

# Basic Science for Sustainable Marine Development

## PROCEEDING

INTERNATIONAL SEMINAR 2015

Ambon, 3-4 June 2015

Organized by  
Faculty of Mathematics and Natural Sciences  
Pattimura University



# PROCEEDINGS

1<sup>st</sup> International Seminar of Basic Science, FMIPA Unpatti - Ambon  
June, 3<sup>rd</sup> – 4<sup>th</sup> 2015

**ISBN : 978-602-97522-2-9**

- Organizing Committee : PANITIA DIES NATALIES XVII  
Fakultas Matematika dan Ilmu Pengetahuan Alam  
Universitas Pattimura
- Advisory : Prof . Dr. Pieter Kakisina, M.Si
- Scientific Comitte : Prof. Dr. Th. Pentury, M.Si (Mathematic)  
Prof. Dr. Pieter Kakisina, M.Si (Biology)  
Dr. Yusthinus T. Male, M.Si (Chemistry)  
Dr. Catherina M. Bijang, M.Si (Chemistry)  
R. R. Lakollo, S.Si., M.Si (Physic)  
Grace Loupatty, S.Si., M.Si (Physic)  
M. W. Talakua, S.Pd., M.Si (Mathematic)
- Obligator : Dr. A. Netty Siahaya, M.Si  
Pieldry Nanlohy, S.Si., M.Si
- Editors : Dr. Ir. R. Hutahalung, M.Si  
Dr. La Eddy, M.Si  
Zeth Arthur Leleury, S.Si., M.Si  
Nelson Gaspersz, S.Si., M.Si  
Lady Diana Tetelepta, S.Si., M.Si  
Yunita Latupeirissa, S.Si., M.Si  
Sunarti, S.Si
- Cover Design : D. L. Rahakbauw, S.Si., M.Si  
Lexy Janzen Sinay, S.Si.M.Si

Mathematic and Natural Science Faculty  
Pattimura University  
Ir. M. Putuhena St.  
Kampus Poka-Ambon  
Pos Code 97233  
Email:fmipa\_unpatti@gmail.com

October 2015

© 2015 Mathematic and Natural Science Faculty, Pattimura University

All rights reserved

Republication of an article or portions thereof in original form or in translation, as well as other types of reuse require formal permission from publisher.

# PROCEEDINGS

1<sup>st</sup> International Seminar of Basic Science, FMIPA Unpatti - Ambon  
June, 3<sup>rd</sup> – 4<sup>th</sup> 2015

## **Welcoming Address by The Organizing Committee**

The honorable, the rector of Pattimura University

The honorable, the vice rector of academic affair, Pattimura University

The honorable, the vice rector of administration and financial affair, Pattimura University

The honorable, the vice rector of planning, cooperation and information affair, Pattimura University

The honorable, all the deans in Pattimura University

The honorable, the key note speakers and other guests.

We have to thank The Almighty God for the blessings that allow this International seminar can be held today. This is the first seminar about MIPA Science in which the Faculty of MIPA Pattimura University becomes the host. The seminar under the title Basic Science for Sustainable Marine Development will be carried out on 3 June 2015 at Rectorate Building, the second floor. There are 250 participants from lecturers, research institute, students, and also there are 34 papers will be presented.

This International seminar is supported by the amazing people who always give financial as well as moral supports. My special thanks refer to the rector of Pattimura University, Prof. Dr. Thomas Pentury, M.Si, and the Dean of MIPA Faculty, Prof. Dr. Pieter Kakissina, M. Si. I also would like to express my deepest gratitude to Dr. Kotaro Ichikawa, the director of CSEAS Kyoto University, Prof. Bohari M. Yamin, University of Kebangsaan Malaysia, Prof. Dr. Budi Nurani Ruchjana (Prisident of Indonesian Mathematical Society/Indo-MS), Dr. Ir. A. Syailatua, M.Sc (Director of LIPI Ambon), and Hendry Ishak Elim, PhD as the key note speakers. We expect that this international seminar can give valuable information and contribution especially in developing basic science for sustainable marine development in the future.

Last but not least, we realize that as human we have weaknesses in holding this seminar, but personally I believe that there are pearls behind this seminar. Thank you very much.

Chairman

Dr. Netty Siahaya, M.Si.

# PROCEEDINGS

1<sup>st</sup> International Seminar of Basic Science, FMIPA Unpatti - Ambon  
June, 3<sup>rd</sup> – 4<sup>th</sup> 2015

## **Opening Remarks By Dean of Mathematic and Natural Science Faculty**

I express my deepest gratitude to The Almighty God for every single blessing He provides us especially in the process of holding the seminar until publishing the proceeding of International Seminar in celebrating the 17<sup>th</sup> anniversary of MIPA Faculty, Pattimura University. The theme of the anniversary is under the title Basic Science for Sustainable Marine Development. The reason of choosing this theme is that Maluku is one of five areas in Techno Park Marine in Indonesia. Furthermore, it is expected that this development can be means where the process of innovation, it is the conversion of science and technology into economic value can be worthwhile for public welfare especially coastal communities.

Having the second big variety of biological resources in the world, Indonesia is rich of its marine flora and fauna. These potential resources can be treated as high value products that demand by international market. Basic science of MIPA plays important role in developing the management of sustainable marine biological resources.

The scientific articles in this proceeding are the results of research and they are analyzed scientifically. It is expected that this proceeding can be valuable information in terms of developing science and technology for public welfare, especially people in Maluku.

My special thanks refer to all researchers and reviewers for your brilliant ideas in completing and publishing this proceeding. I also would like to express my gratefulness to the dies committee-anniversary of MIPA Faculty for your creativity and hard working in finishing this proceeding, God Bless you all.

Dean of Mathematic and Natural Science Faculty

Prof. Dr. Pieter Kakisina, M.Si.

# PROCEEDINGS

1<sup>st</sup> International Seminar of Basic Science, FMIPA Unpatti - Ambon  
June, 3<sup>rd</sup> – 4<sup>th</sup> 2015

---

## Contents

	<i>Page</i>
<b>Cover</b> .....	i
<b>Editor page</b> .....	ii
<b>Welcoming Address by The Organizing Committee</b> .....	iii
<b>Opening Remarks by Dean of Mathematic and Natural Science Faculty</b> ....	iv
<b>Contents</b> .....	v–vii
<b>Papers</b>	
1. Studies on Habitat Use and Vocal Activities of Dugongs by Using Acoustical Analysis <i>Kotaro Ichikawa, Nobuaki Arai</i> .....	1–4
2. Complexation and Structural Studies of 5,5,7,12,12,14-hexamethyl-1,4,8,11-tetraazacyclotetradeca-7,14-dienium Bromide Complexes with Copper Salts <i>Bohari M. Yamin</i> .....	5–10
3. Spin Wave Excitation in YFeO <sub>3</sub> Crystal Investigated with Magnetic Component of Terahertz Pulse <i>Runze Zhou and Guohong Ma</i> .....	11–13
4. Development on Theoretical and Application of Space Time Autoregressive Modeling <i>Budi Nurani Ruchjana</i> .....	14–17
5. The Importance of Basic Science for Sustainable Marine Development in Indonesia <i>Augy Syahailatua</i> .....	18–20
6. Fabrication of Novel Fibers from Rejected Ocean Materials and Their Potential Applications <i>Hendry Izaac Elim</i> .....	21–27
7. Synthesis 3-benzo[1,3]dioxol-5-yl-propenal as a Precursor Asymmetric Curcumin Analogues from Kulit Lawang Oils <i>Immanuel Berly D. Kapelle, Tun Tedja Irawadi, Meika Syahbana Rusli, Djumali Mangunwidjaja, Zainal Alim Mas'ud</i> .....	28–34
8. Metathesis of Ethylolate <i>Nawwar Hanun A. Malek, Nor Wahidah Awang, Kitohiro Nomura, Bohari M. Yamin</i> .....	35–40
9. The Use of Fish as Carbon Sources for The Production of Riboflavin (Vitamin B2) Using <i>Eremothecium Gossypii</i> <i>Syarifuddin Idrus, Marni Kaimudin, Joice P. M. Kolanus</i> .....	41–49
10. The Effect of Sampling Scheme in The Survey of Deposition of Heavy Metals in Ambon Bay by Using Spons (Porifera) Biomonitoring <i>Netty Siahaya, Alfian Noor, Nunuk Suekamto, Nicole de Voogd</i> .....	50–54

# PROCEEDINGS

1<sup>st</sup> International Seminar of Basic Science, FMIPA Unpatti - Ambon  
June, 3<sup>rd</sup> – 4<sup>th</sup> 2015

---

11.	Synthesis and Modification of Ni-N-TiO <sub>2</sub> /Ti for Chemical Oxygen Demand Sensor with Visible Light Response Flow <i>Ruslan, Baharuddin Hamzah, Mohamad Mirzan, Musafira</i> .....	55–62
12.	$\alpha$ -Glucosidase inhibition activity of several compounds of Fatty Acids <i>Edward J. Dompeipen, Maria A. Leha</i> .....	63–69
13.	Chemical–Physics Composition Analysis of Pearl Seashells and Utilazation Possible as Import Nucleus Substitution <i>Voulda D. Loupatty</i> .....	70–74
14.	Thermal Analysis in Geothermal Prospect Suli-District Central Maluku <i>J.R. Kelibulin, N.H. Pattiasina, R.R. Lokolo</i> .....	75–85
15.	Characteristics Interpretation of Alteration Minerals of Waiyari Geothermal Manifestation Area, Central Maluku <i>Helda Andayani</i> .....	86–89
16.	Rainfall and Rainy Days Prediction in Ambon Island Using Vector Autoregression Model <i>Lexy Janzen Sinay, Salmon Notje Aulele</i> .....	90–98
17.	Applied of Backpropagation Algorithm to Analyzing and Forecasting of Currency Exchange Rate Rupiahs and Dollar <i>Dorteus Lodewyik Rahakbauw</i> .....	99–108
18.	Analysis Correspondence of Data Crime in Polres Pulau Ambon dan Pulau-Pulau Lease <i>Y. A. Lesnussa, J. Pentury</i> .....	109–115
19.	The Hypothetical Learning Trajectory on Place Value Concept in Realistic Mathematics Education Approach <i>Christi Matitaputty</i> .....	116–124
20.	Mortality of Coral Reef in the Coastal Waters of the Hila Village Leihitu District Central Maluku <i>Deli Wakano, Dece Elisabeth Sahertian</i> .....	125–128
21.	Histological of Haemocyte Infiltration During Pearl Sac Formation in <i>Pinctada maxima</i> oysters Implanted in The Intestine, Anus and Gonad <i>La Eddy, Ridwan Affandi, Nastiti Kusumorini, Yulvian Tsani, Wasmen Manalu</i> .....	129–134
22.	Effect Of Ethanol Leaf Extract Gambir Laut ( <i>Clerodendrum inerme</i> L. Gaertn) Malformations On Motion To External Equipment Fetal Development Mice ( <i>Mus musculus</i> ) <i>Chomsa Dintasari Umi Baszary, Maria Nindatu, Tony Marchel Lolonlun</i> ...	135–139
23.	Development of Integrated Poso Lake Tourism through Community Based <i>Tabita R. Matana, Gitit IP Wacana</i> .....	140–144
24.	Life Skills in Sector Marine Product Processing through Nonformal Education Approach In Maluku Province <i>Abednego</i> .....	145–148

# PROCEEDINGS

1<sup>st</sup> International Seminar of Basic Science, FMIPA Unpatti - Ambon  
June, 3<sup>rd</sup> – 4<sup>th</sup> 2015

---

25.	<i>Pistia stratiotes</i> and <i>Limnocharis flava</i> as Phytoremediation Heavy Metals Lead and Cadmium in The Arbes Ambon <i>Muhammad Rijal</i> .....	149–155
26.	Effect to used consentartion dose fertilizer Bokshi leaf of lamtoro to growth of <i>Solanum melongena</i> L <i>Cornelia Pary, Wa Atima, Hanisu</i> .....	156–160
27.	Analysis The Maturity Level of Plantain Fruit ( <i>Musa paradisiaca</i> ) by Using NIR Spectroscopy <i>Efraim Samson</i> .....	161–166
28.	Morphological Diversity of Numege Mother Trees and Seedlings in Lilibooi Village, Ambon Island <i>Helen Hetharie, Simon H.T. Raharjo, Kosmas Rahado, Meitty L. Hehanussa</i> .....	167–173
29.	Sustainability Analysis Management Coral Reef Ecosystem in The Water of The Bay Of Ambon <i>Pieter Th. Berhitsu, Sahala Hutabarat, Supriharyono, Djoko Suprpto</i> .....	174–185
30.	The Environmental Management Philosophy of Indigenous Peoples in Coastal Marine Area in Maluku <i>Reveny Vania Rugebregt</i> .....	186–195

# PROCEEDINGS

1<sup>st</sup> International Seminar of Basic Science, FMIPA Unpatti - Ambon  
June, 3<sup>rd</sup> – 4<sup>th</sup> 2015

---

## The Use of Fish as Carbon Sources for The Production of Riboflavin (Vitamin B2) Using *Eremothecium Gossypii*

Syarifuddin Idrus\*, Marni Kaimudin, Joice P. M. Kolanus

Institute of Research and Standardization Industry of Ambon  
Kebun Cengkeh Street - Ambon, Mollucas-Indonesia  
\*Email: syarif.idrus@gmail.com

### ABSTRACT

Riboflavin or vitamin B2 is a vitamin which used as a nutritional, therapeutic, and livestock feed supplements. In techno economy, the cost of riboflavin production by fermentation were cheaper, less waste generated, and lower the energy required compared to the chemical and fermentation-chemical processes. In addition, the fermentation process also uses substrate from renewable sources. This study aims to produce riboflavin using fish as a substrate. Fish that contain fatty acids and proteins are expected to be a source of carbon for the production of riboflavin by fermentation using *Eremothecium gossypii*. The fermentation process was observed at 0-120 hours to obtain an optimal time and substrate for the production of riboflavin. Riboflavin was tested using a spectrophotometer at a wavelength of 445 nm. The results showed that the optimal time for the production of riboflavin was 72 hours. Glucose as a carbon source, optimal at 10 g/L with a number of riboflavin obtained amounted to 40.5 mg/L. The use of fish as a source of carbon, optimal at 10 g/L with the amount of riboflavin was 24.8 mg/L. The use of a mixture of glucose and fish as carbon sources, optimal at 10 g/L with the amount of riboflavin was 51.2 mg/L.

Keywords: Riboflavin, vitamin B2, *Eremothecium gossypii*, fermentation

### INTRODUCTION

Riboflavin or more popularly known as vitamin B2, is used as a nutritional, therapeutic and also as a livestock feed supplement. Humans who lack this vitamin will experience hair loss, skin inflammation, and growth failure. This vitamin has also been successful as the treatment of migraine disease and malaria (Shrikant, 2006). Riboflavin is also required for the metabolism of tryptophan, an amino acid that is essential for growth in childhood. Riboflavin plays an important role in the transfer of electrons and it is the precursor of coenzyme flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), that is needed for the enzymatic oxidation-reduction reaction (Sybesma, et.al., 2004).

Every year, an estimated 3,000 tons of riboflavin are produced worldwide, 2500 tons are produced through fermentation (Choe, et al, 2005). In general, the production of riboflavin can be categorized into three methods, namely chemical synthesis, fermentation, and through biotransformation of glucose into D-ribose which is a combination of fermentation and chemical synthesis. Techno-economical environmental studies showed that riboflavin production through fermentation processes continues to increase due to lower production costs, less waste produced and lower energy requirement (Shrikant, 2006).

Several studies have shown that the two fungi from the classes ascomycetes, namely *Eremothecium ashbyii* and *Eremothecium (Ashbya) gossypii*, can produce more riboflavin



# PROCEEDINGS

1<sup>st</sup> International Seminar of Basic Science, FMIPA Unpatti - Ambon  
June, 3<sup>rd</sup> – 4<sup>th</sup> 2015

---

through the fermentation process than other microorganisms such as *Saccharomyces cerevisiae*, *Candida famata*, or *Bacillus subtilis* (Kato et al., 2012; Suzuki et al., 2012). *Eremothecium ashbyii* and *Eremothecium (Ashbya) gossypii* can produce riboflavin as much as 20 g/L by using molasses and glucose as the carbon source. Thus, the number is much larger when compared with *Bacillus subtilis* (0.1 g/L), *Candida flareri* (0.6 g/L) and *Candida guilliermondii* (0.2 g/L) (Alosta, 2007). More interestingly, a sewage Active Bleaching Earth (ABE), the results of bleaching CPO (crude palm oil), can be used as a carbon source for the production of riboflavin by fermentation using *Ashbya gossypii* without having to waste the ABE (Park, et al, 2006).

The use of soybean oil as carbon source was able to increase the production of riboflavin 1.6-fold compared with no use of it. Several other carbon sources used for the production of riboflavin namely vegetable oil, whey and peanut seed cake (Lim, et al, 2003). Fish that contain fatty acids is expected to be a good source of carbon for the production of riboflavin by fermentation, given *Eremothecium (Ashbya) gossypii* capable of cleaving lipid (fat) as a carbon source through the flow of fatty acids and store them as well as the formation of the riboflavin through the flow of glucose (Lim, et.al., 2001). This research was conducted for the production of riboflavin (vitamin B2) with a substrate of fish for consumption as a carbon source. Riboflavin fermentation was done by using *Eremothecium gossypii*.

## RESEARCH METHODS

### *Microorganism*

*Eremothecium gossypii* (Ashby et Nowell) Kurtzman (ATCC 10 895) was grown on potato dextrose agar (PDA) medium. 19.53 g of PDA were dissolved in 500 mL of sterile aquabidest to get a PDA medium. The pH was made to 6.8 before autoclave session. Then, the medium is poured into a 5 pieces of 4 mL glass tube respectively and the rest of the medium was poured in the sterilized reagent bottle from autoclave at a temperature of 121°C, 103 kPa for 30 minutes. *Eremothecium gossypii* grown on medium, then incubated for 5 days at 37 ° C and stored at 4°C for two months of use.

### *Composition of Medium Production*

Medium production was consisted of peptone 5.0 g/L; yeast extract 5.0 g/L; malt extract 5.0 g/L; potassium hydrogen phosphate (K<sub>2</sub>HPO<sub>4</sub>) 0.2 g/L; and magnesium sulphate (MgSO<sub>4</sub>.7H<sub>2</sub>O) 0.2 g/L (Ozbas and Kutsal, 1986). Glucose as the carbon source used at a concentration of 10.0 - 20.0 g/L. Simultaneously, fish meat will be added as many as -20.0 10.0 g/L in the production process.

### *Production of Riboflavin*

The determination of the growth curve was performed to observe the mid-log phase of *Eremothecium gossypii*. A total of 30 mL *Eremothecium gossypii* culture was inoculated into 270 ml medium in 1 L erlenmeyer. The carbon source of glucose, then incubated at 30 ° C in an incubator which rotated at 120 rpm. A total of 5 mL samples were analyzed every 24 hours for biomass monitoring.

Riboflavin production was using the same growth medium with added glucose and fish meat. For comparison, the riboflavin production uses also glucose only medium and then only use fish meat as well. Biomass will be analyzed after a constant interval at 24 hours using gravimetric method.

# PROCEEDINGS

1<sup>st</sup> International Seminar of Basic Science, FMIPA Unpatti - Ambon  
June, 3<sup>rd</sup> – 4<sup>th</sup> 2015

---

## **Analysis of Riboflavin**

To determine the concentration of riboflavin, 0.5 ml of the culture broth was mixed with 4.5 ml of distilled water and centrifuged at 1000 g for 10 minutes. Supernatant was separated and mixed with 0.8 ml of 1N NaOH and 5 ml of 50 mM phosphate buffer (pH 7.0) and then centrifuged at 9000 g for 5 minutes. Supernatant was separated, and tested using a spectrophotometer at a wavelength of 445 nm.

## **RESULTS AND DISCUSSION**

### **Growth curve of *Eremothecium gossypii***

*Eremothecium gossypii* growth was done with biomass as early as 0.06 g/L with a growth rate of a maximum of 0.03 mg/L. Calculation of the growth rate was based on a formula:

$$\mu = \frac{2.3 (\log X - \log X_0)}{t} \quad (1)$$

With  $\mu$  is the growth rate, X is the biomass at a given time,  $X_0$  is the initial biomass, and t is the determination time of the biomass.

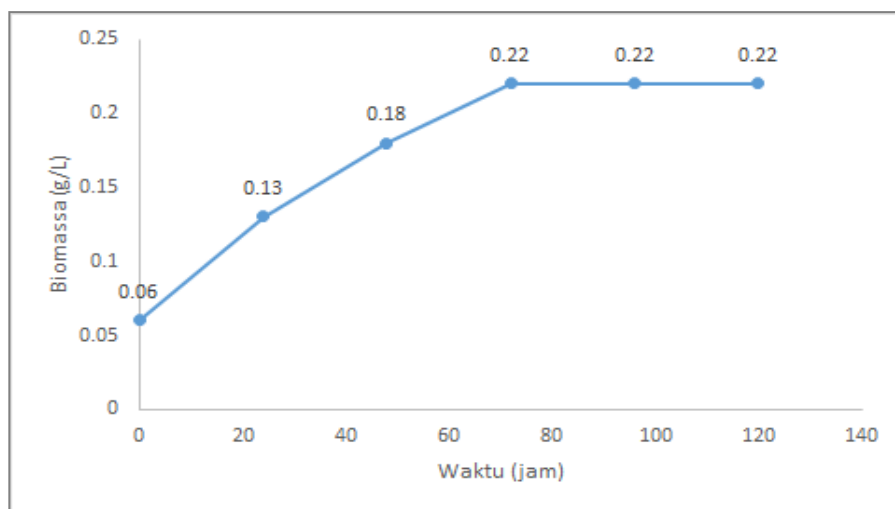


Figure 1. Graph of *Eremothecium gossypii* growth for 120 hours

Growth chart (Figure 1) shows that the lag or starters phase are not on the curve, as the chart patterns of growth in general. The absence of lag phase is due to the occurrence of *Eremothecium gossypii* adaptation with the medium, so that it immediately follow a process of growing, given the utilized medium is specific for growth of *Eremothecium gossypii*. Stationary phase are in the 72 hours up to 120 hours. This time frame is used as a reference for the production of riboflavin in the range of 3-5 days. From the data it is known that the growth charts mid-log phase between the initial phase of growth until the end of the log phase is 36 hours. This time is used as a reference for manufacturing the inoculum that is used for the production of riboflavin. The best growing conditions is in the mid growth duration, because

# PROCEEDINGS

1<sup>st</sup> International Seminar of Basic Science, FMIPA Unpatti - Ambon  
June, 3<sup>rd</sup> – 4<sup>th</sup> 2015

---

the enzymes that play a role in growth has been producing primary metabolites that are needed to grow the microbes.

## **The standard curve of Riboflavin**

Riboflavin analysis was performed with spectrophotometer at a wavelength of 445 nm. the wavelength was obtained from the standard riboflavin at a concentration of 50 mg/L. Standard curve (Figure 2) is made to measure the concentration of fermented riboflavin, obtained from the linearity calculation with  $R^2$  value of 0.9989 which indicates a very high level of curve accuracy. Riboflavin concentration value calculation was done using the formula:

$$y = mx + c \tag{2}$$

With  $y$  as absorbance of riboflavin,  $m$  is the coefficient of riboflavin concentration of 0.0394,  $x$  is the concentration of riboflavin and  $c$  is a constant with a value of 0.0177.

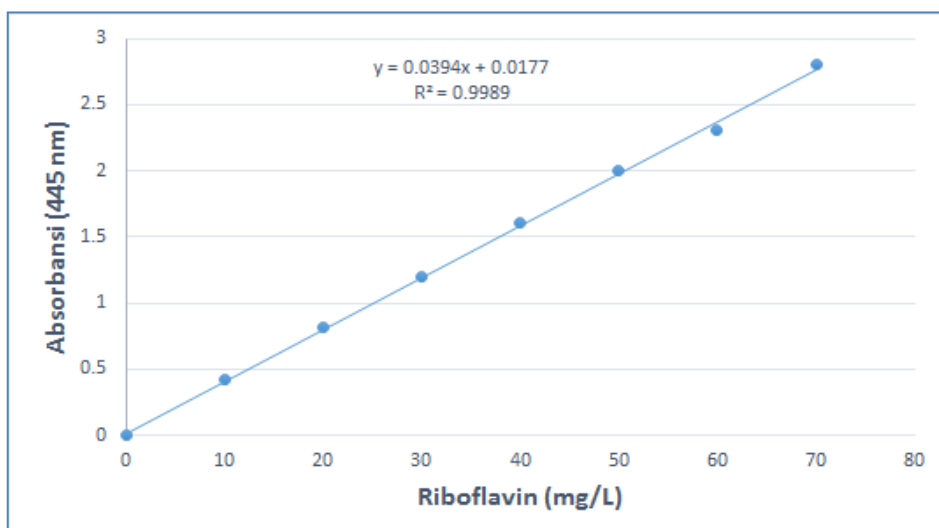


Figure 2. Standard curves of riboflavin in 0.02 M acetic acid with  $R^2=0.9989$  which will be used to calculate the concentration of fermented riboflavin.

## **Production of Riboflavin Using Glucose**

Riboflavin production with carbon sources of glucose can be seen in Figure 3. At the beginning of the fermentation there is just a little bit concentration of riboflavin, this can be due to the utilized inoculum was in the mid-log phase so the *Eremothecium gossypii* has produced secondary metabolites.

# PROCEEDINGS

1<sup>st</sup> International Seminar of Basic Science, FMIPA Unpatti - Ambon  
June, 3<sup>rd</sup> – 4<sup>th</sup> 2015

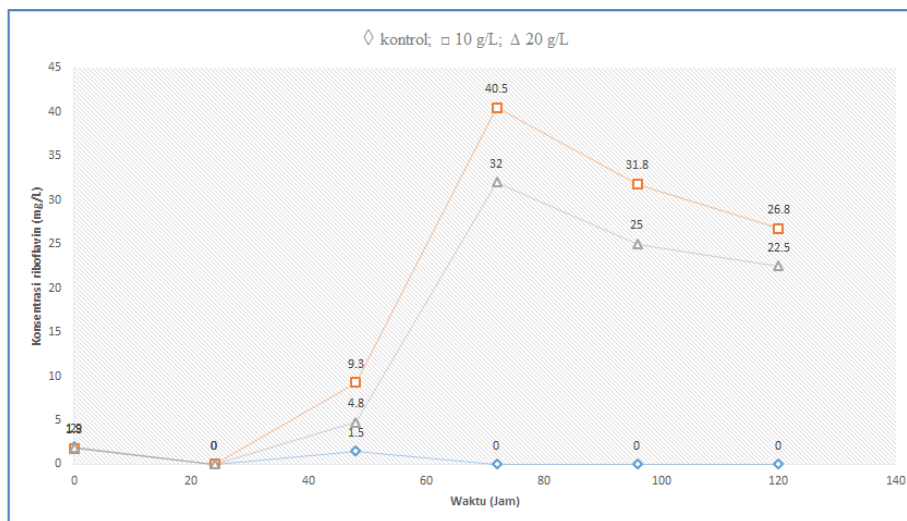


Figure 3. Production of riboflavin by *Eremothecium gossypii* using a carbon source of glucose with different concentrations.  $\diamond$  without glucose control;  $\square$  glucose 10 g/L;  $\Delta$  glucose 20 g/L.

At 24 hours fermentation time, the riboflavin was not found. It is because riboflavin was since 0 hours reused by *Eremothecium gossypii* to grow. At 48 hours fermentation time, it began to find little bit of riboflavin concentration. The optimum time of production of riboflavin is found at 72 hours which has the highest concentration of 40.5 mg/L with the use of 10 g/L glucose. Thus, the use of 20 g/L glucose only reached 32 mg/L, and glucose control was seen without visible signs of riboflavin. The high production of riboflavin at 10 g/L of 20 g/L glucose is too excessive so as the time to achieve stationary conditions for producing secondary metabolites will become longer. At the controls without glucose, riboflavin production will be re-used to be a source of carbon when riboflavin is not found.

At 96 hours and 120 hours fermentation time, it appears that riboflavin production begins to decline and this is because riboflavin was produced from reused by *Eremothecium gossypii* as an additional source of carbon as the glucose fermentation depleted over time.

## **Production of Riboflavin Using Fish**

Riboflavin production with carbon source of fish at the start of fermentation to 24 hours appears that there was a small amount of riboflavin and none at all (Figure 4). At 48 hours riboflavin production control looks no different from the controls on the production of riboflavin with glucose as the carbon source. Visible difference to the number of fish substrate was between 10 g/L and 20 g/L. Thus, the production of riboflavin is much smaller due the use of glucose and this is because the process of adaptation to break the fish into a carbon source. The process of decomposition of fish can occur because *Eremothecium gossypii* has protease and lipase enzymes to degrade proteins and fats.

# PROCEEDINGS

1<sup>st</sup> International Seminar of Basic Science, FMIPA Unpatti - Ambon  
June, 3<sup>rd</sup> – 4<sup>th</sup> 2015

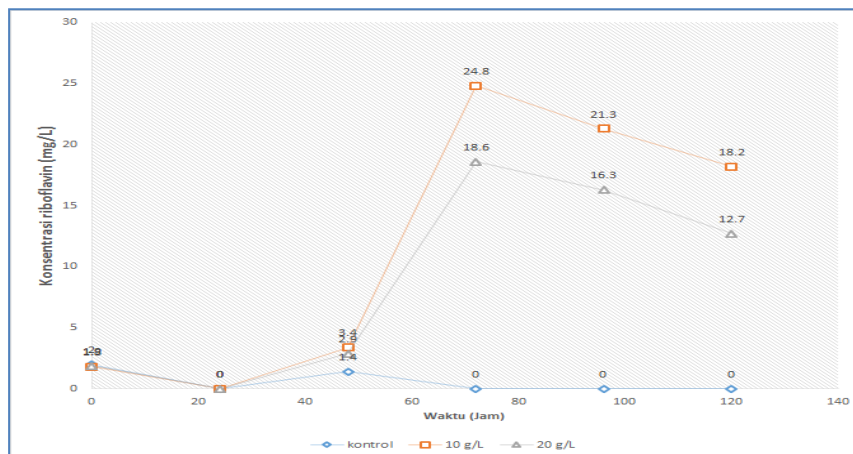


Figure 4. Production of riboflavin by *Eremothecium gossypii* using carbon sources of fish with different concentrations.  $\diamond$  control without fish;  $\square$  fish 10 g/L;  $\Delta$  fish 20 g/L

The optimum fermentation time is at 72 hours with a greater amount of riboflavin at 10 g/L of fish amounted to 24.8 mg/L, whereas at 20 g/L of fish reached 18.6 mg/L. At 96 hours and 120 hours fermentation time, it appears that the production of riboflavin becomes less and this is because of riboflavin production by *Eremothecium gossypii* was reused as an additional source of carbon considering carbon source of fish began to diminish over time of fermentation.

## Production of Riboflavin Using Glucose and Fish

The use of carbon sources of glucose and fish together turned out to produce riboflavin more than the utilization of glucose and fish separately. As shown in Figure 5, the optimum time is at 72 hours fermentation time with the amount of riboflavin produced amounted to 51.2 mg/L for the number of fish were added by 10 g/L. This result is greater than the result of the addition of the fish at 5 g/L, 15 g/L and 20 g/L. This is due to parse the fish as a source of carbon necessary for protease and lipase enzyme which of course would be optimal at 10 g/L of fish.

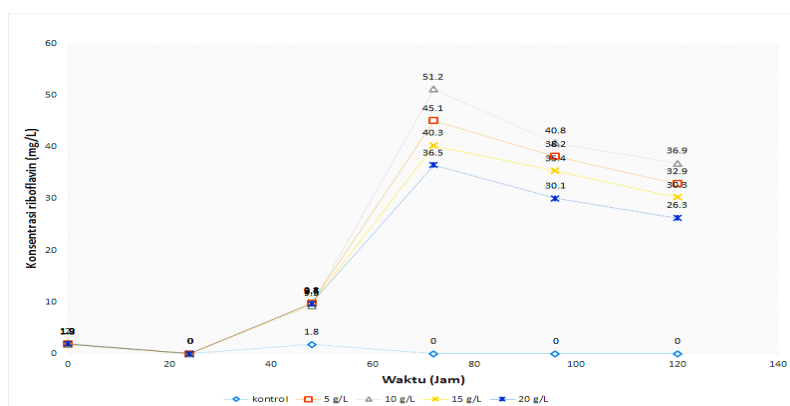


Figure 5. Production of riboflavin by *Eremothecium gossypii* using carbon sources glucose 10 g/L and fish with different concentrations.  $\diamond$  control;  $\square$  fish 5 g/L;  $\Delta$  fish 10 g/L;  $\times$  fish 15 g/L;  $*$  fish 20g/L

# PROCEEDINGS

1<sup>st</sup> International Seminar of Basic Science, FMIPA Unpatti - Ambon  
 June, 3<sup>rd</sup> – 4<sup>th</sup> 2015

At 96 hours and 120 hours fermentation time it appears that riboflavin production begins to decline and this is because riboflavin is produced from by *Eremothecium gossypii* as an additional source of carbon considering carbon sources of glucose and fermented fish depleted over time.

When viewed from the pathway by *Eremothecium gossypii* riboflavin formation, there are two lines of riboflavin formation (Figure 6), the first track of the pathways of glucose to be converted into 3-phosphoglycerate (3PG) and then through a pathway in mitochondrial pyruvate is converted into guanine triphosphat (GTP) which is a starter of riboflavin formation. The second path is from the path of triglycerides (fats) by a lipase that will be converted into fatty acids, through the cytoplasm of the fat will be taken to peroxisome converted into malate and then becomes guanine triphosphat.

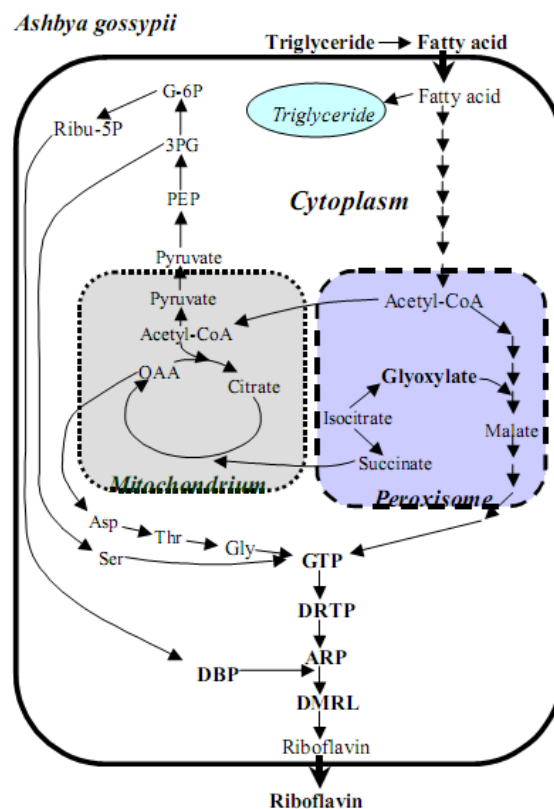


Figure 5. The model of metabolism riboflavin production using *Ashbya gossypii*. Abbreviations: G-6P, Glucose-6-phosphate; 3PG, 3-phosphoglycerate; PEP, Phosphoenolpyruvate; Ribul-5P, Ribulose-5-phosphate; OAA, Oxaloacetate; Asp, Aspartate; Thr, Threonine; Gly, Glycine; Ser, Serine; GTP, Guanosine triphosphate; GMP, Guanosine monophosphate; XMP, Xanthine monophosphate; IMP, Inosine monophosphate; DRTP, 2, 5-diamino-6-ribosylamino-4 (3H)-pyrimidinedione 5-phosphate; ARP, 5-amino-6-ribitylamino-2, 4 (1H, 3H)-pyrimidine; DBP, L-3, 4-dihydroxy-2-butanone-4-phosphate; DMRL, 6, 7-dimethyl-8-ribityllumazine (Lim, et al, 2001)

# PROCEEDINGS

1<sup>st</sup> International Seminar of Basic Science, FMIPA Unpatti - Ambon  
June, 3<sup>rd</sup> – 4<sup>th</sup> 2015

---

These two pathway of riboflavin production can be improved by mixing the glucose and fish substrates, so it appears that the optimum time of 72 hours of use of the glucose and fish substrate will produce riboflavin (51.2 mg/L) greater than the optimum time of 72 hours with the substrate glucose ( 40.5 mg/L) and fish (24.8 mg/L).

## CONCLUSIONS AND SUGGESTIONS

### Conclusion

The use of fish substrates as a carbon source for the production of riboflavin can be done through a fermentation process using *Eremothecium gossypii*. Optimal time needed for the production of riboflavin is at 72 hours. The use of glucose as a source of carbon substrates was optimally at 10 g/L with a number of riboflavin obtained at 40.5 mg/L. The use of fish as the optimal carbon source at 10 g/L with the amount of riboflavin 24.8 mg/L. The use of a mixture of glucose and fish substrates as carbon source optimally at 10 g/L with the amount of riboflavin produced amounted to 51.2 mg/L.

### Suggestions

Riboflavin production is necessary to be scaled for greater capacity to see the time and the optimum substrate to approach industrial conditions. To increase the production of riboflavin, it needs to conduct *Eremothecium gossypii* mutations to optimize and stabilize enzymes that play a role in the production of riboflavin.

## ACKNOWLEDGMENTS

This research is support by Baristand Industri Ambon. The authors are grateful to Dr. rer. nat. Arli Aditya Parikesit, Departement of Cheminty, University of Indonesia, for his critical comments and excellent assistance in proof-reading the manuscript.

## REFERENCES

- Alosta H.A. (2007). *Riboflavin Production by Encapsulat Candida Flareri*. Oklahoma State University. Kato T., Park E.Y., (2012). Riboflavin production by *Ashbya gossypii*. *Biotechnol Lett.* 34:611–618.
- Bacher A., Eberhardt S., Fischer M., Kis K., Richter G., (2000). Biosynthesis of Vitamin B2 (Riboflavin). *Annu. Rev. Nutr.* 20:153–67.
- Choe E., Rongmin H., David B. M., (2005). Chemical Reactions and Stability of Riboflavin in Foods. *Journal of Food Science*—Vol. 70, Nr. 1.
- EFSA Panel, (2010). Riboflavin related health claims. *EFSA Journal.* 8(10):1814, 28 pp.
- Guenther C., Saling P., (2006). Sustainable production of vitamin B2. *Feed Mix.* 14(2). 22-24.
- Kato T., Park E.Y., (2012). Riboflavin production by *Ashbya gossypii*. *Biotechnol Lett.* 34:611–618.
- Lim S.H., Choi J.S., Park E.Y., (2001). Microbial Production of Riboflavin Using Riboflavin Overproducers, *Ashbya gossypii*, *Bacillus subtilis*, and *Candida famate*: *An Overview.* *Biotechnol. Bioprocess Eng.*, 6: 75-88.
- Park E.Y., Kato A., Ming H., (2006). Utilization of waste activated bleaching earth containing palm oil in riboflavin production by *Ashbya gossypii*. *Journal of the American Oil Chemists' Society.* 57-62.
- Saarela U., Leivika K., Juuso E., (2003). Modelling of fed-batch fermentation process. Report A No. 21, Control Engineering Laboratory, University Oulu, Finland. ISBN 951-42-7083-5.

## PROCEEDINGS

1<sup>st</sup> International Seminar of Basic Science, FMIPA Unpatti - Ambon  
June, 3<sup>rd</sup> – 4<sup>th</sup> 2015

---

- Shrikant A. Survase, Ishwar B. Bajaj and Rekha S. Singhal (2006). Biotechnological Production of Vitamins. *Food Technol. Biotechnol.* 44 (3) 381–396.
- Suzuki G.T., Fleuri L., Macedo G.A., (2012). Influence of Nitrogen and Carbon Sources on Riboflavin Production by Wild Strain of *Candida* sp. *Food Bioprocess Technol.* 5:466–473.
- Sybesma W., Burgess C., Starrenburg M., Sinderen van D., Hugenholtz J., (2004). Multivitamin Production in *Lactococcus Lactis* Using Metabolic Engineering. *Metabolic Engineering* 6:109–115.
- Torne Research, (2008). Riboflavin. *Alternative Medicine Review.* 13(4): 334-340.