The 4<sup>th</sup> South East Asian echnical University Consortium (SEATUC) Symposium

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HYBRID TWINNING PROGRAM 2009

### PREFACE

Since establishment in 2006, SEATUC (South East Technical University Consortium) has created valuable and attractive opportunities for member institutions by offering chances for faculty members to get PhD degrees at SIT, and organizing scientific symposium, attracting participation of increasing number of lecturers and students year by year. The symposium this time is the 4<sup>th</sup> symposium, held on 25<sup>th</sup> -26<sup>th</sup> February 2010, and at Shibaura Institute of Technology (SIT), Japan.

Symposium 2010 continues to focus on 6 main fields including energy and environment, information technology, architecture-urban planning-design, bioscience and engineering, robotics and mechanism, and materials science. The total number of papers printed is 101, which over exceed those of the 3<sup>rd</sup> symposium and nearly triple those of the 2<sup>nd</sup> symposium. The increasing number of papers submitted over the 4 symposiums is an absolute evident for the popularity of the proceeding among professors and students of the member institutions and within regional and international scientific community as well. It is also an eloquent proof for the continuous success of symposiums over the years.

The symposium this time is strongly believed to continue promoting exchange of advanced scientific and technological information among speakers and participants beyond the research topics and strengthen the successful collaboration in education and research among member institutions.

Finally, on behalf of Organizing Committee, I would like to express high appreciation to Shibaura Institute of Technology for facilitating the organizing task and providing symposium venue, and Hanoi University of Technology for their great effort with organization duties. My appreciation is also to scientists, researchers, and students who have submitted papers and actively participated in the symposium, contributing to the success of the symposium 2010.

Prof. Dr. Ha Duyen Tu SEATUC President 4th SEATUC Symposium Vice- President of HUT

Date		Room 30	1	Room 302					
Date	Time	Session / Field	Presenter	Time	Session / Field	Presenter			
	9:45 12:30	9:45- Opening Address							
	Afternoonsession								
	13:00	Session A-1	A-01 (KMUTT) Prasitchai Promliphonkul A-02 (SIT) Takashi Ishidou	13:00	Session B-1	B-09 (KMUTT) Pravate Tuitemwong B-10 (KMUTT) Kornkanok Aryusuk			
Feb. 25	15:40	Architecture,Urban Planning and Design (Chairpersons: SIT, UTM)	A-03 (SI.) Akbar Adhuitama A-04 (SI.) Aswin Indraprastha A-05 (SIT) Nafisa Binti Hosni A-06 (SIT) Makoto Itoh A-07 (UTM) Kei Saito A-08 (UTM) Norliza bt. Mohd Isa	15:40	Bioscience and Engineering (Chairpersons: SIT,KMUTT)	B-11 (KMUTT) Panthip Boonsong B-12 (SIT) Akira IZUMIYA B-13 (SIT) Heizo KAJIWARA B-14 (SIT) Azham Zulkhamain B-15 (SIT) Tomohisa Kato B-16 (SIT) Yoshihiko Ito			
		1		Break					
1	16:00	Session A-2	A-09 (UTM) Ismail Bin Said	16:00	Session B-2	B-17 (SIT) Nobutaka MAEZAKI			
	18:00	Architecture,Urban Planning and Design (Chairpersons: SIT, UTM)	A-10 (UTM) Mohd. Hamdan Bin Ahmed A-11 (UTM) Ismail Bin Said A-12 (UTM) Sumaiyah binti Othman A-13 (UTM) Sapura Mohamad A-14 (UTM) Ismail Bin Said	18:00	Bioscience and Engineering (Chairpersons: SIT,KMUTT)	<ul> <li>B-18 (SIT) Nobuo Watanabe</li> <li>B-19 (UTM) Eraricar Salleh</li> <li>B-20 (UTM) Fong Wan Heng</li> <li>B-21 (UTM) Roshafuna Rasit Ali</li> <li>B-22 (UTM) Siti Hamidah Mohd-Setapar</li> </ul>			
1									
	18:40		C	losing for D	ay 1				
1	18:40		the second se	losing for D Reception Pa	and the second sec				
			1	and the second second	urty				
	19:00	Session A-3	I A-15 (UTM) Hamidah Ahmad	Reception Pa	urty	R-09 (SIT) Makoto Mizukawa			
	19:00 9:00	Session A-3 Architecture,Urban Planning and Design (Chairpersons: SIT, UTM)		Reception Pa	arty sions	R-09 (SIT) Makoto Mizukawa R-02 (SIT) Kanlaya Rattanyu R-03 (SIT) Ngo Trung Lam R-04 (SIT) Masaru Ide R-05 (SIT) Weerachai Skulkittiyut			
	19:00 9:00 9:00	Architecture, Urban Planning and Design	A-15 (UTM) Hamidah Ahmad A-16 (UTM) Mohd Hisyam Rasidi A-17 (KMUTT) Viroat Srisurapanon	Reception Pa	arty sion Session R-1 Robotics	R-09 (SIT) Makoto Mizukawa R-02 (SIT) Kanlaya Rattanyu R-03 (SIT) Ngo Trung Lam R-04 (SIT) Masaru Ide			
-eb. 26	19:00 9:00 9:00	Architecture, Urban Planning and Design (Chairpersons: SIT, UTM)	A-15 (UTM) Hamidah Ahmad A-16 (UTM) Mohd Hisyam Rasidi A-16 (UTM) Viroat Srisurapanon k M-01 (KMUTT) Weerasuk Surareungchai	Reception Pa	arty sion Session R-1 Robotics	R-09 (SIT) Makoto Mizukawa R-02 (SIT) Kanlaya Rattanyu R-03 (SIT) Ngo Trung Lam R-04 (SIT) Masaru Ide R-05 (SIT) Weerachai Skulkittiyut			
Feb. 26	19:00 9:00 9:00	Architecture,Urban Planning and Design (Chairpersons: SIT, UTM) Brea	A-15 (UTM) Hamidah Ahmad A-16 (UTM) Mohd Hisyam Rasidi A-17 (KMUTT) Viroat Srisurapanon	Reception Pa	arty sion Session R-1 Robotics	R-09 (SIT) Makoto Mizukawa R-02 (SIT) Kanlaya Rattanyu R-03 (SIT) Ngo Trung Lam R-04 (SIT) Masaru Ide R-05 (SIT) Weerachai Skulkittiyut R-06 (SIT) Kazuo Naito Break R-07 (SIT) Katsuhiro MAYAMA			
Feb. 26	19:00 9:00 9:00	Architecture,Urban Planning and Design (Chairpersons: SIT, UTM) Brea	A-15 (UTM) Hamidah Ahmad A-16 (UTM) Mohd Hisyam Rasidi A-17 (KMUTT) Viroat Srisurapanon k M-01 (KMUTT) Weerasuk Surareungchai M-02 (SIT) Nor Akmal Fadil	Reception Pa 10-ning/Sess 9:00 11:00	arty sion Session R-1 Robotics (Chairpersons: SIT, HUT)	R-09 (SIT) Makoto Mizukawa R-02 (SIT) Kanlaya Rattanyu R-03 (SIT) Ngo Trung Lam R-04 (SIT) Masaru Ide R-05 (SIT) Weerachai Skulkittiyut R-06 (SIT) Kazuo Naito Break			

### Time Table of 4th SEATUC Symposium, Japan Feb. 25-26, 2010

\*. The first alphabet of each field is used to mark different session (Architechture, Bioscience, Energy, Information, Materials, Robotics)

# Feb. 25-26, 2010

0		Room	303	Room 307			
Date -	l'ime	Session / Field	Presenter	Time	Session / Field	Presenter	
	9:45 12:30	Opening Session of SEATUC Symposium & Intensive Workshop Program "Engineering education to foster human resources for innovation" 9:45- Opening Address 10:00- Keynote Lecture & Panel Discussion					
		and the second second		Afternoon ses	sion		
	13:00	Session E-1	E-01 (ITE) Edwan Kardena 	13:00	Session I-1	1-01 (SIT) Khrisna Ariyanto I-15 (SIT) Shigeki Nakamura	
Feb. 25	15:40	Energy and Environment (Chairpersons: SiT, HUT)	E-03 (KMUTT) Suchapa Netpradit E-04 (KMUTT) Kritika Tanprasert E-05 (KMUTT) Nucharin Luangsa-ard E-06 (SIT) Nguyen Duc Tuyen E-07 (SIT) Arwindra Rizqiawan E-08 (SIT) Nafisab ABDUL RAHIMAN	15:40	Information Technology (Chairpersons: SIT, UTM)	1-03 (SIT) Phat Nguyen Huu 1-04 (SIT) Keita Nabeta 1-06 (SIT) Yuuki Kuribara 1-07 (SIT) Shunsuke Kobayashi 1-08 (SIT) Satoshi Aoki 1-05 (SIT) Sittapong Settapat	
1				Break		Letter Sis service and	
	16:00	Session E-2	E-09 (SIT) Nobuaki Nishimura	16:00	Session I-2	1-09 (SIT) Hiroyuki Kawamura	
	18:40	Energy and Environment (Chairpersons: SIT, ITB)	<ul> <li>E-10 (SIT)Goro Fujita</li> <li>E-11 (SIT) Satoshi Matsumoto</li> <li>E-12 (SIT) Suharyanto</li> <li>E-13 (UTM) Rubita Sudirman</li> <li>E-14 (UTM) Lee Yoke Lai</li> <li>E-15 (UTM) Makbul Anwari</li> <li>E-16 (UTM) Ha Chin Simma</li> </ul>	18:00	Information Technology (Chairpersons: SIT, UTM))	<ul> <li>I-10 (SIT) Shin Hasegawa</li> <li>I-11 (SIT) Hung Yu Shih</li> <li>I-16 (SIT) Keizaburo Nishina</li> <li>I-13 (SIT) Kaoru Koshimizu</li> <li>I-14 (SIT) Tomoki Takemura</li> </ul>	
ł	18:40	E-16 (UTM) Ho Chin Siong Closing for Day 1					
Ì	19:00	Reception Party					
	9:00	N.		Morning Suss			
1				9:00	Session I-3	1-02 (SIT) Tran Minh Quang	
Feb. 26				10:40	Information Technology (Chairpersons: SIT, UTM))	— I-12 (SIT) Nurzal Effiyana binti Ghazali I-17 (SIT) Hiroki Murata I-18 (SIT) Akira Aiba I-19 (SIT) Eiji Kamioka	
				58		Break	
				11:00	Session I-4	I-20 (SIT) Michiko Ohkura I-21 (SIT) Masaomi Kimura	
				12:40	Information Technology (Chairpersons: SIT, UTM))	I-21 (SII) Masadini Ksinuta I-22 (SII) Takumi Miyoshi I-23 (UTM) Abu Sahmah Mohd Supaat I-24 (UTM) Khairul Anuar Abdullah	

\*. The first alphabet of each field is used to mark different session (Architechture, Bioscience, Energy, Information, Materials, Robotics)

### Time Table of Intensive Workshop, Japan Feb. 25, 2010

Date	Time	Room 306		
	9:45 12:30	Opening Session of SEATUC Symposium & Intensive Workshop Program "Engineering education to foster human resources for innovation" 9:45- Opening Address 10:00- Keynote Lecture & Panel Discussion		
	Viernoel Sesson			
	14:00	Introduction to the Workshop		
Feb. 25	14:20			
-	14:20	<b>Presentation for 6 persons</b> The talk is 15 minutes for the presentation and 10 minutes for the Q&A.		
	16:50			
	16:50 17:30	Discussion		

Program for Fostering Human Resources with Sigma-Type Integrative Ability SHIBAURA INSTITUTE OF TECHNOLOGY

## **Timetable of Intensive Workshop**

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	🖬 Date	February 25th (Thursday) 14:00 –17:15
	🖬 Venu	e Room 5, Shibaura Campus, Shibaura Institute of Technology
	*) The ta	alk is 15 minutes for the presentation and 10 minutes for the Q&A.
4:00-1	4:20	Introduction to the Workshop
14:20-1	4:45	
		"Mobile Context-Wareness for Traffic Estimation"
		Tran Minh Quang and Eiji Kamioka, Shibaura Institute of Technology
14:45-1	5:10	
		"Radical Oxygen Species Change Cancer Treatment
		-Synthesis of New Type of Anticancer Drug -"
		Kohei Imai <sup>1</sup> , Asao Nakamura <sup>1</sup> , Haruhiro Okuda <sup>2</sup> and Kiyoshi Fukuhara <sup>2</sup> ,
	' Sh	ibaura institute of Technology, <sup>2</sup> National Institute of Health Sciences.
15:10-	15:35	
		"User-Centric Soft Handovers between UMTS-WIMAX for Voice Service Using
		SCTP with Two Thresholds"
		Nurzal Effiyana binti Ghazail <sup>1</sup> , Eiji Kamioka <sup>1</sup> , Abu Sahmah Mohd, Supa' at <sup>2</sup> and
		Sharifah Hafizah Syed Ariffin <sup>2</sup>
		<sup>1</sup> Shibaura Institute of Technology, <sup>2</sup> Universiti Teknologi Malaysia.
15:35-1	6:00	
		"A Progressive Developing of Torque Transferring Mechanism for Superconducting Mixer"
		Atikorn Wongsatanawarid, Yotaro Shimpo, S. Kobayashi, Hironori Seki
		Shibaura Institute of Technology.
16:00-1	6:25	
		"Demands of Medical Robotics Field and Relevance to Our Researches"
		Masaru Ide <sup>1</sup> , Makoto Mohri <sup>2</sup> , Pierluigi Beomonte Zobel <sup>3</sup> , Hiroyuki Koyama <sup>1</sup> , Shin-ichiro
	,	Yamamoto <sup>1</sup> and Takashi Komeda <sup>1</sup>
		ra Institute of Technology, <sup>2</sup> Mohri Hospital, <sup>3</sup> University of L'Aquila
16:25-1	6:50	
		"Protein Movement Visualization Advantages in Medical Science and Biotechnology"
		Naoto Kawasaki, Shibaura Institute of Technology.
16:50-1	7:15	Discussion.

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### Architecture, Urban Planning and Design

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A-01	Integrated Design Strategies in Energy - Efficiency of Industrial Buildings in Tropical Climate	
	(KMUTT) Acharawan Chutarat and Prasitchai Promliphonkul	01
A-02	Analysis of Urban Form by Traffic Network in Tokyo	
	(SIT) <u>Takashi Ishidou</u> and Makoto Itoh	06
A-03	Barrier of Diffusion and Adopter Categories of Electronic Bidet Toilet in Indonesia	
	(SIT) <u>Akbar Adhuitama</u>	10
A-04	Approximate Enclosed Space Using Autonomous Agent	10
	(SIT) Adswin Indraprastha	13
A-05	Visual Landscape Assessment: Objective Assessment using Physical Landscape Data for Cameron Highlands Municipal	13
	(SIT) <u>Nafisa Binti Hosni</u>	17
A-06	Method to Support Restoration and Reconstruction of Historical Buildings Using Virtual Environment Study on Theater Interior Through Impression Estimation	17
	(SIT) <u>Makoto ITOH</u> , Haruhi MIENO, Takahide FUJINUMA, Takashi ISHIDOU, Michiko OHKURA and Yoko WATANABE	20
A-07	Approach to Design Method for Micro-Scale Landscape in Hot and Humid Climate: A Case Study of New Housing Area in Johor, Malaysia	20
	(UTM) Kei Saito, Ismail Said, Mohd Hisyam Rasidi1 and Michihiko Shinozaki	24
A-08	Mosque Expansion and Renovations in Middle Class Housing Area	24
	(UTM) Norliza bt, Mohd Isa and Mohd Tajuddin Rasdi	28
A-09	Establishing a greenway network for university campus: A Case Study At Universiti Teknologi Malaysia	20
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	(UTM) Mohd. Hamdan Bin Ahmed, Rumana Rashid, Mohd. Hamdan Bin Ahmed and Md. Sayem Khan	36
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	(UTM) Ismail Bin Said and Zumahiran Binti Kamarudin	40
A-12	Revitalizing the People's Life in Buffer Zone Area in Melaka: a Case Study at an Urban Village, Melaka	10
	(UTM) Sumaiyah binti Othman and Ismail Said	44
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	(UTM) Sapura Mohamad	47
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	(UTM) Ismail Bin Said and Nor Zalina Harun	51
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	(UTM) Hamidah Ahmad and Muhammad Lutfi	55
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	(UTM ) Mohd Hisyam Rasidi	60
A-17	Interregional Freight Demand Model by Transport Mode	00
	(KMUTT) Viroat Srigurapanon, Sukit Wongnoppadoldeena	17
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	(HUT) Do Thi Hoa Vien and Vu Thi Lan Quyen		74
B-03	Optimization of liquefaction process by enzyme in ethanol production from dry cassava powder	ing.	, ,
	(HUT) Minh Thu Nguyen, Van Anh Mai and Thanh Hang Nguyen		78
B-04	Research on Tan Cuong green tea trademark protection system		, 0
	(HUT) Dang Thi Minh Luyen and Nguyen Thi Tuyet Mai		82
B-05	Study Social Representation of Green Bean Cake in Vietnam using the method of focus group	ι.H.	02
	(HUT) Vu Minh Hang, Tu Vict Phu and Nguyen Thi minh Tu		86
B-06	Preliminary study on lycopene content of Vietnam-grown tomato varieties		00
	(HUT) Nguyen Thi Hong Minh, Nguyen Thi Thuy Ninh and Ha Duyen Tu		90
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Energ	gy and Environment	

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### Biosurfactant Produced from Azotobacter vinelandii and its application for Enhanced Oil Sludge Biodegradation Process

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#### ABSTRACT

The problem of petroleum waste management is giving a due consideration of the national level. Large quantity of dehydrated oil sludge, generated in the disposal process of oilcontaining sewage in Indonesia that needs to be rendered harmless to human and to the environment. Microbial degradation has been accepted as an important method for the treatment of oil sludge by employing indigenous or extraneous microbial flora. The purpose of this study was to investigate the performance of biosurfactant in its attempt in enhanced biodegradation of oil sludge process. Measurement of biosurfactant production indicated that the maximum production occurred at the end of exponential growth phase (48 h). In the oil sludge biodegradation assay, it was found that addition of petrofilic consortia increased the removal efficiency up to 55%, while addition of biosurfactant in this reactor increased the total efficiency of 70% after 70 days of incubation. These results suggest that both petrofilic consortia and biosurfactant addition stimulate the biodegradation and overcome the limitation of petroleum hydrocarbon degradation process.

Keywords: A.vinelandii, biosurfactants, biodegradation, oil sludge

#### **1. INTRODUCTION**

Contamination of soils, groundwater, sediments, surface water, and air with hazardous and toxic chemicals is one of the major problems facing the oil and gas industry in Indonesia. Recent accidents attribute to oil spillages in Tarakan (East Kalimantan), Sorong (Papua), Indramayu (West Java) and Bojonegoro (East Java) should giving a due consideration of the national level. Petroleum hydrocarbon continues to be used as the principle source of energy and hence a large global environmental pollutant. Apart from accidental contamination of ecosystem, one of the most encountered pollutants in petroleum production companies is the formation of oil sludge that is entrapped with the effluents during treatment and conditioning of the wells produced crude oil through treatment process facilities.

Biodegradation is a treatment technology used to remediate a variety of contaminants, including soils contaminated with petroleum hydrocarbons. Bioremediation is an engineered process where the natural biodegradation of petroleum hydrocarbons by indigenous soil bacteria, fungi, and protozoa is accelerated. Since the vast majority of hydrocarbons in crude oils and refined biodegradable, products are and hydrocarbon-degrading microbes are ubiquitous, biodegradation can be อก environmentally acceptable way of eliminating oil sludge (Helmy et al., 2008). So far, biodegradation suggests an effective (Boopathy, 2000). method During biodegradation, hydrocarbon containing in oil sludge is used as an organic carbon source by microbial process, resulting in а the breakdown of oil sludge components to low molecular weight compounds. However, the bioavailability of weakly soluble hydrophobic compounds for microbial conversion is usually low and thus limits their degradation rate in aqueous medium (Genouw et al., 1994; Vasudevan and Rajaram. 2001). The use of surfactants has been found to enhance degradation of crude oil (Abalos et al., 2004; Urum and Pekdemir, 2004) or other hydrocarbons (Olivera et al., 2000). In this

paper, we investigated the performance of petrofilic consortia in degrading oil sludge and surfactant addition to enhance biodegradation process.

In this paper, we report the possible application of biosurfactant produced from *A*. *vinelandii* in the oil industry, enhancing the oil sludge biodegradation process.

#### MATERIAL AND METHODS

#### Reagents.

All chemicals were of reagent grade, purchased from Merck, J.T. Baker and Sigma Chem Co. Growth media were purchased from Oxoid ltd. Crude oil and oil sludge samples was obtained from Duri Oil Field Pekanbaru and Balongan Oil Field Indramayu Indonesia, respectively

#### Bacterial strain and Culture Conditions.

Azotobacter vinelandii AV01 was used in producing biosurfactant, while the petrofilic consortia containing Bacillus cereus BL01, Pseudomonas stuzeri BL02, Acinetobacter sp. BL03 and Bacillus sp BL04 were used in the biodegradation assay of oil sludge. All bacteria were obtained from the Culture Collection of Environmental Biotechnology Laboratory-Environmental Engineering Department, Institute Technology of Bandung, Indonesia. A. vinelandii was maintained at 4°C on mannitol enrichment agar slants containing (1-1): 20 g mannitol, 20 g yeast extract, 20 g tryptone, and 15 g of agar. While each petrofilic bacteria was maintained at 4°C on Nutrient Agar covering with 1 drop of crude oil. Sub-cultures were made to fresh agar slants every 1 month to maintain viability.

#### Biosurfactant Production.

Cultures of *A. vinelandii* were grown on a minimal basal medium (MB) which composed the following components (l<sup>-1</sup>) of distilled water: 1.5 g of K<sub>2</sub>HPO<sub>4</sub>; 0.5 g of KH<sub>2</sub>PO<sub>4</sub>; 0.2 g of MgSO<sub>4</sub>; 0.25 g of (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub>; and 20 g glucose as substrate. 10 ml Trace Element solution was added per liter of MB medium. The compositions of this trace element (l<sup>-1</sup>) are 12 g of Na<sub>2</sub>EDTA<sub>2</sub>.H<sub>2</sub>O; 1 g of CaCl<sub>2</sub>; 0.4 g of Z<sub>11</sub>SO<sub>4</sub>.7H<sub>2</sub>O; 10 g of Na<sub>5</sub>O<sub>4</sub>; 0.4 g of MnSO<sub>4</sub>.4H<sub>2</sub>O; 0.1 g of CuSO<sub>4</sub>.5H<sub>2</sub>O; 0.5 g of Na<sub>2</sub>MoO<sub>4</sub>.2H<sub>2</sub>O. The medium was sterilized by

autociaving at  $121^{\circ}$ C for 15 min. The inoculums of *A. vinelandii* was prepared by transferring cells grown on a slant to 250 ml Erlenmeyer flasks containing 50 ml of MB broth. Culture was incubated in an orbital shaker at room temperature, 110 rpm for 2 days. The MB containing 10<sup>6</sup> cells/ml was used to initiate growth using 2% (v/v) inoculums. Biosurfactant production was carried out in 2.000 ml Erlenmeyer flasks containing 800 ml MB at room temperature (27°C) with shaking at 110 rpm for 2 days in an orbital shaker.

#### Crude Biosurfactant Isolation

The fermentation broth was centrifuged at 13.000 rpm for 30 minute to obtain a cell free broth. After centrifugation, the supernatant was then dissolved in a 4 N hydrochloric solution and allowed to stand overnight at 4°C, followed by the biosurfactant extraction step with a chloroform solvent at room temperature (Makkar and Cameotra, 1998). The organic layer was transferred to a roundbottom flask and the aqueous layer was reextracted two times for complete recovery of biosurfactant. The organic phases were combined yielding a viscous brown-colored crude biosurfactant product and then evaporated to remove the solvent; the residue was collected and weighted. Vermani et al. (1995) method was used to determine the exopolysaccharide fraction of biosurfactant. A mixture of 1:2 (v/v) biosurfactant and chilled acetone were agitated and stand overnight to precipitate. Formed precipitate were filtered and gravimetrically analyzed.

#### Emulsification Index (E24).

To determine the emulsification index, Batista et al., (2006) method was applied. Centrifugation at 13,000 rpm to separate biosurfactant from microorganism cells yielding a *cell free broth*. A mixture of 1:1 between biosurfactant and crude oil is agitated for about 2 minute then stabilized for 24 hour. Emulsification index (%) determined by measuring the column height of emulsified oil against its total height multiplied by 100 times.

#### Total Petroleum Hydrocarbon (TPH) Measurements.

Measurement of TPH was conducted with gravimetric method as described by Mishra et

al. (2001). Sample was extracted with *n*-hexane, the organic layer were pooled and dried by evaporation of solvents. After evaporation, the amount of residual TPH recovered was weighted.

#### **Biodegradation** Assay

To determine the performance of petrofilic bacteria in degrading oil sludge, a preliminary biodegradation assay developed and set up as follows:

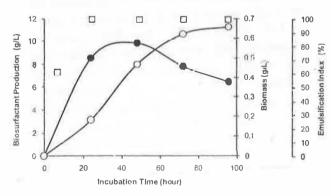
- Control-l: without addition of both petrofilic inoculums/P and biosurfactant/B.
- Control-2: without P; add 2% (v/v) B.
- Reactor-1: add 2% (v/v) P; without B.
- Reactor-2: add 2 % (v/v) each of P and B.
- Oil sludge initial concentration was 10% TPH and incubation time for biodegradation assay was 70 days.

Total petroleum hydrocarbon (TPH) concentration and growth of petrofilic bacteria were observed on certain time.

#### **RESULT AND DISCUSSIONS**

#### **Biosurfactant Production**

Growth and biosurfactant production from *A. vinelandii* with 2% glucose as sole carbon source was described in the **Figure 1**.



**Figure 1.** Growth (open circle symbol), biosurfactant production (solid circle symbol) and emulsification activity (open square symbol) profiles of *A. vinelandii* grown in minimal basal medium with 2% (v/v) glucose as a carbon source at  $27^{\circ}$ C.

The biosurfactant production started to increase during the exponential phase, reaching its maximum after about 48 h (9.81 g/h. These results indicate that the maximum

of biosurfactant biosynthesis from glucose occurred predominantly at the end of the exponential growth phase. The emulsification activity of the cell free broth increased up to 90% in the first 24 hour of incubation, whereas surfactant accumulation increased during this period and start to decrease after reaching its maximum synthesis. This might be due to biosurfactant were used as carbon source by A. vinelandii. Similar result reported by Sarubbo et al. (2007), that grown C.lipolytica with 10% canola oil and 10% glucose. Biosurfactant concentration reaching its maximum production after 48h at the end of exponential phase and start to decrease in a longer incubation time.

#### **Biosurfactant Enhanced Biodegradation**

Biosurfactant is a well known surface active agent that generally used in improving the viability of contaminant to the microbial The biosurfactant affect attack. the biodegradation process by increasing the solubility and dispersion of the compound (Desai and Banat, 1997). There are two ways in which biosurfactant affect which is increasing surface area of hydrophobic water the insoluble substrate. Secondly is increasing the bioavailability of hydrophobic water-insoluble substances. A laboratory scale of biosurfactant enhanced biodegradation of oil sludge was conducted. Effects of addition of biosurfactant from A. vinelandii in the biodegradation process were shown in Table 1. We used crude biosurfactant in the form of cell free directly broth without purifying the biosurfactant first for the simplicity reason of the experiments.

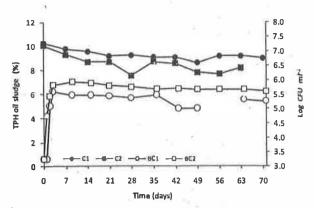
**Table 1.** TPH removal efficiency of oil sludge biodegradation in batch reactor. C1/Control 1 without addition both Petrofilic inoculums/P and Biosurfactant/B; C2/Control 2 (-P, +B); R1/Reactor 1 (+P, -B); R2/Reactor 2 (+P, +B).

Biodegra dation system	TPH Removal Efficiency (%) <sup>1</sup>	Increased Removal Efficiency (%)
C1	12.4	6.4 (C1-C2)
C2	18.8	55.6 (C1-R1)
R1	68.0	20.9 (R1-R2)
R2	88.9	70.1 (C2-R2)

<sup>1</sup> means values from triplicate measurement.

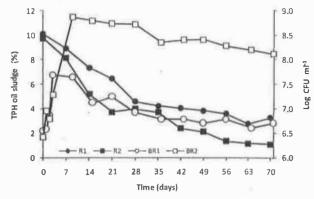
The water-solubility low of manv hydrocarbons reduces their availability to microorganisms and limits the biodegradation process. It has been assumed that biosurfactant can be used to enhance the bioavailability of hydrophobic compounds. On the other hand this low water-solubility increases sorption of compound to surface and limits their availability to biodegrading microorganisms (Abalos et al., 2004). Once again, biosurfactant can enhance growth on bound substrates by desorbing them from surfaces or by increasing their apparent water solubility. Figure 2 showed the microbial growth and TPH profile of control reactor. It was noticed that changes in oil sludge environmental condition from its originally slurry phase into more aqueous phase in the reactor, triggering the indigenous bacteria in it grow. For the total plate count to measurement, the CFU values increased from 10<sup>3.2</sup> (CFU/ml) at day 0 and reach its maximum to 105.4 (CFU/ml) in the first week of incubation. Similar pattern occurred in the control reactor-2 (without addition of petrofilic inoculants, added by 2% v/v of biosurfactant only). Biosurfactant addition make the oil sludge become more soluble in the reactor, this shown by increase in the microbial growth from 103.2 (CFU/ml) at day 0 and reach its maximum to 106 (CFU/ml) in the first week of incubation. However, the degradation process of oil sludge by mean of indigenous bacteria predicted small enough/neglect able throughout the experiment. TPH losses in control reactors mainly due to weathering/physical influences (Eweis et al., 1998; Venosa and Zhu. 2003) such as temperature shift, shaking condition, and volatilization of low molecular weight of hydrocarbon.

**Figure 3** shows that after 70 days of incubation, a significant reduction of TPH (68%) occurred in the biodegradation system supplemented with petrofilic consortia/R-1. This positive result suggests that bio-augmented bacteria could degrade TPH significantly.



**Figure 2.** The indigenous microbial growth (open symbol) and TPH degradation (solid symbol) profiles in batch control reactor system with 0 (circle/C-1) and 2(square/C-2) % v/v of biosurfactant addition at 27°C.

Bioaugmentation also can be used to increase the biodegradative capabilities of the indigenous microbial population. Compared with control reactor/C1, addition of petrofilic consortia increased the removal efficiency up to 55%. Non biological degradation (physical transformation) also occurred in the process; however the biological transformation dominated the process based on the growth of bacteria observed during the process. For the total plate count measurement, the CFU values increased from 106 (CFU/ml) at day 0 to 107.5 and 106.7 (CFU/ml) in the first week and day 70 respectively. The presence of biosurfactant in biodegradation system (R-2) increased the removal efficiency up to 20% compared to those without addition of biosurfactant/R-1. The present of biosurfactant also increased the microbial growth from 106.5 (CFU/ml) at day 0 to 108.8 (CFU/ml) in the first week of incubation and 108.1 (CFU/ml) at day 70. Similar result by Whang et al. (2007), that the effect rhamnolipid examined of biosurfactant to diesel/water degradation from 0 to 80 mg/l significantly increases biomass growth and diesel biodegradation percentage from 1000 to 2500 mg VSS/l and 40-100%, respectively.



**Figure 3**. The petrofilic consortia growth (open symbo!) and TPH degradation (solid symbol) profiles in batch reactor system with 0 (circle/R-1) and 2 (square/R-2) % v/v of biosurfactant addition at 27°C.

Our findings show that the addition of both petrofilic consortia and biosurfactant favors the biodegradation of the oil sludge. The limiting condition in the degradation of hydrocarbon and other PAH is their insolubility, thus decreasing the efficiency and rate of degradation. This limitation can be overcome either by addition of surface-active compounds surfactant to the growing culture, thus making hydrocarbons more water-soluble and available for the cell to degrade, or by production of its own surfactant by the augmented organisms to facilitate uptake. The presence of biosurfactant also stimulate the catabolism of hydrocarbon by mean of cometabolism process since biosurfactant are organic compound and readily degradable to microorganism.

#### ACKNOWLEDGEMENT

This research was supported and financed by ITB Research Grant and Directorate General of Higher Education (DIKTI), Government of Indonesia. QH thank J.F. Nadapdap for contributing in part of the research.

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