2nd KRIPIK-SciFiMaS-2018
(*Scientific Communication in Fisheries and Marine Sciences*)
FISHERIES AND MARINE SCIENCE FACULTY AND
CENTER FOR MARITIME BIOSCIENCE STUDIES
JENDERAL SOEDIRMAN UNIVERSITY, PURWOKERTO

ACCEPTANCE LETTER

12 April 2018

Dear Karseno, PhD
Jenderal Soedirman University, Indonesia

The 2nd Scientific Communication in Fisheries and Marine Science (SCiFiMaS 2018) committee inform you that the abstract entitled:

EFFECT OF MEDIUM AND LIGHT QUALITY ON PINK PIGMENT PRODUCTION OF CYANOBACTERIA *Oscillatoria* sp. BTCC/A0004

has been accepted for Oral Presentation at SCiFiMaS 2018, which will be held on 7-9 May, 2018 at Java Heritage Hotel, Purwokerto - Indonesia. The abstract will be appeared in the book of abstracts and be available for all participants at the conference. We would like to thank for your participation in the SCiFiMaS 2018 and look forward to seeing you in Purwokerto - Indonesia.

Best Regards,

Dr. Norman Arie Prayogo
Chairman of SCiFiMaS Committee

Meeting Schedule of SciFimaS, May 7-9th, 2018
Java Heritage Hotel, Purwokerto, Indonesia

Time	Monday, May 7th 2018	Room
18.30-21.30	Registration	Lobby
18.30-21:30	Welcome Dinner for all Participants	Ambalika Restaurant
Time	**Tuesday, May 8th 2018**	**Room**
07.00-17.00	Registration	Hastinapura CC
08:00-08:30	Traditional Dance (Banyumas Dance) National Anthem "Indonesia Raya" Prayer 1. Opening Remark by Chairman, Dr. Norman Arie Prayogo 2. Welcoming Remark by the Rector of Jenderal Soedirman University 3. Mou Signing Ceremony by the Rector of Jenderal Soedirman University and Dr. (Hc) Susi Pudjiastuti, Minister of Marine Affairs and Fisheries, Republic of Indonesia 4. Photo session National Song “Bagimu Negeri”	Hastinapura CC
08:30-09:45	***Keynote Speaker:*** Dr. (Hc) Susi Pudjiastuti, Minister of Marine Affairs and Fisheries, Republic of Indonesia	Hastinapura CC
09:45-09:50	National Song” Indonesia Subur”	Hastinapura CC
09:50-10:00	Coffee Break	Hastinapura CC
10:00 -10:45 10:45 – 11:15	**Guest Speakers** 1. Prof Ocky Karna Radjasa, Ph.D The Director of Research and Community Empowerment, Ministry of Research, Technology and Higher Education the Republic of Indonesia 2. M. Zulficar Mochtar, M.Sc Head of Agency for Research and Human Resources,	Hastinapura CC

11:15 – 11:45	Ministry of Marine Affairs and Fisheries, Republic of Indonesia 3. Mr.Wahyono The Pioneer of Mangrove Conservation in Segara Anakan Lagoon	
11:45 – 12:45	**Lunch**	Ambalika Restaurant
12:45 – 13:00	Traditional Dance (Saman Dance)	Hastinapura CC
13:00 – 13:20 13:20 – 13:40 13:40 – 14:00 14:00 – 14:20 14:20 – 14:40 14:40 – 15:00 	**Expert Speakers I :** 1.Prof. Xuelei Zhang (First Institute of Oceanography, China) 2.Prof. Pierre Doumenq (Aix Marseille University, France) 3.Associate Prof Agung Dhamar Syakti (UMRAH, Indonesia) Discussion **Expert Speakers II :** 1. Associate Prof. Ryutaro Akiyama (Nara Institute of Science and Technology,Japan) 2. Prof. Il So Moon (Dongguk University, College of Medicine, South Korea) 3. Associate Prof. Purnama Sukardi (Jenderal Soedirman University, Indonesia) Discussion	Hastinapura CC
15:00 – 15:15	**Coffe break**	Hastinapura CC
15:15 – 15:45 15:45 – 16:15 16:15 – 16:45 	**Expert Speakers III :** 1. Dr. Nils Moosdorf (Leibniz Centre for Tropical Marine Research/ZMT, Germany) 2. Associate Prof. Le Cong Tru (Nong Lam University, Vietnam) 3. Prof. Wei Dong (National Marine Enviromental Forecasting Center, State Oceanic Administration, C Discussion	Hastinapura CC
16:45 – 17:15	**Next Step For Publication (Only for Oral Presenter) by Dr. Norman Arie P,M,Si**	Hastinapura CC
19:00 – 21:00	**Gala Dinner for Guest & Expert Speakers only**	Ambalika Restaurant
Time	**Wednesday, May 9th, 2018**	**Room**
7:00-8:00	Registration	Arjuna Ballroom
8:00 – 10:00	**Parallel I,II, III, IV, V & VI (See detail parallel section)**	Arjuna Ballroom
10:00 – 10:10	**Coffee break**	Arjuna Ballroom
10:10 – 12:20	**Parallel I,II, III, IV, V & VI (See detail parallel section)**	Arjuna Ballroom

12:30 – 13:00	**Lunch**	Ambalika Restaurant
13:00 – 14:00	**Closing Ceremony** Announcement of best presenter award Closing remark by Dean of Fisheries and Marine Science Faculty, Jenderal Soedirman University	Arjuna Ballroom
14:30 –17:00	**Sightseeing around Purwokerto (Jenderal Soedirman University and Baturadden area)**	

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Effect of medium and light quality on pink pigment production of cyanobacteria *Oscillatoria* sp. BTCC/A0004

Karseno[1*], *Kazuo* Harada[2], and *Kazumasa* Hirata[2]

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Kampus Karangwangkal, Purwokerto 53123 Indonesia
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Abstract. Cyanobacteria are well known as promising source of valuable chemicals for human usage. Especially, cyanobacteria in tropical area are very wide in diversity and they are potent producers of unique metabolites which exhibit interesting bioactivities. *Oscilatoria* sp. BTCC/A0004 produce pink pigments extracellularly (OsPP). The effects of various environmental factors on the production of cyanobacteria metabolites were well documented. In this research, the effect of medium and light quality on cell growth and OsPP production were investigated. In case, three different culture media, named No 18, C, and modified C media, in which nutrient compositions are different, and light quality (white, blue, green, pink) were tested. The highest cell growth and OsPP production were obtained in modified C medium. The nitrogen concentration in modified C medium is higher (5 g/L) than in No 18 medium (1.5 g/L) or C medium (1 g/L). In addition, cell growth and OsPP production were significantly stimulated by pink light radiation.

1 Introduction

Cyanobacteria are well known as promising source of valuable chemicals for human usage. They have been identified as one of the most attractive group of organisms for novel bioactive natural products [1]. Especially, cyanobacteria in tropical area are very wide in diversity and they are potent producers of unique metabolites which exhibit interesting bioactivities. However, most of them remain unexplored. In this study, we found tropical freshwater cyanobacterial strains, *Oscillatoria* sp.

[1]*Corresponding author*: karseno@unsoed.ac.id

BTCC/A0004 produce pink pigments extracellularly (OsPP) and it was identified as phycoerytrin like pigment [2].

In previous study, OsPP exhibited physical properties similar to phycoerytrin (PE). PE has gained tremendous interest due to excellent spectroscopic properties. It has been extensively investigated and exploited commercially for usage such as for fluorescent dyes in bio-assay [3, 4]. In this regards, OsPP might be substituted chemicals of PE. In addition, OsPP has potent growth inhibitory activity against green algae and cancer cells with function as photosensitizer. These results indicated that OsPP is promising substance for application in various fields.

In many papers, the effects of nutrient, light, pH, temperature and co-cultivation with competitive organisms on the synthesis of cyanobacterial metabolites were well documented [5-7]. It was expected that those factors might stimulate the production of OsPP. Therefore, in this research the effects of nutrient and light quality on stimulate cell growth and OsPP production were investigated.

2 Materials and methods

2.1. Algal cultivation condition

Oscillatoria sp. was sub cultured in test tubes (3 cm i.d. x 20 cm) containing 50 ml of modified C medium at pH 7.5 with the following composition: (per liter) 5 g KNO_3, 0.1 g KH_2PO_4, 0.05 g $MgSO_4.7H_2O$, 0.005 g $FeCl_2$, 2.86 mg H_3BO_3, 1.81 mg $MnCl_2.4H_2O$, 0.22 mg $ZnSO_4.7H_2O$, 0.018 mg $(NH_4)_6Mo_7O_{24}.4H_2O$, and 0.075 mg $CuSO_4.5H_2O$. The cells were cultivated with aeration (10 ml min^{-1}, 1 % CO2) at 25°C under 50 µmol photons m^{-2} s^{-1} of continuous illumination using white fluorescence lamps. For measurement of cell growth and pigment production, 4-day-old seed cultures of *Oscillatoria* sp. was transferred to fresh media and adjusted to 0.1 of OD_{680}. Incubation conditions was the same as for seed cultures except for light intensity was 100 µmol photons m^{-2} s^{-1}.

2.2. Effect of different media

Three different culture media named C, modified C and No 18 media, in which nutrient compositions are different were tested (Table 1). The cultivation methods were same to basal cultivation condition.

Table 1. Chemical composition of three culture media (L^{-1})

Components	No 18	C	Modified C
NaCl (mg)	70	-	-
$MgSO_4$ $7H_2O$ (mg)	380	40	50
$CaCl_2$ H_2O (mg)	106	-	-
$Fe_2(SO_4)_3$ nH_2O (mg)	10	-	-
Na_2EDTA $2H_2O$ (mg)	27	1000	-
K_2HPO_4 (mg)	600	-	100

H_3BO_3 (mg)	3	-	2.86
$MnSO_4$ $4H_2O$ (mg)	2	-	-
Na_2MoO_4 $2H_2O$ (mg)	8	2.5	-
$ZnSO_4$ $7H_2O$ (mg)	0.3	22	0.22
$CuSO_4$ $5H_2O$ (mg)	0.08	-	0.075
$CoCl_2$ $6H_2O$ (mg)	0.03	4	-
Titriplex III (g)	0.07	-	-
$Ca(NO_3)$ $4H_2O$ (mg)	-	150	-
KNO_3 (g)	-	0.1	5
$FeCl_3$ (mg)	-	196	-
$MnCl_2$ $4H_2O$ (mg)	-	36	1.81
Tris (hydroxymethyl) aminomethane (mg)	-	500	-
NaH_2PO_4 $2H_2O$ (mg)	-	50	-
Vitamin B_{12} (µg)	-	0.1	-
Biotin (µg)	-	0.1	-

2.3. Effect of light quality

To investigate the effects of light quality on cell growth and pink pigment production, the cells were cultivated in 100 ml of modified C medium in Petri dishes (6 and 9 cm in depth and diameter, respectively). The dish was placed in a transparent cultivation box and illuminated continuously with 30 µmol photons m^{-2} s^{-1} fluorescence light quality and aeration with 1 % CO_2 in air, 25°C. White, blue and green light were supplied with three 10 W fluorescence lamps (National, Japan). The pink light was supplied with three 10 W fluorescence lamps (NEC, Japan).

3 Results

3. 1. Effect of different culture media

The effects of different media on cell growth and OsPP production were presented in Figure 1. The highest cell growth and OsPP production were obtained in modified C medium. Modified C medium contains more nitrogen (KNO_3) than two other media evaluated (Table 1). It is well known that nitrogen is an essential element required for the growth and synthesis of various metabolites [5]. This might be one of the reasons why cell

growth and OsPP production in modified C medium were higher than those in the other media.

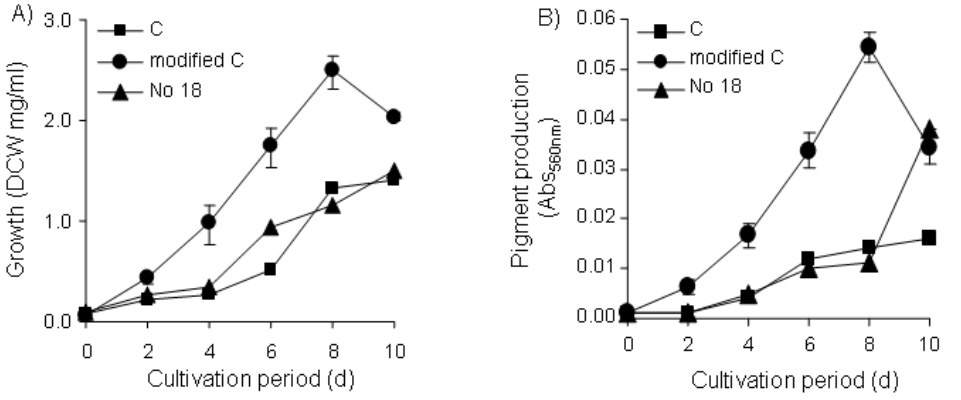

Fig. 1 Effect of different media on growth (A) and pigment production (B) of *Oscillatoria* sp**.** The cell was cultivated in various media in the light (100 µmol photons m^{-2} s^{-1}) and aeration of 1 % CO_2 in air at 25°C. DCW is dry cell weight. Values are the mean ± SDs of three independent experiments.

3. 2. Effects of light quality

Light is the primary energy source and play an important role in photosynthetic organisms including cyanobacteria. There are several reports that light quality as well as light intensity showed significant effects on growth and metabolism in cyanobacteria [8-11]. The effects of light quality on cell growth and OsPP production are shown in Figure 2. The cell growth and OsPP production were stimulated significantly by pink light irradiation.

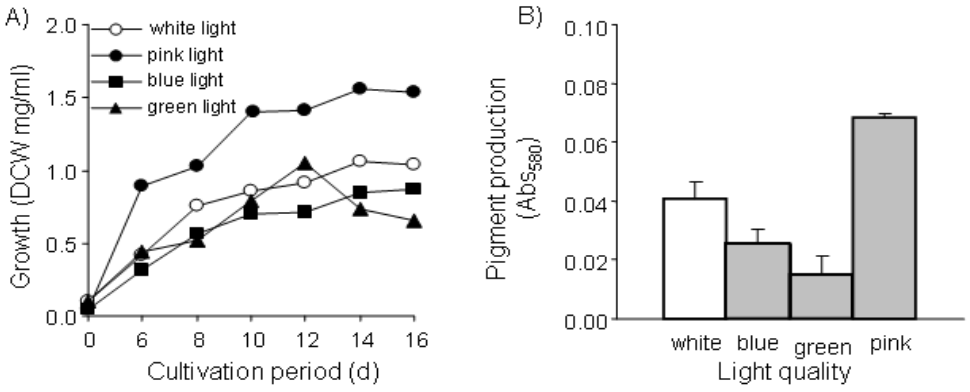

Fig. 2 Effects of light quality on growth (A) and pigment production (B) of *Oscillatoria* sp. Cells were cultivated under different wavelengths of light at 30 µmol photons m^{-2} s^{-1}. The production of OsPP in the medium was measured for 16-day-old *Osillatoria* sp. DCW is dry cell weight. Values are the mean ± SDs of three independent experiments.

4 Discussion

The effects of different media and light quality show significant effect on cell growth and OsPP production. Three different media C, modified C and No 18 are known as culture media for cultivation of freshwater cyanobacteria. These media were also used for cultivation of several freshwater cyanobacteria. Under modified C medium, the pink pigment in culture media was clearly observed on 6-day-old *Oscillatoria*, which is correspond to the absorbance value (Abs_{560}) of 0.02. On the other hand, the pigments from grown cells in No 18 and C media were observed on 10-day-old *Oscillatoria*, respectively.

The highest cell growth and OsPP production were obtained from grown cell in modified C medium (Fig. 1). The nitrogen concentration in modified C is higher (5 g L^{-1}) than C medium (0.1 g L^{-1}) or in No 18 (0 g L^{-1}). The correlations between nitrogen concentration and cells growth as well as on the pigment production were well documented. Nitrogen is an essential major element required for the synthesis of primary and secondary amino acids, proteins, nucleic acids, coenzymes, chlorophyll and other accessory photosynthetic pigment such as phycobiliproteins in cyanobacteria [5]. It was reported that the concentration of KNO_3 as nitrogen source was found to be an essential factor influencing the growth of *Synechocystis* sp. PCC 6701. In contrast, the growth and phycobiliproteins content in several cyanobacteria decreased during nitrogen starvation/limitation [12].

On the other hand, K_2HPO_4 as phosphorus source is also known as essential to algal growth because it was important roles in many cellular processes and it is maintaining pH of culture media by it buffering capacity [13]. Modified C medium containing phosphorus (100 mg L^{-1} as K_2HPO_4) might be appropriate concentration for growth of *Oscillatoria* strain. BG-11 medium supplemented with phosphorus (10 mg L^{-1} as K_2HPO_4) was significantly stimulated the cell growth and phycoerythrin production of *Nostochopsis lobatus* [13]. In contrast, high levels of phosphorus in medium was inhibited the growth of *Anabaena variabilis* [14]. It was reported that nitrate (KNO_3) and phosphate (K_2HPO_4) were identified as major factors of cell growth *Synechocystis* sp. PCC 6701 [12]. In this study, high cell growth and OsPP production obtained from modified C medium were agreed with these phenomena.

Light qualities as well light intensity play an important role on the cell growth and metabolite production in photosynthetic organisms, including cyanobacteria. For example, in *Rhodella reticulate,* the production of algal biomass is enhanced by about 5-6 times in response to increased light intensities from 18 to 215 μE m^{-2} s^{-1} under white, green or red light. At low light intensity, B-phycoerythrin content is preferentially enhanced to 27 % under the influence of green light compared with red light [9]. The phycocyanin content in the cyanobacteria *Synechococcus* sp. NKBG 042902 grown under green or blue light is markedly low, while that of the cells grown under red light is high [10]. The production of marennine, a blue pigment produced by the diatom *Halsela ostrearia*, is controlled by blue-light radiation [32]. In addition, production of micosporine-like amino acids (MAAs) and scytonemin in cyanobacteria, phytoplankton and macroalgae has been observed as a response to counteract the damaging effect of UV-radiation [11]. Therefore, the individual light regimen becomes a predominant factor in affecting the productivity of algae.

It was reported that longer wavelength (540 nm) may not suitable for chlorophyll and carotene synthesis but phycocyanin and PE significantly accumulated under such wavelength. The chlorophyll synthesis was suited under white light and blue light irradiation. Longer wavelengths such as green, yellow and red lights are the main light adsorbed by photosystem II to produced highest phycocyanin and phycoerythrin content in *Spirulina fussiformis.* This is because at higher light wavelength phycobilisome is a highly efficient system for transferring energy to PS II reaction center [15]. In addition, under red

or pink light radiation, most of the photosynthetic energy can be diverted into new biomass due to high carbon investment efficiency [8]. In conjunction to these phenomena, cell growth and extracellular pink pigment production of *Oscillatoria* strain were also stimulated proportionally under pink light radiation.

In conclusion, this research provides information that high cell growth and OsPP production were obtained from cultivation of *Oscillatoria* strains in modified C medium and under pink light irradiation. Modified C medium is simple in both chemical composition and preparation than the other media. Therefore, this medium has advantage for practical application on pink pigment production of *Oscillatoria* strain.

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