THE SOLUBILIZATION OF LIGNITE
BY SELECTIVE STRAINS OF BACTERIA

The world demand for restoring technologies of anthropogenically disturbed soil fertility and increasing crop yields emerges primarily from the active transition to a new paradigm in the field of agro-technologies. In this regard, many countries of the world are potential markets for new technologies of humus production and creation of eco-black-earths (chernozems). For the production of humus, it is intended to use lignite (brown coal) as the raw material, which is rich in humic acids. By familiarizing ourselves with the coal nature, we selected lignite (oxidized) samples for further experiments. Based on the understanding of coal and microorganisms, we selected lignite which are from different areas of Kazakhstan coal-mines, and different types of bacteria. FTIR was used to analyze the content of humic substances treated by different bacteria. A bacterial strain RBK 7 was used to decompose a coal sample, as a result, the humic acid was extracted and applied to the planting process of coriander, and it was found that a certain concentration of humic acid could improve soil fertility and the germination rate of this plant.

Key words: lignite, bacteria, humic acid, FTIR.
Солюбилизация бурого угля селективными штаммами бактерий

Глобальный спрос на технологии восстановления плодородия антропогенно-измененных почв и повышения урожайности сельскохозяйственных культур возникает прежде всего в результате активного перехода к новой парадигме в области агротехнологий. В связи с этим многие страны мира являются потенциальными и конкурентоспособными производителями гуминовых веществ и эко-черноземов. Бурый уголь используется в качестве сырья для производства гуминовых веществ. Нами были отобраны штаммы бактерий и образцы (окисленного) бурого угля из разных Казахстанских месторождений. Для анализа состава функциональных групп гуминовых веществ был использован метод FTIR. Перспективный штамм бактерии RBK 7 использовался для солюбилизации пробы угля, в результате была выделена гуминовая кислота и применена к процессу посадки кориандра. Было установлено, что определенная концентрация гуминовой кислоты способна улучшить плодородие почвы и скорость прорастания растения.

Ключевые слова: лигнит, бактерия, гуминовая кислота, FTIR.

Abbreviations

FTIR – Fourier-transform infrared spectroscopy; HAs – Humic acids; HSs – Humic substances.

1. Introduction

Soil provides human beings with food, vegetables, and fruits and is necessary for the development of agriculture. Under proper management, soil can be used as a renewable resource, while under improper management, soil fertility will gradually weaken or even degenerate into deserts. The world demand for restoring technologies of soil fertility and increasing crop yields emerges primarily from the active transition to a new paradigm in the field of agro-technologies, which is unfolding before us. Land degradation is a process in which the value of the biophysical environment is affected by a combination of human-induced processes acting upon the land [1]. It is viewed as any change or disturbance to the land perceived to be deleterious or undesirable [2]. In addition to the usual types of land degradation that have been known for centuries (water, wind and mechanical erosion, physical, chemical and biological degradation), four other types have emerged in the last 50 years [3]. 1. Chemical pollution, often, due to agricultural, industrial, mining or commercial activities; 2. Loss of arable land due to urban construction, road building, land conversion, agricultural expansion, etc.; 3. Artificial radioactivity, sometimes accidental; 4. Land-use constraints associated with armed conflicts. The main factor in demand for soil fertility restoration technologies is the catastrophic loss of quality and volume of soil as a result of its barbaric exploitation over the past 50 years.

A third of the planet’s land is severely degraded and fertile soil is being lost at the rate of 24 billion tonnes a year, according to a new United Nations-backed study that calls for a shift away from destructively intensive agriculture. The alarming decline, which is forecast to continue as demand for food and productive land increases, will add to the risks of conflicts such as those seen in Sudan and Chad unless remedial actions are implemented. As the ready supply of healthy and productive land dries up and the population grows, competition is intensifying for land within countries and globally. There are 197 Parties in the United Nations Convention to Combat Desertification (UNCCD), and 169 countries have declared that they are affected by desertification. More than 1.5 billion people directly depend on land that is slowly being degraded. By April 2013, every year 75 billion tons of fertile soil is lost to land degradation. Similarly 12 million hectares of land are lost every year to desertification and drought alone [4]. This is an area that could produce 20 million tons of grain. Desertification and land degradation cause USD 42 billion in lost earnings each year. In this regard, many countries of the world are potential markets for new technologies of humus production and creation of chernozems.

In order to solve these problems, it is necessary to determine the initial physicochemical parameters of experimental soils for understanding their condition; to study the microbial landscape of soils; to...
isolate cultures of microorganisms – candidates for inclusion in the mix-consortium; to screen commercial microbial bioproducts. For the production of humus, it is intended to use lignite as the raw material, which is rich in humic acids.

Lignite, often referred to as brown coal [5] is a soft, brown, combustible, sedimentary rock formed from naturally compressed peat. It is a low-rank coal with a lignin-like structure. It is considered the lowest rank of coal due to its relatively low heat content. It has a high inherent moisture content, sometimes as high as 45 percent. It has a carbon content around 60–70 percent [5]. It is mined all around the world, is used almost exclusively as a fuel for steam-electric power generation, and is the coal which is most harmful to health [6]. Lignite serves as a poor fuel source but as a rich source of humic acid [7].

The Kazakhstan WEC Member Committee reports that at end-2011 the remaining discovered amounts of lignite in place were 37.5 billion tons. Though Kazakhstan production of lignite coal fluctuated substantially in recent years, it tended to increase through 1997 – 2016 period ending at 6.338 thousand short tons in 2016. The consists of lignite, mainly from the Turgay, Nizhne-Iliyskiy and Maikuben basins.

China is the largest producer and consumer of coal in the world. As of the end of 2014, China had 52 billion tons of lignite quality coal. China’s proven lignite reserves are 130 billion tons, accounting for 13 percent of the country’s coal reserves. Another 190 billion tons of lignite resources are predicted. The coal-forming period was dominated by the late Jurassic, mainly distributed in the lignite belt in eastern Inner Mongolia, accounting for 3/4 of the country’s lignite reserves. Southwest China is the second largest lignite coal base in China, next only to north China, and its reserves account for about 1/8 of the country’s lignite, most of which are distributed in yunnan province. However, the lignite in southwest China is almost all younger lignite in tertiary period, while the lignite in north China is mostly old lignite in Jurassic period. Lignite has the advantages of clean, low volatility and low sulfur, but at the same time, it has the disadvantages of high humidity, low ignition point and large carbon dioxide emissions, which is one of the important factors leading to global greenhouse effect. However, in the current situation of increasing global energy tension, the economic value of lignite and its related processing and production technology have been re-emphasized by the world energy community. The quantity of lignite resources in northeast China, south central China, northwest China and east China is small. China ranks third in the world in terms of total coal reserves behind the United States and Russia [8].

Production of lignite coal of China increased from 80.514 thousand short tons in 1997 to 207.206 thousand short tons in 2016 growing at an average annual rate of 5.31%.

Lignite is an energy source as well as a humic acid resource. HAs are the most important organic components found in soil, where they contribute substantially to global soil fertility and agricultural production, as well as playing a key role in protecting soil from degradation and contamination [9]. There are two methods commonly used to extract humic acid from lignite, one is to treat lignite with strong alkali, and the other is to treat lignite with microorganisms. Microorganisms can be used to biosolubilize lignite by cleaving the chemical linkages within the lignite molecules, thereby promoting the extraction of lignite products, such as humic acid, and ultimately increasing the value of the coal [10], [11], [12].

Humic acids (HAs) are macromolecules that comprise humic substances (HSSs), which are organic matter distributed in terrestrial soil, natural water, and sediments resulting from the decay of vegetable and natural residues [13]. Due to the widespread presence of humic acid, it has a great impact on the earth, involving carbon cycle, mineral migration and accumulation, soil fertility, ecological balance and other aspects. So the people who first noticed and studied it were soil scientists, who started doing it about two hundred years ago. Until now, environmental scientists pay more and more attention to it. Humic acid has been widely used in industry, agriculture, medicine, environmental protection and other fields. Humic acid, as a potential organic resource, has attracted more and more attention in the world. With the rapid development of human civilization, the shortage of resources becomes more and more serious. Such trillions of tons of potential organic resources deserve attention. Of course, as a resource utilization perspective, not just any place of humic acid, have the use of value. Soil contains the largest amount of humic acid, but its content is less than 1% on average. Brackish water also contains a large amount of humic acid, but at a lower concentration, it is impossible to develop as a resource. The most promising sources of humic acid for exploitation are low-calorific value coal such as peat, lignite and weathered coal. Among them, humic acid content of 10–80%. In this sense, the production and application of humic acid can also be said to be a coal chemical industry [14]. Global lignite deposits account for 40% of total coal resources; Lignite
contains a large amount of humic acid. Therefore, the extraction of humic acid from lignite has created favorable conditions for the development of coal industry and agricultural industry and has broad application prospects. 

The goal of the research project is to receive biologically active humic products based on brown coals and microbial cells with high target metabolic activity for enrichment of soil. By familiarizing ourselves with the coal basins in Kazakhstan and China, we selected lignite samples for further experiments, including the study of the samples’ physical and chemical parameters, microbial landscape and its classification/genetic characteristics.

On the basis of humus, the special microorganism flora was established, and the bacterial strain had the target metabolic activity. Based on active strains and coal samples, humic acid and/or humic acid components were produced and their biological parameters were studied by means of Fourier Transform infrared spectroscopy (FTIR spectroscopy).

Through a series of experiments, we applied the obtained humic acid in the planting process of coriander, and found that a certain concentration of humic acid can significantly improve the germination rate of seeds. Humic acid can improve soil fertility and germination rate of seeds, which is of great significance to agricultural production.

2. Materials and methods

2.1 The preparation of coal

We ground lignite collected from Oikaragai (OLI) region of Kazakhstan into coal powder with a diameter of less than 2 microns.

2.2 Bacterial preparation

The collective bacteria strains RKB1, RKB7 were used, maintained at 30° in LB medium.

2.3 Humic acid extraction

1. 1 milliliter of bacteria solution was inoculated into 200 ml of LB culture medium; 2. Shaken at 150 rpm for 24 hrs. at 30°; 3. 5 g. of pulverized coal added, shaken at 150 rpm, under 30° for 10 days; 4. Centrifuged at 3500 rpm for 5 min., the sediment removed; 5. 11.6 M of HCL added to the supernatant to adjust the pH to 2.00; 6. Left for 12 hrs. at room temperature; 7. Centrifuged at 3500 rpm for 15 min, the supernatant removed; 8. The distilled water is used to clean the sediment; 9. Dried at 80 ° in a drying box.

2.4 The obtained humic acid was analyzed by FTIR

We treated OLI with five kinds of bacteria, and obtained 5 samples. The coal, which was not treated with bacteria referred as blank control. FTIR analyze used to characterize chemical nature of the samples.

2.5 Application of humic acid in the cultivation of coriander

1. The seeds were soaked with humic acid with concentrations of 0.005%, 0.01% and 0.05%, respectively for 5 hours. 2. Prepare five half-filled baskets, and mark the five baskets with 1-5. The soil in basket 1th was untreated. 3. The soil in basket 2 and 3 was mixed with 50 ml of a 0.05% humic acid solution. 4. And the soil in basket 4 and 5 was mixed with 50 ml of 0.1% humic acid solution. 5. Divide the first basket in half, with unsoaked seeds on the left and soaked seeds in clear water on the right. The other four baskets were divided into three. The first part was the seeds soaked with humic acid, and the humic acid concentration was 0.005%; in the second part, the seeds were soaked with humic acid, and the humic acid concentration was 0.01%; in the third part, the seed soaked with humic acid has a concentration of 0.05%.

On the forty-seventh day, the height of plant has grown to about 5cm. The humic acid solutions with concentrations of 0.01% and 0.05% were prepared in 100ml each. The humic acid solution with the concentration of 0.01% was sprayed on the plants in basket marked 2th and 4th with a spray pot. The humic acid solution with the concentration of 0.05% was sprayed on the plants marked 3th and 5th with the spray pot.

3. Results and Discussion

Fourier-transform infrared spectroscopy (FTIR) is a very effective method to study the surface structure of materials. FTIR analysis can directly understand the chemical structure of functional groups on the surface of coal. FTIR analysis of raw materials and products can obtain the change information of functional groups, especially for HAs containing carboxyl and phenolic hydroxy, FTIR analysis is particularly important. In this experiment, infrared spectrum analysis was carried out on a kind of coal sample and different humic acid products obtained by treating lignite with different microorganisms, focusing on the presence of carboxyl group and phenolic hydroxyl group. The FTIR spectrum of coal and its derivatives can be divided into four regions: 900–700 cm⁻¹ (aromatic substitution), 1800–1000 cm⁻¹ (O-containing groups), 3000–2800 cm⁻¹ (aliphatic structure), and 3700–3000 cm⁻¹ (hydrogen bond regions) [15], [16]. In order to further study the distribution of various oxygen-containing functional groups in original
The solubilization of lignite by selective strains of bacteria

lignite and humic acid, Fourier-transform infrared spectrometer was used to detect and analyze them.

FTIR spectrum of HAs extracted from OLI lignite for control was as following (Figure 1): The absorbance peak at 3444 cm\(^{-1}\), belonging to the absorption of hydrogen bond associated fats and aromatic -OH stretching vibration or -NH stretching vibration. The absorbance peak at 3192 cm\(^{-1}\) is attributed to the stretch of hydroxyl groups. The absorbance peak at 1554 cm\(^{-1}\) is attributed to C=O stretching of the ketone and carbonyl groups. The absorbance peak at 1399 cm\(^{-1}\) is attributed to O-H bending vibration of alcohols or carboxylic acids and C-O stretching vibration of phenols.

![Figure 1 – FTIR spectrum of HAs extracted from OLI lignite (Control)](image1)

FTIR spectrum of HAs extracted from OLI lignite treated with RKB1 showed (Figure 2): The absorbance peak at 1536 cm\(^{-1}\) is attributed to the absorption of hydrogen bond associated fats and aromatic -OH stretching vibration or -NH stretching vibration. The absorbance peak at 1399 cm\(^{-1}\) is attributed to O-H bending vibration of alcohols or carboxylic acids and C-O stretching vibration of phenols. The absorbance peak at 838 cm\(^{-1}\) is attributed to C-C stretch vibration of aromatic hydrogen. Compared with raw coal, there was no hydrogen bond associated fats and aromatic -OH stretching vibration or -NH stretching vibration and the stretch of hydroxyl groups in lignite products treated by RBK1.

![Figure 2 – FTIR spectrum of HAs extracted from OLI lignite treated with bacteria RKB1](image2)
FTIR spectrum of HAs extracted from OLI lignite treated with bacteria RKB7 reveled (Figure 3): The absorbance peak at 3168 cm\(^{-1}\) is attributed to the stretch of hydroxyl groups. The absorbance peak at 1650 cm\(^{-1}\) is attributed to the skeletal vibration of the aromatic rings. The results indicate that HAs have an aromatic structure and contain aliphatic side chains, like C = C absorption and C = O...HO associated vibration absorption. The absorbance peak at 1543 cm\(^{-1}\) is attributed to C=O stretching of the ketone and carbonyl groups.

The absorbance peak at 1402 cm\(^{-1}\) is attributed to O-H bending vibration of alcohols or carboxylic acids and C-O stretching vibration of phenols.

Compared with raw coal, there was no hydrogen bond associated fats and aromatic -OH stretching vibration or -NH stretching vibration in lignite products treated by RBK7.

According to the FTIR analysis, the mainly compounds are hydroxyl groups, ketone and carbonyl groups, alcohols or carboxylic acids and phenols. FTIR analysis showed that the skeleton structure of humic acid treated with RKB1 was simpler than that of raw coal.

Because of the skeleton structure of humic acid treated with RKB7 is similar to that of raw coal, we used humic acid extracted by RKB7 for the cultivation of coriander: seeds were soaked with different concentrations of humic acid, soil was treated with different concentrations of humic acid, and plants were sprayed with different concentrations of humic acid. The results were as follows.

2. The germination rate of coriander, %

According to the germination rate of seeds, we can see that a certain concentration of humic acid can improve soil fertility. Observed by trisection basket 2, 3, 4, 5, by comparing the germination rates of the seeds soaked in different concentrations of humic acid solution, we can see that under the same condition of the soil, the seeds soaked in different concentration of humic acid solution, the germination rate was not significantly, therefore, seeds soaked 5 hours in different concentration of humic acid solution will not affect its germination rate (Table 1);

Comparing basket 1th, the germination rate of the seeds soaked in water for 5 hours was 7% higher than that of the seeds not soaked in water. Observed by the baskets 2th and 3th, the soil treated with a concentration of 0.05% humic acid solution, its seed germination rate appeared a strange phenomenon. Both showed high germination rate in the early stage, and the plants gradually died after a few days, indicating that soil treated with a concentration of 0.05% humic acid solution could improve the germination rate of seeds, but can’t keep coriander alive;

However, the seed germination rates in baskets 4th and 5th were compared with those in basket 1th, we can see that, the seeds were planted in soil treated with 0.1% humic acid solution, on the 32nd day, the seeds have all sprouted and are thriving, namely seed germination rate is 100%. In the untreated soil, the germination rate of the seeds in
basket 1th was only 50%. Thus, the soil fertility was doubled by treatment with a 0.1% humic acid solution. On the 43nd day, soil fertility was about 30% higher in soils treated with a 0.1% humic acid solution than in untreated soils. In a word, seeds germinate quickly and well in soil treated with 0.1% humic acid solution. Soil fertility did improve when treated with 0.1% humic acid solution.

Humic acid spraying on day 47 did not affect seed germination (Table 1).

### Table 1 – The germination rate of coriander

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### Table 2 – The average ground height of each plant, cm

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By observing the average height of coriander, we can find that the optimum concentration of seeds soaked was 0.01%, the soil fertility was significantly improved when treated with 0.1% of humic acid solution.

According to the average ground height of each plant, we can see, in the untreated soil, seeds soaked in water 5h are slightly higher than those not soaked.

Observation every one of the rest of the four baskets, we can find that the concentration of seed soaked in 0.01% of humic acid solution, the height is highest, the second is the concentration of seed soaked in 0.05% of humic acid solution, the shortest is in the concentration of 0.005% humic acid solution soaked seeds. As a result, the optimum of seed soaking concentration was 0.01%.

By comparing basket 2th and 3th, and 4th and 5th, we can find that the spraying of humic acid at different concentrations had no significant effect on the growth of coriander.

Contrast basket 2th and 4th, and contrast basket 3th and 5th, we can find that in the other same conditions, the coriander in the baskets 4th and 5th significantly about 1 time higher than those in basket 3th and 4th. Thus the soil treated by the concentration of 0.1% humic acid solution fertility than treated by the concentration of 0.05% humic acid solution about 1 time higher.

By comparing basket 1th with basket 2th and 3th, we can find that the coriander in basket 2th and 3th are shorter than the soil that untreated, so the soil treated with a concentration of 0.05% humic acid solution will inhibit the growth of coriander.

By comparing basket 1th with basket 4th and 5th, we can find that the coriander in basket 4th and 5th is higher than that in the untreated soil, so the soil treated with 0.1% humic acid solution has a significantly improved fertility.

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**Table 3 – Final measurements of each plant, cm**

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<td>12.35</td>
<td>12.45</td>
<td>7.00</td>
<td>11.33</td>
<td>7.63</td>
<td>5.75</td>
</tr>
</tbody>
</table>

R: The average length of the root of each plant, H: The average ground height of each plant, W: The average total length of each plant, N: The average number of side branches per plant (Table 1).

According to the average length of the root of each plant and the average number of side branches per plant, we can see the length of the root and the number of side branches per plant are not depend on the concentration of humic acid solution.

**Conclusion**

According to FTIR analysis, the humic acid produced from lignite OLI treated by different kinds of
bacteria mainly contains hydroxyl groups, ketone and carbonyl groups, alcohols or carboxylic acids and phenols. The skeleton structure of humic acid treated with RKB1 was simpler than that of raw coal. The skeleton structure of humic acid treated with RKB7 is similar to that of raw coal. Because of the skeleton structure of humic acid treated with RKB7 is similar to that of raw coal, we used humic acid extracted by RKB7 for the cultivation of coriander. The optimum humic acid concentration of soaked seeds was 0.05%, under which coriander grew the fastest and best. The germination rate of seeds and the growth status of coriander were the best when the soil was treated with 0.1% humic acid solution, so the soil fertility could be improved most effectively when the soil was treated with 0.1% humic acid solution. That is, certain concentrations of humic acid solution can indeed improve soil fertility and promote plant growth.

**Conflict of interest**

All authors have read and are familiar with the content of the article and do not have a conflict of interest.

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