ARCHIVES OF ENVIRONMENTAL PROTECTION
vol. 35 no. 3 pp. 3 - 10 2009
PL ISSN 0324-8461

BIODEGRADATION OF METHYL ISOBUTYL KETONE (MIBK) BY *FUSARIUM SOLANI*

KRYSTYNA PRZYBULEWSKA[1], ANDRZEJ WIECZOREK[2]

West Pomeranian University of Technology
[1] Department of Microbiology and Biotechnology of Environment
ul. Słowackiego 17, 71-434 Szczecin, Poland
e-mail: kprzybulewska@agro.ar.szczecin.pl
[2] Institute of Chemistry and Fundamentals of Environmental Protection
al. Piastów 42, 71-065 Szczecin, Poland
e-mail: anwiecz@ps.pl

Keywords: *Fusarium solani*, methyl isobutyl ketone, MIBK degradation, biodegradation, biodegradation kinetics.

Abstract: Fungus *Fusarium solani* able to degrade methyl isobutyl ketone was isolated from the bed of biofilter cleaning exhausting gases from the cable plant "Załom" near Szczecin. This substance was used as the only source of carbon and energy. Confirmation and kinetic tests were performed in 25 cm^3 scrubbers filed with mineral medium which was inoculated with the fungus. *Fusarium solani* degraded MIBK at the rate up to 60 $g \cdot m^{-3} \cdot h^{-1}$ and pollution loading up to 200 $g \cdot m^{-3} \cdot h^{-1}$. Degree of elimination ranged from 40 to 80% and decreased when culture pollution loading increased.

INTRODUCTION

Atmospheric emissions of volatile organic compounds are a serious ecological problem at present. The source of these compounds is most often full-scale metallurgical, chemical, electronic and furniture plants, waste disposal sites and transport [22, 24, 26]. An example of such a compound is methyl isobutyl ketone (MIBK) used mainly as a solvent extractant in pharmacy and alcohol industry as well as chemical synthesis. Most commonly, however, it is used as an additive to universal paints and lacquers containing even to 60% solvents. In 1993, almost 70 000 Mg of the aforesaid compound was emitted to environment in the United States. It is an easily volatilizing and fairly well water-soluble light liquid with a sweet odor. For this reason, MIBK can pollute the air and waters, creating hazard for living organisms [3, 11, 18, 25]. The necessity of protecting the environment against pollution with such substances forces them to be removed from emission streams. The treatment of flue gas containing volatile compounds can be done by physicochemical and biological methods. Unlike commonly used physicochemical methods, biological techniques can be used for a broader group of organic substances ensuring their decom-

Kolumna dofinansowana przez
Wojewódzki Fundusz Ochrony Środowiska i Gospodarki Wodnej w Katowicach

position to non-toxic compounds, such as water and CO_2 [15, 23]. The removal of MIBK by biological methods from emission streams, in particular with the use of bacteria, has been discussed by many researchers, i.e. Aizpuru *et al.* [1], Cai *et al.* [4], Deshusses [6, 7], Geoghegan *et al.* [10], Kim *et al.* [13], and Lee *et al.* [16]. They describe bio-filtration process start-up, bio-filter responses to variable pollution loading and successive installation start-up after the rest period as well as the effect of many other factors. Studies on optimization of the biodegradation process of MIBK, MEK, toluene or xylenes [9, 20, 21] have been also carried out. Apart from bacteria, also other microorganisms are capable of biodegrading atmospheric pollutions, in particular fungi. According to certain authors, they are resistant to low pH and, while being placed on a solid medium (peat, compost, pearlite, etc.), can effectively degrade xenobiotics [12, 17]. Decomposition of MIBK by fungi has been described, among others, by Arriaga and Ravah [2], Lee *et al.* [16] and Qi *et al.* [19].

The study presented below aimed at isolation of fungi capable of biodegrading methyl isobutyl ketone (MIBK).

METHODOLOGY

Microorganisms were isolated from a pilot bio-filter bed treating flue gases from a varnishing division of the "Załom" Cable Plant near Szczecin installed there within an earlier research project [27]. The column was filled with lightly compacted compost made of municipal and industrial wastes. In order to create a mixed culture, 100 cm^3 liquid mineral medium [14] without yeast extract and 5 g compost coming from the top horizon of filtration column were introduced into a 250 cm^3 scrubber (Fig. 1). Methyl isobutyl ketone (MIBK), being the only source of carbon and energy, was fed to liquid medium together with flowing gases. In the experiment, of which the primary goal was to propagate microorganisms, small loadings were applied, from a few to a maximum of 40 $g \cdot m^{-3} \cdot h^{-1}$. After 7 days, 10 cm^3 of culture was transferred to 200 cm^3 fresh medium for further microorganism propagation. After next 3 days, a suspension was sampled for inoculation and for isolation of pure strains. From the sample collected, dilutions were made up to 10^{-12} in sterile distilled water. 1 cm^3 culture from the following dilutions from 10^{-4} to 10^{-12} (inoculum) was brought in Petri dishes and poured with liquid medium at the temperature of 45°C. Medium [14] without yeast extract was used, with MIBK addition in the amount of 0.5, 1.0 and 1.5 $cm^3 \cdot dm^{-3}$ of medium. Incubation was carried out at room temperature in 15 dm^3 desiccators in the presence of vapors of methyl isobutyl ketone placed in 10 cm^3 glass vials. After 8 weeks, a fungus was isolated from colonies grown on media. For further examination, it was propagated by inoculating in liquid mineral medium [14] without yeast extract and agar. Identification of the isolated fungus was carried out by Microbial ID Company (Newark, DE, USA). Analyses were made by DNA sequencing method (28S rRNA) and, in addition, confirmed microscopically. Examination of the kinetics of methyl isobutyl ketone biodegradation was carried out in scrubbers within a set presented below (Fig. 1).

Kolumna dofinansowana przez
Wojewódzki Fundusz Ochrony Środowiska i Gospodarki Wodnej w Katowicach

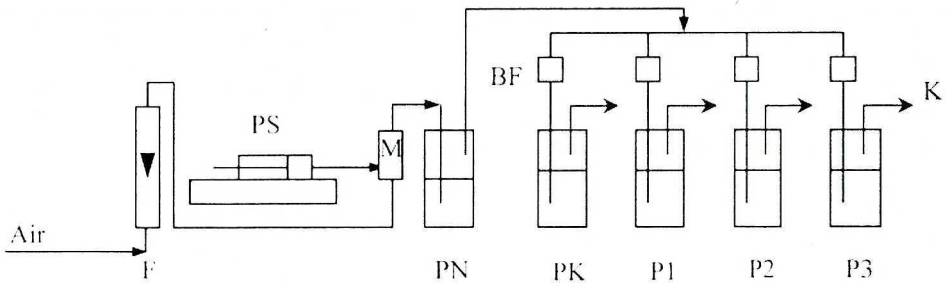

Fig. 1. Schematic representation of the experimental setup
F – flow meter, PS – MIBK dosing pump, M – streams mixer, PN – humidifying scrubber, BF – bacteria filters, PK – control scrubber, P1–P3 – scrubbers with fungi cultures, K – outlet sampling ports

In kinetic tests, 25 cm^3 scrubbers were used, with 10 cm^3 of mineral medium introduced into each of them and 0.5 cm^3 of liquid fungal inoculum added. Through scrubbers, a mixture of MIBK vapors and air was pumped in the amount of 3 $dm^3 \cdot h^{-1}$, with MIBK concentrations ranging from about 60 to 650 $mg \cdot m^{-3}$. In order to protect the culture against infection with airborne microorganisms, Anotop 25 type 0.2 μm (Whatman) bacterial filters were installed within the path of gas stream. During biodegradation kinetics measurements, decisively higher loading were applied, most frequently within the range from several to about 200 $g \cdot m^{-3} \cdot h^{-1}$. The examination was carried out at a constant temperature of 28°C. During kinetic tests, in its main measuring period, strain biodegradation abilities and culture turbidity were determined every hour. The process progress (substrate concentrations) was monitored by the chromatographic method using a Chrom 4 gas chromatograph. Turbidity was measured by means of Spekol 11 spectrophotometer equipped with an attachment for measurements in test-tubes at a wavelength of 480 nm. In order to correlate the degree of culture turbidity with the number of microorganisms, their number in a parallel culture was additionally determined in one of the experiments. For determination of the number of microorganisms inoculations of diluted liquid culture samples were carried out on a PDA medium (BIOCORP).

Based on the results of chromatographic analyses and the data referring to MIBK vapors and air mixture flow through scrubbers, mass scrubber MIBK loading, total biodegradation efficiency (performance) and pollution elimination ability (specific biodegradation rate) were calculated:

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

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

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

Kolumna dofinansowana przez
Wojewódzki Fundusz Ochrony Środowiska
i Gospodarki Wodnej w Katowicach

where: C_i – inlet/outlet MIBK concentration [mg·m^{-3}],
G – flow rate [m^3·s^{-1}],
V – suspension volume [m^3].
M – mass loading of scrubber with MIBK [g·m^{-3}·h^{-1}],
S_u – biodegradation efficiency [%],
EC – elimination capacity (biodegradation rate) [g·m^{-3}·h^{-1}].

Metabolites were identified with GC-MS technique after their extraction from the culture with methylene chloride, using a Hewlett-Packard 6890 chromatograph equipped with MSD 5973 mass detector.

RESULTS AND DISCUSSION

On examinations a fungus was isolated and identified as *Fusarium solni* (Fig. 2). The microorganism developed on mineral medium used methyl isobutyl ketone as the only source of carbon.

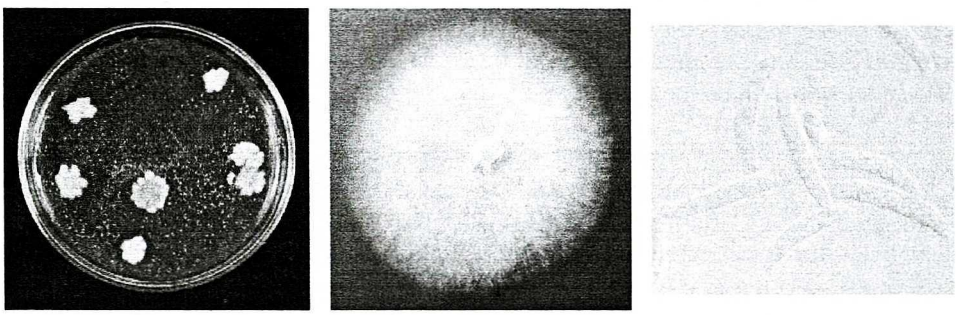

Fig. 2. *Fusarium solani* with spores

While carrying out propagation cultures and making MIBK biodegradation kinetics measurements a visible turbidity of culture media was observed. These changes were most probably caused by an increase in the number of introduced fungus. The growth phase started after a lapse of time, dependent on conditions under which the culture was carried out, in particular, on unavoidable fluctuations in inoculum properties, volatile substrate concentration and culture loading. In some experiments (propagation of microorganisms), in which small MIBK concentrations were applied as well as such culture loadings, a high elimination degree of methyl isobutyl ketone, i.e. over 80%, was observed as early as in the 6th hour after inoculation. Degradation rates obtained just then were small, within 1 to 2 g·m^{-3}·h^{-1}. Such a high efficiency was most probably a result of small substrate culture loading. Prolongation of the culturing time and increase of the loading induced usually an increase in biodegradation rate. However, this was frequently accompanied by a decrease in biodegradation degree. Exemplary results are given in Figures 3–4.

In the course of proper kinetic tests, a maximum value of biodegradation rate recorded was 59.39 g·m^{-3}·h^{-1} at a substrate concentration of 517 g·m^{-3} and a loading of 147.8 g·m^{-3}·h^{-1}. Biodegradation degree corresponding to it was also one of the highest results within a set of kinetics measurements and amounted to 40.18% (maximum 41.24). The values of biodegradation rate obtained in this study are typical for bio-filters colonized by fungi. For example, toluene was degraded at a rate of 77 g·m^{-3}·h^{-1} and 55 g·m^{-3}·h^{-1} by

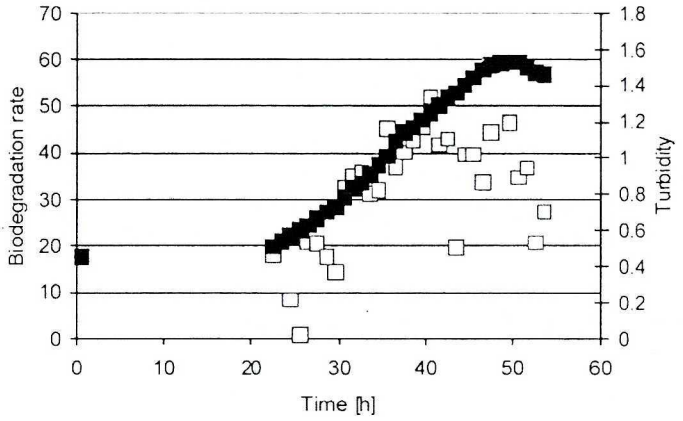

Fig. 3. Degradation of methyl isobutyl ketone by *Fusarium solani*
□ – biodegradation rate [g·m^{-3}·h^{-1}]
■ – turbidity

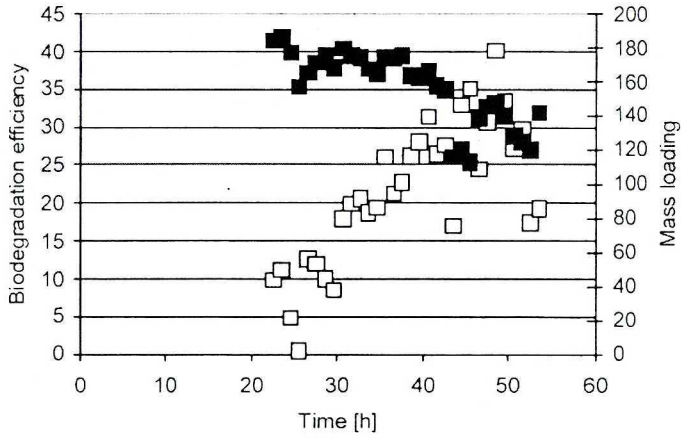

Fig. 4. Degradation of methyl isobutyl ketone by *Fusarium solani*
□ – biodegradation efficiency [%],
■ – mass loading [g·m^{-3}·h^{-1}]

E. oligosperma and *Paecilomyces variotii*, respectively [8], while hexane at a mean rate of 90 g·m^{-3}·h^{-1} and a maximum rate of 130 g·m^{-3}·h^{-1} by *Fusarium solani* [2]. Up to approximately 40th hour of culturing, biodegradation rate clearly depended on the number of microorganisms, finding its reflection in degree of turbidity (Fig. 3). Above the 40th hour, biodegradation rate stopped correlating with turbidity. Most probably, it was a result of the influence of not only living microorganisms but also dead ones on turbidity (Fig. 3). Dead microorganisms (or those in bad condition) induced an increase of turbidity but did not decompose the substrate. At that time, a decrease was observed in the rate of MIBK decomposition by *Fusarium solani* as well as in the degree of its removal (Figs 3–4). The decrease of biodegradation rate could have been also caused by a metabolite produced in

the course of this process, the evidence of which was the second peak showing on chromatographs, not belonging to the tested compound. This metabolite was identified later as 2-methyl-1-propanol. It was found in the medium in concentrations similar to those of primary substrate, i.e. MIBK. Similar events were also observed by other authors, for example by Cinar [5] and Yu *et al.* [28].

In the experiment presented in Figure 5, the number of microorganisms was determined, together with culture turbidity. There was a correlation (R^2) between the number of microorganisms and degree of turbidity (not presented graphically), for biodegradation rate up to about 20 $g \cdot m^{-3} \cdot h^{-1}$. The value of R^2 was equal to 0.8. Linear equation representing this relationship has the following form:

$$CFU = 6801.2 \times Tu + 1629.3$$

where: CFU – number of microorganisms,
Tu – turbidity.

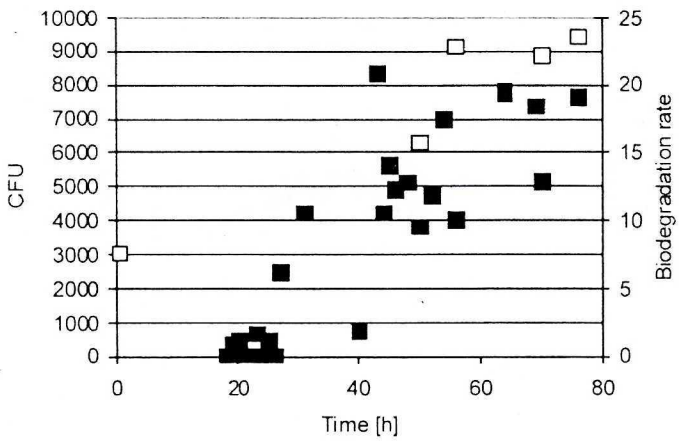

Fig. 5. Degradation of methyl isobutyl ketone by *Fusarium solani*
□ – mean *number* of fungal colony-forming units [$cfu \cdot cm^{-3}$],
■ – biodegradation rate [$g \cdot m^{-3} \cdot h^{-1}$]

CONCLUSIONS

1. Fungus *Fusarium solani* is able to use methyl isobutyl ketone as the only source of carbon and energy.
2. In a liquid culture on mineral medium *Fusarium solani* can effectively decompose methyl isobutyl ketone at decomposition rates up to 60 $g \cdot m^{-3} \cdot h^{-1}$ and pollution loadings up to 200 $g \cdot m^{-3} \cdot h^{-1}$.

REFERENCES

[1] Aizpuru A., L. Malhautier, J.C. Roux, J.L. Fanlo: *Biofiltration of Mixture of Volatile Organic Emissions*, Journal of the Air and Waste Management Association, **51**, 1662–1670 (2001).

[2] Arriaga S., S. Revah: *Removal of n-heksane* by *Fusarium solani with a gas-phase biofilter*, Journal of Industrial Microbiology and Biotechnology, **32**, 548–553 (2005).

[3] Benczek K.M., S. Gorzkowski: *Ocena szkodliwości*, Bezpieczeństwo pracy, **3**, 8–10 (2006).
[4] Cai Z., D. Kim, G. Sorial: *Performance of Trickle-Bed Air Biofilter: A Comparative Study of a Hydrophilic and a Hydrophobic VO*, Water, Air, & Soil Pollution, Focus, **6**(1), 57–69 (2006).
[5] Cinar A.: *Biodegradation of central intermediate compounds produced from biodegradation of aromatic compounds*, Bioprocess and Biosystems Engineering, **26**, 341–345 (2004).
[6] Deshusses M.A.: *Transient behavior of biofilters: Start-up, carbon balances, and interactions between pollutants*, Journal of Environmental Engineering, **123**(6), 563–568 (1997).
[7] Deshusses M., C. Johnson: *Development and Validation of a Simple Protocol to Rapidly Determine the Performance of Biofilters for VOC Treatment*, Environmental Science & Technology, **34**, 461–467 (2000).
[8] Estevez E., M.C. Veiga, Ch. Kennes: *Biofiltration of waste gases with the fungi Exophiala oligosperma and Paecilomyces variotii*, Applied Microbiology and Biotechnology, **67**(4), 563–568 (2005).
[9] Freedman D.L., A.M. Payauys, T. Karanfil: *The effect of nutrient deficiency on removal of organic solvents from textile manufacturing wastewater during activated sludge treatment*, Environmental Technology, **26**(2), 179–88 (2005).
[10] Geoghegan D.P., G. Hamer, M.A. Deshusses: *Effects of unsteady state conditions on the biooxidation of methyl ethyl and methyl isobutyl ketone in continuous flow liquid phase cultures*, Bioprocess and Biosystems Engineering, **16**, 6 (1997).
[11] International Programme on Chemical Safety, Environmental Health Criteria 117, Methyl izobutyl ketone, 1990. http://www.inchem.org/documents/ehc/ehc/ehc117.htm.
[12] Kennes C., M.C. Veiga: *Fungal biocatalysts in the biofiltration of VOC-polluted air*, Journal of Biotechnology, **113**, 305–319 (2004).
[13] Kim D., Z. Cai, G.A. Sorial: *Impact of interchanging VOC's on the performance of trickle bed air biofilter*, Chemical Engineering Journal, **113**(2-3), 153–160 (2005).
[14] Kojima Y., N. Itada, O. Hayaishi.: *Metapyrocatechase: a new catechol-cleaving enzyme*, Journal of Biological Chemistry, **236**, 2223–2228 (1961).
[15] Łebkowska M., A. Tabernacka: *Biotechnologiczne metody usuwania zanieczyszczeń z gazów odlotowych*, Biotechnologia, **50**(3), 141–150 (2000).
[16] Lee T.H., J. Kim, M.J. Kim, H.W. Ryu, K.S. Cho: *Degradation characteristics of methyl ethyl ketone by Pseudomonas sp. KT-3 in liquid culture and biofilter*, Chemosphere, **63**(2), 315–322 (2006).
[17] Moe W.M., B. Qi: *Performance of fungal biofiltr treating gas-phase solvent mixtures during intermittent loading*, Water Research, **38**, 2259–2268 (2004).
[18] NTP Technical Report on the Toxicology and Carcinogenesis Studies of Methyl Isobutyl Ketone (CAS No. 108-10-1) in F344/N rats and B6C3F1 mice (inhalation studies) 1-263, 2005.
[19] Qi B., W. Moe, K. Kinney: *Biodegradation of volatile organic compounds by five fungal species*, Applied Microbiology and Biotechnology, **58**, 684–689 (2002).
[20] Quesnel D., G. Nakhla: *Optimization of the aerobic biological treatment of hermophilically treated refractory wastewater*, Journal of Hazardous Materials, **125**(1-3), 221–230 (2005).
[21] Quesnel D., G. Nakhla: *Removal kinetics of acetone and MIBK from a complex industrial wastewater by an acclimatized activated sludge*, Journal of Hazardous Materials, **132**(2-3), 253–260 (2006).
[22] Reither K.: *Biological waste gas purification in Germany*, International Symposium BIO'94, Świnoujście, Poland, 25–27 Maj 1994, pp. 1–9.
[23] Szklarczyk M.: *Biologiczne oczyszczanie gazów odlotowych*, Wydawnictwo Politechniki Wrocławskiej, Wrocław 1991, pp. 4–33.
[24] Szklarczyk M., M. Czemarmazowicz, W. Adamiak: *Biologiczne oczyszczanie gazów – stan obecny i perspektywy rozwoju*, Biotechnologia, **36**(1), 108–116 (1997).
[25] Toxicological Review of Methyl Isobutyl Ketone (CAS No. 108-10-1), [in:] Support of Summary Information on the Integrated Risk Information System (IRIS), 2003, pp. 1–68.
[26] Warych J.: *Biooczyszczanie gazów odlotowych*, Inżynieria Ekologiczna, **4**, 58–67 (2001).
[27] Wieczorek A.: *Pilot-Scale biofiltration of waste gazes containing aliphatic and aromatic hydrocarbons, phenol, cresols, and other volatile organic compounds*, Environmental Progress, **24**, 60–66 (2005).
[28] Yu H., B.J. Kim, B.E. Rittmann: *The roles of intermediates in biodegradation of benzene, toluene, and p-xylene by Pseudomonas putida F1*, Biodegradation, **12**, 455–463 (2001).

Received: March 6, 2009; accepted: May 19, 2009.

BIODEGRADACJA METYLOIZOBUTYLOKETONU (MIBK) PRZEZ *FUSARIUM SOLANI*

Podczas prac ze złoża biofiltra zainstalowanego w fabryce kabli „Załom" koło Szczecina wyizolowano grzyba *Fusarium solani* zdolnego do wykorzystywania metyloizobutyloketonu (MIBK) jako jedynego źródła węgla i energii. Hodowle namnażające prowadzono w półprzepływowym układzie bazującym na płuczce napełnionej pożywką mineralną, przez którą przetłaczano z zadaną szybkością powietrze domieszkowane metyloizobutyloketonem. Celem ochrony hodowli przed zainfekowaniem na drodze strumienia powietrza zainstalowano filtr bakteryjny. Z tak wzbogaconych hodowli izolowano mikroorganizmy wykonując posiewy na płytkach Petriego zalewanych wspomnianą wyżej pożywką mineralną uzupełnioną agarem. Testy potwierdzające oraz wstępne testy kinetyki biodegradacji MIBK przez wyizolowanego *Fusarium solani* wykonano w analogicznym zestawie badawczym, jak stosowany do hodowli namnażających. W czasie badań mierzono natężenie przepływu gazów oraz oznaczano stężenia metyloizobutyloketonu przed i za płuczkami metodą chromatograficzną. Na bazie tych danych obliczono sprawność i szybkości biodegradacji MIBK przez *Fusarium solani*. Maksymalna szybkości biodegradacji wynosiła około 60 $g \cdot m^{-3} \cdot h^{-1}$, przy obciążeniach do 200 $g \cdot m^{-3} \cdot h^{-1}$, a sprawność zawierała się w przedziale od 40 do 80%, malejąc w miarę wzrostu obciążenia hodowli testowana substancją.