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# Proceedings of the International Conference on Sustainable Biomass (ICSB 2019)

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for the physical and chemical processes along the coast line affected by sea-  
and fresh-water current but also functioning as habitat for many animals,  
including migratory birds. Mangrove in Margasari village or Lampung  
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**Proceedings Article**[Carbon Stock Estimation Due to Changes in Mangrove  
Labuhan Maringgai District, East Lampung Regency](#)[Rh Carina Pertiwi Syafira Salsabilli, Endang L. Widiastuti, Sri Wahyuningsih](#)[The damage to the mangrove ecosystem in Margasari Village District of  
Labuhan Maringgai, East Lampung Regency was predicted to reduce the  
estimated carbon stocks in the ecosystem. This research aims to determine  
the influence of the functioning of the mangrove ecosystem on the total  
biomass, estimated...](#)[+ Article details](#)[+ Download article \(PDF\)](#)

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**Proceedings Article**

## HOME **Policy Cluster: Affirmative Empowerment and Strengthening of Coastal Community Women Home Industries**

PREFACE

~~Novita Tresiana, Noverman Duadji~~

ARTICLES

~~Purpose, The failure of policies and development programs for Home Industry (HI) that have been carried out by the local government is due to the implementation of the previous policies which were carried out sporadically, incidentally, not based on the community learning model. Clusterization is an...~~

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### Proceedings Article

## Isolation and Characterization *Bacillus* sp. Producing Cellulase Enzymes from Hanura Mangrove

Sumardi, Salman Farisi, Christina N. Ekowati, Cahya I. Listiyorini

In the mangrove ecosystem, there are cellulolytic bacteria that act to decompose cellulose in nature. This study aims to obtain cellulolytic bacteria from mangrove forests in Hanura village. Bacterial isolation was carried out using Sea Water Complete Agar (SWCA) media which added Carboxymethyl Cellulose...

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### Proceedings Article

## Potential Taurine Content from Three Different Macroalgae: *Halimeda opuntia* L., *Sargassum* sp. and *Euचेuma cottonii* L.

Ulfa Azzizah, Endang L. Widiastuti, Henni W. Maharani

HOME  
As one of the marine resources, macroalgae presumably contains of free amino acid such as taurine. Taurine is known to be one of the most important amino acid related to hyperosmotic stress for most of living organisms. The study was conducted to explore the taurine contain from the most abundance macroalgae...

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**Proceedings Article**

The Effect of *Bacillus Coagulans* as Feed Probiotics on Non-specific Immunity of Whiteleg Shrimp *Litopenaeus Vannamei*  
S Supono, M Rahayu, M W Yusuf

The study aimed to evaluate the effect of giving a different dosage of *Bacillus coagulans* to feed (feed probiotics) on nonspecific immunity of *Litopenaeus vannamei* (white shrimp). The parameters tested included water quality and shrimp immunity parameters, namely total hemocyte count (THC), differential...

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**Proceedings Article**

Anticancer Potency of Seagrass (*Enhalus acoroides*) Methanol Extract in the *HeLa* Cervical Cancer Cell Culture  
Endang Linirin Widiastuti, Komang Rima, Hendri Busman

Anticancer potential of methanol extracts of seagrass and taurine was proven through cytotoxic and antiproliferation tests by MTT method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) on HeLa cervical cancer cell culture. The results showed that the methanol extract of seagrass



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**Strategies of Development of Marine Ecotourism in Pagarjaya  
Village – District of Pesawaran - Lampung - Indonesia**

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ORGANIZERS

**Supra Jaya Perdana, Abdullah Aman Damai, Endang Linirin Widiastuti**

---

This study aims to determine the perceptions of visitors and the village community of Pagarjaya towards its marine ecotourism and to find out the suitable strategy for the sustainable management of marine ecotourism. The research was conducted in the village of Pagarjaya, Punduh Pedada, Pesawaran District,...

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## Analysis of Heavy Metal Content in the Coral Reef and Foraminifera Benthic in Coastal Regions of Lampung Bay

Endang L. Widiastuti, Benny, R. Supriyanto, Eva Octarianita

Coral reefs and benthic foraminifera are efficient pollution bioindicators for predicting heavy metal pollution due to their great tolerance to high ecological pressures, so that these biota are used as test animals in monitoring the accumulation rate of heavy metals of marine waters. The purpose of...

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**Proceedings Article**

## Perception of Fisherman and Government Officer on Policy Implementation of Cantrang Prohibition in Lampung Bay

Indra Gumay Febryano, Debi Hardian, Supono Supono, Abdullah Aman Damai, Endang L. Widiastuti, Novriyanti Novriyanti

The policy to prohibit cantrang fishing gear is intended to prevent excessive exploitation of fish resources, environmental degradation and conflicts between fisherman, but fisherman and government officers' perceptions of the policy are not the same. The purpose of this study was to analyze the perceptions...

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**Proceedings Article**

## HOME Disaster Preparedness Analysis on Small Island as a Tourist

### Destination

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## Case Study: Pahawang Island, Lampung Indonesia

#### ARTICLES

### Citra Persada, Fadhilah Rusmiati

#### AUTHORS

The Lampung Bay is a coastal area which has become the main destination

for marine tourism in Lampung Province in recent years. After the tsunami

due to the eruption of Mount Anak Krakatau in December 2018, the number of

tourists to the region has decreased. The main reason for this is that there is...

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### Proceedings Article

## Analysis of Suitability of the Mangrove Ecotourism Area Pandan Alas Sriminosari Village Labuhan Maringgai East Lampung

A Herison, A Darmawan, Y Romdania, C Puspaningrum

The Sriminosari village is a coastal village in East Lampung district that had mangrove forests. Now, mangrove forests of Sriminosari used as a mangrove ecotourism area and will be developed as a form of proper management to ensure the conservation and sustainability of rehabilitation while simultaneously...

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### Proceedings Article

## Community-Based Consumable Fresh Water in Coastal Area of Dente Teladas Village, Tulang Bawang District, Lampung

## Province

HOME

Endang L. Widiastuti, Vina O. Indraswati, Indra G. Febryano, Hartoyo, Supono

PREFACE

Community access to consumed water in most coastal areas is still limited, which is one of the causes of the low level of welfare. The aim of the study

was to explain the perceptions of coastal communities in Dente Teladas

Village of Tulang Bawang District-Lampung Province towards the provision of water...

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### Proceedings Article

Identification of Child Problems & Tracking Policies for the Protection and Fulfillment of Children's Basic Rights in South Regency as a Coastal Zone of Lampung Province

Noverman Duadji, Novita Tresiana

The issue of children is a global public problem and is directly related to government programs and activities. To solve children's problems requires commitment and accuracy of action with the intended targets. This research is oriented towards achieving goals, namely identifying children's problems...

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### Proceedings Article

Groundwater Management for Small Islands and Spatial Patterns (Case Study of Banggai Kepulauan Regency)

Jaka Suryanta, Turmudi, Irmandi Nahib

Banggai Kepulauan (Bangkep) Regency with an area of 21,988.56 km<sup>2</sup>, 30% of

[HOME](#)  
its territory is land, namely Peleng Island and several small islands, while the other 70% is in the form of sea. A small portion of the region experiences drought during the dry season and its geological factors, but in the next...

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## Tidal Current Pattern in the Surrounding Bintan Island Waters Based on Model Simulation

Ahmad Bayhaqi, Annisa K. Fajari, Dewi Surinati, Nurin Hidayati

Observation was conducted in the surrounding Bintan Island Waters to collect the ocean surface current data including moored and stationary recording during first transitional monsoon (March-April). Tidal current pattern in all regions of Bintan Island (north, east and southwest) is simulated using 2D...

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## Analysis of Urban Fabric: An Integrated Coastal Zone Management (ICZM) Approach for Sustainable Tourism Development in The Coastal Area of Bandar Lampung

Citra Persada, Yunita Kesuma, Fadhilah Rusmiati

The coastal zone of Bandarlampung which grew as one of the economic development area in southern, is currently experiencing environmental degradation caused by trading activities, warehousing on a large scale. The natural resources in coastal area is holds high economic activity to create the tourism...

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**Lipid Contain of Three Microalgae on Culture with Different pH and Salinity**

SESSIONS

Inas Faridhah, Tia Annisa, Endang L. Widiastuti, Henni W. Maharani

Three species of microalgae were used in this study, namely *Nitzschia* sp., *Porphyridium* sp., and *Tetraselmis* sp., which collected from the Lampung Agency of Maricultures – Ministry of Coastal and Marine Affairs. They were cultured in room temperature with 12:12 day light and given different salinities...

**+ Article details****+ Download article (PDF)****Proceedings Article****Nutrients Distribution and Trophic Status Classification in Coastal Waters of Pulau Pasaran, Lampung**

B Putri, M R Nur Huda, H Yulianto, I G Yudha, N M Noor, M Ali

Research on the characteristics of distribution of phosphate, nitrate and Chlorophyll-a have been carried out in Pulau Pasaran waters Lampung from May to July 2017. The aim of this research was to determine the nutrient distribution and trophic status of Pulau Pasaran waters by chlorophyll-a, nitrate,...

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PREFACE

## Fisherman Attitude on Fisherman Insurance in Bandar Lampung City

ARTICLES

U G Febryano, M Rona, A A Damai, Hartoyo, E Rohana, Novriyanti

As fisherman insurance program is one of the government's policies to protect fisherman in their work. This study aims to explain the attitude of fisherman to fisherman insurance programs. The sampling technique used is non-probability sampling using the method of judgment sampling approach, which is...

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**Proceedings Article**

## The Tourism Concept of Emergency Shelter: Strategies for Community Resilience in The Coastal Area of South Lampung

D Hardilla, K H Basuki, F Rusmiati, C Persada

Facing vulnerability of disaster in the coastal area, a disaster risk reduction planning is evolved in Kalianda as the capital of South Lampung regency. These district areas close to Mount Anak Krakatoa which one of the most active volcano in Indonesia and caused the underwater landslide then turn generated...

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## Estimating the Number of Trees in Margasari Mangrove

## Forests of Lampung Through Aerial Images Using Adaptive Thresholding and Contour Extraction Methods

HOME

PREFACE

Mona A Batubara, Syaiful Alam, Khoirin Nisa, Rizka WA Utami, Helmy

ARTICLES

Fitriawan, Ardian Ulvan

AUTHORS

Estimating number of trees with field plots located in remote or inaccessible areas can be a costly and timely endeavor. Unmanned aerial vehicle (UAV) remote sensing allowing for the estimation of the forest vegetation offers an alternative to traditional ground-based forest measurements. It is an

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### Proceedings Article

## The Technical Concept of Differential Optical Absorption Spectroscopy for SO<sub>2</sub> Gas Spectrum Monitoring on Volcanic Ash of Gunung Anak Krakatau volcano

Ardian Ulvan, Muhamad Rifky Maulana, Mona Arif Muda Batubara, Nora Aditiyan, Melvi

Gunung Anak Krakatau is one of the active volcanoes in Indonesia. In October – December 2018, it erupted 156 times by releasing incandescent materials and toxic gases in the form of sulphur dioxide (SO<sub>2</sub>), carbon dioxide (CO<sub>2</sub>), and hydrogen sulphide (H<sub>2</sub>S) at 2 km radius. The release of SO<sub>2</sub> gas indicates...

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### Proceedings Article

## Characterization of Mixed Biodiesel and Petrodiesel as



## Transportation Fuel

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A Sholihah, S D S Murti, A R Juwita, F M Yanti, H Saputra

PREFACE

The use of biodiesel from palm oil as a substitute for diesel oil is a policy of the Government of Indonesia. In addition to renewable energy, the use of biodiesel as a fuel for transportation and industry is also an effort to reduce imports of diesel oil. Apart from the advantages of renewable biodiesel...

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### Proceedings Article

## Cellulose Microfibrils Filler Contributes to Thermal Stability and Morphology of Bioplastics from Sorghum-Based

Y Darni, N I Hanifah, H Utami, L Lismeri, M Hanif

This research aimed to examine the effect of cellulose microfibrils (CMF) as a filler to thermal characteristics and morphology of bioplastic. Sorghum starch and chitosan, of which 63 micron particle size, were varied by starch to chitosan ratio (g/g) of 10:0; 9.5:0.5; 8.5:1.5; 7.5:2.5; 6,5:3.5; and...

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### Proceedings Article

## Biogas Purification by CO<sub>2</sub> Reduction in Bubble Column Using Ca(OH)<sub>2</sub> and NaOH

E Purba, C N Yogia, P D R Sari

This study is about biogas purification process by reducing the CO<sub>2</sub> content in order to increase the heating value of biogas by using Ca(OH)<sub>2</sub> and NaOH solution as the absorbent in a bubble column. The purpose of this study is to

determine the effect of the sparger hole diameter and the height of Ca(OH)<sub>2</sub>...

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A Brunerova, U Masanudin, D A Iryani, D Herák

Several The following research focuses on analysing waste biomass generated thru the processing of specific tropical fruits, such as Dragon fruit, Duku, Mangosteen, and Snake fruit. The amount (in %) of produced fruit waste biomass (skin, peel) during fruit processing was monitored, and collected waste...

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PREFACE

## The Effect of Alkaline Pretreatment on Biogas Productivity and Kinetic from Cocoa Pod Husk Waste Using Batch Reactor

ARTICLES

U Wijaya, S Nurkhamidah, A Altway, K Laksmi, B Gusdyarto, T Iswanto

Cocoa pod husk (CPH) as a potential plantation waste was used for the biogas production. Pretreatment using NaOH (N), NaOH with addition of H<sub>2</sub>O<sub>2</sub> simultaneously (NHs) and consecutively (NHc) were carried out to remove lignin content in CPH and enhance methane yield. 2% NaOH was used for N pretreatment...

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**Proceedings Article**

## Synthesis and Characterization of NPK Slow Release Fertilizer for Red Onion by Using Empty Fruit Bunch (EFB) Char

E R Finalis, Arfiana, I Noor, S D S Murti, H Suratno, E Rosyadi, H Saputra, R Noda

Red onion (*Allium cepa* L.) is considered one of the most commonly used vegetables in the culinary arts in Indonesia. It has many benefits, one of them is contain antioxidants that promote good health. Conventional cultivation of red onion is depended on the implementation of inorganic fertilizers to...

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**Proceedings Article**

## Upgrading of Palm Oil Empty Fruit Bunches to Solid Fuel

## Using Torrefaction and Hydrothermal Treatment

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D A Rahim, M Yan, R D A Pohan, D Hantoko, H Susanto

PREFACE

To enhance the physicochemical properties of the EFB in the form of biochar, torrefaction and hydrothermal treatment were used. The proximate and ultimate compositions, heating values, and mineral composition of the biochar were all determined. The results showed that after treatment, fixed carbon increased...

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### Proceedings Article

## Effects of Meranti Biochar Addition on the Root Growth of *Falcataria Moluccana* Seedlings

Melya Riniarti, Hendra Prasetya, Ainin Niswati, Udin Hasanudin, Irwan Sukri Banuwa, Aulia Asmara Loka, Jiho Yoo, Sangdo Kim, Sihyun Lee, Wahyu Hidayat

The purpose of this study was to determine the effect of biochar application in growing media on the development of the roots of *Falcataria moluccana* seedlings. Biochar from the waste of meranti wood (*Shorea sp.*) was produced by a slow pyrolysis process using a traditional kiln at a temperature of 600°C....

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### Proceedings Article

## Product Characteristics from the Torrefaction of Bamboo Pellets in Oxidative Atmosphere

HOME **Jacky Michael Pah, Lisman Suryanegara, Agus Haryanto, Udin Hasanudin,  
Dewi Agustina Iryani, Christine Wulandari, Jiho Yoo, Sangdo Kim, Sihyun Lee,  
Wahyu Hidayat**

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**ARTICLES**

**Bamboo is a potential energy crop for the future and an attractive option to  
replace wood due to its fast growth, high productivity, and essential fuel  
characteristics such as low ash content, alkali index, and high heating value.  
This study aimed to evaluate the effects of torrefaction on the physical...**

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**Proceedings Article**

## Effects of Eco-friendly Hot Oil Treatment on the Wood Properties of *Gmelina Aborea* and *Cocos Nucifera*

Shalehudin D. Ma'ruf, Samsul Bakri, Indra Gumay Febryano, Agus Setiawan,  
Agus Haryanto, Intan Fajar Suri, Nam Hun Kim, Wahyu Hidayat

Most of wood in Indonesia is dominated by fast-growing wood such as  
gmelina (*Gmelina arborea*) and materials such as coconut (*Cocos nucifera*).  
Using fast-growing wood natural forests, increase economic growth, and is  
more environment friendly. The objective of this study was to improve the  
wood properties...

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**Proceedings Article**

## Influence of Die Temperature on Unit Density and Calorific Value of Municipal Solid Waste Pellets

S R H Siregar, D Nursani, A Surjosatyo

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The process of waste to energy can solve the problem of waste and produce energy as a by-product. The waste can be used as a raw material in pelletization process which then used as a fuel in the thermal processing technology. In the pelletization process there are operational variables that influence...

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## Improving Fuel Quality of Rice Straw Through Soaking Using Wastewater from Tofu Industry

S Suharyatun, A Haryanto, W Rahmawati, S Triyono, U Zubaidah

Straw is agricultural waste that has enormous potential as an alternative energy source because of its abundant availability. However, straw has poor intrinsic properties as a fuel. Alkali metal content (especially K) is very high, causing problems in the combustion process at high temperatures. One...

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## Emulating Excitation System of a Biomass-Based Micro Steam Turbine Generator

Khairudin, Amirudin, L Hakim, H Gusmedi, U Hasanudin, A Haryanto

An Arduino-driven power supply was applied to control the electromagnetic field of a synchronous machine experimented in this work. Typically, electromagnetic field was controlled by adjusting the field current. In this work, the field was directly controlled by regulating voltage source of the exciter...

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**Effects of Torrefaction Using COMB Dryer/Pyrolizer on the Properties of Rubberwood (*Hevea brasiliensis*) and Jabon (*Anthocephalus cadamba*) Pellets**

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W Hidayat, T Rubiyanti, Y Sulistio, D A Iryani, A Haryanto, Amrul, J Yoo, S Kim, S Lee, U Hasanudin

PUBLISHING INFORMATION

Rubberwood (*Hevea brasiliensis*) and Jabon (*Anthocephalus cadamba*) are two promising species that is widely planted in industrial plantation and community forests in Indonesia. The objective of this study was to improve the properties of rubberwood and jabon pellets through torrefaction. Torrefaction...

**+ Article details****+ Download article (PDF)****Proceedings Article****Decreasing of Environmental Loads in Tapioca Industry Through Utilization of Biogas Digester Effluent for Fertigation During Vegetables Production**

J R Amelia, O Maulidia, A Haryanto, S Triyono, U Hasanudin

In order to lower environmental burden, a tapioca industry make use its wastewater to generate biogas through anaerobic digestion process using a covered lagoon system. However, the effluent from the digester still has not met the quality standards so that it cannot be disposed into the water body. Organic...

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**Cold Model Performance Test of 50 KWe Dual Fluidized Bed Gasification Technology**

SESSIONS

**Egrosyandi, S D S Murti, I Masfuri, J Prasetyo, H Saputra, Y Sudo, R Noda**

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**Research on the development of sustainable alternative fuels to replace fossil fuels has received serious attention because of the increasing world oil prices and environmental concerns. This study focused on the development of Dual Fluidized Bed Gasification technology, especially the cold model performance...**

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**Proceedings Article****Chemical Modifications of Torrefied Palm Kernel Shell Using COMB Method****N Rumbang, Karelius, M Dirgantara, K G Suastika**

Torrefaction is a thermochemical process to improve the properties of biomass as a solid fuel. In addition to solid fuel, the torrefaction process also produces a by-product in the form of liquid smoke from condensation results. In this study, an analysis of lignocellulose and Fourier Transform Infrared...

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# Characterization of Modified Lampung Natural Zeolite with *Cetyl Trimethyl Ammonium Bromide* (CTAB) for Adsorption Industrial Tapioca Wastewater

Darmansyah, S B Ginting, D A Iryani, R P Sari, D Supriyadi

This research was observed on the CTAB modified Lampung Natural Zeolite (ZMS). The aim is to improve the adsorption capacity of tapioca wastewater. This zeolite modification process has several steps, starts with Lampung Natural Zeolite (ZAL or LNZ) pretreatment, LNZ activation by  $\text{NH}_4\text{Cl}$ , and ends

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Aryanto, Melvi, Ardian Ulvan

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HOME P. R Tyas, S D S Murti, S D Nanda

PREFACE Dimethyl Ether (DME) is an alternatives energy that can be synthesized through a direct process from syngas (CO and H<sub>2</sub>) with utilizing Cu-ZnO/Al<sub>2</sub>O<sub>3</sub>-HZSM-5 as a hybrid catalyst. Direct synthesis DME technology is relatively new technology by utilizing syngas that can be derived from gasification of biomass...

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## Zero Waste Concept in Fruit Waste Anaerobic Digester: Case Study of Biogas Plant Gamping, Yogyakarta

A Rahmada, F Marendra, H Rimbawan, C Wulandari, A E Putri, A Mustofa, D A Pramudito, R Millati, R B Cahyono, T Ariyanto

Gamping biogas plant was built in 2011 to solve waste problem from GemahRipah fruit market which generate 4-10 tons waste/day. The digester was design for 4 ton/day and produce biogas and electricity. The electricity is used for lighting the shops and the road. For eightyyears along the installation,...

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## Performance of Biogas Production from Coffee Pulp Waste with Cow Dung and Cattle Rumen Fluid as Inoculum in a Batch Reactor

T Widjaja, Nurkhamidah, A Altway, T Iswanto, E. Grady

**Production of biogas from coffee pulp waste is one of the potential attempts to provide alternative energy in Indonesia. As a leading commodity in Indonesia, the coffee produced the residual waste from its processing such as the pulp waste (include the exocarp, mesocarp, and endocarp). The waste contains...**

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**Pairing Coconut and Candlenut Oil as Feed Supplement in Low Protein Diet with Addition *Se* on Feed Efficiency of Red Nile Fish (*Oreochromis Niloticus*)**

K Haetami, Junianto, Abun

Nutritional Balance in feed is critical to improve the efficiency of feed. The use of oil in omnivore fish can serve as a spare protein-energy provider in addition to carbohydrates. Not only is the adequacy of the quantity and quality of protein that must be guaranteed, often the energy value is not...

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Mahidin, Husni Husin, Khairil, Hamdani, Erdiwansyah, Yanna Syamsuddin, Hisbullah, M Reza Hani, Asri Gani

The biomass gasification is one of the largest chemical transformation procedures of heat effectiveness. Numerical simulation is a significant instrument for studying biomass gasification. The numerical simulation of biomass gasification technologies at work and overseas is evaluated in this article....

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**Mass Yield Improvement of Liquid Fuel from RMSW Using Long Catalyser Downstream in Stage Pyrolysis**

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M. Gani, A Wiyono, Purnawan, Amrul

Thermal degradation at 500 oC to a Real Municipal Solid Waste (RMSW) has leave a footprint of problem with very low mass yields of liquid fuel.

Therefore, in the presence research, it has been attempted to increase the mass yield of liquid fuel by using Long Catalyzer Downstream (LCD) equipped with top...

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**Proceedings Article****Lignocellulosic Analysis of Corncob Biomass by Using Non-Thermal Pulsed Electric Field-NaOH Pretreatment**

A W Putranto, S H Abida, K Adrebi

In recent years, the second-generation bioethanol and advanced bio-based material production from biomass are focused on the pretreatment process by separating cellulose components from other components such as lignin and hemicellulose. Therefore, a physicochemical pretreatment method is needed by applying...

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## Leaching of Potassium from Oil Palm Empty Fruit Bunch (OPEFB) Using Tapioca Wastewater

U M Ningsih, A Haryanto, U Hasanudin, S Triyono

Empty fruit bunch (EFB) of palm oil is an abundant by-product that is resulted from the crude palm oil (CPO) extraction process and has great potential for bioenergy application. However, EFB has a high content of mineral such potassium (K) and silica (Si) that result in a negative impact on boiler like...

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## Biogas Purification Using Chitosan-Impregnated Porous Carbon

D P Rahmadani, A Rahmada, F Marendra, H J Rimbawan, R B Cahyono, Suherman, T Ariyanto

Biogas is produced from the anaerobic digestion of biodegradable organic matter. However, its application is limited because it contains impurities such as carbon dioxide (CO<sub>2</sub>) in high concentrations. Therefore, it is necessary to remove CO<sub>2</sub> to improve the energy content of biogas. Porous adsorbents...

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## Characterization of Cassava Stems as Potential Biomass for Bio-Oil Production via Electromagnetic-Assisted Catalytic

# Liquefaction

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L Lismeri, M Anggraini, A Sudarni, Y Darni

PREFACE

The production of bio-oil from biomass as renewable sources has been trusted as a suitable alternative in supplying future energy needs. Present in

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Amounts as crop residues cassava stems is potential biomass to be utilized. As raw material, it has advantages such as fast and easy growth,

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D A Iryani, A W Cindradewi, S B Ginting, E Ernawati, U Hasanudin

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The hybrid membranes for pervaporation of ethanol-water mixture were

prepared by filled Lampung natural zeolite (LNZ) into cellulose acetate (CA)

casting solution in the varied ratio of Ca:LNZ (30:0, 30:5, 30:10, 30: 20, 20:20 and

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40:10) wt%. The effect of zeolite addition on the resulting membranes...

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Methane Production from Patchouli Oil Distillery Waste Using Wet Semi-Continuous and Dry Batch Anaerobic Digestion

D A Prameswari, A N Rahmah, R Millati, R Wikandari, M N Cahyanto,

Lukitawesa, C Niklasson, M Taherzadeh

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**Patchouli oil distillery waste (PODW)** was used as a substrate for methane production in wet semi-continuous and dry batch anaerobic digestion (AD). In wet semi-continuous AD, the system was operated with recirculation of effluent. Two reactors with 300 mL of working volume were operated for 45 days in...

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## Purification of Biogas by CO<sub>2</sub> Reduction in Biogas Using Potassium Hydroxide Solution (KOH) in a Packed Tower

U N Pamiliani, E Purba

This research is about the absorption of carbon dioxide gas (CO<sub>2</sub>) in biogas processed from tapioca waste using a solution of potassium hydroxide (KOH) as absorbent. The purpose of this research was to determine the effect of gas flow rate and the flow rate of KOH solution to the absorption of CO<sub>2</sub>. The...

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# The Effect of *Bacillus Coagulans* as Feed Probiotics on Non-specific Immunity of Whiteleg Shrimp *Litopenaeus Vannamei*

S Supono<sup>1,2</sup> M Rahayu<sup>1</sup> M W Yusuf<sup>1</sup>

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## ABSTRACT

The study aimed to evaluate the effect of giving a different dosage of *Bacillus coagulans* to feed (feed probiotics) on nonspecific immunity of *Litopenaeus vannamei* (white shrimp). The parameters tested included water quality and shrimp immunity parameters, namely total hemocyte count (THC), differential hemocyte count (DHC) and phagocytosis activity (PA) and carried out for 20 days of culture. The method of administering *Bacillus coagulans* was by spraying it into the commercial feed. White shrimp used were the size of  $13.1 \pm 0.06$  grams in the amount of 10 shrimps per container. The size of the container was  $50\text{cm} \times 40\text{cm} \times 40\text{cm}$  totaling 12 pieces (4 treatments and 3 replications). The treatments consisted of K (control), a bacterial dose of  $10^4$  CFU ml<sup>-1</sup>, a bacterial dose of  $10^6$  CFU ml<sup>-1</sup>, and a bacterial dose of  $10^8$  CFU ml<sup>-1</sup>. The results showed that *Bacillus coagulans* as a probiotic feed was able to increase the nonspecific immunity of white shrimp. The treatment of *Bacillus coagulans* of  $10^8$  CFU ml<sup>-1</sup> produced the best nonspecific immunity in white shrimp.

**Keywords:** immunity, hemocyte, whiteleg shrimp, bacillus coagulans

## 1. INTRODUCTION

Whiteleg shrimp (*Litopenaeus vannamei*) is an introduction shrimp that is currently widely cultivated in Indonesia. The superiority of the white shrimp has led to higher production and demand for these shrimps [1]. The value of white shrimp exports in 2014 reached 197 thousand tons, an increase of 17.34 percent compared to the previous year [2]. Based on these data the value of white shrimp production in Indonesian fisheries has increased every year.

In line with the increased production of white shrimp, intensive cultivation systems characterized by stocking densities and high feeding have been carried out [3]. However, this intensive cultivation system can cause disease and the risk of decreased white shrimp production [4]. The disease is a major obstacle that needs attention besides feed. This causes a general attack of disease suddenly and can lead to shrimp death [5].

One effort in the prevention of disease is through enhancing the body's defense system against shrimp attack pathogens [6] by using immunostimulants [7] [8]. Shrimp body defense

against disease is not only done through feed with a balanced composition but can be accompanied by giving immunostimulants in the feed. Immunostimulants are directly related to cells that can activate the immune system in shrimp [9].

Immunostimulants are now widely used for disease control in aquaculture activities as an alternative to the use of drugs, chemicals, and antibiotics [10] [11]. Immunostimulant can be applied by injection, immersion, or oral methods [12]. Giving immunostimulants through the feed is one method of immunostimulant that is widely studied in shrimp culture.

Much evidence has shown that immunostimulants added to feed can increase fish and shrimp resistance to disease infections through increased non-specific immune responses [13] [14]. In shrimp, hemocyte is a very important factor in a non-specific cellular defense system. The ability of hemocytes in the phagocytic activity that can increase in the incidence of infection shows the body's cellular defense. Increased shrimp body endurance can be known from the increased phagocytic activity of hemocyte cells [15].



The bacterium that has the potential to be immunostimulants is *Bacillus coagulans* [16] *Bacillus coagulans* can produce lactic acid, is resistant to high temperatures, tolerates acidic environment, is antagonistic to pathogenic bacteria, and produces anti-disease compounds [17]. Under these conditions, it is necessary to research to evaluate the use of *B. coagulans* to enhance nonspecific immunity in whiteleg shrimp.

## 2. MATERIALS AND METHODS

### 2.1. Materials

The research was conducted at the Fisheries Laboratory, Department of Fisheries and Marine, Faculty of Agriculture, University of Lampung, Indonesia. Whiteleg shrimp used were sized  $13.1 \pm 0.06$  gr as many as 10 shrimps/container. Previously shrimp acclimatized for 5 days. Shrimp culture was carried out for 20 days by feeding 4 times a day at (08.00 am, 12:00 am, 4.00 pm, 8.00 pm).

### 2.2. Container Preparation

The container for culture was the aquarium, as many as 12 units measuring  $50 \text{ cm} \times 40 \text{ cm} \times 40 \text{ cm}$  and covered with black plastic to prevent stress on the shrimp. The aquariums were filled with seawater until it reaches the desired salinity (25 ppt) of 30 liters. Strong aeration is installed using a blower that is drained using a 0.5-inch pipe that has been perforated and placed on the bottom of the aquarium.

Bacterium *Bacillus coagulans* was isolated from shrimp ponds in Lampung Province. Bacteria were prepared by the re-culture of *Bacillus coagulans* on 70% seawater tilted TSA (Tryptone Soy Agar) to get a younger bacterial culture. Furthermore, the bacteria were cultured on 70% seawater TSB (Tryptone Soy Broth) so that it could be stored until it was used. Bacteria were cultured on the media until they reached the desired density ( $10^4 \text{ CFU ml}^{-1}$ ,  $10^6 \text{ CFU ml}^{-1}$ , and  $10^8 \text{ CFU ml}^{-1}$ ).

### 2.3. Feed Preparation

The feed used in this study is commercial feed. The process of preparation of the test feed involved mixing the diluted bacterial isolates with a dilution dose of  $10^4 \text{ CFU ml}^{-1}$ ,  $10^6 \text{ CFU ml}^{-1}$ ,  $10^8 \text{ CFU ml}^{-1}$  and then mixed into the feed by spray technique. After being mixed evenly, the feed is dried for 5 minutes after which the feed is put into a container and ready to be given to the test shrimp by feeding it by 2% of shrimp biomass every day.

## 2.4. Observed parameters

### 2.4.1. Differential hemocyte count (DHC)

Hemocytes that have been taken from test shrimp are dripped on glass and made a review. The samples are dried in the air and fixed with 100% methanol for 5 minutes. After that, it was dried in the air again and colored by soaking it in 10% giemsa solution for 10 minutes dried in the air, washed in running water for 30 seconds and allowed to dry. The preparations were observed using a light microscope with a magnification of 40 times and distinguished according to its type namely hyaline cells and granular cells [18].

### 2.4.2. Total hemocyte count (THC)

Fresh hemocyte (20  $\mu\text{L}$ ) was diluted with PBS (40  $\mu\text{L}$ ), then samples were diluted using a micropipette and placed on the surface of the hemocytometer, then observed under a microscope. Hemocyte seen on a microscope was then calculated the number of hemocytes (total hemocyte count / THC) [18].

### 2.4.3. Phagocytic activity (PA)

As much as 0.1 ml of hemocyte taken from the test shrimp was put into a microplate and then added 25  $\mu\text{l}$  of *Staphylococcus aureus* ( $10^6 \text{ CFU ml}^{-1}$ ) mixed evenly and incubated for 20 minutes. Hemocyte as much as 5  $\mu\text{l}$  was dropped on a glass object and made to be prepared and then dried. The preparations were fixed into 100% methanol for 5 minutes and stained with giemsa solution for 15 minutes. Phagocytic activity was measured based on the percentage of phagocytic cells that carry out phagocytosis [18].

### 2.4.4. Water quality

Water quality parameters observed during the study included temperature, pH, dissolved oxygen, and ammonia. Types of equipment used to control water quality were a thermometer, pH paper, DO meter and spectrophotometer. Media water quality was analyzed descriptively.

## 2.5. Data Analysis

Nonspecific immunity performance data were analyzed using Anova with a 95% confidence interval using the SPSS software. If there were significant differences in the results followed by the LSD test. While the water quality data were analyzed descriptively.

### 3. RESULTS

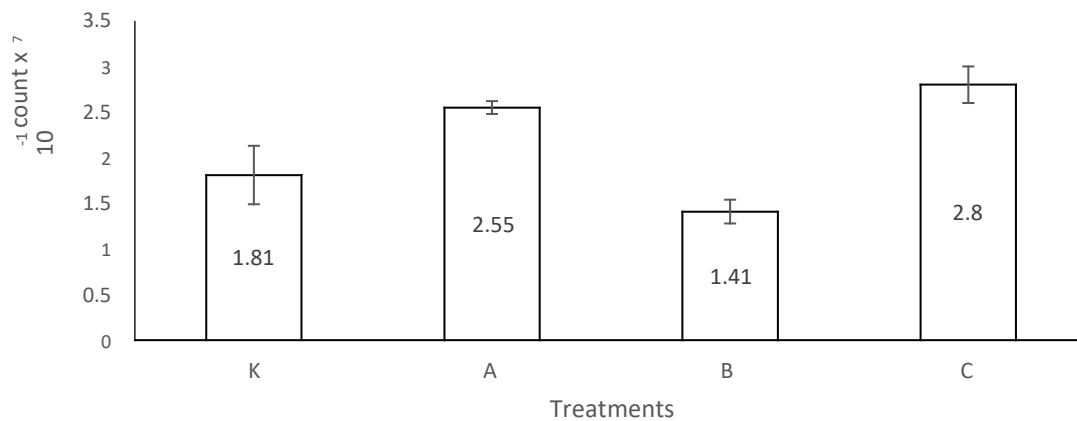
The results of measurements of water quality parameters such as temperature, dissolved oxygen (DO), pH and ammonia were still in the range of whiteleg shrimp culture to grow normally (Table 1)

**Table 1.** Water quality during the culture of whiteleg shrimp treatments

	K	A	B	C
Temperature (°c)	28	28	28	28
DO (ml <sup>-1</sup> )	5.63	5.29	5.50	5.27
pH	7	7	7	7
Ammonia (ml <sup>-1</sup> )	0.003	0.004	0.003	0.003

#### 3.1. Total Hemocyte Count (THC)

Data on total hemocyte count of white leg shrimp for 20 days of culture is displayed in Figure 1.



(K) Control; (A) Treatment of *B. coagulans* dose of 10<sup>4</sup> CFU ml<sup>-1</sup>; (B) Treatment of *B. coagulans* dose of 10<sup>6</sup> CFU ml<sup>-1</sup>; (C) Treatment of *B. coagulans* dose of 10<sup>8</sup> CFU ml<sup>-1</sup>

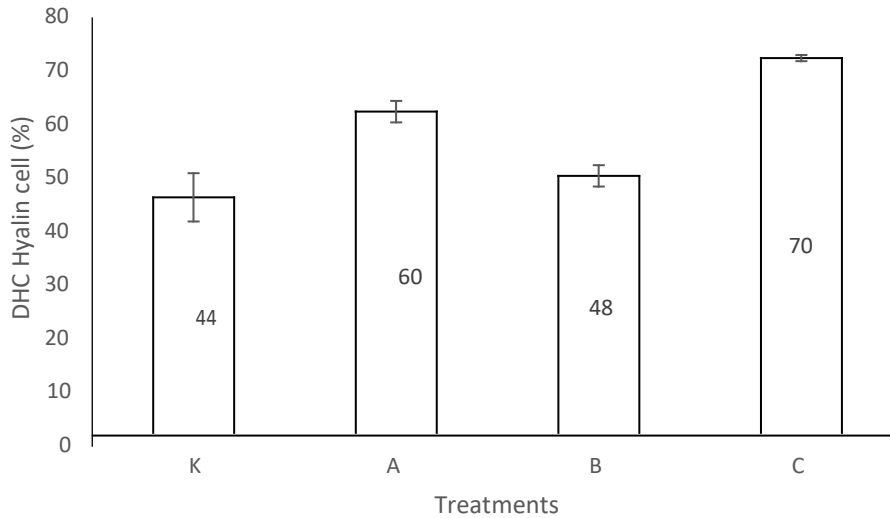
**Figure 1** Total hemocyte count of whiteleg shrimp

Based on data analysis (Anova), the addition of *B. coagulans* to feed had a significant effect on the total hemocyte count of whiteleg shrimp at a 95% confidence level. Treatment of *B. coagulans* dose of 10<sup>6</sup> CFU ml<sup>-1</sup> was different in the treatment of *B. coagulans* dose of 10<sup>4</sup> CFU ml<sup>-1</sup>, treatment of *B. coagulans* dose of 10<sup>8</sup> CFU ml<sup>-1</sup> and control. While the treatments of *B. coagulans* dose of 10<sup>8</sup> CFU ml<sup>-1</sup> and treatment of *B. coagulans* dose of 10<sup>4</sup> CFU ml<sup>-1</sup> were significantly different from the controls.

#### 3.2. Differential Hemocyte Count (DHC) Hyalin Cells

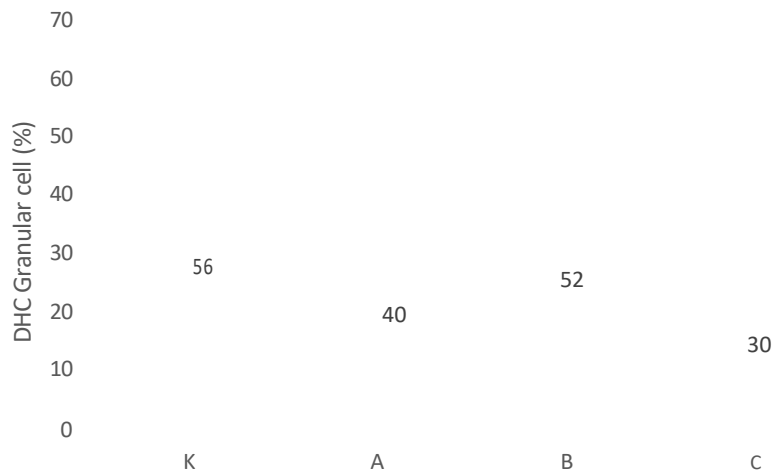
Data on differential hemocyte count (DHC) of hyaline cells of whiteleg shrimp for 20 days of

culture is displayed in Figure 2. Based on data analysis (Anova), the addition of *Bacillus coagulans* to the feed had a significant effect on the differential hemocyte count of hyaline cells of whiteleg shrimp at a 95% confidence level. Treatment of *B. coagulans* dose of 10<sup>8</sup> CFU ml<sup>-1</sup> was significantly different (p <0.05) for all treatments. Treatment of *B. coagulans* dose of 10<sup>4</sup> CFU ml<sup>-1</sup> was significantly different (p <0.05) on the treatment of *B. coagulans* dose of 10<sup>6</sup> CFU ml<sup>-1</sup> and control.



(K) Control; (A) Treatment of *B. coagulans* dose of  $10^4$  CFU ml<sup>-1</sup>; (B) Treatment of *B. coagulans* dose of  $10^6$  CFU ml<sup>-1</sup>; (C) Treatment of *B. coagulans* dose of  $10^8$  CFU ml<sup>-1</sup>

**Figure 2** Differential hemocyte count of hyaline cells of whiteleg shrimp



(K) Control; (A) Treatment of *B. coagulans* dose of  $10^4$  CFU ml<sup>-1</sup>; (B) Treatment of *B. coagulans* dose of  $10^6$  CFU ml<sup>-1</sup>; (C) Treatment of *B. coagulans* dose of  $10^8$  CFU ml<sup>-1</sup>

**Figure 3** Differential hemocyte count (DHC) of granular cells of whiteleg shrimp

While treatment of *B. coagulans* dose of  $10^6$  CFU ml<sup>-1</sup> was not significantly different from control.

### 3.3. Differential Hemocyte Count (DHC) Granular Cells

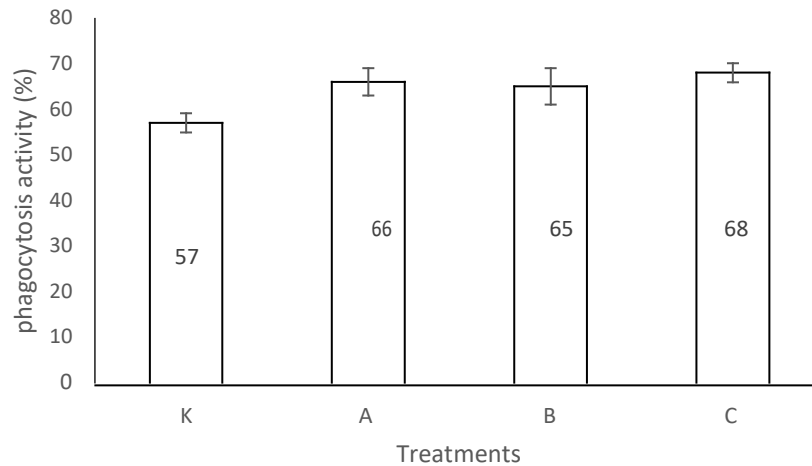
Data on differential hemocyte count (DHC) of granular cells of whiteleg shrimp for 20 days of culture is shown in Figure 3.

Based on data analysis (Anova), the addition of *Bacillus coagulans* to the feed had a significant effect on DHC granular cells. Treatment of *B. coagulans* dose of  $10^8$  CFU ml<sup>-1</sup> treatment was significantly different for all treatments.

Treatment of *B. coagulans* dose of  $10^4$  CFU ml<sup>-1</sup> was significantly different from the treatment of *B. coagulans* dose of  $10^6$  CFU ml<sup>-1</sup> and control.

### 3.4. Phagocytosis Activity

Data on the phagocytosis activity of whiteleg shrimp for 20 days of culture is shown in Figure 4. The results of data analysis (Anova) showed that the addition of *B. coagulans* to the feed affects the phagocytic activity of whiteleg shrimp. Treatment of *B. coagulans* dose of  $10^8$  CFU ml<sup>-1</sup> was significantly different in all treatments.



(K) Control; (A) Treatment of *B. coagulans* dose of  $10^4$  CFU ml<sup>-1</sup>; (B) Treatment of *B. coagulans* dose of  $10^6$  CFU ml<sup>-1</sup>; (C) Treatment of *B. coagulans* dose of  $10^8$  CFU ml<sup>-1</sup>

**Figure 4** Whiteleg shrimp phagocytosis activity

However, treatment of *B. coagulans* dose of  $10^4$  CFU ml<sup>-1</sup> and treatment of *B. coagulans* dose of  $10^6$  CFU ml<sup>-1</sup> did not show significantly different results on controls.

#### 4. DISCUSSION

Total hemocyte count (THC), differential hemocyte count (DHC) and phagocytosis activity (AP) are immune parameters related to hemocyte used to evaluate the immunostimulatory effect of probiotics on shrimp (19). Shrimp defense mechanism depends on the hemocyte process, the total value of hemocyte obtained in this study ranges from  $1.41$  to  $2.8 \times 10^7$  CFU mL<sup>-1</sup>. Total hemocyte in treatment of *B. coagulans* dose of  $10^8$  CFU ml<sup>-1</sup> was significantly different ( $p < 0.05$ ) with other treatments. Total hemocyte count obtained reached  $10^7$  CFU ml<sup>-1</sup> so that the test shrimp was still in normal condition and even tended to increase its immunity after being given the bacterium *B. coagulans*. The total hemocyte count of normal shrimp is  $10^4$  CFU ml<sup>-1</sup> [20].

The increase in total hemocyte in this study means increasing the chances of the formation of hemocyte cells, namely hyaline cells and granular cells. Both of these cells have their respective functions. When the function of each cell increases, the shrimp can defend themselves from incoming pathogen attacks [21].

The results of DHC parameters in hyaline cells ranged from 44% - 70% and granular cells in the range of 30% - 56%. Hyalin and granular DHC cells in the treatment of *B. coagulans* dose of  $10^8$  CFU ml<sup>-1</sup> were significantly different in all treatments. One of the parameters to improve the health status of shrimp with an increase in hyaline

cells and granular cells, but in granular cells, the number is less than in hyaline cells [22]. Hyaline cells have an important role in the activity of phagocytosis in crustacean immunity [23].

The activity of whiteleg shrimp phagocytosis can be seen based on the increase in hemocyte cells that carry out phagocytosis [24] [25]. The mechanism of action of immunostimulants in stimulating the body's immune system is by increasing the activity of phagocyte cells [26].

As the main response mechanism in the shrimp immune system, phagocytic activity parameters indicate how much the shrimp immunity reacts to pathogens that enter the body. An increase in phagocytic activity shows that probiotic bacteria can increase phagocyte cell activity so that when an attack occurs, hemocyte cells are ready to carry out the phagocytosis process [27]. Phagocytosis itself can occur when phagocytic cells are close to the antigen, or the antigen must be attached to the surface of phagocytic cells [28]. The results of the study of phagocytosis activity showed an increase in each treatment ranging from 57% to 68%. The highest value was significantly in the treatment of *Bacillus coagulans* dose of  $10^8$  CFU ml<sup>-1</sup>.

#### 5. CONCLUSION

The administration of *B. coagulans* with different density dosages in feed influences the nonspecific immunity of whiteleg shrimp. Bacterium *B. coagulans* as feed probiotic can increase total hemocyte count (THC), differential hemocyte count granular cells and phagocytosis activity. The dosage of *B. coagulans*  $10^8$  CFU ml<sup>-1</sup> on whiteleg shrimp feed showed the best results.

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