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Biodegradation of selected pollutants in sludge

Key words

Wastewater sludge, contamination, biodegradation, aromatic and polyaromatic hydrocarbons

Abstract

The objective of the project was a laboratory check of biodegradation of sludge from ÚČOV (Central Wastewater Treatment Plant) in Ostrava (Czech Republic) contaminated by PAH/s/, PCB/s/, GRO and DRO. For the laboratory check, pure bacterial cultures of *Rhodococcus sp.* and *Pseudomonas putida* have been used. It is apparent from the laboratory experiments results that after one-month bacterial leaching, applying the bacterium of *Rhodococcus sp.* there is a 93% removal of GRO and DRO, a 97% removal of PAH/s/ and a 64% removal of PCB/s/. Applying a pure culture of *Pseudomonas putida* there is a 94% removal of GRO and DRO, a 93% removal of PAH/s/ and a 51% removal of PCB/s/.

Introduction

Human activities lead to the contamination of our planet by organic and inorganic pollutants. The pollution is spreading and it represents a real threat to a healthy development of mankind, animals and plants. One of the most questionable is the group of persistent — exceptionally resistant substances which have been produced by man in significant amounts in the course of last 50 years.

Biodegradation of hazardous harmful substances in the environment embody significant prospective methods, when complex and ecologically unsound pollutants are decomposed into simpler substances (sound ones) by the action of microorganisms. The principle of

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biodegradation technologies is an optimization of nutrient ratios (to support the growth of selected microorganisms able to degrade the target contaminants) and an application of suitably selected isolated microorganism strains with relevant degradation abilities.

Currently, biodegradation technologies are being improved in an intense way. In the Czech Republic, this trend began to develop after 1989. These are mainly biodegradation technologies designed for the decontamination of soil and water contaminated by oil substances and their derivates, for the decontamination of coal tar-phenol pollutions and last but not least for the decontamination of persistent organic pollutants (xenobiotics). Since 2000 a number of companies dealing with this issue have been active in the Czech Republic (Sezima 2003).

1. Potential biodegradation of aromatic and polyaromatic hydrocarbons

Degradation of organic substances by means of microorganisms makes part of the natural carbon cycle in nature. The process of biodegradation is based on the abilities of microflora to use the present harmful substances as a source of carbon and energy for their own growth. The ability of microorganisms to degrade hydrocarbons has been known since 1895, when growth of yeast fungus on paraffin was described.

More than 200 microorganism species capable of hydrocarbon degradation have been identified. They follow in the order of importance: heterotrophic bacteria, fungi, aerobic bacteria, actinomycetales, phototrophes and oligotrophic bacteria. The most applied bacteria rank in the genera of *Pseudomonas, Arthrobacter, Acinetobacter, Flavobacterium, Alcaligenes, Micrococcus* and *Corynebacterium* (Masák et al. 1992). Intense research in this area confirms that besides bacteria, other microorganisms, including fungi and algae, can be used.

2. The characteristics of *Pseudomonas* bacteria

Pseudomonas bacteria are gram-negative, chemoorganotrophic, aerobe obligate, with aerobically respiratory metabolisms. Some species are facultative chemolithotrophic. They are straight or curved rods. Their dimensions range between 0.5 and 1.0 μm × 1.5—4.0 μm. They move by one or more polar-located flagella. They are arranged mainly individually or in small clusters or chains. They grow under strict aerobic conditions in common culture media, on which they form irregularly large colonies producing water-soluble exopigment (pyocyanine and fluoresceine), which diffuses into the atmosphere and dyes it yellow or blue-green. Older cultures dye dark brown. The temperature range of their growth is 0—42°C; the optimum temperature is 35°C. The enzymatic activity is dependent on ecological conditions out of which the individual strains were isolated. They make use of some sugars, out of which they form acids, but not gas. Many strains oxidize glucose into gluconic acid, 2-keto-gluconic and other acids. The majority of the studied strains reduce

nitrates down to nitrites. They live saprophytically in soil and water. There appears a high affinity with the Vibrio and Xantomonas genera. In total, there are approximately 29 species (Masák et al. 1992).

3. The characteristics of *Rhodococcus* bacteria

These are gram-negative, chemoorganotrophic, aerobe obligate, with aerobically respiratory metabolisms. The cells are of spherical shapes, the average size of the cells fluctuates between 0.5 and 3.5 μm; they appear individually or two and more cells aggregate into irregular clusters, sometimes tetrads or bundles. They grow under aerobic conditions in common culture media, under the optimum temperature of 25—35°C. On the culture media they form shiny colonies with the dimensions of 2—4 μm. Many colonies precipitate pigments of various colours (pink, yellow, orange). In nature, they occur as saprophytes (Masák et al. 1992).

4. The methodology of experimental work

The experimental biodegradation of the selected harmful substances — PAH/s/, PCB/s/, GRO and DRO — was carried out with samples of sludge from Ostrava Wastewater Treatment Plant (ÚČOV), namely by means of pure cultures of *Pseudomonas putida* and *Rhodococcus sp.* bacteria. These microorganism cultures were acquired from the Czech collection of microorganisms with the Natural Science Faculty at the Masaryk University in Brno.

In the course of the cultivation works the following media were made use of:

— liquid medium M1 (Beef extract Broth, peptone, NaCl, distilled water, pH 7.2),
— liquid medium M96 (Mineral Medium with Vitamins, Media, Bacteria, pp 123),
— liquid medium M65 (Mineral Medium for Chemolitotrophic Growth H-3, Media, Bacteria, pp 120).

The check of bacteria viability and an approximate determination of their number were done by means of a microscope. For this task we used the Carl Zeiss Jena "Amplival" microscope and Cyrus I, a cell with a raster for reading the number of bacteria. The enlargement ratio of the microscope ranged from 400 (reading the number of bacteria) up to 1000-fold enlargement (observation of bacteria viability).

Post treatment, the mineralogical composition of the sample was determined by an X-ray diffraction analysis in the laboratory of the Institute of Geological Engineering at VŠB-TU Ostrava. The measurement was carried out using a modernized, fully-automated diffractometer URD-6 (Rich. Seifert-FPM, SRN). With the given samples, the following phases were identified: calcite, dolomite, kaolinite, microcline, muscovite, plagioclase, quartz, vivianite.

The determination of GRO and DRO, PAH/s/ and PCB/s/ was carried out in an accredited laboratory for fuels, waste and water of VÚHU, a.s. Most.

In total, the laboratory experiment lasted four weeks. 100 g of sample, 100 ml of bacterial solution and 500 ml of culture medium were inserted into 1-litre glass vessels which were following closed. Aeration was secured by means of aquarium pumps. The required volume was gradually filled with distilled water. Samples for analyses were taken after one and four weeks.

5. Results of bacterial biodegradation

The results of bacterial leaching with a pure culture of *Rhodococcus sp.* imply that after one-month leaching it is possible to remove 93% of GRO and DRO, 97% of PAH/s/ and 64% of PCB/s/ from the sample. The results are stated in Table 1. It is apparent from the obtained values that application of this bacterial culture is suitable for the degradation of GRO and DRO and PAH/s/. Degradation of PCB/s/ is lower.

The results of bacterial leaching applying a pure bacterial culture of *Pseudomonas putida* imply that after one-month leaching it is possible to remove 94% of GRO and DRO, 93% of PAH/s/ and 51% of PCB/s/ from the sample. The results are stated in Table 2. On the basis of the acquired values, it is clear that application of this bacterial culture is suitable for the degradation of GRO and DRO and PAH/s/. However, degradation of PCB/s/ is lower.

TABLE 1

Course of degradation of selected pollutants by means of *Rhodococcus sp.* bacteria

TABELA 1

Sposób degradacji wybranych zanieczyszczeń za pomocą bakterii *Rhodococcus sp.*

	GRO + DRO [mg/kg solid]	Σ PAH/s/ [mg/kg solid]	Σ 7 congeners of PCB/s/ [mg/kg solid]
Input	3 300	418.6	0.209
Week	880	33.4	0.088
4 weeks	240	12.8	0.075

TABLE 2

Course of degradation of selected pollutants by means of *Pseudomonas putida* bacteria

TABELA 2

Sposób degradacji wybranych zanieczyszczeń za pomocą bakterii *Pseudomonas putida*

	GRO + DRO [mg/kg solid]	Σ PAH/s/ [mg/kg solid]	Σ 7 congeners of PCB/s/ [mg/kg solid]
Input	3 300	418.6	0.209
Week	395	64.6	0.123
4 weeks	200	29.1	0.102

Conclusion

The objective of the project was a laboratory check of biodegradation of PAH/s/, PCB/s/, GRO and DRO from a sample of sludge from ÚČOV (Central Wastewater Treatment Plant) in Ostrava. It is apparent from the acquired results that both bacterial cultures can be used for the given sample. Higher values were obtained in case of *Rhodococcus sp.* bacteria. Better results could be reached by prolonging the period of biodegradation or by more intense biodegradation — adjusting the "magic six" in the bioreactor, or by an application of adapted bacterial cultures.

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RADMILA KUČEROVÁ

BIODEGRADACJA WYBRANYCH ZANIECZYSZCZEŃ W SZLAMACH

Słowa kluczowe

Szlam ze ścieków, zanieczyszczenie, biodegradacja, aromatyczne i policykliczne węglowodory

Streszczenie

Celem przedstawianej pracy było sprawdzenie na skalę laboratoryjną możliwości biodegradacji szlamów z Centralnej Oczyszczalni Ścieków w Ostrawie (Czechy) zanieczyszczonych policyklicznymi węglowodorami aro- matycznymi (PAH), polichlorowanymi biofenylami (PCB), benzynami (GRO) oraz olejami mineralnymi (DRO). Do badań laboratoryjnych wybrano czyste kultury bakterii *Rhodococcus sp.* oraz *Pseudomonas putida.*

Jak wynika z przeprowadzonych doświadczeń, po jednym miesiącu ługowania z użyciem bakterii *Rhodococcus sp.* uzyskano 93% obniżenie zanieczyszczeń benzynami i olejami mineralnymi, 97% obniżenie zanieczyszczeń węglowodowami aromatycznymi oraz 63% obniżenie zanieczyszczeń polichlorowanymi biofenylami. Przy zastosowaniu czystych kultur bakterii *Pseudomonas putida* uzyskuje się 94% redukcję zanieczyszczeń benzynami i olejami mineralnymi, 93% redukcję zanieczyszczeń węglowodowami aromatycznymi oraz 51% redukcję zanieczyszczeń polichlorowanymi biofenylami.