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Changes in the microbial consortium during the disposal of aged post-fermentation distillery grain using the aerobic composting method

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Abstract. The change in the consortium of microorganisms during the disposal of aged post-fermentation distillery grain by aerobic composting using the biopreparation-oil destructor "Lenoil"® SHP containing microorganisms of the genera *Ochrobactrum sp.* and *Acinetobacter sp.* has been assessed. In the initial waste, microorganisms of the genera: *Acidothermus sp.*, *Proteus sp.*, *Pseudomonas sp.*, *Bacillus sp.*, *Escherichia sp.*, *Staphylococcus sp.*, and *Enterobacter sp.* were isolated. After the application of the biopreparation, the following genera of microorganisms were identified: *Ochrobactrum sp.*, *Acinetobacter sp.*, *Pseudomonas sp.*, *Bacillus sp.*, *Escherichia sp.*, *Staphylococcus sp.,* and *Enterobacter sp.* The use of the biopreparation promoted the development of existing microbial populations as well as the introduction of new species. The application of the biopreparation has made it possible to increase the number of microorganisms that contribute to more effective decomposition of organic compounds and reduction of inorganic nitrogen concentration during the disposal of aged post-fermentation distillery grain using the aerobic composting method.

Keywords: post-fermentation distillery grain, bioremediation, waste disposal, biopreparations

Authors' contribution. *Y.M. Russkikh* – development of the research methodology, conducting the research, writing the initial draft; *D.N. Sherstobitov* – development of the research idea, writing and editing the manuscript; *Z.E. Mashchenko* – conducting the research, formal analysis; *V.V. Ermakov* – scientific supervision of the research; *O.V. Tupitsyna* – project administration. All authors have read and approved the final version of the manuscript.

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Изменение микробного консорциума в процессе утилизации застарелой послеспиртовой барды методом аэробного компостирования

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Аннотация. Оценено изменение консорциума микроорганизмов при утилизации отходов застарелой послеспиртовой барды методом аэробного компостирования в результате использования биопрепарата-нефтедеструктора «Ленойл»® СХП с содержанием микроорганизмами родов *Ochrobactrum sp.* и *Acinetobacter sp.* В первоначальном отходе были выделены микроорганизмы родов: *Acidothermus sp.*, *Proteus sp.*, *Pseudomonas sp.*, *Bacillus sp.*, *Escherichia sp.*, *Staphylococcus sp.* и *Enterobacter sp.* После применения биопрепарата были идентифицированы следующие рода микроорганизмов: *Ochrobactrum sp.*, *Acinetobacter sp.*, *Pseudomonas sp.*, *Bacillus sp.*, *Escherichia sp.*, *Staphylococcus sp. и Enterobacter sp.* Применение биопрепарата способствовало развитию существующих популяций микроорганизмов, а также интродукции новых видов. Использование биопрепарата позволило увеличить количество микроорганизмов, способствующих более эффективному разложению органических соединений и снижению концентрации неорганического азота при утилизации отходов застарелой послеспиртовой барды методом аэробного компостирования.

Ключевые слова: послеспиртовая барда, биоремедиация, утилизация отходов, биопрепараты

Вклад авторов. *Русских Я.М.* – разработка методологии исследования, проведение исследования, создание черновика рукописи; *Шерстобитов Д.Н.* – разработка идеи исследования, создание рукописи и ее редактирование; *Мащенко З.Е.* – проведение исследования, формальный анализ; *Ермаков В.В.* – научное руководство исследованием; *Тупицына О.В.* – администрирование проекта. Все авторы ознакомлены с окончательной версией статьи и одобрили ее.

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Introduction

Post-fermentation distillery grain is a waste, which is a liquid (suspension) of light-brown color with dry matter content – 7–9%. Distillery grain is rich in fiber, carbohydrates, proteins and microelements [1]. The formation of post-fermentation distillery grain occurs in the production of ethyl distillery at the stage of rectification, which is accompanied by the formation of at least 13 litres of grain per 1 litre of ethanol.

Post-fermentation distillery grain contains amino acids, dissolved acid fermentation products, vegetable fat and nitrogen-free extracts [2]. The post-fermentation distillery grain contains dead yeast cells, ethanol derivatives, organic acids, vitamins, micro- and macro-elements [3]. The hydrogen content (pH) of fresh alcohol bars is 4.9 units, the composition includes reducing agents – 0.33%, raw protein – 37.0%, ammonium nitrogen – 1200 mg/l, phosphorus oxide (V) – 1480 mg/l, potassium – 688 mg/l, calcium – 130 mg/l, magnesium – 300 mg/l and others [4].

Before the implementation of regulatory acts regulating the disposal of post-fermentation distillery grain at enterprises, wastes were placed in earth storage tanks, and after the introduction of requirements for the treatment of such wastes, their placement was not authorized. Similar situation was created during the work of the Rozhdestvensky spirits factory, closed in 2013 due to a lawsuit by the Samara inter-district prosecutor’s office.

The tanks of the distillery grain are located at a distance of 110 m from the nearest residential development v. Rozhdestveno, within the boundaries of “Samarskaya Luka National Park”. According to engineering surveys, the area of the disturbed areas was 42 hectares, and the depth of contamination of the soil with waste from the post-fermentation distillery grain was at least 10 m. The spread of pollutants from the waste occurred not only in the soil and ground, but also in underground and surface water [5].

In 2024, work began on the excavation and decontamination of an aged post-fermentation distillery grain. For a faster and more effective waste disposal, the technology of aerobic composting was applied. The work was carried out in temporary hangars on site in close proximity to the waste disposal site. The composting of waste was carried out in trapezoidal storage pits 100 m long, up to 2 m high, with a volume of about 400 m^3 each. The formation of the compost mixture occurred from the initial removal of aged distillery grain (30–35% about.), immature compost after 7–10 days of composting

(30–35% about.) and wood sawdust (30–35% about.). The application of immature compost at the thermophilic stage contributed to a faster warming of the compost, and wood sawdust acted as a pollutant for maintaining aerobic conditions. Due to the limited time of production and large amount of post-fermentation distillery grain, which needs to be recycled (25,000 m^3), it was decided to apply "Lenoil"® SHP for further intensification of the waste recycling process with an aged post-wash bar. "Lenoil"® SHP developed by CBA NPP "Biomedchim", protected by the patent of the Russian Federation 2553540.[1]

The waste from aged distillery grain is characterized by high ammonium (up to 3700 mg/kg) and nitrite (up to 37 mg/kg) nitrogen content. The use of aerobic composting method in the recycling of post-fermentation distillery grain waste allows to intensify the process of nitrification, which reduces ammonium and nitrite nitrogen. In addition, during the composting process, the distillery grain waste is mineralized, resulting in an organomineral soil with a low organic matter content [6].

Materials and methods

To analyze the effectiveness of "Lenoil"® SHP as a pharmaceutical agent for intensification of waste composting process with aged post-fermentation distillery grain, a qualitative evaluation of microbial consortium was carried out.

Samples of both the untreated and the bacterial treated grain with "Lenoil"® SHP were taken for analysis.

This agent includes microorganisms belonging to the genera *Ochrobactrum sp.* and *Acinetobacter sp.*, which play a key role in the process of the breakdown of hydrocarbons for the intended purpose of the bioagent.

The microorganisms of these genera are known for their high adaptability to diverse ecosystems and ability to efficiently degrade hydrocarbons and other organic compounds [7-10]. The use of the agent "Lenoil"® SHP for waste disposal of aged distillery grain is justified by the fact that microorganisms of the genus *Acinetobacter sp.* have excellent ability to reduce inorganic nitrogen [11–13]. Integration of the genera of *Ochrobactrum sp.* and *Acinetobacter sp.* in a microbial consortium can accelerate the decomposition of residues of distillary production, which is important for reducing environmental impact.

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[1] *Patent RU 2 553 540 C2. Consortium of strains of Acinetobacter sp. and Ochrobactrum sp., used for cleaning water and soil from oil and petroleum products : intro. 2012151289/10, 29.11.2012* ; publ. 20.06.2015 / patent holder: Closed joint-stock company "Biomedchim" (CBA NPP "Biomedchim"), Federal state budget science institution Institute of Biology of the Ufa Scientific Center RAN (FGBON IB UNC RAN).

The biopharmaceutical agent shows potential for the biodegradation of petroleum products and hydrocarbons, as well as in wastewater treatment. The effectiveness of the agent is due to the activity of specially selected strains of bacteria, which can effectively decompose a wide range of organic compounds. The use of this agent contributes not only to an improvement of the environmental situation, but also to a significant reduction in the costs of waste disposal and sewage treatment compared with traditional methods [14; 15].

The process of micro-organisms from the post-fermentation distillery grain was carried out on a nutritious farm followed by cultivation at temperatures of 30 and 50°C for 24 hours.

To study the morphological and physio-biochemical characteristics of microorganisms, the following methods were applied: description of the morphology of colonies of microorganisms in a dense nutrient medium, Gram's method staining, Nesser's method staining, Cil-Nielsen's method staining, Dugid's method staining, Gray's method staining, glycaemic and lipid addition staining, determination of enzymatic activity including proteolytic, catalytic activity, oxidative activity and ability to break down carbohydrates.

Results and discussion

In this study, a detailed comparative study of the microbial consortium of post-fermentation distillery grain was carried out before and after treatment with the bioagent.

The traditional microbiological methods described in the Bergey's Manual Handbook were used to establish the origin of isolated crops (Tables 1–3)[2].

Table 1. **Morphology of colonies of microorganisms isolated from post-fermentation distillery grain**

Code		Morphology of colonies on solid nutrient medium
Before treatment	A.1.1	Fine colony with a smooth surface and curved profile. It has a shiny white surface with a smooth edge. The structure is homogeneous, and the consistency is cream-like. The growth of colonies is observed on the surface of the nutrient medium
	A.1.2	Medium matte colony with a smooth curved surface. The structure of the colony is homogeneous, the consistency is paste-like, and the color is creamy. Colony growth is observed on the surface of the nutrient medium
	C 1.1	Medium irregularly shaped colony. The profile of the colony is flat with a shiny appearance. The color of the colony is yellow-white, and the edge is wavy. The structure of the colony is slender, and the consistency is paste-like. Colony growth is observed on the surface of the nutrient medium
	C.2.1	Large round colony with a frilled edge. The surface is smooth, and the profile is flat. The colony is shiny and white-yellow in color. The structure is homogeneous, the consistency is paste-like, with colony growth observed on the surface of the nutrient medium

[2] Berkeley R et al. Bergey's *Manual of Determinative Bacteriology*: in 2 volumes. Holt J (ed.); translated from English under the supervision of Corresponding Member of the Russian Academy of Sciences. Zavarzin GA. 9th ed. Moscow: Mir; 1997. Vol. 1, 429 p.; Vol. 2, 500 p. (In Russ.).

Ending of the Table 1

Code		Morphology of colonies on solid nutrient medium
	C.3.1	Large round colony. The surface is smooth, and the profile is flat. It is white-gray in color. The structure is homogeneous, the consistency is paste-like. Colony growth is observed on the surface of the nutrient medium
	C.3.2	Medium opaque colony of creamy-yellow color. The colony is smooth, even, and convex with clear edges. Colony growth is observed on the surface of the nutrient medium
	C.4.1	Large round colony with a frilled edge. The surface is smooth, and the profile is flat. It is matte and gray-white in color. The structure is coarse-grained, the consistency is paste-like, with growth on the surface of the nutrient medium
After treatment	B.4.1	Large matte colony with a smooth surface. The color of the colony is yellow-white with an uneven edge. The structure of the colony is homogeneous, and the consistency is paste-like. Colony growth is observed on the surface of the nutrient medium
	D.1.1	Medium irregularly shaped colony. The profile of the colony is flat with a shiny appearance. The color of the colony is yellow-white, and the edge is wavy. The structure of the colony is slender, and the consistency is paste-like. Colony growth is observed on the surface of the nutrient medium
	D.2.2	Large round colony with a frilled edge. The surface is smooth, and the profile is flat. The colony is shiny and white-yellow in color. The structure is homogeneous, the consistency is paste-like, with colony growth observed on the surface of the nutrient medium
	D.2.3	Large round colony with a frilled edge. The surface is smooth, and the profile is flat, and the color is white. The structure is wavy, and the consistency is paste-like. Colony growth is observed within the nutrient medium
	D.3.1	Large round colony. The surface is smooth, and the profile is flat. It is white-gray in color. The structure is homogeneous, the consistency is paste-like. Colony growth is observed on the surface of the nutrient medium
	D.3.2	Medium opaque colony of creamy-yellow color. The colony is smooth, even, and convex with clear edges. Colony growth is observed on the surface of the nutrient medium
	D.4.2	Large round colony with a frilled edge. The surface is smooth, and the profile is flat. It is matte and grayish-white in color. The structure is coarse-grained, and the consistency is paste-like, with growth on the surface of the nutrient medium

Source: compiled by Ya.M. Russkikh based on data from: Berkeley R et al. *Bergey's Manual of Determinative Bacteriology*: in 2 volumes. Holt J (ed.); translated from English under the supervision of Corresponding Member of the Russian Academy of Sciences. Zavarzin GA. 9th ed. Moscow: Mir; 1997. Vol. 1, 429 p.; Vol. 2, 500 p. (In Russ.).

Table 2. **Morphological characteristics of microorganism cultures isolated from post-fermentation distillery grain**

Code		Cell Shape	Gram Staining	Presence of Flagella	Presence of Spores	Presence of Inclusions			
						Glycogen	Volutin	Lipids	Capsules
Before treatment	A. 1.1	Bacilli	–	–	–	–	–	–	–
	A. 1.2	Bacilli	–	+	–	–	–	–	–
	C. 1.1	Bacilli	–	+	–	–	+	–	–
	C. 2.1	Bacilli	+	+	+	–	–	–	–
	C. 3.1	Bacilli	–	+	–	–	–	–	+
	C. 3.2	Cocci	+	–	–	–	–	–	–
	C. 4.1	Bacilli	–	+	–	–	–	–	–
After treatment	B. 4.1	Bacilli	–	+	–	–	+	–	–
	D. 1.1	Bacilli	–	+	–	–	+	–	–
	D. 2.2	Bacilli	+	+	+	–	–	–	–
	D. 2.3	Bacilli	–	–	–	–	–	–	–
	D. 3.1	Bacilli	–	+	–	–	–	–	+
	D. 3.2	Cocci	+	–	–	–	–	–	–
	D. 4.2	Bacilli	–	+	–	–	–	–	–

Source: compiled by Ya.M. Russkikh based on data from: Berkeley R et al. *Bergey's Manual of Determinative Bacteriology*: in 2 volumes. Holt J (ed.); translated from English under the supervision of Corresponding Member of the Russian Academy of Sciences. Zavarzin GA. 9th ed. Moscow: Mir; 1997. Vol. 1, 429 p.; Vol. 2, 500 p. (In Russ.).

Table 3. **Physiological and biochemical properties of microorganism cultures isolated from post-fermentation distillery grain**

Code		Temperature tolerance	Oxygen requirement	Proteolytic activity	Catalase activity	Oxidase activity	Carbohydrate fermentation ability				Genus
							Glu-cose	Mal-tose	Suc-rose	Lac-tose	
Before treatment	A.1.1	Thermophiles	Aerobes	–	+	+	+	+	+	–	*Acidothermus sp.*
	A.1.2	Thermophiles	Facultative anaerobes	–	+	–	+	+	+	–	*Proteus sp.*
	C.1.1	Mesophiles	Aerobes	+	+	+	–	–	–	–	*Pseudomonas sp.*
	C.2.1	Mesophiles	Facultative anaerobes	+	+	–	+	+	+	+	*Bacillus sp.*
	C.3.1	Mesophiles	Facultative anaerobes	–	+	–	+	+	+	+	*Escherichia sp.*
	C.3.2	Mesophiles	Facultative anaerobes	+	+	–	+	+	–	–	*Staphylococcus sp.*
	C.4.1	Mesophiles	Facultative anaerobes	–	+	–	+	+	+	–	*Enterobacter sp.*
After treatment	B.4.1	Thermophiles	Aerobes	–	–	–	+	–	–	–	*Ochrobactrum sp.*
	D.1.1	Mesophiles	Aerobes	+	+	+	–	–	–	–	*Pseudomonas sp.*
	D.2.2	Mesophiles	Facultative anaerobes	+	+	–	+	+	+	+	*Bacillus sp.*
	D.2.3	Mesophiles	Aerobes	–	+	–	+	–	–	–	*Acinetobacter sp.*
	D.3.1	Mesophiles	Facultative anaerobes	–	+	–	+	+	+	+	*Escherichia sp.*
	D.3.2	Mesophiles	Facultative anaerobes	+	+	–	+	+	–	–	*Staphylococcus sp.*
	D.4.2	Mesophiles	Facultative anaerobes	–	+	–	+	+	+	–	*Enterobacter sp.*

Source: compiled by Ya.M. Russkikh based on data from: Berkeley R et al. *Bergey's Manual of Determinative Bacteriology: in 2 volumes*. Holt J (ed.); translated from English under the supervision of Corresponding Member of the Russian Academy of Sciences. Zavarzin GA. 9th ed. Moscow: Mir; 1997. Vol. 1, 429 p.; Vol. 2, 500 p. (In Russ.).

At the initial stage of analysis, the following types of microorganisms were identified: *Acidothermus sp., Proteus sp., Pseudomonas sp.*, *Bacillus sp.*, *Escherichia sp.*, *Staphylococcus sp.* и *Enterobacter sp*.

After the application of the biopharmaceutical agent in samples of post-fermentation distelliry grain bacteria *Ochrobactrum sp.* and *Acinetobacter sp.* were found. These results indicate that the use of a biopharmaceutical agent not only supports existing populations of microorganisms, but also promotes infestation of the genera contained in the agent, that can significantly improve the functional characteristics of the microbial community towards intensification of microbial processes and survival. Studies have shown that the use of microorganisms *Ochrobactrum sp.* and *Acinetobacter sp.* has the potential to significantly reduce the time required for the organic compound decomposition process [11; 13; 16-18].

Conclusion

The biopharmaceutical agent “Lenoil”® SHP is effective for improving the functional characteristics of microbial communities involved in the biodegradation of organic substances. The use of the agent in the post-fermentation distillery grain samples has increased the diversity of microorganisms by invading new genera of bacteria such as *Ochrobactrum sp.* and *Acinetobacter sp.*

The use of the biopharmaceutical agent is a promising approach for the recycling of post-fermentation distillery grain, as it contributes not only to the maintenance of existing populations of micro-organisms, but also to the introduction of new species that accelerate the decomposition processes of organic wastes. This reduces the environmental burden while reducing waste disposal costs.

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