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Mariusz DUDZIAK[1]

REMOVAL OF ZEARALENONE FROM WATER BY MEANS OF OZONATION AND INTEGRATED SYSTEM OF OZONATION/NANOFILTRATION

USUWANIE ZEARALENONU Z WODY W PROCESIE OZONOWANIA I W UKŁADZIE OZONOWANIE/NANOFILTRACJA

Abstract: Results of the study on the effectiveness of zearalenone removal via ozonation and integrated ozonation/nanofiltration water treatment are presented. The influence of ozone dose, contact time, pH and water properties on ozonation performance was investigated. The study shows that application of integrated system of ozonation and nanofiltration is advantageous according to the effectiveness of zearalenone and other water contaminants removal as well as membrane capacity.

Keywords: zearalenon, organic micropollutants, ozonation, nanofiltration, water treatment

Ozone was found to be a very strong oxidizer already at the beginning of XIX century. The redox potential of ozone in the acidic environment is equal to 2.07 V while in basic to 1.27 V. Theoretically, it assures the amount of energy sufficient to oxidize organic and inorganic compounds present in water. Ozone is usually applied for removal of color, taste and smell of water as well as for its disinfection [1].

The elimination of organic micropollutants by means of ozonation is also discussed in the literature [2-4]. The effectiveness of the process depends on ozone dose, contact time, pH and water properties [1-4]. The ozone dose sufficient for total oxidation of organic compounds (determined as *dissolved organic carbon* DOC) is quite high and equal to 8 mgO$_3$/1 mgDOC [1]. The decrease of required ozone dose can be obtained by integration of ozonation with other unit operations eg activated carbon adsorption [5]. The application of membrane processes is also possible, and additionally the polishing of water is performed [6].

The effectiveness of ozonation and integrated water treatment system ie ozonation/nanofiltration for removal of zearalenone was investigated. Zearalenone (ZON) [6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcylic acid lactone] is a compound from mycotoxins group of estrogenic properties which are produced by fungi of *Fusarium* type [7]. Nowadays, it is found to be present in surface water as a result of environment pollution [7, 8].

Materials and methods

Simulated solutions prepared on two water matrices ie deionized water and tap water with and without addition of humic acids and constant zearalenone concentration equal to 500 μg/dm^3 (Table 1) were used in the study. Humic acids and zearalenone standards were supplied by Sigma-Aldrich. The content of high-molecular weight organic compounds in

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[1] Faculty of Energy and Environmental Engineering, Silesian University of Technology, ul. Konarskiego 18, 44-100 Gliwice, phone 32 237 16 98, email: mariusz.dudziak@polsl.pl

water was determined via absorbance measurements (at wavelength λ = 254 nm) using UV VIS Cecil 1000 spectrophotometer by Jena AG, while inorganic substances concentration via conductivity measurements with laboratory multiparameter analyzer inoLab® 740 by WTW. The determination of zearalenone concentration was made by *solid phase extraction* SPE and *high performance liquid chromatography* HPLC analysis. Supelclean™ ENVI-18 tubes (volume - 6 cm^3, phase - 1.0 g) by Supelco were used. The tube phase was firstly conditioned with acetonitrile (5 cm^3) and next washed with distilled water (5 cm^3). The separated compound was washed out with acetonitrile (4 cm^3). Quantitative and qualitative analyses of zearalenone in obtained extract were performed by means of HPLC (UV detector, wavelength λ = 235 nm). Microsorb 100 C18 column of length 25 cm, diameter - 4.6 mm and granulation - 5 µm. Methanol by POCH was used as a mobile phase.

Ozonation process was carried out at 20°C in cylindrical reactor of volume 1000 cm^3 in which treated solution was constantly mixed with the use of magnetic stirrer. Ozone was generated in Ozoner FM 500 (by WRC Multiozon, Poland) and introduced to reactor via ceramic diffuser. The concentration of ozone was determined by iodometric method. In order to remove ozone from post-reaction mixtures 24 mM/dm^3 Na_2SO_3 (analytical grade, P.P.H. Stanlab) was added. Next, samples were filtered through 0.45 µm membrane made from cellulose acetate by Millipore.

Flat-sheet, composite membrane by Dow Filmtec (USA) of producer symbol NF-270 and molecular weight cut-off 200 Da was used for nanofiltration. The process was carried out under transmembrane pressure 2.0 MPa in steel membrane cell (volume - 350 cm^3, membrane area 38.5 cm^2) enabling dead-end process configuration.

The study determining effectiveness of zearalenone removal from water using integrated system ozonation/nanofiltration comprised of water treatment in ozonation process after which nanofiltration was performed. In the part of study discussing ozonation, the influence of ozone dose, contact time, pH and water matrix properties on the degree of zearalenone removal was investigated.

Table 1

Physicochemical characteristics of the waters

Waters	**pH**	**Conductivity [µS/cm]**	**Absorbance UV_{254} [1/cm]**
Deionized water	5.4 and 7.0*	5.180	0.000
Tap water	7.0	1064	0.004
Tap water with humic acids (15 mg/dm^3)	7.0	1112	0.170

*correction of water pH was made by addition of 0.1 M HCl or 0.2 M NaOH

Results

Ozonation

The degree of zearalenone removal depended on ozone dose and as the dose increased the degree of compound removal also increased (Fig. 1). Moreover, the elongation of ozone contact time with treated water also improved the effectiveness of zearalenone removal (Fig. 2).

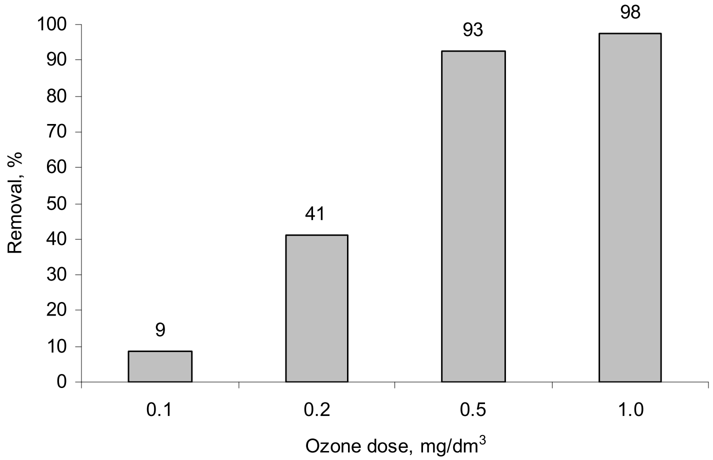

Fig. 1. The influence of ozone dose on degree of zearalenone removal (deionized water, contact time 1 min, pH = 5.3)

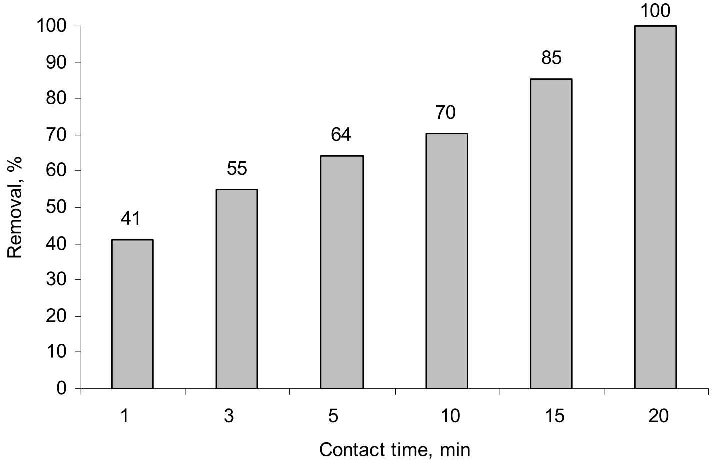

Fig. 2. The influence of contact time on effectiveness of zearalenone removal (deionized water, ozone dose 0.2 mg/dm^3, pH = 5.3)

It was found that the increase of water pH resulted in decrease of zearalenone removal degree (Fig. 3). It proved the greater reactivity of molecular ozone (direct oxidation) in comparison with free radicals $OH^{\bullet}$ formed during ozonation [1]. The lower process effectiveness was also observed in cases when except from zearalenone also other compounds ie inorganic and high-molecular weight organic substances were present in water (tap water with and without humic acids addition). The phenomenon is probably caused by decrease of ozone concentration reacting with low-molecular weight zearalenone, which is used for oxidation of inorganic and organic compounds present in water.

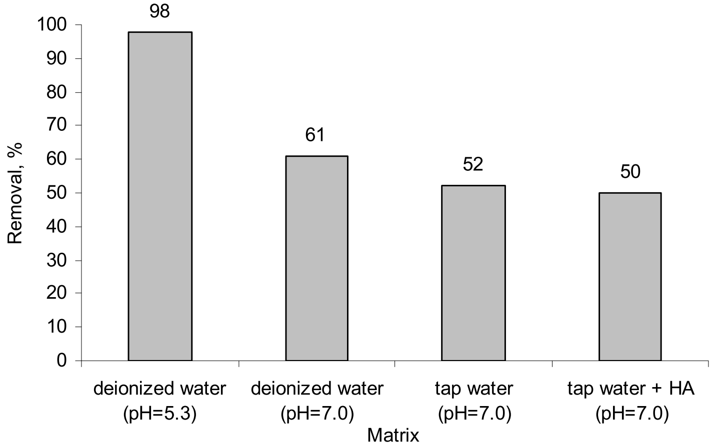

Fig. 3. The influence of water properties on effectiveness of zearalenone removal (ozone dose 1 mg/dm^3, contact time 1 min)

Integrated system ozonation-nanofiltration

Nanofiltration was considered as a method of polishing of water treated via ozonation (tap water with humic acids, ozone dose 1 mg/dm^3). It was found that introduction of nanofiltration to water treatment system improved not only removal of zearalenone, but also other water contaminants (decrease of conductivity and absorbance). Results of the study are shown in Table 2.

Table 2

The effectiveness and the capacity of nanofiltration performed as a unit process or as a part of integrated system with ozonation used for zearalenone removal from water (tap water with humic acids, pH = 7.0)

	Process/system	
	Nanofiltration (membrane NF-270)	**Ozonation+Nanaofiltration (ozone dose 1 mg/dm^3, time 1 min)**
	Removal (decrease*) [%]	
Zearalenone	85.9	89.7
Conductivity*	51.7	58.4
Absorbance*	100	100
Parameter		
Relative permeability of the membrane α^a, -	0.64	0.80

[a] calculated as a ratio of simulated water permeate flux (J_v) to deionized water flux (J_w), where $J_v(J_w) = V/F \cdot t$: V - volume [dm^3], F - membrane area [m^2], t - filtration time [s]

The degree of removal of zearalenone and conductivity decrease in integrated system ozonation - nanofiltration was equal to 89.7 and 58.4%, respectively. Moreover, the total removal of high-molecular weight organic substances determined during absorbance measurements was obtained. Similar results were obtained for water treatment via single nanofiltration, however for such a treatment solution lower membrane capacity was observed ($\alpha = 0.64$).

Conclusions

The study allows to conclude that the effectiveness of removal of zearalenone in ozonation process depends on ozone dose and contact time. The lower degree of compound removal was observed in case when inorganic and high-molecular weight organic substances were present in water or pH of water increased.

The application of nanofiltration after ozonation (integrated system) improves zearalenone removal in comparison with single ozonation treatment. The total removal of high-molecular weight compound and sufficient decrease of inorganic substances concentration are also obtained for integrated system. The combined solution also improves the capacity of applied membrane.

Acknowledgements

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USUWANIE ZEARALENONU Z WODY W PROCESIE OZONOWANIA I W UKŁADZIE OZONOWANIE/NANOFILTRACJA

Wydział Inżynierii Środowiska i Energetyki, Politechnika Śląska

Abstrakt: Zaprezentowano wyniki badań dotyczące efektywności usuwania zearalenonu w procesie ozonowania i w zintegrowanym układzie oczyszczania wody ozonowanie/nanofiltracja. W trakcie ozonowania badano wpływ dawki ozonu, czasu kontaktu, pH i rodzaju wody na stopień usunięcia zearalenonu. Wyniki wskazują, że zastosowanie układu zintegrowanego kojarzącego ozonowanie z nanofiltracją jest korzystne pod względem efektywności usuwania zearalenonu oraz innych wskaźników zanieczyszczenia wody, a także biorąc pod uwagę wydajność membrany.

Słowa kluczowe: zearalenon, mikrozanieczyszczenia organiczne, ozonowanie, nanofiltracja, oczyszczanie wody

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Marie KONEČNÁ[1], Ľubica POSPÍŠILOVÁ[1] and Petr ŠKARPA[1]

DETERMINATION OF ELEMENTS BY ATOMIC ABSORPTION SPECTROMETRY AND ENERGY DISPERSIVE X-RAY SPECTROSCOPY IN HUMIC ACIDS AND SOIL SAMPLES

OZNACZANIE PIERWIASTKÓW ZA POMOCĄ ATOMOWEJ SPEKTROMETRII ABSORPCYJNEJ I SPEKTROMETRII RENTGENOWSKIEJ ENERGII ROZPROSZONEJ W PRÓBKACH KWASÓW HUMINOWYCH ORAZ GLEBY

Abstract: Determination of various elements in humic acids and soil samples is important with regard to their biological availability. This work was focused on the identification of trace elements presented in soil samples and humic acids preparates. The object of our study was the samples of grassland soil classified according to FAO as Eutric Cambisol (locality Vatín, Czech-Moravian Upland). Labile and total several element contents in the studied samples were determined. The labile forms were measured in the 0.01 M $CaCl_2$ extract by using high resolution continuum source atomic absorption spectrometer. Total element contents were determined after extraction in *aqua regia*. Basic soil properties were measured by commonly used methods - soil reaction and conductivity by potentiometric method, texture by pipette method, CEC according to Kappen method and C_{ox} by titrimetric oxidation. Soil *humic acids* (HA) were isolated according to standard IHSS method. Elemental composition of HA was determined by CHSN/O analyzer. Energy dispersive X-ray spectroscopy allowed us to detect elements *in situ* in HA molecule and following elements were found: Fe, Cu, Zn, Ti, Ca, K, S, P, Si and Br. The presence of mentioned elements in humic acids preparates was compared with the determined amounts of labile forms and total contents in the soil samples.

Keywords: humic acids, labile forms, atomic absorption spectrometry, energy dispersive X-ray spectroscopy

Quality and content of soil organic matter, together with processes of mineralization and microbial activity are the main factors affecting the health/quality of agricultural soils. Many authors dealt with evaluation of selected soil parameters influencing mobile and potential mobile heavy metals content showed that humic substances quality strongly affected metals mobility [1-3]. Most of metals in plants originate in uptake by roots from soils. Metals of anthropogenic origin are considered as more available from soils than those from parent rock. The way of uptake influences metal distribution in plant. Some metals are accumulated in roots, which is again the case especially for Pb, while others are well mobile in plants [4]. Humic acids (HA), the main component of humic substances are considered to be very active in interaction with metals and their sorption properties are given by their structure and chemical properties [5-7]. HA consist mainly of hydroxyphenols, hydroxybenzoic acids, and others aromatic structures with linked peptides, amino compounds, and fatty acids as pointed by Hayes and Malcolm [8]. Frequently is given their active interaction with copper, zinc, lead and cadmium. The highest stability constant for copper(II) binding was determined. Stability constant values were decreasing in order: $Cu^{2+} > Ba^{2+} > Pb^{2+} > Cd^{2+} > Ca^{2+}$ [9].

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[1] Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic, email: marie.konecna@mendelu.cz

Our work represents a part of research carried out in Bohemian-Moravian Upland and dealing with total and water-soluble levels of some micronutrients in soils of permanent grassland. The aim of this study was to identify total and labile trace elements presented in the soil, isolate humic acids and give their chemical characterization. We try to characterize portion of selected elements strongly bound into humic acids molecule and show distinct differences for the various metals.

Materials and methods

The monitoring of total and labile elements content in grassland was carried out at locality Vatín (Mendel University Enterprise) - Bohemian-Moravian Upland (altitude 530 m a. s. l., yearly average of air temperature 6.9°C, yearly sum of precipitation 620 mm). Soil type was classified according to FAO as Eutric Cambisol. Grassland was sown by clover-grass mixture (*Sanguisorba-Festucetum comutatae* association). Part of the re-established area was left for spontaneous grassing. Two modes of management were used - mulching once a year and mulching 3 times a year. The experimental variant was established in four replicates, the size of individual plots was 12 m x 10 m. Soil was sampled in 20 cm upper layer twice a year (in spring and autumn).

Soil analysis

Basic soil properties were determined by commonly used methods. Soil reaction was determined by the potentiometric method in distilled water and in 1 M KCl solution. Particle size analysis was determined by the pipette method. The soil was extracted using the Kappen method for *cation exchange capacity* (CEC) determination. Total organic carbon content (C_{ox}) was determined by wet digestion according to Nelson and Sommers [10]. Fractional composition of humic substances was determined according to Kononova and Beltchikova [11]. *Humic substances* (HS) quality was assessed by humic acids (HA)/*fulvic acids* (FA) ratio, humification degree and by *colour indexes* (Q4/6). The labile forms of elements were measured in the 0.01 M $CaCl_2$ extract by high resolution continuum source atomic absorption spectrometry (ContrAA 700, Analytik Jena). Total element contents were determined after extraction in *aqua regia* after microwave extraction (Milestone Ethos 1 microwave system) by *inductively coupled plasma mass spectrometry* (Agilent 7500 ce ICP-MS spectrometer).

Humic acids isolation

The isolation of soil HA was made according to the standard international method (IHSS). Hundred grams of air-dried soil sample was sieved at the mesh size of 1 mm, washed with 10% HCl and stirred for 1÷2 h (decalcination process). After the negative reaction for CO_2 (detected visually) was achieved, the soil rest was washed with 0.05 M HCl. After the negative reaction for Ca^{2+} (detected by ammonium oxalate) was obtained, the soil rest was washed with distilled water. After the negative reaction for Cl^- (detected by $AgNO_3$), was obtained, the soil rest was shaken in 0.1 M NaOH for 7÷8 h. We allowed it to precipitate overnight and centrifuge for 15 min at 5000 rpm. The elution with 0.1 M NaOH and centrifugation was carried out twice and the supernatants were combined. The dark-brown solution of HS was precipitated with concentrated HCl added to pH = 1.

The coagulated HA were decanted, washed several times, extensively purified with 0.5% mixture of HCl + HF, dialysed against distilled water until chloride-free, and finally freeze-dried [8]. Humic acids elemental analysis was made by the standard method of Carlo Erba and elementary CHNS/O analyser - Thermo Finnigan was used. Results of elemental analysis were compared with IHSS Elliot HA standard 1S102H, (IHSS, 2010). Energy dispersive X-ray spectrofluorimeter XEPOS allowed us to detect elements *in situ* in HA molecule.

Results and discussion

Eutric Cambisol under permanent grassland had acid soil reaction, soil was loamy sands textured, with medium CEC, and low C_{ox} content. Quality of HS given by colour index (Q4/6) was less than 1. Humification degree was low (about 20%). Average amount of HS, HA and FA was 7.6, 3.6 and 4 mg/kg, respectively. Isolated HA contain in their molecule 32.73% C, 46.48% H, 2.52% N, and 18.27% O (in atomic %). ^{13}C NMR study showed that isolated HA contain more aliphatic and less aromatic part in their molecule [12].

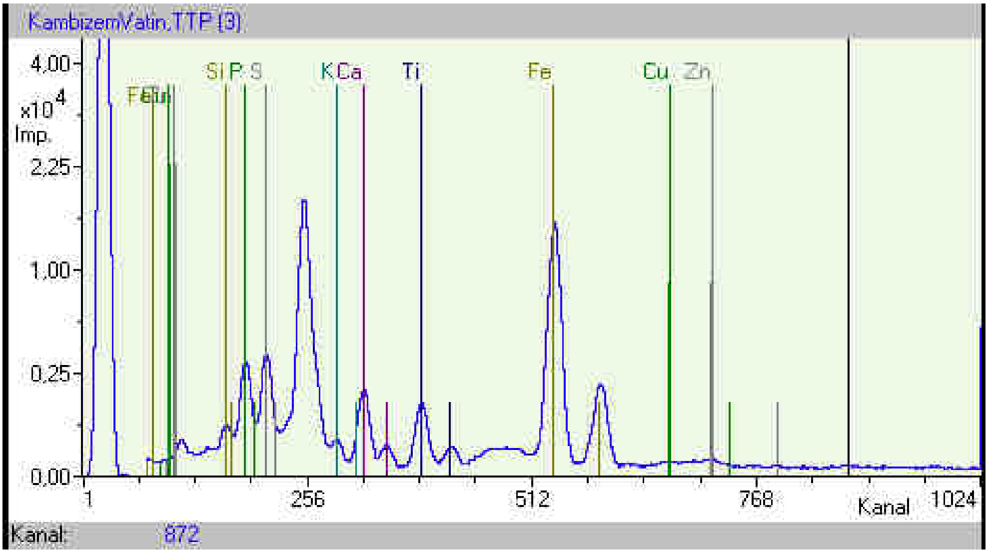

Fig. 1. EDX-ray spectra of humic acids isolateds from Eutric Cambisol (Vatín)

The amount of the most of elements was decreasing in time. The significant decrement was observed in the case of Ca, Cd and Mn (54.0, 40.9 and 34.8%, respectively). Results of total content of selected elements determined in *aqua regia* showed the highest content of Fe in both soil and HA samples. Higher content of K, Fe, V, Cu, Cr and Mo was determined in HA sample to compare with soil. Measured values are typical for this soil type [13]. Limits given for hazardous elements in soils were not exceeded [4, 14, 15]. The correlation between labile form and total element contents was not significant ($p < 0.05$). The most significant dependence was observed in the case of Co ($r = 0.921$). Within labile forms measured in the 0.01 M $CaCl_2$ extract the highest content of Fe and K was determined in both HA and soils samples. HA contain hundred times more Fe, twice more Cu and Co to compare with soil sample. High adsorption of Cu and Co by HA form is also confirmed by literature data [9]. Higher values to compare with background content were found. Also Pb and Zn are usually highly adsorbed by HA, but our measurements not confirmed it. We can conclude that this area is not polluted by atmospheric deposition of heavy metals, because all of determined hazardous elements were in the range of background content. Results of EDX-ray spectroscopy confirmed the presence of Fe, Cu, Zn, Ti, Ca, K, S, P, Si and Br in HA molecule (Fig. 1).

Conclusions

The most important conclusion was that all of determined hazardous elements were in the range of background content. Both mineral soil samples and isolated HA contained the highest amount of *iron* and *potassium*. HA samples contained twice higher amount of *copper* and *cobalt* to compare with soil samples. Statistically significant dependence for *cobalt* (r = 0.921) was found.

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Michał KOZIOŁ[1]

EFFECT OF STORAGE TIME OF WASTEWATER SLUDGE ON ITS MOISTURE CONTENT, CALORIFIC VALUE AND CONTENT OF AGGRESSIVE SUBSTANCES

WPŁYW CZASU SKŁADOWANIA OSADÓW ŚCIEKOWYCH NA ICH WILGOTNOŚĆ, WARTOŚĆ OPAŁOWĄ ORAZ ZAWARTOŚĆ SUBSTANCJI AGRESYWNYCH

Abstract: The problem of the issue which still remains unresolved in many countries - including Poland - related to the management of wastewater sludge derived from municipal wastewater treatment plants was outlined. Later the possibility of solving the problem of the growing amount of generated sludge with the application of, *inter alia*, thermal methods is discussed and the origin of the research presented in the paper is explained. The paper presents results of research conducted on two samples of sludge stored for the period of 5 months each in conditions similar to natural ones. In the research the stabilized by anaerobic fermentation processes and dehydrated wastewater sludge was used. Sludge originated from large municipal wastewater treatment plants. From among a large number of parameters set during the research, the paper focuses on the presentation of the results regarding: moisture content, calorific value as well as phosphate and nitrate contents. Data obtained in the presented research demonstrates the presence of continuous processes taking place in the stabilized, stored sludge, which significantly affect its fuel properties.

Keywords: wastewater sludge fuel properties, calorific value, moisture content, sludge storage

As a result of wastewater treatment by wastewater treatment plants the substance known as wastewater sludge is formed. The amount and composition of the formed sludge depend on a significant number of factors, however, mainly on the parameters of wastewater treatment plants and their treatment technologies (technological solutions of wastewater treatment).

In case of biological municipal wastewater treatment plants, the quantity of formed sludge before it is subject to stabilization processes may be estimated to 60÷80 g of sludge dry matter per resident a day [1]. The value of this index usually increases, *inter alia*, with the level of social wealth and the size of the serviced agglomeration.

The popularization of wastewater treatment results in the problem of the formation of ever larger amounts of sludge. In 2008 in Poland there was the total of 567.3 thousand Mg of sludge dry matter produced in municipal treatment plants. In the next few years, further increase of quantity of the generated sludge should be expected, among others in relation to the application of the significant part of UE funds for investments within the scope of the sewage system development as well as the construction and expansion of wastewater treatment plants. In the "National Waste Management Plan 2010" it is predicted that in 2018 there will be over 706 000 Mg of sludge dry matter produced [2].

It can be estimated that the theoretical amount of sludge which needs to be removed in Poland, with the assumption that 90% of the Polish population is serviced by biological wastewater treatment plants, is about 900 000 Mg of dry matter. It should be also noted that

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[1] Department of Technologies and Installations for Waste Management, Silesian University of Technology in Gliwice, ul. Konarskiego 18, 40-100 Gliwice, phone 32 237 11 23, email: michal.koziol@polsl.pl

the sludge generated is mostly characterised by moisture content at the level of over 75%. Thus, the quantity of substance which needs to be neutralised amounts to over 4,000,000 Mg.

The average flow capacity of Polish municipal wastewater treatment plants classifies them as small treatment plants since in 2008 it amounted to about 1.5 thousand m^3/day (over 77% had flow capacity lower than 1000 m^3/day) [2]. It should be expected that along with the growing popularity of wastewater treatment, the average daily flow capacity of plants will continue to decrease.

In highly developed countries thermal methods belong to one of the basic ways of wastewater sludge neutralisation. Thus, eg in 2006 in Switzerland thermal methods were used to process as much as 90% of sludge (by volume), in the Netherlands almost 70%, in Germany nearly 50% (own calculations based on [2]).

In Poland thermal methods have been used to manage only very little amount of sludge. Since the second half of the 1990s, there has been operating a plant of sludge thermal utilization in Poland, located on the premises of the Joint Wastewater Treatment Plant in Gdynia-Debogorze. According to [2], in the years 2000-2008 only 1.5÷2% of produced sludge dry matter (1.7÷6.2 Mg/year of sludge dry matter) were managed by means of thermal methods.

In the “National Waste Management Plan 2010” [3] it was predicted that in 2018 over 50% of produced sludge would be subject to thermal transformation. Despite the projects of sludge wastewater incineration plant construction which are currently carried out and prepared for execution, the implementation of this objective appears to be very unlikely. The achievement of the intended goal would be possible in case of the implementation of the co-combustion process of sludge wastewater with coals in the existing facilities of the power system. One of the countries with exceptionally rich experience in co-combustion of sludge is Germany, where ten-odd percent of the produced sludge is neutralized in the result of co-combustion. In Poland, in the industrial facilities there were de facto only tests for the sludge co-combustion process conducted. There is no information on the officially implemented sludge co-combustion processes on the industrial scale.

A reason supporting the implementation of co-combustion processes in Poland is the fact that hard coal is still the basic fuel here and its share in the structure of covering the demand for primary energy will continue to be significant - at least until 2030 [4]. Moreover, across the country there is a large number of coal-fired power boilers with relatively small capacity - up to ten-odd MW. Such capacity is completely sufficient for the utilization of wastewater sludge derived even from 90% of the national wastewater treatment plants. Additionally, these boilers are relatively equally distributed across Poland. An argument against the implementation of the discussed solution in Poland is the technical condition of boilers, the fact that the overwhelming majority of low and medium capacity boilers do not possess the flue-gas purification equipment other than the dust extraction equipment.

Origin of testing

One of the most important issues during the implementation of the co-combustion process of sludge with coal is the stability of their properties. There is a considerable number of parameters determining sludge properties. The basic ones, significant in case of submitting wastewater sludge to neutralization by means of thermal methods, include: moisture content, calorific value, elemental composition, fraction of combustible and volatile matter, chlorine and fluorine content, characteristic ash softening points, heavy metal content.

Sludge properties depend on a number of factors, and above all, on the properties of wastewater and applied technologies of their treatment. Types of sludge from individual wastewater treatment plants differ significantly between one another. Even sludges produced in the same wastewater treatment plant in different periods of time demonstrate quite significant differences.

In case of using thermal methods of sludge neutralization, many a time there is the necessity of medium or long term storage of the produced sludge. For conducting the sludge co-combustion process in boiler plants of medium and low capacities, this necessity may result, *inter alia*, from the fact of storing the sludge, eg in summertime, when these plants do not operate or operate with significantly limited capacities. Whereas in case of sludge co-combustion in the plants designed solely for this process, this necessity is the result of the periodic plant stoppages (eg due to overhauls or modernisations).

Current observations performed among others at the JWTP Debogorze, allow to conclude that there is significant influence of the storage time of wastewater sludge on the progress of its combustion.

Testing methodology

Further in this paper, test results of two batches of sludge derived from two different wastewater treatment plants are presented.

The following sludge was used for testing:

- Sludge I - sludge from a large municipal biological wastewater plant with the capacity of approx. 60,000 m^3/day - sludge after the anaerobic fermentation process.
- Sludge II - sludge from a municipal industrial wastewater plant (located near a paper plant) with the capacity of approx. 1500 m^3/day of industrial sludge and 1000 m^3/day of municipal sludge - sludge after the anaerobic fermentation process.

Within the framework of presented tests, sludge provided from the treatment plant was placed in containers made for this purpose. During testing, the conditions of sludge storing in a pile were simulated. For this purpose, the containers were opened at the top and exposed to the atmospheric conditions. Sludge subject to testing was stored in layers of about 1 m thick. Within the framework of presented tests, samples for the conducted measurements were taken from the depth of about 80 cm - measured from the surface of the stored sludge.

Samples were taken at monthly intervals for the period of 4 months. Altogether, there were five samples taken - at the beginning of the period and in the subsequent 4 months. In the figures in the further part of the study, these samples were marked with subsequent numbers from 1 to 5.

Within the framework of conducted tests, ten-odd parameters were determined, including: gross calorific value, moisture content, fraction of combustible and volatile matter, elemental composition, aggressive substance content. Further in the paper only the results of the following analyses are presented:

- moisture content (according to the PN-80/G-04511 standard [5]);
- calorific value (established based on the determination of gross calorific value according to the PN-ISO 1928:2002 standard [6]).

Moreover, the results of determining the content of aggressive compounds marked as nitrogen dioxide and orthophosphates are indicated.

The study refers to test results obtained within the execution of works [7, 8].

Obtained test results underwent statistical and substantial analysis. It should be stated here that in case of each sample taken from laboratory stands, at least three repetitions of each measurement were performed. Based on these repetitions the values of standard deviations and relative errors were determined. Obtained average values were used to determine a trend line in the form of a straight line. Such adoption of the trend line was the result of a relatively small number of measurement points and lack of basis for the adoption of a trend line in a different form. Further on, the assessment of the existing correlation between the tested parameters and time was made (a linear regression coefficient was compared with the critical regression coefficient for the significance level 0.05 and amounting to $R_{kr} = 0.878$).

Test results

Figure 1 presents the results of moisture content measurement for tested sludge. Apart from the moisture content changes at the depth of 80 cm, this figure shows also moisture content changes of analysed sludge in the upper part of the pile (at the depth of about 15 cm).

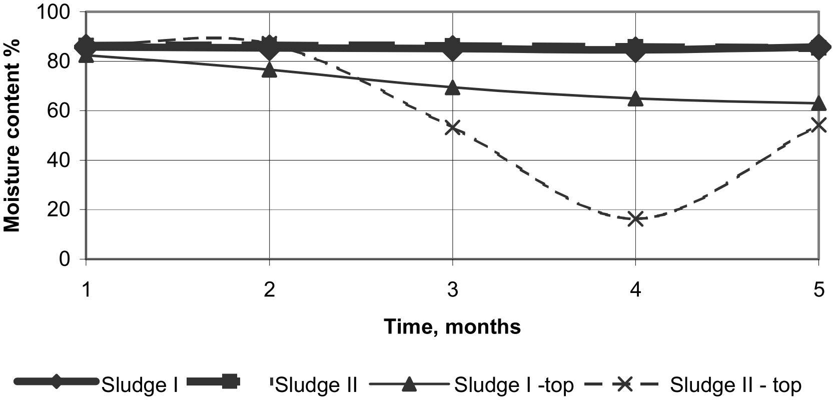

Fig. 1. Moisture content changes of the stored sludge (in upper and lower layers)

As Figure 1 shows, in case of samples taken from the depth of approx. 80 cm, for both analysed types of sludge there were practically no changes in their moisture content discovered. In case of both analysed types of sludge, the moisture content amounted to approx. 85% for the entire period of time. At the same time, in case of the upper layer of the sludge, significant moisture content changes could be observed. These changes were caused by the influence of atmospheric conditions. Significant differences in moisture content changes between the samples Sludge I - top and Sludge II top, were caused, among others, by the fact of conducting tests on different dates.

Figure 2 presents changes of calorific values of tested sludge types (calculated for dry matter).

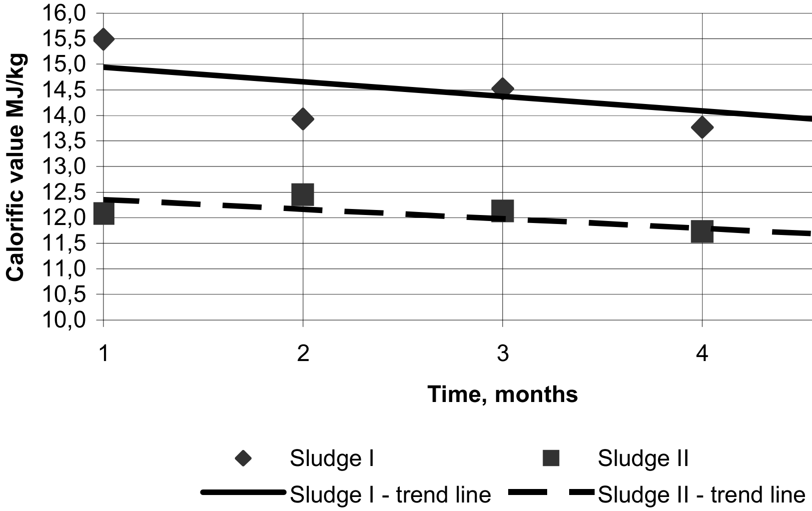

Fig. 2. Changes of calorific value of sludge dry matter

As Figure 2 shows, a falling tendency of the sludge calorific values in time for the presented tests can be observed. At the same time, in case of the first sample the tendency has clearer character. It should also be noted that according to the accepted criteria for both samples no statistical correlation between the sludge storage time and the tested parameter was demonstrated.

Calorific value in case of:

- "Sludge I" varied from 13.8 MJ/kg (fourth measurement) to 15.5 MJ/kg (first measurement);
- "Sludge II" varied from 11.5 MJ/kg (fifth measurement) to 12.5 MJ/kg (second measurement).

Conclusions

As the first part of the study shows, wastewater sludge management continues to be a significant problem in Poland. This problem could be solved *inter alia* by a broad application of the thermal method of sludge utilization. Thermal methods are currently commonly applied in a number of highly developed countries. A method which could be more broadly used in Poland is the co-combustion of wastewater sludge with coal, eg in the existing power facilities.

One of the issues related to thermal methods of sludge utilisation is the change of sludge fuel parameters during storage (induced eg by a periodical change of power facility capacity or a stoppage in the operation of sludge incineration plant). The second part of this study is devoted to the presentation of a small section of the conducted tests on this issue.

Conducted tests prove that the properties of stored sludge may be significantly different along the height of the pile vertical section. Sludge parameters in the upper layer are subject to considerable changes caused by the influence of atmospheric conditions. In the lower layer, the same parameters are not subject to change or their changes are relatively slow. The above relationship was characterized on the example of sludge moisture content changes.

During conducted tests it was also discovered that there is a falling tendency of calorific values in the lower layers of stored sludge. However, eg in case of nitrogen dioxide a growing tendency was discovered. In case of orthophosphates in “Sludge I” a growing tendency was found and in “Sludge II” a falling one.

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WPŁYW CZASU SKŁADOWANIA OSADÓW ŚCIEKOWYCH NA ICH WILGOTNOŚĆ, WARTOŚĆ OPAŁOWĄ ORAZ ZAWARTOŚĆ SUBSTANCJI AGRESYWNYCH

Katedra Technologii i Urządzeń Zagospodarowania Odpadów, Politechnika Śląska w Gliwicach

Abstrakt: Przedstawiono wciąż nierozwiązane w szeregu krajów - w tym w Polsce - zagadnienia zagospodarowania osadów ściekowych pochodzących z komunalnych oczyszczalni ścieków. W dalszej części omówiono możliwość rozwiązania problemu narastającej masy wytwarzanych osadów ściekowych przy wykorzystaniu m.in. metod termicznych oraz wyjaśniono genezę badań prezentowanych w pracy. Przedstawiono wyniki badań przeprowadzanych dla dwóch próbek osadów składowanych każdorazowo przez okres 5 miesięcy w warunkach zbliżonych do naturalnych. W badaniach wykorzystano ustabilizowane w procesach fermentacji beztlenowej i odwodnione osady ściekowe. Osady pochodziły z dużych komunalnych oczyszczalni ścieków. Spośród dużej liczby oznaczanych w trakcie badań parametrów skupiono się na prezentacji wyników dotyczących: wilgotności, wartości opałowej oraz zawartości fosforanów i azotanów. Uzyskane wyniki badań wskazują na procesy wciąż zachodzące w ustabilizowanych, składowanych osadach, istotnie wpływające na ich właściwości paliwowe.

Słowa kluczowe: osady ściekowe, właściwości paliwowe, wartość opałowa, wilgotność, składowanie osadów

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Yevgeniy KUZMINSKIY[1], Kateryna SHCHURSKA[1], Iryna SAMARUKHA[1]
and Grzegorz ŁAGÓD[2]

DIFFERENT TYPES OF ENERGY CONVERSION FOR BIOHYDROGEN PRODUCTION PROCESSES

SPOSOBY KONWERSJI ENERGII DO PRODUKCJI WODORU Z WYKORZYSTANIEM PROCESÓW BIOCHEMICZNYCH

Abstract: Increased environmental problems as well as growing fuel and energy demand encourage the international community to effectively search for new energy technologies that would ensure an acceptable level of pollution and, simultaneously, would not limit economical growth. The key position in solving this problem is occupied by hydrogen energy, ie hydrogen production and use of fuel cells in industry, construction, transportation, housing and other sectors of the economy. So it is possible to say that hydrogen becomes a promising alternative energy carrier to fossil fuels, since it is clean, renewable, contains high energy content and does not contribute to greenhouse effect. Biological hydrogen production is one of the most challenging areas of technology development for sustainable generation of gaseous energy. The present study critically updates various biohydrogenation processes with special references to their advantages and disadvantages. Different approaches towards improvement of the bioprocesses are also outlined. The presented study reviews biohydrogen systems, molecular and genetic aspects of hydrogen production and technologies of biohydrogen production.

Keywords: biohydrogen, biophotolysis, dark fermentation, energy conversion efficiency, biofuel, microbial fuel cells, electrochemically active microorganisms

Increased environmental problems as well as growing fuel and energy demand encourage the international community to effectively search for new energy technologies that would ensure an acceptable level of pollution and, simultaneously, would not limit economical growth. The key position in solving this problem, is occupied by hydrogen energy, ie hydrogen production and use of fuel cells based in industry, construction, energy, transportation, housing and other sectors of the economy according to many experts [1]. From the energetic point of view hydrogen is the only alternative to oil and natural gas because it has high heat of combustion, it is clean and it can replace the traditional fuels in many applications.

There are many methods of hydrogen production from various raw materials. The fossil fuels (natural gas, bituminous coal and brown coal, shale oil) and water resources which are unlimited and renewable can serve for hydrogen production, but these methods are energy-consuming and harmful to the environment. Therefore the search of alternative methods of obtaining hydrogen is a crucial issue in modern *research and development* (R&D).

Among alternative methods of obtaining hydrogen, biotechnology is the most promising because it allows to obtain clean fuel and can help solve some of waste management problem, because wastes are the raw material for this process.

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[1] Chair of Ecobiotechnology and Bioenergy, National Technical University of Ukraine “Kyiv Polytechnic Institute”, Peremogy Ave 37, 03056 Kyiv, Ukraine, email: kuzminskiy@fbt.ntu-kpi.kiev.ua

[2] Faculty of Environmental Engineering, Lublin University of Technology, ul. Nadbystrzycka 40B, 20-618 Lublin, phone 81 538 43 22, email: G.Lagod@wis.pol.lublin.pl

According to energy sources that are used by organisms' microbiological processes can be divided into the following:

- dark anaerobic biohydrogen production when the energy of chemical bonds of substrate molecules is converted into energy of chemical bonds of hydrogen;
- photo production of hydrogen, in which microorganisms converting light energy into chemical bond energy of hydrogen molecules;
- hydrogen production using microbial fuel cells, in which electrical energy, and energy of chemical bonds in organic substrates are accumulated in hydrogen molecules.

In Table 1 the information about bacterial hydrogen production processes is generalized depending on the type of energy conversion.

Table 1

Comparison of microbiological methods of producing hydrogen

Forms of energy conversion	**Type of process**	**Process name**	**Microorganisms - producers**	**Hydrogen producing reaction**
solar → chemical	Light-sensitive selection of biohydrogen	Photoautotrophic hydrogen production - biophotolysis	*Synechococcus* *Scenedesmus* *Chlamydomonas*	$4H_2O$ + "Light-energy"→ $2O_2 + 4H_2$
		Photo-heterotrophic production of hydrogen - Enzymatic photo-degradation of organic acids	*Rhodopseudomonas* *Rhodobacter* *Rhodospirillum* *Rubrum Rhodovulum*	$C_2H_4O_2$liq. + $2H_2O$liq. + "Light-energy" → $2CO_2$gas + $4H_2$gas
chemical → chemical	Dark anaerobic biohydrogen producing	Fermentation	*Clostridium* *Eubacterium* *Sarcina* *Ruminococcus* *Selenomonas* *Enterobacter* *Escherichia* *Clostridium* *Streptococcus*	$C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3COO^- + 2HCO_3^- + 4H^+ + 4H_2$
		Shift-reaction	*Methanosarcina* *Citrobacter* *Rhodopseudomonas*	$CO + H_2O \rightarrow CO_2 + H_2$
bioelectrical → chemical	Biohydrogen production in MFC	Bio-electrochemical hydrogen production	*Geobacter* *Shewanella* *Pseudomonas*	Anode: $C_2H_4O_2 + 2H_2O \rightarrow 2CO_2 + 8e^- + 8H^+$ Cathode: $8H^+ + 8e^- \rightarrow 4H_2$

Bioelectrochemical hydrogen production

Because it is doubtful whether dark fermentations and photoheterotrophic fermentations will ever become economically feasible [2], there exists a demand for alternative processes for hydrogen production from wastewaters.

Hydrogen production via bacterial fermentation is currently limited to a maximum amount of 4 mole of hydrogen per mole of glucose, and under these conditions results in a fermentation end product that bacteria are unable to further convert to hydrogen. It is shown here that this biochemical barrier can be circumvented by generating hydrogen gas from acetate using a completely anaerobic *microbial fuel cell* (MFC). By augmenting the electrochemical potential achieved by bacteria in this MFC with an additional voltage of 250 mV or more (up to 850 mV), it was possible to produce hydrogen at the cathode directly from the oxidized organic matter.

More than 90% of the protons and electrons produced by the bacteria from the oxidation of acetate were recovered as hydrogen gas, with a high efficiency. Production of hydrogen by this anaerobic MFC process is not limited to carbohydrates, as in a fermentation process, as any biodegradable dissolved organic matter can theoretically be used in this process to generate hydrogen from the complete oxidation of organic matter. Various researchers named this process differently. The researchers Liu et al [3] published their preliminary findings on the technology and refer to it as "*electrochemically assisted microbial production of hydrogen*". Rozendal describe this technology "*biocatalyzed electrolysis*" [4]. In this study we call it "bioelectrochemical hydrogen production".

Bioelectrochemical hydrogen production, a recently discovered technology, related to the microbial fuel cell, overcomes thermodynamic barrier by means of a small input of electrical energy. This makes the process independent of the reactor surface area, which benefits the economic feasibility. Bioelectrochemical hydrogen production achieves this feasibility by utilizing electrochemically active microorganisms, which convert dissolved organic material to bicarbonate, protons and electrons. Either by direct contact with an electrode surface [5] or aided by (excreted) redox mediators [6] these microorganisms release the produced electrons to the surface of electrode. In this way, a biological anode is created. By coupling this biological anode to a proton reducing cathode by means of a power supply, a direct conversion of dissolved organic material to hydrogen is accomplished. The complete process takes place in an electrochemical cell in which oxidation of dissolved organic material and proton reduction are separated in two chambers (Fig. 1). The separation between these chambers is established by means of a cation exchange membrane. Externally, the anode and the cathode are connected to the power supply using an electrical circuit. While the power supply drives the released electrons from the anode to the cathode, an equal number of protons permeate through the membrane. At the cathode, protons and electrons combine to form pure hydrogen gas.

Acetate is used as a model compound for the biocatalyzed electrolysis experiments presented in this study, as acetate cannot be directly converted to hydrogen through dark fermentation:

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + H^+ + 4H_2$$

Essential to the working principle of bioelectrochemical systems is the application of mixed consortia of electrochemically active microorganisms. Electrochemically active microorganisms are capable of exocellular electron transfer, ie, electron transfer from the inside to the outside of the cell. This enables them to grow on an electrode surface while using the electrode as an electron acceptor for the oxidation of dissolved organic compounds (eg, in wastewater). As the electrochemically active microorganisms release the electrons at a high energy level, a low electrode potential is established at the electrode, which can be utilized for hydrogen production in the biocatalyzed electrolysis process.

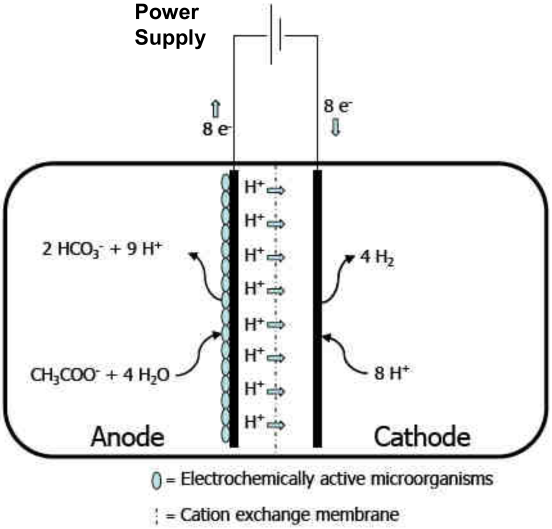

Fig. 1. Schematic representation of bioelectrochemical hydrogen production from acetate

In a typical MFC, the open circuit potential of the anode is ~ –300 mV. If hydrogen is produced at the cathode, the half reactions occurring at the anode and cathode, with acetate oxidized at the anode, are as follows:

$$\text{Anode: } CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^-$$

$$\text{Cathode: } 8H^+ + 8e^- \rightarrow 4H_2$$

Producing hydrogen at the cathode requires a potential of at least $E^0 = -410$ mV (NHE) at pH 7.0, so hydrogen can theoretically be produced at the cathode by applying a circuit voltage greater than 110 mV (ie 410÷300 mV). This voltage is substantially lower than that needed for hydrogen derived from the electrolysis of water, which is theoretically 1210 mV at neutral pH. In practice, 1800÷2000 mV is needed for water hydrolysis (under alkaline solution conditions) due to overpotential at the electrodes. Thus, by deriving the protons and electrons from organic matter instead of water, we can directly generate hydrogen at a low voltage using a type of MFC device that does not require oxygen. The application of a completely anaerobic process should increase the efficiency of the MFC process using mixed cultures as oxygen diffusion through the cathode is avoided [3].

Conclusions

Hydrogen gas is thought to be the promising fuel for a world in which air pollution has been alleviated, global warming has been stopped, and the environment has been protected in an economically sustainable manner. This paper presents three types of energy conversion for biohydrogen production processes: dark anaerobic biohydrogen production, photoproduction and bioelectrochemical in MFC.

The most promising of the currently known biological hydrogen production technologies from wastewaters is dark fermentation, which is characterized by high production rates. Dark fermentation, also referred to heterotrophic hydrogen production, is conducted out by a wide variety of microorganisms. In nature, hydrogen production is a way for these microorganisms to dispose of excess reducing equivalents that are produced under anaerobic conditions. Unfortunately, his reaction is unfavorable to sustain microbial life under hydrogen accumulating conditions.

Photobiological production of hydrogen can be performed by photoautotrophic and photoheterotrophic microorganisms. In photoautotrophic hydrogen production the solar energy captured by the photosystem is used to produce hydrogen and oxygen from water (biophotolysis of water). The main drawback of the process is that hydrogen and oxygen are produced simultaneously, which causes oxygen inhibition of the hydrogen producing enzymes.

Bioelectrochemical hydrogen production has been discovered as a completely new path to renewable energy production. The advantages of these bioelectrochemical systems over production of hydrogen using conventional water electrolysis and non-renewable sources of electricity are multifold, particularly when the possibilities for hydrogen production from waste biomass sources are considered. First, the microbial oxidation of the organic matter provides a renewable source of energy for hydrogen production, and overall greatly reduces the energy needed to produce hydrogen compared with using electricity produced by other means. With this bioelectrochemically assisted reactor, hydrogen can be produced from any type of biodegradable organic matter, although the Coulombic efficiencies and power densities will vary depending on the substrate. Combining hydrogen production and wastewater treatment should result in a more economical venue for hydrogen generation as the infrastructure needed for wastewater treatment can be used to effectively subsidize the cost of the hydrogen generation. This bioelectrochemical microbial system, if combined with hydrogen fermentation that produces 2÷3 mol H_2 per mol of glucose, has the potential to produce ca 8÷9 mol H_2 per mol of glucose at an energy cost equivalent to 1.2 mol H_2 per mol glucose.

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SPOSOBY KONWERSJI ENERGII DO PRODUKCJI WODORU Z WYKORZYSTANIEM PROCESÓW BIOCHEMICZNYCH

[1] Katedra Ekobiotechnologii i Bioenergetyki, Narodowy Uniwersytet Techniczny Ukrainy
[2] Wydział Inżynierii Środowiska, Politechnika Lubelska

Abstrakt: Narastające problemy środowiskowe, a także wzrastające zapotrzebowanie na energię oraz jej nośniki w postaci paliw zmuszają do wzmożonych badań nad nowymi technologiami energetycznymi. Technologie takie z jednej strony powinny zapewnić akceptowalny poziom emisji zanieczyszczeń, z drugiej zaś nie ograniczać jednocześnie wzrostu ekonomicznego. Jednym z kluczowych sposobów rozwiązania problemów energetycznych wydaje się wykorzystanie wodoru jako nośnika energii. W powiązaniu z tym zagadnieniem rozważane są kwestie odnoszące się do produkcji wodoru oraz wykorzystania zawierających go ogniw paliwowych w przemyśle, budownictwie, transporcie, gospodarstwach domowych oraz wielu innych sektorach gospodarki. Wodór staje się obiecującym alternatywnym nośnikiem energii, zdolnym w przyszłości zastąpić paliwa kopalne z uwagi na swój wysoki potencjał energetyczny, odnawialność oraz „czystość" generowanej energii, której wykorzystanie nie powoduje efektu cieplarnianego. Produkcja wodoru za pomocą metod biologicznych jest jednym z obszarów rozwoju technologii, szczególnie ważnym w kontekście zrównoważonej produkcji energii. Prezentowane opracowanie zawiera przegląd ważniejszych metod i procesów biologicznych, umożliwiających produkcję wodoru, korzystających z różnych mechanizmów konwersji energii. W pracy przedstawiono różne podejścia mające na celu udoskonalenie wspomnianych biotechnologii, omówiono również molekularne i genetyczne aspekty produkcji wodoru.

Słowa kluczowe: wodór, biofotoliza, wydajność energetyczna, biopaliwa, mikroorganizmy aktywne elektrochemicznie

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Jolanta MOLAS[1]

COMPARISON OF THE CONTENT OF SOME NITROGEN COMPOUNDS IN WHITE HEAD CABBAGE CULTIVATED IN SOIL POLLUTED WITH HYDRATED NICKEL AND CHELATE NICKEL

PORÓWNANIE ZAWARTOŚCI NIEKTÓRYCH ZWIĄZKÓW AZOTU W KAPUŚCIE GŁOWIASTEJ BIAŁEJ UPRAWIANEJ NA GLEBIE ZANIECZYSZCZONEJ HYDRATEM I CHELATEM NIKLU

Abstract: A pot experiment was study the effects of hydrate ($NiSO_4 \cdot 7H_2O$) and chelate (Ni-EDTA) nickel on white head cabbage crop and on the content of N-total, free amino acids, protein and exogenous amino acids. Added to soil whose pH was 6.2 in the amount of 75 mg · kg^{-1} nickel in both chemical forms decreased the crop of dry mass of cabbage, the content of total nitrogen and the analysed compounds of this element. It also reduced the nutritious value of protein, measured by the content of exogenous amino acids. The level of reduction of the crop of dry mass of cabbage and the content of the analysed nitrogen compounds was much bigger in the presence of hydrated nickel ($NiSO_4 \cdot 7H_2O$) than in the presence of chelate form of this element, ie Ni-EDTA and corresponded to the content of nickel in the plants.

Keywords: amino acid, cabbage, nickel toxicity, nitrogen, soil pollution, protein

At the end of the last century nickel was included into the group of microelements, mainly because it is a component of urease (EC 3.5.1.5) and participates in nitrogen metabolism in plants [1-4]. Therefore, nickel is considered to be an important element mainly for plants applied with urea as a N source [3-5]. Nickel has a positive influence on N assimilation and its metabolism when it occurs in trace quantities [1, 3-5], but disturbs the course of these processes when it occurs in excessive amounts [6, 7]. As a result, the qualitative and quantitative composition of nitrogen compounds in plants changes [6, 7], which, in turn, determines the nutritional value of consumer plants [8].

Studies conducted so far have shown that phytoavailability and phytotoxicity of nickel depend mainly on its speciation in soil [9-12]. As a transition metal, nickel tends to form chelate compounds in soil solution and is accessible to plants in the form of complex ions [9-11]. Furthermore, it is introduced to the soil in chelate compounds together with organic fertilizers and waste deposit [11]. Taking this fact into account, the objective of this study was to compare the effect of hydrated ($NiSO_4 \cdot 7H_2O$) and chelatic (Ni-EDTA) forms of nickel applied to the soil with excessive amount on the content of N-total and some nitrogen compounds in white head cabbage cv. Gloria di Enkhouizen 2.

Materials and methods

White head cabbage cv. Gloria di Enkhouizen 2 was grown in pot cultures (3 plants per pot containing 12 kg of soil), on average-quality soil, whose granulometric composition was that of silt. The soil had the following properties: float particles - 28%, organic

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[1] Department of Plant Biology, Faculty of Agricultural Sciences in Zamosc, University of Life Sciences in Lublin, ul. Szczebrzeska 102, 22-400 Zamość, phone 84 677 27 32, email: jolanta.molas@up.lublin.pl

C - 0.8 mg · kg^{-1}, cation exchange capacity - 11.8 cmol (+) kg^{-1}, pH 6.2, total content of Ni - 11.8 mg · kg^{-1}, content of soluble Ni in 1 mol · dm^{-3} HCl - 2.4 mg · kg^{-1}. Basic fertilization was applied in the following amount per pot: 1g of N in the forms of KNO_3 and $Ca(NO)_2 \cdot H_2O$ (nitrate form), 0.5 g of P - $Ca(H_2PO_4)_2$, 0.8 of K - KCl and 0.3 mg of Mg in the form of $MgSO_4$. The plants were grown in green house at 20°C and at relative humidity of 75% for a long (16-hour) photoperiod with light intensity of 180 µmol · m^{-2} · s^{-1}. Nickel was added to the soil at the stage of initiation of the leaf curling in the forms of $NiSO_4 \cdot 7H_2O$ and Ni-EDTA (Me:L 1:1) in the amount which corresponds to the 2nd degree of pollution of average soil with this element, ie in the amount of 75 mg Ni · kg^{-1} of soil.

The plants were harvested at the stage of loose head. Next, the crop of fresh and dry mass of head was measured. Ni content in dry mass of cabbage was determined using an atomic absorption spectrophotometer (Perkin Elmer, model 1100), according to the method described by Ostrowska et al [13]. Total-N content in dry mass of cabbage head was estimated by Kjeldahl method [13] on the Kjeltec Tecador automatic apparatus. Total protein content was calculated as a product of total-N concentration and protein coefficient (total-N x 6.25). The content of exogenous amino acids was determined on the automatic amino acid analyser (*Amino Acid Analyser T* 339 *M*) after a prior hydrolysis of the samples in 6 mol · dm^{-3} HCl for 24 h at 110°C. Sulphur amino acids were not determined. The content of free amino acids was determined according to the Weibull et al method [14], after deprotenization of the dried leaf tissues with 5% sulphosalicylic acid.

Statistical analysis: Variance analysis (ANOVA) of experimental plant parameters was carried out, followed by a test checking the significant difference with probability of 0.05 (LSD).

Results and discussion

Added in excessive amounts to average soil, whose reaction was slightly acid (pH 6.2), nickel caused reduction of the crop of dry mass of head of white cabbage cv. Gloria di Enkhouizen 2; this element also affected the content of dry mass in fresh mass of head (Tab. 1). The effect depended on the chemical form of this element applied to the soil. When the soil was polluted with hydrated and chelate form of nickel, the crop of head dry mass was smaller by 36.9 and 16.9% respectively, than when the soil was not polluted with this metal. However the percentage of dry mass in the fresh mass of head was bigger than in control; by 9.8% and by 2% in the presence of hydrated nickel and chelate Ni-EDTA respectively (Tab. 1). These and earlier results imply that the observed increase in the content of dry mass in the fresh mass of cabbage head was connected with plant adaptation to growth in nickel stress conditions. As it has been shown earlier in excessive amount of nickel tissue density of the leaf increases and this organ acquires some xeromorphic properties [15].

Nickel used in the cultivation of cabbage in both chemical forms affected the content of N-total, free amino acids and protein (Tab. 1). It also affected the nutritional value of protein, defined by the content of endogenous amino acids (Tab. 2). In the conditions of excessive amount of inorganic nickel and Ni-EDTA chelate the content of N-total and total protein was reduced by 36.6 and 14.8%, respectively, as compared with the content of this element in control plants. The pool of free amino acids also decreased by as much as 65.5%

it the presence of $NiSO_4 \cdot 7H_2O$ and by 12.2% in the presence of Ni-EDTA. Results of studies have shown that the observed differences in the effect of applied chemical forms of nickel on the level of N-total and its analysed organic compounds corresponded to the content of this metal in plants. When the soil was polluted with nickel in the form of $NiSO_4 \cdot 7H_2O$, the content of nickel in cabbage was bigger and the content of nitrogen and its compounds smaller than when the soil was polluted with Ni-EDTA chelate (Tab. 1). Inorganic nickel also reduced the nutritional value of protein to a greater extent than Ni-EDTA chelate (Tab. 2).

Table 1

The crop of dry mass of head and the content of some nitrogen compounds in head of white cabbage

Parameter	**Control object**	**$NiSO_4 \cdot 7H_2O$**	**Ni-EDTA**	**$LSD_{0.05}$**
Crop of head dry mass [g per pot]	5.37	3.02	4.39	0.68
Dry mass of head [% of f.m.]	6.49	7.34	6.62	0.57
Ni content in head [$mg \cdot kg^{-1}$ of d.m.]	19.64	237.36	163.19	17.05
Total-N [% of d.m.]	2.57	1.63	2.19	0.29
Total protein [% of d.m.]	16.06	10.19	13.69	1.78
Total exogenous amino acids [% of protein]	36.39	28.53	31.95	3.21
Total of free amino acids [$mg \cdot g^{-1}$ of d.m.]	46.13	15.91	29.67	4.73

Table 2

The content of exogenous amino acids in head of white cabbage

Amino acid [g per 100 g of protein]	**Control object**	**$NiSO_4 \cdot 7H_2O$**	**Ni-EDTA**	**$LSD_{0.05}$**
Lys	4.68	4.18	4.37	0.35
Phe	4.25	3.96	4.03	0.21
Ile	3.84	2.75	3.24	0.32
Leu	8.29	6.16	6.67	0.57
Thr	4.48	3.15	3.39	0.29
Val	4.44	3.63	4.07	0,30
His*	2.04	2.28	2.76	0.19
Arg*	4.37	2.42	3.42	0.64
EAAI**	0.75	0.58	0.65	-

* exogenous amino acids for children aged below 12; ** EAAI - *essential amino acid index* (standard protein - chicken egg) [8].

Results of the studies conducted so far [3-5, 7, 16] have shown that the effect of nickel on the content of nitrogen compounds in plants depend on its content in the substrate (soil/water medium). Applied in high (excessive) concentrations, this metal depressed of nitrate assimilation and content of nitrogen compound in plants [7, 16]. Results of the conducted experiments have confirmed a negative effect of excess of nickel in the soil on the content of nitrogen and its organic compounds in cabbage plants. These results have also shown that both inorganic and chelatic nickel have this negative effect on the content of nitrogen and its analysed organic compounds in cabbage. However, chemical form of nickel determines the content of this metal in plants, and the level of reduction of content of nitrogen and its organic compounds.

Conclusion

Nickel added in excessive amounts (75 mg Ni · kg^{-1} of soil) to average soil whose pH was 6.2 both in its hydrate ($NiSO_4 \cdot 7H_2O$) and chelate (Ni-EDTA) forms caused a reduction of the crop of dry mass of white head cabbage cv. Gloria di Enkhouizen 2 and a reduction of the content of N-total, free amino acids and protein. It also reduced the nutritional value of protein, determined on the basis of the content of exogenous amino acids. Hydrate nickel reduced cabbage crop, the content of nitrogen and of its analysed compounds to a much greater extent than Ni-EDTA chelate. These differences resulted from a bigger phytoassimilation of nickel from soil polluted with nitrogen sulphate than from soil polluted with Ni-EDTA.

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PORÓWNANIE ZAWARTOŚCI NIEKTÓRYCH ZWIĄZKÓW AZOTU W KAPUŚCIE GŁOWIASTEJ BIAŁEJ UPRAWIANEJ NA GLEBIE ZANIECZYSZCZONEJ HYDRATEM I CHELATEM NIKLU

Katedra Biologii Roślin, Wydział Nauk Rolniczych w Zamościu, Uniwersytet Przyrodniczy w Lublinie

Abstrakt: W doświadczeniu wazonowym badano wpływ zanieczyszczenia gleby niklem w formie nieorganicznej ($NiSO_4 \cdot 7H_2O$) i chelatowej (Ni-EDTA) na plon kapusty głowiastej białej oraz zawartość azotu, białka, egzogennych i wolnych aminokwasów. Nikiel dodany do gleby o pH 6,2 w ilości 75 $mg \cdot kg^{-1}$ w obu formach chemicznych obniżał plon suchej masy kapusty oraz zawartość azotu i analizowanych jego związków. Obniżał także wartość odżywczą białka mierzoną zawartością egzogennych aminokwasów. Poziom redukcji plonu suchej masy kapusty i zawartości analizowanych związków azotu był znacznie większy w obecności niklu nieorganicznego $NiSO_4 \cdot 7H_2O$ niż chelatu Ni-EDTA i dodatnio korelował z zawartością tego metalu w roślinach.

Słowa kluczowe: aminokwasy, azot, białko, kapusta, toksyczność niklu, zanieczyszczenie gleby

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Ewa B. MOLISZEWSKA[1]

ASPECTS OF DECREASE OF SOME SOIL PATHOGENS OF SUGAR BEET BY *Bacillus subtilis* AND STREPTOMYCETES

BADANIE MOŻLIWOŚCI OGRANICZANIA ROZWOJU WYBRANYCH GLEBOWYCH PATOGENÓW BURAKA CUKROWEGO PRZEZ *Bacillus subtilis* ORAZ PROMIENIOWCE

Abstract: Soil-borne pathogens of sugar beet (*Beta vulgaris* L.) are often responsible for its poor quality and yield loss. Protection against the group of pathogens is possible only in the early stage of plant development, later there is no possibility for application of chemicals into the soil. This is a reason of the importance of biological methods of plant protection as well as the natural occurrence in the soil of some potentially antagonistic microorganisms. In the laboratory experiments some interactions between pathogens and potentially antagonistic microorganisms were tested as well as their influence on the sugar beet seedlings. Pathogens (*Aphanomyces cochlioides*, *Rhizoctonia solani*, *Fusarium oxysporum* and *Streptomyces scabies*) were isolated from diseased sugar beet root tissues. The antagonists were *Bacillus subtilis*, *Streptomyces griseoviridis* and two isolate of streptomycetes A-2003 and II-2003. Antagonistic microorganisms could not or could only slightly limit development of pathogens. The best result was observed in the case of *R. solani* which was limited by *S. griseoviridis*. In vitro *B. subtilis* and *S. griseoviridis* could decrease also growth of *S. scabies*. *F. oxysporum* showed very low pathogenic ability against sugar beet seedlings, but isolates of A-2003 and II-2003 decreased the number of diseased seedlings in the soil inoculated by *F. oxysporum*. The microorganisms tested as the antagonists did not injury seedlings of sugar beets. The strongest pathogenicity showed *A. cochlioides* and any one of antagonistic bacteria could not protect the seedlings against it. They also could not protect seedlings against *R. solani*, but in the case of soil infected by *S. scabies* they showed the possibility to protect seedlings.

Keywords: sugar beet, biological methods of plant protection

The use of the antagonistic properties of microorganisms, including different *Streptomyces* spp. and other bacteria in the biological control of many plant diseases has been the subject of many studies. Actinomycetes represent 7% of the total number of bacteria in the wheat rhizosphere. Numbers of actinomycetes are higher in the rhizosphere and root-free soil than in the endorhizosphere [1]. Mayfield et al [2] studied the ecology of actinomycetes in soil and found that they exist in soil for much of the time as spores unevenly distributed throughout the soil. Only 4% of spores germinated in natural soil but in sterile and glucose supplemented soil germination increased. Spores were more stable than mycelia and more growth was observed in sterile than in non-sterile soil [1]. *Streptomyces griseoviridis*, the representative of actinomycetes has been reported as an antagonist to the plant pathogens *Alternaria brassicicola, Botrytis cinerea, Fusarium avenaceum, F. culmorum, F. oxysporum, Rhizoctonia solani, Sclerotinia sclerotiorum. In vitro* tests reveled that *S. griseoviridis* suppressed the growth of these fungi [3]. Moussa and Rizk [3] reported also that 70% culture filtrate of *S. aureofaciens* inhibited spore germination, mycelium development and spore production of *Fusarium solani*. They found that seed-coating was more effective than soil pre-inoculation.

Some strains of beneficial microorganisms are marketed as biopesticides: Kodiak (*Bacillus subtilis* GB03), Epic (*Bacillus subtilis* GB07), Dagger G (*Pseudomonas*

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[1] Chair of Biotechnology and Molecular Biology, Opole University, ul. kard. B. Kominka 6a, 45-032 Opole, phone 77 401 60 40, email: ewamoli@uni.opole.pl

fluorescens), Mycostop (*Streptomyces griseoviridis*), F-stop (*Trichoderma harzianum*), Deny (*Burgholderia cepacia*) [4]. Beneficial plant bacteria which are associated with the surfaces of plant roots may increase plant yield. Both *in vitro* and *in vivo* experiments with *Streptomyces griseoviridis*, the active ingredient of Mycostop, effectively inhibits the growth of a wide range of fungal pathogens. The antagonistic effect seems to be based on several modes of action: rhizosphere competence, lysis of the pathogen cell wall followed by hyperparasitism, antifungal metabolites, auxin excretion (IAA), competition for living space and nutrients and general growth stimulation of the plant [4].

Soil-borne pathogens of sugar beet (*Beta vulgaris* L.) are often responsible for its poor quality and yield loss. Protection against the group of pathogens is possible only in the early stage of plant development, later there is no possibility for application of chemicals into the soil. This is a reason of the importance of examination of biological methods for plant protection as well as natural occurrence of some potentially antagonistic microorganisms in the soil.

Materials and methods

In the laboratory experiments, some interactions between pathogens and potentially antagonistic microorganisms were tested *in vitro* as well as their influence on the sugar beet seedlings in the pot test. Pathogens used in the experiments were originally isolated from diseased sugar beet root tissues. They were: *Aphanomyces cochlioides*, *Rhizoctonia solani*, *Fusarium oxysporum* and *Streptomyces scabies*. As the antagonists four isolates of bacteria were used: *Bacillus subtilis*, *Streptomyces griseoviridis* and two isolate of streptomycetes coded as A-2003 and II-2003. The last two isolates were obtained from sugar beet root tissues with the symptoms of scab.

The interactions between pathogens and the antagonistic bacteria were tested on Petri dishes filled with PDA medium. Each dish was inoculated in the centre with 5 mm disc of the medium overgrown by one-week old mycelium of appropriate microorganism and than bacteria were transferred on the two opposite lines situated on the margin of the PDA medium. For *Streptomyces scabies* liquid medium with the pathogen was used. The medium was transferred to the crack in the solid medium, and the antagonists were inoculated on the medium surface. The combinations without antagonists were used as controls. The experiment was done in five replications, dishes were kept in a room temperature, in the dark. The inhibition of growth of tested pathogens was observed.

The influence of the same microorganisms on the sugar beet seedlings was examined in the laboratory pot test. Fungal pathogens were grown on corn-sand medium during three weeks. Pots (500 cm^3) were filled with the sterilized soil, and after that inoculated with a portion (5 cm^3) of corn-sand medium overgrown by pathogen mycelium. The medium was covered with 1 cm (depth) of the soil and after two days ten non-pelleted sugar beet seeds were sown in each pot. Before sowing, seeds were surface sterilized, dried and soaked in the liquid culture of antagonists (15 min). For this experiment actinomycetes were grown on the liquid *oat-malt broth* (OMB) and *Bacillus subtilis* was grown on the liquid malt medium. The controls were not infected pots as well as pots inoculated only by one type of microorganism (pathogen or antagonist). The experiment was done twice in three replications and it was finished when plants developed the first pair of true leaves. In this experiment the disease development was observed. Each diseased seedling was transferred to the sterilized water, incubated for 24 hours and microscopically examined to confirm the appropriate pathogen presence. In the end of the experiment the dry mass of seedlings grown in each pathogen/antagonist combination was measured.

Results

Antagonistic microorganisms could not or could slightly limit development of pathogens. The best result was observed in the case of *R. solani* which was limited by *S. griseoviridis*. In vitro *B. subtilis* and *S. griseoviridis* could decrease also growth of *S. scabies*. The growth of *Aphanomyces cochlioides* was even stimulated slightly (Fig. 1).

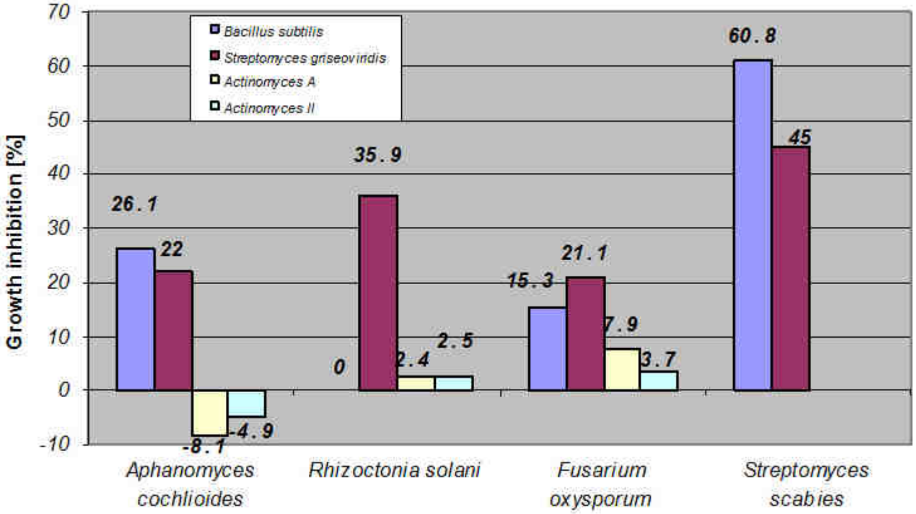

Fig. 1. Interactions between pathogens and antagonists *in vitro* ("–" means growth stimulation)

Fusarium oxysporum showed very low pathogenic ability against sugar beet seedlings, but isolates of A-2003 and II-2003, as well as *B. subtilis* decreased the number of diseased seedlings in the soil inoculated by *F. oxysporum*. The same effect was observed in the case of pots infected with *S. scabies* (Fig. 2). The microorganisms tested as the antagonists did not injury seedlings of sugar beets. The strongest pathogenicity showed *A. cochlioides* and any one of antagonistic bacteria could not protect the seedlings against it (Fig. 2). They also could not protect seedlings against *R. solani*, but in the case of soil infected by *S. scabies* they showed the possibility to protect seedlings (Fig. 2).

The dry mass content of sugar beet seedlings is the inverse of the tissue hydration. Plants with more hydrated tissues have better resistance possibility against pathogens [5]. Tested antagonists showed the possibility to decrease of dry mass content in sugar beet seedlings, in some cases, especially in case of pots infected with *A. cochlioides*, but this effect do not correlated with disease development (Figs. 1 and 3). Antagonists added alone to the soil without pathogens increased the dry mass content in sugar beet seedlings (Fig. 3).

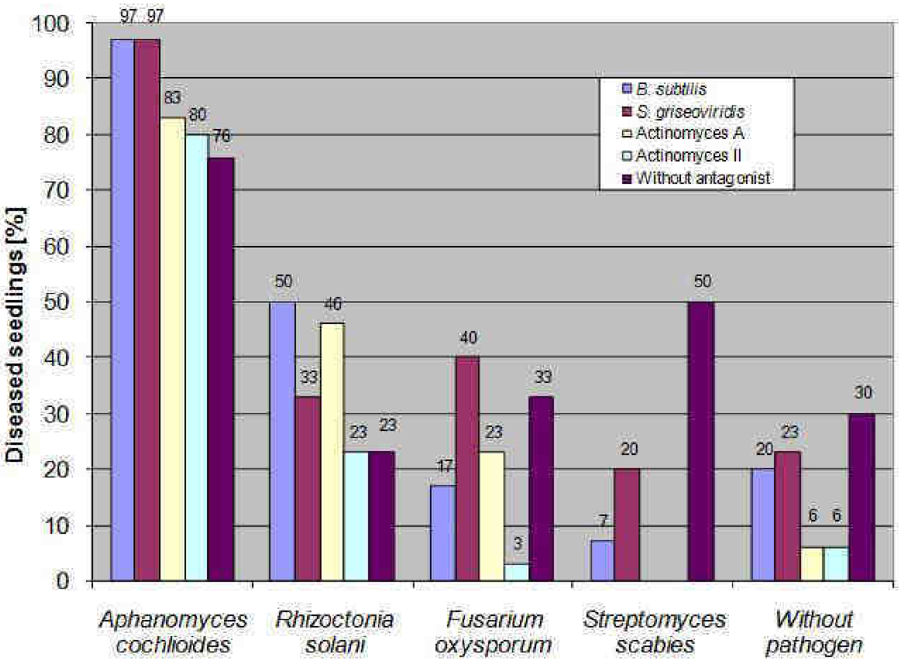

Fig. 2. The influence of antagonists on the disease development on sugar beet seedlings

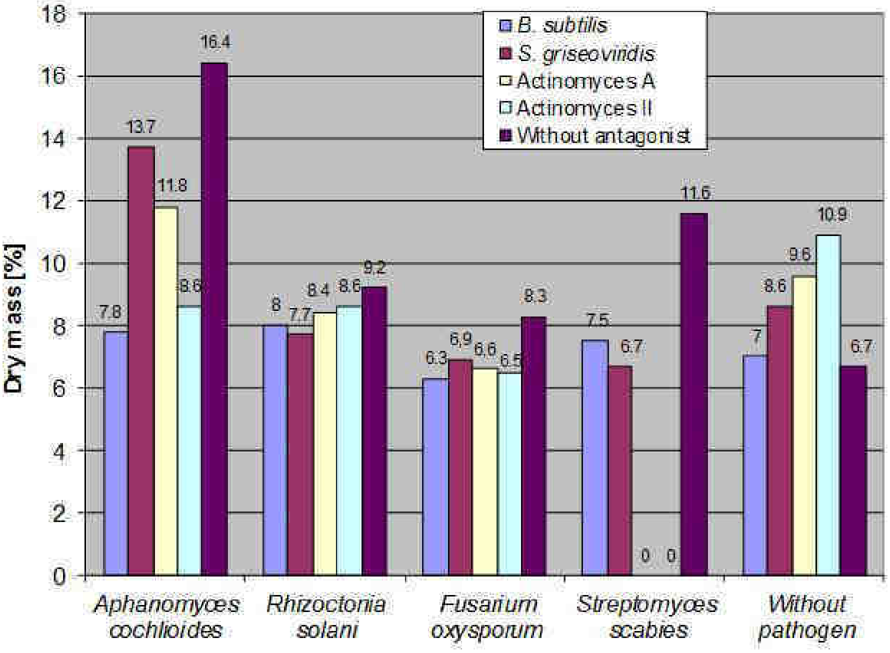

Fig. 3. The influence of antagonists on the dry mass content in sugar beet seedlings

Conclusions

Tested antagonists showed no destructive abilities against sugar beet seedlings, they were also not sufficiently effective against most of tested pathogens. *B. subtilis* as well as *S. griseoviridis* have protected sugar beet seedlings against *S. scabies*. Antagonistic isolate of *B. subtilis*, Actinomyces A and Actinomyces II could also decrease the seedlings injury made by *F. oxysporum*. The mode of action of antagonists is due to some extent with the tissue hydration.

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BADANIE MOŻLIWOŚCI OGRANICZANIA ROZWOJU WYBRANYCH GLEBOWYCH PATOGENÓW BURAKA CUKROWEGO PRZEZ *Bacillus subtilis* ORAZ PROMIENIOWCE

Samodzielna Katedra Biotechnologii i Biologii Molekularnej, Uniwersytet Opolski

Abstrakt: Choroby korzeni buraka cukrowego (*Beta vulgaris* L.) powodują znaczne obniżenie ilości oraz jakości plonu tej rośliny. Zwalczanie patogenów glebowych ograniczone jest tylko do fazy siewek buraka, w późniejszym czasie nie ma możliwości chemicznej walki z patogenami glebowymi. Dlatego bardzo ważna jest obecność w glebie naturalnych antagonistów mikroorganizmów patogennych. Organizmy antagonistyczne można także wprowadzać sztucznie do środowiska glebowego, co umożliwia rozwój metod biologicznej ochrony roślin. W przeprowadzonych doświadczeniach, w warunkach laboratoryjnych, zbadano wzajemne oddziaływanie, w warunkach *in vitro*, pomiędzy patogenami otrzymanymi z zainfekowanych korzeni buraka cukrowego: *Aphanomyces cochlioides*, *Rhizoctonia solani*, *Fusarium oxysporum* oraz *Streptomyces scabies* a potencjalnymi antagonistami: *Bacillus subtilis*, *Streptomyces griseoviridis* oraz dwoma wybranymi izolatami promieniowców (A-2003 i II-2003) otrzymanymi z ranek parchowych na korzeniach buraka. Zbadano także ich wpływ na rośliny buraka w infekcyjnym doświadczeniu wazonowym. Stwierdzono, że wpływ antagonistów na rozwój patogenów był niejednakowy. Obserwowano niewielki stopień ograniczenia wzrostu patogenów przez *S. griseoviridis* i przez *B. subtilis*, szczególnie w odniesieniu do *R. solani*. *F. oxysporum* wykazywał bardzo nikłe właściwości patogenne wobec siewek buraka, a badane promieniowce A-2003 i II-2003 w niewielkim stopniu ograniczały rozwój jego grzybni oraz w odniesieniu do kombinacji kontrolnej zmniejszały liczbę chorych roślin w teście wazonowym. Wykazano, że żaden z potencjalnych antagonistów nie powodował uszkodzeń siewek buraka. Siewki hodowane w wazonach zainokulowanych antagonistami oraz *A. cochlioides* nie były chronione przed tym patogenem, podobnie jak w przypadku wazonów infekowanych *R. solani*, natomiast *B. subtilis* i *S. griseoviridis* dobrze chroniły siewki przed *S. scabies*.

Słowa kluczowe: burak cukrowy, biologiczna ochrona roślin

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Małgorzata NABRDALIK[1] and Katarzyna GRATA[1]

ANTIFUNGAL ACTIVITY OF BACTERIAL SPECIES *Pseudomonas*

AKTYWNOŚĆ PRZECIWGRZYBOWA BAKTERII Z RODZAJU *Pseudomonas*

Abstract: The aim of the research was to determine a fungistatic activity of bacteria *Pseudomonas* against *Fusarium* sp. The antagonistic properties were assessed with a culture-plate method on Czapek and PDA growth media for *Pseudomonas* sp. cultures after 4, 6, 8, 10 and 24 hours of culturing. The culturing process was conducted at 22ºC for 14 days. The fungistatic activity of *Pseudomonas* sp. strains was determined against the growth rate index. Obtained results proved that tested strain of *Fusarium* sp. was sensitive to supernatants of *Pseudomonas* spp. The linear growth of the mycelium of *Fusarium* sp. was inhibited most actively by *Pseudomonas* sp. strain BK1. The highest decrease, amounting 77%, in the value of the growth rate index was obtained after 10 hours of culturing on Czapek medium. The highest reduction of the growth rate index for *Pseudomonas* sp. strain KF1 was also noted for 10-hour culture, but it amounted only 42% in this case. Conducted research confirm fungistatic activity of *Pseudomonas* spp. strains against *Fusarium* sp. and prove that growth inhibition of the mycelium depends not only on the type of metabolites produced by a specific bacterial strain but also on time they were cultured for.

Keywords: fungistatic activity, growth rate index, *Pseudomonas* sp., *Fusarium* sp.

There has been a general concern about the amount of chemical substances applied in the process of plants protection. Therefore natural biological preparations containing non-pathogenic microorganisms have become more and more popular due to their high efficiency which allows to use them in place of fungicides. Investigations into properties of microorganisms in order to find the most suitable bacterial strains brought the attention to the activity of bacteria *Pseudomonas*.

Many non-pathogenic species of bacteria *Pseudomonas* belong to PGPR group (ie *Plant Growth Promoting Rhizobacteria*) and have an advantageous influence on plants by producing biologically active metabolites and growth regulating substances. Moreover, some of *Pseudomonas* spp. strains developed different mechanisms which limit pathogenic activity of fungi. According to literature sources [1, 2], bacteria possess the ability to synthesize different substances which are biologically active, ie hydrogen cyanide, siderophore, salicylic acid and lytic enzymes, proteases.

The aim of conducted research was to determine how metabolites produced by strains of *Pseudomonas* sp. KF1 and BK1 affect the growth of *Fusarium* sp.

Materials and methods

For the experiment, test strains of *Pseudomonas* sp. marked KF1 and BK1 isolated from the natural environment were used together with an indicator strain *Fusarium* sp.

Fungistatic activity of tested strains was determined with the culture-plate method on Czapek medium consisting of [g/dm^3]: sucrose 30.0, $MgSO_4 \times 7H_2O$ 0.5, KH_2PO_4 1.0, KCl 0.5, $NaNO_3$ 3.0, $Fe_2(SO_4)_3 \times 7H_2O$ 0.01, agar 15.0 and on PDA medium consisting of [g/dm^3]: glucose 20.0, potato extract 4.0, agar 15.0. In bacterial cultures which were control

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[1] Biotechnology and Molecular Biology Department, University of Opole, ul. kard. B. Kominka 6, 45-035 Opole, phone 77 401 60 56, email: mnabrdalik@uni.opole.pl

trials, *Pseudomonas* sp. was added to the media as supernatants obtained after 4, 6, 8, 10 and 24 hours of culturing. Next, the media were inoculated with fungi discs of 10 mm diameter. The control trial contained *Fusarium* sp. cultures and aseptic broth medium in place of the supernatant. All plates were cultured at 22ºC for 14 days. After 4-2 days the diameters were measured until *Fusarium* sp. on the plate with the control trial grew over the whole surface of the plate. The experiment was conducted in three trials, where one trial was represented by one plate with the growth medium and one mycelial disc.

The influence of metabolites produced by *Pseudomonas* sp. on the growth of *Fusarium* sp. was determined against the growth rate index, calculated according to the formula below [3]:

$$T = \frac{A}{D} + \frac{b_1}{d_1} + \frac{b_2}{d_2} + ... + \frac{b_x}{d_x}$$

where: T - growth rate index, A - mean value of diameter measurements, D - the length of the experiment [number of days], b_1, b_2, b_x - increase in a diameter size since the last measurement, d_1, d_2, d_x - number of days since the last measurement.

Results

In the experiment, 2 bacterial strains *Pseudomonas* (KF1 and BK1) were tested against their ability to synthesize exocellular metabolites possessing fungistatic abilities in relation to *Fusarium* sp. On the basis of obtained results, it has been observed that fungistatic activity of tested *Pseudomonas* is varied and depends on the type of the growth medium and the length of culturing.

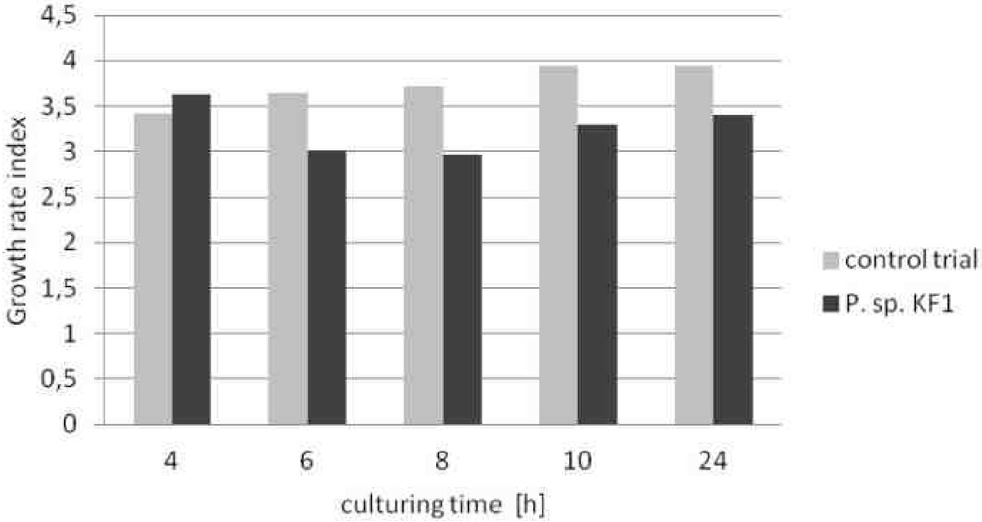

Fig. 1. The influence of *Pseudomonas* sp. KF1 on mycelial growth of *Fusarium* sp. on Czapek medium

The tests showed that strain *Pseudomonas* KF1 was least active in terms of its fungistatic activity on Czapek medium (Fig. 1). For the supernatant obtained after 4 hours of culturing, a decrease in the growth rate index has not been recorded in comparison with the control trial. Only after supplementing the growth medium with 6, 8, 10 and 24-hour cultures, the drop of the growth rate index was noticeable and reduction amounted between 14 and 20%. Linear growth of the mycelium was inhibited most actively by adding 8-hour culture to the growth medium.

The analysis of *Fusarium* sp. growth on PDA medium with an addition of supernatants of *Pseudomonas* sp. KF1 revealed much more intensive drop of the growth rate index than on Czapek medium (Fig. 2). The highest value of the growth rate index was noted for 8 and 10-hour cultures and obtained reduction amounted almost 40 and 42%. The lowest reduction in the value of the growth rate index was obtained in case of 4-hour culture supernatant.

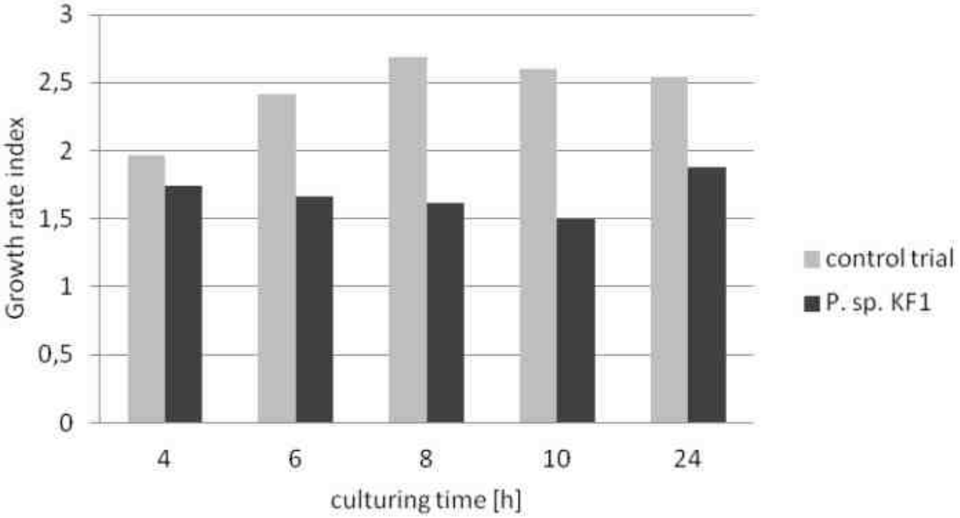

Fig. 2. The influence of *Pseudomonas* sp. KF1 on mycelial growth of *Fusarium* sp. on PDA medium

In presented tests, fungistatic activity in relation to *Fusarium* sp. was also determined for strain *Pseudomonas* sp. BK1. Recorded values of the growth rate index allowed to state that strain *Fusarium* sp. showed the fastest linear growth (Fig. 3) on Czapek medium. Only after 8 days from the beginning of the experiment, plates with control trials were grown over with mycelium, which covered its whole surface and the growth rate index for the consecutive trials flactuated between 4.121 and 4.735. At the same time, the test trials were characterised by much lower value of the growth rate index, which resulted in the highest reduction of the mycelial growth rate. The reduction value was recorded as follows: 77% for the supernatant obtained from 10-hour culture and 70% for 24-hour culture. For the remaining cultures, the reduction in the growth rate index did not exceed 60%.

In case of *Pseudomonas* sp. BK1 cultured on PDA medium, a linear growth of the mycelium of *Fusarium* sp. was inhibited most actively when the supernatant obtained from

4-hour culture was added (Fig. 4). The reduction in the growth rate index amounted almost 60% when compared with the control trial. For the remaining trials, a lower reduction in the growth rate was recorded: from 25% for 24-hour culture to ca. 44% for 8-hour culture.

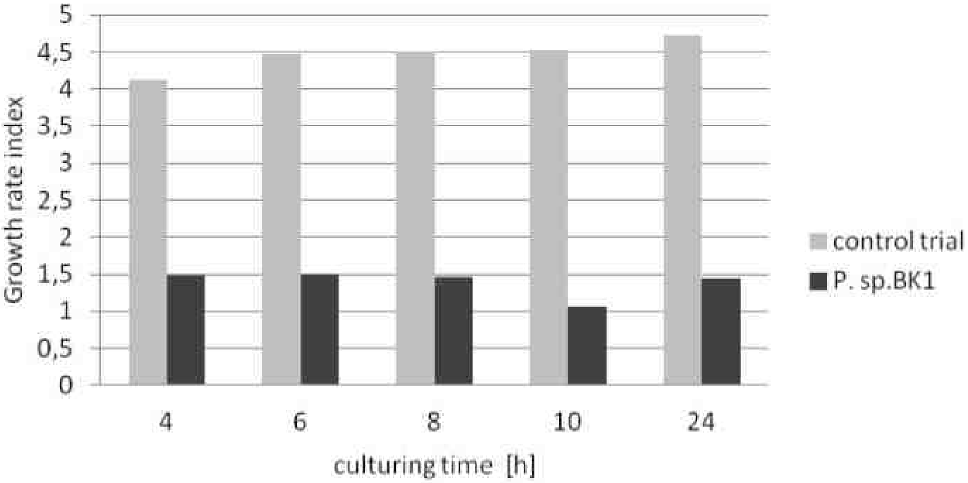

Fig. 3. The influence of *Pseudomonas* sp. BK1 on mycelial growth of *Fusarium* sp. on Czapek medium

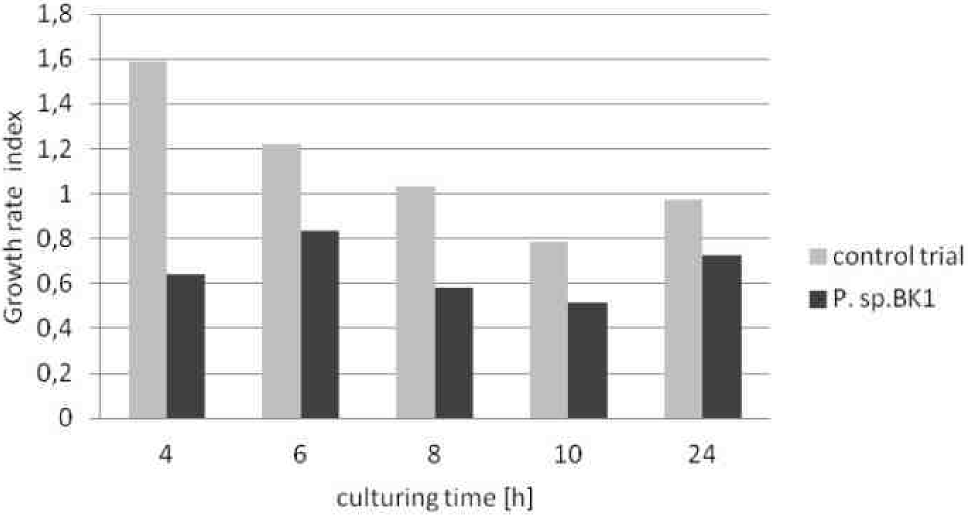

Fig. 4. The influence of *Pseudomonas* sp. BK1 on mycelial growth of *Fusarium* sp. on PDA medium

It is worth noticing that obtained values of the growth rate index for the control trials on Czapek medium were several times higher in comparison with the ones obtained on PDA medium for the both *Pseudomonas* sp. KF1 and *Pseudomonas* sp. BK1 strains. It may probably result from different sources of carbon in the growth medium (glucose or sucrose) and different preference towards them showed by tested strain of *Fusarium* sp. Moreover, for most of the trials the linear growth of the mycelium of *Fusarium* sp. was increasing with the time of culturing.

Summary and conclusions

Conducted research showed significant diversity of fungistatic activity among *Pseudomonas* sp. strains. The growth of *Fusarium* sp. was inhibited most actively by the exocellular metabolites produced by *Pseudomonas* sp. BK1. The most optimal results have been obtained on Czapek medium where sucrose is a source of carbon. The lowest fungistatic activity was observed for *Pseudomonas* sp. KF1 and the growth medium for which the results were satisfactory was PDA medium with glucose. Judging from recorded results, an addition of the supernatants obtained from 8 and 10-hour cultures may significantly inhibit linear growth of *Fusarium* sp.

Strains of *Pseudomonas* sp. may be applied to protect plants against diseases caused by *Fusarium* sp. However, the difference in fungistatic activity between tested strains revealed in the experiment may be the evidence that it is typical for a specific strain, which depends not only on the time of culturing, but also on the type of the growth medium or the environment it is designed for.

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AKTYWNOŚĆ PRZECIWGRZYBOWA BAKTERII Z RODZAJU *Pseudomonas*

Samodzielna Katedra Biotechnologii i Biologii Molekularnej, Uniwersytet Opolski

Abstrakt: Celem badań było określenie aktywności fungistatycznej bakterii z rodzaju *Pseudomonas* wobec *Fusarium* sp. Ocenę właściwości antagonistycznych metabolitów przeprowadzono metodą hodowlano-płytkową na podłożu Czapka oraz PDA dla 4-, 6-, 8-, 10- i 24-godzinnych hodowli *Pseudomonas* sp. Hodowle prowadzono w temperaturze 22°C przez 14 dni. Na podstawie indeksu tempa wzrostu określono aktywność fungistatyczną szczepów *Pseudomonas* sp. Wyniki doświadczenia wskazują, że badany szczep *Fusarium* sp. był wrażliwy na działanie supernatantów *Pseudomonas* sp. Największą inhibicję rozrostu liniowego grzybni *Fusarium* sp. powodował szczep *Pseudomonas* sp. BK1. W tym przypadku najwyższy 77% spadek indeksu tempa wzrostu grzybni uzyskano w przypadku 10-godzinnej hodowli na podłożu Czapka. Natomiast dla szczepu *Pseudomonas* sp. KF1 największą redukcję indeksu tempa wzrostu odnotowano dla hodowli 10-godzinnej i wynosiła ona jedynie 42%. Przeprowadzone badania potwierdzają fungistatyczne działanie szczepów *Pseudomonas* sp. wobec *Fusarium* sp. Ponadto wykazują, że inhibicja wzrostu grzybni uzależniona jest nie tylko od rodzaju metabolitów wydzielanych przez dany szczep bakterii, ale również od wieku jej hodowli.

Słowa kluczowe: aktywność fungistatyczna, indeks tempa wzrostu, *Pseudomonas* sp., *Fusarium* sp.

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Matúš PEŠKO[1] and Katarína KRÁĽOVÁ[1]

PHYTOTOXIC EFFECTS OF HEXAVALENT CHROMIUM ON RAPESEED PLANTS

FITOTOKSYCZNOŚĆ CHROMU SZEŚCIOWARTOŚCIOWEGO DLA ROŚLIN RZEPAKU

Abstract: Rapeseed (*Brassica napus* L. *subsp. napus*) plants were exposed to six different concentrations (12, 24, 60, 120, 240, 480 μmol dm^{-3}) of $K_2Cr_2O_7$ for 7 days. Dry mass of shoots and roots decreased rapidly with increasing external Cr(VI) concentration. Application of Cr(VI) concentrations ≥ 120 μmol dm^{-3} caused that leaves were strongly chlorotic and some of them even desiccated. Roots of these plants where subtile and brownish. Notable decrease in chlorophyll content was observed already at the lowest (12 μmol dm^{-3}) used concentration. Content of soluble proteins in leaves decreased rapidly within the studied concentration range, whereby the lowest protein content was observed after application of 240 μmol dm^{-3} Cr(VI). Lipid peroxidation expressed as a content of malondialdehyde in leaves was notable already after application of 12 μmol dm^{-3} Cr(VI). At lower applied Cr(VI) concentrations (12÷120 μmol dm^{-3}) the bioaccumulation factors related to Cr accumulation in roots were higher then those determined for shoots. Treatment with higher Cr(VI) concentrations (240 and 480 μmol dm^{-3}) had an opposite effect and BAFs for the shoots exceeded those determined for the roots. The portion of Cr allocated in shoots related to the total Cr amount accumulated by plant ranged from 23.3% (12 μmol dm^{-3}) to 94.7% (480 μmol dm^{-3}). In the case of higher applied external Cr(VI) concentrations (120÷480 μmol dm^{-3}) the defence mechanisms of plants were evidently impaired and uncontrolled Cr translocation within the plant occurred.

Keywords: bioaccumulation and translocation factors, chlorophyll content, chromium, lipid peroxidation, rapeseed

Chromium is a toxic carcinogen and it is released into the soil mainly from leather tanning, textile, carpet and electroplating industries [1]. It occurs in soil mainly in two oxidation states, as Cr(III) or Cr(VI) ions. Chromium(VI) remains stable for several months in the soil without changing its oxidation state. In fact, oxidative behaviour of the chromium in soils is of ecological significance, since Cr(VI) is found to be more toxic to plants and animals than Cr(III) [2]. Chromium is bioaccumulated by plants and its accumulation is biomagnified at different trophic levels through the food chain [3].

Symptoms of Cr phytotoxicity include decrease of seed germination, reduction of root growth, induction of leaf chlorosis, reduction of biomass, induction of biochemical changes and it was demonstrated that chromium inhibit photosynthetic and mitochondrial electron transport in higher plants [4]. Further, plants growing in chromium-stressed environment face a potential risk from *reactive oxygen species* (ROS) like superoxide, hydroxyl radicals and hydrogen peroxide. Their presence cause oxidative damage to the biomolecules such as lipids, proteins and nucleic acids [5]. In the plants, metal induced lipid peroxidation has been reported [6], which profoundly alters the structure of membranes and consequently modifies their enzymatic and transport activities. Plants have developed complex defence mechanisms by which they mediate the deleterious effects of the ROS. Such defence systems involve both enzymatic and non-enzymatic antioxidants [7]. The enzymatic

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[1] Faculty of Natural Sciences, Comenius University Bratislava, Mlynská dolina CH-2, SK-842 15 Bratislava, Slovakia, phone +421 2 60296340, fax +421 2 65429064, email: pesko@fns.uniba.sk

protective mechanism operates by sequential and simultaneous action of a number of antioxidant enzymes such as catalase, superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase and glutathione reductase [7].

Rapeseed (*Brassica napus* L. *subsp. napus*) is known to be able to accumulate substantial amounts of metals; moreover, this plant has a high biomass, various genotypes are easily available and the plant belongs to Brassicaceae family [8] that has received considerable attention [9] based on the capacity of these plants to uptake and accumulate Cr and other heavy metals in amounts higher than those of other plant species [10].

The aim of this study is to investigate the effect of Cr(VI) on plant biomass as well as Cr accumulation and translocation in plant organs of hydroponically cultivated *B. napus* plants (cv. Verona). In addition, content of soluble proteins, *malondialdehyde* (MDA), chlorophyll *a* and *b* as well as carotenoids in leaves was determined.

Material and methods

For experiments $K_2Cr_2O_7$ of analytical purity was used (Slavus, Bratislava). For cultivation of experimental plants the seeds of *Brassica napus,* cv. Verona (Centre for Research of Crop Production, Research Institute of Crop Production, Piešťany) were used. Three-weeks old rapeseed plants were exposed in hydroponics for seven days in controlled conditions (mean air temperature: 25 ±0.5°C, relative air humidity: 80% and photosynthetic active radiation: 80 μmol m^{-2} s^{-1}): control variant in Hoagland solution and metal-treated variants in Hoagland solution containing 12, 24, 60, 120, 240 and 480 μmol dm^{-3} $K_2Cr_2O_7$. Then the length and dry mass of shoots and roots were determined. Flame *atomic absorption spectroscopy* (AAS Perkin Elmer Model 1100, USA) was used for determination of Cr content in shoots and roots of rapeseed plants. Protein content in leaves was determined spectrophotometrically (Genesys 6, Thermo Scientific, USA) according to Bradford [11]. Content of malondialdehyde in leaves was determined spectrophotometrically as a content of *thiobarbituric acid reactive substances* (TBARS) according to method described in [12]. Photosynthetic pigments were determined spectrophotometrically after extraction into 80% acetone [13].

Results and discussion

Shoot and root dry mass as well as length of both plant organs of rapeseed plants treated with Cr(VI) are summarized in Table 1. Significant reduction of dry biomass (both plant organs) was observed after application of concentrations higher than 60 μmol dm^{-3}. Shoot length was affected by applied concentrations of Cr(VI) to a greater extent this of root. General response of decreased root growth due to Cr toxicity could be due to inhibition of root cell division/ root elongation or to the extension of cell cycle in the roots. The reduction in plant height might be mainly due to the reduced root growth and consequent lesser nutrients and water transport to the above parts of the plant. In addition to this, Cr transport to the aerial part of the plant can have a direct impact on cellular metabolism of shoots contributing to the reduction in plant height. The overall adverse effect of Cr on growth and development of plants could be serious impairment of uptake of mineral nutrients and water leading to deficiency in the shoot [4].

Chlorosis was notable already after application of the lowest Cr(VI) concentration (12 µmol dm^{-3}). Concentrations of Cr(VI) higher than 60 µmol dm^{-3} caused that leaves of plants were strongly chlorotic, some of them even desiccated and fell off (240 and 480 µmol dm^{-3}). Roots of plants treated with two highest concentrations were subtile and brownish. Content of chlorophyll *a* and carotenoids (Fig. 1) was affected by Cr(VI) to a greater extent than that of chlorophyll *b*. Significant decrease of chlorophyll *b* was observed after application of 60 µmol dm^{-3} Cr. The decrease in the chlorophyll *a*/*b* ratio caused by Cr indicates that Cr toxicity possibly reduces size of the peripheral part of the antenna complex [14]. The decrease in chlorophyll *b* due to Cr could be due to the destabilization and degradation of the proteins of the peripheral part. The inactivation of enzymes involved in the chlorophyll biosynthetic pathway could also contribute to the general reduction in chlorophyll content in most plants under Cr stress. It could be assumed that chromium toxicity is not located at the level of δ-aminolevulinic acid synthesis, but, probably at the δ-aminolevulinic acid dehydratase (ALAD activity which was more severely affected during chlorophyll biosynthesis). Finally, impaired chlorophyll biosynthesis results in reduced total chlorophyll content [4].

Table 1

Shoot and root length and dry mass of rapeseed plants treated with different concentrations of Cr(VI). Mean ± S.E., n = 5. Means followed by different letters are significantly different at the 0.05 probability level

c [µmol dm^{-3}]	**Shoot dry mass [mg]**	**Root dry mass [mg]**	**Shoot length [cm]**	**Root length [cm]**
0	417.3 ± 43.7^{a}	29.8 ± 2.9^{a}	21.2 ± 1.2^{a}	16.1 ± 1.2^{a}
12	424.8 ± 64.1^{a}	22.5 ± 3.0^{b}	20.4 ± 0.5^{a}	16.8 ± 1.6^{a}
24	378.5 ± 33.4^{a}	22.3 ± 2.6^{b}	18.3 ± 1.1^{b}	16.6 ± 1.0^{a}
60	247.8 ± 26.7^{b}	17.3 ± 2.1^{c}	17.0 ± 1.3^{bc}	17.2. ± 0.8^{a}
120	213.3 ± 28.2^{bc}	16.0 ± 1.8^{c}	17.7 ± 0.9^{bc}	16.1 ± 1.4^{a}
240	168.0 ± 13.0^{c}	13.3 ± 2.2^{c}	16.9 ± 0.3^{c}	16.2 ± 0.7^{a}
480	111.0 ± 9.2^{d}	7.5 ± 1.7^{d}	16.4 ± 1.1^{c}	16.5 ± 0.8^{a}

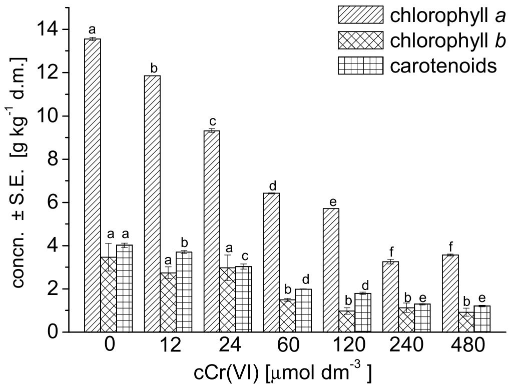

Fig. 1. Effect of applied Cr(VI) concentration on chlorophyll *a*, chlorophyll *b* and carotenoids concentration in leaves of rapeseed plants. Mean ± S.E., n = 3. Means followed by different letters are significantly different at the 0.05 probability level; d.m. - dry mass

Application of Cr(VI) concentrations higher than 24 μmol dm^{-3} caused strong decrease of protein content in leaves (Table 2). Degradation of proteins in rapeseed plants by Cr result in the inhibition of *nitrate reductase* activity (NR), whereby the correlation between NR activity and proteins has been well documented in plants [15]. A decline in amino acid cysteine may result in the degradation of sulphate-reducing enzymes leading to toxic effects [16].

Heavy metal toxicity is considered to induce the production of reactive oxygen species and may result in significant damage to cellular constituents. Membrane lipids and proteins are especially sensitive to attack by free radicals and they are considered to be reliable indicators of oxidative stress in plants [17, 18]. Content of proteins and malondialdehyde (MDA) in leaves of rapeseed plants is presented in Table 2.

Chromium induced oxidative stress in rapeseed plants was evident from the increased lipid peroxidation (content of MDA) in leaves, which was notable already after application of 12 μmol dm^{-3} Cr(VI). This finding is in agreement with the results of other studies carried out with plants cultivated in hydroponics [19, 20].

Table 2

Protein and MDA concentrations in leaves of rapeseed plants treated with different concentrations of Cr(VI). Mean ± S.E., n = 3. Means followed by different letters are significantly different at the 0.05 probability level; d.m. - dry mass

Cr(VI) conc. [μmol dm^{-3}]	**Protein conc. [g kg^{-1} d.m.]**	**MDA conc. [mmol kg^{-1} d.m.]**
0	45.0 ± 2.7^{a}	1.89 ± 0.03^{a}
12	37.4 ± 6.1^{ab}	1.94 ± 0.05^{a}
24	36.4 ± 4.00^{ab}	2.18 ± 0.07^{b}
60	31.1 ± 2.2^{bc}	2.19 ± 0.02^{bc}
120	23.3 ± 2.9^{cd}	2.32 ± 0.04^{cd}
240	17.5 ± 2.00^{d}	2.40 ± 0.06^{d}
480	18.7 ± 3.7^{d}	2.82 ± 0.03^{e}

Table 3

Chromium concentrations in shoots and roots of rapeseed plants treated with different concentrations of Cr(VI), corresponding values of bioaccumulation (BAF) and translocation (TF) factors and fraction of accumulated Cr allocated in shoots related to the total amount of Cr accumulated by plants (in [%]); d.m. - dry mass

c [μmol dm^{-3}]	**Cr conc. [mg kg^{-1} d.m.] BAF**		**TF [% Cr in shoot]**
	Shoot	**Root**	
0	0.3	6	-
12	36 **29.1**	2259 **1810.3**	0.016 **23.3**
24	92 **36.9**	2463 **986.9**	0.037 **38.8**
60	508 **81.4**	2462 **394.4**	0.206 **74.7**
120	1740 **139.4**	2587 **207.3**	0.673 **90.0**
240	3161 **126.7**	2615 **102.3**	1.209 **93.9**
480	3931 **78.8**	3251 **65.2**	1.209 **94.7**

Chromium content in shoots and roots of rapeseed plants treated with different metal concentrations, the corresponding *bioaccumulation* (BAF) and *translocation* (TF) *factors* as well as the fraction of accumulated Cr allocated in shoots related to the total amount of Cr accumulated by plants are summarized in Table 3.

Bioaccumulation factor (BAF) express the ratio of the metal concentration in the biological material [μmol or μg g^{-1} dry mass] to the metal concentration in external solution in [μmol or μg dm^{-3}]. In the concentration range 12÷120 μmol dm^{-3} chromium, BAF values of roots were higher than those of shoots. After application of 240 and 480 μmol dm^{-3} of Cr the BAFs determined for shoots exceeding those for roots reflect more effective mobility of Cr in the plants (Table 3). The translocation factor corresponds to the ratio of accumulated Cr amount in shoots and roots. Translocation factor values higher than 1 correspond to higher Cr concentration [mg kg^{-1} dry mass] in the shoots than in the roots. However, the total Cr content occurring in individual plant organs is affected not only by Cr concentrations in shoots and roots but also by actual dry mass of these plant organs. While after application of 12 and 24 μmol dm^{-3} Cr(VI) the higher portion of Cr (76.7 and 61.2%) was accumulated in roots, treatment with higher Cr(VI) concentrations (60÷480 μmol dm^{-3}) had an opposite effect and the majority of Cr accumulated by rapeseed plants (74.7 to 94.7%) was allocated in shoots (Table 3).

Chromium uptake by plants is mainly non-specific, probably as a result of uptake of essential nutrients and water [21]. It has been reported that translocation of chromium from roots to shoots was inhibited in the presence of toxic levels of the metals [22]. Rapeseed appeared to accumulate Cr mainly in the root system, whereas the shoots content is relatively low. The reason of the high accumulation in roots of the plants could be because Cr is immobilized in the vacuoles of the root cells, thus is less toxic, which may be a natural toxicity response of the plant [23]. The dependence of accumulated Cr content in shoots on the applied concentration of Cr(VI) showed practically linear increase up to application of 240 μmol dm^{-3} Cr(VI). On the other hand, root tissue was gradually saturated by the studied metal. In the case of higher applied external Cr(VI) concentrations (120÷480 μmol dm^{-3}) the defence mechanisms of plants were evidently impaired and uncontrolled Cr translocation within the plant occurred. Considerably higher biomass of shoots (more than one order) in comparison with that of roots as well as higher Cr concentration in the shoots than in the roots observed after treatment with 240 and 480 μmol dm^{-3} Cr significantly contributed to very high values of the fraction of accumulated Cr allocated in the shoots related to the total amount of Cr accumulated by plants.

Acknowledgements

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Iwona PUCZYŃSKA[1], Jerzy SKRZYPSKI[1], Mirosław IMBIEROWICZ[1]
and Maria TROSZKIEWICZ[1]

STUDY OF HEAT EFFECT MEASUREMENT METHOD DURING OXIDATION OF ORGANIC COMPOUNDS IN SUBCRITICAL WATER

OPRACOWANIE METODYKI POMIARU EFEKTÓW CIEPLNYCH UTLENIANIA ZWIĄZKÓW ORGANICZNYCH W ŚRODOWISKU WODY PODKRYTYCZNEJ

Abstract: Combination of calorimetry and the oxidation process at elevated temperature and pressure to determine heat effects can be a good method to study mechanisms of chemical reactions.

Keywords: calorimetry, wet oxidation, reaction calorimety, glucose oxidation

Calorimetry (Lat. *calor* = heat) is applied for measurement of heat evolving during chemical reactions or physical changes. Indirect calorimetry is used for calculation of heat that living organisms generate during producing of carbon dioxide and nitrogen waste or during oxygen consumption. The Dynamic Energy Budget theory support this theory and adds that heat generated by living organisms may be measured by direct calorimetry, in which the entire organism is placed inside the calorimeter for the measurement.

The specific heat formula is as follows:

$$q = mc\Delta T \tag{1}$$

where: q - heat, m - mass, c - specific heat capacity, ΔT - change in temperature.

Calorimetry is based on the fact that all chemical reactions and physical transformations generate or consume heat. The basis for calculation of accurate calorimetric data is the balancing of heat and mass flow.

Oxidation of organic compounds at elevated temperature and pressure

A new technological innovation is the calorimetry at elevated temperature and pressure. Determination of heat effects during oxidation of glucose using oxygen, elevated temperature and pressure is the aim of the studies.

Glucose is often considered as useful chemical resource that could be used as a starting material in chemical or biochemical processes [1]:

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + \Delta H_r \tag{2}$$

The calorimetric measurements, researched in calorimetric reactor RC1, were used for these studies. A Mettler-Toledo reaction calorimeter with 2 dm^3 jacketed batch reactor equipped with a gas type stirrer was used as a pressure reactor (Fig. 1).

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[1] Faculty of Process and Envirronmental Engineering, Technical University of Lodz, ul. Wólczańska 213, 90-924 Łódź, phone 42 631 37 09, email: skrzypsk@wipos.p.lodz.pl

The calorimetric principle applied in RC1 apparatus is based on a continuous measurement of the temperature difference between the reactor content (T_r) and the heat transferred to fluid in the outer jacket (T_j). At any given time, the heat transferred between the fluid in the jacket and the reactor content, q_{flow}, is given by:

$$q_{flow} = UA(T_r - T_j) \tag{3}$$

where U is the heat transfer coefficient and A is the heat exchange area. These parameters are determined by the calibration process.

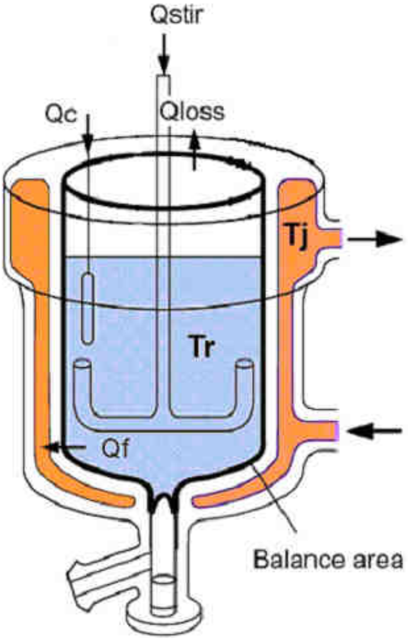

Fig. 1. Calorimetric reactor RC1 made by Mettler Toledo Company

The heat flow balance over the reactor is as follows:

Inflow = Accumulation + Outflow of the heat

$$(Qr + Qc + Qstir) = (Qa + Qi) + (Qf + Qdos + Qloss + Qreflux + ...) \tag{4}$$

Qr - heat generation rate of chemical or physical process, Qc - calibration power, Qstir - energy input due to stirrer (heat production in the reaction mass), Qa - heat storage (accumulation) by the reaction mass, Qi - heat storage (accumulation) through the inserts, Qf - heat flow through the reactor wall, Qdos - heat input due to dosing. Power that is needed to bring the inflow from Tdos to Tr, Qloss - heat flows through the reactor head assembly (radiation, conduction), Qreflux - quantity of heat dissipated in the reflux condenser.

Qr is the sum of all the individual heat effects in the reaction medium that result from simultaneous chemical reactions and phase changes such as evaporation, crystallization, dissolution and mixing. In the evaluation of an experiment, this unknown heat generation rate must be determined using the measurable heat effects.

To do this, the heat balance equation is rearranged, the term Qstir neglected and several terms combined:

$$Qr = Qflow + Qaccum + Qdos + Qloss + Qreflux \tag{5}$$

where: Qflow - Qf – Qc (heat flow through the reactor wall - calibration power), Qaccum - Qa + Qi (heat accumulation by the reaction mass and inserts), Qdos - heat input due to dosing. Power that is needed to bring the inflow from Tdos to Tr, Qloss - heat flows through the reactor head assembly (radiation, conduction), Qreflux - quantity of heat dissipated in the reflux condenser.

The evaluation cannot calculate each individual contribution to Qr separately if several chemical reactions/physical transformations occur at the same time. The calculation, eg of heats of mixing or heats of vaporization therefore requires particular experimental measures to separate the individual effects from one another. In general, it is advisable to make all the disturbing heat effects (eg due to stirring, evaporation or dispensing) as small as possible or keep them as constant as possible. Heat effects that remain constant during the reaction under investigation appear in the baseline of Qr and make no contribution to the integration of the reaction enthalpy. Usually, this applies to the contribution of Qstir, which is the reason why it is not specially specified in the basic equation.

Our calculations rest on calorimetric rules. The reaction enthalpy is determined by integrating the peak of the heat generation rate Qr. In the evolution we define the start and end point of possible reaction and its baseline (we use a pure water as baseline). Between the start and end pairs for the reaction limits, the integral is calculated as the area between Qr curve and the baseline Qb (Fig. 2).

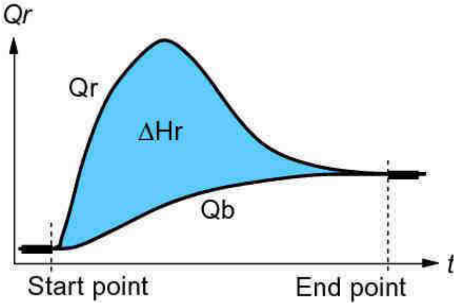

Fig. 2. Heat flow curves of possible reaction and its baseline

Overall heat generated during the process ΔH_r was estimated from the following equation:

$$\Delta H_r = \int_S^E (Q_r - Q_b) \cdot dt$$

where: ΔH_r - reaction enthalpy [J/mol], Q_r - heat generation rate, calculated from the sum of the selected heat flow terms [W], Q_b - baseline for Q_r [W].

As an area between recorded thermal curves Q_r and baseline Q_b.

Calibration

The first important aim was the calibration because of the possible process' disturbances. The calorimetric reactor had to be calibrated due to the extreme conditions of the reaction such as the diphase system (gas-liquid) and increased temperature and pressure. To avoid measurement mistakes and to determine the heat effects correctly it was necessary to calibrate the system.

A. Firstly, the value of U (*the heat transfer coefficient*) and A (*the heat exchange area*) was calculated. UA is the function of stirrer rotation velocity.
B. To avoid disturbances resulting from additional processes (adsorption and desorption heat, the heat losses from rotation's energy) it was necessary to create the baseline. That is why the same experiments for water (in the same conditions and by using the same parameters as in the main experiments) were carried out.

Results

Figure 3 shows the correlation between U, A and T (temperature), using different velocity of the stirrer rotation (R). The parameter UA depends on temperature and the velocity of stirrer rotation (R). If the velocity of stirrer rotation increases, UA increases too.

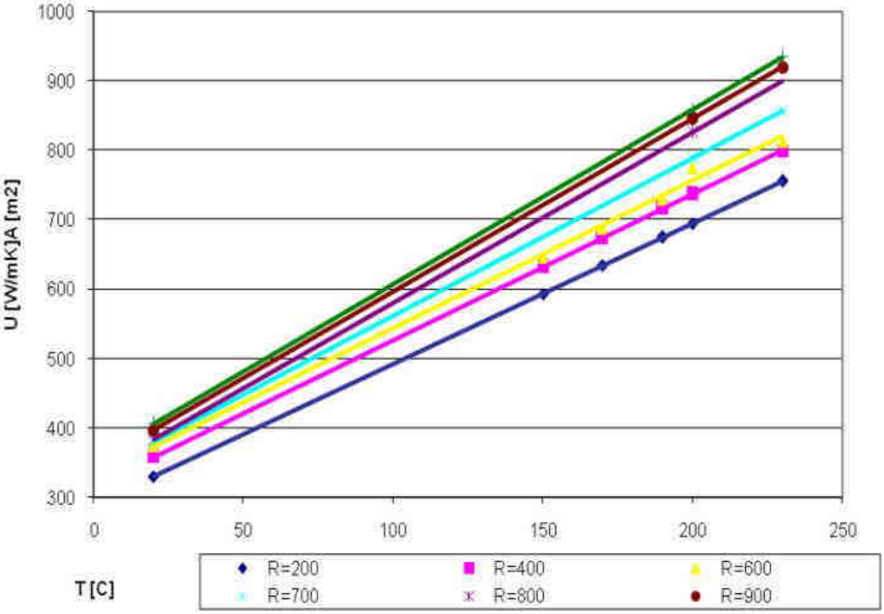

Fig. 3. The correlation between UA and T, using different velocity of the stirrer

Figure 4 shows the comparison of correlations between UA and R in minimum and maximum temperatures of experiments: 20 and 200°C.

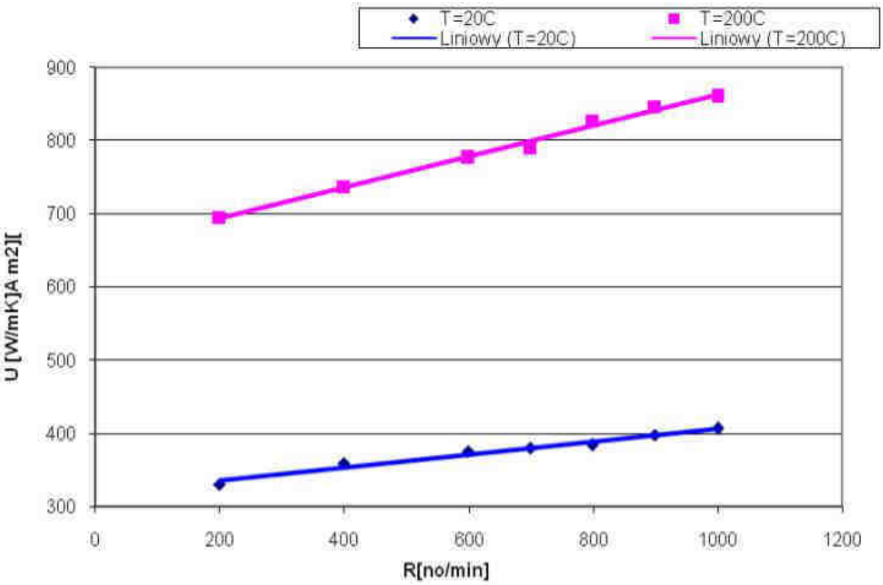

Fig. 4. The comparison of UA for different velocities of the stirrer in T = 20ºC and T = 200ºC

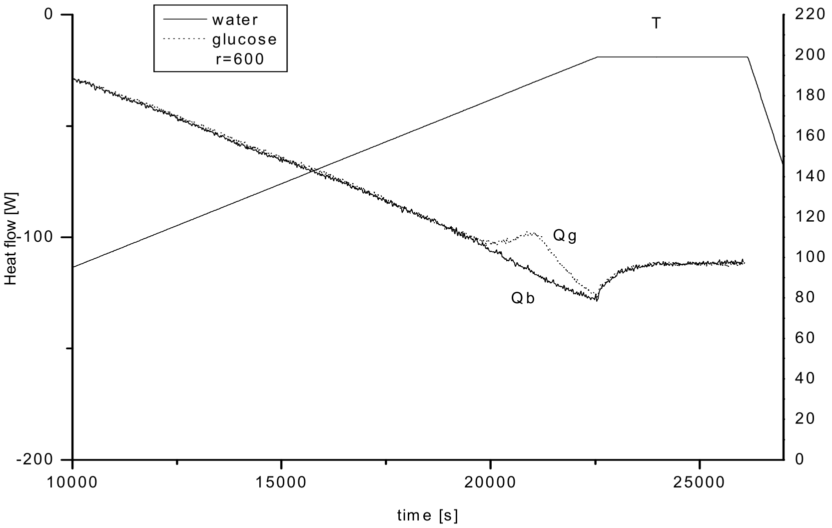

Fig. 5. Changes of water and glucose solution heat flow in RC-1 reactor (C_0 = 1600 ppm of glucose)

Figure 5 shows curves of water (baseline-Q_b) and glucose solution (Q_g) heat flow in time and temperature. We do not observe any reaction during the first 18000 s of the reaction. When the temperature reaches 200°C, we can see the area between q_g curve and the baseline curve (Q_b). It is the result of heat effects. The area between these curves is heat of glucose decomposition in wet oxidation reaction. We can calculate a decrease of *total organic carbon* (TOC) in glucose solution too. The area converted into decreasing TOC unit gives us minor heat of reaction.

Conclusions

This method applied for determination of heat effects in binary system (gas - liquid) at elevated temperature and pressure can be suitable for the research on chemical reactions' mechanism.

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OPRACOWANIE METODYKI POMIARU EFEKTÓW CIEPLNYCH UTLENIANIA ZWIĄZKÓW ORGANICZNYCH W ŚRODOWISKU WODY PODKRYTYCZNEJ

Wydział Inżynierii Procesowej i Ochrony Środowiska, Politechnika Łódzka

Abstrakt: Zaproponowana metodyka wyznaczania efektów cieplnych reakcji utleniania związków chemicznych może stać się dobrym narzędziem do badania mechanizmów ich przebiegu.

Słowa kluczowe: kalorymetria, mokre utlenianie, kalorymetria reakcyjna, utlenianie glukozy

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Kinga SKALSKA[1], Jacek S. MILLER[1] and Stanisław LEDAKOWICZ[1]

REACTIONS OF NITROGEN OXIDES AND OZONE IN THE GAS PHASE FOLLOWED BY ABSORPTION PROCESS

REAKCJE TLENKÓW AZOTU I OZONU W FAZIE GAZOWEJ ORAZ ABSORPCJA PRODUKTÓW REAKCJI

Abstract: The growing emissions of nitrogen oxides into atmosphere endanger the human health and degrade our environment. The presence of nitrogen oxides in the atmosphere is responsible for troposphere ozone and urban photochemical smog. NO and NO_2 (NO_x) are one of the major contributors to harmful acid rains. Various methods exist to reduce NO_x emission. Combustion modification and selective catalytic reduction (SCR) are probably the most widely used techniques to control NO_x emissions from industry. New technologies such as nonthermal plasma and pressure swing adsorption appear to be efficient for the removal of higher concentrations of NO_x but they are still expensive for the treatment of huge volumes of flue gases. Thus, there is a need for environmentally friendly and cost effective methods for treatment of flue gases. One of the solutions to NO_x emission problem can be to couple ozonation process in the gas phase with SCR or absorption process. In this work the application of ozone for the oxidation of nitrogen oxides present in exhaust gases was presented. The main concept of the process is to oxidize nitrogen oxide (NO) and nitrogen dioxide (NO_2) into nitrogen pentoxide (N_2O_5) which reacts with water giving nitric acid. This way NO_x can be absorbed in water with good effectiveness. In presented studies the influence of nitrogen(II) oxide initial concentration on the NO conversion was studied. Furthermore, the two stage process composed of the ozonation process of NO_x and the absorption of reaction gases in the 0.01 M solution of NaOH was performed. These experiments proved that the yield of absorption process is higher for gases containing N_2O_5.

Keywords: nitrogen oxides, emission control, ozonation

Nitrogen oxides are considered to be dangerous air pollutants. They contribute to the occurrence of several environmental problems, ie photochemical smog, acid rain, tropospheric ozone, ozone layer depletion and even greenhouse effect. NO_x (NO and NO_2) can lead to health problems in humans exposed to high doses of these gases. The wide range of NO_x control technologies, eg techniques such as absorption, adsorption or electric discharge were developed. However, all these methods have its limitations and drawbacks.

Therefore, attempts to develop new technologies of reduction of NO_x emission are still carried out all around the world. Most of them as literature survey suggests is focused on NO_x emission control from power plants and mobile vehicles [1]. One of the solutions of the NO_x emission problem can be to couple ozonation process in the gas phase with *selective catalytic reduction* (SCR) or absorption process. In 2001 the process called Low-Temperature NO_x absorption (LoTOx) won the Kirkpatrick Award. In this method the 90% efficiency of NO_x emission reduction can be obtained [2]. High amounts of ozone are needed in this application to obtain N_2O_5. Nitrogen pentoxide (N_2O_5) has high solubility in water around 500 $g \cdot dm^{-3}$ [3], which is significantly higher than solubility of nitric oxide or even nitrogen dioxide. The obtained N_2O_5 gives HNO_3 in the absorption column through reaction with water. The process can be described by sequence of reactions:

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[1] Faculty of Process and Environmental Engineering, Technical University of Lodz, ul. Wólczańska 213, 90-924 Łódź, phone 42 631 37 39, fax 42 631 37 38, email: kinga.skalska@wipos.p.lodz.pl

$$NO + O_3 \longrightarrow NO_2 + O_2 \quad (1)$$

$$NO_2 + O_3 \longrightarrow NO_3 + O_2 \quad (2)$$

$$NO_2 + NO_3 \longrightarrow N_2O_5 \quad (3)$$

$$N_2O_5 + H_2O \longrightarrow 2HNO_3 \quad (4)$$

The main objective of this paper is to describe the influence of NO initial concentration in reaction gases on the nitrogen oxides ozonation process. Furthermore, series of experiments were performed to confirm that the absorption of higher nitrogen oxides is more effective than absorption of NO_2.

Material and methods

Series of experiments were conducted for nitrogen oxides commonly occurring in flue gases from nitric acid plant. Various initial concentrations of nitric oxide ($1.5\div7.7\cdot10^{-5}$ mol dm^{-3}) were mixed with oxygen-ozone mixture under different mole ratios of O_3/NO : $0.5\div2.5$. The ozonation process was conducted in the temperature of 298 K (25°C) and the reaction gases flow rate equal to 1.5 dm^3 min^{-1} was applied. An overview of the experimental set-up was described in previous papers [4-6]. The second series of experiments were conducted as a two stage NO_x removal process for concentrations of NO_x presented in Table 1. The process consists of the ozonation part and the absorption part. The absorption process of reaction gases was conducted in the bubble reactor filled with 0.01 M solution of NaOH. The absorption of gases was conducted for 30 minutes. After the absorption of nitrogen oxides was finished the ozonation reactor was purged with nitrogen for 100 minutes. The nitrogen gases were also absorbed in the solution. The solution was analyzed for the NaOH loss.

Table 1

Specification of the experiments conducted in the two stage NO_x removal process with the initial substrates concentrations and the results obtained

	Experiment		
	1	**2**	**3**
Concentration of NO+NO_2 [mol dm^{-3}]	$2.09\cdot10^{-5}$	$2.11\cdot10^{-5}$	$2.2\cdot10^{-5}$
Mole ratio O_3/NO_x	1.2	2.3	2.3
Total selectivity of NO into N_2O_5 and HNO_3 [%]	23.6	61.2	58.1
NO_x absorption yield [%]	21.2	47.4	38.5

Results and discussion

The influence of initial NO concentration on the ozonation process was investigated (Fig. 1). The correlation between the initial NO concentration and the NO conversion can be observed. The increase of the NO concentration results in the increase of NO conversion. The NO conversion increases because higher initial concentration of NO influences the reaction rate. However, this can be observed only for the mole ratios of

O_3/NO lower than 1. Above this value the conversion of NO is almost complete and further reactions occur in the reaction space. Dora et al [3] also studied the influence of initial NO concentration on the NO conversion. In their experiments they applied constant ozone concentration and changed only the initial NO concentration. Therefore, it is difficult to directly compare their results with the ones obtained in our studies. Nevertheless the fact that Dora et al [3] obtained lower effectiveness of NO oxidation in spite of increasing mole ratio O_3/NO suggests that NO conversion is dependent on initial NO concentration.

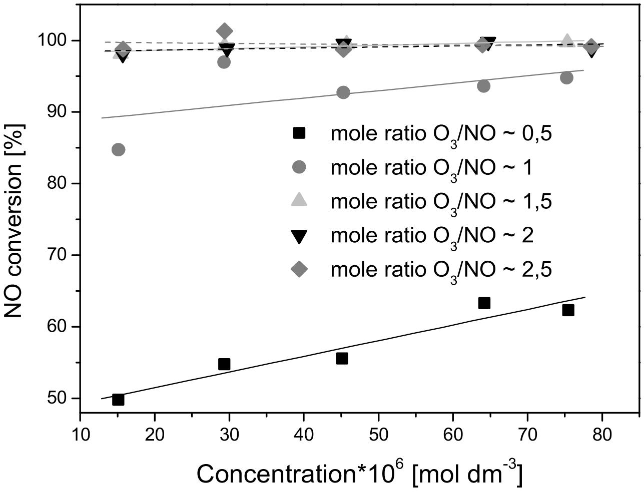

Fig. 1. The influence of initial NO concentration on the NO conversion

In order to elaborate the studies aimed at the development of nitrogen oxide removal technology additional experiments were conducted. The main objective of this series of experiments was to confirm that ozonation leads to better removal of higher nitrogen oxides from exhaust gases than NO_2 when the absorption process is applied. The experimental data are presented in Table 1. The selectivities of NO ozonation into N_2O_5 and HNO_3 were calculated according to:

$$S_{NOtoN_2O_5} = \frac{([N_2O_5]_t - [N_2O_5]_0)\cdot 2}{[NO]_0 - [NO]_t}\cdot 100\% \tag{5}$$

$$S_{NOtoHNO_3} \frac{[HNO_3]_t - [HNO_3]_0}{[NO]_0 - [NO]_t}\cdot 100\% \tag{6}$$

where: $[NO]_0$ - initial concentration of NO, $[NO]_t$ - final concentration of NO, $[N_2O_5]_0$ - initial concentration of N_2O_5, $[N_2O_5]_t$ - final concentration of N_2O_5, $[HNO_3]_0$ - initial concentration of HNO_3 and $[HNO_3]_t$ - final concentration of HNO_3.

Total selectivity of NO conversion into N_2O_5 and HNO_3 obtained during experiment 1 was around 24%, whereas the absorption yield was around 21%. This experiment was carried out with the mole ratio O_3/NO_x = 1.2. In this process large amount of NO is oxidized into NO_2 which is soluble in water. In experiments 2 and 3 the mole ratio $O_3/NO_x > 2$ was applied. Therefore, the observed total selectivity of NO conversion into N_2O_5 and HNO_3 was higher than in the case of experiment 1 and was equal to 61.2% and 58.1%, respectively. The absorption yields obtained during these two experiments were around 47 and 39%, respectively.

Conclusions

Results presented in this paper confirm that the combination of ozonation of flue gases with the absorption process is a promising method for control of NO_x emission from chemical plants. The NO conversion is dependent on the NO initial concentration only for processes carried out with the mole ratio $O_3/NO \leq 1$. Results of these studies confirmed that absorption yield is higher for gases ozonated with the mole ratio $O_3/NO_x > 2$ than for gases ozonated with mole ratio O_3/NO_x close to 1 when the main component of reaction gases is NO_2. Therefore, this method seems to be especially valuable for nitric acid production plants because nitrogen pentoxide forms nitric acid through reaction with water, thus efficiency of nitric acid production might be increased additionally to abatement of NO_x emission.

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REAKCJE TLENKÓW AZOTU I OZONU W FAZIE GAZOWEJ ORAZ ABSORPCJA PRODUKTÓW REAKCJI

Wydział Inżynierii Procesowej i Ochrony Środowiska, Politechnika Łódzka

Abstrakt: Rosnąca emisja tlenków azotu do atmosfery zagraża zdrowiu ludzi, a także przyczynia się do degradacji środowiska naturalnego. Obecność tlenków azotu w atmosferze może prowadzić do powstawania ozonu troposferycznego oraz smogu fotochemicznego. NO_x (NO oraz NO_2) są jednymi z głównych zanieczyszczeń przyczyniających się do występowania kwaśnych deszczów. Modyfikacje procesu spalania oraz *selektywna katalityczna redukcja* (SCR) są prawdopodobnie najczęściej stosowanymi metodami ograniczania emisji tlenków azotu ze źródeł przemysłowych. Nowe technologie, takie jak niskotemperaturowa plazma i adsorpcja zmiennociśnieniowa, wydają się być skuteczne przy usuwaniu wysokich stężeń NO_x, jednak są one nadal drogie w szczególności w przypadku oczyszczania dużych strumieni gazów odlotowych. Dlatego istnieje konieczność opracowania przyjaznych środowisku i tanich metod ograniczania emisji zanieczyszczeń do atmosfery. Jednym z rozwiązań może być zastosowanie technologii łączącej proces ozonowania w fazie gazowej z selektywną katalityczną redukcją (SCR) bądź procesem absorpcji. W niniejszej pracy przedstawiono wykorzystanie ozonu w procesie utleniania tlenków azotu obecnych w gazach odlotowych. Głównym założeniem jest utlenienie monotlenku azotu (NO) i ditlenku azotu (NO_2) do pentatlenku diazotu (N_2O_5), który ulega reakcji z wodą, dając kwas azotowy. W ten sposób NO_x mogą być absorbowane w wodzie z dobrą wydajnością. Zbadano wpływ początkowego stężenia NO oraz stosunku NO/NO_2 w gazach reakcyjnych na stopień konwersji NO oraz selektywności do produktów. Ponadto przeprowadzono eksperymenty w układzie dwustopniowym, składającym się z procesu ozonowania i absorpcji gazów reakcyjnych w 0,01 M roztworze NaOH. Otrzymane wyniki potwierdzają, że wydajność absorpcji jest wyższa w przypadku gazów zawierających N_2O_5.

Słowa kluczowe: tlenki azotu, kontrola emisji, ozonowanie

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Henryk SOBCZUK[1] and Grzegorz ŁAGÓD[1]

SOME DISTANCE MEASURES FOR ESTIMATING THE EFFICIENCY OF WASTEWATER TREATMENT IN ACTIVATED SLUDGE SYSTEMS BY BIOMONITORING

POMIARY ODLEGŁOŚCI I PODOBIEŃSTWA TAKSOCENOZ W ASPEKCIE BIOINDYKACJI SYSTEMÓW OCZYSZCZANIA ŚCIEKÓW OSADEM CZYNNYM

Abstract: The results of bioindication, together with the physical and chemical parameters, may be used in wastewater treatment plant process management. The measurement of saprobiontic microorganisms' population is a relatively easy and cheap method for assessment of wastewater quality in sewage system. It can be also applied at the inflow of the wastewater treatment plant, at the following devices of technological line, as well as at outflow to receiver. Some methods allow to distinguish between distributions of saprobes population on the basis of species abundances. Splitting of a whole population of saprobes onto morphological-functional groups allows to simplify measurement and thus allow to make it *in situ*. The main idea of this type of measurement is to study the biocenosis entire structure. Because it is hardly possible, the approximately identified taksocenosis may be utilized for the needs of bioindication. An open question is how to compare the communities with different abundance of saprobes group. However using the different methods of biodiversity assessment the more or less visible differences among studied objects should be expected. In this paper the authors apply some existing distance measures based on entropy definitions to distinguish distributions of morphological-functional groups measured. The presented measures allow to tell how far - in terms of given measure of distance - are two distributions measured *in situ*, and differentiate them.

Keywords: bioindication, saprobes community, entropy measure

The results of bioindication, apart from the other physical and chemical parameters, may be used in wastewater treatment plant process management. Using the different methods of biodiversity assessment the more or less visible differences among studied objects should be expected [1-3]. The measurement of saprobiontic microorganisms' population is a relatively easy and cheap method for assessment of wastewater quality in sewer system, and can be applied at the inflow to the wastewater treatment plant as well as at outflow [1, 3, 4]. The main idea of this type of measurement is to study the biocenosis entire structure. Because it is hardly possible, the approximately identified taksocenosis may be utilized for the needs of bioindication.

The method described in paper prepared by Lagod et al [2] allows to distinguish between distributions of saprobes population on the basis of group of species abundances. Basically this method allows to draw some conclusions about the current status of wastewater but also on the history of the sewage quality change. If there is an event of microbiologically active pollutant discharge that seriously influences microbial activity at certain moment, the saprobe microorganism population will tend afterwards to the equilibrium state for a certain time. In order to discover such pollutants discharge in the past, the microorganisms distribution measurement and a comparison tool for different distributions are required.

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[1] Faculty of Environmental Engineering, Lublin University of Technology, ul. Nadbystrzycka 40B, 20-618 Lublin, phone 81 538 44 81, email: H.Sobczuk@wis.pol.lublin.pl

The partitioning of a whole community onto morphological-functional groups allows to simplify measurement thus allow to make it even *in situ*. Trained employee can do it basically with an optical microscope [5]. An open question is how to compare populations with different abundance of group of species. The significance of differences amongst measurement results may be determined according to the popular statistical procedures, eg T-Student for distribution comparison. These tests require the specific strategy of the experiment - passive or active, determination of required number of samples for each studied level, rejection of doubtful results and the other actions standard for this type of tests. In case of WWTP (*Wastewater Treatment Plant*) sub-biocenosis object structures measurement, being in principle one of a kind, the single measurement result occurs. Thus, the use of usually applied mathematical tests is difficult.

The idea of entropy was introduced because many measures of this phenomenon may be applied to describe the differences among the described structures. The models similar to the one used in information theory [6] or likewise the others models describing the measure of entropy [7-10] may be useful in quantitative descriptions of different aggregations including sub-biocenosis such as populations of microorganisms settled inside WWTP devices.

The aim of this paper is to select, among the available descriptions of communities diversification, the one which would distinguish the WWTP sub-biocenosis communities the best.

Methods

Used method relies on the application of several available manners of taksocenosis diversification measure to the presented material. In this paper the authors apply some existing distance measures based on entropy definitions to distinguish between distributions of morphological-functional groups measured. Presented measures allow to tell how far - in terms of given measure of distance - are two distributions measured *in situ*, and differentiate them.

Each distance measure between two objects should fulfill three conditions: it should be positively defined ie the distance must be positive for the different objects and equal to zero for the identical objects; it should be symmetrical ie the distance from object A to B should be equal the distance from object B to A; and it should fulfill the triangle inequality. One of the simplest definitions of a distance function is an Euclidean distance function.

For the assessment of distance amongst random objects, like measured distributions of abundance of morphological-functional groups, the entropy-based measures are often applied. These measures usually lack of positive definition.

In mathematical theory of communication [6] there is a general entropy function of order α introduced in a form [8]:

$$H_\alpha = (1-\alpha)^{-1}\ln\sum_i p_i^\alpha \tag{1}$$

for α = 1 this formula reduces to well known Shanon formula:

$$H = -\sum_i p_i \ln p_i \tag{2}$$

where for α = 2 it leads to another metric applied for biological diversity assessment [11]:

$$H = -\ln\sum_i p_i^2 \tag{3}$$

In [8] a mathematical background of application of entropy measures connected with generalized replicator equation was given. We will apply presented theory concerning state of the genetic system to the higher level - state of saprobes population on the basis of the abundances of species groups:

$$\dot{p}_i = h(p)f_i(p_i)\left\{\sum_{j=1}^{n} w_{ij}f_j(p_j) - \theta^{-1}(p)\sum_{j,k=1}^{n} w_{kj}f_k(p_k)f_j(p_j)\right\},\ i = 1,\ldots,n \tag{4}$$

where: $\dot{p}_i$ is a time derivative of probability p_i, $h(p)$ is the function determined by application, $f_i(p_i)$ are response functions with positive slope and starting from zero, $W = (w_{ij})$ is the matrix of interactions of microbial groups under consideration, and:

$$\theta(p) = \sum_{j=1}^{n} f_j(p_j) \tag{5}$$

The system of equations (4) describes the dynamics of the population. As shown in [8] on the basis of the equation (4) there is a possibility to calculate a response function for existing entropy measure and construct an entropy and distance function from response functions. The distance function generated in this way usually does not fulfill all the three requirements for distance function, for population comparison can be however applied.

As an example one can show that linear response function $f_i(p_i) = p_i$ generates distance function of the form:

$$H_{\hat{p}} = \sum \hat{p}_i \ln\left(\frac{p_i}{\hat{p}_i}\right) \tag{6}$$

which is equivalent to relative entropy, whereas logistic response function:

$$f_i(p_i) = 1/\left(b + ce^{-\alpha p_i}\right) \tag{7}$$

causes weighted logistic entropy:

$$H_{\hat{p}} = \sum \hat{f}_i \ln\left(\frac{1 - e^{-\alpha p_i}}{1 - e^{-\alpha \hat{p}_i}}\right) \tag{8}$$

to be an appropriate measure of distance. In this paper we will compare some measures of the distance among measured abundances of microorganisms, and also the distance given by the Euclidean norm.

Results and discussion

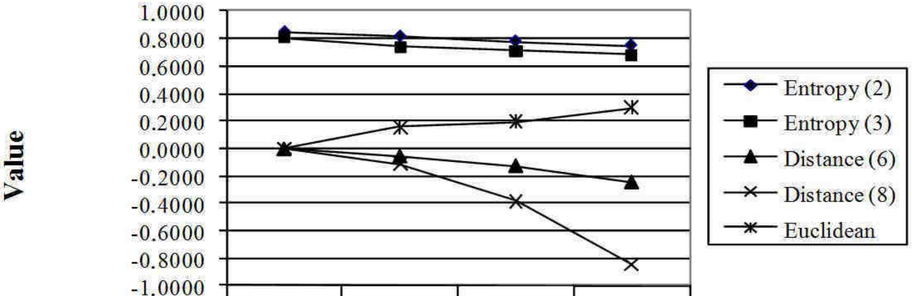
In the calculation we apply data set taken from publication [12], also analyzed in our previous papers [1, 2, 13]. The most important data are presented in Table 1 and used to calculate values of entropy according to equation (2) and (3) and distances to the first data set according to equations (6) and (8).

Table 1

Species numbers and species abundances in specified classes of wastewater purity [1, 2]

BOD$_5$ **organisms**	**0÷10 g O_2/m^3**	**11÷20 g O_2/m^3**	**21÷30 g O_2/m^3**	**> 30 g O_2/m^3**
swimming ciliates	27	25	18	12
	1969	**3712**	**1477**	**1392**
attached ciliates	35	29	26	18
	5862	**5284**	**5198**	**1606**
crawling ciliates	14	14	12	7
	1449	**1535**	**1263**	**1142**
all ciliates	76	68	56	37
	9280	**10531**	**7938**	**4140**
rotifers	45	40	35	24
	6354	**2318**	**503**	**962**
flagellates	13	15	19	26
	4785	**4199**	**4463**	**6399**
amoebas	14	14	14	7
	3914	**3507**	**4385**	**4850**
others	8	9	1	1
	4214	**1328**	**1100**	**50**
all	152	146	125	95
	28547	**21883**	**18389**	**16401**

In rows: S - species richness (number of species), $\mathbf{n_T}$ - total number of individuals in cm^3 of wastewater (after multiplication by 10^3 in dm^3), others - the rest of organisms representing *Metazoa:* nematodes, oligochaetes, tardigrades, gastrotriches, arachnids, copepods, cladocers, turbellarians



	1	2	3	4
◆ Entropy (2)	0.8481	0.8158	0.7803	0.7502
■ Entropy (3)	0.8077	0.7381	0.7123	0.6790
▲ Distance (6)	0.0000	-0.0527	-0.1248	-0.2464
× Distance (8)	0.0000	-0.1114	-0.3863	-0.8477
✱ Euclidean	0.0000	0.1571	0.1947	0.2945

Fig. 1. Comparison of different distance measures for data distributions

Results are presented in Figure 1. It is clearly visible that distance measures presented here much more differentiate the data set than entropy measure. As such, the presented models can be used more effectively to parameterize measurement data distributions than entropy measures. Due to the possibility to generate measures according to the functional form of the response function in (4) one can find the best distance measure for the certain purpose. For the presented data set the measure (8) is differentiating data sets best of all applied methods.

Conclusion

Based on the calculation presented here the following conclusions are offered:

- As a result of the presented method the data sets used in our studies were successfully distinguished, as it was shown at Figure 1.
- Evidently, the most significant differences in microorganisms communities are visible when model described by equation (8) was applied.
- Entropy and distance measures should be appropriately chosen for complex data sets comparison. Equation (4) with accompanying theory allows to generate distance measures according to the data set character. It shows up that the distance measure (8) is best fitted to recognize differences in microbial distribution functions described here.
- In the situation of the necessity of subtle differences in compared taksocenosis structures perception, basing on the distance determination method, the most suitable method seems to be the model presented in equation (8).

Acknowledgement

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POMIARY ODLEGŁOŚCI I PODOBIEŃSTWA TAKSOCENOZ W ASPEKCIE BIOINDYKACJI SYSTEMÓW OCZYSZCZANIA ŚCIEKÓW OSADEM CZYNNYM

Wydział Inżynierii Środowiska, Politechnika Lubelska

Abstrakt: Rezultaty pomiarów bioindykacyjnych mogą stanowić przydatne uzupełnienie, a niekiedy nawet alternatywę dla pomiarów fizykochemicznych wykonywanych w celach kontroli i sterowania procesami oczyszczania ścieków. Pomiary liczebności odpowiednio dobranych populacji organizmów saprobiontycznych zasiedlających poszczególne urządzenia oczyszczalni ścieków mogą być relatywnie proste i tanie. Stąd też wykorzystujące je metody pomiarowe są możliwe do zastosowania zarówno do oceny jakości ścieków dopływających do oczyszczalni, oczyszczanych w kolejnych urządzeniach ciągu technologicznego, jak i odprowadzanych po oczyszczeniu do odbiornika. Niektóre ze wspomnianych metod pozwalają oceniać kompozycję zbiorowisk saprobów na podstawie liczebności poszczególnych gatunków. Jednakże podział całej populacji saprobiontów na grupy funkcjonalno-morfologiczne umożliwia znaczne uproszczenie pomiarów oraz wykonywanie ich *in situ*. Ze względu na fakt, iż pomiar struktury całej biocenozy saprobów jest trudny do realizacji, identyfikacja wybranych taksocenoz może być wykorzystana do celów bioindykacyjnych. Ciągle otwartą kwestią pozostaje, jak najlepiej porównywać pomiędzy sobą zbiorowiska saprobów złożone z grup o różnej liczebności. Używając istniejących metod oceny bioróżnorodności, uzyskać można wartości liczbowe indeksów i wskaźników mniej lub bardziej różnicujące porównywane między sobą zgrupowania organizmów. W niniejszym opracowaniu autorzy wykorzystują sposoby opisu odległości korzystające z definicji entropii w celu jak najlepszego zróżnicowania zbiorowisk, złożonych z grup morfologiczno-funkcjonalnych, do których sklasyfikowano organizmy bytujące w ściekach o różnym stopniu zanieczyszczenia. Prezentowane procedury pozwalają określić, jak daleko - w kategoriach przyjętych miar odległości - znajdują się od siebie rozkłady analizowanych zbiorów i odpowiednio je zróżnicować.

Słowa kluczowe: bioindykacja, zbiorowiska saprobów, pomiary entropii

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Elżbieta SROKA[1] and Joanna PODLASIŃSKA[2]

CONTENT OF HEAVY METALS IN BOTTOM SEDIMENTS OF BINOWSKIE LAKE

ZAWARTOŚĆ METALI CIĘŻKICH W OSADACH DENNYCH JEZIORA BINOWSKIEGO

Abstract: The studies of chemical composition of bottom sediments of Binowskie Lake (located within area of protection zone of Szczecin Landscape park "Fagus Forest") was carried out in 2003 year. The contents of Cr, Zn, Co, Mn, Cu, Ni, Pb, Hg and Fe were examined. Sediments samples from 4 stands have been taken at spring, summer and autumn seasons. Obtained results of heavy metals concentrations do not exceeded permitted values. Bottom sediments of examined lake can be classified as low contaminated.

Keywords: Binowskie Lake, bottom sediments, heavy metals

Water sediments are integrated part of water environment. Their composition and properties have been formed as a result of long-term physical, chemical and biological processes occurring at reservoir itself as well as within its catchment. Sediments are accumulation medium of pollutants occurring in water environment, mainly biogenic compounds, organic matter as well as specific pollutants like heavy metals. Bottom sediments play important role in water systems, both as reservoirs in which pollutants can be deposited and as well as sources for water organisms pollution [1-3].

That is why the chemical composition knowledge, especially the total content of heavy metals in surface layers of bottom sediments, is an essential condition for water ecosystems biogeochemical balance prognosis [4, 5].

Water sediments of Binowskie Lake have been taken and investigated in 2003 year. Binowskie Lake is a postglacial, interior water region reservoir located within area of protection zone of Szczecin Landscape park "Fagus Forest". It belongs to catchments of Plonia-Odra rivers [6]. Water surface of investigated lake has 53 ha, and its average depth is 5.5 m. Formerly this lake had ideal clean water and was a habitat of very rare plant species, big amount of fish and crayfishes. On the lake bottom *Charetea* vast meadows have been formed. This is the most beautiful object of this type in Poland. At present due to biogenic compounds flow from Binowo town progressive eutrophication can be observed [7, 8].

The main aim of our study was determination of heavy metals (Cr, Zn, Co, Mn, Cu, Ni, Pb, Hg, Fe) content in bottom sediments of studied reservoir. These were the first studies of heavy metals content in sediments of Binowskie Lake, so far this type of analysis have not been provided.

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[1] Department of Marine Ecology and Environmental Protection, West Pomeranian University of Technology, ul. K. Królewicza 4D, 71-550 Szczecin, phone 91 449 66 15, email: elzbieta.sroka@zut.edu.pl

[2] Department of Environmental Protection and Management, West Pomeranian University of Technology, ul. J. Słowackiego 17, 71-434 Szczecin, phone 91 449 63 67, email: joanna.podlasinska@zut.edu.pl

Material and methods

Sediments samples have been taken at spring (30.05.2003), summer (08.10.2003) and autumn (03.12.2003) seasons from 4 sites located at following altitudes and longitudes: 1 - N 53°18.423'; E 14°37.758', 2 - N 53°18.509; E 14°38.266', 3 - N 53°18.767'; E 14°38.180', 4 - N 53°18.560'; E 14°38.620' (Fig. 1). Samples have been taken by van Veen grab with buck surface of 0.06 m^2. After drying in air condition samples were sieved by 1 mm sieve and this fraction were used for further analyses. Bottom sediments samples were digested in nitric(V) and chloric(VII) (perchloric) concentrated acids at MILESTONE microwave stone. Content of Cr, Zn, Co, Cu, Mn, Ni, Pb and Fe in mineralized sediment samples were determined using the AAS method on Solaar 929 apparatus. Total mercury content was determined by using of AMA 254 apparatus.

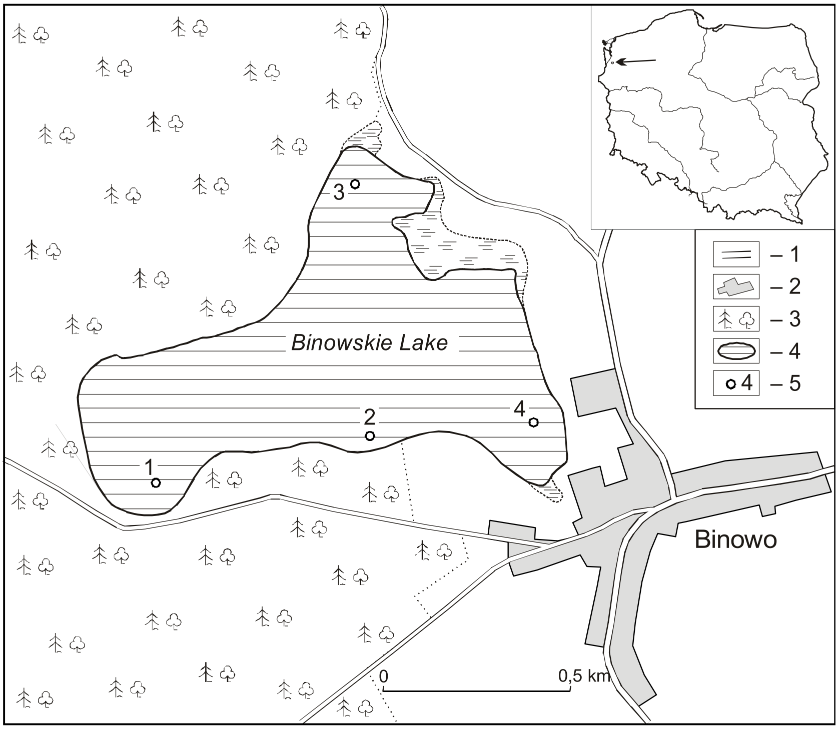

Fig. 1. Location of sampling sites on Binowskie Lake. Explanation: 1 - roads, 2 - urban areas, 3 - forests, 4 - lakes, 5 - sampling sites

Results and disscussion

Obtained results indicate on spatial and seasonal differentiation in heavy metals content. At the 1st and 3rd sites heavy metals concentrations were higher than those at the 2nd and 4th site (Fig. 2). These sites differed between themselves by substratum - on the 1st and 3rd sites sediments were organic origin and on the 2nd and 4th - mineral ones.

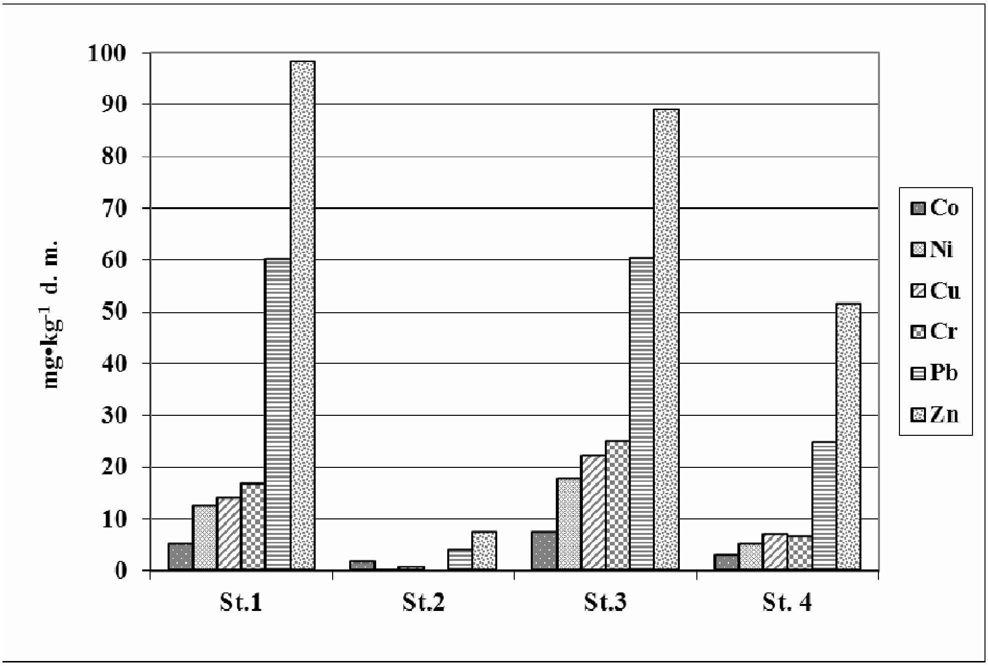

Fig. 2. Average concentration of selected heavy metals in bottom sediments of Binowskie Lake

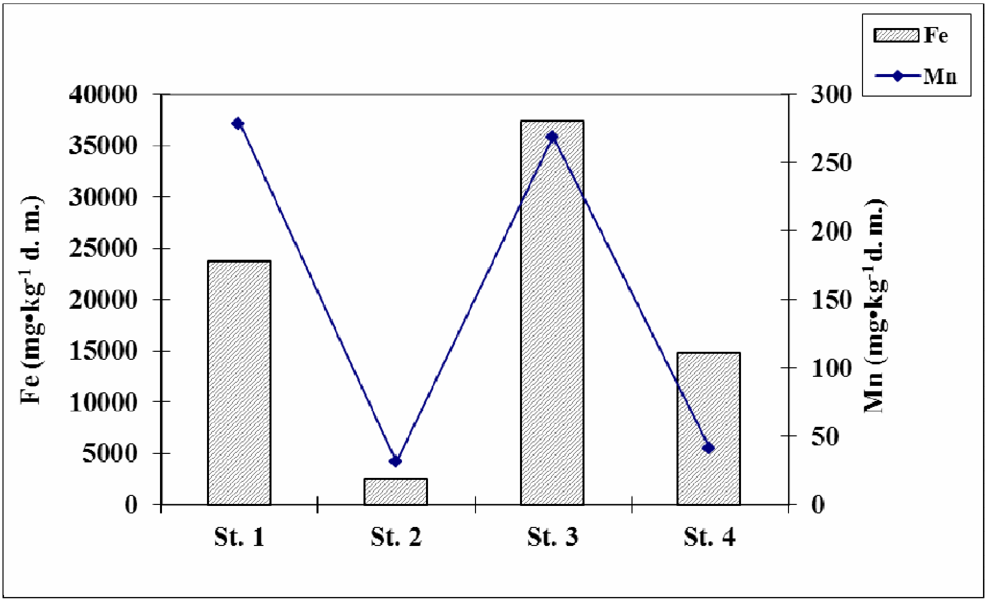

Fig. 3. Average concentration of Fe and Mn in bottom sediments of Binowskie Lake

Iron and manganese dominated among investigated elements (Fig. 3). Iron content was between 2442 and 41 000 mg·kg^{-1} d.m. and manganese from 24.84 to 321 mg·kg^{-1} d.m. Contents of zinc, lead, chromium, copper, nickel, cobalt and mercury were much lower, respectively: Zn 5.55÷133.35 mg·kg^{-1} d.m., Pb 2.85÷76.02 mg·kg^{-1} d.m., Cr <0.01÷26.30 mg·kg^{-1} d.m., Cu 0.01÷25.33 mg·kg^{-1} d.m., Ni 0.15÷18.42 mg·kg^{-1} d.m., Co 0.37÷7.95 mg·kg^{-1} d.m. and Hg 0.0072÷0.2155 mg·kg^{-1} d.m.

Contents of investigated elements in bottom sediments of Binowskie Lake at diminishing sequence were as follows: Fe > Mn > Zn > Pb > Cr > Cu > Ni > Co > Hg.

Heavy metal concentration did not exceed the geochemical background for most taken samples. In Poland there is no uniform classification system for bottom sediments like valid classes of selected pollutants content as it is in case of sewage sludge [9]. That is why obtained results were compared with decree of Minister of the Environment (from 9 September 2002 concerning soil quality standards and ground quality standards) [10], to the most frequent elements content in sediment rocks according to Kabata-Pendias and Pendias [11] as well as to geochemical classes of water sediments worked out by PIG [12].

Comparing obtained results to values given at decree of Minister of the Environment from 9th September 2002, concentrations of chromium, cobalt, copper and mercury were below permissible concentration level. But concentrations of zinc (at sites 1st - taken in spring and autumn time, as well as 3rd and 4th taken in summer) and lead (1st site during all investigation period; 3rd taken in summer and autumn and 4th - taken in summer) were higher than permissible concentration level [10].

According to values given by Kabata-Pendias and Pendias [11] results obtained in our studies indicated that concentration of chromium, zinc, copper, nickel and mercury were below upper level of these elements natural concentration in sediment rocks. Obtained results of zinc content at all investigated sites indicated to considerable higher than natural concentration. In case of iron and lead content - the exceed of natural concentration level were observed, respectively during the summer at 4th site for Fe and 1st and 2nd site during the whole study period and 4th site in summer [11].

According to classification of water sediments worked out by PIG investigated sediments belong to I class - non polluted by chromium, zinc, copper, nickel and mercury, and to second class - mediocre polluted by lead [12].

Comparing obtained in our investigations results of heavy metals concentration from Binowskie Lake to results of these metals content at other lakes from Poland (Goreckie and Swarzedzkie Lakes near Poznan, Sumin Lake on Leczynsko-Wlodawskie Lakeland) no significant differences were found [2, 13, 14].

The concentrations of heavy metals in bottom sediments from Binowskie Lake were compared with these metals concentrations in bottom sediments of other lakes in West Pomerania province (investigated by the Regional Inspectorate of Environment Protection) as well as with sediments from lakes of Waleckie Lakeland and Drawska Plain studied by Cieslewicz and Rozanski [13, 14]. The obtained results of zinc, copper, lead and mercury were similar to the concentrations of these metals listed by WIOS, but were generally lower comparind with data obtained from sediments of lakes located at Waleckie Lakeland and Drawska Plain. Higher concentrations of Ni, Cr, and Co were similar in all compared lakes sediments.

Concentrations of zinc in bottom sediments of Binowskie Lake could be elevated due to surface runoff (corrosion of galvanized roofing sheets and car bodies), and hard coal burning in households [15].

However lead can get into surface waters with suspensions, created as a result of erosion. Other sources of lead occurrence in sediments in higher concentrations may also be local communication and domestic waste. Especially, the domestic wastes were introduced in recent years to the lake by the surrounding Binowo Resorts [4].

Conclusions

The most important factor which differentiated quantity of heavy metals concentration in bottom sediments of Binowskie Lake was the share of individual granulometrical fraction as well as organic matter content. The highest contents of examined elements occurred in organic sediments, which have strong sorption property.

Seasonal differentiation in heavy metals content were cause by natural and anthropogenic factors.

Content of majority examined heavy metals was on the level of none-polluted water reservoirs in Poland.

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ZAWARTOŚĆ METALI CIĘŻKICH W OSADACH DENNYCH JEZIORA BINOWSKIEGO

[1] Zakład Ekologii Wód i Ochrony Środowiska
[2] Katedra Ochrony i Kształtowania Środowiska
Zachodniopomorski Uniwersytet Technologiczny w Szczecinie

Abstrakt: W 2003 r. zbadano skład osadów dennych z Jeziora Binowskiego, położonego w otulinie Szczecińskiego Parku Krajobrazowego „Puszcza Bukowa", oznaczając zawartość metali ciężkich (Cr, Zn, Co, Mn, Cu, Ni, Pb, Hg oraz Fe). Materiał do badań pozyskano z 4 stanowisk w sezonach wiosennym, letnim i jesiennym. Wyniki badań wykazały, że stężenia większości metali ciężkich w osadach Jeziora Binowskiego odpowiadają poziomowi stężeń występujących w niezanieczyszczonych zbiornikach wodnych w Polsce.

Słowa kluczowe: Jezioro Binowskie, osady denne, metale ciężkie

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Zbigniew SUCHORAB[1], Henryk SOBCZUK[1] and Zbyšek PAVLIK[2]

EXAMINATION OF CAPILLARY RISE PHENOMENON IN AERATED CONCRETE BLOCK USING THE SURFACE TDR PROBE

BADANIE ZJAWISKA PODCIĄGANIA KAPILARNEGO W BLOCZKU Z BETONU KOMÓRKOWEGO Z ZASTOSOWANIEM POWIERZCHNIOWEJ SONDY TDR

Abstract: Aerated concrete is the basic building material applied in traditional building industry. It is mainly caused by its thermal parameters - heat conductivity coefficient λ for lighter brands of this material is many times lower than other traditional materials like brick etc. Low value of heat conductivity coefficient is mainly caused by the material structure which is highly porous. This porosity causes capillary forces which are the reason of so called capillary rise phenomenon being the reason of many buildings destruction. This article presents the possibility of monitoring and quantitative valuation of moisture increase in building barriers due to capillary rise with the application of the TDR surface probes enabling quick and noninvasive moisture determination in porous building materials. The analyses conducted using surface TDR probes will be compared with the examinations made using other electrical methods and the results will be presented in the form of moisture profiles changing in time.

Keywords: capillary rise, aerated concrete, surface probes TDR

Aerated concrete is the one of the most popular building materials in the Polish market [1]. It is the artificial material with specific cellular structure which was intentionally designed to obtain suitable bearing and thermal parameters. The structure of aerated concrete consists of the air gaps called pores which can differ in volume and shape within a single sample of the material [2].

With its porous structure the aerated concrete is a very good building material. Especially its thermal properties make it a particularly interesting option from the point of view of ecology and Environmental Engineering. Many literature sources [3-5] and normatives (PN-EN ISO 6946) inform that the value of thermal conductivity λ of aerated concrete varies between 0.1 and 0.25 W/mK depending on apparent density and moisture which makes it even 10 times better than the other materials like red ceramic brick or stones.

It should be also underlined that many of its production technologies utilize ashes from power plants [6] which satisfies the idea of Sustainable Building [7] in two most important fields:

- building materials production,
- building exploitation.

On the other hand, this complicated porous structure of aerated concrete strongly influences its water parameters because the empty pores attract water molecules to fill the

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[1] Faculty of Environmental Engineering, Lublin University of Technology, ul. Nadbystrzycka 40B, 20-618 Lublin, phone 81 538 43 22, email: Z.Suchorab@wis.pol.lublin.pl, H.Sobczuk@wis.pol.lublin.pl

[2] Faculty of Civil Engineering, Czech Technical University, Thákurova 7, 166 29 Praha, Czech Republic phone +420 22 435 43 71, email: pavlikz@fsv.cvut.cz

air gaps. This phenomenon called capillary rise is a serious reason of many objects destruction. Porous building materials like aerated concrete absorb water from the ground, waterfalls, floodwater or sanitary installations fails in the common phenomenon called capillary rise.

All above-mentioned parameters and problems connected with the aerated concrete are the reason to develop a handy method of water presence detection which could enable quantitative moisture determination in building barriers. Determination of moisture and its changes monitoring in particular periods may enable easier finding of the problem source and may give the indications how to remove the problem.

Traditionally used direct, gravimetric methods, despite their accuracy are not perspective in this branch because they are invasive, require sampling and, what is perhaps the biggest problem, are significantly time-consuming. That is why, especially for monitoring and *in-situ* measurements, they are successfully being replaced by the indirect methods which offer less accuracy, but otherwise provide quick results and in some cases give the possibility of constant monitoring of moisture changes phenomena.

Among the indirect methods two electric ones are presented in this paper. They measure one of the the electric parameters of the material which is dependent on water content - dielectric permittivity. Dielectric permittivity of the moist material can be measured in *Frequency Domain* (FD) and *Time Domain* (TD) methods. FD method [8] is a capacitance method, which relies on the determination of condenser capacity. This method enables quick moisture determination with simple, user friendly mobile devices - Figure 1a which are noninvasive and do not require samples preparations. Among the Time Domain methods the most popular is the TDR (*Time Domain Reflectometry*) method which was successfully used for moisture determination of the soils [9-11] and building materials [12-17]. Until now, application of the TDR method with building materials was problematic due to the necessity of probes internal installation which was only possible to apply in laboratory conditions and rather for soft building materials [18, 19].

Since several years the surface TDR probe is developed which seems to be perspective for noninvasive moisture determination in building materials. The idea and parameters of the presented surface TDR probe are presented in the following papers [20-23]. Figure 1b presents the example of the surface TDR probe manufactured at Lublin University of Technology.

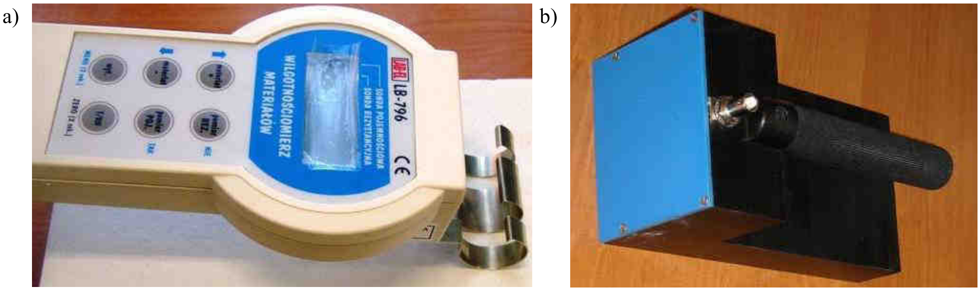

Fig. 1. Probes applied in described experiment: a) capacitance moisture probe, b) surface TDR probe

In this article we present the possibility of monitoring of capillary rise phenomenon using the capacitance and surface TDR probes in blocks of aerated concrete.

Materials and methods

The experimental setup consisted of:

- TDR surface probe presented in Figure 1,
- TDR Soil Multimeter (Easy Test),
- PC computer controlling the TDR device,
- LB-796 capacitance moisture meter (Label, Figure 1),
- aerated concrete sample (SOLBET Lubartow).

As a sample we used the block of aerated concrete produced by the local manufacturer SOLBET Lubartow. The sample dimensions were the following: 240×240×240 mm and its density in dry was about 700 kg/m^3. First the sample was dried in 105°C and put into the water container. The bottom of the sample was one centimeter under water level which was kept constant by the specially prepared device. The reference points were signed every 5 centimetres above water level and during the experiment they shown the measuring points (5, 10, 15 and 20 cm).

The measurements were conducted manually in period of 16 days with the irregular time steps (in most cases three measuring series per day).

Results

The experimental results are presented in Figures 2 and 3 in the form of the process diagrams at the particular heights. Figure 2 presents readouts obtained with capacitance probe and Figure 3 shows the readouts obtained with TDR device. Water content increase at each altitude is expressed by increased value at the ordinate axis.

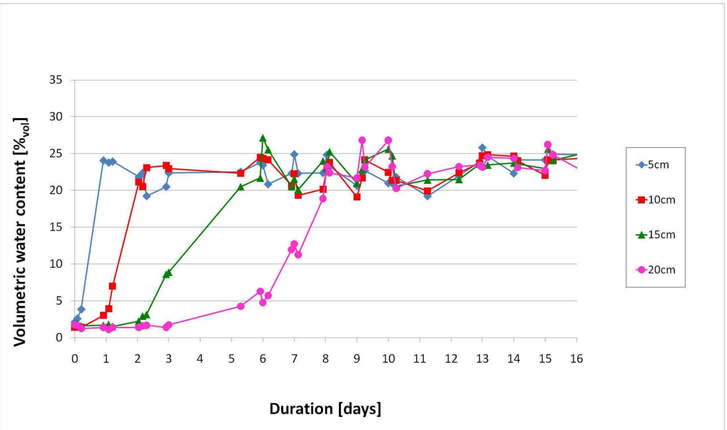

Fig. 2. Moisture changes in aerated concrete determined using capacitance method

The experiment confirms the phenomenon of capillary rise in aerated concrete and enables quantitative determination of its progress. In both diagrams the initial value of moisture is equal 0 which is the result of drying during sample preparations. Both probes show quick moisture increase at 5 cm reference point, the slope of the increase is very steep which proves high capillary forces of the material. After one day of the experiment it reaches the maximum water content value. At higher altitudes moisture increase is shifted in time but also very quick. At 10 cm the first water presence is noticed after one day of the experiment but from that moment it increases quickly and after the next day it reaches the maximal value possible for this material. The rate of water content increase at 15 cm is slower than before. The first readouts are observed after 3 days of the process and the maximal value is noticed about 4 days later. Fourth, the highest reference point, placed at the height of 20 cm shows water presence after the period of 6 days and then it gently rises to reach high values after the next 4 days but its maximum value was noticed at the end of the whole presented experiment.

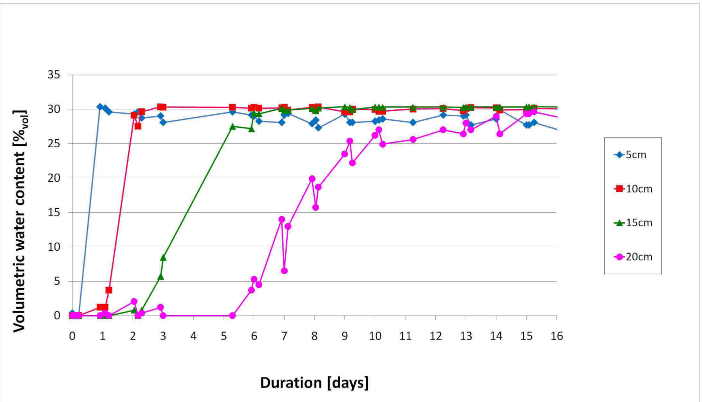

Fig. 3. Moisture changes in aerated concrete sample determined using surface TDR probe

Both experiments show very similar process progress. The major noticed differences are the following:

- Maximum water content read by the capacitance probe was less than TDR surface probe.
- In high water states moisture readouts by the capacitance probe were less stable than TDR readouts.
- In low water content and intermediate states the process readouts determined with capacitance and TDR probe were comparable.

Conclusions

- The experiment confirms the potential of TDR technique for moisture processes monitoring in building materials. Application of the surface TDR probes enables quantitative determination of moisture changes in the material profile without the necessity of invasive probes installation which may be successfully used in *in situ* experiments.

- Comparing with the traditional invasive TDR experiments of capillary rise, surface TDR probes show greater results dispersion which is compensated by the simplicity of application and no drilling is required.
- The reasons of this increased dispersion are the smaller resolution of surface probes and the increased influence of other factors connected with permanent probe repositioning. In case of traditional TDR measurements the probes are buried inside the material. In the described experiment the probes position was permanently moved from one to other reference point which could increase the readouts dispersion.
- Capacitance probe readouts showed underestimated values of maximum water content. Also the readouts dispersion was greater than in presented TDR measurements. This can be explained by the salinity influence on capacitance measurement - especially in high water content states.

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BADANIE ZJAWISKA PODCIĄGANIA KAPILARNEGO W BLOCZKU Z BETONU KOMÓRKOWEGO Z ZASTOSOWANIEM POWIERZCHNIOWEJ SONDY TDR

[1] Wydział Inżynierii Środowiska, Politechnika Lubelska
[2] Wydział Inżynierii Budowlanej, Czeski Uniwersytet Techniczny, Praga, Czechy

Abstrakt: Beton komórkowy jest podstawowym materiałem budowlanym stosowanym w budownictwie tradycyjnym. Wynika to głównie z jego właściwości termoizolacyjnych - współczynnik przewodzenia ciepła λ dla lżejszych jego odmian jest wielokrotnie niższy od wartości tego współczynnika takich materiałów, jak cegła itp. Niska wartość współczynnika przewodzenia ciepła wynika głównie ze struktury materiału, która charakteryzuje się dużą porowatością. Z właściwością tą wiąże się fakt występowania sił kapilarnych, które są przyczyną zjawiska podciągania kapilarnego będącego przyczyną destrukcji wielu budynków dotkniętych nadmiernym zawilgoceniem. Artykuł przedstawia możliwość monitoringu i ilościowej oceny wzrostu wilgotności w przegrodach budowlanych wskutek procesu podciągania kapilarnego przy wykorzystaniu powierzchniowych sond TDR, umożliwiających szybkie i bezinwazyjne wyznaczanie wilgoci w porowatych materiałach budowlanych. Analizy wykonane za pomocą sond powierzchniowych porównano do wyników z innych metod elektrycznych i przedstawiono w postaci profili wilgotnościowych zmieniających się w czasie.

Słowa kluczowe: podciąganie kapilarne, beton komórkowy, powierzchniowe sondy TDR

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Andrzej ŚWIĄTKOWSKI[1], Krzysztof KUŚMIEREK[1] and Monika SANKOWSKA[1]

KINETICS OF SIMULTANEOUS ADSORPTION OF WATER CHLOROORGANIC POLLUTANTS ON ACTIVATED CARBON

KINETYKA ADSORPCJI CHLOROORGANICZNYCH ZANIECZYSZCZEŃ WODY Z ROZTWORÓW WIELOSKŁADNIKOWYCH NA WĘGLU AKTYWNYM

Abstract: A comparative study of adsorption kinetics in the systems multicomponent aqueous solutions of selected chlorophenols (2-CP, 3-CP, 4-CP) and chlorophenoxy herbicides (2,4-D, MCPA) - activated carbon (F-300) has been performed. The concentrations of all adsorbates in the aqueous phase were determined using high performance liquid chromatography (HPLC). The applicability of two kinetic models, the pseudo-first and pseudo-second order models, for the experimental data was examined. The adsorption kinetics was better described by the pseudo-second order model with correlation coefficients $R^2 \geq 0.997$ for all adsorbates.

Keywords: adsorption, activated carbon, chlorophenols, chlorophenoxy herbicides, adsorption kinetics

Chlorophenols are one of the most common environmental contaminants. These compounds cause distasteful taste and odor of drinking water and can exert negative effects on different biological processes. Amongst the several techniques of chlorophenols removal, adsorption has been found to be a proficient and economic method. Large amount commercial systems currently use activated carbon as adsorbent to remove chlorinated phenols in wastewater because of its exceptional adsorption capability. Increasingly stringent legislation on the purity of drinking water has created a growing interest in the decontamination of water, wastewaters and polluted trade effluents using activated carbon. The applicability of this adsorbent for chlorophenols (4-CP, 2,4-DCP, 2,4,6-TCP from aqueous phase was investigated [1-6].

Water pollution by pesticides, particularly herbicides, has been recognized in agricultural areas for many years. A majority of the herbicides used in Europe are acidic herbicides, and phenoxy acid herbicides such as *2,4-dichlorophenoxyacetic acid (2,4-D)*, *2-methyl-4-chlorophenoxyacetic acid (MCPA)*, *dichlorprop (DCPP)* and *mecoprop (MCPP)* are among the 10 most important pesticides. Due to their high solubility in water, phenoxy herbicides easily enter surface or groundwaters through natural drainage or infiltration. Conventional water treatment processes have been reported to be more effective in removing less water soluble and easily degradable pesticides. Since there is a wide variety of herbicides varying in physical and chemical properties, it is difficult to apply a single method. Adsorption on activated carbon is one of the well-established and effective techniques for the removal of herbicides (CPA, 2,4-D, MCPA) from water [7-9].

The objective of the present study is to investigate and describe kinetics of simultaneous adsorption of selected chlorophenols and chlorophenoxy herbicides from aqueous solutions.

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[1] Institute of Chemistry, Military University of Technology, ul. S. Kaliskiego 2, 00-908 Warszawa, phone 22 683 95 61, fax 22 683 95 82, email: a.swiatkowski@wp.pl, kkusmierek@wat.edu.pl, monika075@tlen.pl

Experimental

The commercial *granular activated carbon* Filtrasorb 300 (Chemviron) was chosen for this study. Prior to use, the as-received GAC was washed several times with distilled boiling water. Ash content decreased in that way from 8.05 to 7.85% wt. The main properties characterizing so prepared carbon are as follows: S_{BET} = 938 m^2/g, IN = 927 mg/g, NaOH neutralization ability 0.07 mmol/g, surface oxygen content from EDS - 4.57% wt.

As adsorbates three chlorophenols 2-CP, 3-CP, 4-CP and two chlorophenoxy herbicides 2,4-D and MCPA were selected. The kinetic experiments were conducted for initial concentration of aqueous solution C_o = 100 mg/dm^3 at temperature of 25°C. After putting in Erlenmeyer flask 0.2 g activated carbon and 100 cm^3 solution its content was agitated and hour by hour analyzed concentration of each adsorbate using HPLC with diode array detector (Shimadzu LC-20, Kyoto, Japan). Separation of analytes was performed using a Phenomenex Luna C_{18} (4.6 ×150 mm, 3 µm) column (Torrance, CA, USA).

The amount of adsorption at time t, q_t [mg/g], was calculated by: $q_t = (C_o - C_t)V/m$ where C_t is the adsorbate concentration at any time t [mg/dm^3], V is the volume of the solution [dm^3] and m is the mass of the adsorbent [g]. It was observed, that after about 6-7 hours adsorption equilibrium was achieved (Fig. 1). For description of curves q_t = f(t) equations pseudo-first and pseudo-second order were considered.

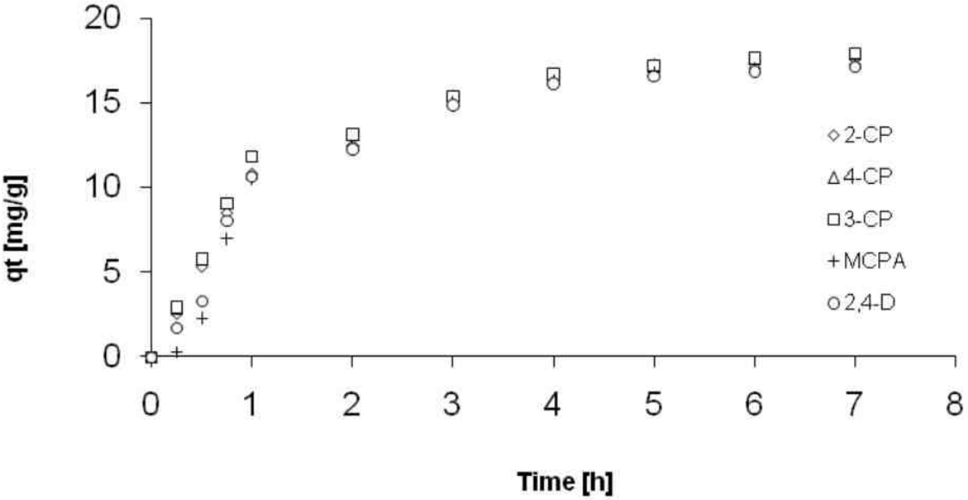

Fig. 1. Adsorption kinetics of selected chlorophenols and chlorophenoxy herbicides on activated carbon at 25°C, initial concentration 100 mg/dm^3

Results and discussion

In order to investigate the kinetics of adsorption of selected chlorophenols and chlorophenoxy herbicides on activated carbon, the rate constants were determined in terms of the pseudo-first order and pseudo-second order models.

The pseudo-first order equation has the form:

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \tag{1}$$

where q_e and q_t are the amounts of adsorption at equilibrium and at time t, respectively, and k_1 is the pseudo-first order rate constant. After integration and applying the initial conditions, the integrated form of Eq. (1) becomes:

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303}t \tag{2}$$

In order to obtain the rate constants, the straight line plots of $\log(q_e - q_t)$ vs t for the selected adsorbates have been tested.

If the sorption follows pseudo-second order mechanism, the equation is expressed as:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \tag{3}$$

where q_e and q_t are the amounts of adsorption at equilibrium and at time t, respectively, and k_2 is the pseudo-second order rate constant. Integrating Eq. (3) and applying the initial conditions we have:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e}t \tag{4}$$

The straight line plots of t/q_t against t have also been analyzed. The values of pseudo-second order rate constants were calculated from these plots.

The experimental results were described by the pseudo-first and pseudo-second order models equations. The rate constants (k_1, k_2) and correlation coefficients R^2 for both kinetic models were calculated and listed in Table 1.

Table 1

The pseudo-first and pseudo-second order rate constants for adsorption of selected chlorophenols and chlorophenoxy herbicides on activated carbon at 25°C, initial concentration 100 mg/dm^3

Compounds	**Pseudo-first order model**			**Pseudo-second order model**		
	k_1 [h^{-1}]	**k_1 [min^{-1}]**	**R^2**	**k_2 [g mg^{-1}h^{-1}]**	**k_2 [g mg^{-1}min^{-1}]**	**R^2**
2-CP	0.689	0.0115	0.989	21.241	0.0008	0.997
3-CP	0.725	0.0120	0.987	17.560	0.0010	0.998
4-CP	0.714	0.0120	0.986	17.316	0.0010	0.997
2,4-D	0.811	0.0138	0.986	18.842	0.0009	0.997
MCPA	0.830	0.0136	0.988	19.172	0.0009	0.997

The correlation coefficient values $R^2 \geq 0.997$ for pseudo-second order equation indicate the applicability of this model to describe the adsorption process of the all selected adsorbates on Filtrasorb 300 activated carbon. Literature survey has shown that pseudo-second order model suitably fitted experimental data and is more suitable than pseudo-first order model [2, 3, 5, 6].

Conclusions

The adsorption kinetics for all studied systems aqueous multicomponent solutions of selected chloroorganic adsorbates - activated carbon was found to follow pseudo-second order model with good correlation. The correlation coefficients of particular adsorbed chlorophenols or chlorophenoxy herbicides were very high. For the pseudo-first order model they are only slightly lower.

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KINETYKA ADSORPCJI CHLOROORGANICZNYCH ZANIECZYSZCZEŃ WODY Z ROZTWORÓW WIELOSKŁADNIKOWYCH NA WĘGLU AKTYWNYM

Instytut Chemii, Wojskowa Akademia Techniczna, Warszawa

Abstrakt: Wykonano badania porównawcze szybkości adsorpcji z wieloskładnikowych wodnych roztworów wybranych chlorofenoli (2-CP, 3-CP, 4-CP) i herbicydów chlorofenoksyoctowych (2,4-D, MCPA) na granulowanym węglu aktywnym (F-300). Stężenia wszystkich adsorbatów w fazie wodnej oznaczano za pomocą wysokosprawnej chromatografii cieczowej (HPLC). Zbadano przydatność dwóch modeli kinetycznych: pseudopierwszego i pseudodrugiego rzędu do opisu wyników eksperymentalnych. Uzyskane rezultaty pokazują, że kinetykę adsorpcji lepiej opisuje równanie pseudodrugiego rzędu, co potwierdziły większe wartości współczynnika korelacji dla wszystkich adsorbatów ($R^2 \geq 0{,}997$).

Słowa kluczowe: adsorpcja, węgiel aktywny, chlorofenole, herbicydy chlorofenoksyoctowe, kinetyka adsorpcji

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Petr ŠKARPA[1]

MONITORING THE CHANGES IN TOTAL CONTENTS OF MANGANESE, COPPER AND ZINC IN SOILS FROM LONG-TERM STATIONARY EXPERIMENTS

MONITORING ZMIAN CAŁKOWITYCH ZAWARTOŚCI MANGANU, MIEDZI I CYNKU W GLEBACH PODDAWANYCH DŁUGOTERMINOWYM DOŚWIADCZENIOM ROLNICZYM

Abstract: The objective of the long-term stationary experiment was to discover the effect of the year, production region, soil kind and soil type on total contents of micronutrients (Mn, Cu and Zn) in the soils. In the years 1982 to 1998 the soil was sampled in 7 selected localities. Analyses and extractions determining the total content of metals were carried out by means of mineralization in the HF-H_2O_2-HNO_3 open system. The AAS method was used to determine the contents of the micronutrients. The content of manganese ranged between 296.2 and 978.6; copper between 6.1 and 25.7 and zinc between 29.5 and 99.8 $mg \cdot kg^{-1}$ of soil. During the experimental period 1982-1998 the total content of Mn and Zn decreased (by 7.9 and 3.6%, respectively), but was not statistically significant ($p < 0.05$). During the 15 years of the experiment the total amount of copper in the soil increased by 7.0%. In comparison with the potato growing region the contents of all the micronutrients in the sugar-beet growing region were higher. The highest and statistically highly significant difference ($p < 0.001$) was that of copper (45.6% increase). In terms of the soil kind the lowest contents of Mn and Cu were monitored on light soil. The total content of zinc on light soil and medium-heavy soil was comparable. With an increasing proportion of clay particles in the soil the contents of the micronutrients increased significantly ($p < 0.001$). In comparison with light soil, in heavy soil the contents of the metals increased; Mn by 38.9; Cu by 48.2 and Zn by 19.4%. The levels of Cu and Zn were also affected by the soil type. The contents of these micronutrients were statistically ($p < 0.001$) the highest in chernozem (24.6 and 71.1 $mg \cdot kg^{-1}$ of soil, respectively). The content of Mn was the highest in brown soil (714.3 $mg \cdot kg^{-1}$ of soil). Graded rates of fertilisers did not have a significant ($p < 0.05$) effect on the total contents of metals. The differences were more marked in the treatment where liming was not carried out; here we monitored the greatest decrease in the contents of Mn, Cu and Zn, ie by 7.3, 23.8 and 9.4%, respectively, compared with the control.

Keywords: long-term experiment, total zinc, total manganese, total copper

Soil is characterised as the most complicated, most dynamic and most reactive component on Earth [1]. Soil is a key part of the environment and irreplaceable source of most of the biochemically active micronutrients influencing human beings through plants and animals. Important micro-biogenic elements are manganese, copper and zinc [2, 3]. Their total levels in the soil are based particularly on the mineral composition of the soil, soil type, content of clay particles (soil kind), content of organic matter in the soil, soil reaction etc. [4-7]. The content of micronutrients in the soil is considerably affected by anthropogenic activities. An important source of micronutrients which increases their levels in the soil are indisputably fertilisers (particularly nitrogenous and phosphorus), calcareous matter, farmyard manure, slurry, pesticides and others [4, 8, 9].

The aim of our study was to evaluate changes in total content of manganese, copper and zinc in soil in long-term experiment.

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[1] Department of Agrochemistry, Pedology, Microbiology and Plant Nutrition, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic, phone + 420 545 133 345, email: petr.skarpa@mendelu.cz

Material and methods

The experiment was established on 7 localities of potato growing regions (altitude 400÷650 m a.s.l., annual average temperature 5÷8°C, annual average precipitation 550÷900 mm, predominate cambisols) and sugar-beet growing regions (altitude 250÷350 m a.s.l., annual average temperature 8÷9°C, annual average precipitation 500÷650 mm, predominate chernozems and haplic luvisols) as a long-term small-plot stationary trial by the Central Institute for Supervising and Testing in Agriculture between 1982 and 1998. Table 1 gives the characteristics of the localities.

Table 1

The characteristic of localities

Locality	**Growing regions**	**Altitude [m a.s.l.]**	**Annual average**		**Reference soil groups**	**Soil textural class**
			temperature [°C]	**precipitation [mm]**		
Horazdovice	potato	470	7.4	573	Cambisols	sandy
Svitavy	potato	460	6.5	624	Cambisols	sandy
Chrastava	potato	345	7.1	798	Luvisols	loamy
Stankov	potato	370	8.3	443	Luvisols	clay
Puste Jakartice	sug.-beet	295	8.0	640	Luvisols	loamy
Uhersky Ostroh	sug.-beet	196	9.2	551	Luvisols	loamy
Zatec	sug.-beet	247	8.3	451	Chernozems	clay

soil type (FAO soil taxonomy), soil textural class [10]

The experiment comprised 6 combinations of fertilization, in 4 replications. Table 2 shows the average contents of N, P and K, which were applied in organic fertilizers (farm manure) and mineral fertilizers at three levels (low - $N_1P_1K_1$, medium - $N_2P_2K_2$, high - $N_3P_3K_3$). Organic fertilizers were applied once every 4 years; 40 Mg ha^{-1} of farm manure was incorporated in the sugar-beet growing region to maize for silage and to sugar-beet and in the potato growing region to potatoes.

Table 2

Treatments of the experiment

Treatments of fertilization	**Average content of nutrients in fertilizers [kg·ha^{-1}·year^{-1}]**					
	Nitrogen		**Nitrogen**		**Nitrogen**	
	PGR[1]	**SGR[2]**	**PGR[1]**	**SGR[2]**	**PGR[1]**	**SGR[2]**
no fertilized	0	0	0	0	0	0
farm. manure	25	25	8	8	35	35
farm. manure + $N_1P_1K_1$	83	83	31	29	92	86
farm. manure + $N_2P_2K_2$	113	112	43	41	125	116
farm. manure + $N_3P_3K_3$	142	140	59	57	166	154
farm. manure + $N_3P_3K_3$ without liming	142	140	59	57	166	154

[1] PGR - *potato growing regions*; [2] SGR - *sugar-beet growing regions*

Soil was sampled on the localities in autumn 1982 and 1998. Soil samples were taken from 0÷30 cm depth. Samples were dried naturely. The total content micronutrients (manganese, copper and zinc) of the soil in its dry state was estimated by the method of Houba et al [11] with the soil extracted in the mixture of HF-H_2O_2-HNO_3 and measured by *atomic absorption spectrometry* (AAS) on the ContrAA 700 spectrometer (Analytic Jena).

The results of chemical soil analyses were computer-processed and set up using the Microsoft Excel editor. The Statistica 7.1 programme was used for the determination of the overall characteristics. Arithmetic means were calculated when evaluating the results. To elaborate the significance of differences among the arithmetic means of each characteristic we used the mono-factor and two-factor analysis of variance followed by testing at a 95% ($p < 0.05$) level of significance using Tukey test.

Results and discussion

Manganese - Manganese is the tenth-most abundant element on the surface of the earth [2]. Its natural content in soils of the Czech Republic ranges between 80 and 2220 mg·kg^{-1} of soil [12]. Bohn et al [13] reported that the average amount of total Mn ranges between 850 and 1000 mg·kg^{-1}. In our experiment the content of manganese ranged between 296.2 and 978.6 mg·kg^{-1}. During the experiment the total content of Mn decreased by 7.9%, but this decrease was not statistically significant [F (1; 164) = 3.312; p = 0.071] (Tab. 3). The reason of the statistically insignificant effect of the production region on the Mn content in the soil [F (1; 164) = 3.646; p = 0.058] were significant differences in the Mn content among the individual localities. Nonetheless its average value in the sugar-beet growing region was by 9.1% higher than in the potato-growing region (Tab. 4). Only the factor of the soil kind was statistically significant [F (2; 163) = 26.536; p < 0.001]. A significant difference was discovered between light soil and the medium-heavy and heavy soils (Tab. 5). Katyal and Sharma [14], Trebichavsky et al [12], Raji et al [15] and Rinaudo [16] reported that due to the content of clay particles the total content of Mn increased. In terms of the soil type the highest statistically significant content of Mn was detected in brown soil [F (2; 163) = 52.607; p < 0.001]. We compared the soils and discovered that the content of manganese was the lowest in cambisols; in chernozem it was by 11% higher (Tab. 6). Wang et al [17] reported the highest content of Mn in chernozem. The total amounts of manganese in the soil after the respective fertiliser treatments did not significantly differ [F (5; 160) = 0.165; p = 0.975]. Podkolzin et al [18] presented similar conclusions. By contrast Kurakov et al [19] reported that the Mn content increased due to long-term application of fertilisers. Table 7 shows that the content of Mn was the highest in the treatment with no fertilisers (treatment 1) and the lowest where the highest amount of mineral fertilisers was applied without liming (treatment 6).

Table 3

Total contents of microelements in soil [mg·kg^{-1} d.m. soil] - Factor: year

Year	Mn	Cu	Zn
1982	636.6 a	15.7 a	61.4 a
rel. %	*100.0*	*100.0*	*100.0*
1998	586.0 a	16.8 a	59.2 a
rel. %	*92.1*	*107.0*	*96.4*

$p < 0.05$ - statistical significance at a 95% level of significance. Variants with identical letters express statistically insignificant differences

Table 4

Total contents of microelements in soil [$mg \cdot kg^{-1}$ d.m. soil] - Factor: growing regions

Growing regions	**Mn**	**Cu**	**Zn**
Potato growing regions	587.2 a	13.6 a	59.0 a
rel. %	*100.0*	*100.0*	*100.0*
Sugar-beet growing regions	640.8 a	19.8 b	62.0 a
rel. %	*109.1*	*145.6*	*105.1*

$p < 0.05$ - statistical significance at a 95% level of significance. Variants with identical letters express statistically insignificant differences

Table 5

Total contents of microelements in soil [$mg \cdot kg^{-1}$ d.m. soil] - Factor: soil textural class

Soil textural class	**Mn**	**Cu**	**Zn**
Sandy soil	471.0 a	13.7 a	56.7 a
rel. %	*100.0*	*100.0*	*100.0*
Loamy soil	676.2 b	14.7 a	56.6 a
rel. %	*143.6*	*107.3*	*99.8*
Clay soil	654.3 b	20.3 b	67.7 b
rel. %	*138.9*	*148.2*	*119.4*

$p < 0.05$ - statistical significance at a 95% level of significance. Variants with identical letters express statistically insignificant differences

Table 6

Total contents of microelements in soil [$mg \cdot kg^{-1}$ d.m. soil] - Factor: reference soil groups

Reference soil groups	**Mn**	**Cu**	**Zn**
Cambisols	471.0 a	13.7 a	56.7 a
rel. %	*100.0*	*100.0*	*100.0*
Luvisols	714.3 b	14.8 a	58.5 a
rel. %	*151.7*	*108.0*	*103.2*
Chernozems	525.3 a	24.6 b	71.1 b
rel. %	*111.5*	*179.6*	*125.4*

$p < 0.05$ - statistical significance at a 95% level of significance. Variants with identical letters express statistically insignificant differences

Table 7

Total contents of microelements in soil [$mg \cdot kg^{-1}$ d.m. soil] - Factor: Fertilization

Variant of fertilization	**Mn**	**Cu**	**Zn**
no fertilized	626.1 a	17.2 a	62.7 a
rel. %	*100.0*	*100.0*	*100.0*
farm. manure	618.5 a	16.7 a	60.5 a
rel. %	*98.8*	*97.1*	*96.5*
farm. manure + $N_1P_1K_1$	601.6 a	16.5 a	59.9 a
rel. %	*96.1*	*95.9*	*95.5*
farm. manure + $N_2P_2K_2$	613.4 a	16.4 a	60.2 a
rel. %	*98.0*	*95.3*	*96.0*
farm. manure + $N_3P_3K_3$	605.1 a	16.1 a	59.8 a
rel. %	*96.6*	*93.6*	*95.4*
farm. manure + $N_3P_3K_3$ without liming	580.1 a	13.1 a	56.8 a
rel. %	*92.7*	*76.2*	*90.6*

$p < 0.05$ - statistical significance at a 95% level of significance. Variants with identical letters express statistically insignificant differences

Copper - The total content of copper in non-contaminated soils ranges between 2 and 200 mg·kg^{-1} of soil [6, 13]. The average contents are quoted to range between 10 and 50 mg·kg^{-1} of soil [4, 20, 21]; in the Czech Republic the average Cu content is 26 mg·kg^{-1} of soil [12]. In our experiment the amount of Cu ranged between 6.1 and 25.7 mg·kg^{-1} of soil, an equivalent to the lower half of its natural content reported by these authors. As Table 3 shows during the experiment the content of copper increased by 7%, but not statistically significantly [F (1; 164) = 1.412; p = 0.236]. However, the influence of the production region on the copper content was statistically significant [F (1; 164) = 56.401; p < 0.001]. In the sugar-beet region the content of copper was by 45.6% higher than in the potato-growing region (Tab. 4). According to Trebichavsky et al [12], Kabata-Pendias and Pendias [4] and Yu et al [22] the soil kind also significantly affects the content of copper in the soil and confirms that the level copper in the soil increases due to the increasing amount of clay particles [F (2; 163) = 23.576; p < 0.001]. While the copper content on medium-heavy soil was by 7.3% higher, the significantly highest content was monitored on heavy soil (Tab. 5). Likewise Wang et al (2003) reported a positive correlation between the copper content and clay particles. Another factor affecting the copper content was the soil type. The statistically significantly highest level of copper was detected in chernozem [F (2; 163) = 60.237; p < 0.001]. In brown soil and cambisol the content was lower by 39.8 and 44.3%, respectively (Tab. 6). Barker and Pilbeam [2] also reported that the level of copper was the highest in chernozem soils. Graded rates of fertilizers had no statistically significant effect on the total amount of soil copper [F (5; 160) = 1.052; p = 0.389] (Tab. 7). Erhart et al [23] arrived at the same conclusions. In the experiment its amount gradually decreased with increasing rates of nutrients contained in the applied organic and mineral fertilisers. Much like Mn, also the copper content was the lowest in treatment 6.

Zinc - Trebichavsky et al [12] reported that the average total content of zinc in agricultural soils of the Czech Republic ranges between 10 and 244 mg·kg^{-1} of soil (82 mg·kg^{-1}) and that the worldwide average ranges between 50 and 60 mg·kg^{-1} [13]. The total content of zinc in our experiment ranged between 29.5 and 99.8 mg·kg^{-1} of soil. Much like manganese the average zinc content decreased during the experiment (Tab. 3), however not statistically significantly [F (1; 164) = 0.874; p = 0.351]. When compared with the other monitored micronutrients the effect of the production region on the zinc content was the same as on Mn (Tab. 4). The zinc supply was higher in the sugar-beet growing region (by 5.1%) [F (1; 164) = 1.696; p = 0.195]. In our experiment the contents of zinc on light soil and on medium-heavy soil were comparable. The level of zinc on heavy soil (Tab. 5) significantly differed from the above soil kinds [F (2; 163) = 11.369; p < 0.001]. Moreno et al [24], Kparmwang et al [25], Valladares et al [26] and Menezes et al [27] also discovered that the effect of clay on the content of soil zinc was significant. The effect of the soil type was similar as in the case of copper (Tab. 6). In cambisol and brown soil 56.7 and 58.5 mg Zn·kg^{-1}, respectively, were detected, whereas the significantly highest Zn content was monitored in chernozem [F (2; 163) = 11.247; p < 0.001]. The results of Wang et al [17] also support this fact. Table 7 shows that fertilisation did not significantly affect the content of Zn or the contents of the other micronutrients [F (5; 160) = 0.345; p = 0.884]. The level of Zn was the lowest when fertilised with the highest rate of mineral fertilisers without liming (treatment 6) and was the highest when not fertilised; the difference was

9.4%. Podkolzin et al [18] also reported that long-term application of organic and mineral fertilisers had no marked effect on the content of soil Zn.

Conclusions

The results of a long-term stationary experiment showed that during the 17 years of the experiment the total contents of Zn and Mn decreased and the amount of Cu increased, but not significantly. The levels of all the micronutrients were higher in the sugar-beet region than in the potato-growing region and only Cu was significantly higher (by 45.6%). In terms of the soil kind the levels of the micronutrients were the lowest in light soil. The levels of metals increased significantly with the increasing content of clay particles in the soil. In heavy soil the contents of Mn, Cu and Zn were by 38.9, 48.2 and 16.4%, respectively, higher than the amounts monitored on light soil. The differences among the soil types were significant. The contents of Cu and Zn were the highest in chernozem; the significantly highest content of Mn was detected in Luvisol. Graded rates of organic and mineral fertilisers did not affect the total content of metals.

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Bohumil VYBÍRAL[1]

AUTOTHIXOTROPY OF WATER AND ITS POSSIBLE IMPORTANCE FOR THE PHYSICS-CHEMICAL THEORY IN ENVIRONMENT

AUTOTIKSOTROPIA WODY I JEJ ZNACZENIE DLA CHEMOFIZYKALNEJ TEORII ŚRODOWISKA

Abstract: It is well known that water is the source of all life and therefore it is an important factor for the chemical-physics theory in environment. The article deals with phenomenon in water that was called „autothixotropy of water“. A history of the discovery of the phenomenon is connected with very fine gravimetric experiments which have been made by the author between the years 1978 and 1986. The independent phenomenon of autothixotropy of water was qualitatively explored in the period from 1991 to 1992 and quantitatively in period from 2003 to 2011. From the physical point of view the autothixotropy of water is a very weak macroscopic phenomenon that is observed in water that has remained at rest for a certain time (in about tens of hours or days) *ie* ‘standing’ water. For the experiments distilled water was used, re-boiled before use. Physically the phenomenon manifests itself by force (mechanical resistance) acting on a body immersed in ‘standing’ water in the direction of the tendency to change its position. The fundamental method of research of phenomenon is static method; it uses a moment of force that is necessary for a very distinctive turn of a stainless steel plate hung up on a very thin filament and immersed in examined water. With a certain angular torsion of the filament a certain moment of force is achieved when a state of stress reaches a critical value in ‘standing’ water. It is demonstrated with an expressive changing of angular position of the plate. When the direction of moment of force in ‘standing’ water is changed, it is reflected in a different angle rotation of the plate (the phenomenon of hysteresis was observed). The phenomenon of autothixotropy disappears for a certain time (in tens of minutes or hours), if ‘standing’ water is mechanically vigorous shaking or re-boiled. It is significant that the phenomenon of the autothixotropy is not present in deionised distilled ‘standing’ water. From the chemical-physics microscopic point of view the autothixotropy of water can be explained by a hypothesis of a cluster formation by H_2O molecules in ‘standing’ water. As the phenomenon of autothixotropy is not present in ‘standing’ deionised water, it may be primarily caused by a presence of ions in water, as it was demonstrated in the experiment with a salt solution (NaCl). Environment and biophysical applications of the autothixotropy of water remain still open. Some possible applications of the phenomenon are outlined.

Keywords: standing water, autothixotropy of water, deionised water, salt solution, super clusters

Autothixotropy of water is a very weak macroscopic phenomenon in water that has remained at rest for a certain time (in about tens of hours or days) ie ‘standing’ water. For experiments distilled water was used, re-boiled before use. The phenomenon is manifested by very small force (mechanical resistance) acting against a body immersed in water and arising when it should change its position. If standing water is vigorously mixed or re-boiled again, the phenomenon disappears and appears again in a matter of about tens of minutes or hours (or days). Macroscopically therefore standing water acts as a very weak gel, which has approximately thixotropical properties. Because it arises spontaneously a phenomenon was called ‘*the autothixotropy of water*’.

The history of discovery of this phenomenon in water is connected with very fine gravimetric measurements which I made in the years 1978 till 1986 [1]. The experiments

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[1] Department of Physics, Faculty of Science, University of Hradec Králové, Rokitanského 62, 500 03 Hradec Králové, Czech Republic, phone +420 493331187, email: bohumil.vybiral@uhk.cz

were not successful: during the experiments unknown molecular properties of water were shown disturbingly and superimposed the expected gravitation of the phenomena very strongly. After a period longer than about ten hours it was not possible to control the torsion pendulum with a rotation of the filament hanging - molecular forces of unknown origin took effect against small gravitational forces. In 1986 I decided to use the dynamic method which an influence of phenomenon of autothixotropy was excluded. The results of qualitative analysis of autothixotropy of water were published [2].

Method and experimental

The fundamental method of research of phenomenon is a static method. The apparatus for measurements with the method is shown in Figure 1. The method uses a moment of force that is necessary for a very distinctive turn of a stainless steel plate hung up on a very thin filament and immersed in examined water. With a certain angular torsion (angle φ_u) of the filament a certain moment of force is achieved when a state of stress reaches a critical value in ‘standing’ water. It is demonstrated with an expressive changing of angular position of the plate (angle φ_d).

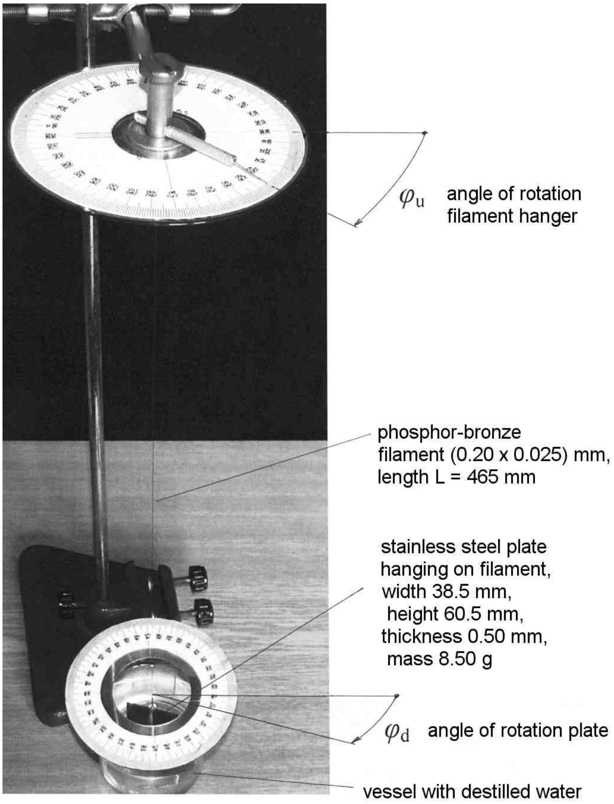

Fig. 1. Photo of apparatus for measurements of autothixotropy of water

Ideal fluid model of water: Then, if we turn the upper end of the filament by angle φ_u, we expect that the plate will follow the rotation of upper, so that approximately is[2] $\varphi_d = \varphi_u$, where φ_d being angle of rotation of the plate (this equality is true for i.e. 'fresh' water).

Basic observations: According to our experiments with 'standing' water, equality $\varphi_d = \varphi_u$ was not achieved. In the experiment, a series of increasing values of angle φ_d is observed, following a very slow - 'step by step' - change of angle φ_u. We measured function $\varphi_d = f(\varphi_u)$. If angle φ_u reaches a critical value $(\varphi_u)_{crit.}$, the rotation of the plate (ie $\dot{\varphi}_d$) becomes relatively quick.

Phenomenon of hysteresis: When the direction of moment of force is changed, it is reflected in the different rotation of the hanged plate - the phenomenon of hysteresis is observed [3]. Measurements for a cyclic change of angle φ_u were carried out.

Experiments were made for this case:

1. distilled water (re-boiled before use),
2. deionised distilled water (re-boiled before use),
3. salt solution (concentration of 1 per mille NaCl in deionised distilled water, re-boiled before use).

Results and discussion

1. *Distilled water.* Typically experimental functions $\varphi_d = f(\varphi_u)$ are on Figure 2; loop **a** is relevant for the experiment with the plate totally immersed in water, while loop **b** is related to the experiment with the plate only half-immersed. The effect is clearly more pronounced for the half-immersed plate than for the completely immersed one. When the direction of moment of force is changed, it is reflected in the different rotation of the hanged plate - *the phenomenon of hysteresis* was observed [3].
2. *Deionised distilled water.* Neither the existence of critical angles $(\varphi_u)_{crit.}$, nor the phenomenon of hysteresis, were found. From the performed experiment [3] we arrived at the important conclusion that the autothixotropy of water, characterized by a non-zero critical angle, and hysteresis, *is caused by the presence of ions in the water.*
3. *Salt solution.* For a period of 7 days no autothixotropy appeared, more to the contrary for angles inequality $\varphi_d > \varphi_u$ was valid (see the curve **a** in the Figure 3). Thus the angle φ_d of turning of plate is bigger than the angle φ_u of a primal turning of the upper hanging of filament. It is a remarkable finding because the plate shows a certain additional automotoric movement. The phenomenon of autothixotropy appeared after 7 days from a preparation of the solution. Afterwards, on the contrary, the measurement results $\varphi_d < \varphi_u$. After the critical angle $\varphi_u = (\varphi_u)_{crit.1} = 70°$ is achieved, the angle φ_d of plate turning starts increasing, even if φ_u, is invariable how it is evident from the Figure 3, loop **b**.

The autothixotropy of water can be explained by a hypothesis [5] of a (super) cluster formation by H_2O molecules in 'standing' water. As the phenomenon of autothixotropy is not present in *deionised* water, it may be primarily caused by a presence of ions in water [2, 3]. In case of the experiment with salt solution NaCl in deionised distilled water dissociation happens (the molecule NaCl breaks up to ions Na^+, Cl^-). Around ions Na^+, Cl^- the clusters of molecule H_2O are formed.

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[2] See too Figure 3 (the curve **a**).

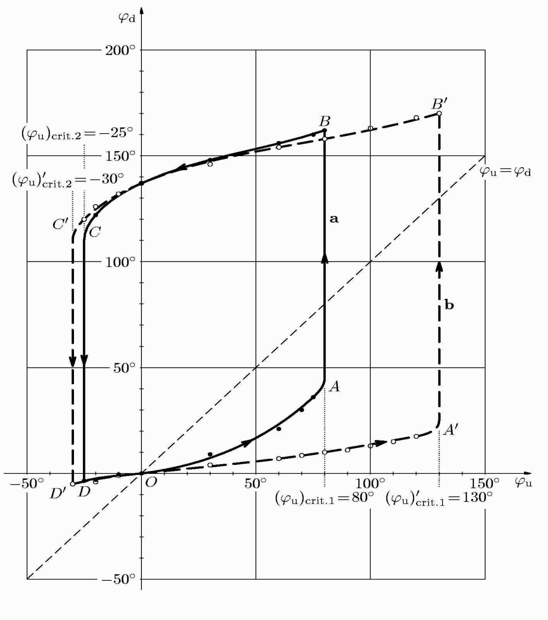

Fig. 2. Autothixotropy in distilled water: loops of hysteresis - the changes of angle $\varphi_d = f(\varphi_u)$): with the completely immersed plate (loop **a**) and with the half-immersed plate (loop **b**) [3]

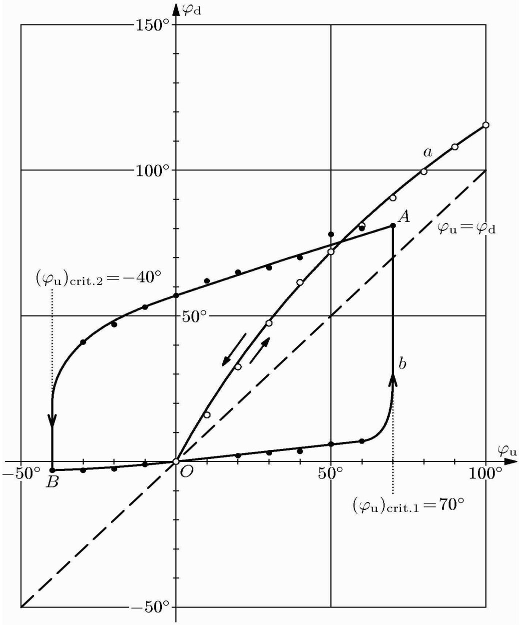

Fig. 3. The results of experiments with salt solution (with the plate totally immersed in solution). The curve **a** describes a dependence $\varphi_d = f(\varphi_u)$ of a fresh salt solution. The curve **b** describes a dependence $\varphi_d = f(\varphi_u)$ after arising of autothixotropy. Evidently a phenomenon of hysteresis appeared

From Figure 2 we see that the loop ***b*** describes the phenomenon of autothixotropy of water more intensive than loop **a**, although the plate was immersed only by half of its area. However, in this case, the plate cut through the surface of water. This suggests that at the surface of the water the network of H_2O super clusters molecules is denser.

Conclusions

- A state of autothixotropy of water is macroscopically demonstrated with very weak mechanical properties analogous to the properties of *solid substances*, such as a certain internal static friction, elasticity and strength.
- Water slightly deviates from an ideal Newtonian viscous fluid, because the autothixotropy appears in the form of a certain internal static friction, although very weak.
- Existence of autothixotropy *is probably caused by super clusters* that are created in standing water after a certain time and evidently fill up the whole vessel with the exploring water (with greater density at the surface level). The kernels of these macroscopic clusters *can be ions* present in water.
- The time interval during which the autothixotropy arises (in case of fresh water or after its re-boiling or vigorous shaking) has not been definitely determined yet. Obviously it depends on the structure and amount of ions contained in water.
- Environment and biophysical applications of the autothixotropy of water remain still open [6, 7]. The autothixotropy can have a considerable significance for biophysics because with the aid of autothixotropy *the rigidity of cytoskeleton* of living cell could be explained. Time depended self-organisation of water molecules into super clusters may play a significant role in the mechanical and electrical stability of this clusters system.

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AUTOTIKSOTROPIA WODY
I JEJ ZNACZENIE DLA CHEMOFIZYKALNEJ TEORII ŚRODOWISKA

Katedra Fizyki, Wydział Przyrodniczy, Uniwersytet w Hradec Králové, Hradec Králové, Czechy

Abstrakt: Woda jest źródłem życia i dlatego jest ona ważnym obiektem badań fizyki chemicznej środowiska. W artykule przedstawiono zjawisko zachodzące w wodzie, nazywane „autotiksotropią wody”. Historia jego odkrycia wiąże się z bardzo precyzyjnymi eksperymentami grawimetrycznymi, które zostały wykonane przez autora w latach 1978 i 1986. Ponadto zjawisko autotiksotropii wody zostało jakościowo zbadane w latach 1991/92 oraz ilościowo od 2003 do 2011 roku. Z fizykalnego punktu widzenia autotiksotropia wody jest bardzo subtelnym zjawiskiem makroskopowym, które obserwuje się w wodzie, pozostającej przez pewien czas w spoczynku (około kilkudziesięciu godzin), czyli wodzie „stojącej”. Zjawisko to przejawia się przez oporność mechaniczną działającą na ciało zanurzone w wodzie „stojącej”. Podstawę badania stanowi metoda statyczna, wykorzystująca moment siły, która umożliwia pomiar kąta skręcenia nierdzewnej blachy stalowej zawieszonej w badanej wodzie. Kiedy kierunek momentu siły w „stojącej” wodzie się zmienia, znajduje to odzwierciedlenie w różnych kątach obrotu tej płyty (zaobserwowano zjawisko histerezy). Zjawisko autotiksotropii znika po pewnym czasie (w kilkadziesiąt minut lub godzin), jeśli „stojąca” woda jest mieszana mechanicznie lub ogrzewana do wrzenia. Znamienne jest, że zjawisko autotiksotropii nie występuje w „stojącej” zdejonizowanej wodzie destylowanej. Zjawisko to może być wyjaśnione przez hipotezę powstawania „w wodzie stojącej” klastrów molekuł H_2O. Zjawisko autotiksotropii nie jest obecne w zdejonizowanej wodzie „stojącej”, co może być spowodowane obecnością jonów w wodzie, jak to wykazano w eksperymencie z roztworem soli kuchennej (NaCl). Środowiskowe i biofizyczne wykorzystanie autotiksotropii wody nadal pozostają otwarte. Niektóre z możliwych zastosowań tego zjawiska są już opisane.

Słowa kluczowe: wody stojące, autotiksotropia wody, dejonizowane wody, roztwory soli, superklastry

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Marcin K. WIDOMSKI[1], Beata KOWALSKA[1] and Anna MUSZ[1]

COMPUTATIONAL FLUID DYNAMICS AND WATER QUALITY MODELING

OBLICZENIOWA MECHANIKA PŁYNÓW A MODELOWANIE JAKOŚCI WODY

Abstract: Modeling of water quality in distribution systems becomes nowadays a very popular tool applied in the processes of systems design and operation. Usually, according to everyday practice and literature reports, the chlorine propagation is one of the mostly reported subjects of modeling. Meanwhile, literature presents many examples of pollutants originated in polymer pipes' material seriously deteriorating the quality of water in distribution systems. In this case, the *computational fluid dynamics* (CFD) may be applied to numerical calculations of simultaneous transport of several organic and non-organic pollutants in drinking water supply systems constructed of metal or polymer pipes. This paper contains the presentation of recognized pollutants migrating to drinking water from plastic pipes, possibilities of CFD application to water quality modeling and basic set of necessary input data as well as range of simulation results. Advantages and disadvantages of CFD as a tool of water quality numerical assessment were also presented.

Keywords: water quality, numerical modeling, computational fluid dynamics, water supply systems

According to the rapid development of computing technologies and availability of various commercial and open source software modeling of water quality flowing through the distribution systems has recently gained wide popularity among scientists, designers and operators of water supply systems. Numerical simulations focused on water quality modeling allow to predict the propagation of selected pollutant, or several pollutants simultaneously, along the whole system of drinking water distribution in various operational conditions. The modeled pollutants of different kinds may enter the water distribution systems in the source (ie disinfectants) or in any given point of distribution system, including wall reactions or migrations from pipe material [1]. Thus, modeling of contaminants transport helps to understand the movement and behavior of pollutants resulting in possibility of proper network planning and management.

The initial water quality, after processes of treatment and disinfection may be changed due to different causes leading to its deterioration - oxygen decay, disinfectant decay, the formation of disinfection by-products (*trihalomethanes* THMs), change of color, smell and turbidity [2] as well as by leakage of organic or inorganic compounds from pipes materials during its contact with water [3-5]. The strict regulations concerning the proper level of disinfectant cause the necessity of studies and monitoring of chlorine propagation and decay along the drinking water distribution systems - the decay of disinfectant agent may result in the degradation of microbial conditions of water supply network, causing thus a possible risk to the consumers' health [6]. The recent literature studies show, however, that many different, organic and inorganic compounds creating possible danger to water quality (eg discoloration and fouling), customers complains as well as threats to public health may migrate from pipe material to drinking water during the distribution process

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[1] Faculty of Environmental Engineering, Lublin University of Technology, ul. Nadbystrzycka 40B, 20-618 Lublin, phone 81 538 41 38, email: M.Widomski@wis.pol.lublin.pl

through the water supply systems consisting of polymer pipes [3-5, 7, 8] - eg metals and heavy metals, organotins compounds, *volatile organic compounds* (VOCs) or thermal stabilizers such as Irganox 10XX series. The mentioned problem becomes more serious due to the rapid increase of polymer pipes (PE, PVC) application to construction of water distribution systems. Recent reports show that plastic pipes make up over 50% of all pipes installed worldwide [5]. In Poland approx. 70% of newly constructed pipelines are made of polymer pipes [9]. The most popular recognized pollutants originated from polymer materials migrating to drinking water and influencing its quality and organoleptic properties [4, 8, 10-12] are presented in Table 1.

Table 1

The most popular recognized pollutants migrating to drinking water from plastic pipes

Metal stabilizers		**Lead, organotins compounds, cadmium**
Antioxidants		Irganox 1010, Irganox 1035, Irganox 1076, 2,6-di-*tert*-butyl-4-methylphenol (BHT)
Products degradation of antioxidants used in polymer production		4-ethylphenol, 4-*tert*-butylphenol, 2,6-di-*tert*-butyl-*p*-benzoquinone, 2,4-di-*tert*-butylphenol, 3,5-di-*tert*-butyl-4-hydroxystyrene, 3,5-di-*tert*-butyl-4-acetophenone, 3,5-di-*tert*-butyl-4-hydroxyacetophenone, 1,5-bis(*tert*-butyl)-4-(2-carboxyethylidene)cyclohexa-1,4-dien-6-on, 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)methylpropanoate, 3-(3,5-di-*tert*-butylo-4-hydroxyphenyl)propanoic acid
VOCs	Esters	Butylacetate, ethylhexanoate, hexylacetate, propylhexanoate, butylhexanoate, ethyloctanoate, hexylhexanoate, hexamethylbutanoate, isobornylacetate, ethyldecadienoate, 2,2,4-trimethyl-1,3-2,2,4-TPD
	Aldehydes	Nonanal, decanal
	Ketones	2-decanon, 2-undecanone, 2-dodecanone
	Terpenoids	Alpha-pinene, delta-carene, limonene, alpha-terpinolene, alpha-farnesene
	Aromatics	Benzene Ethylbenzene, *m*- and *p*-xylene, *o*-xylene, styrene, isopropylbenzene, *n*-propylbenzene, 1,3,5-trimethylbenzene, 1,2,4-trimethylbenzene, *p*-isopropyltoluene, naphthalene

Nowadays, the EPANET-based (by EPA, USA) chlorine propagation modeling, as the main disinfectant applied worldwide, is very popular and is being reported frequently [13-20]. Usually, the following set of assumptions is required: distribution network is consisting of sources, pipes and nodes; flow directions in pipes are fixed, all hydraulic parameters and chlorine concentration in all outer sources are known [17]. Usually, the obtained results cover predicted chlorine concentrations in modeled systems nodes and pipes. However, these models have many disadvantages limiting the proper assessment of water quality in distributional systems (eg in some EPANET-based models propagation of studied pollutant may be studied only in system nodes) or just limiting their usage only to small networks as well as, which seems to be fairly important, simulating changes of narrow group of pollutants of in fixed hydraulic conditions [2] - these may not be problematic for engineers and network operators but may be insufficient for more complicated hydraulic analyses.

Computational fluid dynamics and water quality modeling

More precise numerical calculations of water quality inside distribution systems covering different, not only chlorine, pollutants propagation in various, dynamic flow conditions (laminar or turbulent), chemical reactions inside the water body with different kinetic rates as well as migration of organic and non-organic compounds of pipe material to water are possible when the *computational fluid dynamics* (CFD) methods are applied [eg 10, 21].

The development of CFD was started in the first two decades of XXth century and it has recently reached high popularity, due to the rapid development of computational techniques. The CFD is now being applied in many branches of science and technology, from dynamic hydraulic of water supply and sanitation systems, water and wastewater treatment [22-24] to food industry [25], different technological processes [26], heating and ventilation [27, 28] and modeling of flow in rivers [29]. This popularity is reflected by availability of many open source and commercial CFD software.

One of the most popular commercial CFD software applied to water quality calculations is FLUENT developed by Fluent Inc., which was required by Ansys in 2006. Since then Fluent is a part of ANSYS engineering modeling software package. FLUENT was frequently reported as a successful tool in various simulation calculations of fluid flow [25, 29-32].

Fluid flow modeling in CFD is based on the following governing equations [29, 33]:

Conservation of mass:

$$\frac{\partial \rho}{\partial t} + \mathrm{div}\rho\mathbf{u} = 0$$

For incompressible fluid flow:

$$\mathrm{div}\rho\mathbf{u} = 0$$

Conservation of momentum

$$\rho\frac{\partial u_i}{\partial t} + \rho u_j\frac{\partial u_i}{\partial x_j} = \frac{\partial \tau_{ij}}{\partial x_j} + \rho g_i$$

Navier-Stokes:

$$\rho\frac{\partial u_i}{\partial t} + \rho u_j\frac{\partial u_i}{\partial x_j} = -\frac{\partial p}{\partial x_i} + \frac{\partial p}{\partial x_j}\left[\mu\left(\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i}\right)\right] + \frac{\partial \tau_{ij}}{\partial x_j} + \rho g_i$$

where: ρ - fluid density, t - time, u - velocity, **u** - velocity tensor, $\boldsymbol{\tau}_{ij}$ - stress tensor.

These equations, with the proper set of computational simplifications, boundary conditions and support of numeric techniques provide for comprehensive modeling tool for various laminar and turbulent, compressible and incompressible fluid, steady state and transient fluid (gas, liquid or multi-phase) flows analyses.

Qualitative calculations in CDF covering transport and mixing of reactive and non-reactive chemical species are usually based on the conservation equations of considered species. The mentioned equation general form for i species may be presented as follows [34]:

$$\frac{\partial}{\partial t}(\rho Y_i) + \nabla(\rho \vec{u} Y_i) = -\nabla \vec{\mathbf{J}}_i + R_i + S_i$$

where: Y_i - local mass fraction of i species, $\vec{\mathbf{J}}_i$ - vector of diffusion flux of species i, R_i - production rate of i species by chemical reaction, S_i - creation rate of i species by addition from dispersed phase or any other possible source.

Thus, the CDF calculations of multiple species transport and mixing in different flow conditions reflect convection, diffusion and reaction sources for each component species. The involved chemical reactions of transporting, mixing and migrating may occur in the bulk phase of fluid, on pipe/tank walls or particle surfaces as well as in porous media. So the most important advantages of CFD application to modeling of water quality in distribution networks should be now visible - the wide choice of pollutants, possibility of simultaneous calculations of multiple chemical processes occurring in water body and on pipe walls as well as in porous filtering bed, concentration of studied pollutants may be observed in any, freely selected, points of studied water system.

Usually, to apply CFD to engineering calculations the *finite element method* (FEM) or *finite volume method* (FVM) is required. Thus, the standard regime of the above-mentioned techniques is enabled: preparing the spatial mesh of finite elements/volumes and assignments of medium parameters, initial and boundary conditions in pre-processor, numerical calculations in model processor (solver) and results viewing and exporting in postprocessor. The appropriate skills represented by software users are required. Generally, CDF software is not suitable for beginners - some level of experience in computer modeling in FEM or FVM is necessary.

Below, in Table 2, the exemplary necessary set of basic input data for flow and species transport-mixing model as well as the possible results of calculations by commercial software Ansys, Fluent [34, 35] are presented.

Table 2

Input data and calculation results of CFD

Input data		**Results**
Material properties	**Flow inputs**	
Density, molecular weights Viscosity Mass diffusion coefficients-Fickian or full multicomponent diffusion User defined scalar diffusivity, isotropic or anisotropic	Flow type: laminar/turbulent, single or multiphase flow, turbulences model and its input data, Transport equations Source and sink terms Species transport type: mixing or non-mixing, species volumetric reactions Constant or time and space dependant boundary conditions: inflow, outflow and wall: mass flow, pressure gradient or distribution, temperature, velocity distribution, species inlet mass fraction, wall surface reactions	Pressure and velocity of flow distribution, Mass and mole fraction of species, Concentrations and molar concentrations of species distribution, Laminar and effective diffusion coefficient of species, Kinetic and turbulent rate of reaction

Summary and conclusions

The presented literature review shows a high potential of CFD application to water quality in distribution systems modeling. One may compute different scenarios, from the simplest ones (eg non-mixing transport of species in simple system) to the most complicated cases (eg mass transport of n-species with volumetric and wall surface reactions inside the complex distribution systems). The presented above advantages of CFD application allow numerical modeling of probably all engineering cases and problems encountered in practice. The popularity of CFD modeling among water distribution systems' designers and operators may be limited by its disadvantages: usually complex structure of open source software, high cost of commercial programs, required experience in data management and simulation calculations, high system and hardware requirements, full range of necessary input data (in dependence to the level of problem simplification). Nonetheless, the CFD's capabilities and range of possible application make it a worthy tool in modeling of water quality in distribution systems.

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OBLICZENIOWA MECHANIKA PŁYNÓW A MODELOWANIE JAKOŚCI WODY

Wydział Inżynierii Środowiska, Politechnika Lubelska

Abstrakt: Modelowanie parametrów jakości wody w systemach dystrybucji jest obecnie stosowane zarówno w procesie projektowania, jak i w eksploatacji sieci wodociągowych. Najczęściej stosowanym w modelowaniu wskaźnikiem jakości wody w sieci jest rozkład chloru. Badania literaturowe wskazują, iż istnieje duża grupa zanieczyszczeń pogarszających jakość wody, a pochodzących z materiału przewodów. W celu numerycznego określenia transportu tych zanieczyszczeń w sieciach wodociągowych wykonanych z różnych materiałów może być zastosowana obliczeniowa dynamika płynów (CFD). W artykule przedstawiono najczęściej identyfikowane w wodzie zanieczyszczenia pochodzące z materiału przewodów wykonanych z tworzyw sztucznych, możliwości zastosowania CFD do modelowania jakości wody oraz niezbędne w tym celu dane wejściowe. Przedstawiono także wady i zalety CFD jako narzędzia do modelowania zmian jakości wody w sieciach.

Słowa kluczowe: jakość wody, modelowanie numeryczne, obliczeniowa dynamika płynów, sieci wodociągowe