Removal of PAHs from Sewage Sludge

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Summary

The aim of this research is to reduce the quantities of polyaromatic hydrocarbons (PAHs) in the samples of non-hygienised sewage sludge via laboratory biodegradation. A bacterial mixture of Pseudomonas putida and Rhodococcus sp. has been used. Prior to own biodegradation, a proportion of the samples underwent physical pre-treatment in an ultrasonic bath and microwave oven. The laboratory experiment lasted for 28 days and the acquired values were compared with limits stipulated in Decree 294/2005 Coll. The obtained results imply that biodegradation of such contaminated samples is practicable. The most prominent percentage reduction in PAHs occurred in the sludge sample without the physical pre-treatment.

Keywords: biodegradation, polyaromatic hydrocarbons, wastewater treatment plant, sludge, Pseudomonas putida, Rhodococcus sp.

Introduction

Human activities lead to the contamination of the planet by organic and inorganic pollutants. The pollution spreads and endangers the healthy development of mankind, animals and plants [4]. Among the most objectionable pollutants is the group of persistent, exceptionally resistant substances, which have been produced by man in significant quantities in the course of the last 60 years in the form of herbicides, pesticides, insecticides as well as in many industrial products (e.g. capacitator, transformer or hydraulic fillings).

Biotechnologies incorporate all technological procedures of mining, preparation and processing in which microorganisms or the products of their metabolisms are applied in order to acquire a desired qualitative change in the mineral resources and wastes. In principle, nature itself creates the systems and people have used their products unconsciously from the very beginning. Biotechnological applications combine microbiological, biochemical and chemical knowledge.

Decontamination technologies designed in the past were principally expensive and environmentally unfriendly. The tendency is to propose and apply procedures that are both cheaper as well as more natural. One of the prospective routes is biological decontamination of the environment by means of microorganisms and plants.

Potential biodegradation of hydrocarbons

The capacity of microorganisms to degrade hydrocarbons has been known since 1895, when growth of yeast fungus on paraffin was described. Shortly, bacteria's ability to use methane as a source of carbon was discovered. Gradually, it was demonstrated that they are able to degrade practically all components of crude oil and many other hydrocarbons. At present, more than 200 microorganism species capable of hydrocarbon degradation have been identified. Some use only one hydrocarbon (e.g. methane), but we do not know a microbial strain that would degrade a whole range of hydrocarbons abundant, for example, in crude oil. Therefore, it is rather a microbial community that participates in degradation [2].

The general principle of biological degradation of contaminants is the activity of bacteria and sufficient amount of oxygen (aerobic conditions) which leads to the conversion of pollutants into carbon dioxide, water and biomass [5]. Nu-

merous aerobic microorganisms have been described, such as the representatives of the genera *Pseudomonas, Acinetobacter, Corynebacterium, Rhodococcus, Alcaligenes, Achromobacter, Arthrobacter, Nocardia, Bacillus*, etc. [1]. Under anaerobic conditions pollutants are converted due to metabolic processes into methane and a limited quantity of carbon dioxide and hydrogen [8]. So far, only few anaerobic bacteria have been isolated, most frequently they are microorganisms participating in the biodegradation of highly chlorinated compounds. They rank among the genera of *Desulfomonile, Clostridium, Desulfitobacterium*, etc. [1].

Polyaromatic hydrocarbons

Polyaromatic hydrocarbons belong among persistent organic pollutants. Their molecules are made up of two or more condensed benzene nuclei. Bonding of other substituents (e.g. halogen, sulfo, amino, nitro) onto the benzene nucleus considerably decreases the reactivity of the nucleus' resonance structure towards oxygen and the ring becomes more resistant to breaking [3].

The group is represented by 280 basic hydrocarbons, but the number rises due to the number of isomers [8]. Most frequently 16 polyaromatic hydrocarbons are observed which are listed among the priority pollutants of US EPA: naphthalene, acenaphtylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3,c,d)pyrene, dibenz(a,h)anthracene and benzo(g,h,i)perylene [11].

The characteristics of Pseudomonas bacteria

In total, there are approximately 29 species. *Pseudomonas* bacteria are gram-negative, chemoorganotrophic, aerobe obligate. Some species are facultative chemolithotrophes. They are straight or curved rods. Their dimensions range between 0.5 and 1.0 μm x 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 irregular colonies producing water-soluble exopigment 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 – 42ºC; the optimum temperature is 35ºC. The enzymatic activity depends on the eco-conditions out of which the individual strains were isolated. They make use of some sugars, out of which they form acids, but not gas. The majority of the studied strains reduce nitrates down to nitrites. They live saprophytically in soil and water. Recently, the literature has suggested that some species appear to be potential pathogens [10].

The characteristics of Rhodococcus bacteria

These are gram-negative, chemoorganotrophic, aerobe obligate. The cells are of spherical shapes, the average size of the cells fluctuates between 0.5 and 3.5 μm; they occur 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 ºC. On the culture media they form shiny colonies with the dimensions of 2 – 4 μm. Many colonies precipitate pig-

Fig. 1.Pseudomonas putida [6]

Rys. 1. Monad putida [6]

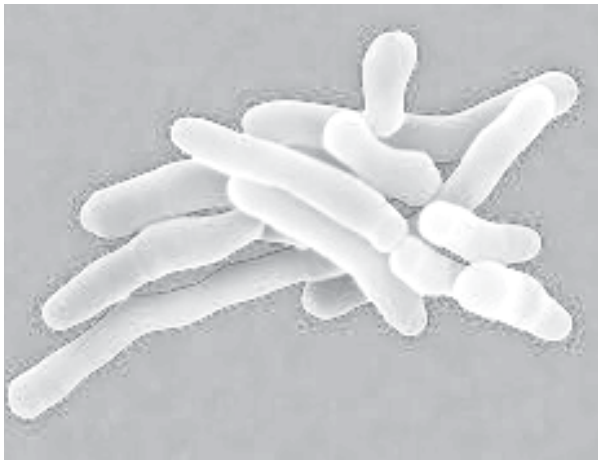

Fig. 2. Rhodococcus sp. [9]

Rys. 2. Rhodococcus sp. [9]

ments of various colours (pink, yellow, orange). Catalase is positive, and glucose is oxidized only partially [10].

Sewage sludge

Sewage sludge is a mixture of inert organic substances (live and dead cells of microorganisms participating in wastewater treatment processes, such as water purification or stabilisation of sludge) and inorganic components [12]. Sludge may be defined as an aqueous suspension of solid and colloid particles of organic and inorganic substances. Sewage sludge contains:

- Non-toxic organic substances amounting to 60% of dry mass (carbohydrates, fats, proteins, waxes, etc.), and nitrogen and phosphorus compounds.
- Toxic substances, such as heavy metals (Zn, Pb, Cu, Cd, Hg, As), or polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pesticides, polyphenols, etc.
- Wastewater treatment and other microorganisms, including pathogenic ones.
- Minerals, such as quartz, feldspar, carbonates, Fe-oxides.
- Water [7].

The Methodology of Laboratory Biodegradation

Samples of non-hygienised sewage sludge underwent laboratory biodegradation in the laboratories of the Institute of Environmental Engineering of VŠB-TUO. Chemical analyses were carried out in the laboratories of T. G. Masaryk Water Research Institute (VUV) in Ostrava. The mineralogical composition of the sludge was determined in the laboratories of the Institute of Geological Engineering of VŠB-TUO.

For sludge biodegradation we used the bacterial mixture of *Pseudomonas putida* and *Rhodococcus* sp. (Figures 1 and 2) in 1:1 proportion. The microorganism cultures were acquired from the Czech collection of microorganisms with the Natural Science Faculty at the Masaryk University in Brno. The nutrients for cultivation were supplied from culture media: M1 pro *Pseudomonas putida* and M96 for *Rhodococcus* sp.

Two-litre beakers were used as 'bioreactors'. Aerobic conditions were ensured by aquarium air pumps. For biodegradation tests the individual bioreactors were filled with 100 g of sludge, 500 ml of culture medium (250 ml : 250 ml), 100 ml of bacterial solution, or a mixture of both the solutions amounting to 50 ml. There was also a control sample representing 100 g of sludge sample and 600 ml (300 ml : 300 ml) of culture media or distilled water.

Prior to biodegradation, a proportion of the samples had undergone physical pre-treatment in the laboratories of VUV. Figure 3 below shows the equipment used for the pre-treatment, namely a focused microwave oven and an ultrasonic bath.

Sample designation:

- Sludge sample + pre-treatment in an ultrasonic bath + bacteria (US+BIO)
- Sludge sample + pre-treatment in a microwave oven + bacteria (MW+BIO)
- Sludge sample + bacteria (BIO)
- Control sample + medium (K with medium)
- Control sample + distilled water (KK with distilled water).

Biodegradation lasted for 28 days. Next, the samples were filtered, dried and sent for analyses to VUV.

Fig. 3. Equipment for sample pre-treatment (ultrasonic bath, focused microwave oven PLASMATRONIC)

Rys. 3. Wyposażenie do obróbki próbki (wanna ultradźwiękowa, ustawiona kuchenka mikrofalowa PLASMATRONIC)

Tab. 1. The analyses results for sludge samples prior to and post the biodegradation process in mg/kg of dry mass

Tab. 1. Analiza wyników próbek odpadów przed i po procesie degradacji w mg/kg suchej masy

Sample designation/Indicators	Sludge - input	US+BIO	MW+BIO	BIO	K with medium	KK with distilled water
Dry mass in %	29.1	30.7	37.1	30.8	22.9	22.7
EOX	13					
PCB 28	0.016					
PCB 52	0.014					
PCB 101	0.003					
PCB 118	0.006					
PCB 153	0.028					
PCB 138	0.027					
PCB 180	0.024					
SUM of PCBs	0.118					
Naphthalene	8.02	1	0.97	1.1	1.41	2.43
Acenaphthene	47.1	23.6	22.7	16	22.9	25.3
Fluorene	14.7	8.62	7.86	4.6	8.09	8.75
Phenanthrene	21.8	8.46	6.45	3.8	7.9	9.56
Anthracene	2.66	1.39	0.87	1.2	2.03	2.01
Fluoranthene	8.59	7.03	6.12	7.3	7.86	7.59
Pyrene	5.11	3.84	3.57	4	4.77	4.76
Benzo(a)anthracene	4.71	5.55	4.27	4.9	4.85	4.99
Chrysene	4.71	4.06	3.25	4	4.46	4.18
Benzo(b)fluoranthene	8.49	8.55	7.48	8.5	8.78	8.86
Benzo(k)fluoranthene	3.96	4.42	3.7	4.3	4.38	4.51
Benzo(a)pyrene	8.04	8.44	7.53	8.7	8.76	8.47
Dibenzo(a,h)anthracene	1.41	5.08	1.08	1.4	1.46	1.31
Benzo(g,h,i)perylene	4.73	1.33	4.34	5.1	4.88	5.12
Indeno(1,2,3,c,d)pyrene	4.02	3.6	3.02	3.5	4.28	4.22
SUM of 15 PAHs	148	95	83.2	78.4	96.8	102
SUM of 12 PAHs	85	61.5	51.6	56.4	64.6	66.8

Tab. 2. The results in the samples of filtrates in ng/l

Tab. 2. Wyniki próbki przesączu w ng/l

Sample	US+BIO	MW+BIO	BIO	K	KK
designation/Indicators				with medium	with distilled water
Naphthalene	<0.005	<0.005	<0.005	0.012	0.005
Acenaphthene	<0.005	<0.005	<0.005	0.01	0.035
Fluorene	<0.005	<0.005	<0.005	0.005	0.004
Phenanthrene	0.011	0.03	0.018	0.036	0.045
Anthracene	<0.002	<0.002	<0.002	0.002	0.007
Fluoranthene	0.033	0.023	0.023	0.073	0.108
Pyrene	0.028	0.021	0.02	0.097	0.11
Benzo(a)anthracene	0.011	0.003	0.003	0.032	0.009
Chrysene	0.011	0.004	0.006	0.03	0.01
Benzo(b)fluoranthene	0.049	0.024	0.028	0.137	0.077
Benzo(k)fluoranthene	0.021	0.014	0.015	0.059	0.04
Benzo(a)pyrene	0.041	0.026	0.032	0.135	0.066
Dibenzo(a,h)anthracene	0.008	0.007	0.004	0.023	0.03
Benzo(g,h,i)perylene	0.035	0.038	0.033	0.106	0.128
Indeno(1,2,3,c,d)pyrene	0.021	0.026	0.025	0.065	0.075
SUM of 15 PAHs	0.278	0.225	0.216	0.822	0.749

Results and Discussion

The mineralogical composition of the sludge implies that the sample predominantly contains amorphous phase.

Evaluation of laboratory biodegradation

The laboratory analyses of the samples concentrated on the input compositions, outputs and identification of the efficiency within 4-week degradation applying a mixed bacterial culture of *Pseudomonas putida* and *Rhodococcus* sp. in 1:1 proportion. With regard to the characteristics of the given sample, only polyaromatic hydrocarbons (PAHs) were determined due to the fact that the input analysis identified their abundances as the highest.

It is apparent from Table 1 that in the input sample the most abundant is acenaphthene (32%), followed by phenanthrene (15%) and fluorene (10%).

The ultrasonic physical pre-treatment of the sewage sludge sample was carried out in a laboratory SONIC cleaner, U-3STH model, ultrasound output 2x180 W, heating output of 330 W, ultrasonic frequency 40 kHz, experiment length of 60 minutes. After degradation of the US+BIO sample, acenaphthene decreased from 32 to 25%, phenanthrene from 15 to 9%, and fluorene from 10 to 9%.

The focused microwave pre-treatment of the sewage sludge sample was executed in a laboratory microwave oven with focusation, PLASMATRONIC, 250 Watt, secondary energy 75 kJ, length of experiment - 5 minutes. After degradation of the MW + BIO sample, the content of acenaphthene decreased from 32 to 27%, phenathrene from 15 to 8%, and fluorene originally amounting to 10% hardly decreased.

The third sewage sludge sample underwent bacterial degradation only, without any pre-treatment. For biodegradation we used the identical mixed culture of Pseudomonas putida and Rhodococcus sp. The results reveal a reduction in the content of acenaphthene from 32 to 21%, of phenanthrene from 15 to 5%, and of fluorene from 10 to 6%.

Having compared the results of the third sample with the two previous sample results, it is clear that the best degradation effect occurred when applying the mixed bacterial culture without any pre-treatment.

The next analysed sample is the control sample and the medium. The percentage abundance of selected PAHs decreased from 32 to 24% in acenaphthene, from 15 to 8% in phenanthrene, and from 10 to 8% in fluorene.

The last analysed sample is the second control sample and distilled water. The percentage abundance of acenaphthene fell from 32 to 25%, of phenathrene from 15 to 9%, and of fluorene from 10 to 9%.

Evaluation of chemical analyses

The following samples were supplied into the laboratory for chemical and physical analyses at VUV:

1. A sample of non-hygienised and dewatered sludge (input).
2. 3 samples of sludge after biodegradation experiments (samples labelled as US + BIO, MW + BIO, and BIO).
3. 2 control sludge samples for biodegradation experiments (samples labelled as K with medium, KK with distilled water).
4. 3 samples of sludge filtrate post biodegradation (samples labelled as US + BIO, MW + BIO, and BIO).
5. 2 samples of control sample sludge filtrate from biodegradation experiments (samples labelled as K with medium, KK with distilled water).

The sample of non-hygienised and dewatered sludge used as input material for the experiments applying biodegradation processes was analysed for the indicators of PAHs, PCBs and EOX as shown in Table 1. This was executed in order to identify the concentrations of analytes characterising the sludge as for contamination by the above mentioned pollutants prior to own biodegradation tests.

The samples after biodegradation applying bacterial microorganisms and after biodegradation preceded by physical-chemical pre-treatment of sewage sludge (by the action of microwave radiation and ultrasound), and parallel control tests were tested for PAHs only, in order to intercept changes in the concentrations of the monitored substances during the biodegradation process. PCBs were not determined in the samples after biodegradation with regard to their low values in the input sample.

In total, 15 polyaromatic hydrocarbons were determined. For the purposes of sludge pollution evaluation the sum of 12 PAHs was evaluated, subject to Appendix 10 of Decree 294/2005 Coll. To evaluate the biodegradation of PAHs, a sum of 15 PAHs was used. Having compared the value of the sum of 12 PAHs in the sludge prior to and post

Tab. 3. Percentage reduction in the concentration of PAHs post biodegradation in contrast to the input

Tab. 3. Procentrowa redukcja stężenia WWA po biodegradacji w porównaniu ze stanem początkowym

Sample designation/Indicators	US+BIO	MW+BIO	BIO	K with medium	KK with distilled water
Naphthalene	87.5	87.9	86.3	82.5	70
Acenaphthene	48.9	51.1	66	51.1	46.8
Fluorene	42.7	47.3	69.3	46	41.3
Phenanthrene	61.4	70.5	82.7	64.1	56.4
Anthracene	48.1	67.8	55.6	25.9	25.9
Fluoranthene	18.6	29.1	15.1	8.1	11.6
Pyrene	25.5	29.4	21.6	5.9	5.9
Benzo(a)anthracene	19.1	8.5	4.3	4.3	6.4
Chrysene	12.8	29.8	14.9	4.3	10.6
Benzo(b)fluoranthene	1.2	11.8	0	3.5	4.7
Benzo(k)fluoranthene	10	7.5	7.5	10	12.5
Benzo(a)pyrene	5	6.3	8.7	10	6.3
Dibenzo(a,h)anthracene	7.1	21.4	0	7.1	7.1
Benzo(g,h,i)perylene	8.5	8.5	8.5	4.3	8.5
Indeno(1,2,3,c,d)pyrene	10	25	12.5	7.5	5
SUM of 15 PAHs	35.5	43.5	47	34.3	31.1
SUM of 12 PAHs	27.6	39.3	33.6	24	21.4

biodegradation, a very high excess of permissible concentration of PAHs was discovered. The input concentration went over the limit 15 times and the concentration after biodegradation exceeded the limit 9.4 times on average. These are very high contamination values.

The sludge filtrates from the experiments and sludge filtrates from the parallel control tests (identical conditions in the reactor, but without any microorganisms or physical-chemical pre-treatment of sludge before biodegradation) were analysed for polyaromatic hydrocarbons only – see Table 2. This analysis was carried out to check or exclude the transfer of analytes from the sample solid phase into the liquid one.

The obtained data of sludge prior to and post biodegradation experiments were compared with the limit concentrations of pollutants in Appendix 10 of Decree 294/2005 Coll. in order to assess the application of sludge as the feed material for the production of solid alternative fuel (SAF).

The results, i.e. concentration values of the observed PAHs in the analysed samples, were evaluated in terms of variations in the concentrations of indicators during the biodegradation process with and without the physical-chemical pre-treatment. The obtained concentration values post biodegradation were compared with the indicator concentration values prior to the process. Table 3 shows the relevant results.

Evaluation of genotoxic risks

To determine a possible genotoxic risk of the input sample of non-hygienised and dewatered sludge, we used the Ames fluctuation test in two variants, i.e. with/without metabolic activation (S9+ and S9-) in order to identify indirect mutagens or promutagens. The detection organisms were the strains of *Salmonella typhimurium* TA 98 and TA 100. The input sample was positive for the presence of genotoxic substances in both test variants. Both the strains registered the presence of genotoxic substances in the sample. The limit concentration to detect mutagenic substances by the strain of *Salmonella typhimurium* TA 98 was 250 ml/l. In the case of the strain TA 100 the positive result was manifested all the way to the concentration of 31.25 ml/l.

After biodegradation of the sewage sludge applying different procedures, five samples were tested for the presence of mutagenic substances, namely US + BIO, MW + BIO, BIO, K with medium, and KK with distilled water.

In the sample labelled US + BIO post biodegradation there was a positive result applying the strain *Salmonella typhimurium* TA 100 in both the test variants, i.e. both S9+ and S9-. The abundance

Tab. 4. Evaluation of genotoxic risks

Tab. 4. Ocena ryzyka genotoksycznego

Sample	Strain of *Salmonella typhimurium*			
	Without S9		With S9	
	TA 98	TA 100	TA 98	TA 100
Non-hygienised and dewatered sludge (Input)	**pos**	**pos**	**pos**	**pos**
Sewage sludge post biodegradation US + BIO	neg	**pos**	neg	**pos**
Sewage sludge post biodegradation MW + BIO	neg	neg	neg	neg
Sewage sludge post biodegradation BIO	neg	**pos**	neg	neg
Sewage sludge post biodegradation K with medium	neg	**pos**	neg	neg
Sewage sludge post biodegradation KK with distilled water	neg	**pos**	**pos**	**pos**

of genotoxic substances was experienced up to the concentration of 31.25 ml/l, which corresponds to the identical results prior to biodegradation.

The sample MW + BIO was negative for the presence of genotoxic substances in both test variants. However, this sample was the only one where mutagenic substances were not registered post sludge biodegradation.

The next sample, labelled as BIO, had a positive reaction for the presence of genotoxic substances in the test variant without the metabolic activation using the strain *Salmonella typhimurium* TA 100.

Similar results in the genotoxicity test were detected with the sample labelled as K with medium. There, the mutagenic substances were manifested in higher concentrations, namely 250 ml/l.

In the sample KK with distilled water using all the 5 tested samples of sludge post biodegradation, genotoxicity was detected both using the S9+ and S9- tests as well as both the salmonella strains. Only the variant without metabolic activation and the strain TA 98 there was a negative result.

Using the strain TA 100 without the metabolic activation mutagenic substances were detected in all the samples, except for sample MW + BIO – see Table 4. The test with metabolic activation using the identical strain was positive only in two cases, i.e. samples US + BIO and KK with distilled water.

The strain *Salmonella typhimurium* TA 98 appeared as less sensitive when applied with the sewage sludge and only a single positive result was detected, namely in the test S9+ and sample KK with distilled water.

Conclusion

Degradation of pollutants, which somehow disrupt the natural life cycle, from the environment belongs among most up-to-date and discussed topics. Biodegradation may be ranked among methods that are frequently used for such purposes. However, own application of biodegradation in the field or industry must be preceded by laboratory research.

The objective of the experimental section was to verify the reduction of the pollutant contents in the samples of non-hygienised sewage sludge. Two sludge samples underwent pre-treatment in an ultrasonic bath and a focused microwave oven prior to own biodegradation. Only the bacterial culture was applied with one of the samples. The remaining samples served as controls, either with medium or with distilled water. For biodegradation we used the mixed bacterial culture of *Pseudomonas putida* and *Rhodococcus* sp. in 1:1 proportion.

The major indicators were the most abundant congeners, namely acenaphthene, phenanthrene and fluorene. The results imply that the most prominent percentage reduction of PAHs occurred in the sample of sludge without physical pre-treatment. A rather low percentage reduction of PAHs using the combined technology may be attributed to a significant drop in the original bacterial activation, which plays a positive role in the biodegradation process. Therefore, physical pre-treatment has substantial hygienisation effects and will be subject to further experimental research.

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Usuwanie wielopierścieniowych węglowodorów aromatycznych z osadów ściekowych

Przedmiotem badań jest redukcja ilości wielopierścieniowych węglowodorów aromatycznych (WWA) z próbek nieoczyszczonych osadów ściekowych w laboratorium biodegradacji. Użyto mieszaniny bakterii Pseudomonas putida *oraz* Rhodococcus sp. *Przed naturalną biodegradacją część próbek poddano fizycznej obróbce w wannie ultradźwiękowej oraz kuchence mikrofalowej. Eksperyment laboratoryjny trwał 28 dni, następnie otrzymane wartości zostały porównane z wymogami w Rozporządzeniu 294/2005 zbioru ustaw. Otrzymane wyniki wskazują, że biodegradacja tak zanieczyszczonych próbek jest możliwa. Największy procentowy spadek WWA zauważono w próbce nie poddanej fizycznej obróbce.*

Słowa kluczowe: biodegradacja, wielopierścieniowe węglowodory aromatyczne, oczyszczalnia ścieków, Pseudomonas putida, Rhodococcus sp.