Editorial Board

Volume 52, Iss. 6, 2017

Journal of Chemical Technology and Metallurgy

52, Iss. 6, 2017
ISSN 1314-7471 (print)
ISSN 1314-7978 (on line)

EDITOR-IN- CHIEF
Prof. Dr. Bogdana Koumanova
Tel: (+ 359 2) 81 63 302
University of Chemical Technology and Metallurgy
8 Kl. Ohridski, 1756 Sofia, Bulgaria
E-mail: journal@uctm.edu

SELECTED ARTICLES

International Conference

"Collaboration Seminar of Chemistry and

Industry (CoSCI-2016)"

5-6 October, 2016

Surabaya, Indonesia

Invited Editor

Dr. Purkan Purkan

Composite beads of chitosan/bentonite as a matrix for phosphate fertilizer controlled-release

Bambang Piluharto, Veinardi Suendo, Ida Maulida, Asnawati

Serum acetaldehyde as a potential biomarker for the detection of pathogenic biofilm formation by *Candida albicans*

Masfufatun, Sumayyah Luqman Bayasud, Mei Shirli Yasinta, Ni'matuzahro, Afaf Baktir

The influence of ascorbic acid, creatine, and creatinine on the uric acid analysis by potentiometry using a carbon paste modified imprinting zeolite electrode

Miratul Khasanah, Muji Harsini, Alfa Akustia Widati, Prihantari Mukti Ibrani

A novel spectrophotometric method for determination of histamine based on its complex reaction with Cu(II) and alizarin red S

Miftakhul Jannatin, Ayu Nabila I.L, Ganden Supriyanto, Pratiwi Pudjiastuti

Application of ionic liquid dispersive liquid-liquid microextraction for analysis of n-nitrosodipropylamine in salted fish

Aning Purwaningsih, Yanuardi Raharjo, Hendarta Agasi

Determination of chlorpyrifos pesticide by effervescence liquid phase microextraction HPLC UV-Vis

Usreg Sri Handajani, Yanuardi Raharjo, Bagas Wantoro

Effect of aliphatic and aromatic hydrocarbons on the oxygenase production from hydrocarbonoclastic bacteria

Sri Sumarsih, Ni'matuzahroh, Fatimah, Miranti Puspitasari, Meilisa Rusdiana

Identification of *Candida* species by assimilation and Multiplex-PCR methods

Hermansyah, Nurmalina Adhiyanti, Julinar, Kemas Yakub Rahadiyanto, Susilawati

Xylanase enzyme from a local strain of *Pseudomonas stutzeri*

Purkan Purkan, Emma Huruniawati, Sri Sumarsih

Study of a catalyst of citric acid crosslinking on locust bean gum

Wuryanto Hadinugroho, Suwaldi Martodihardjo, Achmad Fudholi, Sugeng Riyanto

Production and characterization of sulfonated chitosan-calcium oxide composite membrane as a proton exchange fuel cell membrane

Siti Wafiroh, Abdulloh, Winda Kusuma Wardani

An excellent way to prepare conductive glass using a simple glass plate aiming a promising solar cell

Harsasi Setyawati, Handoko Darmokoesoemo, Hamami, Faidur Rochman, Ahmadi Jaya Permana

Partial oxidative synthesis of fluorescent carbon derived from local bamboo leaves

Ahmadi Jaya Permana, Abdul Haris, Harsasi Setyawati, Mochamad Zakki Fahmi

Stability of coordination compounds obtained by reduction of copper(II) halide and 1,3–bis(diphenylphosphino)propane (DPPP)

Nike Prilil Puspita Sari, Lis Siaturohmah, Effendy, Fariati

Phenolic compounds from Aquilaria microcarpa stem bark

Alfinda Novi Kristanti, Mulyadi Tanjung, Okky P. Rahayu, Erika Herdiana

Electrochemical degradation of naphthol AS-BO batik dyes

Journal of Chemical Technology and Metallurgy

Country	Bulgaria - SIR Ranking of Bulgaria
Subject Area and Category	Chemical Engineering Chemical Engineering (miscellaneous) Engineering Industrial and Manufacturing Engineering
Publisher	University of Chemical Technology and Metallurgy
Publication type	Journals
ISSN	13147978, 13147471
Coverage	2013-2020
Scope	The Journal of Chemical Technology and Metallurgy started originally in 1954 as Annual Journal of the former Higher Institute of Chemical Technology. It ran in Bulgarian. In 2000 its name was changed to Journal of the University of Chemical Technology and Metallurgy. It was published quarterly in English. Since 2013 it has run bimonthly as Journal of Chemical Technology and Metallurgy. Journal of Chemical Technology and Metallurgy is a specialized scientific edition presenting original research results in the field of chemical technology and metallurgy, chemical engineering, biotechnology, industrial automation, environmental protection and natural sciences. The articles published in Journal of Chemical Technology and Metallurgy refer to: Inorganic Chemistry; Organic Chemistry; Analytical Chemistry; Physical Chemistry; Organic Synthesis and Fuels; Polymer Engineering; Textile and Leather; Cellulose; Paper and Polygraphy; Inorganic and Electrochemical Productions; Chemical Engineering; Industrial Automation; Information Technology; Biotechnology; Economics and Management of Chemical and Metallurgical Industry; Sustainable Development and Environmental Protection; Physical Metallurgy; Metallurgy of Iron and Casting; Metallurgy of Non-ferrous and Semiconducting Materials; Technology of Silicates; Nanomaterials.

17

H Index

Homepage

How to publish in this journal

Contact

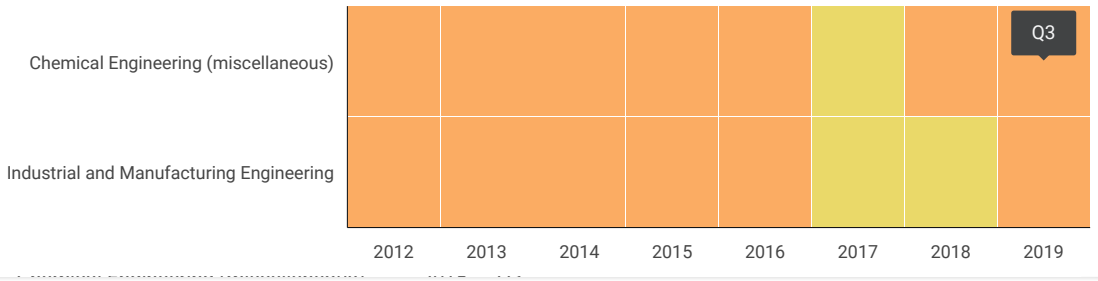
Join the conversation about this journal

Nature Briefing

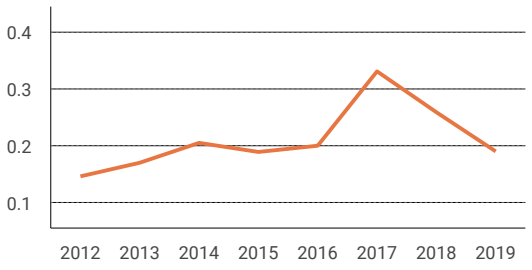
An essential round-up of science news, opinion & analysis, free in your inbox.

nature.com OPEN

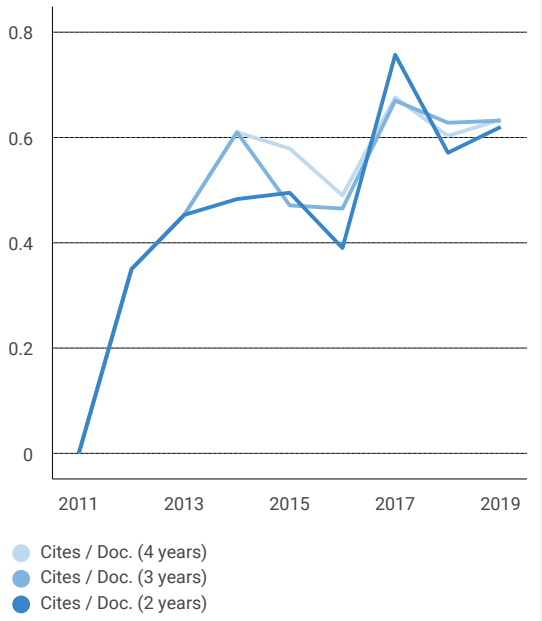
Quartiles



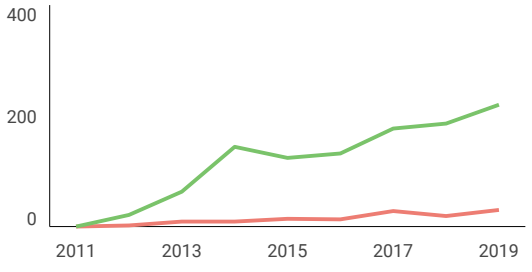
SJR



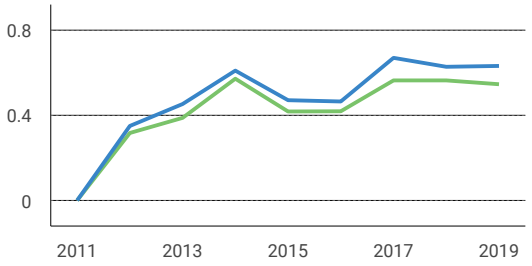
Citations per document



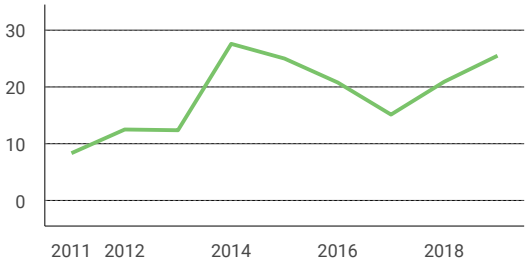
Total Cites Self-Cites



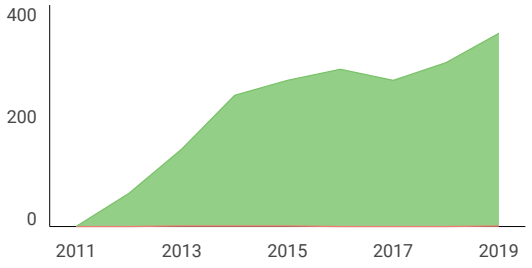
External Cites per Doc Cites per Doc



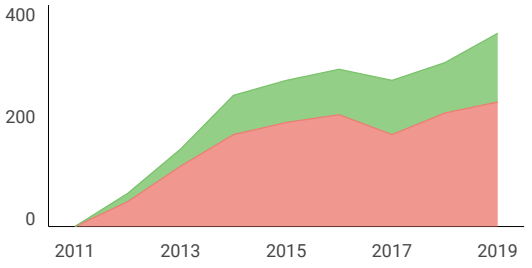
% International Collaboration



Citable documents Non-citable documents



Cited documents Uncited documents



← Show this widget in your own website

Just copy the code below and paste within your html code:

```
<a href="https://www.scimag
```

EFFECT OF ALIPHATIC AND AROMATIC HYDROCARBONS ON THE OXYGENASE PRODUCTION FROM HYDROCARBONOCLASTIC BACTERIA

Sri Sumarsih[1], Ni'matuzahroh[2], Fatimah[2], Miranti Puspitasari[1], Meilisa Rusdiana[1]

[1] *Chemistry Department, Faculty of Sciences and Technology*
Universitas Airlangga
[2] *Biology Department, Faculty of Sciences and Technology*
Universitas Airlangga
Kampus C UNAIR Mulyorejo Surabaya, Indonesia
E-mail: sri-sumarsih@fst.unair.ac.id

Received 05 January 2017
Accepted 20 July 2017

ABSTRACT

This research aims to study the effect of aliphatic and aromatic hydrocarbon on oxygenase production from four hydrocarbonoclastic bacteria, including Actinobacillus sp. P3(7), Bacillus subtilis 3KP, Micrococcus sp. LII(61), and Pseudomonas putida T1(8). The bacterial growth during cultivation in a mineral salt medium, containing 1 % hydrocarbons (hexadecane, toluene, and naphthalene) was observed by measuring the optical density of the culture medium at λ 600 nm. The activity of the intracellular catabolic enzyme was determined by measuring the change of NADH absorbance at λ = 340 nm with an UV/Vis spectrophotometer. The results showed that the addition of aliphatic and aromatic hydrocarbons in the growth media enhanced the growth and production of oxygenase from the four hydrocarbonoclastic bacteria. The addition of 1 % hexadecane and 1 % naphthalene into cell culture of Pseudomonas putidaT1(8) increased the oxygenase activity up to 8.789 times, and 20.770 times, respectivelly. The addition of 1 % toluene into cell culture of Micrococcus sp. LII(61) increased the oxygenase activity up to 15.72 times.

Keywords: bacteria, hydrocarbonoclastic, oxygenase, hexadecane, toluene, naphthalene.

INTRODUCTION

Hydrocarbon-degrading microbes were investigated by researchers and the oil industry for many purposes, such as petroleum exploration, treatment of environmental pollution, oil sludge degradation or oil tanks cleaning. Microbial degradation is an effective and inexpensive approach for degradation of hydrocarbons from oil sludges. An oil sludge is a complex organic material containing aliphatic, aromatic and polycyclic aromatic hydrocarbons (PAHs). The metabolism of PAHs in the human body produces epoxides, which are mutagenic and carcinogenic affecting the skin, blood, immune system, liver, spleen, kidney, lung, fetal development, and also lost weight [1].

Biodegradation of aliphatic and aromatic hydrocarbons can occur in aerobic or anaerobic conditions. The microbial aerobic alkane biodegradation is usually initiated by O_2-dependent monooxygenases. The monooxygenase is the key enzyme of the alkane degradation pathway, that converts alkanes to corresponding alkylalcohols. The alkylalcohol is converted to alkylaldehyde by alcohol dehydrogenase (ADH), and then to fatty acids by the aldehyde dehydrogenase (ALDH). The fatty acids are activated by conjugation to CoA, and further the conversion proceeds by β-oxidation to generate acetyl-CoA. The initial terminal hydroxylation of n-alkanes in bacteria can be carried out by enzymes of different families. Bacteria degrading short chain alkanes (C_2-C_4) have enzymes related to methane

monooxygenase. Bacteria degrading medium chain alkanes (C_5-C_{11}) or long chain alkanes (>C_{12}) frequently have an integral membrane monooxygenase, called AlkB alkane hydroxylase. Some strains have alkane hydroxylases that belong to the family of cytochrome P-450 and are active against C_5-C_{11} alkanes. Aromatic hydrocarbons, such as benzene, toluene, xylene, and naphthalene can also be degraded under aerobic conditions. The degradation of these compounds serves as an initial step in the formation of catechol or structurally related compounds. Catechol compounds formed can be degraded into other compounds that can enter into the citric acid cycle [2 - 4].

The hydrocarbonoclastic bacteria play the key role in the removal of hydrocarbons from polluted environments. Microorganisms-based oil sludge treatment technology has already started and is developed in Indonesia. Various studies have been conducted to improve the solubility and degradation of the oil sludge, such as exploration of potential oil sludge degrading microbes and biosurfactant producing bacteria. Ni'matuzahroh*et al*. have isolated some potential oil sludge degrading bacteria from oil contaminated soil, the so call hydrocarbonoclastic bacteria[5, 6].

This research aims to investigate the effect of aliphatic and aromatic hydrocarbons on the oxygenases production from hydrocarbonoclasticbacteria: *Actinobacillus* sp. P3(7), *Bacillus substilis* 3KP, *Pseudomonas putida* TI(8), and *Micrococcus* sp. LII (61).

EXPERIMENTAL

Materials

The materials used in this research were Nutrient Agar (NA), Nutrient broth (NB), yeast extract, sea salt, hexadecane, naphthalene, toluene, Tris-base, HCl, and NADH.

Bacteria strains

Bacteria strains used in this research, identified as *Actinobacillus* sp. P3(7), *Bacillus substilis* 3KP, *Pseudomonas putida* TI(8), and *Micrococcus* sp. LII (61), were collection of Laboratory of Microbiology, Biology Department Faculty of Science and Technology, Airlangga University.

Bacteria Cultivation

Each bacterium were inoculated on Nutrient Agar (NA) slant and incubated for 24 hours at room temperature, for the next research steps.

Bacterial Growth Curve

The growth of the four bacteria strains was measured in 20 mL sterile Mineral Salt Medium (MSM) containing 1 % hexadecane in 100 mL Erlenmeyer flasks. The other flasks contained MSM supplemented with 0.5 % yeast extract and 1% naphthalene and toluene, instead of hexadecane. All cultures were incubated by shaking 100 rpm at 30^0C for 14 days. Control flasks of each bacteria strain in the MSM medium, without hydrocarbon were incubated in the same conditions as reference for the bacterial growth. The bacterial growth was indirectly assessed by turbidity measurement of optical density at 600 nm ($OD_{600\ nm}$) using an UV/Vis spectrophotometer at 2 days intervals [7, 8].

Preparation of the Intracellular Enzymes

Intracellular oxygenase enzymes were prepared by cultivation of each bacterium in 20 mL sterile MSM supplemented with 0.5 % yeast extract and 1% hexadecane in 100 mL Erlenmeyer flasks. The other flasks contained MSM supplemented with 0.5 % yeast extract and 1% naphthalene and toluene, instead of hexadecane. All cultures were incubated by shaking 100 rpm at 30^0C for 14 days. Control flasks of each bacteria strain in MSM without hydrocarbon, were incubated in the same conditions as the reference for the bacterial enzymes production. Samplings were done in 2 days interval period. Cells were harvested by centrifugation at 5000 rpm, then resuspended in 5 mL of buffer Tris-HCl 20 mM pH 7.4, and disrupted using an ultrasonic disintegrator for 4 minutes. The cells lysate was centrifuged at 8000 rpm at 4ºC for 10 minutes. The cell-free supernatant obtained was the crude intracellular enzyme. The oxygenase activities of the crude enzymes were determined against the corresponding hydrocarbon as substrates [8, 9].

Assay of enzyme activity

The reaction mixture contained 20 mM Tris-HCl buffer (pH 7.4), crude enzymes, 0.1 mM NADH, 1 %

hydrocarbons solution (in 80 % DMSO), and 50 µL crude extract in 1 mL volume. The mixture was then incubated at 30^{0}C for 6 minutes. The reaction mixture containing inactivated enzyme were used as negative controls. Enzymes activities were assessed by measuring the absorbance decrease caused by consumption of NADH and resulting from oxidization of hydrocarbons, at 340 nm with an UV-Vis spectrophotometer. One unit enzyme activity is defined as the amount of enzyme required for consumption of 1 micromole of NADH per minute [7, 8].

RESULTS AND DISCUSSION

Bacterial Growth on a Hydrocarbon Containing Medium

The effect of aliphatic and aromatic hydrocarbons on the bacterial growth was studied by cultivation of four bacteria: *Actinobacillus* sp. P3(7), *Bacillus substilis* 3KP, *Pseudomonas putida* TI(8), and *Micrococcus* sp. LII (61) in the MSM, containing different hydrocarbons (hexadecane, toluene and naphthalene). The results showed that the addition of 1 % hydrocarbons (hexadecane, toluene and naphthalene) into the bacterial culture - hexadecane, naphthalene or toluene, could enhance the hydrocarbonoclastic bacteria growth, as confirmed by an increasing OD_{600nm}, as presented on the bacterial growth curve (Fig. 1). The bacterial growth curves demonstrate that the four bacteria show the same growth pattern in the first 2 days of cultivation. They could grow rapidly in the MSM enriched with yeast extract, both without and with the addition of hydrocarbons. The adaptation phase was not observed from all bacteria. The bacterial cells started multiplying immediately after cultivation and gained exponential phase until second day of cultivation. The four hydrocarbonoclastic bacteria were capable to adapt and used hydrocarbons (hexadecane, naphthalene and toluene) as carbon sources, after 2 days of cultivation. As seen, the growth curve showed an increasing of bacterial growth (OD_{600nm})

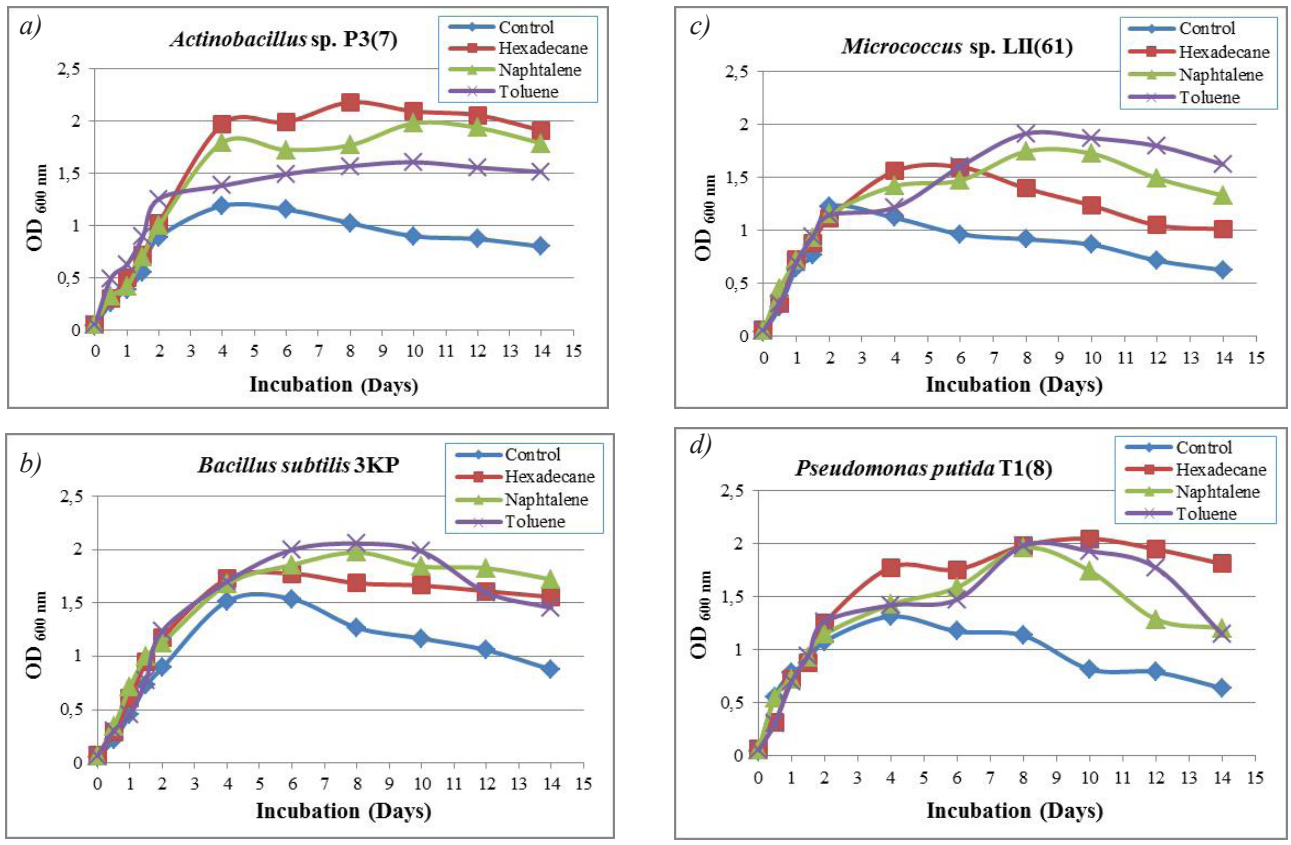


Fig. 1. Bacterial growth curves of hydrocarbonoclastic bacteria in hydrocarbon containing medium: 1a - *Actinobacillus* sp. P3(7); 1b - *Bacillus subtilis* 3KP; 1c - *Micrococcus* sp. LII(61); 1d - *Pseudomonas putida*T1(8).

when compared to the reference growth curve without the addition of hydrocarbons. In the bacteria cultivation on a medium without hydrocarbons, all bacteria realized the stationary or dead phase after 4 days cultivation. However, the bacteria grown in a medium containing hydrocarbons gain the increasing $OD_{600\ nm}$. It was been concluded that *Actinobacillus*sp. P3(7), *Pseudomonas putida* T1(8), *Bacillus subtilis* 3KP, and *Micrococcus* sp. LII(61) were able to degrade and use hydrocarbons (hexadecane, naphthalene and toluene) as a carbon source for the bacterial growth.

Degradation of hexadecane in MSM by *Pseudomonas aeruginosa* PSA5, *Rhodococcus* sp. NJ2 and *Ochrobactrumintermedium* P2, isolated from petroleum sludge, was also investigated [8]. Naphthalene biodegradation has also been reported in bacteria genus *Pseudomonas, Mycobacterium, Corynebacterium, Aeromonads, Rhodococcus*, and *Bacillus* [10].

Hydrocarbons, which are molecules with high energy and carbon content, can be good carbon and energy source for microorganisms. On the other hand, the complexity of the structure, molecular mass, hydrophobicity and solubility of the hydrocarbons are become a limiting factor for bacteria to access the hydrocarbons. Bacteria have a specific mechanism to access and degrade hydrocarbons, to produce an extracellular biosurfactant or bacterial adhesion mechanisms. The adhesion of the hydrocarbon to the cell wall can induce the bacteria to release enzymes that catalyze the oxidation of hydrocarbons. Most bacteria, able to degrade n-alkane, produce and secrete surfactants that allow emulsification of the hydrocarbon [4].

The mechanisms involved in the degradation of hydrocarbon are a specific enzyme-mediated, attachment of microbial cells to the substrate, and biosurfactants production. The process of hydrocarbon degradation by bacteria is influenced by several factors, namely bacterial properties, properties of hydrocarbons and environmental factors. The ability of bacteria to degrade the hydrocarbon depends on the ability for bacterial adaptation to the environment, the

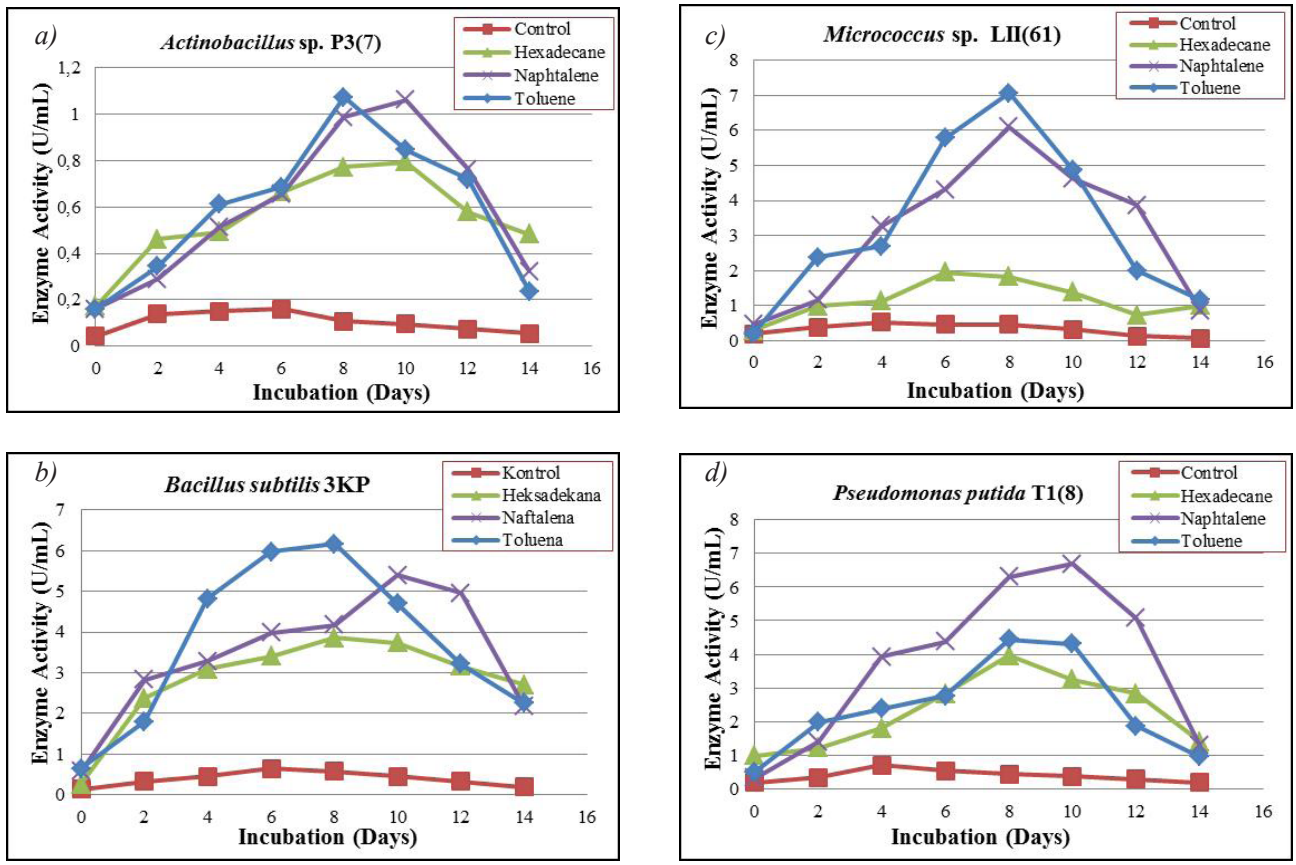


Fig. 2. Effect of hydrocarbons on the oxygenase production by hydrocarbonoclatic bacteria: 2a - *Actinobacillus* sp. P3(7); 2b - *Bacillus subtilis* 3KP; 2c - *Micrococcus* sp. LII(61); 2d – *Pseudomonas putida*T1(8).

toxicity of the substrate and the presence of hydrocarbon degrading enzymes in the bacteria [10].

The Effect of Hydrocarbons on the Oxygenase Production

The effect of hydrocarbons on the oxygenase production was observed to study the induction of hydrocarbon degrading enzymes during hydrocarbons degradation. The oxygenase activity, caused by consumption of NADH and resulting from oxidization of hydrocarbons, was assessed by measuring the absorbance change at 340nm with an UV-Vis spectrophotometer. One unit enzyme activity is defined as the amount of enzyme required for consumption of 1 micromole of NADH per minute [7]. Fig. 2 demonstrates the effect of hexadecane, toluene and naphthalene onto the oxygenases activity of four hydrocarbonoclastic bacteria: *Actinobacillus*sp. P3(7), *Bacillus subtilis* 3KP,*Micrococcus* sp. LII(61), and *Pseudomonas putida* T1(8). The results showed that the production of catabolic enzymes (oxygenases) during cultivation of hydrocarbonoclastic bacteria can be enhanced by addition of hydrocarbons in the growth medium. The four bacterial cell cultures, without the addition of a hydrocarbon in the growth medium, showed very low activity towards the corresponding hydrocarbon as a substrate. It was observed that the addition of a hydrocarbon in the media cultures induced oxygenase production in *Actinobacillus* sp. P3(7), *Pseudomonas putida* T1(8), *Bacillus subtilis* 3KP, and *Micrococcus* sp. LII(61).

The Effect of Hexadecane on the Oxygenase Production

The effect of hexadecane on the production of alkane hydroxylases was observed by measuring the activity of the intracellular crude enzymes from *Actinobacillus* sp. P3(7), *Pseudomonas putida* T1(8), *Bacillus subtilis* 3KP, and *Micrococcus* sp. LII(61). The results showed that the production of alkane hydroxylase during cultivation of the four bacteria, can be enhanced by the addition of hexadecane in the growth medium. Bacterial cell culture without the addition of hexadecane in the medium had a very low activity towards hexadecane as a substrate. It was observed that alkane hydroxylase was induced during the hexadecane degradation in all the four bacterial strains, but with different value and incubation time. Table 1 shows that among the four bacteria, *Pseudomonas putida* T1 (8) exhibited the highest activity of alkane hydroxylase (3.955 U/ mL), which was reached at 8 days cultivation. Whereas the other bacteria gained lower activity of alkane hydroxylase, 3.859 U/mL (*Bacillus subtilis* 3KP), 1.823 U/mL (*Micrococcus* sp. LII(61)), and 0.772 U/mL (*Actinobacillus* sp. P3(7)), respectively. The addition of 1% hexadecane into the medium culture of *Pseudomonas putida*T1(8), induced an alkane hydroxylase activity up to 8.789 times higher than the value for the negative control without hexadecane as an inducer.

The ability of bacteria to degrade hydrocarbons is influenced by the presence of catabolic enzymes, capable of degrading hydrocarbons into metabolites and to enter into the citric acid cycle. The first stages of a catabolism of the alkane compounds by bacteria are initiated by the alkane hydroxylase enzyme [2, 3]. Several enzymes, involved in the biodegradation of petroleum hydrocarbons, have been found in bacteria. The activity of alkane monooxygenases in hexadecane degradation have

Table 1. The effect of hexadecane on the activity of alkane hydroxylase.

Bacteria	Enzyme activity (U/mL)		Induction (times)
	no hexadecane	with hexadecane	
Actinobacillus sp. P3(7)	0.150	0.793	5.286
Bacillus subtilis 3KP	0.579	3.859	6.665
Micrococcus sp. LII(61)	0,343	1.952	5.691
Pseudomonas putida T1(8)	0.450	3.955	**8.789**

been identified for *Pseudomonas aeroginosa* sp. PSA5, *Rhodococcus* sp. NJ2, and *Ochrobactrum intermedium* P2 [8]. Alkane hydroxylases which can degrade C_5-C_{16} alkanes, fatty acids, cycloalkane and alkyl benzene were found in *Pseudomonas, Burkhlderin, Rhodococcus and Mycobacterium*. A bacterial P450 oxygenase system which can degrade C5-C16 and cycloalkane were found in *Acinetobacter* and *Mycobacterium* [11].

The Effect of Toluene on Oxygenase Production

The effect of toluene on the production of oxygenases was observed by measuring the activity of the intracellular crude enzyme from *Actinobacillus* sp. P3(7), *Pseudomonas putida* T1(8), *Bacillus subtilis* 3KP, and *Micrococcus* sp. LII(61) towards toluene as a substrate. The results showed that the production of dioxygenase during cultivation of the four bacteria can be enhanced by the addition of toluene in the growth medium. The bacterial cell culture, without the addition of toluene in the medium, showed very low activity towards toluene as a substrate. It was observed that oxygenase was induced during the toluene degradation in the four bacterial strains, but with different value and incubation time.

Table 2 shows that the addition of 1% toluene into the culture medium effected *Micrococcus* sp. LII(61) has exhibited the highest activity of dioxygenase (7.074 U/mL), which has been reached after 8 days cultivation. Whereas the other bacteria gained lower dioxygenase activities: 6.174 U/mL (*Bacillus subtilis* 3KP), 4.437 U/mL (*Pseudomonas putida*T1(8)), and 1.072 U/mL (*Actinobacillus* sp. P3(7)). The addition of 1 % toluene into the medium culture of *Micrococcus* sp. LII(61) induced the dioxygenase activity up to 15.720 times, as compared to the negative control without toluene as an inducer.

Several bacteria that could degrade and use toluene as carbon source had been reported. They use several pathways in toluene degradation, for example a monooxygenase pathway for *Pseudomonas aeruginosa* UKMP-14T, *Bacillus cereus* UKMP-6G, and *Pseudomonas merdocina* KR1, *Pseudomonas stutzuri* ox1, *Burkhoderia* sp. Strain JS150) [13, 14]. A dioxygenase pathway has been reported for (*Pseudomonas putidu* F1) [13, 14].

Effect of Naphthalene on the Enzymes Production

The effect of naphthalene on the production of oxygenases was observed by measuring the activity of the intracellular crude enzyme from *Actinobacillus* sp. P3(7), *Pseudomonas putida* T1(8), *Bacillus subtilis* 3KP, and *Micrococcus* sp. LII(61)s towards naphthalene as a substrate. The results showed that the production of dioxygenase during cultivation of the four bacteria could be enhanced by the addition of naphthalene in the growth medium. The bacterial cell culture, without the addition of naphthalene in the medium, showed very low activity towards naphthalene as a substrate. It was observed that oxygenase was induced during the naphthalene degradation in the four bacterial strain, but with different values and incubation times.

Table 3 shows that the addition of 1% naphthalene into the culture medium has effected *Pseudomonas putida* T1(8), which has exhibited the highest activity of dioxygenase (6.688 U/mL), reached after 10 days cultivation. Whereas the other bacteria gained lower dioxygenase activities: 6.100 U/mL (*Micrococcus* sp. LII(61)), 5.402 U/mL (*Bacillus subtilis* 3KP), and 1.061 U/mL (*Actinobacillus* sp. P3(7)). The addition of 1%

Table 2. Effect of toluene on the oxygenase activity.

Bacteria	Enzyme activity (U/mL)		Induction (times)
	No toluene	with toluene	
Actinobacillus sp. P3(7)	0,16	1.072	10.018
Bacillus subtilis 3KP	0,643	6.174	9.602
Micrococcus sp. LII(61)	0.450	7.074	**15.720**
Pseudomonas putida T1(8)	0,386	4.309	11.163

Table 3. Effect of naphthalene on the oxygenase activity.

Bacteria	Enzyme activity (U/mL)		Induction (times)
	no naphthalene	with naphthalene	
Actinobacillus sp. P3(7)	0.086	1.061	12.337
Bacillus subtilis 3KP	0.450	5.402	12.004
Micrococcus sp. LII(61)	0.450	6.100	13.555
Pseudomonas putida T1(8)	0.386	6.688	**20.770**

naphthalene into the medium culture of *Pseudomonas putida*T1(8) induced the dioxygenase activity up to 20.776 times, as compared to the negative control without naphthalene as an inducer.

The metabolism of naphthalene by *Mycobacterium* sp. involves both monooxygenation and dioxygenation to form cis- and trans-1,2 dihydrodiols, and is catalyzed by the cytochrome P450 monooxygenase, and forms a 1,2-oxide, which is further converted to a trans-diol by epoxide hydrolase [10].

CONCLUSIONS

The addition of aliphatic and aromatic hydrocarbons into the cell cultures induced the bacterial growth and oxygenase production in hydrocarbonoclastic bacteria. The addition of 1% hexadecane and 1 % naphthalene into the culture of *Pseudomonas putida*T1(8) increased the oxygenase activity up to 8.789 times, and 20.770 times, respectivelly. Whereas the addition of 1 % toluene into the cell culture of *Micrococcus* sp. LII(61) increased the oxygenase activity up to 15.72 times.

Acknowledgements

We thank to the Ministry of Research, Technology, and Higher Education for the funding of this research.

REFERENCES

1. O. Ubani, H.I. Atagana, M.S.Thantsha, Biological degradation of oil sludge: A review of the current state of development, Academis Journals, 20, 47, 2013, 6564-6567.
2. A.A. Olajire, J.P. Essien, Aerobic Degradation of Petroleum Components by Microbial Consortia, J. Pet Environ Biotechnol., 5, 5, 2014.
3. F. Rojo, Mini review: Degradation of alkanes by bacteria, Environmental Microbiology, 11, 10, 2009, 2477-2490.
4. F. Rojo, Enzymes for Aerobic Degradation of Alkanes, in K.N. Timmis (ed.), Hanbook of Hydrocarbon and Lipid Microbiology, DOI 10.1007/978-3-540-77587-4_59, Springer-Verlag Berlin-Heidelberg, 2010, 781-797.
5. Fatimah, Ni'matuzahroh, N.H.Alami,Screening of Biosurfactan Production of Hydrocarbonoclastic Microbes Isolated from Oil Polluted Soil, Proceeding, 10th Congress and International Conference of Indonesian Society for Microbiology, 2009, 317-324.
6. Ni'matuzahroh, H. Muna, Fatimah, S. Sumarsih, The Solubility of Oil Sludge by *Bacillus subtilis 3KP* Biosurfactant and Crude Enzyme, J. Appl. Environ. Biol. Sci., 2, 12, 2012, 645-651.
7. P. Li, L. Wang, L. Feng, Characterization of a Novel Rieske-type Alkane Monooxygenase System in *Pusillimonas* sp. T7-7, J. Bacteriol.,2013, doi:10.1128/JB.02107-12.
8. S. Mishra, S.N. Singh, Microbial degradation of n-hexadecane in mineral salt medium as mediated by degradative enzymes, Bioresource Technology, 111, 2012, 146-154.
9. N. Jauhari, S. Mishra, B. Kumaridan, S.N. Singh, Bacteria-mediated Aerobic Degradation of Hexacosane In Vitro Conditions, Bioresource Technology, 170, 2014, 62-68.
10. A. Mrozik, Z. Piotrowska-Seget, S. Labuzek,

Bacterial Degradation and Bioremediation of Plycyclic Aromatic Hydrocarbon, Polish Journal of Environmental Studies, 12, 1, 2003, 15-25.

11. N. Das, P. Chandran, Microbial Degradation of petroleum hydrocarbon contaminants: An overview, SAGE-Hindawi Access to research: Biotechnology Research International, 2011, 2011, 1-13.
12. A. Hamzah, A. Tavakoli, A. Rabu, Detection of Toluene Degradation in Bacteria Isolated from Oil Contaminated Soils, SainsMalaysiana, 40, 11, 2011, 1231-1235.
13. J. Gerben, Zylstra, D.T. Gibson, Toluene Degradation by *Pseudomonas putida* F1 Nucleotide Sequence of the todClC2BADE Gene and Their Expression in *Escherichia coli*, The American Society for Biochemistry and Molecular Biology, 264, 25, 1989, 14940-14946.
14. R.E. Parales, J.V. Parales, D.A. Pelletier, J.L. Ditty, Diversity of Microbial Toluene Degradation Pathway in: A.I. Lasker, S. Sariaslani, G.M. Gadd (Eds), Advances in Applied Microbiology, 2008, Elsevier, UK.