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KINETIC STUDY OF BIODEGRADATION OF ORGANIC MATTER EXTRACTED FROM BLACK SHALE ORE

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Microbial degradation of organic matter extracted from black shale ores was investigated. The kinetic models of biodegradation of organic compounds were discussed. The effect of chloroform on the rate of biomass growth was described. Our results suggested that the process of biodegradation of organic matter is rather slow. The Haldane kinetic model could be available to describe the kinetic of biodegradation of organic matter.

Keywords; biodegradation, black shale, heterotrophic macroorganism, kinetic, organic matter

INTRODUCTION

The *in situ* biodegradation of organic compounds is a function of the catabolic activity of bacteria. Some toxic organic compounds are chemoatractant for different bacteria species, which can lead to improved biodegradation of these compounds. For instance, crude oil is a complex mixture of hydrocarbons, basically composed of aliphatic, aromatic and asphaltene fractions. Many microbes have ability to utilize hydrocarbons as a sole source of carbon and to transform them to a less toxic form (Kunar et al., 2005).

Asphaltenes is a group of organic compounds extracted from crude oil samples. Their composition is close to the organic matter extracted from black shale. Biodegradation of asphaltenes through the used of a microbial consortium or mixed

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cultures isolated from soil samples has been described (Pineda-Flores et al., 2004). However, the extent of biodegradation was from 0.55 to 35 %.

There have been relatively few studies on the bacterial degradation of metallorganic compounds. Several strains of *Pseudomonas fluorescens* were used to degrade tetracyanonickelate (TCN) as a sole nitrogen source (Baxter Cummings, 2006). An *Acinetobacter* sp. strain isolated from gold mine was cable for degrading gold, silver, cadmium, zinc, copper, cobalt, and iron cyanide complexes.

Microbial degradation of complex organic compounds such as phtalic acid was investigated using bacteria cultures isolated from sewage sludge. More than 99% of phthalic acid at the initial concentration of 4000 mg/dm^3 was degraded within 5 days (Fan et al., 2004).

A biological decolourisation process was used for azo dyes (Beydilli Pavlostathis 2005). The kinetic modelling of azo decolourisation was performed using the data obtained from the tests where the effect of different initial dye concentrations on the decolourisation process was examined. Up to now, some research dealing with the mineralogical analysis showed that very important compounds of organic matter are metalloporphyrins. However, lake petroporphirins, metalloporphyrins from black shale are highly resistance towards biodegradation (Grosjean et al., 2004).

The goal of this research is to understand better biodegradation of complex chemical mixtures such as organic matter by microbial consortium.

KINETICS OF BIODEGRADATION

The mathematical model of homogeneous substrate consumption (biodegradation) should take in consideration the microbial growth, competitive inhibition and the formation of toxic intermediates. Most models have been tested with single or two substrates (Reardon et al., 2002). The initial stapes in model creation was used the simple model for cell growth kinetics and substrate depletion.

$$\frac{dS}{dt} = -\frac{\mu X}{Y_{\frac{X}{S}}} \tag{1}$$

where S is the substrate concentration, t is time, μ is specific growth rate, $Y_{X/S}$ is the biomass yield, and X is biomass concentration. To determine the yield, $Y_{X/S}$ the concentration of cells produced (cells/cm^3) was divided by the concentration of substrate consumed (mM). In the case of single substrate, the growth kinetics is well fitted by the Monod equation:

$$\mu = \frac{1}{X}\left(\frac{dX}{dt}\right) = \frac{\mu_{\max} S_L}{K_S + S_L} \tag{2}$$

in which μ_m is the maximum specific growth rate and K_S is the Monod half-saturation constant. However, in some cases, the substrate inhibition effect has been observed (Andrrews 1968). Haldane has proposed a substrate inhibition model:

$$\mu = \frac{1}{X}\left(\frac{dX}{dt}\right) = \frac{\mu_{\max} S_L}{K_S + S_L + \left(\frac{S_L^2}{K_i}\right)} \tag{3}$$

where K_i is an inhibition parameter.

MATERIALS AND METHODS

ORGANIC MATTER

The organic matter was extracted from black shale sample (middling) from Lubin mine. This material was collected from the flotation circuits. The mineral sample was treated with HCl solution to remove carbonate components, than it was treated with HF solution to decompose clay minerals. The organic matter was extracted in Socslet. The conditions of extraction were as follows: the solid fraction 300 g; chloroform 600 cm^3, temperature 70^0C, time of extraction 24 h. The composition of both "middlings" and organic matter is presented in Table 1.

Table 1. Concentration of metals and organic matter in middlings

Metal concentration [ppm or %]	Cu	Co	Ag	Fe	Ni	V	C_{org}
Lubin middlings	1.96[%]	498	-	1.75 [%]	304	897	8.84
Organic matter	254	31.5	1.1	198	450	259	-

MICROORGANISMS

Microbial degradation of organic matter was investigated using culture of heterotrophyc bacteria enriched from solid samples collected with waste heap. A mixture of bacteria (Consortium C) containing *Pseudomonas fluorescen, Areomonas hydrophilia, Oligella* sp. *Bacillus megaterium, Streptomonas maltophilia, Stenotrophomonas maltophilia, Bacillus amyloliquefaciens, Bacillus circulans, Bacillus pumilus and Burkhordelia capacia* was used for the biodegradation experiments. The media used in this study contained (g/dm^3): $(NH_4)_2SO_4$ – 2g: K_2HPO_4 – 3g; KH_2PO_4-2g; $MgSO_4$ $7H_2O$ 0.5g. The pH value of the medium was adjusted to about 7.0.

ORGANIC MATTER EXTRACTION

The powdered black shale ore samples were exhaustively extracted with chloroform in Soxhlet. The conditions of organic matter extraction were as fellows: solid samples –300g; volume of chloroform 600 cm^3; temperature 70^0C; time of extraction 24 h.

BIODEGRADATION EXPERIMENT

A series of 250 ml sterile Erlenmeyer flasks were used in biodegradation study. Each flask contained 100 cm^3 of sterile mineral salt medium. 20 cm^3 of the water emulsion, contains the organic matter dissolved in chloroform, was added to the flask. Also, 0.2 cm^3 of inoculum was added. The biodegradation of organic matter experiments was carried out in a shaker and the temperature was 30^0C.

RESULTS AND DISCUSSION

Fresh shale contains approximately from 1 to 10 wt. % organic carbon (Fischer and Gaupp, 2005). Table 2 shows the concentration of organic carbon bleached and unbleached black shale.

Table 2. Total organic carbons content of bleached and unbleached black shale ores (Fischer, Gaupp, 2005)

Sample	black	dark grey	pole grey	whitish yello
Total organic carbon [wt%]	8.08	2.42	1.55	1.35

EFFECT OF CHLOROFORM ON THE BACTERIA GROWTH

The rate at which microorganism cells grows during the biodegradation process can be depended on the reagent used for organic mater extraction (chloroform). The effect of the presence of chloroform on the microbial growth was investigated. Figures 1 and 2 show the effect of the addition of 15 % of chloroform on the kinetic of *Bacillus cereus* and *Bacillus laterosporus* growth.

The experimental results indicated that the presence of chloroform could greatly decrease the kinetics of growth of bacteria. The population of bacterial cells dramatically decreased. For support of this conclusion photographs of bacterial colonies have been taken. The surface colonies of *Bacillus cereus* after 6 hours growth with and without chloroform are presented in Figure 3.

BIODEGRADATION OF ORGANIC MATTER

The recommended procedure for estimating biodegradation of organic matter would be to use mixtures of microorganisms. The biodegradation of organic matter was carried out for up to 69 and 76 days for the Lubin and Mansfeld samples, respectively. A special prepared bacteria consortium (consortium C) was used.

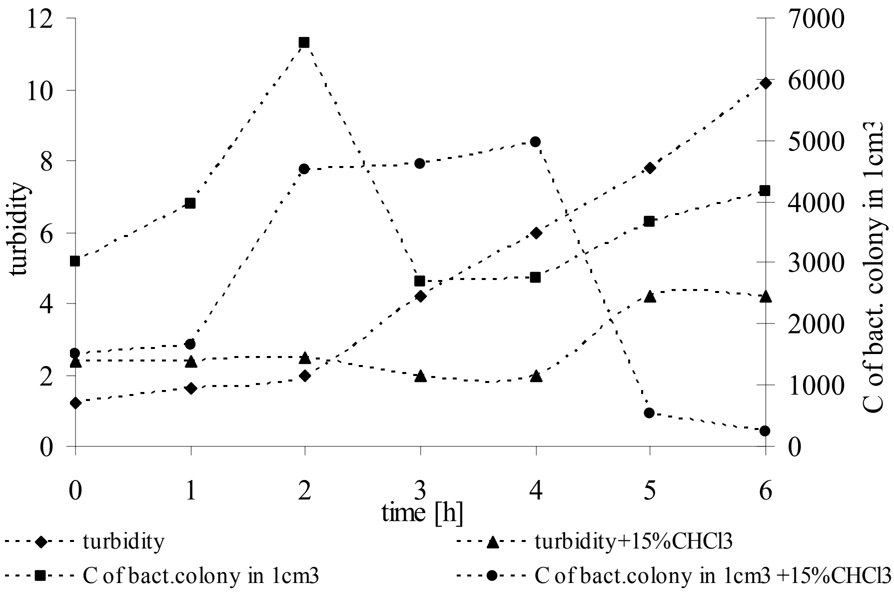

Fig. 1 The growth of *Bacillus cereus* with the presence and absence of chloroform

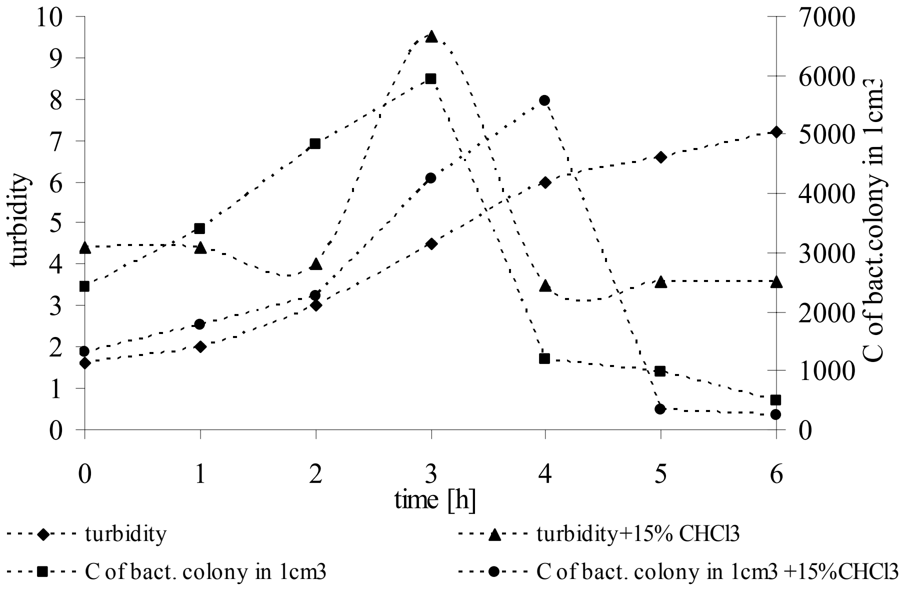

Fig. 2. The growth of *Bacillus laterosporus* in the presence and absence of chloroform

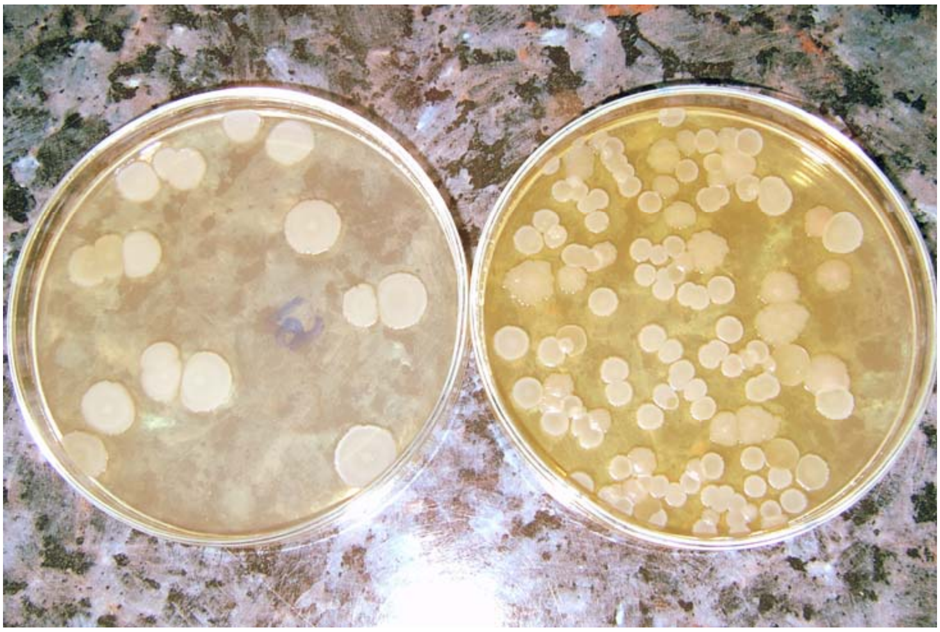

Fig. 3. The colony of *Bacillus cereus* in the presence of chloroform (left) and its absence (right)

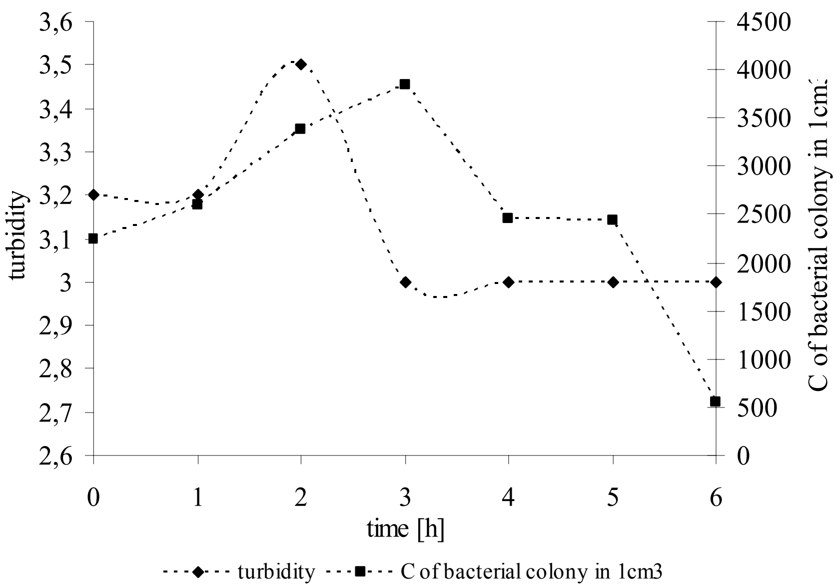

Fig. 4. The growth of *Bacillus cereus* in the presence of organic matter

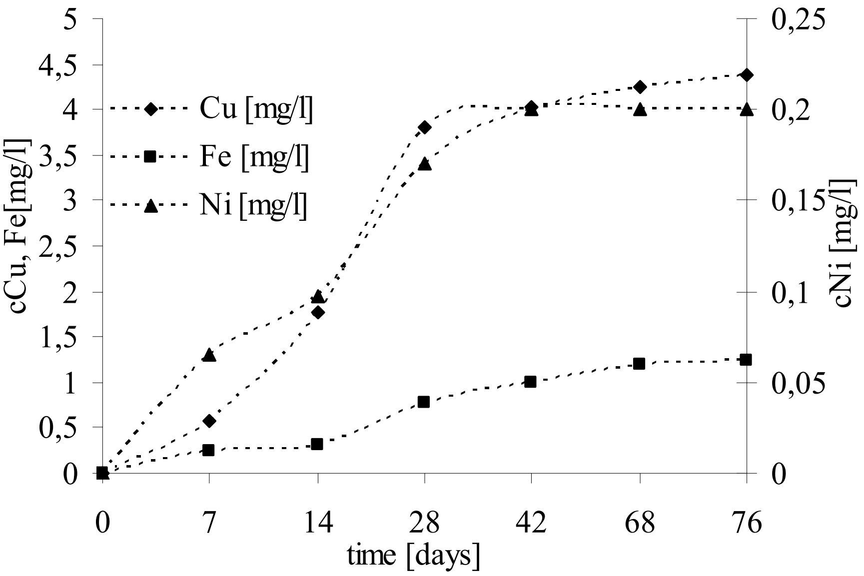

Fig. 5. Biodegradation of organic matter extracted from black shale (Lubin, Poland)

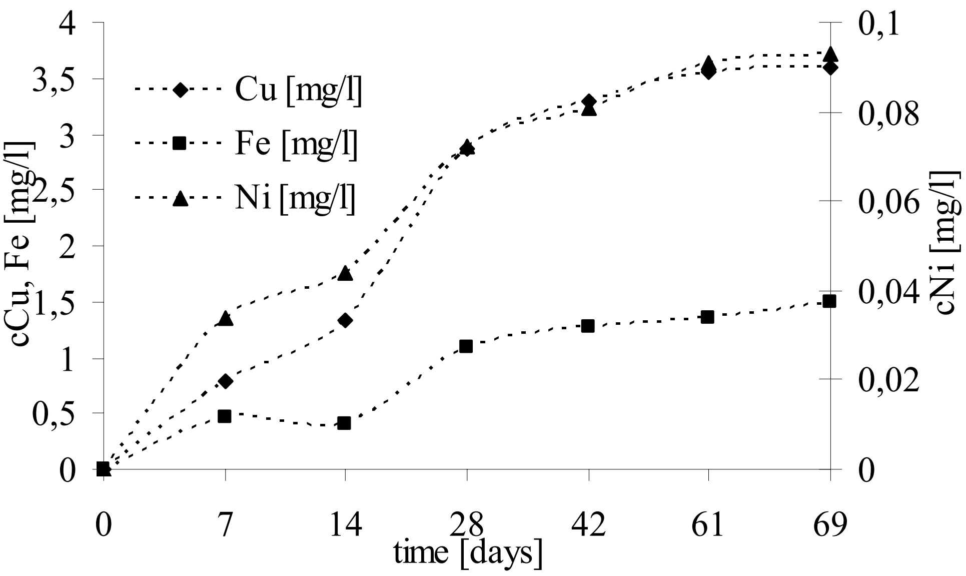

Fig. 6. Biodegradation of organic matter extracted from black shale (Mansfeld, Germany)

The rate of ions production can be involved in biodegradation of organic matter. The biological degradation of metal-organic compounds, contained in organic matter, produced metal ions. The rate of increase of the metal ions concentration should correlate with the biodegradation rate. However, the theory of biodegradation single or mixed organic compounds bases on the rate of substrate decrease. A model biodegradation based on changes of product rate should be developed. The above reported results showed that the biodegradation of organic matter was quite slow. Initial analysis showed that modified Haldane equation would be responsible for biodegradation described. The concentration of substrate should be exchanged for the concentration of products. Further investigations of mechanism of organic matter biodegradation should be done for a discovery of correlation between substrate and product during an organic matter biodegradation process.

CONCLUSIONS

The investigations revealed that organic matter dissolved in chloroform and suspended in water solution could be degraded by bacteria. The presence of chloroform caused a decrease of microbial growth kinetics. Preliminary study showed that a modified kinetic model could be used to describe the biodegradation of organic matter extracted from the black shale ore samples.

ACKNOWLEDGEMENTS

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Farbiszewska T., Farbiszewska-Kiczma J. Jażdżyk E., Sadowski Z. Szubert A., Badania kinetyki procesu biodegradacji matrycy organicznej ekstrahowanej z rudy łupkowej, Physicochemical Problems of Mineral Processing, 40 (2006), 317-325 (w jęz. ang.).

Matryca organiczna, ekstrahowana z próbek mineralnych rudy łupkowej, poddana została procesowi biodegradacji. Do procesu biodegradacji użyto specjalnie przygotowanej mieszaniny bakterii. Na podstawie danych literaturowych przeanalizowano istniejące modele kinetyki procesu biodegradacji i uznano, że zmodyfikowany model zaproponowany przez Haldane będzie najlepszym dla opisu procesu. Modyfikacja wymaga zastąpienie stężenia substratu stężeniem produktu reakcji biodegradacji. To, czy jest możliwe, jako produkty użyć stężeni jonów metali, odpowiedz dadzą następne badania. Zbadano wpływ obecności chloroformu na proces biodegradacji i wzrostu mikroorganizmów. Wykazano, że obecność chloroformu pogarsza kinetykę procesu biodegradacji. Proces biodegradacji jest procesem powolnym.