# PROGEDINGS

# 16<sup>th</sup> ASEAN Regional Symposium on Chemical Engineering

# "Chemical Engineering at the forefront of Global Challenges"

December 1-2, 2009 Manila Hotel Manila, Philippines

Organized by:



Pontifical and Royal UNIVERSITY OF SANTO TOMAS The Catholic University of the Philippines

In cooperation with:





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#### FOREWORD

It is with great pleasure that the Chemical Engineering Department of the University of Santo Tomas (UST), on the occasion of its 75<sup>th</sup> Foundation Anniversary, hosts the 16<sup>th</sup> ASEAN Regional Symposium on Chemical Engineering (RSCE), in cooperation with the Philippine Institute of Chemical Engineers (PIChE) and the PIChE-Metro-Manila Academe Chapter. As UST prepares to celebrate its Quadricentennial in 2011, it opens its doors to the members of the Chemical Engineering Scientific and Professional Community in the ASEAN and Asia-Pacific regions in the spirit of international cooperation and collaboration in order to bring forth technological solutions to pressing global challenges. Thus, the theme of the 16<sup>th</sup> ASEAN RSCE is *"Chemical Engineering at the Forefront of Global Challenges"*.

As this is the first time that the Chemical Engineering Department is hosting an international event as prestigious as the 16<sup>th</sup> ASEAN RSCE, it is keen in keeping the Symposium as a venue for meaningful scientific and professional exchanges among its participants and ensuring that such exchanges become a catalyst of collaboration and innovation in the ASEAN Region. Therefore, in addition to the customary Technical and Poster Sessions, four fora were included in the Scientific Programme where topics that are deemed apt in addressing current regional and global challenges are tackled. It is hoped that this format will accommodate Academic, Industrial and Governmental viewpoints, both in highly scientific discussions and more informal exchanges of ideas, and more effectively synthesize sustainable solutions to pressing global challenges. This Proceeding is a testament of such exhilarating discussions and exchanges.

The Organizing Committee recognizes how the Chemical Engineering discipline is evolving into an almost basic science-like discipline on which allied fields anchor to create new branches of technological disciplines of highly specific applications. It is viewed that this evolution reiterates the fact that Chemical Engineering will continue to be a highly relevant discipline in responding to global challenges that involve science and technology. Therefore, this year, rather than dividing the Technical Sessions according to Chemical Engineering Principles, the Organizing Committee divided the Scope of the Symposium into five areas where different Chemical Engineering principles may be used to address specific issues that require technological intervention: (1) Emerging Technologies, (2) Materials Innovation, (3) Processing: State of the Art, (4) Product Innovation and Entrepreneurship, and (5) Advances in Chemical Engineering Education. More than 100 paper and poster presentations with authors from 15 different countries are showcased in the 16<sup>th</sup> ASEAN RSCE. We are grateful for their participation and the opportunity to host them. Likewise, the Organizing Committee thanks the invited speakers, Technical Session Chairs and all the attendees. The Organizing Committee also wishes to thank the 16<sup>th</sup> ASEAN RSCE sponsors, donors and exhibitors, without whose generosity, holding this event will not be possible.

The historical Manila Hotel was chosen as a venue because of its long history of catering to people and events that had significant impact to the Philippines. The 16<sup>th</sup> ASEAN RSCE, being a milestone for the UST Chemical Engineering Department, deserves to be held in a storied venue such as the Manila Hotel.

**Prof. Philipina A. Marcelo, Ph. D.** Chair, Technical Committee The Secretariat 16<sup>th</sup> ASEAN Regional Symposium on Chemical Engineering Chair, Chemical Engineering Department University of Santo Tomas December 2009

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Nanoscience Research Group Leader at the Researc Center for the Natural and Applied Sciences University of Santo Tomas, Manila, Philippines

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# **UST** History

The University of Santo Tomas (UST) is the oldest existing university in Asia and in terms of student population, the largest Catholic University in the world located in one campus. It was through the pioneering desire of Bishop Miguel de Benavides, O.P., (1550-1605), the third Archbishop of Manila, to establish an educational institution and the donation he bequeathed, that the University of Santo Tomas was founded on April 28, 1611. It was originally conceived as a school to prepare young men for the priesthood. Originally located within Intramuros, the Walled City, UST was first called Colegio de Nuestra Señora del Santisimo Rosario and was later renamed Colegio de Santo Tomas, in memory of the foremost Dominican theologian, St. Thomas Aquinas. The University holds three distinct titles: 'Royal' granted by King Charles III of Spain in 1795, 'Pontifical' bestowed by Pope Leo XIII on September 17, 1902, and 'The Catholic University of the Philippines' conferred by Pope Pius XII in 1947. It transferred to its present location in España, Sampaloc, Manila in 1927.



#### **TABLE OF CONTENTS**

Foreword	ii
International Scientific Committee	iii
Organizing Committee	iv
Technical Consultants	vi
Acknowledgement	vii
UST History	viii

#### PLENARY TALK

Code	Title of Paper/Author	Page
1	Environmental Conerns are Chemical Engineering Opportunities Mohamed Eisa	1
	Membrane Processes in Chemical and Nuclear Industries: Current Scenario and Future	4
2	Challenges Anil Kumar Pabby	
3	<b>Current Status and Future Direction of Chemical Engineering Researches and Education</b> <i>Hiroo Niiyama</i>	9
5	<b>Innovations in the Production of Biodiesel</b> Mohammed M. Farid and Sam Behzadi, The University of Auckland, New Zealand	10
7	<b>Membranes as Sustainable Technologies for Water, Energy, and Biofuel</b> Neal Tai-Shung Chung *, Yan Wang, Bee Ting Low, May May Teoh,National University of Singapore, Singapore	19
8	Nanostructured Polymer Brushes and Layer-by-Layer Films: From Nanocomposites to Ultrathin Smart Coatings Rigoberto C. Advincula University of Houston, USA	24

#### **TECHNICAL KEYNOTE**

Code	Title of Paper/Author	Page
1	<b>Development of Efficient Photocatalysts for H</b> 2 <b>Production from Water Using Visible</b> <b>Light</b> <i>Wei Zhang, Yabo Wang, Rong Xu</i>	27
2	<b>Recent Advances on Carbon Dioxide Capture by Chemical Absorption</b> <i>Meng-Hui Li</i>	31
3	<b>Optimal Deployment of Carbon Dioxide Capture in the Power Sector Using Fuzzy</b> <b>Boolean Programming</b> <i>R. R. Tan, D. K. S. Ng, D. C. Y. Foo, and K. B. Aviso</i>	33
4	<b>Urban River Cleanup Collaboration Project in Ota-Ward, Tokyo</b> Masaaki Suzuki	34
5	Multiscale Modeling for Interfacial Adhesion Enhancement Using Self-Assembled Nanostructures Matthew M. F. Yuen , Haibo Fan, Cell K.Y.Wong	36
6	Role of $\chi$ -phase Contents in Nanocrystalline $\gamma$ -Al <sub>2</sub> O <sub>3</sub> on the Physiochemical and Catalytic Properties of Al <sub>2</sub> O <sub>3</sub> and Pt/Al <sub>2</sub> O <sub>3</sub> Catalysts Piyasan Praserthdam, Jutharat Khom-in, Chatchai Meephok, Joongiai Panpranot, and Okorn Mekasuwandumrong	40
7	<b>Thermal Plasma Processing for Functional Nanoparticle Synthesis</b> Takayuki Watanabe and Manabu Tanaka	47
8	Unraveling and Probing the Current Paradigm of Chemical Engineering as a Basis in Formulating Teaching Strategies W. I. Jose	51
9	Fuel Cell Research Trends: Towards Zero Emission Energy Technology	55

Wan Ramli Wan Daud

 10
 Mesenchymal Cell Seeding on Three-Dimensional Matrices
 63

 James Patrick Abulencia, Dominique J. Griffon, Guillaume R. Ragetly, L. Page Fredericks, and
 63

 Sahraoui Chaieb
 63

#### **EMERGING TECHNOLOGIES**

#### **Renewable Energy and Alternative Energy Technologies** Code **Title of Paper/Author** Page Experimental Investigation of Biodiesel Synthesis from Palm Oil using Reactive **1ET-REAET2** 66 **Distillation Process** Ratna D. Kusumaningtyas, Arief Budiman, Rochmadi, Sutijan, Suryo Purwono **1ET-REAET4** Thermodynamic Study on Vapour-Liquid Equilibrium of Toluene And Several Types of 70 **Oil as Absorbent** Hendriyana, Suhartono and Herri Susanto **1ET-REAET5** Synthesis and Activity Test of Cu/Zno/Al<sub>2</sub>O<sub>3</sub>for the Methanol Steam Reforming 74 IGBN Makertihartha, Subagjo, Melia Laniwati **1ET-REAET7 Gasification of Lignite by Microwaved Steam** 78 M. Djoni Bustan, Rosalina, Gusni Sushanti The Effect of Colocasia Esculenta Leaf as Electrolyte to Hydrogen **1ET-REAET8** 80 Production on Water Electrolysis by Using Electrochemical Reactor Sri Harvati. Widivanto. Yus Donald Chaniaao **1ET-REAET10** Nitrogen Isotope Separation Using Plasma Chemical Method 83 Harunori Nagoya, Shinsuke Mori, Masaaki Suzuki **1ET-REAET14** Review on Agricultural Biomass Utilization as Energy Source in Malaysia 86 Suzana Yusup, Mohamad Taufiq Arpin, Yoshimitsu Uemura, Anita Ramli, Lukman Ismail, Siew Hoong Shuit, Kok Tat Tan, Keat Teong Lee **1ET-REAET15** Engine Performance Characteristics of Using a Low Blend Coconut Methyl Ester(B5) 90 and Coconut - Jatropha Methyl Ester(CJ5) Mixture in an Unadditized Diesel Fuel, for a Single Cylinder, 4 Stroke Diesel Engine Felipe Ronald M. Argamosa, Carlos E. Zapanta, Elexis Edmond D. Lauzon Preliminary Engine Performance Tests and Smoke Emision Analysis Using Jatropha **1ET-REAET16** 96 (Tubang-Bakod) Methyl Ester in a Compression Ignition Engine Hernando E. Enal Jr., Mark Yu Tang P. Lin, Aldrin Kevin G. Tamse and Maria Natalia R. Dimaano **Environmental Science and Technology** Code **Title of Paper/Author** Page **1ET-EST1** Sorption of Chromium Using Dried Biomass of Water Spinach (Ipomoea aquatica) 99

	Daizyree Baran, Rey Eliseo Torrejos, Joann Baconguis and Maria Isabel R. Dumlao	
1ET-EST5	<b>Effect of Preparation Methods on Nanogold Supported TiO</b> 2 Padikkaparambil Silija, Zahira Yaakob, Narayanan Binitha, S.K.Kamarudin,S.M.Tasirin, Viswanathan Suraja	102
1ET-EST6	Photocatalytic Activity of Iron and Niobium Co-Doped TiO2 Towards Perfluorooctanoic Acid (PFOA) Degradation Carl Renan Estrellan, Chris Salim, and Hirofumi Hinode	105
1ET-EST7	<b>Technological Feasibility Study on the Chromium Recovery from a Synthetic Electroplating Effluent</b> <i>R.T. Bachmann, D. Wiemken, A.B. Tengkiat</i>	109
1ET-EST8	Recovery of Phenol from Aqueous Solutions Using Liquid Extraction and Liquid Membranes: A Review Somayyeh Nosrati, Jayakumar Natesan Subramanian Nayagar, Mohd. Ali Hashim	114
1ET-EST9	Characterization of Nano-Titania Prepared by Sol-Gel Method and Photocatalytic Studies in Dye Degradation	118

	Jurex Gallo, Kerry Cabral, Carmela Centeno, Josephine Borja and Susan Gallardo	
1ET-EST11	<b>Substance Flow Analysis of Mercury in Fluorescent Lamps in the Philippines</b> Karl Ivan M. San Luis, John Patrick Y. Tio, Jennerson T. Ong Florinda T. Bacani, Raymond R. Tan	122
1ET-EST14	<b>Utilization of Cross-linked Carboxymethyl κ-Carrageenan as Adsorbent for Hexavalent</b> <b>Chromium (Cr</b> <sup>+6</sup> <b>) Ion</b> Princess Joyce R. Antonio, Mark Emile H. Punzalan, Rochelle Anne B. Saturno and Aristea V. Bayquen	125
1ET-EST15	Influence of Water Content on Biofiltration Performance Daisy B. Badilla, Peter A. Gostomski, Maria Lourdes P. Dalida	129
1ET-EST16	Sorption Selectivity of Mine Drainage Heavy Metals on Coco Peat Dennis C. Ong, Maria Antonia N. Tanchuling, and Augustus C. Resurreccion	133
1ET-EST18	<b>The Photocatalytic Degradation of Lignin from Simulated Recycled Paper Mill Effluent using Nano Titania</b> Susan M. Gallardo, Ria Angelica L. Hermoso, Shaira Sharmaine G. Montero	137
1ET-EST19	Solubility of Carbon Dioxide in the Aqueous Blended Amine System of Triethanolamine and Piperazine Rhoda B. Leron, Pei-Yuan Chung, Allan N. Soriano, Meng-Hui Li	141
1ET-EST20	A Liquid-Phase Batch Adsorption Study of Methyl Violet Dye Removal Using Acid Modified Activated Carbon Azam T. Mohd Din, Henry F. Chee Yew, M.S. Al-Amin A. Malik	145
1ET-EST21	<b>Reduction of Heavy Metal and Microbial Contaminants in Septage via Vermicomposting</b> Jessie O. Samaniego and Louernie F. De Sales-Papa	150
1ET-EST22	Utilization of Sulfonated Polystyrene in the Reduction of Pb+ <sup>2</sup> in Electroplating Industry Wastewater Aries A. Arcega, Justin Micah B. Comia, Aprille M. de Castro, Sherryl A. Perez	154
1ET-EST23	Dye Removal from Simulated Textile WastewaterUsing Pyrolyzed Spent Coffee Grounds Juan Paolo T. Flores, Clyde F. Permalino, Florenze Jesse D.D. Dumandan, and Andrew Benedict Tengkiat	157

#### Analyses and Processing of Food and Pharmaceutical Products

Code	Title of Paper/Author	Page
1ET-APFPP1	Characterization and Performance Studies of a Chloramphenicol Nano-Sensor Based on Molecularly Imprinted Polymer Coated Piezoelectric Quartz Crystal Benilda S. Ebarvia, Isaiah Ubando, and Fortunato B. Sevilla III	160
1ET-APFPP2	Pressurized Microwave-Assisted Extraction of Protoberberine Alkaloids from Coscinium fenestratum Makoto Suzuki, Phengxay Deevanhxay, Nariaki Maeshibu, Sachio Hirose	163
1ET-APFPP3	<b>Development of Industrial Microwave Processing</b> M. Rozainee, A. A. Yussuf , M. M. Mutahharah, M.Sarah, P.S. Ng	166

#### Materials Science and Engineering

Code	Title of Paper/Author	Page
1ET-MSE1	Preparation of Palladium Nanoparticles Supported on Mesoporous Silica for Liquid- Phase Semihydrogenation of Phenylacetylene Napaporn Tiengchad and Joongjai Panpranot	170
1ET-MSE2	Preparation of Composite Membrane Using Atmospheric Pressure Plasma Polymerization Process Taichi Bannai, Tran Thi Dung, Shinsuke Mori, Masaaki Suzuki	174
1ET-MSE3	Characterization of Gold Nanoparticles Prepared by Deposition Precipitation Method on Surfactant Assisted Sol-Gel Co3O4 Viswanathan Suraja, Zahira Yaakob, Narayanan Binitha, S.M.Tasirin, Padikkaparambil Silija	177
1ET-MSE4	Modification of Properties of CeO2-Doped MgO-ZrO2 Ceramic Synthesized at Low	179

#### Temperature

Eufrecina B. Bognalbal and Alberto V. Amorsolo, Jr.

#### MATERIALS INNOVATION

#### **Construction and Indutrial Materials**

Code	Title of Paper/Author	Page
2MI-CIM1	Urea-Formaldehyde Microcapsules by in Situ Polymerization: Effect of pH and Prepolymer concentration Rochmadi, Agus Prasetyo, Wahyu Hasokowati	183
2MI-CIM2	Potential of Virgin Coconut Oil in the Production of Lacquer Enamel Paint Lina D. dela Cruz	187
2MI-CIM3	Preparation of Carbon Molecular Sieve for CO2/CO4 Separation by Pyrolysis of Phenol Formaldehyde Resin Imam Prasetyo, Rochmadi, and Endro Wahyono	193
2MI-CIM4	<b>Crude Glycerol Purification and Treatment for Biolubricant Preparation</b> Manal Ismail, Wan Nor Roslam Wan Isahak , Mohd Ambar Yarmo , Jamaliah Mohd Jahim, Jumat Salimon	197
2MI-CIM5	<b>Effect of Fiber Loading on the Mechanical Strength of NFR Hybrid Composites</b> Terence Tumolva, Masatoshi Kubouchi, Saiko Aoki <sup>1</sup> , Tetsuya Sakai	201
2MI-CIM6	Preparation of Activated Carbon from Bagasse Fly Ash by Chemical Activation Chandra Wahyu Purnomo, Chris Salim, Hirofumi Hinode	205
2MI-CIM7	Performance of Potassium Metavanadate as Low Carbon Steel Corrosion Inhibitor in Chloride and Sulfide Environments Isdiriayani Nurdi, Aditya Arif, Asri Pratiwi, Fikri Putra, Rennie Windyawati	208
2MI-CIM8	Effect of Compatibilizer on Mechanical Properties of Polypropylene/Zinc Oxide Nanocomposites Thitipong Sanitchai, Sirirat Wacharawichanant, and Supakanok Thongyai	213
2MI-CIM9	<b>Aging Behavior of Epoxy Resin in an Inoxidizable Environment</b> Daisuke <u>.</u> Shono, Hidetaka Minagata, Masatoshi Kubouchi, Saiko Aoki	216
2MI-CIM10	<b>Production of Activated Char from Rice Husk for Gasification Wastewater Treatment</b> Frita Yuliati, Dwiwahju Sasongko and Herri Susanto	220
2MI-CIM11	Nonpolar and Polar Solvent Solubilities in Polymer Solution Using Quartz Crystal Microbalance Method Gede Wibawa, Grastayana Suki, Dicky Afrizal and Kuswandi	224
2MI-CIM12	<b>Effects of Low Temperature Synthesis on the Properties of Magnesia-Doped Zirconia</b> <i>Eufrecina B. Bognalbal and Alberto V. Amorsolo, Jr.</i>	229

#### **Biomedical and Pharmaceutical Products**

Code	Title of Paper/Author	Page
2MI-BMPP3	Inline Analysis of Compounds in Lao Medicinal Plant- <i>Kiderm</i>	233
	Nariaki Maeshibu, Phengxay Deevanhxay, Makoto Suzuki, Keooudone Rasphone, Ken Tanaka,	
	Sachio Hirose	

#### **Electronics Materials**

Code	Title of Paper/Author	Page
2MI-EM1	A Study of Polypropylene, Sodium Hydroxide and Quartz Composition on the Capacitive Property of Cu M. Djoni Bustan, Sri Haryati and Dian Kharismadewi	236
2MI-EM2	Study of Resistive Degree of Polypropylene, Hydrofluoric Acid and Quartz Combination on Cu Material M. Djoni Bustan, Sri Haryati, Rahmawaty and Dian Kharismadewi	239

2MI-EM3	Structural and Electronic Properties of OH <sup>.</sup> Passivated Germanium Nanowires	241
	Mahasin Alam SK, Haixia Da, Man-Fai Ng and Kok Hwa Lim	

#### **PROCESSING: STATE OF THE ART**

Mathematical Modeling and Numerical Analyses

Code	Title of Paper/Author	Page
3Pro-MMNA1	<b>Diffusion Coefficients of Ethylene Glycol: Measurements and Correlations</b> Alvin R. Caparanga, Ming-Hung Wang, Allan N. Soriano, Meng-Hui Li	243
3Pro-MMNA2	Molar Heat Capacity and Electrolytic Conductivity of Aqueous Solutions of 1-Butyl-3-methylimidazolium Methylsulfate Allan N. Soriano, Pei-Yin Lin, Alvin R. Caparanga, Meng-Hui Li	246
3Pro-MMNA3	<b>Two Solved Models of Mass and Mass-Heat Transfers for Supercritical CO</b> <sub>2</sub> <b>Extraction of Melaleuca cajuputi oil</b> Shahnaz Mansouri Jajaei, Wan Ramli Wan Daud, Masturah Markom, Asghar Mansouri Jajaei	250
3Pro-MMNA4	Validation and Development of a Dispersion Model with Routine Ambient Concentration Monitoring Data from Petrokimia Gresik Industrial Complex Mohammad Fahrurrozi, Sutijan, Nanang Teguh, Mohammad Syahriari and Wahyudi B. Sediawan	254
3Pro-MMNA5	<b>Effect of Tetrasodium EDTA on the Vapor-Liquid Equilibria of Ethanol-Water System</b> Vergel C. Bungay, Yolanda P. Brondial and Nathaniel P. Dugos	257
3Pro-MMNA6	Modeling of Supercritical Fluid Extraction with Cosolvent Mixtures Masturah Markom, Masitah Hasan and Wan Ramli Wan Daud	261
3Po-MMNA7	Modeling and Simulation of a Separate Line Calciner Fueled with a Mixture of Coal and Rice Husk Sunu Herwi Pranolo, Yazid Bindar, Dwiwahju Sasongko, and Herri Susanto	266
3Pro-MMNA8	Adaptive Wavelet Density Distribution for Modeling Polymerization Processes Jose Co Munoz and Junghui Chen	271
3Pro-MMNA9	Intermittent Hot Air, Dehumidified Air, Heat Pump and Convective Cum Vacuum Microwave Drying Characteristics and Models Chien Hwa Chong, Adam Figiel, Chung Lim Law	275
3Pro-MMNA10	A Combined Model Based on the Pore Blockage and the Cake Deposition for the Permeation Process of Cross-flow Ultrafiltration of Whey Suspension Kensuke Karasu, Shiro Yoshikawa, Shinichi Ookawara and Kohei Ogawa	279
3Pro-MMNA11	Purely Predictive Application of Statistical Associating Fluid Theory for Enhanced Oil Recovery by Miscible Gas Flooding Tjokorde W. Samadhi, Hertanto Adidharma, Sugata P. Tan	282

#### **Catalysis and Reaction Engineering**

Code	Title of Paper/Author	Page
3Pro-CRE1	Kinetics Modeling for Synthesis of Terpineol from Turpentine Herti Utami, Arief Budiman, Sutijan, Roto, Wahyudi Budi Sediawan	286
3Pro-CRE2	<b>The Effect of Unburned Carbon on Coal Fly Ash Toward Cr(VI) Adsorption Capacity</b> Widi Astuti, I Made Bendiyasa, Endang Tri Wahyuni and Agus Prasetya	290
3Pro-CRE3	Kinetic Study on the Hydrolysis of Water Hyacinth to Levulinic Acid Buana Girisuta, L. P. B. M. Janssen, H. J. Heeres	294
3Pro-CRE5	Aerobic Oxidation of Benzyl Alcohol Using Molecular Oxygen Over Surface-Modified Tud-1 Supported Palladium Catalysts Yuanting Chen, Yanhui Yang	297
3Pro-CRE12	Synthesis of Nanosized Platinum Catalysts for Cinnamaldehyde Hydrogenation Reaction Chalisa Kruprasert , Choowong Chaisuk, Okorn Mekasuwandumrong	302
3Pro-CRE13	Immobilization of Phosphoramidite Ligands—Rh Complexes on SBA-15 and its Catalytic Application in Conjugate Addition of Arylboronic Acide to Enones	306

Zhen Guo, Yingshan Tan, Munfong Chan, Yanhui Yang

3Pro-CRE15	Kinetics of Transesterification of <i>Jatropha Curcas</i> -Based Triglycerides with an Alcohol in the Presence of Alkaline Catalyst Azhari M. Syam, Yunus, R., Mohd. Ghazi, T. I., Choong, T. S. Y.	310
3Pro-CRE16	Synergistic Effects of Plasma Chemical Reaction and Electrochemical Reaction using the SOFC Reactor	314

Yuki Tagawa, Shinsuke Mori, Masaaki Suzuki

#### Design, Optimization, Instrumentation and Process Control

Code	Title of Paper/Author	Page
3Pro-DOIPC1	<b>Conservative Versus Optimum: Perspective on Process Design</b> Jed M. Bellen, Kevin Leonard C. Caudal, Herbie Gino S. Vinluan	317
3Pro-DOIPC3	Vapor-Liquid Equilibria (VLE) of Isopropyl Alcohol-Toluene-Water System in the Presence of Magnesium Chloride Nathaniel P. Dugos, Yolanda P. Brondial, Vergel C. Bungay	321
3Pro-DOIPC4	Effect of Time and Temperature on Reflux Extraction from Sea Cucumber (Holothuria scabra J) as Source of Natural Testosterone Kurnia Harlina Dewi, Masturah Markom, Devi Silsia and Laili Susanti	325
3Pro-DOIPC5	Comparison of Different Extraction Techniques for Isolation of Testosterone from Sea Cucumber (Holothuria scabra J) Kurnia Harlina Dewi, Masturah Markom, Wan Ramli Wan Daud, Devi Silsia and Laili Susanti	328
3Pro-DOIPC6	Dynamic Optimization of a Fixed-Bed Reactor System for Methanol Production with Optimized Time Intervals Mohd Nazri Mohd Fuad, Mohd Azlan Hussain, Adam Zakaria	332
3Pro-DOIPC7	<b>Product Quality and Drying Characteristics of Intermittent Heat Pump Drying of</b> <i>Ganoderma tsugae</i> Murrill <i>Chung Lim Law, Siew Kian Chin</i>	335
3Pro-DOIPC9	Application of Solvent Extraction for Neodymium Separation from Mix Rare Earth Rattakrei Hongsupanpang, Weerawat Patthaveekongka	341
3Pro-DOIPC10	Deactivation of Water Gas Shift Catalyst for PEFC Applications under Startup and Shutdown (DSS) Operation, Tomohiko Tagawa, Hirofumi Goshima, Rakunei Ba, Hiroshi Yamada, Yoshimi Kawashima	343
3Pro-DOIPC11	<b>The Exergy Analysis of Modified Flowsheeting Primary Reformer</b> <b>in Pt Pusri II Palembang</b> Sri Haryati, M. Djoni Bustan, I G. Mandera, J. Asnani I and D. Kharismadewi	346
3Pro-DOIPC12	<b>Techno-Economic Comparison of Various Metallic Catalysts for Syngas Production in the Steam Reformer of Pt Pusri II Palembang</b> Sri Haryati, M. Djoni Bustan, Juniarti Asnani I and Dian Kharismadewi	349
3Pro-DOIPC14	Utilization of Adhesion of Fine Crystals for Increasing the Efficiency of NaCl Crystallization Yoshinari Wada, Koji Masaoka, Masakazu Matsumoto and Kaoru Onoe	351
3Pro-DOIPC15	Palladium-Based Inert Membrane Reactor to Improve Productivity of Hydrogen Production from Isopropyl Alcohol Azis Trianto, Pri Januar, Erwan Yonata and Stephanus Adrian	354
3Pro-DOIPC16	Isotope Separation by Condensation of Vibrationally Excited Gas Nga T.A Nguyen, Toyoaki Hayakawa, Shinsuke Mori, Masaaki Suzuki	357
3Pro-DOIPC17	Separation of Rubber Particle from Skim Latex Using Rotating Microfilter in Concentrated Latex Industry Pravit Ployngam, Lim Chin Hock and Chirakarn Muangnapoh	360
3Pro-DOIPC18	Sludge Formation and Removal in Concentrated Latex Industry Arnop Meesupree, Lim Chin Hock and Chirakarn Muangnapoh	362
3Pro-DOIPC20	A Simple Ebulliometer for Accurate Measurement of Vapor Pressure of Alcohol- Isooctane Mixtures Ignatius Gunardi, Vika Amildesi, Rama Oktavian, and Gede Wibawa	365
3Pro-DOIPC21	The Effect Of Alcohol, Catalyst Concentration, And Reaction Time To The Yield Of Neem	369

	<b>Biodiesel And Characteristics</b> Felycia Edi Soetaredjo, Aning Ayucitra, Sindu Wibowo and Hendy Kurniawan	
3Pro-DOIPC23	<b>Dynamic Modeling and Control of a Debutanizer column</b> Nasser M Ramli and Mohd Azlan Hussain	372
3Pro-DOIPC24	<b>Exergy Analysis and Fuel Reduction Strategies For Crude Distillation Unit</b> Nur Izyan binti Zulkafli, Shuhaimi Mahadzir	377
Biotechnology	7	
Code	Title of Paper/Author	Page
3Pro-Biotech1	Investigation of Fungitoxic Activity of Neem Seeds, Leaf and Oil Extracts on the Most Important Soil-Born Phythopathogenic Fungi Under Laboratory Condition Lida Jabbari, Shahnaz Mansoori Jajaie, Homayoom Afshari Azad, Mohsen Morovati, Ahmad Heidari	382
3Pro-Biotech3	Study of the Performance of a Bioreactor System Using Microbial Support Materials Derived from Solid Wastes Lam Van Giang, Franz Furby C. Ramos, Jocelyn B. Toga-on, Ma. Catriona Devanadera, Nguyen Phuoc Dan, Ohtaguchi Kazuhisa, and Wilfredo I. Jose	385
3Pro-Biotech4	Prediction of Fructose Concentration in a Glucose Isomerisation Process using Artificial Neural Network N A. Rahman, M.Hasan, M.A. Hussain, J. Jahim, S.R.S. Abdullah	389
3Pro-Bioteh6	<b>Characteristic Modification of Bacterial Cellulose during the Biosynthesis by</b> <i>Acetobacter Xylinum</i> <i>Siriporn Taokaew, Chattrin Mahaisavariya and Muenduen Phisalaphong</i>	392
3Pro-Biotech7	<b>Ethanol Productivity from Sugarcane Juice and Cane Molasses by Mixed Cultures of</b> <i>Kluyveromyces marxianus</i> DMKU 3-1042 and Saccharomyces cerevisiae M30 Akekasit Eiadphum, Anuchit Rattanapan, Jirawan Mongkolkajit, Savitree Limtong and Muenduen Phisalaphong	396
3Pro-Biotech8	<b>Cellulose Decomposition Using Trichoderma viride and Derived Enzyme</b> Feng Liu, Motoki Kobayashi and Kaoru Onoe	399
3Pro-Biotech9	<b>Cabbage Extract as a Precursor in Xanthan Gum Production using Xanthomonas</b> <i>Campestris</i> Ronny Purwadi, Zulhaj Rizki , Fleuri Paramita Aprianti	402

#### **PRODUCT INNOVATION AND ENTREPRENEURSHIP**

# Product DevelopmentCodeTitle of Paper/AuthorPage4PIE-PD1The Use of Kepok Banana Starch as Soy Milk Ice Cream Stabilizer<br/>Aning Ayucitra, Felycia E Soetaredjo, Trio T Putra, Bob M Hoesan, and Hendy Heriyanto4054PIE-PD4Development of a Personal Water Purification Solution for Rural Communities in the<br/>Philippines<br/>Anne Joan Caraccio, Nithin Susan Abraham, Kevin John McDonnell, Nicholas Alexander Ruffini,<br/>Susan Gallardo, and James Patrick Abulencia408

#### **CHEMICAL ENGINEERING EDUCATION UPDATES**

#### **Emerging Teaching Approaches**

Code	Title of Paper/Author	Page
5CEEU-ETA2	A Proposed Strategy in Teaching "Mass and Energy Balance Calculations" (MEBC) under Steady-State Situation <i>Wilfredo I. Jose</i>	411

Kurnia Harlina Dewi 1\*, Masturah Markom<sup>2</sup>, Wan Ramli Wan Daud <sup>2</sup>, Devi Silsia <sup>1</sup> and Laili Susanti<sup>1</sup>

<sup>1</sup>Department of Agroindustrial Technology, Faculty of Agriculture, University of Bengkulu, Jl. Raya Kandang Limun, Bengkulu, 38371, Sumatera, Indonesia

<sup>2</sup>Department of Chemical and Process Engineering, Faculty of Engineering, National University of Malaysia, UKM Bangi 43000, Selangor Darul Ehsan, Malaysia

\*E-mail: nia\_unib@yahoo.com

**ABSTRACT:** Testosterone, the steroid hormone, is not only produced by sea cucumber but a lot of other organisms as well, which is used to sex reversal and aphrodisiac. This research aimed to comparing the conventional extraction methods for isolation of testosterone from sea cucumber. The solvent is selected from previous study on reflux extraction with solvent of acetone, methanol, methanol- chloroform mixture (1:2) and chloroform solvent. Ratio of material: solvent are 1:1, 1:2 and 1:3 respectively. The highest result is obtained by successively use methanol: chloroform, acetone, chloroform and methanol mean equal to 0.2728 mg / 100g dry weight (dw), 0.2623 mg/100 g (dw), 0.1606 mg/100g (dw) and 0.0920 mg/100 g (dw). Influence of ratio showed increasing usage of solvent increase the result of extraction. The highest materials: solvent ratio is obtained at ratio 1:3, 1:2 and 1:1. The highest extraction in maceration extraction is obtained by using acetone at ratio 1:3 equal to 0.07721 mg/100 g dry weights

Keywords: testosterone, sea cucumber, maceration, soxhlet, reflux

#### INTRODUCTION

ne of marine resources that has high value and potential to become biopharmaceutics is sea cucumber (Holothuria scabra J) which is also known as teat fish, sandfish and sea ginseng. Currently, sea cucumber in Indonesia is exported in dried form (beche-de-mer), konoko (dried gonad), konowata (salted intestines) (Martoyo et al. 2004). This product is popular as food for health because it can increase men's vitality due to the testosterone content (Kustiariah. 2006). Utilization of extraction as aphrodisiac on human has been done and tested on mice, as an ingredient for sex reversal on commodities where male has more economical value than female such as on lobster and gapi fish (Nurjanah, 2008). Economically, sea cucumber has two important values, which are as marine biopharmaceutical source and as food for health. In addition, sea cucumber is also used as raw material in various industries in China (Kerr, 2000). Chemical contents of wet sea cucumber consist of 44-55% protein, 3-5% carbohydrate and 1.5% fat. According to Fredalina, important fatty acid contents in sea cucumber such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) are involved in wound healing agent and anti-thrombotic (Fredalina et al. 1998). In addition, sea cucumber also contains antibacterial active ingredients (Haug et al. 2002; Villasin & Christopher, 2000; Ridzwan, 2005), antifungal (Muray, A. P. 2002), anticoagulant (Mulloy, 2000), as protease producer (Xue-Yan Fu et al. 2005a) and arginine kinase (Xue-Yan Fu et al. 2005b).

Problem in producing steroid from sea cucumber extraction is the low yield of extraction, thus sea cucumber has not yet been utilized in industrial scale biopharmaceutical source for fulfilling natural testosterone need. Correct extraction method will produce high steroid content (Goat & Toshihiro, 1997). This paper emphasize research on factors which affect conventional extraction, such as the conventional methods (maceration,soxhlet and reflux), solvent type, and material : solvent ratio.

#### MATERIALS AND METHODS

#### **Characterization of Sea Cucumber**

Early stage of the research was characterizing the sea cucumber to be extracted including type and age of sea cucumber

based on characteristics and criteria (length and weight) which reflect the age of the sea cucumber. Raw material used was matured sea cucumber (*Holothuturia scabra J*) caught by the fishermen in Bengkulu province (Indonesia) weighing 200-500 grams each. The characteristics were the same as a sea cucumber (Wibowo *et al.* 1997). The characteristics of sea cucumbers used in the research were the same as the characteristics of the steroid producing sea cucumbers identified by Riani *et al* (2007) and also the same as sea cucumbers used by Kustiariah (2005) and Nurjanah (2008).

#### Chemical analysis of Sea Cucumber

Suitable sea cucumber fulfilling the criteria was cleaned and the flesh was separated from the entrails, washed and milled. Then proximate analysis was carried out by using ether, NaOH,  $H_2SO_4$  and alcohol.

#### **Conventional Extraction**

*Maceration extraction.* Fresh sea cucumber that has been milled was weighed 100 gr, put into erlemeyer, soaked in several type of solvents and several ratio (material:solvent) and then put into refrigerator for 24 hours.

*Soxhlet extraction.* Fresh sea cucumber that has been milled was weighed 100 gr, wrapped by using filtering paper and then put in sampling container on soxhlet equipment. Heating device of the equipment was set at 50°C for 4 hours.

**Reflux extraction.** Reflux extraction is an extraction by soaking and heating the material and solvent simultaneously. Fresh sea cucumber that has been milled was weighed 100 gr, put into Erlenmeyer and soaked in solvent with several ratios. Chemicals used in conventional steroid extraction are methanol, acetone, methanol: chloroform and chloroform.

#### **RESULTS AND DISCUSSION**

#### **Characterization of Sea Cucumber**

The sea cucumbers were round in shape, long like a cucumber with the back were dark grey with white or yellow spots and whole body surface were covered by lime. The sea cucumbers bodies were rough, muscular with spots on the skin. An illustration of a sea cucumber used in the research is shown in **Figure 1**.



Figure 1. Steroid raw material of a sea cucumber (*Holuthuria scabra J*).

#### **Conventional Extraction**

#### Maceration extraction of sea cucumber

In sequence, the most polarized solvent used in the research was methanol, acetone, solvent mixture of methanol:chloroform and chloroform with dielectric constant of 32.6, 20.7, ±14.07 and 4.8 deybe respectively. Solvent's ability in extraction is very much determined by the suitability of polarization level of the material with the solvent. Polarized solvent will dissolve polarized components and non-polarized solvent can extract non-polarized components (Ashton, N.F and McDermott, C. 2004). Maceration extraction results can be seen in **Figure 2** in details.

According to Riddick and Burger (1970), lower dielectric constant of a solvent means the solvent is more polarized (Riddick J. A. and William B.B. 1970). Lowering dielectric constant from 32.6 (methanol) to 20.7 (acetone) shows improved result. However, lowering dielectric constant will decrease the amount of obtained result.



Figure 2. Sea cucumber's testosterone weight by maceration extraction.

Differentiation test showed that extraction result by using methanol, acetone, methanol: chloroform and chloroform solvent noticeably different at 5% level. This is due to differences in polarization level of the solvents affect the solvents ability to extract the material. Polarized solvent will dissolve polarized components and non-polarized solvent can extract non-polarized components (Agilera, 2003). Thus, it can be seen that sea cucumber's steroid differs from the common fats which are easily dissolved in non-polarized solvents. High solubility of sea cucumber's steroid in acetone (semi-polarized solvent) shows that sea cucumber is categorized as semi-polarized fat with –OH group at C-17.

Ratios effect of material:solvent in maceration extraction show the similarity of extraction characteristics for all kind of solvents, i.e. more solvent used or lower ratio will give more results obtained. Ratio of material:solvent which yields highest extraction is 1:3. In the case, more solvent caused higher contact between material and solvent, thus solubility was increased. At ratios of 1:2 and 1:1, solvent used was decreased; hence lowering the contact between them and the solubility was decreased.

Further testing showed that ratio 1:1 noticeably different from 1:3. This is because in maceration extraction which is grouped in batch system using single stage without mixing, contact between the material and solvent is only affected by the amount of solvent used (Tzia & Goerge, 2003). This result clarifies the contact between material and solvent in maceration extraction depends on the amount of solvent used. Nevertheless, solvent usage is limited by the contents of material compound and the cost of solvent with its effects on the environment. Maceration extraction produces highest extraction result by using acetone as solvent with material: solvent ratio at 1:3.

#### Soxhlet extraction of Sea Cucumber

Soxhlet extraction of sea cucumber by using soxhlet equipment showed fluctuating results as seen in **Figure 3**. This is due to the high water content (88.99%) of the fresh raw material, so that the water covered the solvent's surface in extraction result. The water layer blocked reflux process. At solvent ratio 1:1 and 1:2, water and solvent ratio was almost reaching ± 89 ml: 100 mL and ±89 mL:200 mL, circulation was very slow therefore the contact of material and solvent was very low. At solvent ratio 1:3, water and solvent ratio was ±89 ml:300 mL, therefore reflux process could occur. This shows that more solvent used will reduce water on the surface and hence lowering the blockage to solvent circulation, thus reflux cycle can occur normally.



Figure 3. Sea cucumber's testosterone weight by soxhlet extraction.

In soxhlet extraction method, solvent's ability in extracting is not only determined by the suitability of polarization level of the solvent and material, but is also determined by solvent's boiling point. Methanol chloroform solvent is able to extract steroid more than acetone due to the boiling points of methanol chloroform is higher than acetone. The evaporation of methanol chloroform is not as fast as acetone with the solvent's circulation is slower and contact time is longer, therefore extraction is higher.Highest extraction was obtained by methanol chloroform compound solvent at material: solvent ratio of 1:3.

#### **Reflux extraction of Sea Cucumber**

Sea cucumber's steroid extraction by reflux is shown in **Figure 4**. Solvent's influence in reflux extraction showed highest result by using chloroform; solvent's mixture of methanol chloroform, acetone, and the lowest is methanol. Tests showed that by using chloroform with the result of 7.9547 mg/100 gr (dw) did not show significant difference from the result by using mixture of methanol chloroform which was 7.6142 mg/100 gr (dw).

Effects of material: solvent ratio showed that more solvent used produced higher extraction. However, further testing showed that ratio of 1:2 did not cause significant difference from 1:3. Therefore, the best solvent used in the next scale is the mixture of methanol chloroform with ratio of 1:2. The testing also showed that chloroform usage could be reduced, thus lowering the cost (chloroform is more expensive than methanol) and the environmental effects of chloroform could be reduced as well.



Figure 4. Sea cucumber's testosterone weight by reflux extraction.

#### Comparison

Comparison of extraction methods, solvents' type and material: solvent ratio can be seen in **Figure 5**. Highest extraction by maceration method was obtained by using acetone as the solvent with the material: solvent ratio of 1:3 and the result was 0.0772 mg/100 gr (dw). In soxhlet extraction, solvent mixture of methanol chloroform with ratio of 1:3 produced highest extraction of 0.6220 mg/100 gr (dw). While in reflux extraction, solvent mixture of methanol chloroform with the ratio of 1:2 produced highest extraction of 13.9547 mg/100 gr (dw)



Figure 5. Testosterone weight comparisons

Further testing of the three extraction methods (maceration, soxhlet and reflux) showed significant differences, where extraction by reflux produced extraction containing highest testosterone, followed by soxhlet extraction and the lowest was maceration extraction. Differences in methods, solvents and ratio could improve results obtained from 0.97% (maceration) to 7.82% by soxhlet and even to 95.72% by reflux.

#### CONCLUSION

Effects of the solvent's type and material: solvent ratios were not the same in the extraction methods. However, the effects of solvent's type on every ratio showed the same trend. Lower dielectric constant of the solvent (more non-polarized) caused more testosterone extraction. This was due to the testosterone steroid extracted was in the group of semi-polarized fat. The effect of material: solvent ratio in maceration extraction showed that when more solvent was used, testosterone extraction was higher.

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