Eco. Env. & Cons. 26 (3) : 2020; pp. (1420-1423)
Copyright@ EM International
ISSN 0971–765X

Development and evaluation of bacterial consortia for biodegradation of polycyclic aromatic hydrocarbons

E.R. Faizulina, S.A. Aitkeldiyeva, L.G. Tatarkina, K. Ashimuly, A.V. Alimbetova and A.K. Sadanov*

Research and Production Center for Microbiology and Virology, Almaty, Kazakhstan

(Received 4 April 2020; accepted May 31, 2020)

ABSTRACT

Based on PAH-degrading bacteria, consortia have been created, which showed active growth on a mixture of naphthalene, fluorene, and anthracene. Their biomass increased by a factor of 6.6-14. Gas chromatography analysis showed that naphthalene was the most degrading; the degree of its destruction during seven days of cultivation was 94.6-97.7%. Fluorene degradation was 57.1-70.4%. Anthracene was the most resistant to microbial impact. The degree of its degradation by the studied consortia was 19.2-39.8%. The results showed that effective bioremediation of different ecosystems contaminated with mixed organic compounds can be achieved by using microbial consortia.

Key words : *Biodegradation, PAH-degrading bacteria, Consortium, Polycyclic aromatic hydrocarbons, Naphthalene, Fluorene, Anthracene.*

Introduction

At the present time, polycyclic aromatic hydrocarbons (PAH) are a serious problem for the environment and people's health (Downward *et al.*, 2014; Abdel-Shafy and Mansour, 2016). Due to their hydrophobic nature, they remain in the environment for quite a long time and can be a source of secondary pollution (Duran and Cravo-Laureau, 2016). Many compounds are carcinogenic, mutagenic, and, therefore, teratogenic (Balachandran *et al.*, 2012).

Bioremediation with the use of microorganisms can be an effective and economical solution for remediation of contaminated areas. The majority of types of contamination, as a rule, include not a single source but multiple contaminants (Andreoni and Gianfreda, 2007). Therefore, bioremediation involving one type of microorganisms is often ineffective due to low degradation activity, adaptability, and viability of the microorganisms used (Rayu, Karpouzas and Singh, 2012; Herrero, and Stuckey 2015). These limitations can be easily overcome by applying a microbial consortium consisting of several strains with different biodegradation abilities and physiological properties that ensure survival under different conditions (Li Zhao and Adam, 2016; Gurav *et al.*, 2017). It has been established that the effectiveness of PAH degradation is higher if a consortium of bacteria is used due to their synergism and coordinated metabolic actions (Xu *et al.*, 2013; Tauler *et al.*, 2016). The use of several microorganisms capable of biodegradation of the same organic pollutants increases the probability of successful bioremediation since it is more likely that at least one microorganism will show good adaptability and viability in a particular contaminated site (Dastgheib *et al.*, 2012).

The objective of this study was to create bacterial consortia and study their PAH biodegradation efficiency.

Materials and Methods

Chemicals and culture media

Naphthalene, fluorene, and anthracene were provided by Sigma-Aldrich (purity 98-99%). Nutrient agar (Titan Biotech Ltd, India) and mineral medium of the following composition, g/L: NH_4NO_3 – 1.0, K_2HPO_4 – 1.0, KH_2PO_4 – 1.0, $MgSO_4$ – 0.2, $CaCl_2.6H_2O$ – 0.02, $FeCl_3$ – traces, NaCl – 1.0, pH =7.0-7.2.

Consortia growth on the PAH mixture

PAH-degrading bacteria isolated from contaminated soil of the oil field in Western Kazakhstan were used to create consortia. Bacterial strains were grown on nutrient agar slant. Then, with sterile water, the cells were washed away in separate sterile flasks. The consortium was received by mixing suspensions of separate strains in equal amounts.

Hydrocarbon-oxidizing ability of the created consortia of microorganisms was studied in the flasks with the liquid mineral medium of 100 mL. As a source of carbon and energy were added the PAH mixture ‾ naphthalene 1.0 g/L, fluorene 0.5 g/L, and anthracene 0.5 g/L. The PAH mixture was previously dissolved in acetone and added to the required final concentration. The flasks with PAH were left for a day on a shaker to evaporate the solvent; after that, a cell suspension in the amount of 5 ml was added. They were incubated under aeration conditions on shaker ISF1-X model SMX1501 at 180 rpm for 7 days. The control was a bacteria-free mineral medium with the addition of the studied substrates. The activity of the created consortia was assessed based biomass growth, which was determined by changing the optical density on the PD-303 spectrophotometer (Japan) at 540 nm wavelength.

Biodegradation analysis of the PAH mixture

To determine the content of the PAH mixture 100 mL of sample was extracted with 40 mL of chloroform for 20 min. After extraction, the organic phase was separated using a separatory funnel. The samples were analyzed using gas chromatography-mass spectrometry. Using a microsyringe for the autosampler, the sample was injected into a device for injecting gas chromatograph samples with a mass spectrometer detector 7890D/5977A (Agilent, USA) of 0.5 µL in Split 10:1 mode, sample input temperature 240°C, solvent delay of 5 min. The separation was performed using a DB-35MS chromatographic capillary column 30 m long, with an inner diameter of 0.25 mm and a film thickness of 0.25 µm at a constant carrier gas (helium) rate of 1 mL/min. The temperature of chromatography from 50 °C to 300 °C with a heating speed of 20 °C/min. Detection is performed in SCAN m/z 35-650 mode. Agilent MSD Chem Station software (version 1701EA) was used to control the gas chromatography system, register, and process the obtained results and data. Wiley 7th edition and NIST'02 libraries (the total number of spectra in the libraries is more than 550 thousand) were used for decoding the obtained mass spectra

Statistical analysis

The statistical analysis was performed using Microsoft software (Redmond, Washington, USA), Excel. Differences with $p<0.05$ were considered statistically significant.

Results and Discussion

Selection of consortia of PAH microorganism-destructors

To increase the efficiency of biodegradation of oil hydrocarbons, it is advisable to use mixed cultures consisting of two or more microorganisms. It should be noted that no clear criteria for the preparation of microorganisms' consortia have been proposed so far, and biopreparations include strains on the principle of their compatibility and high hydrocarbon-oxidizing activity (Vetrova, 2010).

Consortia of PAH microorganisms-destructors were composed of 9 bacterial strains growing on different hydrocarbons. Strains of *Ps. aeruginosa* P1-naf2-1 and *Ps. frederiksbergensis* P1-naf2-2 grew well on medium with naphthalene, strains of *Ps. songnenensis* 1-2naf, *Ps. songnenensis* 1-5naf, *B. cereus* 1/6 fl, *B. megaterium* 1/10 fl, *B. thuringiensis* 1/13 fl, and *Rh. marinonascens* 24 ‾ on medium with fluorene. The *B. haynesii* 21WDT strain grew equally well on both substrates. Besides, strains growing on fluorene, except for *Ps. songnenensis* 1-5 naf, also used anthracene as the only source of carbon and energy.

Fifteen consortia of hydrocarbon-oxidizing microorganisms were formed. Primary screening on the ability to grow in a liquid mineral medium with the PAH mixture (naphthalene 1.0 g/L, fluorene 0.5 g/L, anthracene 0.5 g/L) was performed. The activity of the created consortia was assessed based on biomass growth. The results showed that four consortia 1 (P1-naph2-1, 1-2-naph, 1/6-fl, 24), 6 (P1-naph 2-1, 1-5-naph, 1/6-fl, 21WDT), 9 (P1-naph2-2, 1-5-naph, 1/13-fl), and 10 (P1-naph2-2, 1/6-fl, 1/10-fl) showed the highest activity among all the studied consortia.

It was established that the growth character of consortia 1 and 6 was practically the same (Figure 1). The cells were adapting to the substrates during the day, then their biomass sharply increased in 4 days and the stationary phase of growth occurred. At the same time, the biomass increased by a factor of 14.2 and 11, respectively.

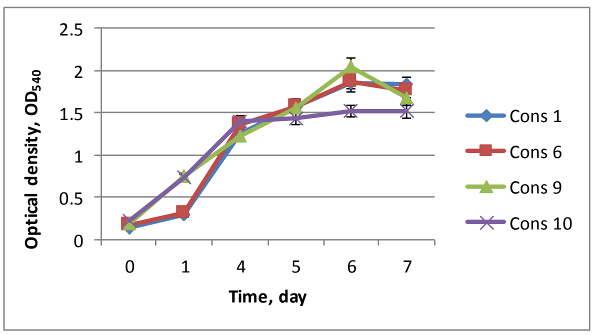

Fig. 1. Consortia growth on PAH mixture

Consortia 9 and 10 had a shortened lag phase; after a day of cultivation, their biomass increased by a factor of 4 and 3.2, respectively. The exponential phase of growth of consortium 9 lasted 6 days and of consortium 10 ‾ 4 days, while the biomass increased by a factor of 11 and 6.6, respectively.

Destruction of the PAH mixture by selected bacterial consortia

Many studies have been devoted to the biodegradation of polycyclic aromatic hydrocarbons, and most of their degradation processes are associated with the use of certain species of microbial populations. These species usually use a specific target pollutant. However, they have limited capacity to degrade other hydrocarbons. Therefore, a mixed consortium of microorganisms is better suited for treating areas contaminated with a mixture of different types of PAH (Oberoi, Philip and Bhallamudi 2015).

The destruction of the PAH mixture under the influence of four selected consortia was studied. It was found out that naphthalene was the most degrading; the degree of its destruction during seven days of cultivation was 94.6-97.7% (Figure 2). Fluorene was more effectively oxidized under the influence of consortium 1; its residual content in the medium was 29.6%. Other consortia utilized 57.1-67.5% of hydrocarbons. Anthracene was the most resistant to microbial impact. The degree of its destruction by the studied consortia was 19.2-39.8%. This is consistent with the data obtained by Yuan *et al.* (2000). They showed that naphthalene was the most easily degrading compound, anthracene was the most difficult to decompose. The reason may be low bioavailability for microbes, which depends on the complexity of the PAH chemical structure (Bauer and Capone 1988). Thus, Akashdeep S. Oberoi et al. showed that the time of the PAH degradation increased with the number of rings and the degree of condensation of the ring. Studying the biodegradation of various PAH by enriched bacterial cultures, they found that the degradation followed the following order: naphthalene (2-rings)>acenaphthenes (2-rings with an ethylene bridge) > phenanthrene (3-rings) (Oberoi, Philip and Bhallamudi 2015).

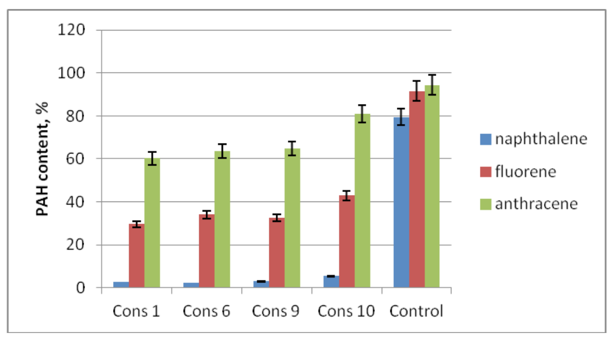

Fig. 2. The destruction of the PAH mixture by active consortiums

Abiotic losses of the studied substrates in control were also determined. The residual content of fluorene and anthracene in the medium in 7 days was 91.6% and 94.4% respectively, while naphthalene remained 79.5%.

Conclusion

The consortia were obtained that effectively degrade polycyclic aromatic hydrocarbons. In mineral media with naphthalene, fluorene, and anthracene, their biomass increased by a factor of 6.6-14. It was established that consortium 1 was the most active. Under its influence, naphthalene content decreased by 97.3%, fluorene ˉ by 70.4%, and anthracene ˉ by 39.8% after 7 days. These results support the idea that effective bioremediation of different ecosystems contaminated with mixed organic compounds can be achieved by using microbial consortia consisting of several microorganisms.

Acknowledgments

This study was supported and funded by the Ministry of Education and Science of the Republic of Kazakhstan (Grant no. AP05132069).

References

Abdel-Shafy, H.I. and Mansour, M.S.M. 2016. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation Egypt. *J. Petrol*. 25 : 107-123.

Andreoni, V. and Gianfreda, L. 2007. Bioremediation and monitoring of aromatic-polluted habitats. *Appl. Microbiol. Biotechnol.* 76 : 287–308.

Balachandran, C., Duraipandiyan, V., Balakrishna, K. and Ignacimuthu, S. 2012. Petroleum and polycyclic aromatic hydrocarbons (PAHs) degradation and naphthalene metabolism in *Streptomyces* sp. (ERI-CPDA-1) isolated from oil contaminated soil. *Bioresour. Technol.* 112 (2012) : 83-90.

Bauer, J. E. and Capone, D. G. 1988. Effects of co-occurring aromatic hydrocarbons on degradation of individual polycyclic aromatic hydrocarbons in marine sediment slurries. *Applied and Environmental Microbiology.* 54 (7) : 1649-1655.

Dastgheib, S.M.M., Amoozegar, M.A., Khajeh, K., Shavandi, M. and Ventosa, A. 2012. Biodegradation of polycyclic aromatic hydrocarbons by a halophilic microbial consortium. *Appl Microbiol Biotechnol*. 95 : 789–798.

Downward, G.S., Hu, W., Rothman, N., Reiss, B., Wu, G., Wei, F., Chapman, R.S., Portengen, L., Qing, L. and Vermeulen, R. 2014. Polycyclic aromatic hydrocarbon exposure in household air pollution from solid fuel combustion among the female population of Xuanwei and Fuyuan Counties China. *Environ. Sci. Technol.* 48 : 14632-14641.

Duran, R. and Cravo-Laureau, C. 2016. Role of environmental factors and microorganisms in determining the fate of polycyclic aromatic hydrocarbons in the marine environment. *FEMS Microbiol. Rev.* 40 : 814-830.

Gurav, R., Lyu, H. H., Ma, J. L., Tang, J. C., Liu, Q. L. and Zhang, H. R. 2017. Degradation of n-alkanes and PAHs from the heavy crude oil using salt-tolerant bacterial consortia and analysis of their catabolic genes. *Environ. Sci. Pollut. Res.* 24 : 11392–11403.

Herrero, M. and Stuckey, D. C. 2015. Bioaugmentation and its application in wastewater treatment: a review. *Chemosphere*. 140 : 119–128.

Li, X. F., Zhao, L. and Adam, M. 2016. Biodegradation of marine crude oil pollution using a salt-tolerant bacterial consortium isolated from Bohai Bay. *China. Mar. Poll. Bull.* 105 : 43–50.

Oberoi, A. S., Philip, L. and Bhallamudi, S. M. 2015. Biodegradation of Various Aromatic Compounds by Enriched Bacterial Cultures: Part A–Monocyclic and Polycyclic Aromatic Hydrocarbons. *Appl Biochem Biotechnol.* 176 : 1870-1888.

Rayu, S., Karpouzas, D. G. and Singh, B. K. 2012. Emerging technologies in bioremediation: constraints and opportunities. *Biodegradation*. 23 : 917–926.

Tauler, M., Vila, J., Nieto, J.M. and Grifoll, M. 2016. Key high molecular weight PAH-degrading bacteria in a soil consortium enriched using a sand-in-liquid microcosm system Appl. *Microbiol. Biotechnol*. 100 : 3321-3336.

Vetrova, A.A. 2010. *Biodegradation of oil hydrocarbons by plasmid-containing microorganisms-destructors*: dis. ... Ph.D. Moscow, pp. 169.

Xu, N., Bao, M., Sun, P. and Li, Y. 2013. Study on bioadsorption and biodegradation of petroleum hydrocarbons by a microbial consortium. *Bioresour. Technol.* 149 : 22-23.

Yuan, S., Wei, S. and Chang, B. 2000. Biodegradation of polycyclic aromatic hydrocarbons by a mixed culture. *Chemosphere.* 41 : 1463-1468.