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CAPABILITY OF *KOCURIA* SP. IN MTBE BIODEGRADATION

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Methyl *tert*-butyl ether (MTBE) is one of most commonly used oxygenates added to replace toxic compounds from gasoline and to reduce air pollutants emission. Due to intensive use and persistence, MTBE has become one of the most important environment pollutants. Presented research shows the capability of bacterial isolate *Kocuria sp*. 27/1 of biodegradation of MTBE as a additional source of carbon and energy. The obtained results shows that *Kocuria sp*. 27/1 was capable of utilizing of different initial concentrations of MTBE added as additional carbon and energy source. After 86 days of incubation on rotary shaker at 110 rpm and 27 °C, degradation rates of initial 25; 125 and 250 μg concentrations MTBE x ml^{-1} were 32; 28 and 62%, respectively. Highest degradation rate at all examinated concentrations was noticed in the beginning of incubation period. During the incubation increasing of bacterial number in all treatments was noticed, but this increasing was very slow, probably because of intermedier(s) during degradation and recalcitrance of C-atom. This research confirms the potential use of *Kocuria sp*. 27/1 in bioremediation of MTBE-contaminated environments.

Key words: Biodegradation, *Kocuria* sp., MTBE

INTRODUCTION

Methyl *tert*-butyl ether (MTBE) is the most commonly used oxygenate, added to replace the toxic components from gasoline, to improve fuel combustion and reduce air pollution. Because of its useful properties, MTBE was one of the organic chemicals with the highest world production (SCHMIDT *et al.*, 2004). Production of MTBE has risen in 1999 to 21 million tons, with 3.3 million tons in the countries of EU (KRAYER VON KRAUS and HARREMOËS, 2001). Nevertheless, its widespread use has led to discharge into environment (HATZINGER *et al.*, 2001), i.e. to pollution of surface soils, groundwaters and sediments. Due to its high water solubility and persistence, MTBE become one of the most frequently detected contaminants in Europe (KLINGER *et al.*, 2002) and USA (BAEHR *et al.*, 1999). The consequence of environment contamination with MTBE was development of different physical, chemical and biological methods for its removal. The biological methods are more efficient compared with physical or chemical treatments (MILLIOLI *et al.*, 2009). From these biological methods, one of most efficient is bioremediation, based on activity of microorganisms (VIDALI, 2001; JOVANOVIĆ *et al.*, 2005).

Until the present time, biodegradation of MTBE added as a sole or additional carbon and energy source has been reported by a pure bacterial cultures (HATZINGER *et al.*, 2001, LALEVIC *et al.*, 2012a; LALEVIC *et al.*, 2012b) or microbial consortium (FORTIN *et al.*, 2001) under oxic and anoxic conditions (SCHMIDT *et al.*, 2004) or in river sediments (BRADLEY *et al.*, 2001). However, according to increasing of MTBE worldwide accidence (HANSEN *et al.*, 2002) and low rate of its natural attenuation (JOHNSON *et al.*, 2000), it is necessairy to find microorganisms with similar potential efficiency in biodegradation of MTBE. The aim of this research was to examinate the biodegradation of MTBE added as additional source of carbon and energy by pure cultures of *Kocuria sp*.

MATERIALS AND METHODS

Pure cultures of *Kocuria sp.* 27/1 were isolated from oil hydrocarbons-contaminated wastewaters from «HIP Petrohemija», Pancevo district (Serbia), and previously identified by API STAPH and APIWEB system (bioMerieux, Inc., France) and by sequence analyzes of 16S rDNA (Lalevic *et al.*, 2012b).

The culturing of 72h-old *Kocuria sp.* pure cultures was conducted in 250 ml flasks containing 100 ml of nutrient broth with 25; 125 and 250 μg MTBE x ml^{-1}. All flasks were incubated on orbital shaker at 110 rpm and 27 °C for 86 days in a dark. All experiments were performed in triplicate. The number of bacteria was determined at time zero, and subsequently after 2; 6; 11; 17; 28; 49; and 86 days of incubation on nutrient agar and expressed using the logarithmic function. In control variant, nutrient broth was inoculated with pure cultures of *Kocuria sp.*

The concentration of MTBE during incubation was measured by sampling of 0.2 mL of headspace (Agilent 7694E Head Sampler) and analyzed by GC system (Agilent Technologies 6890N Network) linked to a flame ionization detector and DB-624 column (JandW Scientific, 30 m x 0.53 mm ID). During incubation, MTBE concentration was measured in gas phase above the suspension and compared with initial MTBE concentration. The injector temperature was 170°C, the column temperature was initially 50°C for 2 minutes and was ramped to 100°C at 8°C/min, and temperature of detector was 300°C. Nitrogen was carrier and makeup gas (4.5, and 25 ml/min, respectivelly). The concentration of MTBE was measured at time zero, and subsequently after 11; 17; 28; 49; and 86 days.

RESULTS

Results of this research suggest that *Kocuria sp*. 27/1 was capable of utilization of MTBE as a additional source of carbon and energy. During the incubation period of 86 days, decreasing of MTBE concentrations was noticed. Degradation rate of MTBE was controlled by incubation time and initial MTBE concentrations (Tab. 1).

Table 1. - Degradation of MTBE by Kocuria sp. 27/1

Initial MTBE concentrations (ppm)	MTBE concentrations				
	after 11 days	after 17 days	after 28 days	after 49 days	after 86 days
22.12	19.10	16.72	17.60	14.86	15.00
131.65	113.70	109.90	106.53	92.30	94.60
251.23	123.50	121.52	111.60	106.19	95.40

As can be seen from table 1, a rapid degradation of MTBE using the *Kocuria sp*. 27/1 was noticed, with highest decreasing of initial MTBE concentrations at 250 μg MTBE x ml^{-1}.

Table 2. - Degradation rate of MTBE during incubation

Initial MTBE concentrations (ppm)	MTBE degradation rate (%)				
	after 11 days	after 17 days	after 28 days	after 49 days	after 86 days
22.12	13.7	24.4	20.4	32.8	32.2
131.65	13.6	16.5	19.1	29.9	28.1
251.23	50.9	51.6	55.6	57.7	62.0

The highest degradation rate of initial 25 and 125 μg MTBE x ml^{-1} concentrations was notices in the beginning of experiments (between time zero

and 11th days of incubation). Final degradation rates of these initial concentrations were 32.2 and 28.1%, respectively. Highest concentration of MTBE was rapidly degraded during initial incubation period (until 11 days) and final degradation rate was 62%.

Results also showed that *Kocuria sp*. 27/1 was capable to grow in MTBE-containing nutrient broth, but characteristic of growth was controlled by initial MTBE concentration. Increasing in the MTBE initial concentration (25 and 125 μg x ml^{-1}) resulted in decreasing of bacterial number in initial incubation period of 17 days. After this period rapid bacterial growth was noticed compared with control (Table 3). The highest number of bacteria in control was noticed after 28 days of incubation, while in the presence of MTBE highest values was detected at the end of incubation.

Table 3. - Number (log) of Kocuria sp. 27/1 in medium containing MTBE

MTBE concentrations (ppm)	Time (days)							
	0	2	6	11	17	28	49	86
control	7.81	8.17	8.08	8.17	8.15	8.33	8.01	7.94
22.12	7.81	8.11	8.05	7.91	7.93	7.91	8.18	8.27
131.65	7.81	8.05	8.07	8.16	8.23	8.21	8.22	8.26
251.23	7.81	7.80	7.80	7.81	7.80	7.80	7.80	7.82

DISCUSSION

Use of microorganisms in biodegradation is continually increasing, because of their biodiversity and catabolitic potential (BEŠKOSKI *et al.*, 2011). The role of microbial populations in biodegradation of organic pollutants is well described (LALEVIĆ *et al.*, 2006; BAGHERZADEH-NAMAZI *et al.*, 2008; TALAIE *et al.*, 2010). Biodegradation of MTBE by different pure cultures of bacteria as *Mycobacterium* (SMITH *et al.*, 2003), *Pseudomonas* (LALEVIC *et al.*, 2012b), *Aquincola* (MULLER *et al.*, 2008) etc. was observed. Different bacterial strains of *Kocuria sp*. play important role in degradation of some polycyclic aromates (AHMED *et al.*, 2010) or some BTEX compounds (JUN and CHO, 2004), as well as MTBE added as a sole energy and carbon source (LALEVIC *et al.*, 2012a).

Bacterial strain *Kocuria sp*. 27/1 was capable to partially and slowly degrade the initial concentration of 25 and 125 μg MTBE x ml^{-1}. Dynamics of MTBE degradation in this research differs from data presented by LALEVIC *et al.* (2012b), during the biodegradation of MTBE, added as a unique source of carbon and energy, using same bacterial strains, and LALEVIC *et al.* (2012a), concluded that the highest degradation rate at the end of incubation period was reached by using the *Staphylococcus saprophyticus* and *Pseudomonas sp*. bacterial strains.

Presented results of MTBE degradation rate were confirmed by research of bacterial number during incubation. Increase of bacterial number in liquid culture indicates the adaptation of *Kocuria sp*. 27/1 to different MTBE concentration. However, this increase of bacterial number during incubation was very slow. Similar conclusion was previously reported by HATZINGER *et al.* (2001) and is probably result of recalcitrance of tertiary/quaternary C-atom (SUFLITA and MORMILE, 1993) or metabolite(s) production during degradation (LIU *et al.*, 2001). Also, some reports indicate that MTBE is potential inhibitor of microbial metabolism (MO *et al.*, 1997).

CONCLUSION

The conclusion gained according to the results of this investigation is that bacterial strain *Kocuria sp*. 27/1, previously isolated from oil hydrocarbons-contaminated wastewaters, is capable of degrading MTBE, that was added as an additional carbon and energy source. The degradation rate of different initial MTBE concentrations ranged from 28-62%. During incubation period the increase of bacterial number was noted, which confirms the potential usrd of this strain in bioremediation of MTBE-contaminated ecosystems.

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SPOSOBNOST *KOCURIA* SP. U BIODEGRADACIJI MTBE-A

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I z v o d

Metil tercijarni butil etar (MTBE) je jedan od najčešće korišćenih oksigenata koji je dodat benzinu u cilju zamene toksičnih komponenti i redukcije aerozagađenja. Zbog intenzivne upotrebe i otpornosti, MTBE je postao jedan od najvažnijih polutanata u čovekovoj sredini. Ova istraživanja pokazala su potencijal bakterijskog izolata *Kocuria sp*. 27/1 u biodegradaciji MTBE-a kao dounskog izvora ugljenika i energije. Dobijeni rezultati pokazuju da je *Kocuria sp*. 27/1 bila sposobna da koristi različite početne koncentracije MTBE-a dodatog kao dopunski izvor ugljenika i energije. Posle 86 dana inkubacije u orbitalnom šejkeru pri 110 rpm i 27 °C, stepen degradacije početnih koncentracija MTBE-a (25, 125 i 250 μg x ml^{-1}) iznosio je između 28 i 62%. Najveći stepen degradacije u svim ispitivanim koncentracijama zabeležen je na početku inkubacionog perioda. Tokom inkubacije konstatovan je porast broja bakterija, ali je ovaj porast bio veoma slab, što je verovatno povezano sa nastankom intermedijera tokom degradacije i otpornošću C atoma. Ova istraživanja potvrđuju potencijalnu primenu bakterije *Kocuria sp*. 27/1 u bioremedijaciji ekosistema kontaminiranih MTBE-om.

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