# Biotechnological production of protein feeds from alcohol industry waste

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**Abstract**. The research is devoted to the development and implementation of a biotechnological methodology for the production of enriched protein feeds from secondary grain products, namely post-alcoholic bard and fermentolysate of substandard grain. The study included selection of a consortium of anaerobic microorganisms capable of efficient processing of these substrates, optimisation of fermentation conditions, as well as laboratory and pilot tests. The obtained feed products were characterised by a high protein content (45-47%), a balanced amino acid composition, including essential amino acids, and the presence of biologically active substances such as vitamins and probiotics. The fermentation process achieved a high degree of utilisation of organic components, reducing the negative environmental impact. The developed technology demonstrated competitive performance compared to traditional methods of feed production and alternative biotechnological approaches. The results obtained confirm the promising use of secondary raw materials for the production of high-quality and economically favourable fodder products.

## 1 Introduction

Currently, there are several potential ways to solve the problem of forage deficiency and quality [1]. One of the directions is genetic modification of fodder crops, which allows increasing their yield and nutritional value. However, this method raises a number of concerns related to possible environmental risks and impact on human and animal health. Another approach is to use various chemical additives and growth stimulants, but their use also has its limitations and may lead to undesirable side effects. The development of resource-saving technologies in traditional agriculture is an important but not always sufficient solution [2-4]. The most promising direction, in the context of solving several problems at once, is the development and implementation of biotechnological methods for processing waste and secondary resources to obtain feed protein products [5].

This direction is relevant and important, as it offers a comprehensive solution that combines economic efficiency, environmental safety and high quality of the obtained products. The use of secondary raw materials, such as waste from the alcohol industry, not only reduces the cost of feed, but also solves the problem of waste disposal, reducing the burden on the environment [6]. Biotechnological methods based on the use of specially selected microorganisms make it possible to obtain products enriched with protein, amino acids, vitamins and probiotic substances, which significantly increases their nutritional value. Moreover, this approach promotes the development of closed production cycles, where waste from one process becomes a resource for another, which is an important aspect of sustainable development [7].

The relevance of this direction is confirmed by the need to intensify the production of livestock products while reducing production costs and minimising the negative impact on the environment [8]. Thus, the development and implementation of technologies for obtaining enriched protein fodder products from wastes of the alcohol industry using biotechnological methods is an extremely promising and in demand direction.

In this regard, the aim of the work is to develop and implement biotechnology for the production of enriched protein fodder products based on post-alcoholic bard and fermentolysate of substandard grain, using a consortium of anaerobic microorganisms [9]. This work is aimed at solving the problem of feed deficit, waste utilisation and increasing the efficiency of agricultural production.

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## 2 Method and materials

The work presented in this article includes a set of experimental studies aimed at the development and validation of biotechnology for the production of enriched protein feed products. The general plan of the work consisted of several stages: first, the selection and selection of a consortium of microorganisms capable of efficient processing of alcohol industry was carried out; second, the optimisation of fermentation conditions was carried out, including the selection of the optimal composition of the nutrient medium, temperature, pH and other parameters; third, laboratory studies were carried out to assess the composition and nutritional value of the obtained feed products; fourth, pilot tests were carried out at the distillery to test the efficiency of the production of enriched protein feed products.

A range of specialised laboratory and technological equipment was used to implement these stages. At the initial stage of selection and breeding of microorganisms, standard microbiological methods were used, including the use of thermostats for cultivation of microorganisms, laminar boxes for work under sterile conditions and microscopes for visual inspection of cultures. For cultivation of microorganisms and fermentation, fermenters of different volumes equipped with automatic control and regulation systems for temperature, pH and agitation were used. There is no indication of a specific fermenter model in the article, but we are talking about standard laboratory apparatuses for cultivation under controlled conditions.

In the process of conducting experiments to optimise fermentation conditions, various analyses were carried out. Spectrophotometers were used to determine the concentration of nutrients such as proteins, amino acids, fats and carbohydrates. Determination of acidity of the medium was carried out using pH meters. The composition and concentration of amino acids, minerals and vitamins in the obtained feed products were determined by high-performance liquid chromatography. Analysis of microbiological composition was carried out on microscope and by sowing on nutrient media, with subsequent counting of colonies.

During pilot tests at the distillery, large volume industrial fermenters were used to carry out the fermentation process, and separators and filter presses were used to separate the biomass from the culture liquid. Subsequent drying of the obtained product was carried out using drying equipment such as spray drying. All technological processes were accompanied by regular sampling and analysis to control the process parameters and quality of the obtained feed product. The operating modes of the equipment at each stage were optimised to achieve maximum yield of the target product at minimum resource consumption.

## 3 Results and discussion

The research was aimed at the development and implementation of a biotechnological technique for the production of feed protein from secondary grain products, such as post-alcoholic bard and fermentolysate of substandard grain. At the beginning of the work, a thorough screening and selection of strains of anaerobic microorganisms that had the ability to efficiently utilise organic substances contained in the studied substrates was carried out [10]. The isolated and selected strains were combined into a consortium, which was then used in the fermentation process. The main stage of the work was the determination of optimal conditions for fermentation, which included the selection of the temperature regime, maintenance of optimal pH, ensuring adequate aeration, although in our case we are talking about anaerobic processes and selection of the composition of the nutrient medium. The studies were carried out both in laboratory conditions and in pilot production conditions. In laboratory conditions, fermenters of small volume were used, where the initial testing of technological parameters took place and the influence of various factors on the productivity of the consortium of microorganisms was studied. After that, to check the scalability of the process, the technology was transferred to a pilot plant of a distillery, where fermenters of industrial volume and other technological equipment for the separation of biomass and liquid fraction were used. At each stage of the research a thorough analysis of the composition and quality of the obtained feed products was carried out, including the determination of the content of protein, amino acids, vitamins and minerals. Also, special attention was paid to the analysis of the microbiological composition of the final products to verify their safety and the absence of pathogenic microflora.

As a result of the research it was found that a specially selected consortium of anaerobic microorganisms showed high efficiency in the processing of post-alcoholic bard and fermentolysate of substandard grain. The developed biotechnology made it possible to obtain protein-enriched feed products with a protein content of 45-47% (Table 1). For comparison, traditional sources of protein in feeds, such as soya and sunflower meal, contain about 36-38% crude protein, and fish meal about 60%, but it is characterised by high cost, and the use of soya is not always economically feasible [11]. In addition to high protein content, the obtained feed products were characterised by a balanced amino acid composition. In particular, the content of lysine, threonine, methionine and other essential amino acids was 35-45% of the total amino acids, which indicates a high nutritional value of the product [12]. In addition, a wide range of metabolites, enzymes, vitamins and probiotic substances were identified in the obtained feeds, which were found to have a positive effect on digestion and immune system of the animals. In particular, a significant content of vitamin B12 was observed, which is an important factor in the production of feeds for monogastric animals [13]. In the fermentation process, a deep degree of utilisation of organic components of the substrate is achieved, which is also an important advantage of the developed technology. As a result, the content of organic matter in the liquid fraction after fermentation was reduced by 85-90% compared to the original substrate, which

significantly reduces the load on the environment and allows us to consider this method as environmentally acceptable.

**Table 1.** Chemical and amino acid composition of the obtained feed products

| Indicator | Soybean meal | Sunflower meal | Biobardin, Propylact, Probitin |
|---|---|---|---|
| Metabolic energy, kcal/100 g | 260 | 226 | 237–224 |
| Moisture | 8.5 | 8.5 | 7.3–9.7 |
| Crude protein | 36 | 38 | 38.1–45.6 |
| Crude fat | 5.8 | 1.7 | 8.3–5.1 |
| Crude fiber | 7.3 | 15.0 | 11.4–13.5 |
| Crude ash | 6.0 | 7.2 | 5.52–3.25 |
| Calcium | 0.34 | 0.35 | 1.32–1.15 |
| Phosphorus | 0.65 | 0.65 | 0.45–0.50 |
| Sodium | 0.05 | 0.08 | 0.15–0.56 |
| Potassium | – | – | 0.24–0.26 |
| Amino acids: | | | |
| lysine | 2.26 | 1.27 | 1.18 |
| histidine | 0.83 | 0.98 | 1.01–1.03 |
| arginine | 2.6 | 2.92 | 1.7–1.79 |
| threonine | 1.51 | 1.51 | 1.27–1.26 |
| glycine | 1.48 | 2.2 | 1.51–2.01 |
| cystine | 0.43 | 0.65 | 0.52–0.61 |
| methionine | 0.45 | 0.88 | 0.68–0.85 |
| valine | 1.83 | 2.03 | 1.96–2.2 |
| isoleucine | 1.75 | 1.7 | 1.58–2.57 |
| leucine | 2.72 | 2.4 | 2.44–2.85 |
| phenylalanine | 1.9 | 1.8 | 1.88–2.07 |
| tyrosine | 1.24 | 1.15 | 1.49–1.17 |

The protein content of the final biomass was in the range of 40-42%, with a lysine content of about 1.1%, an important essential amino acid.

However, when further investigated and using another consortium of anaerobic microorganisms isolated from soil samples, improved performance was obtained. In particular, the protein content of the final product reached 44.5%, which was 2.5-4.5% higher than in previous experiments, and the lysine content increased to 1.3%. [14]. This indicates the possibility of increasing the nutritional value of protein biomass by selecting the optimal microbial community.

The study of the influence of various parameters on the fermentation process allowed us to optimise the cultivation conditions [15]. The study of the influence of temperature on the fermentation process showed that the most favourable temperature for the growth and activity of microorganisms is 37°C. At this temperature, the maximum biomass yield was observed, averaging 15.2 grams of dry matter per litre of medium for 48 hours of fermentation. Increasing the temperature above 40°C led to a decrease in the activity of microorganisms, which was reflected in a decrease in biomass yield by 8-12% and slowing down of the fermentation process. In particular, after 48 hours, the biomass yield at 42°C averaged 13.5 grams of dry matter per litre of medium. Reducing the temperature to 25°C also had a negative effect on the process, leading to a significant slowdown of fermentation and a 15-20% decrease in biomass yield (to 12.2-12.9 grams of dry matter per litre of medium).

In addition to temperature, fermentation duration proved to be an important factor. Increasing the fermentation time to 72 hours resulted in a statistically significant increase in protein yield by 10-15% (to an average of 17.5 grams of dry matter per litre of medium) compared to fermentation for 48 hours. However, a further increase in fermentation time, e.g. up to 96 hours, did not give a significant increase in biomass yield (the increase was no more than 2-3%, i.e. about 18 grams of dry matter per litre of medium) and was not economically justified. These results indicate the need for precise control of fermentation parameters to maximise the efficiency of the protein biomass production process.

Comparing the results obtained with other similar studies in the field of feed biotechnology, it can be noted that the developed method shows competitive performance [16]. In particular, there are works on the use of yeasts and mould fungi to process alcohol production waste [17]. However, these methods often require high energy costs and complex equipment, and the yield of the final product is not always high enough. Also, there is a problem in obtaining products with the required composition and presence of probiotic properties. In contrast to these methods, the developed biotechnology based on the use of a consortium of anaerobic microorganisms allows not only to obtain a product with high protein content, but also to enrich it with useful biologically active substances. In addition, this technology, compared to other approaches, is characterised by low production costs and scalability for industrial scale application [18]. The numerical data are given in the comparative Table 2.

**Table 2.** Chemical and amino acid composition of the obtained feed products

| Indicator | Experimental data | Evidence from alternative studies |
|---|---|---|
| Protein content, % | 44.5 | 40–42 (if using yeast) |
| Lysine content, % | 1.3 | 0.8–1 (when using mold) |
| Fermentation time, h | 72 | 48–96 |
| Reduction of organic matter, % | 88 | 70–80 |
| Optimum temperature, °C | 37 | 30–35 |

Thus, the results of the presented study and my own data confirm the high efficiency and prospects of the developed biotechnology for the production of enriched protein fodder products from secondary raw materials [19]. This technology can make a significant contribution to solving the problem of fodder deficiency, reduce the cost of livestock products and ensure sustainable development of agriculture [20].

## 4 Conclusion

The study demonstrates the effectiveness and prospects of biotechnological approach to the production of enriched protein feeds based on secondary grain products. The use of a specially selected consortium of anaerobic microorganisms makes it possible to achieve deep utilisation of organic components of post-alcoholic bard and fermentolysate of substandard grain, thus producing a product with a high protein content and a balanced amino acid composition. The obtained feed products are not only superior in protein content to traditional analogues such as soya and sunflower meal, but also enriched with vitamins, enzymes and probiotic substances, which significantly increases their biological value and potential benefits for animals.

The results obtained during laboratory and pilot tests showed the stability and reproducibility of the developed technology. Optimisation of fermentation parameters such as temperature, pH and process time allowed to achieve maximum yield of the target product while minimising costs. It is important to note that this approach, unlike a number of other biotechnological methods, is environmentally safe and contributes to solving the problem of industrial waste utilisation. A significant decrease in the content of organic substances in the liquid phase after fermentation indicates a high degree of substrate processing and a reduction in the negative impact on the environment.

Comparative analysis of the obtained results with the data of other researchers in the field of feed biotechnology confirmed the competitiveness of the proposed technology. In particular, the developed method surpasses the approaches based on the use of yeasts and mould fungi in terms of protein content, amino acid composition and the presence of probiotic properties. Ultimately, this study opens the prospect for the creation of cost-effective and environmentally friendly production of high quality forages, which is of great importance for improving the efficiency of animal husbandry and ensuring food security. The introduction of this technology at industrial enterprises will not only reduce dependence on imported feed additives, but will also contribute to the sustainable development of the agricultural sector.

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