

## THE INFLUENCE OF RADICAL REACTIONS ON THE KINETICS OF POLYCYCLIC AROMATIC HYDROCARBONS BIODEGRADATION

### Summary

Progressing from the eighteenth century industrial development, transport and urban areas expansion contribute to the accumulation of polycyclic aromatic hydrocarbons (PAHs) in different components of the environment and their deposition into soils. Due to the established, highly adverse PAHs effects on living organisms, there is a tendency to develop effective remediation technologies which can result in PAHs removing from the environment. The aim of this study was to determine the effect of ozone-initial radical reactions on biodegradation kinetics of selected PAHs by an environmental consortium. The consortium was composed of microorganisms isolated from soil permanently contaminated with organic substances such as PAHs. The decomposition reactions were carried out under aerobic conditions at 25°C for 240h in model systems containing water and PAH (naphthalene, phenanthrene or pyrene) at a concentration of 50 mg/l. The ozonation process was conducted before microbial inoculation and lasted 60 minutes (yield 2-5 g of ozone/h). The final PAHs concentrations were measured by photochemiluminescent method. It was found that pre-ozonation does not result in the death of microorganisms. The biodegradation kinetics was evaluated by a comparison of PAH half-life degradation. The results indicate that radical reactions increase the biodegradation efficiency by 25%. These results demonstrate a good efficiency of combined techniques - ozonation and biodegradation - and can provide the possibility of their application in the protection of agricultural land against degradation.

**Key words:** Polycyclic aromatic hydrocarbons (PAHs), ozonation process, bioremediation process, radical reactions

## WPŁYW REAKCJI RODNIKOWYCH NA KINETYKĘ BIODEGRADACJI WIELOPIERŚCIENIOWYCH WĘGLOWODORÓW AROMATYCZNYCH

### Streszczenie

Postępujący od wieku XVIII rozwój przemysłu i transportu samochodowego oraz ekspansja aglomeracji miejskich przyczyniają się do akumulacji związków z grupy wielopierścieniowych węglowodorów aromatycznych (WWA) w różnych komponentach środowiska oraz ich późniejszej depozycji do gleb. Z uwagi na stwierdzone, wysoce niekorzystne oddziaływanie WWA na organizmy żywe dąży się do opracowania technologii ich efektywnej remediacji. Niniejsza praca miała na celu określenie wpływu reakcji rodnikowych indukowanych ozonowaniem na kinetykę biodegradacji wybranych WWA przez konsorcjum środowiskowe wyizolowane z gleb trwale zanieczyszczonych substancjami organicznymi. Reakcje rozkładu prowadzono w warunkach tlenowych, w temperaturze 25°C przez 240h w modelowych mineralnych układach wodnych z dodatkiem odpowiednio: naftalenu, fenantrenu lub pirenu w stężeniach 50 mg/l. Proces ozonowania wykonano przed mikrobiologicznym zaszczerpieniem układów i trwał on 60 minut (wydajność 2-5 g ozonu/godz.). Pomiaru ubytków WWA dokonano stosując metodę fotochemiluminescencyjną z uprzednią ekstrakcją heksanem. Stwierdzono, iż wstępne ozonowanie nie wpływa letalnie na konsorcjum środowiskowe. Ocena kinetyki biodegradacji dokonana na podstawie porównania czasu połowicznego rozkładu WWA świadczy, iż reakcje rodnikowe zwiększają o średnio 25% efektywność biodegradacji. Powyższe wyniki świadczące o dobrej efektywności kombinowanych technik - ozonowania i biodegradacji - wskazują na możliwości aplikacyjne w ochronie gruntów rolnych przed degradacją.

**Słowa kluczowe:** wielopierścieniowe węglowodory aromatyczne (WWA), ozonowanie, bioremediacja, reakcje rodnikowe

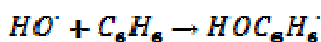
### 1. Introduction

Polycyclic aromatic hydrocarbons are a group of hydrophobic xenobiotics closely related to human activity. Due to the reported mutagenic, carcinogenic and genotoxic properties, PAH may contribute to the imbalance in nature and pose a threat to human health [1]. According to SNAP classification, the main sources of PAHs in Poland are combustion processes in the municipal and housing sectors (87,2%), production processes in industry (11,11%) and

road transport (0,99%) [2]. The distributed nature of the emission indicates that the problem of PAH contamination is not only limited to areas with a high intensity of human activity. This problem is connected also with arable lands thereby contributing to changes in their habitat functions, progressive degradation and reduced agricultural soils suitability [3]. Evaluation of agricultural soils contamination with reference to 13 PAHs carried out by GIOS in 2010 indicated that 35 measurement points were characterized by a low level of contamination (class 2

involves the need to reduce the cultivation of plants for food production designed for children and infants). As many as 18 measurement points were characterized by a high risk of crops (especially root leafy vegetables) contamination (class 3). In the general classification, 8.3% of agricultural soils in Poland are among the group contaminated with polycyclic aromatic hydrocarbons [4].

The European Parliament and the Council of the European Union decided to state the problem of PAHs deposition as one of the priorities of the EU sustainable development. Therefore, it is extremely important to place emphasis on developing remediation technologies. Recently, the ozonation technology as one of the method of in situ PAHs degradation has becoming more and more popular in literature reports. It is based on the oxidation potential of hydroxyl radicals HO<sup>•</sup>. Hydroxyl radicals react by electrophilic addition with benzene rings of polycyclic aromatic hydrocarbons:



The organic radicals with unpaired electron on the carbon atom (carboradicals) are converted to superoxide radicals in the reaction with oxygen:



The above reaction is the first crucial step in organic pollutants oxidation, which further can undergo a mineralization to carbon dioxide, water and mineral acids [5].

Despite many benefits of ozonation, this technique involves high overhead costs, which is particularly economically disadvantageous in the case of soils with low contamination [6]. It has been found that certain compounds of PAHs are not degraded in a chemical way (pyrene, chrysene and benzo(a)pyrene). In addition, oxidants abusing can result in clogging of the aquifer by precipitation of minerals in the pores. Therefore, more and more researchers underline the potential of selected microorganisms to degrade organic contaminants. Enzymatic transformation of the aromatic ring to catechol, protocatechuic acid, gentisic acid or hydroquinone, their conversion to the aliphatic compounds and decomposition into the Krebs cycle intermediates, provides direct distribution of PAH to carbon dioxide and water [7]. With the aid of molecular techniques it was possible to identify a number of bacterial strains capable of degrading PAHs. In order to overcome the natural barrier (extremely low bioavailability) of the PAHs biological degradation, it is important to place emphasis on combined methods.

## 2. Material and methods

### 2.1. Microorganisms

K23 bacterial consortium was isolated from soil in south-eastern Poland. The sample of soil was characterized by persistent petroleum products pollution. The consortium had the ability of metabolizing polycyclic aromatic hydrocarbons as the sole carbon source.



Fig. 1. Distribution of agricultural soils in 2010 in the context of the PAH contamination degree [4]

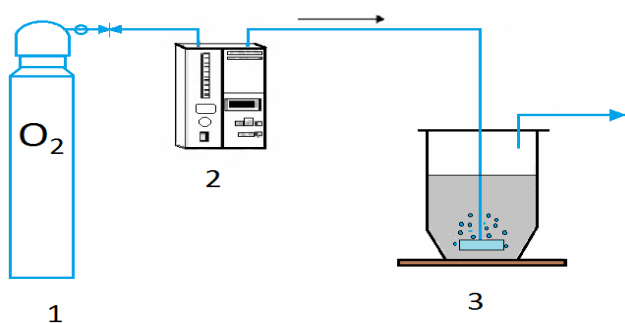
Rys. 1. Przestrzenny rozkład profili gleb użytkowanych rolniczo w 2010 roku w kontekście stopnia zanieczyszczenia sumą 13 związków WWA [4]

## 2.2. Chemical reagents

The standard mineral medium M9 supplemented with micronutrients solution 4ml/l  $MgSO_4 \times 7H_2O$  (50,00 g/l),  $FeSO_4 \times 7H_2O$  (5,00 g/l),  $MnSO_4 \times 4H_2O$  (2,50 g/l),  $ZnCl_2$  (3,20 g/l),  $CaCl_2 \times 6H_2O$  (0,50 g/l),  $BaCl_2$  (0,30 g/l),  $CoSO_4 \times 7H_2O$  (0,18 g/l),  $CuSO_4 \times 5H_2O$  (0,18 g/l),  $H_3BO_3$  (3,25 g/l), EDTA (5,00 g/l), HCl 2 N (73,00 ml/l) PAH solutions (naphthalene, phenanthrene and pyrene) in methanol at concentrations of 5 mg/l were used in all experiments. The 30% hydrogen peroxide was applied into ozonized systems in order to intensify the production of hydroxyl radicals  $OH \cdot$ . All reagents were characterized by the highest commercially available purity and were purchased from commercial producers.

## 2.3. Biodegradation conditions

Biodegradation processes were carried out in an aqueous system (model systems). Bottles were spiked with 50 ml of M9 medium, micronutrients solution and PAH solutions (in a final concentration of PAH - 50 mg/l). Before inoculation, an ozonation process (lasted one hour) was performed with the use of ozone generator KORONA L MED with yield of 2-5 g / h and with hydrogen peroxide in an amount of 1.7 g / l. In order to increase the surface contact between a solution and gas, the ozone was added through a septum of sintered glass (Figure 2).



Source: own work / Źródło: opracowanie własne

Fig. 2. System for ozonation: 1- cylinder stress with oxygen, 2- ozone generator KORONA L MED, 3- rinses containing PAH solution (suspension)

Rys. 2. Układ do ozonowania roztworów WWA: 1- butla ciśnieniowa z tlenem, 2- ozonator KORONA L MED, 3- płuczka z perlatozem zawierająca roztwór (zawiesinę) WWA

Prepared systems were inoculated with consortium K23 (250  $\mu$ l (0.5% v / v)). Biodegradation was carried out for 10 days at 25°C under aerobic conditions. Shaking (rotation of 200 rev/min) was used in order to increase aeration of the medium. The processes were carried out in identical bottles. Furthermore, an additional variant without microorganisms was used as an internal standard.

## 2.4. Determination of the PAHs biodegradation kinetics

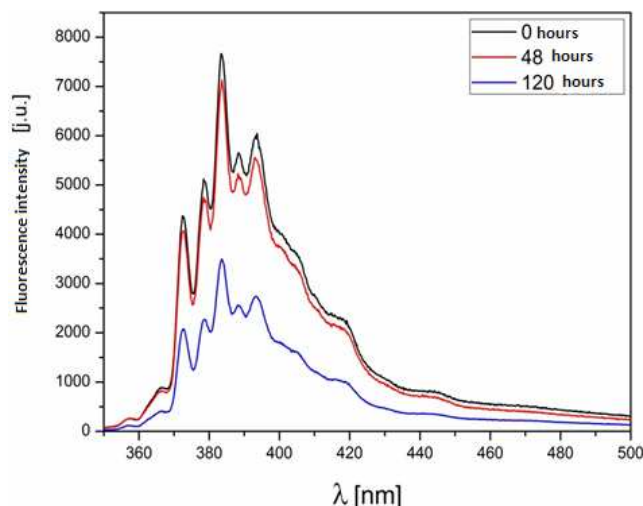
PAHs biodegradation kinetics were determined by measuring the PAHs loss with the use of Hitachi F-7000 Spectrofluorometer. In order to determine the emission wavelength, appropriate spectra were performed. Due to the

fact that concentrations of PAHs in experimental samples were above their solubility (microemulsion systems) three-time extraction with hexane were conducted. The assessment of PAHs concentrations were carried out with reference to the calibration curves.

## 3. Results and discussion

The excitation spectra for naphthalene, phenanthrene, and pyrene allowed to determine the maximum values of the absorption spectra of the studied PAHs (275 nm for naphthalene, 348 nm for pyrene and 340 nm for phenanthrene). Fluorescence spectra of internal standards (systems without microorganisms) proved the absence of the PAHs degradation not connected with biological activity. It can therefore be clearly stated that changes in the concentration of hydrocarbons in the test systems resulted from the activity of microorganisms or the pre-ozonation.

PAHs concentrations in systems with or without pre-ozonation indicate a decrease in fluorescence intensity with biodegradation time. Figure 3 shows an example of fluorescence spectra of pyrene in ozone-free system, whereas Figure 4 presents the spectrum of phenanthrene system with pre-ozonation.



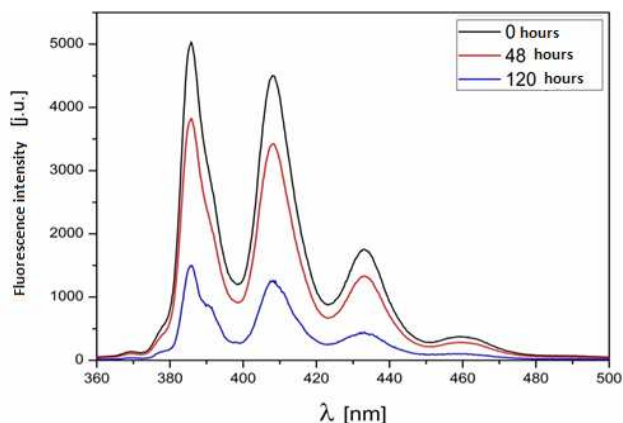
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Fig. 3. Fluorescence spectra of pyrene after 0, 48 and 120 hours from the start of biodegradation

Rys. 3. Widma fluorescencji pirenu ekstrahowanego heksanem po upływie 0, 48 i 120 godzin od momentu rozpoczęcia biodegradacji

Decreases in fluorescence intensity over time indicate the PAHs decreasing in both systems. The pre-ozonation process does not affect lethally on microbial consortium. It appears, therefore, that the products of radical reactions are not a toxic factor towards microorganisms. This statement is in agreement with results obtained by Haapea and Tuhkanen (2005) (ozonolysis in the soil system) [8]. Model ozonation soil samples performed by Bernal-Martinez et al. (2007) also confirm lack of toxic effect towards to microbial consortia [9].

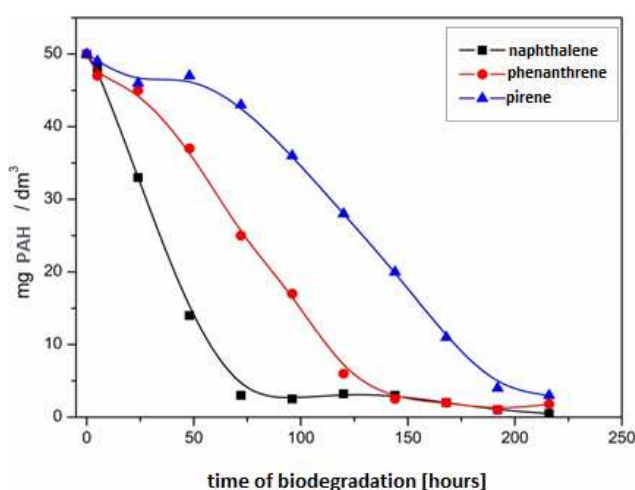
Biodegradation kinetics of PAHs concentrations which were read out based on calibration curves are shown in Figure 5, and the biodegradation kinetics of PAH concentrations in the systems with pre-ozonation are presented in Figure 6.



Source: own work / Źródło: opracowanie własne

Fig. 4. Fluorescence spectra of phenanthrene after 0, 48 and 120 hours from the start of biodegradation

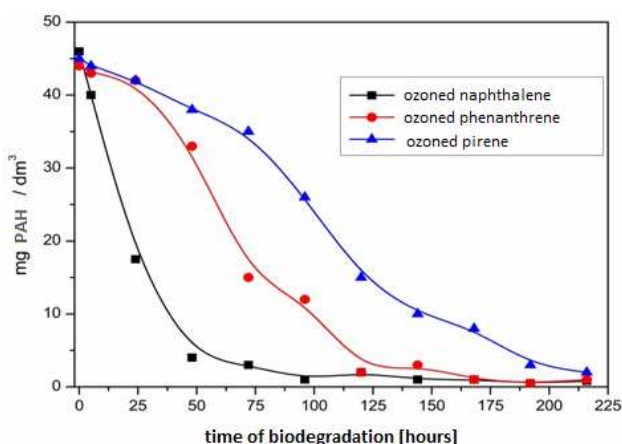
Rys. 4. Widma fluorescencji fenantrenu ekstrahowanego heksanem po upływie 0, 48 i 120 godzin od momentu rozpoczęcia biodegradacji w układzie ozonowanym



Source: own work / Źródło: opracowanie własne

Fig. 5. Decline of PAHs concentration during biodegradation in the aquatic system

Rys. 5. Zanik stężenia badanych WWA w czasie biodegradacji w środowisku wodnym



Source: own work / Źródło: opracowanie własne

Fig. 6. Decline of PAHs concentrations during the biodegradation process in an aqueous system after the initial ozonation

Rys. 6. Zanik stężenia badanych WWA podczas procesu biodegradacji w układzie wodnym po wstępnym ozonowaniu

Obtained results indicate different rates of individual hydrocarbons biodegradation. Naphthalene is the most, while pyrene - the least susceptible to biodegradation by the consortium K23. Tikilili and Nkhalambayausi-Chirwa (2011) [10] present similar trends with regard to PAHs degradation kinetics. It is generally accepted that the rate of PAHs biodegradation decreases with increasing number of aromatic rings. This phenomenon is attributed to the decrease in the bioavailability of the compounds which directly results from the limited solubility in water. It is worth noting that the PAHs concentrations decreased in the systems with ozonation process. The loss of 4-6 mg/l is a direct result of induced radical reactions. In order to quantification and comparison of the decomposition kinetics of the two systems the half-time values of pyrene, naphthalene, phenanthrene biodegradation were defined. The obtained results are shown in Table 1.

Table 1. Half-time degradation of naphthalene, phenanthrene and pyrene in aqueous solutions without and with ozonation process

Tab. 1. Czas połowicznej degradacji naftalenu, fenantrenu i pirenu w roztworach wodnych bez udziału ozonu oraz poddanych ozonowaniu

PAH	Half-time biodegradation [h] (without ozonation)	Half-time biodegradation [h] (after ozonation)	PAH solubility in water [mg/dm <sup>3</sup> ]*
naphthalene	35	26	30
phenanthrene	75	56	1,2
pyrene	130	105	0,076

Source: own work / Źródło: opracowanie własne

Half-time of naphthalene, pyrene and phenanthrene biodegradation in the case of pre-ozonation systems is approximately 25% shorter than in variants without ozonation process. There is also a clearly correlation between PAHs bioavailability and biodegradation rate mentioned above. Relatively soluble naphthalene is degraded (in both variants) almost four times faster than the hardest soluble pyrene.

The positive effect of ozonation on the degradation rate can be explained by the increase in medium aeration which results in improvement of the optimal conditions for microorganisms. The consortium K23 was isolated from soil contaminated with petroleum permanently and thus microorganisms were adapted to the environment characterized by high concentrations of PAHs. Moreover the limited bioavailability of the xenobiotic did not induce toxic effects.

#### 4. Conclusions

Based on the carried out experiments, below are presented main conclusions:

- Ozonation products of polycyclic aromatic hydrocarbons do not exhibit toxicity against a K23 consortium, and thus they do not reduce the biological potential for the enzymatic mineralization of xenobiotic
- The rate of selected PAHs biodegradation by an environmental consortium depends on the number of

aromatic rings, and thus also on solubility in water (closely associated with the bioavailability).

- Short-term ozonation of medium with PAHs slightly contributed to the change in the total content of xenobiotic and improves aerobic conditions. This results in a significant increase of the PAHs mineralization.
- Application of a short-term ozonation and subsequent biodegradation as a combination technique results in the mineralization increase by 25%. Thus, described combine method is worth considering as an alternative to conventional, full ozonation technology.

## 5. References

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