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The Aplication of Plasma Technology As Nutrient Source for The *Porphyridium* Growth

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ABSTRACT

Porphyridium are indispensable in the commercial rearing of various species of marine animals as a food source. *Porphyridium* are also used directly in the larval tanks and play an important role in stabilizing the water quality, nutrition of the larvae, and microbial control. *Porphyridium* are spherical to obovoid unicelluler of Rhodophyte with stellate chloroplast and prominent central pyrenoid, 5-10 µm diametre in exponential, 7-16 µm in stationary phase. Cells solitary, but often grouped into irregular colonies with ill-defined mucilaginous matrix. Cells become dormant during prolonged darkness. Species vary in tolerance of salinity fluctuations. Plasma technology capable of producing a compound of free radicals, ions, and radical atoms. The corona discharge plasma may induce the nitrogen gas of the air become ions. The microalgae can be iradiated by the corona discharge plasma for nutrient supplement. The aim of this research was to determine the potencial of corona discharge plasma as source of nutrient for *Porphyridium* growth. In the petridisc, *Porphyridium* was irradiate with corona plasma for 10 minutes then cultured in the 1 L saline water. This will compare with medium culture of Walne, and marine water. Its population was counted everyday for 7 days. The population growth of irradiaton *Porphyridium* with corona plasma was higher than culture in the marine water, but lower tha Walne medium. The plasma technology could be applied in the microalgae culture, but require added with micronutrients that require by microalgae to enhance its population growth. Research had to be continued to determine the concentration of micronutrients addition for optimal growth of microalgae, particularly *Porphyridium.*

Keywords: *Porphyridium*, plasma technology,nutrient, microalgae

1. INTRODUCTION

All algal species are not equally successful in supporting the growth and survival of a particular filter-feeding animal. Suitable algal species have been selected on the basis of their mass-culture potential, cell size, digestibility, and overall food value for the feeding animal. Various techniques have been developed to grow these food species on a large scale, ranging from less controlled extensive to monospecific intensive cultures. However, the controlled production of micro-algae is a complex and expensive procedure. A possible alternative to on-site algal culture is the collection of algae from the natural environment where, under certain conditions, they may be extremely abundant. Furthermore, in order to overcome or reduce the problems and limitations associated with algal cultures, various investigators have attempted to replace algae using artificial diets either as a supplement or as the main food source. These various aspects of the production, use and substitution of micro-algae in aquaculture will be treated within the limits of this chapter. (fao,2012)

The red microalga Porphyridium is a source of biochemicals that possess nutritional and therapeutical value. These biochemicals include a high content of polysaccharides, long-chain polyunsaturated fatty acids, carotenoids such as zeaxanthin, and fluorescent phycobiliproteins. The polysaccharides of Porphyridium exhibited impressive antiviral activity (Huleihel et al. 2001, 2002; Huang et al. 2005).

Porphyridium has been shown to contain four biliproteins with the following amounts (percentage): allophycocyanin (5%), R-phycoerythrin (11%), b-phycoerythrin (_42%), and B-phycoerythrin (BPE) (_42%) (Bermejo et al. 2001). Among the four biliproteins, BPE is particularly useful due to its high molar absorptivity and great fluorescence properties (Ayyagari et al. 1995). It has been also reported that BPE can be used as a pigment in the food, cosmetic, and pharmaceutical industries. Porphyridium cruentum is a red microalga (Rodophyta), with spherical cells that lack a cell wall (Arrad,1992). Phycoerythrin, an accessory pigment,provides red colour characteristic of the cells Cells excrete a sulphurized polysaccharide which causes the cultures to become viscous, especially under limiting conditions. The polysaccharide is used commercially (Arrad,1992)

On cultured microalgae needed anoraganik elements such as N, K, P, Na, Si, Ca, and also micro-nutrients such as Fe, Zn. Element N, P and S role in the formation of proteins and the K function in the metabolism of carbohydrates. Fe and Na contributes to the formation of chlorophyll. Microalgae growth is also influenced by environmental factors such as light, temperature, ph, Osmose pressure, and concentration of nutrients.

Porphyridium cruentum spherical with a diameter of 4-9 µm. Cell structure consists of a nucleus (core), chloroplasts, Golgi bodies, mitochondria, starch and vesicles. Each cell has a chloroplast with pirenoid in the middle (Lee 1989). Porphyridium can live in a variety of natural habitats such as sea water, fresh water, or on the surface of moist soil and form a layer of reddish very interesting. Native habitat of Porphyridium cruentum probably derived from the sea as it can live well in liquid media and solid media seawater (Borowitzka dan Borowitzka 1988).

Giving the name of the red alga Porphyridium cruentum based on excess and dominance of the red pigment r (red)-fikoeritrin and r (red)-fikosianin owned. Its kind of chlorophyll is chlorophyll a while chlorophyll b does not exist and is replaced by chlorophyll d (Sharma 1986). Kumar and Shingh (1979) states that the red pigment of the color cover other photosynthetic pigments. Pigment-fikoeritrin r, r-fikosianin, and alllofikosianin contained in fikobillin of red algae (Sharma 1986). Fikobillin important role in photosynthesis as the light receiver pigment especially in photosystem II (PSII) in phycobillisome (Arylza 2005).

2. MATERIALS AND METHODS

2.1. Microorganism

A starter culture of Porphyridium was obtained from the collection of cultures of microalgae, BPPBAB jepara. Medium prepared from sea water and in Diponegoro University the seawater was sterillized until the patogen microorganism didn't contamination the microalgae, all glass and tools have sterilized to with hot water and bayclin to killed patogen microorganism. The sea water have salin condition 32ppm, the temperature of the culture was controlled at 21-26 celcius degress but optimum in 25 celcius degress The photobioreactor consisted of a airlift pump that drove the culture ¯uid through a horizontal tubular solar receiver. Total culture volume in glass was 1 Liter and the air was continuously supplied

2.2 Pengaruh Faktor Lingkungan *Porphyridium cruentum*

Growth of Porphyridium cruentum meeliputi influenced by environmental factors light, temperature, salinity, pH, and nutrient use (carbon, nitrogen, sulfur, and phosphorus).

a) light

Light has a great influence on the chemical composition of algal photosynthesis. Generally decrease in intensity of light will increase the chlorophyll a and other pigments (chlorophyll b, chlorophyll c, fikobilliprotein, and carotenoids), while high-intensity light will degrade chlorophyll a and other pigments (Richmond 2004).
Growth of Porphyridium cruentum in sea water also depends on the light intensity of light but tolerate relatively larger. The increase in light intensity causes chloroplast size reduction and increased granulation on cell content (Borowitzka dan Borowitzka 1988).

b) temperature

Temperature is one of the factors that influence the biochemical composition of algae. Temperature effect on membrane lipid composition and content. The reduced temperature growth under optimal conditions increase the unsaturated fatty acids in membrane systems. Increased stability and fluidity of cell membranes especially the thylakoid membrane (increase of unsaturated fatty acids in membrane lipids) protects photosynthesis from fotoinhibisi at low temperature (Richmond 2004).

Tues Porphyridium can be grown in the temperature range 10-35 ° C with an optimum temperature of 25 ° C. Optimum photosynthetic activity of Porphyridium cruentum cultures occurred at 25 ° C (Vonshak 1988). The optimum temperature for growth is 21-26 ° C Porphyridium and at temperatures below 13 ° C while the slow growth at temperatures above 31 ° C are stunted (Richmond 1988).

c) Salinity and pH

Porphyridium can survive the salinity range is large enough that 0.5 to 2 times the concentration of sea water (Borowitzka and Borowitzka 1988). In conditions of salinity less than 3.5%, Porphyridium not compete live with other microalgae when grown in open culture. Salinity of 4.6% does not inhibit the growth process. Nevertheless, with a range of 3.5-4.5% salinity can stimulate optimal growth (Richmond 2004).

Porphyridium cruentum can grow well in the range of pH 5.2 to 8.3. The degree of acidity (pH) optimum for photosynthesis Porphyridium cruentum was 7.5. The growth will be hampered if the pH is less than 5 (Borowitzka and Borowitzka 1988).

d) Nutrient

microalgae takes the form of nutrient and micronutrient makronutrient. Examples of macro nutrients for the growth of Porphyridium are organic compounds such as N, K, MG, S, P and CL. While the micro nutrients are Fe, Cu, Zn, Mn, B . Each nutrient has specific functions for the growth and density of organisms dikuktur without neglecting the influence of the environment.

2.3 Methods

The tools used in this study is the size 3L glass jars, fluorescent lights, aerators, hose, microscopes, glass objects, glass cover, SRC, pipettes, paper, pumps, hand counter, paper labels, markers, refrigerators, Natural food Chlorella sp obtained from pure stock that has been done over and over and have developed purification seedlings cultured in the laboratory at the Center for Development of Brackish Water Aquaculture (BBPBAP) Jepara. Sea water as a medium of life Porphyridium sp obtained after sterilization by boiling stage. The treatment used by the Culture Media Walne and second treatments using sea water that has been on fire with the plasma and beam. Planting seeds of Chlorella sp. made after calculating stock density by taking samples of plankton from the media stock and then counted under a microscope with the SRC. Data taken include growth patterns Chlorella sp ie lag phase, specific growth konstantan (Specific Growth Rate), the peak population (Maximum Cell Density) and Density end (Final Cell Density). Compiled as much as 3 glass with plasma treatment and 3 glass with walne treatment and 1 glass as a control.

Seeds Porphyridium incorporated into each glass culture containing sea water and also contains media to existing volumes reached a liter. Cultures were observed in the span of two weeks to see the growth of Porphyridium.Semuanya placed into a rack that has been prepared and has been given a lamp as a light source. Porphyridium growth observed by taking samples every day using a micro pipette, and then inserted into the SRC, then counted the number of cells directly using a microscope. The results of the calculation value converted into logarithmic values and made the growth curve.

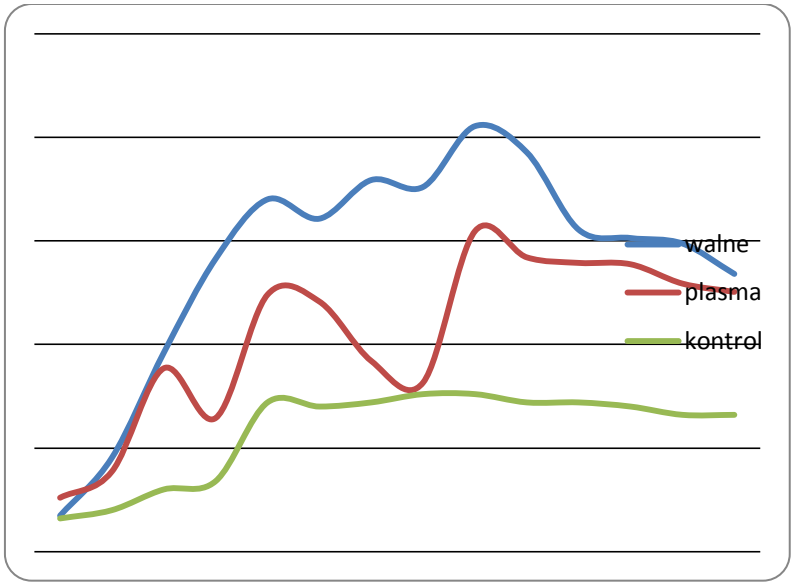
3. RESULTS

Growth of microalgae in culture can be characterized by increased cell size or magnitude of the increase of the number of cells. The development of microalgae cells in culture consists of five phases, namely phase lag (adaptation), exponential phase (logarithmic) growth rate decline phase (declination), stationary phase and death phase. The first phase is the phase lag or adaptation phase where the new population transferred decreased metabolic rate due to uneven phase inoculum and the process of adaptation to the culture medium. The second phase is the phase of exponential (logarithmic) where the acceleration of growth and biochemical component concentration ratio becomes constant (Fogg 1975).

Phase declination occurred with the end of the logarithmic phase with no growth. This happens due to lack of nutrients (nitrogen and phosfat). Stationary phase is the end of the production. biomass becomes constant. In this phase, the maximum concentration of biomass is reached. Phase is characterized by the occurrence of death biomass production decreased due to cell lysis (Aprimara 2010).

hari ke	rata-rata		
	walne	plasma	kontrol
1	173.333	260.000	160.000
2	453.333	386.667	200.000
3	960.000	884.667	300.000
4	1.413.333	644.667	340.000
5	1.700.000	1.240.000	720.000
6	1.606.667	1.206.667	700.000
7	1.793.333	920.000	720.000
8	1.760.000	816.000	760.000
9	2.053.333	1.546.667	760.000
10	1.926.667	1.420.000	720.000
11	1.553.333	1.393.333	720.000
12	1.513.333	1.386.667	700.000
13	1.486.667	1.293.333	660.000
14	1.340.000	1.253.333	660.000

Tabel. the growth of Porphyridium



Grafik. the growth of Porphyridium

From the observational data can didimpulkan that plasma is only good for the first day because the plasma there are many elements of N compared to walne. However microalgae not only require N for growth elements but also elements of P good growth data using wakne because nutrient fertilizer which is in very memadahi walne notice of microalgal growth. Porphyridium aakan optimum on day nine and growth will continue to decline until the fourteenth day

Porphyridium can use KNO3 and ammonium as nitrogen source. Sources of nitrogen required for cell growth and the formation of polysaccharide synthesis enzymes. Excess nitrogen is usually reduces production of extracellular polysaccharides. If grown in a medium with a limited nitrogen source, the production polisakaridanya higher (Borowitzka and Borowitzka 1988).

Nitrogen is needed cells in large quantities as a component of proteins, nucleic acids and other cellular components. Nitrogen is generally used by the organism in the form of nitrate (NO3-) and ammonium (NH4 +). Ammonium is a form of nitrogen that most assimilated by phytoplankton. Ammonium does not require reduction prior to assimilation into amino acids (Kurniawati 2006). Nitrogen can affect cellular metabolism which causes a decrease in the efficiency of energy transfer to photosystem II in photosynthetic reaction due to the concentration of nitrogen is too high or too low (Aprimara 2010).

Phosphorus is another major macronutrient that is important to the process of cellular metabolites to form many structural and functional components for the growth and development of microalgae. When the excess nutrients that light is the limiting factor of growth (Richmond 2004). Phosphorus is used by algae in the form of H2PO4-or-HPO42. The optimum concentration of phosphorus in the media varies by species. Average phosphorus tolerance range for most algae is 50 mg / l to 20 mg / l (Becker 1994).

Phosphorus decreased with nitrogen. In eukaryotic and prokaryotic cells, the content of chlorophyll a decreased when the carbohydrate content increases. However, in contrast to nitrogen, phycobillisome damage reduced slightly when phosphorus. Decrease phycobillisome cause cell division and synthesis occurs phycobillisome new (Richmond 2004).

Phosphorus acts as a structural element of nucleic acids, and phospholipids tifosfat adenosine and energy metabolism mainly to produce ATP during photosynthesis (Raynods 1994). Phosphorus is one of the elements that play a role in the formulation of carbohydrates and nitrogen compounds (Bold and Wynne 1985). Phosphates can degrade chlorophyll in the cell. Phosphorylated energy-rich sugars appear in the process of photosynthesis (Lombardi and Wangersky 1991).

Non Thermal Plasma technology especially plasma begun to be exploited for the benefit of biological, medical and agriculture. This is based on the utilization of nature's own plasma is capable of producing a compound of Free Radicals, ions, and radical atoms. Non-Thermal Plasma can be generated through the crack of the corona plasma generated by two electrodes.

In collaboration with the laboratories of Physics UNDIP FSM has made use of plasma is applied to the field of biology that influence population growth of Porphyridium rasiasi plasma beam. Corona glow discharge plasma to ionize the gases that exist between the electrodes in the study using free water, and 80% of outdoor air that exists in nature is also needed by the nitrogen in particular microalgae Porphyridium for growth,
Walne is good for medium because have the compotition such as

(1) Trace metal solution (TMS)
ZnCl2 2.1 g
CoCl2.6H2O 2.0 g
(NH4)6Mo7O24.4H2O 0.9 g
CuSO4.5H2O 2.0 g
Make up to 100 ml with distilled water. This solution is normally
cloudy. Acidify with a few drops of conc. HCl to give a clear solution.
(2) Vitamin solution
Vitamin B12. (Cyanocobalamin) 10.0 mg
Vitamin B1 (Thiamine.HCl) 10.0 mg
Vitamin H (Biotin) 200.0 µg
Make up to 100 ml with distilled water.
per litre
(3) Nutrient solution
FeCl3.6H20 1.3 g
MnCl2.4H20 0.36 g
H3BO3 33.6 g
EDTA(Disodium salt) 45.0 g
NaH2PO4.2H2O 20.0 g
NaNO3 100.0 g
TMS (1 above) 1.0 ml
Make up to 1 litre with distilled water.
Medium per litre
Nutrient solution (3) 1.0 ml
Vitamin solution (2) 0.1 ml
Sterilised seawater 1.0 litre
Dispense nutrient and vitamin solutions separately into 10 ml and 1 ml respectively

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The preferred spelling of the word “acknowledgment” in American English is without an “e” after the “g.” Use the singular heading even if you have many acknowledgments.

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