UDK 622.332/333(497.115)
Original scientific article/Izvirni znanstveni članek

ISSN 1580-2949
MTAEC9, 44(5)271(2010)

THE MICROFLORA AND PHYSICO-CHEMICAL PROPERTIES OF LIGNITE FROM THE MIRASH MINE, NEAR KASTRIOT

MIKROFLORA IN FIZIKALNO-KEMIJSKE LASTNOSTI LIGNITA IZ RUDNIKA MIRASH PRI KASTRIOTU

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Prejem rokopisa – received: 2010-03-08; sprejem za objavo – accepted for publication: 2010-04-29

Coal, as an important source of energy, is very often the subject of study for scientists from the areas of chemistry, physics, technology, and biology, etc. In Kosovo, large deposits of coal, in the form of lignite, can be found. Lignite is mostly used to produce electrical energy; however, the interest of the country of Kosovo is to study many of the aspects of lignite.

In this paper an analysis of the microflora of lignite from the mine in the locality of Mirash, near Kastriot, is presented. With modern microbiological methods, the density, assortment, and some of the morphological and physiological characteristics of bacteria (heterotrophic, proteolytic, amilolytics, lypolytic and celulolytic) were determined and aerobic bacteria and fungi (yeasts and moulds) were investigated also. The samples of lignite were analyzed for physic–chemical attributes, for example, a determination of ash, and of C, O, H, N, S, carbonates, silicates, etc. The biomass of the isolated microflora from lignite was submitted for a chemical analysis and an astounding number of bacterial microflora and fungal was found. A composition chemical link between the coal and the microbial biomass was observed: the values of C, O, N, and H were found to be approximately similar in the coal and the bacterial mass.

Key words: coal, lignite, bacterial and fungal microflora, energy, carbonates, silicates, biomass

Premog kot pomemben vir energije je pogosto predmet študija znanstvenikov s področja kemije, fizike, tehnologije, biologije in drugih ved. Na Kosovu so velike količine premoga v obliki lignita. Največ lignita se uporablja za proizvodnjo električne energije, zato je razumljiv interes kosovske družbe in znanosti za njegovo vsestransko preučevanje. V tem prispevku je predstavljena analiza mikroflore lignita iz rudnika Mirash pri Kastriotu. Z modernimi mikrobiološkimi metodami so bile določene gostota, razvrstitev, morfološke in fiziološke značilnosti bakterij (heterotrofnih, proteolitičnih, alilolitičnih, lipolitičnih in celulitičnih). Istočasno so bile preiskane tudi aerobne bakterije in glive (kvasovke in plesni). Vzorci lignita so bili analizirani in določene so bile fizikalno-kemične lastnosti, kot so: vsebnost pepela, vsebnosti C, O, H, N, S, karbonatov, silikatov in podobnega. Biomasa izolirane mikroflore iz lignita je bila kemijsko analizirana. Najdeno je osupljivo veliko število bakterijske mikroflore in gliv v lignitu in opažena je bila povezava med kemijsko sestavo premoga in biomaso mikrobov. Vsebnosti C, O, N in H so bile približno enake v premogu in v masi bakterij.

Ključne besede: premog, lignit, mikroflora bakterij in gliv, energija, karbonati, silikati, biomasa

1 INTRODUCTION

Coal is considered to be a very important source of energy, and it has been used since the earliest times (before the new era). The economic development of society during the past 1000 years was based on coal as a source of energy (1). Oil and its derivatives are also an important source of energy, and by the beginning of the second half of the 20th Century it had achieved primacy as a source of energy. Nowadays, an interest in coal has once again emerged, because 80 % of fossil fuel reserves are coal as compared to 18 % for oil.

Actually, 45 % of the globally generated energy is generated with coal, as compared to 30 % from oil and 14 % from natural gas (2). Coal is a fossil fuel; it is an organic sedimentary rock; and it is formed by the accumulation of organic matter (plant debris).

This process usually takes place in waterlogged environments (water basins, lakes, marshes, swamps) (3). Coalification covers a number of geological periods, which can be used to determine the geological history of the Earth's crust (4). For this reason, the process of coal formation took place as a result of a number of different factors (pressure, humidity, lack of air, microbiological reactions, physical and chemical reactions, etc.). The best way to describe this complex process, which took place over millions of years, is the accumulation of dead organic (plant) matter that subsequently forms coal. The composition of coal is very complex; however, it consists mainly of carbon (between 60 % and 95 %). Thus, it has a very wide range of uses and a characteristic structure. Coal contains the following biogenic elements: carbon, hydrogen, oxygen and sulfur. From the biochemical point of view coal contains the same elements as did the tropical plants from which it was formed: oils, resins, etc. It ranges in color between black and brownish-black. Its strength is between 0.5 kg/cm^2 and 2.5 kg/cm^2 and its specific weight is 1–1.7.

Coals are divided into humic (formed by the degradation of land plants) and sapropelic (algae and fungi) (5) and it is found in the form of sedimentary rocks. After the fossilization the organic matter was submitted to degradation and in specific circumstances a new matter was formed, which served as the skeleton for the formation of coal. The following can be found in plant cells: cellulose, hemicellulose, lignin, proteins, oils, waxes, resins, cutin, etc. Through the interaction of micro-biological, physical and chemical, as well as geological factors, the organic matter turns into a mass called torv (peat), lignite and other kinds of coal. The coalification process has two phases: humification and carbonization. During the process of humification, organic materials, under the influence of the above-mentioned factors, turn into torv (peat) – humus. The peat is then degraded by microorganisms. Bacteria are known to degrade organic matter and cellulose, on which occasion enzymes and biocatalyzers are produced and affect the oxidoreduction process, as well. Microorganisms affect the inorganic matter by increasing the mineral solubility (7).

According to several authors (8, 9, 10), upon the degradation of peat, microorganisms reduce their activity as a consequence of the formation of antiseptic substances, such as phenols (8).

During the second phase, torv or sapropel will turn into anthracite, lignite, coal, etc (9).

In aerobic conditions, 80 % of the organic matter is lost, whereas in anaerobic conditions only 5 % of the organic matter is lost (10). During the degradation of the organic matter proteolytic microorganisms degrade proteins. Then the degradation of the oils by lipolytic microorganisms and that of cellulose by cellulolytic microorganisms follows and afterwards, the degradation of hemicellulose, lignin, cutin and eventually resins, occurs.

The influence of physical and chemical factors in the formation of coal is characterized by increased pressure and the catalytic influence of minerals and gases together with water, temperature and pH. From the petrographic point of view lignite is xylitic. Geological data show that the Kosovo Coal Basin dates back to the Pliocene (approximately 2.5 million years ago). This tectonic basin was formed during the Oligocene (other layers were sedimented on top of it during the late Tertiary Period). Its reserves are estimated to 2.2 billion tons and could be as high as 10 billion tons. According to (11), the ecoregions in the Kosovo basin during the Pliocene consisted of wetlands, hills and mountains. Based on the ecological conditions, in which certain groups of plants lived, the following wetland vegetation zones existed (12):

1) Submerged aquatic plants (Myriophyllum)
   a) floating plants
   b) emergent plants (Graminea and Cyperaceae)
2) Mountain vegetation zone (Taxodiaceae)
3) Shrub zone (Polipodiaceae)
4) Dry forest zone (Sequoia forests) (14)

II. Hilly region

Forest zone: Salix, Alnus, Populus (15)
Deciduous forest zone: Querqus, Fagus, Carpinus, Tilia, Castanea, Acer, etc.

III. Mountainous region: Pinus, Picea, Abies, Laryx (16).

From these groups of plants the coal was formed. According to several references, coal and different microorganisms co-existed since the time the deposit was formed, around 2.5 million years ago. They have survived in a latent or dormant state, which slowed down their metabolism in order to survive the unfavorable conditions. The other form is called spores or endospores, and many scientific papers have dealt with coal and microorganisms.

It was established that certain species of bacteria can clean coal and improve its quality, e.g., leptospirillum, ferroxidans and thiobacillus ferroxidans. These species have been bred to use phenol as their only source of food (17) and pseudomonas and arthrobacter have degraded coal molecules by "eating" sulfur and other contaminants.

Bacteria increase the intensity of methane degradation (attested in the labs of the US Energy Department). The digestive system of the above-mentioned bacteria eliminates harmful pollutants and helps the production of clean coal (18). Certain strains (Pseudomonas) can degrade or ferment, coal derivatives (fenatren) producing CO_2 and H_2O: Fenatren – 1 – hydroxyl 2 – naphtoic acid – salicylic acid – catechol – CO_2+H_2O (19). The bacteria digest the carbon in the rocks and produce natural gas for a period of about 10–1000 years (21) and lock methane at the bottom of the sea in anaerobic conditions. The aim of this study was to determine the presence of the bacterial and fungal microflora in the lignite from the Mirashi-Kastrioti Coal Mine. The presence of heterotrophic, proteolytic, amilolytic, lipolytic, cellulolytic, aerobic bacteria and fungi was studied in order to get an idea about the existing microflora at the time when the lignite was formed.

Furthermore, the aim of this study was to determine the composition of the ash, biogens and bacterial biomass in the lignite from the Mirashi Coal Mine. The authors were motivated by the claim (6) that hopanoids, the compounds in the membranes of prokaryotic cells (as well as bacteria), can be found in great abundance here (as well as by the data on the amount of carbon in the coal of the Kosova basin). It must be stressed that hopanoids are the main constituents of coal (90 %). Scientific data show that the membranes of prokaryotic cells (bacteria, cyanobacteria) have the most hopanoids.

Since the organic material (in coal) consists largely of bacteria in the form of kerogen, which is the organic precursor of petroleum, it becomes obvious that hopanoids or bacteriohopanetetrol are isolated from kerogen. In order to verify the relation between the accumulation of kerogens and bacterial activity, the authors (20, 23) have demonstrated the importance of bacteria in the formation of fossil fuels and the degradation of organic matter.

2 MATERIAL AND METHODS

Lignite samples from the Mirashi Coal Mine in the vicinity of Kastriot were used as study material and samples of dry and wet coal were examined. For the physico-chemical analysis of the coal (lignite), gravimetric, chromatographic and spectrophotometric methods were used. The following parameters were determined: moisture, ash, combustible volatile matter, lower calorific value, fixed carbon, total sulfur, organic sulfur, inorganic sulfur, SiO_2, Al_2O_3, Fe_2O_3, CaO, magnesium oxide and sulfur oxide.

A lump of coal was first examined. Later it was subjected to grinding and mixed with sterilized tap water in order to determine the presence of microflora on its surface and interior. Modern methods were applied to determine the presence of microflora (Standard Methods for the Examination of Water and Wastewater, APHA – last edition). The presence of biogens was determined using a sophisticated apparatus (VARIO MACRO CHNS). Initially, wet coal was dried at a temperature of 105 °C for 2 h. A sample of coal with a weight of 75 mg to 125 mg was used. The minimum weight for a reliable analysis was 40 mg. First, the content of biogens present in the lignite, the ratio between C and N, C and H as well as C and S were determined. Then the presence and the amount of biogens in the bacterial biomass isolated from the lignite were determined using the same apparatus.

3 RESULT WITH DISCUSSION

3.1 Microbiological analysis of lignite

The microbiological, physical and chemical results were obtained in the three-month period of March, April and May. The results represent the arithmetical average of three measurements. Wet coal was used due to the fact that it had a higher density of microorganisms.

Table 1: Heterotrophic bacteria and physiological groups isolated from lignite
Tabela 1: Heterotrofne bakterije in fiziološke skupine, izolirane iz lignita

Bacterial group	Unit	Number	*x*/%
Heterotrophic bacteria (saprophytic)	mL	28,855,000	100
Proteolytic	mL	3,375,000	11.49
Amilolytic	mL	4,900,000	16.98
Lipolytic	mL	2,380,000	8.24
Cellulolytic	mL	3,200,000	11.08
Sporogenic (microaerophilic)	mL	15,000,000	51.98
Total		28,855,000	99.97

(The amount of substance fraction, *x*/%)

Table 2: Fungal microflora isolated from the lignite of the Mirashi Coal Mine.
Tabela 2: Mikroflora gliv, izoliranih iz lignita iz premogovnika Mirashi

Fungi type	Unit	Number	*x*/%
Mold	mL	16,000,000	66.66 %
Yeast	mL	8,000,000	33.33 %
Total		24,000,000	99.99

Table 3: Some physical parameters of the lignite from the Mirashi Coal Mine (March, April and May 2008)
Tabela 3: Nekaj fizikalnih parametrov lignita iz premogovnika Mirashi (marec, april in maj 2008)

Month	*w*(M)/%	*w*(A)/%	*w*(Vm)/%	*Hu*/(MJ/kg)
March	41.30	12.84	41.27	8.563
	44.64	16.49	38.87	7.809
	42.35	18.9	35.94	6.889
April	41.30	19.20	37.35	7.332
	42.20	15.1	39.37	7.966
	41.25	15.15	38.95	7.834
May	42.40	12.80	39.31	7.947
	42.32	17.79	35.67	6.804
	42.30	19.60	36.88	7.184
	40.28	13.74	36.82	7.165
Average value	42.03	16.16	38.04	7.549

Legend: M – moisture content, A – ash content, Vm – volatile matter, *Hu*/(MJ/kg) – lower calorific value.

The data show that the sporogenic bacteria (51.98 %) dominate the microflora, followed by the proteolytic bacteria (11.49 %) and amilolytic (16.98 %). The results of the analysis prove that the physiological groups listed in **Table 1** were present in the plant matter from which the lignite was formed. Based on the results in **Table 1** (according to which a single group may contain up to 28,855,000 bacteria), we can conclude that 1 t of lignite contains about $28.8 \cdot 10^{12}$ microorganisms, as was stated by other authors as well (6).

According to **Table 2**, just 1 g of lignite with moisture content contains 16 million mold cells (66.6 %). The remaining 33.33 % are yeast cells.

3.2 Physical and chemical analysis

The above-mentioned lump of coal was used to determine the content of moisture and ash, combustible volatile matter as well as the lower calorific value of the coal (**Table 3**).

The lower calorific value of lignite present in **Table 4** was 8027 MJ.

CaO and SiO_2 are the main components in the ash of the coal from the Mirashi Coal Mine. The large concentration of CaO shows that it originates from limestone sedimentary rock. The large presence of SiO_2 shows that the lignite originates from silica algae (silica rich soil) (**Table 5**).

Table 4: Content of components important for the caloric value of lignite (Mirashi Coal Mine March, April and May 2008)
Tabela 4: Vsebnost komponent pomembnih za kalorično vrednost lignita (premogovnik Mirashi, marec, april in maj 2008)

No	Analyzed parameters	Content, *w*/%
1	Coke	34.06
2	Fixed carbon	18
3	Vaporizing matter	20.2
4	Volatile matter	39.64
5	Total sulfur	0.78
6	Other	7.52

Table 5: Chemical composition of ash of lignite from the Mirashi Coal Mine (March, April and May 2008)
Tabela 5: Kemijska sestava pepela v lignitu iz premogovnika Mirashi (marec, april in maj 2008)

Components	Content, *w*/%
SiO_2	27.48
Al_2O_3	11.82
Fe_2O_3	7.46
CaO	37.33
MgO	3.2
SO_2	9.6
Other	3.11

Table 6: Content of biogens (C, N, S, H) in lignite from the Mirashi Coal Mine
Tabela 6: Vsebnost biogenov (C, N, S, H) v lignitu iz premogovnika Mirashi

	Sample weight	Unit	Sample	*m*(C)/*m*(N) ratio	Content, *w*/%
First measurement	40.4	mg	lignite	43.06	*w*(N) = 1.096 *w*(C) = 47.19 *w*(S) = 1.268 *w*(H) = 4.383
Second measurement	40.0	mg	lignite	45.55	*w*(N) = 1.032 *w*(C) = 47 *w*(S) =1.347 *w*(H) = 4.392
Third measurement	40.8	mg	lignite	49.23	*w*(N) = 1.055 *w*(C) = 51.92 *w*(S) = 1.381 *w*(H) = 4.405
Average value				45.94	*w*(N) = 1.061 *w*(C) = 48.73 *w*(S) = 1.332 *w*(H) = 4.36

Table 7: Presence of biogens (C, N, S, H) in the microbial biomass isolated from lignite of the Mirashi Coal Mine
Tabela 7: Prisotnost biogenov (C, N, S, H) v biomasi mikrobov, izoliranih iz lignita iz premogovnika Mirashi

Measurement	Sample weight m/mg	Sample	C/N ratio *w*(C)/*w*(N)	Content, *w*/%
First measurement	84–95	Microbial biomass *w*(C)/*w*(N) = 2.07	N: C: S: H:	21. 34 44.36 0.77 7.18
Second measurement	84–95	*w*(C)/*w*(N) = 2.07	N: C: S: H:	21.53 44.75 0.72 7.31
Third measurement	84–95	*w*(C)/*w*(N) = 2.08	N: C: S: H:	21.42 44.68 0.70 7.24
	w(AV)/*w*(N) = 21.43; *w*(C) = 44.59; *w*(S) = 0.73; *w*(H) = 7.24			

3.3 The presence of biogens (C, N, S, H) in the analyzed coal and in the microbial biomass isolated from the same

Data in **Table 6** show that carbon is the main component in biogenic coal (48.73). The average value of $m(C)/m(N)$ ratio is 45.94.

The data in **Table 7** confirm, once again, the domination of carbon in the microbial biomass isolated from the lignite of the Mirashi Coal Mine. The results show that there is a resemblance in the constitution of the biogens in the coal and those in the bacterial biomass. It has been confirmed that during the Pliocene the vegetation in the Kosovo basin consisted of 132 different types of plants with numerous sub-types, grasses, aquatic plants, trees, etc. A number of authors (11, 12, 13, 14, 15) have reconstructed the vegetation of that period by comparing the fossil pollen grains and spores from the Pliocene with those from the present day. The same plants can nowadays be found in the area of the Kosovo coal basin.

4 CONCLUSION

Based on the investigations performed, the following conclusions can be drawn:

- The lignite from the coal mine in Mirash (Kastriot) has a high density of microorganisms, i.e., 28,855,000 microorganisms per gram or $28 \cdot 10^{12}$ microorganisms per ton.
- Among the isolated microorganisms we have determined the presence of bacteria, molds and yeasts (gram positive, negative and variable). The most common shapes were rods, spheres and spirals.
- Forty pure cultures were isolated (with typical representatives).
- Due to it physical and chemical characteristics the lignite from the Kosovo basin is of a better quality than that of other basins in the region.
- The presence of biogens (C, N, H, S) in the coal and the microbial biomass provides evidence of their functional interaction during the process of coal formation.
- The number of microorganisms in 1 g of lignite is evidence that most of the microorganisms are deposits of hopanoids, respectively carbon.
- The fossil pollen grains and spores found in different horizontal and vertical layers of the Kosovo coal basin give us a picture of the vegetation of the area from which the lignite was formed.

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