JPPIPA 9(11) (2023)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

The Growth Test of Marine Microalgae *Porphyridium cruentum* in Different Salinity on Small Scale Culture

Indrian Rizka Amalia[1*], Abidin Nur[1], Lisa Ruliaty[1], Ratna Indria Sari[1]

[1]Research Center for Fishery, National Research and Innovation Agency, Bogor, Indonesia.

Received: September 13, 2023
Revised: November 12, 2023
Accepted: November 25, 2023
Published: November 30, 2023

Corresponding Author:
Indrian Rizka Amalia
indr059@brin.go.id

DOI: 10.29303/jppipa.v9i11.5301

Abstract: This study aimed to know the growth response and proximate value of *Porphyridium cruentum* cultured in different salinities of 30 ppt, 15 ppt and 0 ppt. Culture media of 30 ppt and 15 ppt salinity were prepared with sterile seawater, while 0 ppt was from sterile aquades. Fertilizer used was KW21 (Daiichi Seimo Co., Ltd., Kumamoto, Japan) with a dose of 1 $ml.l^{-1}$. All treatments were cultured for 11 days or until the cell reached exponential-stationer phase, then being growth observed and proximate analyzed. The result showed that all treatments gave the same growth pattern. Control 30 ppt had the highest cell density compare to treatments 0 ppt and 15 ppt, while 0 ppt had higher cell density to 15 ppt. The result of ANOVA (95% of significancy) showed that there was no significant different of cell density with value of 0.232. Proximate analysis showed that all treatments had high water content above 95%. The highest value of energy, carbohydrates and crude fiber were found in 0 ppt treatment, while the highest value of protein and ash were found in 30 ppt. The result from ANOVA showed that there was significant difference in proximate value with significancy less than 0.05.

Keywords: Cell density; *Porphyridium cruentum;* Proximate analysis; Salinity

Introduction

Microalgae *Porphyridium cruentum* has potency in food and feed industry as it contains protein, exopolysaccharide sulfates, and unsaturated fatty acids (PUFA), as well as the pigment phycoerythrin which gives a red color expression (Plaza et al., 2009). *P.cruentum* has nutritional content similar to soybean flour so it has potency as food substitute (Guil-Guerrero et al., 2004). The biomass content of the dry weight of *P.cruentum* is 34% protein, 32% carbohydrates, 6.5-7%% lipids, and some content of EPA (eicosapentaenoic acid), palmitic acid, arachidonic acid and linoleic acid (Rebolloso Fuentes et al., 2000, Chronakis & Madsen, 2011), as well as vitamin E in the form of tocopherols (Durmaz et al., 2007). The nutritional content of *P.cruentum* can be optimized by regulating its culture or environmental conditions. Micronutrients and macronutrients such as nitrogen and phosphate can affect the carbohydrate content of *P.cruentum* (Razaghi et al., 2014, Morales-Sánchez et al., 2014).

Exopolysaccharide extract that encapsulates the cell wall of P. cruentum plays a role in the cosmetic and health industry. This characteristic makes *P.cruentum* easily dissolved and has high viscosity (Usov, 2011) . Exopolysaccharide in *P.cruentum* consists of several sugar groups such as xylose, glucose, galactose, mannose, methylated galactose, and pentose (Geresh et al., 2002; S. Y. Li et al., 2019). In terms of carbohydrate content, glucose is the largest composition, around 16-17% of dry weight, while the smallest composition is xylose and galactose (Kim et al., 2017). The presence of lipopolysaccharide and exo-cellular polysaccharide (EPS) (Balti et al., 2018), PUFAs, polyunsaturated fatty acids, and phycoerythrin (S. Li et al., 2019) makes *P.cruentum* useful as antioxidants (Agustini & ., 2017) and antivirals (Dewi et al., 2018).The exopolysaccharide extract from *P.cruentum* can be used as an immunostimulant for vibrio-infected vannamei shrimp by rapidly increasing the immune parameters of shrimp to prevent re-infection of vibrio in shrimp (Risjani et al., 2021).

How to Cite:
Amalia, I.R., Nur, A., Ruliaty, L., & Sari, R. I. (2023). The Growth Test of Marine Microalgae Porphyridium cruentum in Different Salinity on Small Scale Culture. *Jurnal Penelitian Pendidikan IPA*, *9*(11), 9693–9697. https://doi.org/10.29303/jppipa.v9i11.5301

The essential contents of *P.cruentum* make this microalgae potential as functional live food, especially in fishery commodities. Generally, *P.cruentum* is cultured under saline conditions within a range of 5-30 ppt. However, to optimize the use of *P.cruentum* in various fishery commodities, it has to adapt to different ranges of salinity. Therefore, this study tested the growth response and proximate value of P. cruentum cultured in three different salinities, which were 0 ppt, 15 ppt, and 30 ppt.

Method

Inoculant *P.cruentum* was obtained from the Laboratory of Live Feed, Main Center of Brackish Water Aquaculture Jepara (BBPBAP Jepara), Ministry of Marine Affairs and Fisheries Indonesia. The e different salinity in culture media was designed as salinity 30 ppt as control while 15 ppt and 0 ppt as the test treatments, with three replications each.

The culture media was prepared by flowing sterile sea water into 1 liter of erlenmeyer. As the salinity of sterile sea water was 33 ppt, then the aquades was flowing into the sterile seawater until the salinity reached 30 ppt and 15 ppt with total volume of 1 liter. Meanwhile, the culture media for 0 ppt treatment was filled up with aquades. Fertilizer of KW21 (Daiichi Seimo Co., Ltd., Kumamoto, Japan) with a dose of 1 ml.l^{-1} was being added into each treatment. Inoculant of *P.cruentum* was being added with initial density of 1.000.000 cell.ml^{-1}. All treatments were being aerated and placed inside laboratory with temperature of 15-16°C, light intensity of 3.000-3.500 lux with 24 hours of photoperiod.

All treatments were cultured for 11 days or until the cell reached exponential-stationer phase. The growth of *P.cruentum* was observed by counting the cell density daily by haemacytometer. The salinity level was being controlled daily to maintain the treatment consistently. At the end of observation, the proximate value of protein, fat, crude fiber, carbohydrates and energy were analyzed.

Result and Discussion

Cell Density of P.cruentum

The result showed that between control and all treatment were having the same growth pattern, which was lag phase in the beginning, followed by exponential phase and later with stationer phase (Figure 1). The death phase in treatments and control were not being observed because the stocks was directly analyzed for proximate after reached exponential-stationer phases. In microalgae culture, phase determination is important because it is the optimum period for cell growth. In this late exponential to early stationer phase, phytoplankton culture is recommended to be harvested or rejuvenated. The late stationary phase is not recommended as a harvest period because the large number of dead cells can trigger the emergence of bacteria including the class *Vibrio* spp. (Creswell, 2010).

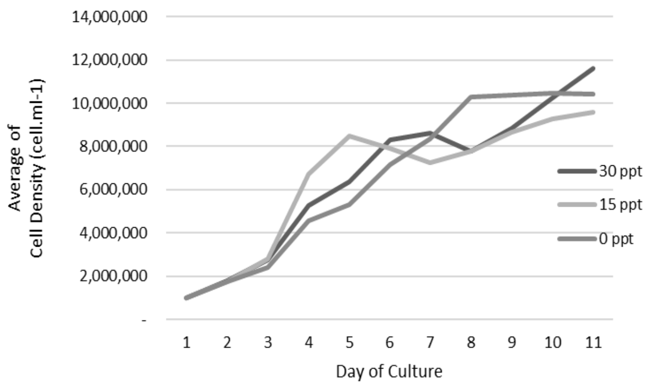

Figure 1. Growth pattern of *P.cruentum* from both treatments (15 ppt and 0 ppt) and control (30 ppt)

At the end of culture period (day 11th) when cell growth was in late-exponential phase to early stationer, the highest cell density of *P.cruentum* was at control (30 ppt) compare to treatments (15 ppt and 0 ppt). The average cell density of control was 11.600.000 cell.ml^{-1} while treatment 15 ppt at 9.600.000 cell.ml^{-1} and 0 ppt at 10.400.000 cell.ml^{-1}. However, if two treatments were compared to each other, then treatment 0 ppt was showing higher cell density than 15 ppt.

The normality test showed that the data was normally distributed, then the analysis was continued to ANOVA. The result from ANOVA (95% of confidence level) showed that there was no significant different of cell density among all treatments with the value of significancy was 0.232. Thus, the different of salinity did not much affect the cell density of *P.cruentum*.

P.cruentum that is cultured in seawater grew faster than *P.cruentum* cultured in fresh water at the start of rearing, but after the 10th day of rearing there was a lot of cell death in *P.cruentum* cultured in seawater media compared to fresh water (Kim et al., 2017). Kim et al. (2017) states that P. cruentum can optimally be cultured in seawater and freshwater media by considering the specific harvest time to get maximum biomass. The optimum cell density and total biomass produced are parameters for the successful production of P. cruentum culture (Razaghi et al., 2014). Salinity, radiation or lumination and the availability of nutrients in the culture media can affect the biomass, as it may lead to specific metabolites production that cannot be found in other organisms(Plaza et al., 2009).

Proximate analysis of P.cruentum biomass

The results showed that each treatments produced different proximate values. As *P.cruentum* is a marine microalga then it had high water content in all treatments which was above 95% (Figure 2.). Among of all proximate, energy was the highest content in all treatments with range of 4.2-9%, then followed by ash, protein, crude fiber, carbohydrate and fat (Figure 3.).

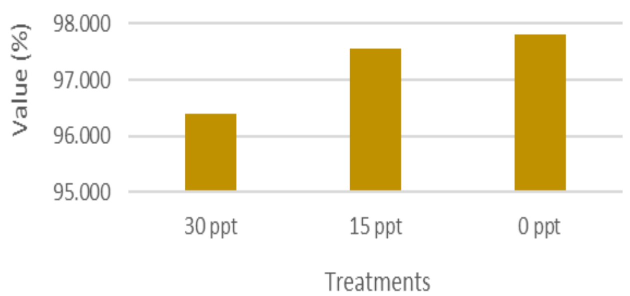

Figure 2. Water content of *P.cruentum* in all treatments

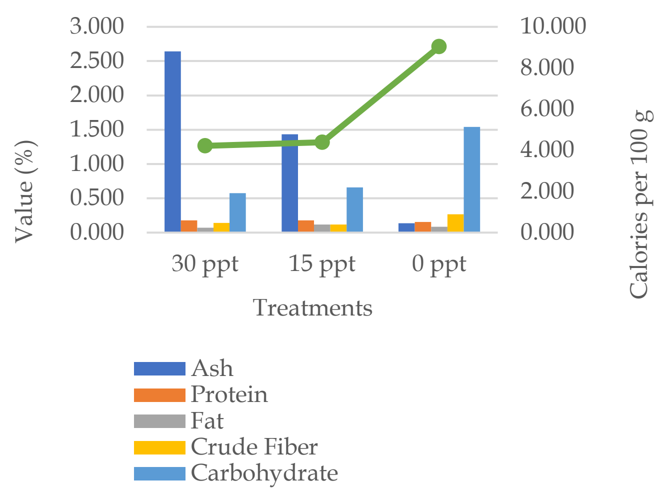

Figure 3. Proximate values of *P.cruentum* in all treatments

The highest proximate value of energy content, carbohydrates and crude fiber were found in 0 ppt treatment with value was 9.034 cal per100 g, 1.539%, and 0.268% respectively. Meanwhile the highest value of protein and ash content were found in 30 ppt control with a value of 0.181% and 2.656% respectively. In order to examine the difference between all treatments applied, all the proximate values were analyzed further with statistic. Normality test was applied to all data with significancy more than 0.05, meant that the data was distributed normally and could be analyzed with ANOVA (95% of confidence level). The results of ANOVA showed that there was significant difference in the proximate value among all treatments with a significancy less than 0.05.

P.cruentum has high content of fatty acids, especially from the EPA group which is widely used in the pharmaceutical industry (Plaza et al., 2009). This microalga is also known for its polysaccharide sulfate content in its cel which is useful in the drug and cosmetic industry (Geresh et al., 2002), even as an immunomodulator for *vanamei* shrimp in the face of *Vibrio* contamination (Risjani et al., 2021). The polysaccharides contained in *P.cruentum* generally consist of glucose, galactose, xylose, glucuronic acid and methyl-glucuronic acid as sugar monomers (Heaney-Kieras & J. Chapman, 1976).

Kim et al.(2017) stated that in 100 grams of *P.cruentum* cultured in fresh water media obtained 16.9 g of glucose, 5.3 g of galactose, 4.7 g of xylose while 100 grams of *P.cruentum* cultured in seawater media obtained 16. 6 g glucose, 5.5 g galactose and 6.4 g xylose. This can be used to facilitate the condition of P. cruentum culture media which quickly adapts to variations in salinity. The proximate results of the 15 ppt treatment were not as high as the percentage of the 0 ppt treatment and the 30 ppt control. However, in terms of cell density, the 15 ppt culture medium still potential to be applied.

Conclusion

The result showed that during 11 days of culture, the highest cell density was on control 30 ppt at 11.600.000 cell.ml^{-1}, while treatment 15 ppt at 9.600.000 cell.ml^{-1} and 0 ppt at 10.400.000 cell.ml^{-1}. The result from ANOVA (95% of confidence level) showed that there was no significant different of cell density among all treatments with the value of significancy was 0.232.
The result from proximate analysis showed that energy was the highest content in all treatments with range of 4.2-9%, then followed by ash, protein, crude fiber, carbohydrate and fat. The highest proximate value of energy content, carbohydrates and crude fiber were found in 0 ppt treatment with value was 9.034 cal per100 g, 1.539%, and 0.268% respectively, while the highest value of protein and ash content was found in 30 ppt control with a value of 0.1806% and 2.65635% respectively. The results of ANOVA showed that there was significant difference in the proximate value between all treatments with significancy less than 0.05.

Acknowledgements
We thank all colleagues of Laboratory of Live Feed, Main Center of Brackish Water Aquaculture Jepara (BBPBAP Jepara), Ministry of Marine Affairs and Fisheries Indonesia for the inspiration and assist on this research.

Author Contributions
All authors have contributed with their respective tasks which are equally important for the completion of the writing of this paper.

Funding
This research received no external funding.

Conflicts of Interest
The authors declare no conflict of interest.

References

Agustini, N. W. S., & Kusmiati (2017). Potency of Endo-Exopolysaccharide from *Porphyridium cruentum* (S.F.Gray) Nägeli as Antioxidant (DPPH) and Biological Toxicity (BSLT). *KnE Life Sciences*, *3*(4), 147. https://doi.org/10.18502/kls.v3i4.699

Balti, R., Le Balc'h, R., Brodu, N., Gilbert, M., Le Gouic, B., Le Gall, S., Sinquin, C., & Massé, A. (2018). Concentration and purification of *Porphyridium cruentum* exopolysaccharides by membrane filtration at various cross-flow velocities. *Process Biochemistry*, *74*, 175–184. https://doi.org/10.1016/J.PROCBIO.2018.06.021

Chronakis, I. S., & Madsen, M. (2011). Algal proteins. In Phillips. G., O & Williams, P., A (Eds.), *Handbook of Food Proteins* (pp. 353–394). Woodhead Publishing Series in Food Science, Technology and Nutrition. Sawston, UK: Woodhead Publishing. https://doi.org/10.1533/9780857093639.353

Creswell, L. (2010). *Phytoplankton culture for aquaculture feed* (Issue 5004). Mississippi, USA: Southern Regional Aquaculture Center.

Dewi, I. C., Falaise, C., Hellio, C., Bourgougnon, N., & Mouget, J. L. (2018). Anticancer, Antiviral, Antibacterial, and Antifungal Properties in Microalgae. *Microalgae in Health and Disease Prevention*, 235–261. https://doi.org/10.1016/B978-0-12-811405-6.00012-8

Durmaz, Y., Monteiro, M., Bandarra, N., Gökpinar, Ş., & Işik, O. (2007). The effect of low temperature on fatty acid composition and tocopherols of the red microalga, *Porphyridium cruentum*. *Journal of Applied Phycology*, *19*(3), 223–227. https://doi.org/10.1007/S10811-006-9127-6/METRICS

Geresh, S., Adin, I., Yarmolinsky, E., & Karpasas, M. (2002). Characterization of the extracellular polysaccharide of *Porphyridium* sp.: molecular weight determination and rheological properties. *Carbohydrate Polymers*, *50*(2), 183–189. https://doi.org/10.1016/S0144-8617(02)00019-X

Guil-Guerrero, J. L., Navarro-Juárez, R., López-Martínez, J. C., Campra-Madrid, P., & Rebolloso-Fuentes, M. M. (2004). Functional properties of the biomass of three microalgal species. *Journal of Food Engineering*, *65*(4), 511–517. https://doi.org/10.1016/J.JFOODENG.2004.02.014

Heaney-Kieras, J., & J. Chapman, D. (1976). Structural studies on the extracellular polysaccharide of the red alga, *Porphyridium cruentum*. *Carbohydrate Research*, *52*(1), 169–177. https://doi.org/10.1016/S0008-6215(00)85957-1

Kim, H. M., Oh, C. H., & Bae, H.-J. (2017). Comparison of red microalgae (*Porphyridium cruentum*) culture conditions for bioethanol production. *Bioresource Technology*, *233*, 44–50. https://doi.org/10.1016/j.biortech.2017.02.040

Li, S., Ji, L., Shi, Q., Wu, H., & Fan, J. (2019). Advances in the production of bioactive substances from marine unicellular microalgae *Porphyridium* spp. *Bioresource Technology*, *292*, 122048. https://doi.org/10.1016/J.BIORTECH.2019.122048

Li, S. Y., Shabtai, Y., & Arad, S. (2019). Production and composition of the sulphated cell wall polysaccharide of *Porphyridium* (Rhodophyta) as affected by CO2 concentration. *Phycologia*. *39*(4), 332–336. https://doi.org/10.2216/I0031-8884-39-4-332.1

Morales-Sánchez, D., Tinoco-Valencia, R., Caro-Bermúdez, M. A., & Martinez, A. (2014). Culturing Neochloris oleoabundans microalga in a nitrogen-limited, heterotrophic fed-batch system to enhance lipid and carbohydrate accumulation. *Algal Research*, *5*(1), 61–69. https://doi.org/10.1016/J.ALGAL.2014.05.006

Plaza, M., Herrero, M., Alejandro Cifuentes, A., & Ibáñez, E. (2009). Innovative natural functional ingredients from microalgae. *Journal of Agricultural and Food Chemistry*, *57*(16), 7159–7170. https://doi.org/10.1021/JF901070G

Razaghi, A., Godhe, A., & Albers, E. (2014). Effects of nitrogen on growth and carbohydrate formation in *Porphyridium cruentum*. *Central European Journal of Biology*, *9*(2), 156–162. https://doi.org/10.2478/S11535-013-0248-Z/MACHINEREADABLECITATION/RIS

Rebolloso Fuentes, M. M., Acién Fernández, G. G., Sánchez Pérez, J. A., & Guil Guerrero, J. L. (2000). Biomass nutrient profiles of the microalga *Porphyridium cruentum*. *Food Chemistry*, *70*(3), 345–353. https://doi.org/10.1016/S0308-8146(00)00101-1

Risjani, Y., Mutmainnah, N., Manurung, P., Wulan, S. N., & Yunianta. (2021). Exopolysaccharide from *Porphyridium cruentum* (purpureum) is not toxic and stimulates immune response against vibriosis: the assessment using zebrafish and white shrimp *Litopenaeus vannamei*. *Marine Drugs*, *19*(3), 133. https://doi.org/10.3390/md19030133

Usov, A. I. (2011). Polysaccharides of the red algae.In

Horton., E (Ed), *Advances in Carbohydrate Chemistry and Biochemistry*, *65*, (pp. 115–217). Massachusetts, USA: Academic Press. https://doi.org/10.1016/B978-0-12-385520-6.00004-2