Environment Protection Engineering

Vol. 34 2008 No. 4

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ISOLATION AND KINETIC CHARACTERISTICS OF METHYL ISOBUTYL KETONE (MIBK) DEGRADING MICROORGANISMS

From a biofilter bed used for cleaning the MIBK-doped air, nine bacteria strains decomposing this substance, being used as the only source of carbon and energy, were isolated. The most active strains were identified as *Rhodococcus globerulus, Gordonia terrae, Gordonia bronchialis* and *Bacillus subtilis*. The rate of MIBK decomposition by these microorganisms was tested within its concentration ranging from approx. 0.1 to 3 g m^{-3}. They degraded MIBK at a maximum rate ranging from approx. 15 to approx. 50 g $m^{-3}\cdot h^{-1}$. The highest biodegradation rate in suspension (EC), i.e. 163 g $m^{-3}\cdot h^{-1}$, was observed in *Gordonia terrae.* The number of micoorganisms reached the value of 10^7 cfu in 1 cm^3 of liquid culture.

1. INTRODUCTION

The problem of the elimination of pollutants discharged into the atmosphere with industrial waste gases has become a particularly important issue today. Main anthropogenic sources of the emission of these compounds are, among others, transport, paint and varnish, chemical, petrochemical, coke, pharmaceutical, and dyestuff industries. The harmfulness of these compounds results not only from their primary toxicity, but also from the fact that many of them undergo complex transformations in air leading frequently to a significant increase in emission toxicity. Harmful emissions can be reduced in many ways. From an environmental and economic points of view, better methods are those based on natural abilities of microorganisms to biodegrade organic substances.

The aim of this study was to search for new bacteria strains capable of decomposing methyl isobutyl ketone (MIBK) and to describe their activity.

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2. RESEARCH METHODS

2.1. MICROORGANISM ISOLATION

Microorganisms used in the study were isolated from a spent bed of a large-scale laboratory biofilter used for MIBK removal and prepared from municipal-industrial waste compost coming from a composting plant in Racula and pine bark, mixed at the ratio of 1:2 [1]. Filter bed samples, about 1 g each, were introduced into 150 cm^3 liquid mineral medium (culture medium according to KOJIMA [2] without agar and yeast extract supplement) placed in a 250 cm^3 scrubber. A schematic drawing of the experimental system used is presented in figure 1. After several days of supplying the cultures placed in scrubbers with a mixture of air and MIBK at a concentration of 500 mg/m^3, an increase in turbidity was observed as well as a significant decrease in the concentration of MIBK leaving the scrubbers. From suspensions collected in the scrubbers, a microbiological culture was prepared on Kojima culture medium with one source of carbon – MIBK (0.5, 1.0 and 1.5 $cm^3 \cdot dm^{-3}$ culture medium) [2]. Incubation was carried out for 21 days in a 10 dm^3 desiccator, where a bottle with 5 cm^3 MIBK was additionally placed. Thereafter, the microorganisms differing in their morphology were isolated and proliferated on MPA culture medium (Biocorp), and the isolated bacteria strains were stored on agar slants.

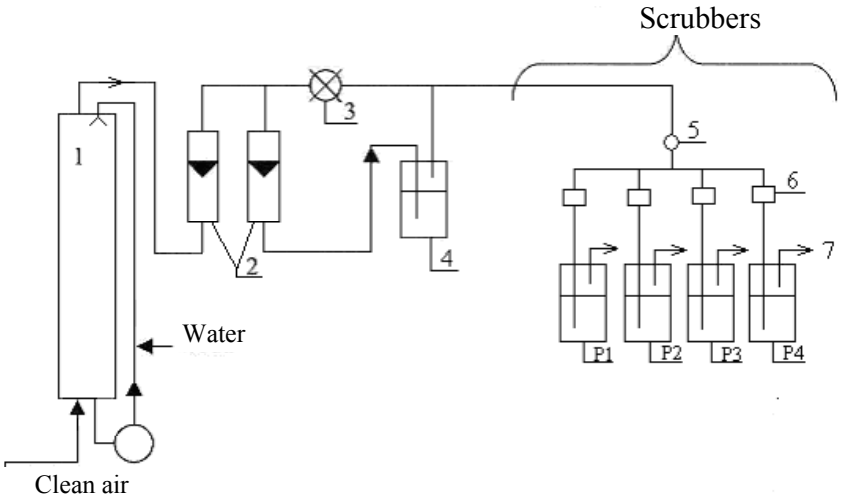


Fig. 1. Schematic drawing of the experimental system; 1 – moistener, 2 – air flowmeters, 3 – valves, 4 – scrubber with MIBK, 5 – inlet sampling ports, 6 – bacterial filters, 7 – scrubber outlets/outlet sampling ports, P1 to P4 – scrubbers with bacterial cultures

2.2. EXAMINATION OF DEGRADATION ACTIVITY – SCRUBBER EXPERIMENT

The biodegradation of MIBK vapours in mixtures with air was examined using an experimental system, the core of which was composed of a set of parallel fed reactors (figure 1). At the stage of microorganism isolation, 250 cm^3 scrubbers were used for this purpose, whereas 25 cm^3 ones were used in kinetic tests. Into each of them, 150 or 10 cm^3 of mineral medium were inserted and 2 or 0.5 cm^3 of inoculum were added, obtained by washing bacterial slants with 3 cm^3 of physiological salt solution. A mixture of MIBK vapours and air, in the amount of 3–10 $dm^3 \cdot h^{-1}$, was pumped through the scrubbers at the concentrations ranging from 400 to 2700 $mg \cdot m^{-3}$. In order to protect the cultures in the scrubbers against infection with aerobic microorganisms, 0.2 μm Anotop 25 bacterial filters (Whatman, Maidstone, UK) were installed before the scrubbers within the path of gas stream. The progress of the whole process was monitored with chromatographic method, using a Chrom 4 gas chromatograph (Laboratory Instruments, Prague, Czech Republic). In the kinetic analysis, culture turbidity was also measured by means of a Spekol 11 spectrophotometer (Carl Zeiss Jena, Germany). Based on the results of chromatographic analyses and the data referring to the flow of MIBK vapours and the air mixture through the scrubbers, the mass loading of scrubber with MIBK, the total effectiveness (efficiency) of biodegradation and pollutant elimination capacity (specific biodegradation rate) were calculated according to the following equations:

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

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

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

where:

C_i – the inlet/outlet MIBK concentration, $mg \cdot m^{-3}$;
G – the flow rate, $m^3 \cdot s^{-1}$;
V – the suspension volume, m^3;
M – the mass loading of scrubber with MIBK; $g \cdot m^{-3} \cdot s^{-1}$,
Su – the biodegradation efficiency, %;
EC – the elimination capacity (biodegradation rate), $g \cdot m^{-3} \cdot s^{-1}$.

In kinetic analyses, culture turbidity was also measured by means of a Spekol 11 spectrophotometer (Carl Zeiss Jena, Germany) with a test-tube measuring attachment. At the same time, the count of proliferating inoculum was done by culturing the solutions of the samples collected on MPA medium.

3. RESULTS AND DISCUSSION

3.1. MICROORGANISM ISOLATION

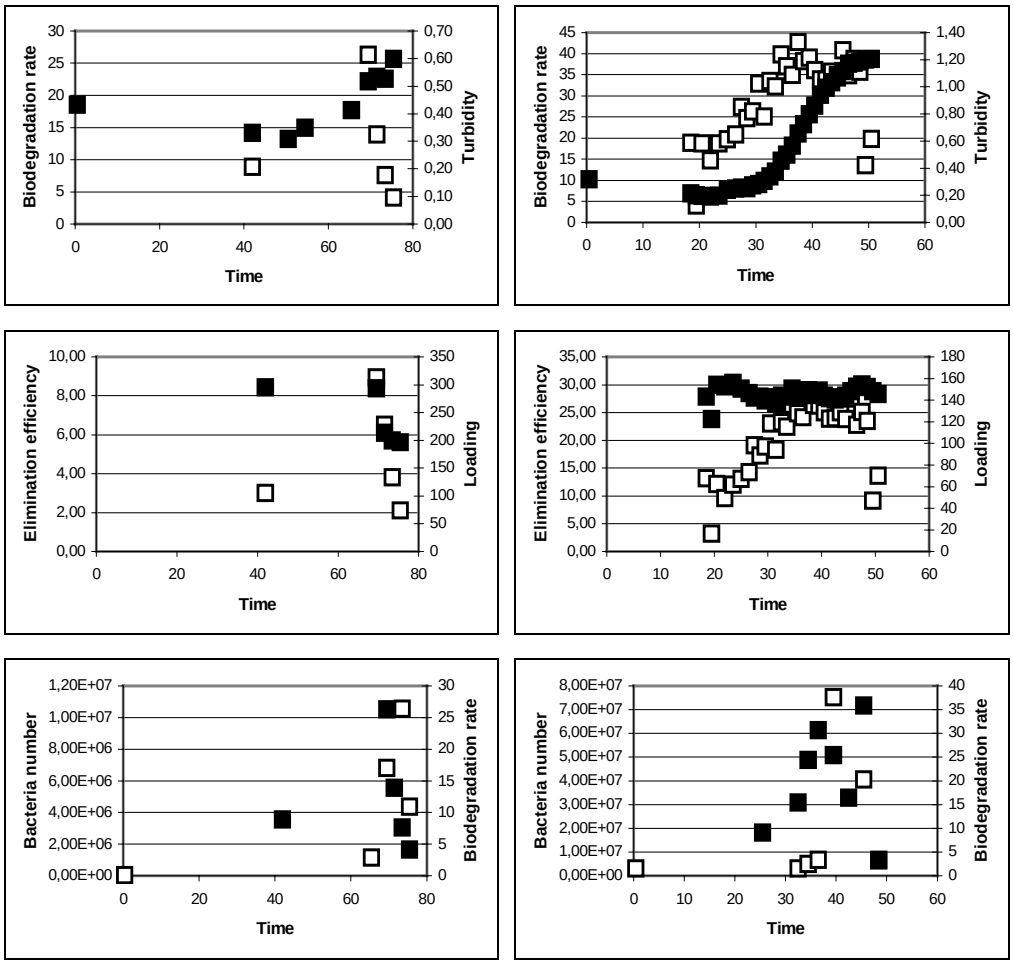
During the study, 9 bacteria strains were isolated, being capable of using MIBK as the only source of carbon and energy. The most active microorganisms were identified as *Gordonia terrae, Gordonia bronchialis*, *Rhodococcus globerulus* and *Bacillus subtilis* with two methods, i.e. MIDI and 16S rRNA sequencing, by Microbial ID laboratory (Newark, DE, USA).

3.2. EXAMINATION OF DEGRADATION ACTIVITY

The results of preliminary biodegradation kinetics measurements made in "large scrubbers" at the early stage of microorganisms isolation differed significantly from those carried out in "small scrubbers". Maximum biodegradation rates recorded for *Gordonia terrae, Gordonia bronchialis* and *Rhodococcus globerulus* strains in large reactors were 163, 128 and 134 g $m^{-3}\cdot h^{-1}$, respectively, at a MIBK concentration of approx. 2700 $mg\cdot m^{-3}$, whereas 49, 54 and 19 $g\cdot m^{-3}\cdot h^{-1}$, respectively, at MIBK concentrations of 901, 527 and 865 $mg\cdot m^{-3}$ in small ones. One could expect quite different results with respect to biodegradation rate, since a practically linear correlation should exists between it and the culture loading with substrate below a certain loading, called the critical one [3]–[4]. The maximum loading applied in this study, being a derivative of concentration, gas flow volume and substrate (culture medium) volume, was by approx. 1.5 times larger in small scrubbers. Most likely, the loadings applied during our experiments in small scrubbers were high enough to be outside the critical point, which resulted in a decrease in the rate and degree of biodegradation.

Similar results were obtained by ZIEMIAŃSKI et al. [5]. They found that microbiological decomposition of butyl acetate and butyl alcohol depended on the initial concentration of the compounds examined and on the substrate loading. The increase of this last parameter aggravated the effects of biodegradation of these compounds. On the other hand, a definitely higher elimination degree in our experiments in large scrubbers may be explained by a slower depletion of culture medium components, smaller concentration of accumulated metabolites or both these factors together, and vice versa. FREEDMAN et al. [6] confirmed in their study that nitrogen and phosphorus deficiency affected the biodegradation of such compounds as MIBK, MEK, toluene or *p*-xylene. Addition of these chemical elements improved the elimination of the xenobiotic substances mentioned above. The next reason of the lower activity of bacteria strains at the measurement stage in small reactors could have been the proliferation of bacteria strains after isolation and their accumulation on culture medium with easily available carbon. The bacteria strains kept under such conditions could lose in part their capability of

synthesizing certain necessary enzymes, whereas its restoration would require their conditioning under appropriately selected conditions [7]. This phenomenon was frequently observed by numerous scholars [8]–[9]. This effect could have been also the reason for the practical loss of MIBK decomposing ability by *Bacillus subtilis* after storage.



a b

Fig. 2. *Gordonia terrae* (column a) and *Gordonia bronchialis* (column b):
□ (the left *y*-axis) – biodegradation rate, $g \cdot m^{-3} \cdot h^{-1}$, elimination efficiency, %; bacteria number, cfu cm^{-3};
■ (the right *y*-axis) – turbidity; loading, $g \cdot m^{-3} \cdot h^{-1}$; biodegradation rate, $g \cdot m^{-3} \cdot h^{-1}$

The analysis of correlations presented in figure 2 also shows that biodegradation rate and elimination degree decreased after a certain time, i.e. in the final period of the culture life, in spite of maintaining the loading on a rather invariable level.

These changes were correlated with the number of microorganisms in the culture, which also decreased at the end of respective experiments. At the same time, the turbidity did not decrease in the final part of the experiment, which most probably proved that some part of microorganisms present in the culture at this stage was dead or was not capable of growing.

4. CONCLUSIONS

1. Methyl isobutyl ketone (MIBK) is a compound that can be relatively easily biodegraded under aerobic conditions by bacteria which naturally occur in biofilter beds, including for instance the bacterial strains of the genus *Gordonia* sp., *Rhodococcus* sp. or *Bacillus* sp.

2. The isolated bacteria species, i.e. *Gordonia terrae, Gordonia bronchialis* and *Rhodococcus globerulus*, degraded MIBK with maximum rates, i.e. 163, 128 and 134 $g \cdot m^{-3} \cdot h^{-1}$, respectively, at a MIBK concentration of approx. 2700 $mg \cdot m^{-3}$.

REFERENCES

[1] WIECZOREK A., OSÓCH M., KARCZ J., *Eksperymentalna analiza procesu biofiltracji gazów odlotowych w kolumnie ze złożem naturalnym*, Inż. Ap. Chem., 2006, 5, 139–141.

[2] KOJIMA Y., ITADA N., HAYAISHI O., *Metapyrocatechase: a new catechol-cleaving enzyme*, J. Biol. Chem., 1961, Vol. 236, 2223–2229.

[3] WIECZOREK A., *Biofiltracja lotnych nierozpuszczalnych ksenobiotyków na złożach kompostowych – czynniki limitujące*, ZPPNR, PAN, Warszawa, 2005, 505, 503–511.

[4] DESHUSSES M., JOHNSON C., *Development and validation of a simple protocol to rapidly determine the performance of biofilters for VOC treatment*, Environ. Sci. Technol., 2000, Vol. 34, 461–467.

[5] ZIEMIAŃSKI K., PIELECH-PRZYBYLSKA K., SZOPA J.St., *Mikrobiologiczny rozkład wybranych węglowodorów stanowiących zanieczyszczenie powietrza*, Rozkład i Korozja Mikrobiologiczna Materiałów Technicznych, Łódź, 2001, 274–279.

[6] FREEDMAN D.L., PAYAUYS A.M., KARANFIL T., *The effect of nutrient deficiency on removal of organic solvents from textile manufacturing wastewater during activated sludge treatment*, Environ. Technol., 2005, Vol. 26(2), 179–88.

[7] PRZYBULEWSKA K., WIECZOREK A., *Wykorzystanie procesów biodegradacji styrenu do oczyszczania gazów odlotowych*, Post. Mikrobiol., 2006, Vol. 45, 1, 51–65.

[8] ŁABUŻEK S., KŁAPACIŃSKA B., MROZIK A., RADECKA I., WOŹNICA A., *Pozyskiwanie wysoko aktywnej mieszanej populacji drobnoustrojów zdolnych do biodegradacji związków fenolowych*, Arch. Ochr. Śr., 1996, Vol. 1–2, 49–63.

[9] MROZIK A., ŁABUŻEK S., *A comparison of biodegradation of phenol and homologous compounds by Pseudomonas vesicularis and Staphylococcus sciuri strains*, Acta Microbiol. Pol., 2002, Vol. 51, 367–378.

WYIZOLOWANIE I KINEMATYCZNA CHARAKTERYSTYKA MIKROORGANIZMÓW ROZKŁADAJĄCYCH KETON METYLOWO-IZOBUTYLOWY

Ze złoża biofiltra służącego do oczyszczania powietrza domieszkowanego ketonem metylowo-izobutylowym (KMIB) wyizolowano dziewięć szczepów bakterii rozkładających tę substancję wykorzystywaną jako jedyne źródło węgla i energii. Najaktywniejsze z nich zostały zidentyfikowane jako *Rhodococcus globerulus, Gordonia terrae, Gordonia bronchialis* i *Bacillus subtilis.* Szybkość rozkładania KMIB przez te mikroorganizmy testowano w zakresie jego stężeń w powietrzu od około 0,1 do 3 $g \cdot m^{-3}$. Degradowały one KMIB z szybkością, której wartości maksymalne mieściły się w zakresie od około 15 do około 50 $g \cdot m^{-3} \cdot h^{-1}$. Największą szybkość biodegradacji w suspensji (*EC*), tzn. 163 $g \cdot m^{-3} \cdot h^{-1}$, zaobserwowano w przypadku *Gordonia terrae.* Liczebność mikroorganizmów osiągała wartości rzędu 10^7 j.t.k. w 1 cm^3 hodowli płynnej.