

Volume 2055

 **Conference collection**

# Proceedings of the Europe/Africa Conference Dresden 2017 – Polymer Processing Society PPS



**Dresden, Germany**  
27-29 June 2017

**Editors**

Udo Wagenknecht, Petra Pötschke, Sven Wiessner and Michael Gehde

**AIP** | Conference Proceedings

[proceedings.aip.org](http://proceedings.aip.org)



AIP Conference Proceedings  
**The 18th International Conference  
 on Positron Annihilation**

ORDER PRINT EDITION

# AIP Conference Proceedings

HOME

BROWSE

MORE ▼

To support global research during the COVID-19 pandemic, [AIP Publishing is making our content freely available](#) to scientists who register on Scitation.

To gain access, please [log in](#) or [create an account](#) and then [click here](#) to activate your free access. You must be logged in to Scitation to activate your free access.

## • Table of Contents

### PROCEEDING OF INTERNATIONAL BIOLOGY CONFERENCE 2016: Biodiversity and Biotechnology for Human Welfare

< PREV NEXT >



Conference date: 15 October 2016

Location: Surabaya, Indonesia

ISBN: 978-0-7354-1528-7


Editors: Michael Murkovic, Gianfranco Risuleo, Endry Nugroho Prasetyo, Maya Shovitri and Gibson S. Nyanhongo

Volume number: 1854

Published: Jun 26, 2017

DISPLAY : 20 50 100 all

## PRELIMINARY


 Free . June 2017

### **Preface: Proceeding of International Biology Conference 2016 Biodiversity and Biotechnology for Human Welfare**

AIP Conference Proceedings **1854**, 010001 (2017); <https://doi.org/10.1063/1.4985390>

---



 Free . June 2017


### **The Committee of 3rd International Biology Conference (IBOC) 2016 & 10th Korea-ASEAN Biomass Symposium**

AIP Conference Proceedings **1854**, 010002 (2017); <https://doi.org/10.1063/1.4985391>

---



## ARTICLES

 Free . June 2017

### **Lipase production in lipolytic yeast from Wonorejo mangrove area**

Nur Hidayatul Alami, Liziyatin Nasihah, Rurin Luswidya Artaty Umar, Nengah Dwianita  
Kuswytasari, Enny Zulaika and Maya Shovitri

AIP Conference Proceedings **1854**, 020001 (2017); <https://doi.org/10.1063/1.4985392>

---

SHOW ABSTRACT



## The production and activity test of cellulases using bagasse substrate on *Aspergillus niger* isolated from Clove field, Kare, Madiun


Muh. Waskito Ardhi, Ani Sulistyarsi and Pujiati

AIP Conference Proceedings **1854**, 020002 (2017); <https://doi.org/10.1063/1.4985393>

---

SHOW ABSTRACT



 Free . June 2017

## Lead (Pb) bioaccumulation; genera *Bacillus* isolate S1 and SS19 as a case study


Achmad Arifiyanto, Fitria Dwi Apriyanti, Puput Purwaningsih, Septian Hary Kalqutny, Dyah Agustina, Tini Surtiningsih, Maya Shovitri and Enny Zulaika

AIP Conference Proceedings **1854**, 020003 (2017); <https://doi.org/10.1063/1.4985394>

---

SHOW ABSTRACT



 Free . June 2017

## Local community knowledge and participation for animal diversity conservation in SSWP IV Sidoarjo, East Java, Indonesia

Nova Maulidina Ashuri, Dwi Oktafitria, Indra Wirawan, Zainul Muttaqin, M. Ulya Alfarisy, Abdul Azis, Sherly Eka Argiyanti and Via Nur Fadilah

AIP Conference Proceedings **1854**, 020004 (2017); <https://doi.org/10.1063/1.4985395>

---

SHOW ABSTRACT



## Potency of *Nicotiana tabacum* as anti - microfouling


Aunurohim, Dian Ahmada Nurilma and Nengah Dwianita Kuswytasari

AIP Conference Proceedings **1854**, 020005 (2017); <https://doi.org/10.1063/1.4985396>

---

SHOW ABSTRACT



 Free . June 2017

## The effect of pomelo citrus (*Citrus maxima* var. Nambangan), vitamin C and lycopene towards the number reduction of mice (*Mus musculus*) apoptotic hepatocyte caused of ochratoxin A


Badriyah and Utami Sri Hastuti

AIP Conference Proceedings **1854**, 020006 (2017); <https://doi.org/10.1063/1.4985397>

---

SHOW ABSTRACT



 Free . June 2017

## Potency of bio-charcoal briquette from leather cassava tubers and industrial sludge


Nita Citrasari, Tety A. Pinatih, Eko P. Kuncoro, Agoes Soegianto, Salamun and Bambang Irawan

AIP Conference Proceedings **1854**, 020007 (2017); <https://doi.org/10.1063/1.4985398>

---

SHOW ABSTRACT



 Free . June 2017

## Distribution study on migratory bird (*Scolopacidae: Numenius*) in Surabaya, Indonesia: Estimating the effect of habitat and

BROWSE VOLUMES

## climate change


Iska Desmawati, Indah Trisnawati D. T., Ory Kurnia, Albi Hamdani, Mahsun Fahmi and Mirza Fahmi

AIP Conference Proceedings **1854**, 020008 (2017); <https://doi.org/10.1063/1.4985399>

---

SHOW ABSTRACT



 Free . June 2017

### **Effect of gamma 60Co irradiation on the lipid content and fatty acid composition of *Nannochloropsis* sp. microalgae**


Dini Ermavitalini, Ika Puspita Sari, Endry Nugroho Prasetyo, Nurlita Abdulgani and Triono Bagus Saputro

AIP Conference Proceedings **1854**, 020009 (2017); <https://doi.org/10.1063/1.4985400>

---

SHOW ABSTRACT



 Free . June 2017

### **Effects of gamma irradiation dose-rate on sterile male *Aedesaegypti***


Beni Ernawan, Usman Sumo Friend Tambunan, Irawan Sugoro and Hadian Iman Sasmita

AIP Conference Proceedings **1854**, 020010 (2017); <https://doi.org/10.1063/1.4985401>

---

SHOW ABSTRACT



 Free . June 2017


### **Bioremediation of oil sludge using a type of nitrogen source and the consortium of bacteria with composting method**

AIP Conference Proceedings **1854**, 020011 (2017); <https://doi.org/10.1063/1.4985402>

---

SHOW ABSTRACT



 Free . June 2017

## **Analysis of *Hylocereus* spp. diversity based on phenetic method**


Hamidah, Husnus Tsawab and Rosmanida

AIP Conference Proceedings **1854**, 020012 (2017); <https://doi.org/10.1063/1.4985403>

---

SHOW ABSTRACT



 Free . June 2017

## **Briquettes of rice husk, polyethylene terephthalate (PET), and dried leaves as implementation of wastes recycling**


Sucipto Hariyanto, Mohammad Nurdianfajar Usman and Nita Citrasari

AIP Conference Proceedings **1854**, 020013 (2017); <https://doi.org/10.1063/1.4985404>

---

SHOW ABSTRACT



 Free . June 2017

## **Histopathological assessment of cadmium effect on testicles and kidney of *Oreochromis niloticus* in different salinity**


Alfiah Hayati, Hanna Pratiwi, Inayatul Khoiriyah, Dwi Winarni and Sugiharto

AIP Conference Proceedings **1854**, 020014 (2017); <https://doi.org/10.1063/1.4985405>

---

SHOW ABSTRACT



 Free . June 2017

## The influence of snakehead (*Channa striata*) fish extract to increase hyperglycemic mice fertility based on spermatogenic cell composition


Dewi Hidayati, Nurlita Abdulgani, Nova Maulidina Ashuri, Noor Nailis Sa'adah and Maharani Lukitasari

AIP Conference Proceedings **1854**, 020015 (2017); <https://doi.org/10.1063/1.4985406>

---

SHOW ABSTRACT



 Free . June 2017

## Analysis of protein profiles in diabetic rat blood plasma that induced by alloxan


Dewi Hidayati, Nurlita Abdulgani, Hengki Setiyawan, Indah Trisnawati, Nova Maulidina Ashuri and Noor Nailis Sa'adah

AIP Conference Proceedings **1854**, 020016 (2017); <https://doi.org/10.1063/1.4985407>

---

SHOW ABSTRACT



 Free . June 2017

## Effect of methionine and lactic acid bacteria as aflatoxin binder on broiler performance

Lusty Istiqomah, Ema Damayanti, Hardi Julendra, Ade Erma Suryani, Awistaros Angger Sakti and Ayu Septi Anggraeni


AIP Conference Proceedings **1854**, 020017 (2017); <https://doi.org/10.1063/1.4985408>

---



SHOW ABSTRACT



 Free . June 2017

## **Growth and physiological responses of some *Capsicum frutescens* varieties to copper stress**


Nurul Jadid, Rizka Maziyah, Desy Dwi Nurcahyani and Nilna Rizqiyah Mubarokah

AIP Conference Proceedings **1854**, 020018 (2017); <https://doi.org/10.1063/1.4985409>

---

SHOW ABSTRACT



 Free . June 2017

## **Antioxidant activities of different solvent extracts of *Piper retrofractum* Vahl. using DPPH assay**


Nurul Jadid, Dewi Hidayati, Sylviana Rosyda Hartanti, Byan Arasyi Arraniry, Rizka Yuanita Rachman and Wiwi Wikanta

AIP Conference Proceedings **1854**, 020019 (2017); <https://doi.org/10.1063/1.4985410>

---

SHOW ABSTRACT



 Free . June 2017

## **Adsorption of cadmium from aqueous solution using algae waste based adsorbent**


Eko Prasetyo Kuncoro, Thin Soedarti, Trisnadi Widyalaksono Catur Putrato and Nurul Alvia Istiqomah

AIP Conference Proceedings **1854**, 020020 (2017); <https://doi.org/10.1063/1.4985411>

---

SHOW ABSTRACT



 Free . June 2017

## **Mixing of acacia bark and palm shells to increase caloric value of palm shells white charcoal briquette**


Edy Wibowo Kurniawan, Rudianto Amirta, Edy Budiarmo and Enos Tangke Arung

AIP Conference Proceedings **1854**, 020021 (2017); <https://doi.org/10.1063/1.4985412>

---

SHOW ABSTRACT



 Free . June 2017

## **Keratinase from newly isolated strain of thermophilic Bacillus for chicken feed modification**


Ditya Larasati, Nur Tsurayya, Maharani Pertiwi Koentjoro and Endry Nugroho Prasetyo

AIP Conference Proceedings **1854**, 020022 (2017); <https://doi.org/10.1063/1.4985413>

---

SHOW ABSTRACT



 Free . June 2017

## **Revegetation increase bird diversity in coastal area of Socorejo, Tuban, East Java – Indonesia**

Yeni Indah Lestari, Wasito Edi, Alkautsar Alivvy, Acib Setia Ibadah, Fadina Yuliana Sari, Finda Nuraini, Ahmad Yanuar, Agus Satriyono, Citra Fitrie Riany, Dian Saptarini and Farid Kamal Muzaki

AIP Conference Proceedings **1854**, 020023 (2017); <https://doi.org/10.1063/1.4985414>

---

SHOW ABSTRACT



## Growth of vegetative explant *Moringa oleifera* on different composition of auxin and cytokinin and its synthetic seed germination


Wirdhatul Muslihatin, Nurul Jadid, Ika D. Puspitasari and Chusnul E. Safitri

AIP Conference Proceedings **1854**, 020024 (2017); <https://doi.org/10.1063/1.4985415>

---

SHOW ABSTRACT



 Free . June 2017

## Community structure of fish larvae in mangroves with different root types in Labuhan coastal area, Sepulu – Madura


Farid Kamal Muzaki, Aninditha Giffari and Dian Saptarini

AIP Conference Proceedings **1854**, 020025 (2017); <https://doi.org/10.1063/1.4985416>

---

SHOW ABSTRACT



 Free . June 2017

## Biodegradation of naphthalene and phenanthren by *Bacillus subtilis* 3KP


Ni'matuzahroh, N. Trikurniadewi, A. R. A. Pramadita, I. A. Pratiwi, Salamun, Fatimah and Sri Sumarsih

AIP Conference Proceedings **1854**, 020026 (2017); <https://doi.org/10.1063/1.4985417>

---

SHOW ABSTRACT



 Free . June 2017

## **Manganese (Mn) stress toward hyperaccumulators plants combination (HPC) using *Jatropha curcas* and lamtoro gung (*L. leucocephala*) in mychorrizal addition on soybean (*Glycine max*) seedling stage**


Tania Sylviana Darmawan, Tata Taqiyyatuz Zahroh, Mirza Merindasya, Ririn Masfaridah, Dyah Ayu Sri Hartanti, Sekar Arum, Sri Nurhatika, Anton Muhibuddin, Tini Surtiningsih and Achmad Arifiyanto

AIP Conference Proceedings **1854**, 020027 (2017); <https://doi.org/10.1063/1.4985418>

---

SHOW ABSTRACT



 Free . June 2017

## **The potency of curing fish waste pellet for growth and protein level of African sharptooth catfish (*Clarias gariepinus*)**


Awik Puji Dyah Nurhayati and Asti R. Febiyani

AIP Conference Proceedings **1854**, 020028 (2017); <https://doi.org/10.1063/1.4985419>

---

SHOW ABSTRACT




 Free . June 2017

## **Reducing the level of leaves damage of (*Brassica rapa*) caused by armyworm (*Spodoptera litura* F.) through liquid bioinsecticide formulation of bintaro (*Cerbera odollam*) leaves extract**

Kristanti Indah Purwani, Sri Nurhatika, Dini Ermavitalini, Triono Bagus Saputro and Dwi Setia Budiarti

AIP Conference Proceedings **1854**, 020029 (2017); <https://doi.org/10.1063/1.4985420>

---

 Free . June 2017

## **Molecular characters of melon (*Cucumis melo* L. “Tacapa”) in response to karst critical land**


Yuanita Rachmawati, Budi Setiadi Daryono and Ganies Riza Aristya

AIP Conference Proceedings **1854**, 020030 (2017); <https://doi.org/10.1063/1.4985421>

---

SHOW ABSTRACT



 Free . June 2017

## **Analysis of lipid profile and atherogenic index in hyperlipidemic rat (*Rattus norvegicus* Berkenhout, 1769) that given the methanolic extract of Parijoto (*Medinilla speciosa*)**


Noor Nailis Sa’adah, Kristanti Indah Purwani, Awik Puji Dyah Nurhayati and Nova Maulidina Ashuri

AIP Conference Proceedings **1854**, 020031 (2017); <https://doi.org/10.1063/1.4985422>

---

SHOW ABSTRACT



 Free . June 2017

## **White syndrome on massive corals: A case study in Paiton power plant, East Java**


Farid Kamal Muzaki, Dian Saptarini and Aida Efrini Riznawati

AIP Conference Proceedings **1854**, 020032 (2017); <https://doi.org/10.1063/1.4985423>

---

SHOW ABSTRACT



 Free . June 2017

## **Genetic diversity of improved salt tolerant calli of maize (*Zea mays* L.) using RAPD**


Triono Bagus Saputro, Siti Dianawati, Nur Fadlillatus Sholihah and Dini Ermavitalini

AIP Conference Proceedings **1854**, 020033 (2017); <https://doi.org/10.1063/1.4985424>

---

SHOW ABSTRACT



 Free . June 2017

## **Spicule size variation in *Xestospongia testudinaria* Lamarck, 1815 at Probolinggo-Situbondo coastal**


Iwenda Bella Subagio, Edwin Setiawan, Sucipto Hariyanto and Bambang Irawan

AIP Conference Proceedings **1854**, 020034 (2017); <https://doi.org/10.1063/1.4985425>

---

SHOW ABSTRACT



 Free . June 2017

## **Soil burial method for plastic degradation performed by *Pseudomonas* PL-01, *Bacillus* PL-01, and indigenous bacteria**


Maya Shovitri, Risyatun Nafi'ah, Titi Rindi Antika, Nur Hidayatul Alami, N. D. Kuswytasari and Enny Zulaikha

AIP Conference Proceedings **1854**, 020035 (2017); <https://doi.org/10.1063/1.4985426>

---

SHOW ABSTRACT



 Free . June 2017

## **Production of secondary metabolites *trimethyl xanthina* by *Camellia sinensis* L suspension culture**


Sutini, Mochamad Sodik, Wirdhatul Muslihatin and Mochamad Rasjad Indra

AIP Conference Proceedings **1854**, 020036 (2017); <https://doi.org/10.1063/1.4985427>

---

SHOW ABSTRACT



 Free . June 2017

## **Effects of the combination between bio-surfactant product types and washing times on the removal of crude oil in nonwoven fabric**


Agus Triawan, Ni'matuzahroh and Agus Supriyanto

AIP Conference Proceedings **1854**, 020037 (2017); <https://doi.org/10.1063/1.4985428>

---

SHOW ABSTRACT



 Free . June 2017

## **The effectiveness of habitat modification schemes for enhancing beneficial insects: Assessing the importance of trap cropping management approach**


Indah Trisnawati and Abdul Azis

AIP Conference Proceedings **1854**, 020038 (2017); <https://doi.org/10.1063/1.4985429>

---

SHOW ABSTRACT



 Free . June 2017

## The effect of ripening stages on the antioxidant potential of melon (*Cucumis melo* L.) cultivar Hikapel


Puji Wulandari, Budi Setiadi Daryono and Supriyadi

AIP Conference Proceedings **1854**, 020039 (2017); <https://doi.org/10.1063/1.4985430>

---

SHOW ABSTRACT



 Free . June 2017

## Improving properties of sweet potato composite flour: Influence of lactic fermentation


Neti Yuliana, Siti Nurdjanah, Sri Setyani and Dini Novianti

AIP Conference Proceedings **1854**, 020040 (2017); <https://doi.org/10.1063/1.4985431>

---

SHOW ABSTRACT



 Free . June 2017

## Viability of *Azotobacter* consortium in auxin production


Enny Zulaika, Farihatu Solikhah, Nur Hidayatul Alami, Nengah Dwianita Kuswytasari and Maya Shovitri

AIP Conference Proceedings **1854**, 020041 (2017); <https://doi.org/10.1063/1.4985432>

---

SHOW ABSTRACT



 Free . June 2017

## Diversity of heavy metal resistant bacteria from Kalimas Surabaya: A phylogenetic taxonomy approach

Enny Zulaika, Andry Prio Utomo, Adisya Prima, Nur Hidayatul Alami, Nengah Dwianita



SHOW ABSTRACT



## Resources

AUTHOR

LIBRARIAN

ADVERTISER

---

## General Information

ABOUT

CONTACT

HELP

PRIVACY POLICY

TERMS OF USE

FOLLOW AIP PUBLISHING:



Website © 2020 AIP Publishing LLC.

Article copyright remains as

[BROWSE VOLUMES](#)



## Academic paper editing

Get Professional academic paper editing by Subject Area Native English Editors Today!

[authorassists.com](http://authorassists.com)

OPEN

# AIP Conference Proceedings

**Country** [United States](#) - [SIR Ranking of United States](#)

**Subject Area and Category** [Physics and Astronomy](#)  
[Physics and Astronomy \(miscellaneous\)](#)

**Publisher** [American Institute of Physics](#)

**Publication type** Conferences and Proceedings

**ISSN** 0094243X, 15517616

**Coverage** 1974-1978, 1983-1984, 1993, 2000-2001, 2003-2020

**Scope** Today, AIP Conference Proceedings contain over 100,000 articles published in 1700+ proceedings and is growing by 100 volumes every year. This substantial body of scientific literature is testament to our 40-year history as a world-class publishing partner, recognized internationally and trusted by conference organizers worldwide. Whether you are planning a small specialist workshop or organizing the largest international conference, contact us, or read these testimonials, to find out why so many organizers publish with AIP Conference Proceedings.



[Homepage](#)

[How to publish in this journal](#)

[Contact](#)



[Join the conversation about this journal](#)

# 64

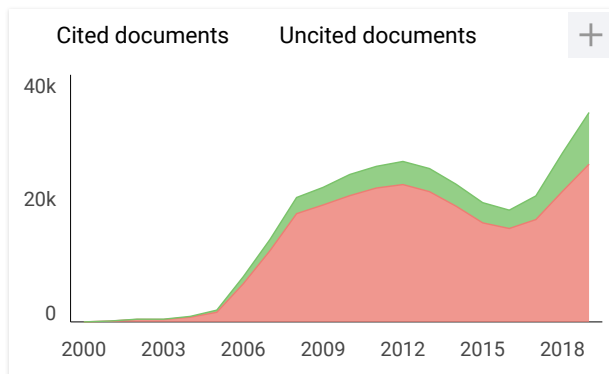
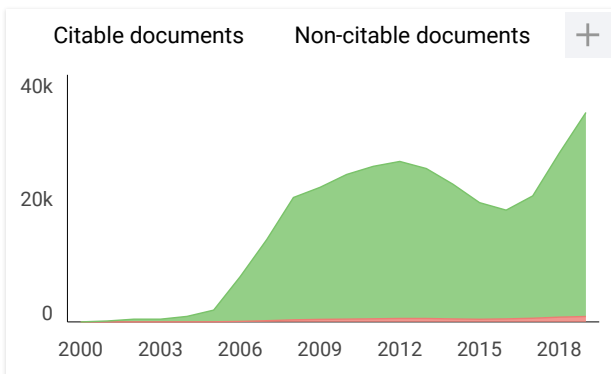
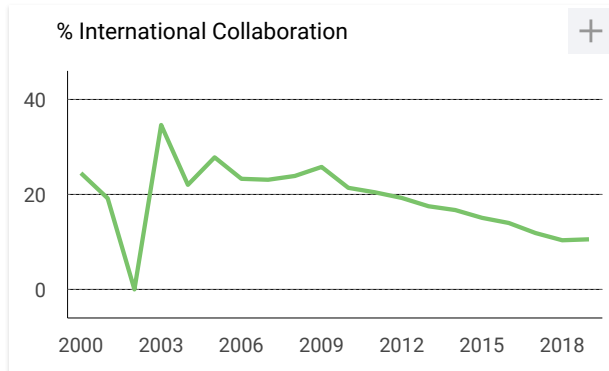
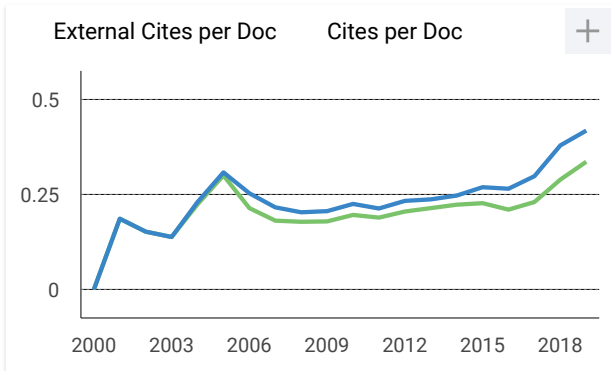
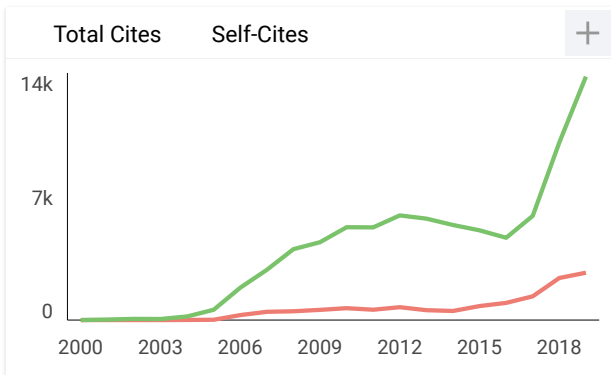
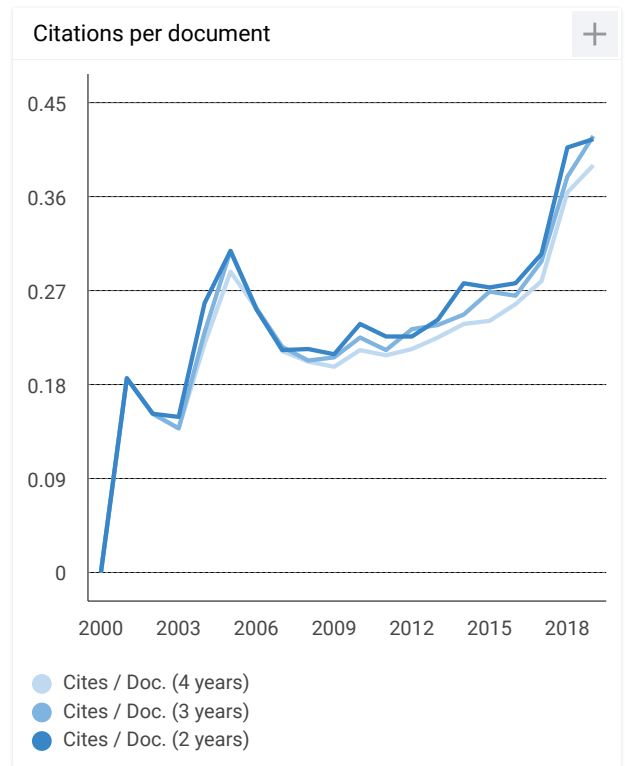
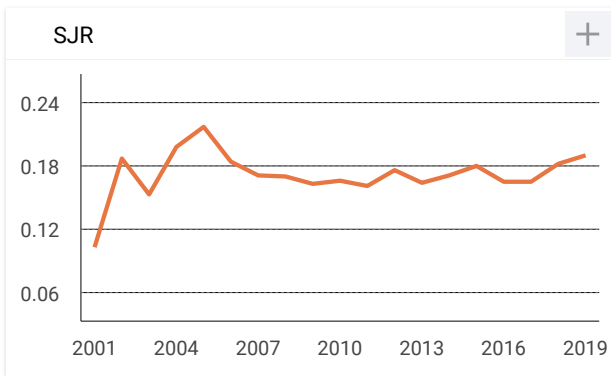
H Index

## Free Grammar Checker

Eliminate grammar errors instantly and enhance your writing with Grammarly

Grammarly

DOWNLOAD



**AIP Conference Proceedings** ← Show this widget in your own website

Not yet assigned quartile

**SJR 2019**  
0.19

powered by scimagojr.com

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

```
<a href="https://www.scimagojr.com" style="border: 1px solid #ccc; padding: 2px 5px; display: inline-block;">https://www.scimagojr.com
```

# Effects of the combination between bio-surfactant product types and washing times on the removal of crude oil in nonwoven fabric

Cite as: AIP Conference Proceedings **1854**, 020037 (2017); <https://doi.org/10.1063/1.4985428>  
Published Online: 26 June 2017

Agus Triawan, Ni'matuzahroh, and Agus Supriyanto



View Online



Export Citation

## ARTICLES YOU MAY BE INTERESTED IN

[Bioremediation of oil sludge using a type of nitrogen source and the consortium of bacteria with composting method](#)

AIP Conference Proceedings **1854**, 020011 (2017); <https://doi.org/10.1063/1.4985402>

[Oil removal from petroleum sludge using bacterial culture with molasses substrate at temperature variation](#)

AIP Conference Proceedings **1718**, 030002 (2016); <https://doi.org/10.1063/1.4943312>

[Biodegradation of naphthalene and phenanthren by Bacillus subtilis 3KP](#)

AIP Conference Proceedings **1854**, 020026 (2017); <https://doi.org/10.1063/1.4985417>

Lock-in Amplifiers  
up to 600 MHz



Watch



# Effects of the Combination between Bio-surfactant Product Types and Washing Times on the Removal of Crude Oil in Nonwoven Fabric

Agus Triawan<sup>a)</sup>, Ni'matuzahroh<sup>b)</sup>, Agus Supriyanto

Department of Biology, Faculty of Science and Technology,  
Airlangga University, Mulyorejo Kampus C, Surabaya, 60115, Indonesia

<sup>a)</sup>Corresponding author: agustriawan@yahoo.co.id

<sup>b)</sup>nimatuzahroh@yahoo.com

**Abstract.** This research aimed to characterize bio-surfactants produced by *Bacillus subtilis* 3KP, *Pseudomonas putida* T1-8, *Micrococcus* sp. L II 61 and *Acinetobacter* sp. P 2(1) and to investigate its combination's effects on the removal of crude oil in nonwoven fabric with different washing times vary from 12, 24 to 36 hours. The production of bio-surfactants was done on Synthetic Mineral Water mixed with molasses 4% within four days. The bio-surfactant products were characterized by measuring the Surface Tension (ST) (mN/m) and Emulsion Activity (EA) (%). Oil removal experiment was done by mixing 10 mL bio-surfactant with nonwoven fabric that contains crude oil into 50 mL bottle inside a shaker. The removed crude oil was extracted with n-hexane and measured gravimetrically. The results were then being analyzed with two ways ANOVA and Duncan test. Bio-surfactant produced by four bacteria has variations of Surface Tension and Emulsion Activity values. Bio-surfactant produced by *Bacillus subtilis* 3KP and *Pseudomonas putida* T1-8 showed the increasing of crude oil removal as washing times increase, while bio-surfactant produced by *Micrococcus* sp. L II 61 and *Acinetobacter* sp. P2(1) showed the decreasing result at 36 hours. However, the combination that showed the best result was *Acinetobacter* sp. P 2(1) at 24 hours valued 65,3%.

**Keywords:** Bio-surfactant, Crude Oil, Nonwoven Fabric, Surface Tension, Emulsion Activity.

## INTRODUCTION

According to the data of Group of Expert on Scientific Aspects of Marine Pollution (GESAMP) 6,44 million tons of hydrocarbon compounds from crude oil have been contaminating the ocean every year. Crude oil waste contains complex compounds such as benzene, toluene, ethyl benzene, and xylene isomer (BTEX) that are harmful for the marine ecosystem including corals, mangroves, and water organisms. Various methods have been developed to overcome the oil spillage problems, including the usage of oil sorbent, polypropylene nonwoven fabric. However, most of the nonwoven fabrics were eventually wasted and caused another source of pollutant. Thus, another solution is needed to reduce the nonwoven fabric waste. The used nonwoven fabric can be re-used through separating process between the oil content and the fabric itself.

One of the effective alternatives to remove oil content from nonwoven fabric is by using bio-surfactant. Bio-surfactants, biologically produced, have been increasingly used in soil washing and oil removal from contaminated areas (Mulligan *et al.*, 1999, 2001). Furthermore, bio-surfactant has low toxicity and highly bio-degradable (Batista *et al.*, 2006). Hence, hydrocarbon bacteria that have the ability to produce bio-surfactant are highly recommended in crude oil degradation process in a short time (Kumar *et al.*, 2006). Result of Integrated Research (Ni'matuzahroh *et al.*, 2004) and National Grant Research (Ni'matuzahroh *et al.*, 2009) have successfully identified potential bacteria in

producing bio-surfactant such as *Bacillus subtilis* 3KP, *Pseudomonas putida* T1-8, *Micrococcus* sp. L II 61 and *Acinetobacter* sp. P 2(1).

This research aims to characterize bio-surfactants produced by *Bacillus subtilis* 3KP, *Pseudomonas putida* T1-8, *Micrococcus* sp. L II 61 and *Acinetobacter* sp. P 2(1) and to investigate its combination's effects on the removal of crude oil in nonwoven fabric with different washing times vary from 12, 24 to 36 hours.

## MATERIALS

### 1. Bacteria Cultures

*Bacillus subtilis* 3KP, *Pseudomonas putida* T1-8, *Micrococcus* sp. L II 61 dan *Acinetobacter* sp. P 2(1) are owned by the Microbiology Laboratory of Biology Department, Faculty of Science and Technology, Airlangga University.

### 2. Crude Oil

Crude oil is owned by the Microbiology Laboratory of Biology Department, Faculty of Science and Technology, Airlangga University which was taken from oil plant in Bojonegoro regency, Indonesia.

### 3. Media and Other Materials

The media that was used is synthetic mineral water and molasses (Pruthidan Comeotra, 1997). Other materials that were used are n-hexane, Tween-20, spirituous, alcohol 70 %, aluminum foil, and cotton

### 4. Nonwoven Fabric

TABLE 1. Specifications of Nonwoven Fabric

| Specification                    | Detail                  |
|----------------------------------|-------------------------|
| Color                            | White                   |
| Raw Material                     | Meltblown Polypropylene |
| Basic Weight (g/m <sup>2</sup> ) | 346.3 – 353.8           |
| Thickness (mm)                   | 2.35 – 2.45             |
| Tensile Strength (N/50 mm)       | MD: 26.4 – 35.7         |
| Elongation (%)                   | 16 – 22                 |

## METHODS

### Bio-surfactant Production Media

Synthetic Mineral Water according to Pruthi and Comeotra (1997) contains (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (3 g), MgSO<sub>4</sub>.7H<sub>2</sub>O (0,2 g), NaCl (10 g), CaCl<sub>2</sub> (0,01 g), MnSO<sub>4</sub>.H<sub>2</sub>O (0,001 g), H<sub>3</sub>BO<sub>3</sub> (0,001 g), ZnSO<sub>4</sub>.7H<sub>2</sub>O (0,001 g), CuSO<sub>4</sub>.5H<sub>2</sub>O (0,001 g), CoCl<sub>2</sub>.6H<sub>2</sub>O (0,005 g), and Na<sub>2</sub>MoO<sub>4</sub>.2H<sub>2</sub>O (0,001 g) diluted in 1 L of water with pH 7. Furthermore, 4% of substrate molasses (v/v) were then added. After being sterilized, 4.8 mL of nutrient stock of KH<sub>2</sub>PO<sub>4</sub> (1 g), K<sub>2</sub>HPO<sub>4</sub> (1 g) and 4.8 mL of FeSO<sub>4</sub>.7H<sub>2</sub>O (1 g), were added aseptically.

### Bio-surfactant Production

4% starter of *Bacillus subtilis* 3KP, *Pseudomonas putida* T1-8, *Micrococcus* sp. L II 61, and *Acinetobacter* sp. P 2(1) with OD= 0,5 at  $\lambda = 650$  nm were added into 100 mL of media in each bottle. Incubate in a shaker with 90 rpm speed for 4 days. Centrifuge for 20 minutes with 450 rpm speed to produce supernatant without cells. This supernatant considered as bio-surfactant product and then used in the treatment.

### Characterization of Bio-Surfactant

Characterization of bio-surfactant products was done by measuring the surface tension (ST) (mN/m) and emulsion activity (EA) (%). Surface tension measurement was using Tensiometer Du-Nouy. Pour 10 mL of supernatant into a clean and fat-free petri dish then set to the Tensiometer Du-Nouy. The measured surface tension value is the scale value when the ring detaches from the sample (Ni'matuzahrohet *al.*, 2009). Distilled water and molasses were used as control. Each sample uses 3 replications, and the result was determined using following formula:

$$r = r_o \times \frac{\theta}{\theta_o}$$

where:

|                |   |  |
|----------------|---|--|
| r              | = | sample surface tension                         |
| r <sub>o</sub> | = | aquadest surface tension at t° C               |
| θ              | = | sample surface tension read from tensiometer   |
| θ <sub>o</sub> | = | aquadest surface tension read from tensiometer |

Emulsion activity (%) was measured using a method based on Suryatmana *et al.* (2006). Pour 1 mL of supernatant and 1 mL of diesel fuel into a tube, the vortex for 2 minutes. The value of emulsion activity (%) was measured after 1 hour and 24 hours by measuring the emulsion phase layer (cm) compared to the total solution (cm) (PruthidanCameotra, 1997) as follows:

$$\frac{\text{Emulsion layer (cm)}}{\text{Total layer (cm)}} \times 100\%$$

### **Preparation of Nonwoven Fabric and Crude Oil**

Nonwoven fabric was cut into 3 cm x 1 cm then submerged into crude oil. The weight of crude oil absorbed by nonwoven fabric was measured by deducting the weight after submergence to the weight before submergence. Nonwoven fabric containing crude oil was then put into 50 mL sample bottles with 10 mL of supernatant. All of the sample bottles were put into a shaker according to the treatment of washing time.

### **Treatment**

Tween-20 was used as positive control and distilled water as negative control. Washing time treatments were 12 hours, 24 hours, and 36 hours. During the washing process inside a shaker, samples were kept in an aseptic condition to avoid contamination that could possibly affect the result of crude oil removal.

### **Crude Oil Removal**

#### *Extraction*

Extraction was done by using n-hexane which functioned to bind the water phase crude oil. N-hexane was added into the bottle samples. After that, the sample solutions were moved into sample tubes before being vortex-ed for 1 minute. The vortex process resulted 3 layers. Upper layer is n-hexane mixed with crude oil and lower layer is water while emulsion layer in between. Upper layer which contains crude oil was then moved to another tube for the next process, evaporation.

#### *Evaporation*

Rotary evaporator was used to separate the n-hexane and crude oil. During the evaporation process at 65° C, n-hexane evaporated while the crude oil that was bound by the n-hexane remained inside the tube. Finally, the volume of crude oil removed from the washing process could be calculated gravimetrically.

### **Data Analysis**

The data of crude oil removal (%) was analyzed statistically using Two Ways Analysis of Variance (ANOVA) (significance degree 5%) and continued with Duncan test to know which group of treatment that has significantly different result among all group of treatments.

## RESULT AND DISCUSSIONS

### Surface Tension Measurement

Distilled water, molasses, and tween-20 were used as the control group. Based on the Table 2 below, the samples that have the highest result were *Acinetobacter* sp. P 2(1) and *Bacillus subtilis* 3KP valued  $55.8 \pm 2.79$  mN/m and  $55.8 \pm 0.87$  respectively, while the sample that has the lowest result was *Pseudomonas putida* T1-8 valued  $43.4 \pm 0.46$  mN/m. This means that bio-surfactant produced by *Pseudomonas putida* T1-8 has the highest value of surface tension reduction towards molasses and distilled water valued 16,8 mN/m and 27,56 mN/m respectively, comparable with synthetic surfactant tween-20's surface tension reduction value which are 18.47mN/m and 30.23mN/m, respectively.

Willumsen and Karlson (1997) stated that good bio-surfactant products are shown by their ability to decrease the surface tension value towards its growth media. From the data, it could be concluded that there was activity of bio-surfactant that is able to reduce the surface tension value.

**TABLE 2.** Result of Surface Tension (ST) Measurement

| Materials                       | Average ST Value (mN/m) | Reduction Value of ST to |                        |
|---------------------------------|-------------------------|--------------------------|------------------------|
|                                 |                         | Molasses (mN/m)          | Distilled Water (mN/m) |
| Distilled Water                 | $71.96 \pm 2$           | -                        | -                      |
| Molasses                        | $60.2 \pm 1.35$         | -                        | 11.76                  |
| Tween-20                        | $41.73 \pm 0.17$        | 18.47                    | 30.23                  |
| <i>Bacillus subtilis</i> 3KP    | $55.8 \pm 0.87$         | 4.4                      | 16.16                  |
| <i>Pseudomonas putida</i> T1-8  | $43.4 \pm 0.46$         | 16.8                     | 27.56                  |
| <i>Micrococcus</i> sp. L II 61  | $55.3 \pm 3.11$         | 4.9                      | 16.66                  |
| <i>Acinetobacter</i> sp. P 2(1) | $55.8 \pm 2.79$         | 4.4                      | 16.16                  |

### Emulsion Activity Measurement

Emulsion activity was measured by using the method from Suryatmana *et al.* (2006). The result showed that the emulsion activity value decreased at 24 hours (Table 3). However, the result of emulsion activity measurement showed that there were relatively stable emulsion activities. This indicates that those bio-surfactant products could be categorized as bio-emulsifiers. The occurrence of emulsion activity in the samples was indicated by the forming of foam that creates layers inside the tube. The foam layer was then measured to calculate the value of emulsion activity.

**TABLE 3.** Result of Emulsion Activity (EA) Measurement

| Materials                       | Emulsion Activity (EA) (%) |                  | Reduction Value of Emulsion Activity (%) |
|---------------------------------|----------------------------|------------------|--|
|                                 | 1 Hour                     | 24 Hours         |  |
| Tween-20                        | $47.1 \pm 13.99$           | $46.3 \pm 13.04$ | 0.8                                      |
| <i>Bacillus subtilis</i> 3KP    | $30.09 \pm 8.02$           | $20.9 \pm 3.66$  | 10                                       |
| <i>Pseudomonas putida</i> T1-8  | $35.2 \pm 10.87$           | $27.1 \pm 13.12$ | 8.1                                      |
| <i>Micrococcus</i> sp. L II 61  | $23.6 \pm 10.05$           | $20.8 \pm 6.78$  | 2.8                                      |
| <i>Acinetobacter</i> sp. P 2(1) | $9 \pm 2.57$               | $10.1 \pm 1.19$  | -1.1                                     |

### Crude Oil Removal from Nonwoven Fabric

Based on the Table 4 below, each control group and treatment group showed positive result in oil removal but with different percentages. Negative control using distilled water had the lowest oil removal percentage among other samples valued 5,2 % at 12 hours, 9,5% at 24 hours and 12,2% at 36 hours. This happened because negative control used distilled water which did not have surface active compound or emulsifier and the removed crude oil was estimated as the result of mechanical process while shaking. Positive control using synthetic surfactant tween-20 had



the highest oil removal percentage among other samples valued 51,3% at 12 hours, 67,1% at 24 hours, and 71,2% at 36 hours.

**TABLE 4.**Percentage of Oil Removal from Nonwoven Fabric using Different Treatments and Washing Times

| Materials                       | Crude Oil Removal (%)       |                            |                               |
|---------------------------------|-----------------------------|----------------------------|-------------------------------|
|                                 | 12 Hours                    | 24 Hours                   | 36 Hours                      |
| Distilled Water                 | 5,2 ± 5 <sup>a</sup>        | 9,5 ± 10,35 <sup>a</sup>   | 12,2 ± 3,53 <sup>a</sup>      |
| Tween-20                        | 51,3 ± 10,9 <sup>c</sup>    | 67,1 ± 10,42 <sup>b</sup>  | 71,2 ± 26,67 <sup>d</sup>     |
| <i>Bacillus subtilis</i> 3KP    | 36,4 ± 7,5 <sup>b,c</sup>   | 34,36 ± 9,3 <sup>a,b</sup> | 43,8 ± 1,19 <sup>b,c,d</sup>  |
| <i>Pseudomonas putida</i> T1-8  | 33,4 ± 11,33 <sup>b,c</sup> | 44,9 ± 24,14 <sup>b</sup>  | 54,7 ± 12,84 <sup>c,d</sup>   |
| <i>Micrococcus</i> sp. L II 61  | 19,7 ± 1,6 <sup>a,b</sup>   | 37,3 ± 7,97 <sup>a,b</sup> | 22,8 ± 8,48 <sup>a,b</sup>    |
| <i>Acinetobacter</i> sp. P 2(1) | 50,2 ± 15,15 <sup>c</sup>   | 65,3 ± 29,79 <sup>b</sup>  | 35,3 ± 23,95 <sup>a,b,c</sup> |

The result of *Analysis of Varian* (ANOVA) showed that data of each treatment of crude oil removal has real difference, hence  $H_0$  is rejected which means the combination between bio-surfactant product types and washing times indeed affected the crude oil removal. The results of Duncan Test are shown by notations of a, b, c, and d. Every different notation indicates significant difference of the result. However, notation of ab means it has no significance to either a or b.

Bio-surfactant from *Acinetobacter* sp. P 2(1) had the ability in oil removal relatively equal with synthetic surfactant tween-20 at 12 hours and 24 hours and also the highest among other bio-surfactant products. This result correlated with previous research by Widodo (2010) that reported the effectiveness of crude oil mobilization by bio-surfactant of *Acinetobacter* sp. P 2(1) is higher than *Pseudomonas putida* T1-8. Another research by Pratiwi (2012) reported that the characteristics of bio-surfactant from *Acinetobacter* sp.P 2(1) are similar with synthetic surfactant *tween-20* at CMC concentration. However, oil removal percentage by bio-surfactant from *Acinetobacter* sp.P 2(1) decreased at 36 hours. This could be correlated with the instability of the bio-surfactant itself as shown at the emulsion activity result in Table 4.3 where emulsion activity at 24 hours increased, while it was supposedly decreased. Bio-surfactant's instability was presumably caused by incubation time that took too long. Changjun (2014) stated that the optimum incubation time for *Acinetobacterbaylyi* is 12 to 36 hours, while in this research the incubation time was 48 hours and might cause the decreasing of product's quality.

Several other researchers reported different results. Ekpo dan Udofia (2008) reported that some species such as *Pseudomonas aeruginosa*, *Micrococcus varians*, and *Bacillus subtilis* have the ability to produce bio-surfactants that are able to degrade crude oil up to 97,2%, 85,7% dan 72,3%, respectively at 10 incubation time. This explains that each and every different bacteria has different optimum incubation time in producing bio-surfactant. Wei *et al.* (2004) reported that the ability of bio-surfactant in crude oil removal increase as the washing time increases.

## CONCLUSIONS

This research had shown that the bio-surfactant produced by *Bacillus subtilis* 3KP, *Pseudomonas putida* T1-8, *Micrococcus* sp. L II 61, and *Acinetobacter* sp. P 2(1) could be used to remove crude oil form nonwoven fabric. The reduction of surface tension and emulsion activity indicated that these bio-surfactants have the potential in emulsifying hydrocarbon compound of crude oil. The combination between bio-surfactant product types and washing times affected the removal of crude oil where oil removal increases along with the increasing of washing time until it reaches maximum limit. However, the combination showed the best result was produced by *Acinetobacter* sp. P 2(1) at 24 hours, valued 65,3% ± 29,79.

## REFERENCES

1. Batista S.B., Mounter A.H., Amorim F.R., Totola M.R., Isolation and Characterization of Biosurfactant/bioemulsifier Producing Bacteria from Petroleum Contaminated Sites. *BioresourTechnol* 2006; 97(6):868–75.
2. ChangjunZou, Meng Wang, Yu Xing, GuihongLan, TingtingGe, Xueling Yan, Tong Gu. 2014. Characterization and Optimization of Biosurfactants Produced by *Acinetobacterbaylyi* ZJ2 Isolated from Crude Oil Contaminated Soil Sample Toward Microbial Enhanced Oil Recovery Applications. *Biochemical Engineering Journal*.

3. Ekpo MA, Udofia VS. Rate of Biodegradation of Crude Oil by Microorganism Isolated From Oily Sludge Environment. *Afr J Biotechnol* 2008;7 (24): 4495–9.
4. Kumar, M., Leon, V., Materano, A.S, Ilzins O.A., Enhancement of Oil Degradation by Co-culture of Hydrocarbon Degrading and Biosurfactant Producing Bacteria. *Pol J Microbiol* 2006; 5(2):139–46.
5. Mulligan, C.N., Yong, R.N., Gibbs, B.F., 1999. Removal of heavy metals from contaminated soil and sediments using the biosurfactantsurfactin. *Journal of Soil Contamination* 8 (2), 231–254.
6. Mulligan, C.N., Yong, R.N., Gibbs, B.F., 2001. Remediation technologies for metal-contaminated soils and groundwater: an evaluation. *Engineering Geology* 60 (1–4), 193–207.
7. Ni'matuzahroh, Fatimah, AgusSupriyanto, Moch. Affandi. 2009. Beoremediasi Tanah TercemarMinyakmenggunakanKonsorsiumMikroba. *LaporanPenelitianHibahRiset*. Surabaya, Indonesia.
8. Ni'matuzahroh, Surtiningsih, Tini., Isnaeni. 2004. KemampuanBakteriHidrokarbonoklastikdariLingkunganTercemarMinyakt dalamMemproduksiBiosurfaktan: UpayaBioremediasiLingkungan. *LaporanPenelitian RUT VIII.3*. LembagaPenelitianUniversitasAirlangga. Surabaya
9. Pratiwi, Intan. 2012. PengaruhPenambahan Crude Enzyme (EkstrakKasarEnzim) Lipase *Micrococcus sp.* L II 61 PadaBiosurfaktan*Acinetobacter sp.* P 2(1) TerhadapPeningkatanPersentaseKelarutan*Oil Sludge*. *Skripsi*. FakultasSainsdanTeknologi. UniversitasAirlangga. Surabaya
10. Pruthi, V. dan S.S. Cameotra, 1997. Rapid Identification of Biosurfactant Producing Bacterial Strain Using a Cell Surface Hydrophobicity Techniques. *Biotechnol Technique*.
11. Suryatmana, P., E., Kardena, E., Ratnaningsih, danWisnuprpto. 2006. KarakteristikBiosurfaktandari*Azotobacterchroococcum*. *JurnalMikrobiologi Indonesia*. Vol. 11 No. 1: 30-34.
12. Wei, Q.F., Mather, R.R., Fotheringham, A.F. 2004. Oil Removal from Used Sorbents Using a Biosurfactant. *Biosource Technology* 96 (2005) 331-334
13. Widodo. 2010. UjiEfektivitasBiosurfaktandari*Acinetobacter sp.* P2-1 dan*Pseudomonas putida*T1(8) dalamMemobilisasiMinyakMentahMenggunakan*Sand Pack Column*. *Skripsi*. FakultasSainsdanTeknologi. UniversitasAirlangga. Surabaya.
14. Willumsen, P.A., and Karlson, U. 1997. Screening of Bacteria, Isolated from PAH-Contaminated Soil, for Production of Biosurfactant and Bioemulsifiers. *Biodegradation*. Vol. 7: 415-423