamic acid (Glu)	9.43	15.45	8.30	14.9
Proline (Pro)	2.78	4.55	1.51	2.71
Glycine (Gly)	4.03	6.6	3.18	5.71
Tyrosine (Tyr)	2.29	3.75	1.7	3.06
Alanine (Ala)	4.94	8.09	4.74	8.51
Cystine (Cys)	0.44	0.72	0.57	1.02
Arginine (Arg)	3.67	6.02	2.51	4.51
Valine (Val)	3.36	5.51	3.87	6.95
Total number of substituted amino acids	42.48	69.6	37.38	67.11
Essential amino acids				
Methionine (Met)	1.31	2.14	1.25	2.24
Isoleucine (Iie)	2.35	3.85	2.66	4.78
Leucine (Leu)	4.82	7.9	3.84	6.89
Phenylalanine (Phe)	2.89	4.74	2.02	3.63
Histidine (His)	1.14	1.89	0.8	1.44
Lysine (Lys)	3.24	5.31	4.66	8.37
Total amount of essential amino acids	15.75	25.83	15.23	27.35

Source: *compiled by the authors*

The amino acid composition of microbial raw materials obtained in different variants of microbial cultivation indicates some differences in the content of individual amino acids. Thus, a significant decrease of arginine and glutamic acid was observed in the final product in the co-synthesis of methanotrophs with *Bacillus subtillis* compared to the cultivation of *Methylococcus capsulatus*. Whereas for other substitutable amino acids, their levels were at the same level or not significantly higher than the protein obtained from the initial synthesis. The introduction of 5% hay bacillus culture into the substrate for cultivation allowed increasing the concentration of most essential amino acids, which are limited in protein compounds of grain crops (wheat, barley, maize). The greatest increase was observed in the concentration of lysine, isoleucine, valine, and asparagic acid. These amino acids are the most demanded amino acids in ruminant diets when using maize silage. Therefore, the addition of *Bacillus subtillis* in the industrial production of microbial protein will allow obtaining a feed product maximally suitable for cattle.

In the process of primary cultivation of *Methylococcus capsulatus* microorganisms, a significant amount of carbon dioxide is produced, which in small concentrations stimulates the growth of methanotrophic bacteria and completely inhibits their cultivation at high concentrations. Biosynthesis produces about 3.5 litres of carbon dioxide from 10 litres of methane. The removal of the gas phase from the bioreactor used in the research is done in a degassing chamber, due to the pressure difference. After analysing the concentration of ambient gases, the necessary additional injection of methane and oxygen up to the specified values was pumped back into the bioreactor.

All known methods of cultivation of methanotrophic bacteria provide, as a prerequisite, the need to remove carbon dioxide from the gas-air mixture of the bioreactor. It is achieved either by chemical methods, which significantly affects the final cost of the product; or by discharging unspent methane into the atmosphere together with oxygen and carbon dioxide. Therewith, several microorganisms require carbon dioxide for growth, *Chlorella vulgaris* being one such species. In the improved model of the bioreactor, the purification of the gas mixture from carbon dioxide is performed by cultivation of Chlorella algae on carbon dioxide, which is produced by methanotrophic organisms as a waste. The gas phase separated in the degassing chamber passes through a tank with growing chlorella before being returned to the bioreactor, where it is cleared of carbon dioxide and saturated with oxygen. In preliminary studies conducted on an additional source of protein animal feed derived from the accumulation of microbial biomass of the algae *Chlorella vulgaris*. more than 700 g of oxygen was obtained from one kilogram of carbon dioxide, allowing this approach to be used in biotechnological production. As a result of this improvement, atmospherically harmful carbon dioxide emissions were utilised to grow valuable plant protein and generate oxygen for the cultivation of methanotrophic bacteria. Therefore, future studies are planned to explore the possibility of obtaining additional feed protein from *Chlorella vulgaris* biomass and to analyse the qualitative and quantitative characteristics of this product.

In addition, future research is planned to explore the possibility of stimulating microbial growth by increasing or decreasing the concentration of individual ions that are the foundation of the mineral nutrient medium and obtaining the optimal concentration of macro- and microelements in the microbial biomass for animal feeding. In this block of research is planned to explore the influence of trivalent iron and calcium ions, and the introduction of these metals in the form of different salts of sulphates, phosphates or in the form of chlorides. A separate area of research will be the examination of the effect of different concentrations of copper salts on the activity of enzyme systems of *Methylococcus capsulatus* and the rate of methane oxidation.

DISCUSSION

The main trend of the 2015-2023 years in agricultural production in the world is the annual growth of animal population with a relative constancy of areas under fodder crops. This situation forces either to search for fodder crops with higher yields or to search for alternative sources of fodder for the growing needs of animal husbandry. The most promising method of obtaining high-grade protein feeds for animals is industrial biotechnological approaches using microbiological synthesis. In addition to the main purpose – to obtain dry microbial biomass, which can replace part of grain feed in the diet of farm animals, and thereby improve the situation with food safety, another positive factor of such production is to reduce the adverse impact of animal husbandry on the ecological situation of the planet. Using the wastes accumulated during animal housing and products of their vital activity in the process of microbiological production of fodder protein contributes to increasing the profitability of animal husbandry and reducing the environmental impact on nature.

Based on the analysis of scientific publications and preliminary studies, the most promising method appeared to be using methanotrophic bacteria as protein biomass producers, which combines high-intensity bacterial mass synthesis with using one of the most dangerous greenhouse gases – methane – as a nutrient. Therefore, similar conclusions were reached by several other authors: B. Valverde-Pérez *et al.* (2020), exploring the products of microbiological synthesis of methanotrophic bacteria, P.A. Zenkovich *et al.* (2022), when feeding Siberian sturgeon with dry feed obtained from the cultivation of *Methylococcus capsulatus*, and M. Rajesh *et al.* (2022) in a study conducted on rainbow trout individuals. All authors noted positive growth dynamics of fish when feed products obtained from microbiological synthesis were introduced into their diet. These additives were found to be on par with fish meal in terms of nutritional quality. In Kazakhstan, biotechnological approaches using methanotrophic microorganisms are only at the stage of research and implementation in limited volumes in industrial production, while in other countries these methods are already in full use mainly for wastewater treatment. R. Salem *et al.* (2021) indicate that wastewater treatment has yielded important products such as ectoine, polyhydroxyalkanoates and methanobactins, which are actively used in biotechnology and biomedicine.

Using methanotrophic bacteria *Methylococcus capsulatus* for industrial production of feed protein for ruminants using biotechnological methods was positively confirmed in the studies described in this publication. As a result of the laboratory experiment, an average of 7-8 g of dry microbial protein was obtained from each litre of nutrient medium. Therewith, the feed product was characterised by an increased content of essential amino acids and basic macronutrients. In addition, the results obtained were confirmed by the publication of A. Kuźniar *et al.* (2019), indicate that as a result of the cultivation of methanotrophic bacteria of *Methylocystis* and *Methylosinus* species, the dry biomass of microorganisms contained high concentrations of potassium, 9.6-19.1, magnesium, 2.2-7.6, and iron, 2.4-6.6 g/kg, and it could be considered as a feed additive for the enrichment of animal diets with macronutrients and some trace elements. In addition, this mass had a high content of unsaturated fatty acids in its composition.

E.O. Sadykova *et al.* (2023) report that their studies of comparative evaluation of the amino acid profile of *Methylococcus capsulatus* protein demonstrated a balanced content of most amino acids, the level of which was comparable to chicken egg protein, which is a conventional benchmark of high-quality complete protein. All these results allow confirming with certainty that the *Methylococcus capsulatus* culture selected as a producer of protein biomass can be used for the industrial production of feed for animals of different species.

However, the industrial use of such a method of microbiological synthesis, according to X. Huang *et al.* (2022), is limited by the relatively low efficiency of carbon processing by methanotrophic cells. For this purpose, the studies conducted utilised symbiotic microorganisms, in particular the culture of the probiotic bacteria *Bacillus subtillis*. It was possible due to preliminary studies conducted by A.G. Kisten *et al.* (2022) on solid nutrient media. In this work, it was indicated that the activity of methane utilisation began to increase on the 2nd-4th day after the co-cultivation of microorganisms. Within the framework of the conducted research, *Bacillus subtillis* culture was used under the conditions of a mineral medium, which allowed obtaining positive results of symbiotic use of two microbiological cultures. This approach allowed microbiological mass production and improved the quality of the obtained fodder product. In comparison with the monocultivation of *Methylococcus capsulatus*, the addition of symbiont culture allowed, on the one hand, to increase the content of "crude" fat by 3.8% and limited essential amino acids by increasing the share of lysine, isoleucine and glutamic acid. On the other hand, this approach allowed reducing the amount of "crude" fibre and increasing its digestibility by changing the ratio of bacterial composition of the finished product. The increase in microbial biomass yield and quality of the obtained protein was also described by J.J. Nunes *et al.* (2016) when methanotrophs were co-cultured with heterotrophic bacteria *Alcaligenes acidovorans*, resulting in a 265% increase in biomass with a cell concentration of 10.3 g/l compared to 2.8 g/l in monoculture. Heterotrophic bacteria did not grow independently on mineral media with methane addition but increased biomass concentration when added to *M. capsulatus* culture.

No less dangerous than methane greenhouse gas is carbon dioxide, which is actively produced by methanotrophic microorganisms in the process of their cultivation (Zheng *et al.*, 2023). Modern production facilities do not allow the utilisation of carbon dioxide produced as a result of methane oxidation. It is in most cases released into the atmosphere, and the processing of 10 litres of methane produces 3-3.5 l CO_2 in the environment. Preliminary studies conducted within the framework of the research work programme to explore the effect of adding a tank with microscopic algae of *Chlorella vulgaris* species to the fermentation system allowed reducing both the amount of carbon dioxide emitted and increasing the level of effective carbon utilisation. Thus, when one kilogram of CO_2 was utilised, up to 700 g of oxygen was released into the environment, which was used for further oxidation of methane. Thus, such a minor modernisation of industrial feed protein production will allow obtaining an autonomous system without the need to control the gas mixture in the bioreactor and additional oxygen pumping. It, in turn, will allow reducing the cost of production of biotechnological feed products.

Future studies are planned to investigate the growth of microbiological biomass of algae and the quality of such a product as animal feed. In studies by N. Roberts *et al.* (2020) in the joint cultivation of *Chlorella sorokiniana* with methanotrophic bacteria *Methylococcus capsulatus* in comparison with microalgae monoculture, it was possible to obtain an increase in biomass production by 120%, improved assimilation of total nitrogen and total phosphorus by 71 and 164%, respectively, with the same amount of biogas produced. Therefore, in future studies, it is expected that in addition to the autonomy of the biotechnological system, there will be an additional increase in dry animal feed products. In addition, several studies are planned shortly to explore the influence of the concentration of different constituent elements of mineral nutrient medium for the cultivation of methanotrophs and the addition of copper ions, which can increase the activity of microbial enzyme systems responsible for methane oxidation.

CONCLUSIONS

Based on the results obtained during the experimental work on the cultivation of methanotrophic microorganisms *Methylococcus capsulatus and their co-cultivation with probiotic bacteria Bacillus subtillis* to obtain protein biomass for ruminant feeding, the following conclusions and suggestions for future scientific work can be made: using of *Methylococcus capsulatus* culture on depleted mineral medium in methane-oxygen atmosphere allowed obtaining microbial biomass yield with cell concentration of 7-8 g/l of nutrient medium and content of "crude" protein at the level of 60.9%, which corresponds to the indicators of high-protein animal feeds and significantly exceeds the protein feeds of plant origin.

The addition of the probiotic bacteria *Bacillus subtillis* to the bacterial culture of *Methylococcus capsulatus* in the bioreactor allowed improving the quality characteristics of the feed product obtained as a result of the initial microbial synthesis. It was expressed in an increase in the amount of essential amino acids by 1.5%, "crude" fat by 3.8% and the main macronutrients – calcium and phosphorus. Therewith, due to the partial replacement of the microbiological composition of the product, the content of "crude" fibre decreased and its biological value increased. The inclusion of a tank with *Chlorella vulgaris* microalgae culture in the fermenter design allowed reducing the concentration of carbon dioxide in the bioreactor gas mixture and saturating it with oxygen. In the process of algae life activity, more than 700 grams of oxygen were obtained from one kilogram of carbon dioxide, which will allow establishing an autonomous bioreactor without the need for constant monitoring of carbon dioxide levels.

In future studies, it is planned to conduct a comprehensive study of the qualitative characteristics of the feed product obtained by the cultivation of *Methylococcus capsulatus* with symbiotic cultures of *Bacillus subtillis* and *Chlorella vulgaris*. In addition, to examine the effect of different concentrations of media components on the quantitative characteristics of the feed product and the possibility of activation of the enzyme system of *Methylococcus capsulatus* by increasing the concentration of copper ions in the nutrient medium.

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CONFLICT OF INTEREST

None.

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Отримання білкової маси *Bacillus subtillis* на мікробній масі *Methylococcus capsulatus*

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Анотація. Зростаючий попит на харчові продукти тваринного походження сприяв щорічному збільшенню чисельності сільськогосподарської популяції продуктивних тварин у світі, що, в свою чергу, призвело до суттєвого дефіциту кормів та зростання їх вартості. Тому метою роботи було дослідження якісного складу кормових продуктів, отриманих шляхом культивування метанотрофних мікроорганізмів *Methylococcus capsulatus* з пробіотичними бактеріями *Bacillus subtillis*. Під час проведення досліджень використовували арбітражні методи визначення якості кормів, які регламентуються нормативними документами. Результати досліджень свідчать, що білковий продукт, отриманий при вирощуванні культури *Methylococcus capsulatus*, має високий вміст «сирого» протеїну на рівні 60,9 %, що за своїми технічними характеристиками відповідає високобілковим кормам тваринного походження. Додавання в середовище для культивування пробіотичних мікроорганізмів *Bacillus subtillis* дало змогу значно поліпшити якісні показники синтезованого білка за рахунок збільшення на 1,5 % кількості незамінних амінокислот, підвищення на 3,8 % жирних кислот і зростання концентрації кальцію та фосфору. Збільшення концентрації лізину, ізолейцину, валіну та аспарагінової кислоти внаслідок спільного культивування двох мікроорганізмів дало змогу збільшити їхню поживну цінність для великої рогатої худоби, оскільки ці амінокислоти є найзатребуванішими в раціонах жуйних тварин під час використання кукурудзяного силосу та інших рослинних кормів. Додавання культури *Bacillus subtillis* дало змогу знизити вміст «сирої» клітковини та поліпшити її біологічні характеристики за рахунок заміни мікробіологічного складу отриманого продукту. Використання в подальшому біотехнологічного методу отримання кормового білка для тварин дозволить знизити залежність тваринництва від вирощування кормових культур і погодних умов

Ключові слова: метанотрофи; мікробна культура; пробіотична культура; співкультивування; кормовий продукт; амінокислотний склад