International Journal of Advanced Science and Technology
Vol. 29, No.02, (2020), pp. 273-278

Bioconversion of low-rank coal into humic substances by Acinetobacter sp. RKB

[1]Zhubanova A.A., [2]Akimbekov N.S., [3]Qiao X., Tastambek K.T., [4]Abdieva G.Zh., [5]Ualieva P.S., [6]Kayirmanova G.K.
Al-Farabi Kazakh National University, Almaty, Kazakhstan

Abstract: *Over recent years, the area of coal bioprocessing has been growing steadily due to its negative consequences for the environment, as fossil coal mainly harnessed as an energy source. The current state of the research on low-rank coal processing is aimed at improving its efficiency and environmental safety, as well as at obtaining high-demand chemical products, such as humic substances. This study was conducted that took into consideration the nature of coal low-rank coal biosolubilizaticonversion processes. The results presented herein show that the bacterial strain could be used as an effective aid for bioutilization efficiency to produce humic substances from low-rank coal.*

Index Terms *: Acinetobacter, bioconversion, biosolubilization, humic substances, low-rank coal*

I. INTRODUCTION

Oxidation of various fossil coals during their weathering occurs on the huge scale and adversely affects the coal properties, as well as its composition and structure, contributing to their degradation, concentration, and dispersion [1,2]. As a result of oxidation, there is a deterioration in the quality characteristics of coal as a fuel, while in some cases it is such great that these coals do not even find energy-fuel use, due to the low heat of combustion and extreme fragmentation. Such coals are not taken into account when calculating economic profits or reserves and refer to the so-called “off-balance” or “low-rank” coals (LRC) [3].When extracting solid fuel by an open method, a significant part of it goes to dumps in the form of waste heaps, which are generally unstable and unsuitable for use in the national economy. The reserves of oxidized brown and black coal reach billions of tons. They are scattered over large areas, which makes them difficult to selective-mining and processing [4].However, LRC could be used for the production of chemical raw materials, including fertilizers and the recultivation of polluted and disturbed lands [5]. Weathered and metamorphosed coals contain a huge amount of humic acids, which in their properties and composition are similar to humic substances contained in agricultural backgrounds, i.e. fertile soils. This circumstance was the decisive basis for a detailed study of the possibility of obtaining humic acids for their use in the production of humus, a natural fertilizer for vegetable crops. Currently, a significant number of studies show the positive effect of humic acids on soil fertility and crop yields [6-8]. Biotechnological processes significantly contribute to receive valuable products from various types of raw materials. Recent achievements in biotechnology have begun to attract increased attention and are now broadly used in extracting and processing of coal. Biotechnological transformation of coal can be aimed at obtaining solid, liquid and gaseous types of products, as well as improving its consumer-oriented characteristics. Depending on the method of bioprocessing of coal and the strains of microorganisms used in this process two main technological methods are distinguished: aerobic and anaerobic [9]. In aerobic conditions, due to the supply of oxygen-containing gas, oxidative processes develop predominantly, contributing to the partial destruction of the structure of coal and its transfer to a suspended state; while under anaerobic

ISSN: 2005-4238 IJAST

conditions, the metabolic processes lead to the formation of methane and carbon dioxide with the production of solubilized coal particles.
The aim of this study is to examine the biosolubilization of LRC samples in a bioreactor under aerobic condition using a selected strain of Acinetobacter sp.

II. MATERIALS AND METHODS

A. Coal sampling

Two LRC samples were received from the coal beds of "Kiyakty" (LRC-1) and "Lengerskoe" (LRC-2), Kazakhstan. The samples were collected according to Dai et al [10], ground (<0,2 mm) and stored at 4°C for further analyses.

B. LRC biosolubilization test

The selection of microorganisms with a target activity for biosolubilization was tested using the agar-diffuse method. The sterile coal was applied to the surface of the bacterial lawn at a rate of 1 g/cm^2 and cultured at 30°C for 5 days. Agar medium without inoculation served as control.

C. LRC bioprocessing in a batch reactor

Bioprocessing of LRC was performed under aerobic condition using a liquid culture of Acinetobacter sp. RKB. The process was conducted in a bioreactor with mechanical agitation (150 rpm) and aeration at 28°C (Fig. 1). A daily inoculum (OD_{600}=0.1) adapted to the coal was introduced into a reactor with a working volume of 1 l, containing powdered LRC at a concentration of 5% (w/v). The culture aliquots were collected every day under aseptic conditions, centrifuged at 10,000 rpm for 15 minutes, and filtered through membrane filters with a pore size of 0.22 μm. The resulting cell-free supernatants were measured at A_{450} using a LabTech UV-Vis spectrophotometer (UV-1000, China) to assess the biosolubilization rate.

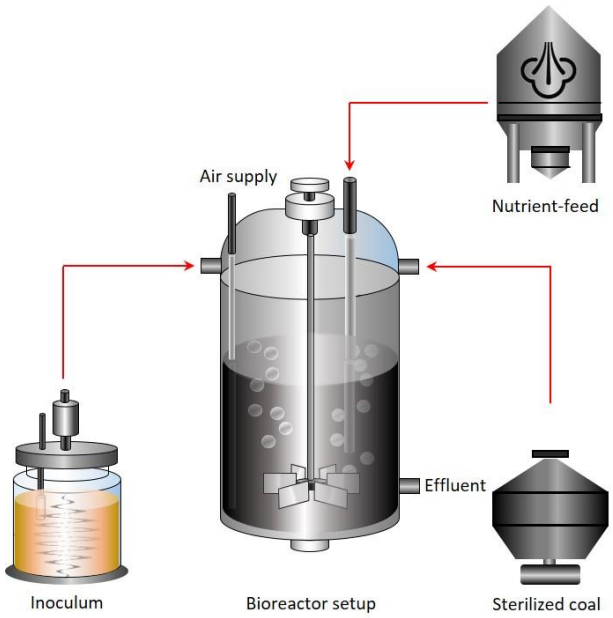


Fig. 1. Schematic diagram of bioreactor setup for LRC bioprocessing

D. Extraction of humic substances

Humic substances (HS) from treated and untreated LRC-1 and LRC-2 samples were extracted according to [11]. After biosolubilization the LRC was filtered through Whatman No. 1 paper and pH

was adjusted to 2.0 using 11.6 M HCl. The precipitant was centrifuged at 10 000 rpm for 15 min, washed with distilled water and dried at 60°C.

E. Fourier Transform Infrared (FTIR) analysis

FTIR of both HS was analyzed using Nicolet 6700 FT-IR spectrometer. IR spectrum of lignite samples were recorded in the range of 400 to 4.000 cm^{-1}.

F. Elemental analysis

Elemental composition of all HS was determined using a Vario EL cube Elemental Analyzer. The difference to 100% was calculated to the oxygen content.

III. RESULTS

A. Coal characterization

The results of the ultimate and proximate analyses of coals are shown in Table 1. As can be seen, both samples belong to the low-rank coal.

Table 1. Results of proximate and ultimate analyses of coal samples*

Parameters	LRC-1	LRC-2
Ultimate analysis (db, wt%)		
C	74.5 ± 0.2	63 ± 0.7
H	4.12 ± 0.1	3.4 ± 0.1
N	0.74 ± 0.4	0.88 ± 0.1
S	0.75 ± 0.1	2.11 ± 0.3
$O^{diff.}$	19.89 ± 0.1	30.61 ± 0.2
Proximate analysis (ar, wt%)		
Moisture, W	9.8 ± 0.8	9.3 ± 1.0
Ash, A	11.5 ± 0.5	22 ± 1.2
Volatile matter, V	41.8 ± 0.4	42.1 ± 0.1
Calorific value, Q (kJ/kg)	21 000	7 800

* Mean values ± standard deviation (n=5)

B. Selection of microbial strains with metabolic activity

The metabolic activity of the bacterial isolate was determined by the agar-diffusion method. The sterile dry coal was applied to an agar medium with a bacterial lawn, then incubated for 5 days. At the end of the incubation period, the presence or absence of biosurfactants was visually determined. As it can be seen from Fig. 2 bacteria cultures quickly solubilized the LRC samples on solid LB culture medium after 2 days. In control the brown strip around the coal was not observed due to the lack of inoculum. Thus, it can be assumed that the isolated culture has a high solubilized activity with respect to LRC.

ISSN: 2005-4238 IJAST

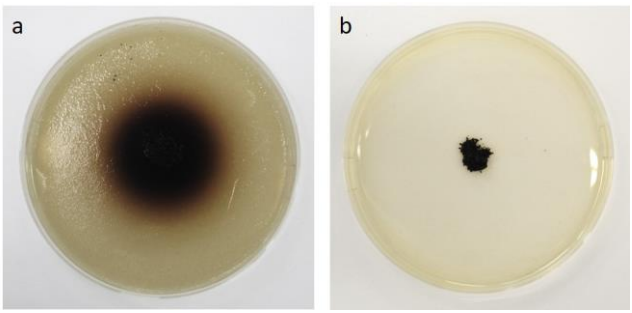


Fig. 2. Biosolubilization of LRC samples on a solidified medium. a – Bacterial strain growing on medium with LRC, b – control (sterile medium with LRC)

C. Bioprocessing reactor for the production of HS

The isolate was used to solubilize coal samples in a batch mode. At a coal concentration of 5%, the degree of the solubilization increased significantly with an increase in incubation time. Higher metabolic activity was shown after 6 days since spectral absorption of biosurfactants in the supernatant was 3.75±0.8. However, no significant changes were observed after 8 days of incubation (Fig. 3). However, the LRC-2 appears to be more resistant to biosolubilization than LRC-1.

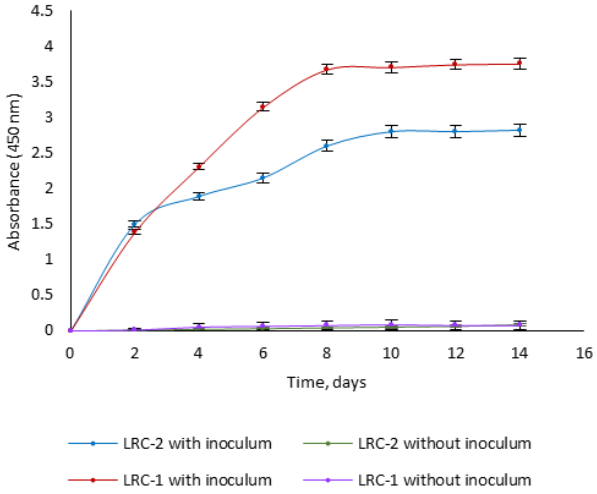
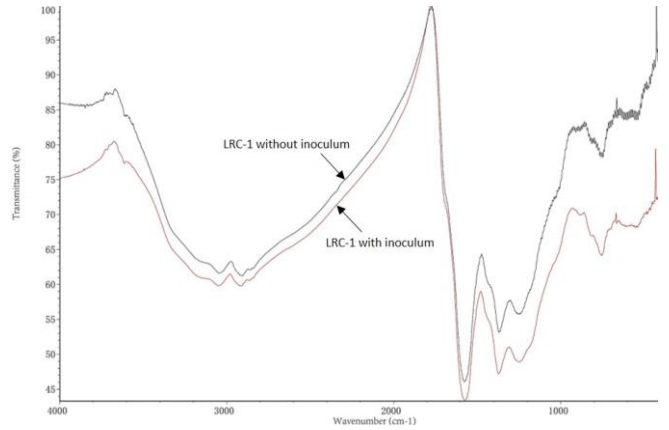


Fig. 3. The degree of biosolubilization of LRC samples

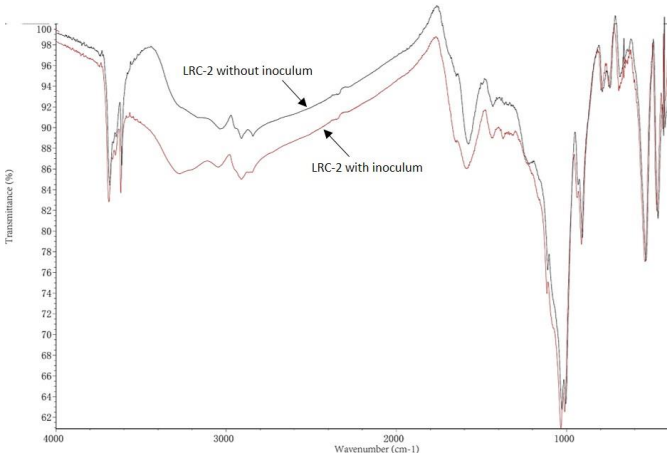
D. FTIR characterization

The IR spectra of samples have showed various absorption bands, indicating the multi-functionality of their molecules. The characteristic frequencies of 2980-2845 cm^{-1} in all samples indicate the presence of aliphatic groups. The absorption bands between 1600–1585 and 1500–1400 cm^{-1} regions indicate skeletal vibrations (C-C) (Fig. 4). Frequencies between 1250-1020 cm^{-1} of the LRC-1 can be associated with the absorption bands of C-N bonds characteristic for the amino groups of aliphatic amines.The bands of stretching vibrations of 1200–1100 cm^{-1} (C–O bond) in the IR spectra of the LRC-2 supernatant indicate the presence of secondary or α-unsaturated alcohol groups (-OH). The broad absorption bands in the region of 3670-3230 and 1410-1310 cm^{-1} indicate the presence of -OH-groups in their molecules, which are linked by intermolecular bonds. The bands of the C–O bond in the region of 1050–1000 cm^{-1} show the presence of primary alcohol groups. Absorption bands in the region of 700–600 cm^{-1}, corresponding to stretching vibrations related to the C–S bond, and absorption bands in the region of 500–400 cm^{-1}, corresponding to stretching vibrations of the S–S bonds.

ISSN: 2005-4238 IJAST



a



b

Fig. 4. FTIR spectra of LRC-1 (a) and LRC-2 (b) samples

So, the FTIR spectra demonstrated that under the influence of metabolic activity the slight alterations of LRC samples caused by appearing of various bands. This means, that the biosolubilization products can be heterogeneous with diverse chemical groups.

E. Elemental analysis

The residues of biosolubilization were subjected to elemental analysis (Table 2).

Table 2. Ultimate analysis of HS samples*

Sample	Element (%)					
	C	H	N	S	$O^{diff.}$	Ash
LRC-1 with inoculum	53.5	4.0	3.8	0.2	35.4	3.1
LRC-1 without inoculum	56.1	4.1	1.6	0.1	33.6	4.5
LRC-2 with	59.1	3.5	3.1	1.5	30.2	2.6

inoculum						
LRC-2 without inoculum	58.1	3.6	1.3	1.1	29.6	6.3

* Mean values ± standard deviation (n=5)

Bacterial treatment of LRC reduced the carbon and hydrogen content for HS-1 and increased the content of oxygen (from 33.6 to 35.4%), as well as nitrogen (from 1.3 to 3.1%). LRC-2 biosolubilization products were characterized by elevated levels of carbon, nitrogen and sulfur.
The elemental analysis revealed that the chemical composition of the biosolubilized products was changed after bioprocessing.

CONCLUSION

The aerobic biotransformation of LRC by Acinetobacter sp. RKB significantly changes the chemical and structural characteristics of the organic mass of coal. The generated HS through LRC bioprocessing could be used as a source of biofertilizers.

REFERENCES

[1] D. Banerjee, B.K. Niyogi, "Prediction of acid drainage from coal mining wastes", Indian J Environ Ecoplan, vol. 10, no. 3, pp. 911-916, 2005.
[2] K. Singh, B. K. Tewary, A. Sinha, "Environmental geochemistry and quality assessment of mine water of Jharia coalfield India", Environ Earth Sci, vol. 65, no. 1, pp. 49-65, 2012.
[3] M. J. Ghani, M. I. Rajoka, Kalsoom Akhtar, "Investigations in fungal solubilization of coal: Mechanisms and significance," Biotechnology and Bioprocess Engineering, vol. 20, no. 4, pp. 634-642, 2015.
[4] R.M. Fakoussa, M. Hofrichter, "Biotechnology and microbiology of coal degradation," Appl. Microbiol. Biotechnol., vol. 52, pp. 25-40, 1999.
[5] B.J. Baker, J.F. Banfield, "Microbial communities in acid mine drainage"., FEMS Microbiol. Ecol., vol. 44, pp. 139-152, 2003.
[6] B. D. Faison, "Microbial conversions of low rank coals," Bio/Technology, vol. 9, pp. 951-956, 1991.
[7] H. Machnikowska, K. Pawelec, A. Podgórska, "Microbial degradation of low rank coals," Fuel Process. Technol. Vol. 77-78, pp. 17-23, 2002.
[8] F. Jiang, Z. Li, Z. Lv, T. Gao, J. Yang, Z. Qin, H. Yuan, "The biosolubilization of lignite by Bacillus sp. Y7 and characterization of the soluble products," Fuel. Vol. 103, pp. 639-645, 2013.
[9] N. Kwiatos, M. Jędrzejczak-Krzepkowska, B. Strzelecki, S. Bielecki, "Improvement of efficiency of brown coal biosolubilization by novel recombinant Fusarium oxysporum laccase," AMB Express, vol. 8, p. 133, 2018.
[10] S. Dai, X. Wang, V. V Seredin, J.C. Hower, C.R. Ward, J.M.K. O'Keefe, W. Huang, T. Li, X. Li, H. Liu, W. Xue, L. Zhao, "Petrology, mineralogy, and geochemistry of the Ge-rich coal from the Wulantuga Ge ore deposit, Inner Mongolia, China: New data and genetic implications," Int. J. Coal Geol., vol. 90-91, pp. 72-99, 2012.
[11] L. Dong, Q. Yuan, H. Yuan, "Changes of chemical properties of humic acids from crude and fungal transformed lignite," Fuel, vol. 85, pp. 2402-2407, 2006.