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# Technology of a Creation of a Combined Microbiological Fertilizer Based on Soil Microorganisms

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**Were carried out the results of a research aimed on the production of biofertilizers from waste coal industry with adding of soil microorganisms to the content of consortium. Microbiological analysis of components of complex microbiologically-fertilizer mixtures (CMFM) showed that all groups of microorganisms can proliferate on the surface of the marked substrate. To create CMFM were selected strains of *Bacillus mesånteriñus, Bacillus megatherium, actinomycetes - Streptomyces candidus*, isolated from the waste coal industry. The fermentative and nitrogen-fixing activity, growth stimulating and destructive properties of the isolated strains were taken into the consideration while creating CMFM. The total number of microorganisms was stabilized after 2 months of composting, remaining at a high level, in another word, a stable microbial community was created by this period. This indicates for the readiness of the compost. In the process of growth and a development of wheat plants was marked an inhibitory effect of bio-fertilizers on root rot pathogens of wheat. According to the results of a research the promising *Bacillus mesånteriñus, Bacillus megatherium, actinomycetes - Streptomyces candidus* were deposited in the "Republican collection of microorganisms" of Central Museum of microorganisms for creation of CMFM. Were developed regulations for the establishment of complex microbiologically-fertilizer mixtures (CMFM) with the microbiological way from the carbonaceous waste.**

**Keywords:** composting, soil microbial consortia, coal waste.

The economy of the country depends upon the following main issues of agriculture, like the increasing of the yield and fertility of soils, which are dependent on humus content. During the years of the development of virgin lands and cultivation of land without taking into account the climatic conditions of the Northern region, farming methods and agronomic practices that are typical for the other regions of Kazakhstan, there was a significant decrease in the content of humus in the soil. To increase crop yields and preserve the soil fertility requires the use of fertilizers, including the use of local fertilizer resources. One of these ways that can be rather successfully and effectively be used to regulate the biological status of arable soils, is a further enrichment of the topsoil organic matter and nutrients with the fertilizers and a subsistence part of the harvest of crops[1]. The application of manure, coal mining waste and green manure fertilizers compensates for a deficiency of humus, improves the water, the food regime of soils, and increasing crop yields[2].

According to calculations of scientific institutions, even the increasing of the amount of organic matter is unable to provide self-supporting balance of humus in the soil. So, there is an urgent

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need to maximize the production of all types of organic fertilizers, including non-traditional[1]. The lack of traditional forms of organic fertilizers is forcing to find new types of organic materials and include them in modern agricultural technologies. For enhancing of soil fertility it is necessary to use fertilizers systematically. According to the results of agrochemical researches conducted in 2000-2010 in Kazakhstan, the share of land with a low content of humus in the non-irrigated soil consisted of 63%, and for irrigated – 98%, which is directly connected with a reduction in the amount of fertilizer[2].

However, in recent decades the volume of use of manure and composts decreased sharply as a result of the increased costs of their adding to the soil and the reduction of livestock numbers. It is possible to ensure the highest yield not only through the traditional system of fertiliser, but also due to the use of waste of coal production, processed by the association of specially selected micro-organisms[3].

Scientific studies have shown that biofertilizers can be created on the basis of the composting of carbonaceous substances, mineral clay-loamy rocks and modern organic matter by the fermentation with the use of soil microflora. This approach to the creation of fertilizers is due to the fact that non-carbonaceous compounds produce humic and other soil substances during a long period of time. Clay-loamy components improve water-physical and properties of absorption; on the plane of the crystals of rocks the humic acids are polymerized and converted into soil humus. Soil microflora performs fermentation of organic-mineral-carbonaceous mass, making it biologically active and bringing its properties to the properties of the soil.

The aim of the research was to develop a new fertilizer using soil microorganisms and coal mining waste as non-traditional fertilizers, improving the nutritional regime of soils, as well as stimulating the growth and a development of plants, improving the quality of their crop, reducing the harmful effects of pollution and hazardous biological objects.

## MATERIALS AND METHODS

In 2013-2015 were carried out research works in the laboratory of microbiology of the S. Seifullin Kazakh Agrotechnical University on the creation of a combined microbiological fertilizer mixture (CMFM) based on soil, microbial culture and a coal mining waste.

The starting material for the preparation of bio-fertilizers was a coal mining waste. In laboratory conditions were isolated 14 strains of microorganisms from a coal mining waste of the Maikuben and Ekibastuz coal basins. Identification of strains was performed by PCR analysis[4]. To create CMFM, as the leaven of the selected *Azotobacter chroococcum* Strain. No. 1, *Bacillus megatherium* strain No. 77, *Bacillus mesånteriñus* strains No. 71 and No. 81, *Streptomyces candidus* strain No. 137 and No. 139, with phosphate mobilizing, growth-stimulating, cellulolytic, nitrogen-fixing and antifungal properties. The titer of microorganisms according to the study with the photocolorimeter - CMFM No.4 – is $6.2\times10^6$, CMFM No. 5 - $6.4\times10^6$, CMFM No. 6 – $7,1\times10^6$.

The decomposition by microorganisms of the coal residue with the formation of humus and aerobic bacteria was determined on silica gel plates by the method of Vinogradsky[5]. The composting of carbonaceous substances with the addition of sterile soil and the consortium of microorganisms was carried out according to the proportion - 4.5 t/ha (4.5 g per 1 kg soil).

Microbiological analysis was performed according to the standard techniques[6]; the intensity of decomposition of cellulose modified application technique was established by the method of I. S. Vostrov[7, 8].

The test properties of the developed bio-fertilizers were conducted on dark chestnut soils of a country farm "Niva" in Tselinograd district of Akmola region.

The experiment was repeated four times, a placing of plots was randomized. The area of each plot was 1 $m^2$.

Was sown spring soft wheat of a variety - Akmola 2. Sowing time –24-25 of May. The seeding depth is 6-7 cm, seeding rate of 350 seeds/$m^2$. The soil of the experimental plot is light brown, light loam. The contents in the layer 0-20 cm: clay physical — 20,1%; humus — 0,9; total nitrogen - 0.09; total phosphorus — 0,15%; pH 7.5.

Scheme of experiment included 4 options:

**Control**

CMFM No. 4 (soil, ash, the consortium

of microorganisms *Bacillus mesånteriñus* Strain. ¹81+*Streptomyces* candidus Strain. No. 139+*Azotobacter chroococcum* Strain ¹1);

CMFM No. 5 (soil, Maikuben coal mining waste, the consortium of microorganisms *Bacillus megatherium* Strain. ¹77+*Streptomyces candidus* Strain. ¹137+*Azotobacter chroococcum* Strain No. 1);

CMFM No. 6 (soil, Ekibastuz coal mining waste, the consortium of microorganisms *Bacillus mesånteriñus* Strain No. 71+ *Streptomyces candidus* Strain. ¹139+ *Azotobacter chroococcum* Strain No. 1).

During the vegetation period were carried out phenological observations over growth and development of wheat, was determined the spread of a root rot, the biological effectiveness of the conducted activities.

## RESULTS AND DISCUSSION

At present, worldwide has dramatically increased an interest for the fertilizers of a humic type. Humic compounds as physiologically active substances, regulate and intensify the metabolic processes in plants and soil[9]. Complex humic microbiological preparations in contrast to the humic products on the basis of lignite or of peat a priori have a broader spectrum of action both on the soil and on plants[10].

In the works of S. M. Shaheen et al[11] and R. S. Blissett[12] are discussed the potential use of a fly ash (waste coal industry) as the melioration agent in agriculture. Over the past four decades many studies on the use of a fly ash as the ameliorants for the improvement of soil quality have been carried out worldwide. The similarity with the

**Table 1.** Results of chemical analysis of samples of a coal mining waste

| Coal waste | Acidity, pH | Elements content, mg/kg | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | P | K | N | S | Cu | Co | Mn |
| Ekibastuz coal waste | 8,39 | 18,8 | 147 | 12,3 | 6,0 | 1,3 | 0,2 | 16,3 |
| Maikuben coal waste | 7,65 | 4,8 | 221 | 5,6 | 6,0 | 0,6 | 0,5 | 27,4 |
| Ash | 8,57 | 21,4 | 46,6 | - | 6,3 | 1,1 | 0,3 | 26,2 |

**Table 2.** Influence of CMFM for germination of wheat cultivars - Akmola 2 in the field in 2013-2015, %

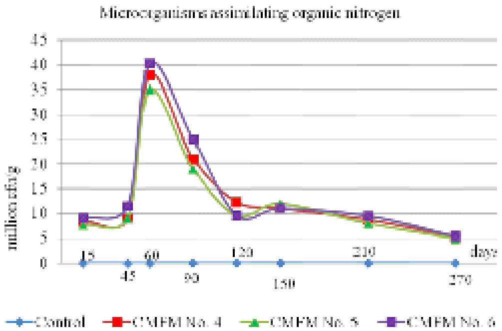
| Variants | 2013 | | 2014 | | 2015 | | On average over three years | |
|---|---|---|---|---|---|---|---|---|
| | Germin-ation | In relation to control | Germin-ation | In relation to control | Germin-ation | In relation to control | Germin-ation | In relation tocontrol |
| Control | 74,8 | - | 60,3 | - | 72,0 | - | 69,0 | - |
| CMFM No. 4 | 83,1 | +11 | 63,1 | +4 | 88,0 | +22 | 78,1 | +13 |
| CMFM No. 5 | 85,4 | +14 | 66,1 | +9 | 87,0 | +21 | 79,5 | +15 |
| CMFM No. 6 | 85,3 | +14 | 78,7 | +13 | 87,0 | +21 | 83,7 | +22 |
| HCP $_{0,5}$ | 2,1 | | 2,0 | | 2,1 | | 2,0 | |

**Table 3.** Biological activity of a model-experimental ecosystem in the field in 2013-2015

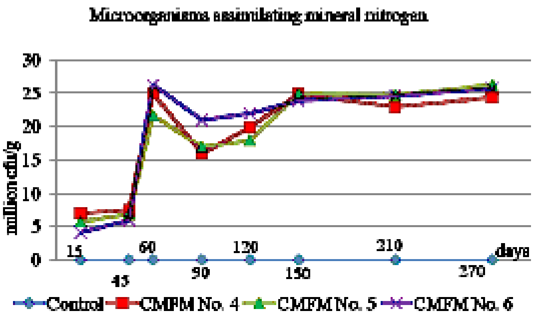
| Variants | The decomposition of flax fibers, % | | | |
|---|---|---|---|---|
| | 2013 | 2014 | 2015 | On average over three years |
| Control | 27,9 | 22,6 | 18,7 | 23,1 |
| CMFM No. 4 | 59,6 | 61,9 | 65,0 | 54,2 |
| CMFM No. 5 | 63,7 | 63,5 | 57,7 | 53,0 |
| CMFM No. 6 | 49,1 | 63,7 | 62,7 | 49,4 |

**Table 4.** Distribution of root rot in wheat cultivars Akmola 2, processed CMFM on average for 2013-2015, %

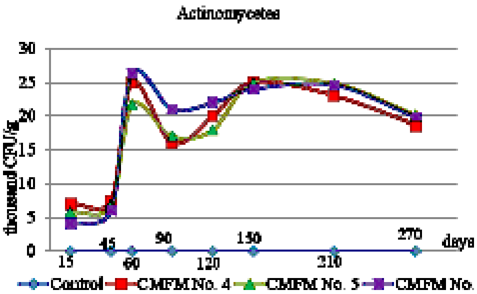
| Variants | Seedlings – tillering | | Earing-flowering | | Full ripeness | | On average over 3 years | |
|---|---|---|---|---|---|---|---|---|
| | The spread of root rot | Biological efficiency CMFM | The spread of root rot | Biological efficiency CMFM | The spread of root rot | Biological efficiency CMFM | The spread of root rot | Biological efficiency CMFM |
| 2013 | | | | | | | | |
| Control | 43,3 | - | 28,3 | - | 13,0 | - | 28,0 | - |
| CMFM No. 4 | 33,8 | 22,0 | 18,8 | 33,6 | 9,7 | 25,4 | 21,0 | 27,0 |
| CMFM No. 5 | 37,9 | 12,5 | 26,9 | 5,0 | 7,1 | 45,4 | 24,0 | 21,0 |
| CMFM No. 6 | 28,6 | 34,0 | 21,4 | 24,4 | 9,0 | 30,8 | 20,0 | 30,0 |
| 2014 | | | | | | | | |
| Control | 12,0 | - | 36,0 | - | 28,0 | - | 25,0 | - |
| CMFM No. 4 | 12,0 | 0 | 24,0 | 33,3 | 20,0 | 28,6 | 19,0 | 21,0 |
| CMFM No. 5 | 8,0 | 33,3 | 20,0 | 44,4 | 20,0 | 28,6 | 16,0 | 35,0 |
| CMFM No. 6 | 8,0 | 33,3 | 16,0 | 55,5 | 20,0 | 28,6 | 15,0 | 39,0 |
| 2015 | | | | | | | | |
| Control | 26,0 | - | 28,0 | - | 48,0 | - | 34,0 | - |
| CMFM No. 4 | 4,0 | 84,6 | 12,0 | 57,1 | 28,0 | 41,7 | 15,0 | 61,0 |
| CMFM No. 5 | 8,0 | 69,2 | 16,0 | 42,9 | 28,0 | 41,7 | 17,0 | 51,0 |
| CMFM No. 6 | - | 100 | 8,0 | 71,4 | 24,0 | 50,0 | 11,0 | 74,0 |




a) Meat-peptone agar environment: microorganisms metabolizing organic nitrogen, million CFU/g,




b) Starch ammonium agar environment: the microorganisms assimilating mineral nitrogen




c) Gauze medium: actinomycetes, thousand CFU/g

**Fig. 1.** A proportion of species of microorganisms that was isolated from the model ecosystem in the observation period (a – MPA environment: microorganisms metabolizing organic nitrogen, million CFU/g, b – SAA environment: the microorganisms assimilating mineral nitrogen, million CFU/g, c – Gauze medium: actinomycetes, thousand CFU/g)

sludge particles, low bulk density, high water retaining capacity, favourable pH and the presence of nutrients, makes a fly ash potentially useful for soils.

With the aim of creating of microbiologically-fertilizer mixture was studied the chemical composition of the coal mining waste. It is established that in the Ekibastuz coal mining waste a phosphorus content was 18.8 mg/kg, potassium – 14.7 mg/kg, easy hydrolyzed nitrogen - 12.3 mg/kg, and in Maikuben coal mining waste a phosphorus content was 4.8 mg/kg, potassium – 22,1 mg/kg, easy hydrolyzed nitrogen – 2.6 mg/kg, i.e., there are all the necessary elements for plant nutrition (table 1).

According to the results of chemical analysis the following elements are contained in the ash: phosphorus – 21,4 mg/kg, potassium – 46.6 mg/kg, sulfur – 6.3 mg/kg, copper 1.1 mg/kg, cobalt – 0.3 mg/kg, manganese – 26,2 mg/kg.

Thus, the chemical composition of the coal waste confirms the high content of nutrients, and can be used as a component for the development of a microbial biofertilizer method.

In the scientifical literature there is a significant number of works devoted to the bioconversion of the coal waste with the aim of creating environmental technology of disposal, but the environmental aspects of the ongoing processes is almost not lit. The most effective way of recycling wood waste from this point of view is their use as fertilizer.

Currently, in connection with the intensification of the processes of dehumification of soils of agrolandscapes, it is important to study the conditions of humus in the natural environment with the purpose of modelling them in agrocenoses[13, 14].

Microorganisms are not only the creators of soils, but are also the main factors of determining their fertility and preservation. Causing a mineralization of fresh organic residues entering the soil, the organic matter of the soil, microorganisms are enriching it with mineral nutrients for plants. At the same time, the speed of microbial degradation of soil organic matter under certain conditions can exceed the speed of its synthesis, that is also carried out by microorganisms[15].

During the experiment, it was empirically determined that composting for at least 45 days is not sufficient for complete microbial decomposition of raw materials and waste, and after 60 days there is a maturation of the compost and as a consequence, the decrease in microbial activity.

The results of studies of the development of the microorganisms included in the CMFM with the use of a coal waste on the silica gel plates by the method of Vinogradsky showed that the microbes that are using the organic forms of nitrogen, are most actively developing at the 60-th day (figure 1). The total number of microorganisms was stabilized after 2 months of composting, remaining at a high level, that is, to this period was created a stable microbial community that can serve as an indicator of the readiness of the compost.

In a composting substrate, on the 60th day, the number of bacteria detected in a meat-peptone agar, in the variant, where the Ekibastuz coil waste is presented, slightly exceeds the figure of proteolytics in the other variants, and this indicates the increasing consumption of organic forms of nitrogen by microbes of all experimental samples. The greatest number of bacteria was identified in CMFM No. 6 on the 60-th day of incubation, the lowest in CMFM No. 5 on the 270-th day of incubation. Overall, the number of bacteria increased from the beginning of composting, reaching a maximum on the 60-th day. After 120 days of composting the combined microbial-substrate fertilizer is declining the ammonitella activity of microorganisms, which is associated, rather, with the advent of inhibitory factors - the lack of organic nutrients in the substrate.

In SAA is observed a significant increase in the number of the mineralizer microorganisms on the 60th day of composting of microbial-carbonaceous mass with a consequent reduction of the development of microbes in dynamic with 25 CFU million/g to 15 CFU million/g on 90th day of cultivation.

However, after 5 months of cultivation of compost from the beginning of experiment is observed an increase in the number of microorganisms mineralizing coil waste, to a maximum value without a significant reduce in the number of microbes in dynamic up to 270-th day.

The impact of the CMFM on the dynamics of microorganisms on the Gauze environment was visible in the early stages of decomposition, in the period between 60-th and 90-th days. In this period on the embodiments with all types of CMFM was observed a large number of cellulose destructive aerobic actinomycetes. A stimulating effect of CMFM on the growth of cellulolytic microflora was manifested the most noticeably in the period from 90-th to 150-th days.

Thus, proteolytics, amylolytics and cellulosolytica are the most actively developing on the 60-th day of decomposition of coal mining waste. An optimal development of amylolytic bacteria and microorganisms of indigenous groups that are mineralizing lignin and humic substances can be observed at 150-th day of composting.

Numerous studies[16, 17] confirmed the increase in seed germination after adding of coal mining waste in the soil, due to their sorption capacity in relation to macro - and microelement fertilizers (including organic fertilizers), which prevents their rapid leaching from the topsoil.

In average over the 3 years the germination on the experimental variants exceeded the control for 13-22 % (table 2). Germination of a wheat seed ranged from 78,1 to 83.7%. The most beneficial effect of a seed processing was observed in a variant with application of CMFM No. 6 during all the experimental years.

Thus, it was experimentally proved the influence of the activity of complex microbial fertilizer mixtures for the plant development at early stages of the growing season.

One of the main indicators of soil biological activity is a cellulose destructive capacity of the soil. In natural conditions, cellulose decomposition occurs only as a result of a vital activity of microorganisms, producing a set of hydrolytic enzymes – cellulase [18].

The results of the research of the activity of decomposition of cellulose have shown (table 3) that the percentage of decomposition of linen in 2013 on a control variant amounted to 27.9%. Cellulase activity of microorganisms in the CMFM No. 5, increased this ratio to 63.7%. The level of microbial activity in a variant - CMFM No. 4 was 2 times higher than the control – 59,6%.

The overall pattern of degradation of the flax linen depending on the application of CMFM remained in 2014, but the degree of decomposition of cellulose was slightly lower, apparently, due to a dry year. On the variants with the adding of CMFM was observed an average rate of decomposition of the linen.

In 2015 it was observed a high intensity of decomposition of the linen on the experimental variations because of the favorable conditions for the development of microbial activity, due to an optimal temperature and a water regime.

Thus with the adding of the experimental samples of biofertilizers was increased a cellulose degradational capacity of the soil, which undoubtedly plays an important role in the transformation of organic matter into the humus compounds, thereby helping to improve a soil fertility.

In modern agroecosystems, more than 60% of arable lands are infected with the causative agents of root rot above the economic threshold. As a result, spring wheat and barley are cultivated annually in a large area on the infectious background, affected with a root rot, dark brown spotting and alternaria[19].

The use of biological preparations, immuno stimulators and growth stimulators for the protection of crops is very promising, especially for the improvement of immunity to pathogens. A biological control over the development of root rot, for example, can be achieved by the use of such biological products like Trichodermin, Bactofit, Planriz, Agat-25K, especially with a moderate contamination of soil and seeds [20].

Studies have shown that the samples of spring wheat among the variants, in varying degrees were affected by a root rot (table 4). Analysis of three years data of infestation of studied plants by the pathogens of a root rot in the phase of seedlings-tillering showed that the spread of the disease on a control variant amounted to 29.0 %. In the variants with the use of CMFM No. 6 was observed the lowest percentage of infected plants – 11,0-20,0%.

Despite of the low prevalence of root rot on the embodiments with adding of CMFM in the phase of tillering, at the flowering there was a slight increase in lesions of the micromycetes. During all phases of the growing season at the experimental embodiments the infestation of wheat plants with this disease has significantly decreased compared to control in 1.5-2 times.

Thus, when using complex

microbiological fertilizers is observed the tendency of reducing a root rot incidence of a soft spring wheat, by suppressing of a development of pathogenic microflora by the microbes-antagonists comprising samples of combined bio-fertilizers.

## CONCLUSION

Throughout the world it is recognized that biological preparations of plant protection compared to the chemical nature of the pesticides are more environmentally friendly[21]. The advantages of the biopreparations added into the soil, are very diverse: they contribute to the improvement of mineral nutrition of plants, reduce the activity of pathogens, increase the yield and improve the quality of cultivated products, and without disturbing the ecological balance in the biosphere.

Prototypes CMFM, developed in the laboratory, contain a complex of effective microorganisms for the degradation of coil waste complexes and their subsequent transformation into the humic-like and humic substances. Their application will allow creating high concentration of useful forms of microorganisms that are able to compete successfully with the native microflora of the soil, occupying the corresponding ecological niches. When processing the soil with these fertilizers, the microorganisms participating in transformation, form a humification trophic chain (the effect of information transmission from the biological product to the soil microflora).This provides a better integration of microbial-fertilizer complex in humus forming processes.

During the research it was revealed that proteolytics, amylolytics and cellulosolytics are the most actively developing on the 60-th day of decomposition of coal mining waste. An optimal development of amylolytic bacteria and microorganisms of indigenous groups, mineralizing lignin and humic substances can be observed at 150-th day of composting.

When adding experimental samples of bio-fertilizers there was an increase in cellulose destructive ability of the soil, which undoubtedly plays an important role in the transformation of organic matter in humus compounds, thereby helping to improve the soil fertility.

The results of these studies show that the soil adding of CMFM exerted a protective effect on plants, increasing their resistance to root rots throughout the growing season.

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