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SCREENING OF MICROORGANISMS ABLE TO BIODEGRADE ETHYLBENZENE

New bacteria able to decompose ethylbenzene were searched for. Four active strains were isolated from used compost biofilters. They degraded styrene and exhausting gases from wire production plant as well as fresh compost. Their suspensions in mineral medium degraded ethylbenzene at the rates of about 6–56 g/m^3/h. The most active bacteria were identified as *Bacillus pumilus* and *Rhodococcus rhodochrous*. The decomposers of styrene isolated in our previous research were not able to degrade ethylbenzene.

1. INTRODUCTION

More and more attention has been recently paid to the hazardousness of volatile organic substances emitted into the environment. Transport, dye and lacquer production, chemical, oil, coke, pharmaceutical industries, etc., are main anthropogenic sources of emitting these compounds. The pollutants are danger not only because of their primary toxicity, but also because of the fact that many of them are converted in the air which often leads to a significant increase in the toxicity of the pollutants emitted.

Those hazardous emissions may be reduced in many ways, both traditional and new. In general, they are expensive and often affect natural environment. One of the environmentally friendly and economical methods is the use of natural ability of microorganisms to biodegrade organic substances.

The aim of the research was to search for new bacteria strains able to decompose ethylbenzene and to characterize their activity.

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2. METHODS

2.1. ISOLATION OF MICROORGANISMS

At first, the styrene-decomposing microorganisms that had been isolated from compost playing a role of biofilter bed installed in paint shop of wire production plant "Załom" near Szczecin were used [1]. The microorganisms used in a subsequent research were isolated from used composts of two biofilters: a) degrading styrene and b) removing contaminants from exhaust gases in wire production plant. Also bacteria isolated from fresh compost composed of municipal and industrial wastes (achieved from compost production plant in Racula) were applied. Aliquots of 2 g of compost samples were introduced into 150 cm^3 of nutrient solution according to KOJIMA et al. [2] (with no agar and yeast extract addition) and placed in 250 cm^3 scrubber. The scheme of the system applied is presented in figure 1. The solution turbidity increased considerably and ethylbenzene concentration in the air stream leaving the scrubber decreased markedly after several days of passing the mixture of air with ethylbenzene, the former in 500–600 mg/m^3 concentration, through the scrubber. Then suspensions were taken from scrubbers and inoculated onto Koijma's solid medium [2] with ethylbenzene as a single source of carbon (0.5, 1.0 or 1.5 cm^3/dm^3 of medium). Bacterial cultures were incubated for 10 days in 30 dm^3 desiccator, where a vial filled with 5 cm^3 of ethylbenzene was also placed. After 10 days, the population size was measured and then the microorganisms that differed morphologically were isolated. Suspensions from scubbers were also used as inoculum in degradation activity tests.

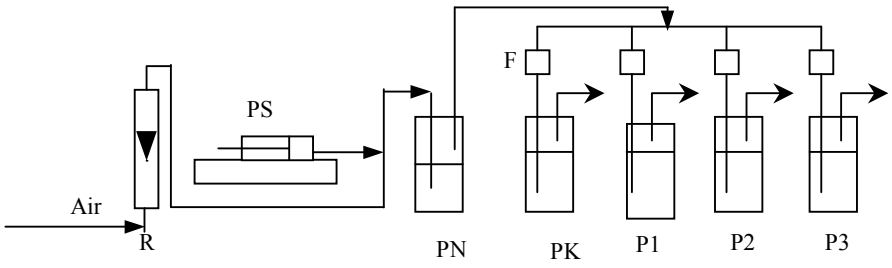

Fig. 1. Schematic representation of the experimental setup; R – air flowmeter; PS – ethylbenzene syringe pump; PN – moistener (scrubber filled with water); F – bacteria filters; PK – control scrubber, P1–P3 – scrubbers with bacterial culture; S – outlet sampling ports

2.2. DEGRADATION ACTIVITY TEST – SCRUBBER EXPERIMENT

The biodegradation of ethylbenzene vapours in the mixture with air was tested in the set consisting of 4 reactors filled with microorganism suspension in mineral me-

dium (figure 1). Four parallel scrubbers (reactors), each of 250 cm^3 capacity, were used. Volumes of 150 cm^3 of mineral medium plus 2 cm^3 of the suspension of microorganism consortium or of the strain tested (inoculum) were introduced into each reactor. Vaccines containing microorganism consortia were the suspensions taken from scrubbers at the stage of bacteria isolation (chapter 2.1). Inoculum consisting of bacteria strains was obtained by means of washing away bacteria present on slants with 3 cm^3 of 0.85% physiological salt (NaCl). The mixture of ethylbenzene and air in various concentrations were passed through each reactor at the rate of 9–14 dm^3/h. In the case of microorganism consortia, the ethylbenzene concentrations ranged from 400 to 680 mg/m^3; for strains, the concentrations were changed from 525 to 2090 mg/m^3. In order to protect cultures in scrubbers against infection by microorganisms present in the air, the bacterial filters Anotop 25 (0.2 μm, Whatman) were installed before reactors. The process progress was assessed based on chromatographic analysis (gas chromatograph system Chrom 4) which consists in determining the ethylbenzene concentrations in air stream after a control scrubber and the scrubbers containing cultures [1]. The gas chromatograph was equipped with a flame ionization detector and a stainless steel column packed with Chromosorb W-HP, 60/80 mesh. In the column, whose length and inside diameter were 1.5 m and 3 mm, respectively, a temperature reached 110 °C. Nitrogen supplied to the column at the rate of 40 cm^3/min was used as carrier gas. Based on chromatographic results and the data referring to the flow of air and ethylbenzene mixture through scrubbers, it was possible to calculate the mass loading of a scrubber with ethylbenzene, total biofiltration efficiency and the Chromosorb ability to eliminate pollutants (specific biofiltration rate) according to following formulae:

$$M = \frac{G \cdot C_1 \cdot 10^{-3}}{V}, \tag{1}$$

$$S_u = \frac{(C_1 - C_2)}{C_1} \cdot 100, \tag{2}$$

$$EC = \frac{G \cdot (C_1 - C_2) \cdot 10^{-3}}{V} \tag{3}$$

where:

C_1, C_2 – the concentration of ethylbenzene at the inlet to/outlet from the column (mg/m^3),

G – the flow rate (m^3/s),

V – the suspension volume (m^3),

M – the mass loading of the scrubber with ethylbenzene ($g/m^3/s$),

S_u – the biodegradation efficiency (%),

EC – the biodegradation rate ($g/m^3/s$).

3. RESULTS AND DISCUSSION

3.1. ISOLATION OF MICROORGANISMS

The results of determining the size of microorganism population cultured in the scrubbers with a liquid nutrient solution depended on the conditions of determination. The size of the populations of bacteria inoculated onto a solid medium enriched with ethylbenzene in the concentration of 0.5 and 1.5 cm^3/dm^3 of medium ranged from 130 to 150 thousands of CFU per 1 cm^3 of culture. The size of the population inoculated onto the medium with 1 cm^3/dm^3 of ethylbenzene addition was about twice the size of the above one. In total, four bacteria strains able to utilize efficiently ethylbenzene as the only carbon and energy source were isolated from fresh and used composts. Two most active strains i.e., *Bacillus pumilus* and *Rhodococcus rhodochrous*, were identified by means of MIDI method and 16S rRNA sequencing at the Microbial ID Laboratory (Newark, DE, USA).

3.2. DEGRADATION ACTIVITY TEST – SCRUBBER EXPERIMENT

Culturing bacteria onto nutrient solutions in the scrubbers being flushed by the air with ethylbenzene vapours was possible. No microorganism growth was observed in a control scrubber. None of 15 strains able to decompose styrene degraded structurally similar ethylbenzene. Probably the microorganisms degrading styrene were not capable of opening the benzene ring, but they could attack its unsaturated side chain [3]. Up to 40% of substrate were decomposed in scrubbers (reactors) inoculated with microorganism consortia and isolated strains that degraded ethylbenzene; maximum biodegradation rate exceeded 55 $g/m^3/h$. Detailed results for two selected consortia are presented in figure 2, and for identified strains – in figure 3. Mean rates of ethylbenzene biodegradation were about 6 and 9 $g/m^3/h$ for consortia occupying biofilter from wire production plant and from fresh compost, respectively. Microbial consortia originated from biofilter degrading styrene behaved in the way similar to those from wire production plant biofilter. Bacterial isolates degraded ethylbenzene more efficiently than microorganism consortia. In the latter case, mean values of bio-degradation rates and its efficiencies for both strains, i.e., *Bacillus pumilus* and *Rhodococcus rhodochrous*, were 15 $g/m^3/h$ and 20%, respectively. It is possible that higher rates of ethylbenzene decomposition by isolates, at least partially, resulted from higher inlet concentrations of substrate when testing single strains. Higher concentrations at similar flow rates affected the load increase, which stimulated an increase in degradation rate [4]. Mean values of biodegradation rate achieved for all strains tested that decompose ethylbenzene, and especially for the strains identified, are similar or higher than those found by other authors for ethylbenzene and similar substrates [5]–[10]. Optimization

of such process conditions as the choice of an appropriate medium and making the process continuous, which would ensure a constant medium renewal and removal of non-decomposed metabolites, should improve significantly the biodegradation rate and efficiency. However, these issues were not within the range of present research. Based on the results achieved by NEAL and LOEHR [11] as well as our own experiments, we can expect that the biodegradation rates close to the maximum values obtained by us may be also predicted for gaseous ethylbenzene-degrading biofilters.

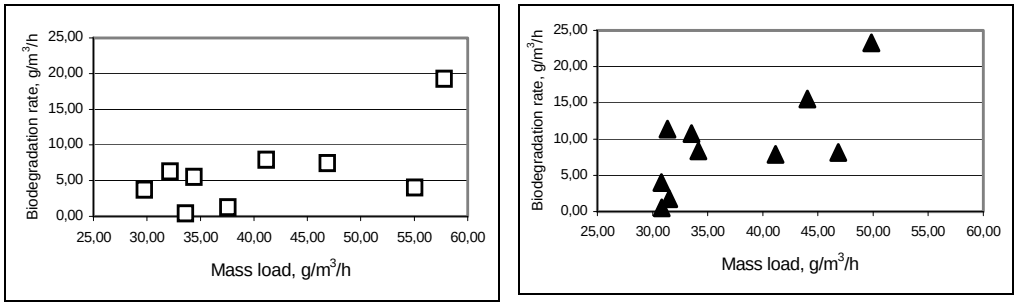

Fig. 2. The rate of ethylbenzene removal versus the mass load of microorganism culture:
a – microorganisms from biofilter installed at wire plant, b – microorganisms from fresh compost

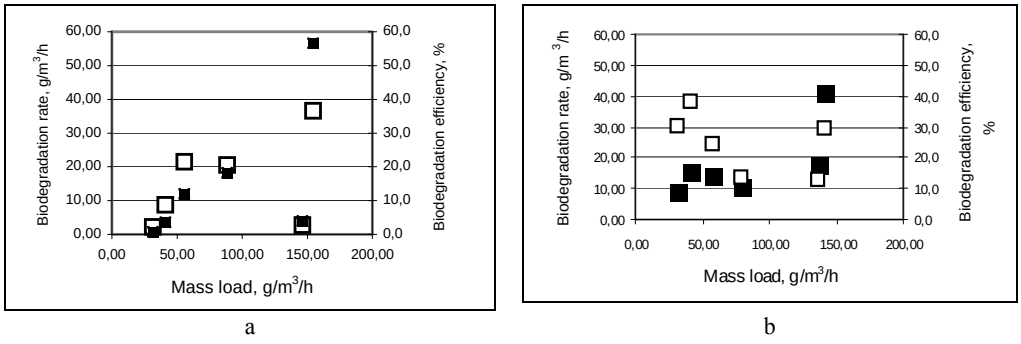

Fig. 3. The rate of ethylbenzene removal and its efficiency versus the mass load for *Bacillus pumilus* (a) and *Rhodococcus rhodochrous* (b), ■ – rate, □ – efficiency

4. CONCLUSIONS

1. Ethylbenzene is a compound that can be relatively easily biodegraded under aerobic conditions by bacteria living in compost, including *Bacillus pumilus* and *Rhodococcus rhodochrous.*

2. Isolated bacteria strains degrade ethylbenzene in liquid mineral medium at mean

rates ranging from about 6 to about 18 g/m^3/h and the maximum rate exceeding 56 g/m^3/h.

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SKRINING MIKROORGANIZMÓW ZDOLNYCH DO BIODEGRADACJI ETYLOBENZENU

Poszukiwano nowych bakterii zdolnych do rozkładu etylobenzenu. Z przepracowanych złóż kompostowych biofiltrów degradujących styren i zanieczyszczenia gazów odlotowych z fabryki kabli oraz ze świeżego kompostu wyizolowano cztery aktywne szczepy. Ich zawiesiny w pożywce mineralnej rozkładały etylobenzen z szybkością od około 6 do około 56 g/m^3/h. Najbardziej aktywne z nich zidentyfikowano jako *Bacillus pumilus* i *Rhodococcus rhodochrous.* Wyizolowane we wcześniejszej pracy bakterie rozkładające styren nie rozkładały etylobenzenu.