Adaptation and Growth of Botryococcus braunii on Acid Mine Drainage

Fransiscus Benhardi Wastuwidya, Setyo Sarwanto Moersidik

Abstract: ***Phycoremediation of acid mine drainage (AMD) is an alternative to AMD treatment but has limited applications. The obstacle in the application of AMD phycoremediation is that the characteristic of wastewater which limits the growth of microalgae, where AMD has a high metal content and low pH. In this study, Botryococcus braunii was cultured on media with variations in the addition of AMD of (v/v) 0%, 2.5%, 3%, 3.5%, 4% and had a pH of 7.2, 5.6, 5.1, 4.8, 4.3, respectively, on the photobioreactor. Botryococcus braunii growth rate was analyzed, as well as the effect of metal exposure and pH variations on the growth of Botryococcus braunii. Botryococcus braunii showed different growth rates, sequentially from the highest rate in the media with addition of AMD (v/v) 2.5%, 3%, 0%, 3.5%, 4% is 1.403 d^{-1},1.374 d^{-1},0.0862 d^{-1},0.0738 d^{-1}, and 0.0616 d^{-1}. It is known that the highest growth rate of Botryococcus braunii is obtained in media with 2.5% (v/v) AMD with an initial pH of 5.6, and Fe and Mn concentrations of 2.15 mg.L^{-1} and 0.62 mg.L^{-1}, respectively. It is also known the ability of Botryococcus braunii to adapt to acidic conditions with Fe and Mn content, where Botryococcus braunii plays a role in increasing media pH and is able to remove Fe and Mn with the highest values of 84.28% and 98%, respectively.***

Keywords**:** ***AMD, bioremediation, microalgae, mining, phycoremediation.***

I. INTRODUCTION

Acid mine drainage (AMD) is wastewater from mining or mine processing activity that has high acidity and are formed as a result of oxidation of sulfide minerals exposed to the atmosphere in excavation and stockpiling. AMD is caused by mining residual products that are rich in sulfides, usually found in coal mining or deposits of sulfide rock in large quantities, which are exposed to water and oxygen [1]. AMD is a common problem in mining industry which if not handled properly can last long into the post-mining period. Contamination with AMD plays an important role in modifying natural channels ' physical, chemical, and biological character. Large amounts of metal ion and sulphate that flow into uncontaminated stream can cause the channel to degrade chemically. Although the concentration of toxic substances will diminish along the flow of water, AMD pollution will move to sediment in the river due to precipitation from these toxic elements and increase the potential for toxicity. Rivers affected by AMD can store heavy metals in sediments even though the water has a pH that is not too low and metal concentrations are low [2].

One of AMD's processing techniques is the phycoremediation method, which is Defined as the use of microalgae or macroalgae to extract and biotransform contaminants including nutrients and xenobiotics from flue gases in wastewater and CO2 along with the cultivation of algal biomass [3]. In general, removal of nutrients, heavy metals, and minerals in algae occur through the absorption and bioaccumulation mechanism. Along with the removal of elements from wastewater, algae will grow using these nutrients. Some elements will enter the cell surface, while others will be brought into the cell [4]

Phycoremediation technology in AMD is a developing field. Most research in this area uses microalgae for remediation of AMD indirectly, but combined with other technologies or utilizes dead algal biomass as biosorbent for heavy metal adsorption on AMD [4][5][6]. The drawback of AMD phycoremediation is limited by the growth rate of microalgae under acidic conditions. The growth rate limitations can reduce the effectiveness and efficiency of the remediation method, which makes other methods more feasible to be applied. In addition to an acidic environment, media with high heavy metal concentrations can damage microalgae cells, which causes inhibition of the microalgae growth [7].

Microalgae are known to be able to build resistance under acidic conditions and excessive heavy metal concentrations through the process of adaptation and acclimatization. *Chlamydomonas acidophila* microalgae strains were found in AMD ponds in disused copper mines with high acidity (pH 1.6) and high PO_4-P concentrations, which indicate the adaptability of microalgae to extreme conditions. Another study shows microalgae colonies that built acid resistance present on an aquatic system affected by AMD [8], [9].

Microalgae acclimatization can be done by culturing microalgae in a controlled environment. A study was conducted to develop Scenedesmus dimorphus acid resistance through nonlethal exposure to acids. The study showed that *Scenedesmus dimorphus* were able to build acid resistance after being exposed to acidic media with ideal adaptive pH was pH 4.0 and ideal adaptation time was 1 h.[10].

Botryococcus braunii is a green colonial planktonic alga and has been known for its ability to remove metal. *Botryococcus braunii* is

Revised Manuscript Received on January 15, 2020.
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known as microalgae with slow growth, and for that reason, research on its ability on wastewater is limited and considered to be quite challenging.[11], [12].

The purpose of this study is to examine the ability to grow *Botryococcus braunii* acclimatized in the AMD environment so that AMD can be used as a growth medium for *Botryococcus braunii* while removing excess metals in AMD. In this study, the growth kinetics of *Botryococcus braunii* were examined, and also the response of *Botryococcus braunii* to different variations of AMD concentrations, based on the observed parameters. Growth rate determination is useful for evaluating cell growth, which can also be used to design large scale bioreactors. It is expected through the results obtained from this study, it can contribute to the problem of AMD using *Botryococcus braunii*.

II. MATERIALS AND METHOD

A. Materials

Botryoccocus braunii was collected from Surfactant and Bioenergy Research Center (SBRC) Institut Pertanian Bogor (IPB). Experiment was conducted using 5 photobioreactors (PBR) with capacity of 2 l (10 cm diameter, 30 cm height). Aeration was given continuously using air (Resun Pump AC-9906). Two 21watt 1750 lumen LED (Philips T5 essential) was used as light source with 18:6 light:dark intervals. The AMD gold mine effluent, obtained from J Resources Bolaang Mongondow mining site, Sulawesi, Indonesia.

B. Microalgae concentration determination

Microalgae growth rate was determined by optical density at 680nm. Sample was diluted with aquadest to produce absorbance in the range of 0.1-1.0 and analyzed using UV-Vis Spectrophotometer (Shimadzu UV-1800). The solution was then filtered using Whatman filter paper No. 1 and dried using an oven for gravimetric analysis producing algal concentrations ($g.L^{-1}$). Microalgae biomass are calculated using the equation [5], [10], [13]:

$$DW = (m_1\text{-}m_2)/V \quad (1)$$

where DW ($gr \cdot L^{-1}$) is the biomass concentration m_1 dan m_2, is biomass weight before and after drying in gram (g), respectively, and V is sample volume in litre (L). Results of optical density and microalgae concentration are plotted on a graph to find a linear relationship, which will be used as a basis for determining algal concentrations in solution. Botryoccocus braunii's relation between optical density and concentration of biomass was:

$$y = 1.5118x - 0.1216 \quad (2)$$

Specific growth rate μ (d^{-1}) was calculated from the following equation [14]:

$$\mu = (\ln N_2 - \ln N_1) / (t2\text{-}t1) \quad (3)$$

where μ (d^{-1}) is specific growth rate, N_1 and N_2 is biomass concentration ($g.L^{-1}$) on day t_1 dan t_2, respectively.

C. Fe and Mn concentration determination

Fe and Mn were analyzed with Logam Elmer – USA Analyst 800 Atomic Absorption Spectrophotometer (AAS). Removal efficiency was calculated using the [15]:

$$\text{Removal Efficiency} = ((C_0 - C_t)/C_0) \times 100\% \quad (4)$$

where C_0 metal concentration on initial time (t_0) and C_t is metal concentration on observed time (t).

D. Batch Cultivation

Experiments were carried out by cultivating microalgae with different concentrations of AMD in each PBR, thus producing a medium with initial pH: 5.6, 5.1, 4.8, and 4.3. Nutrition is given through 2g N: P: K fertilizer in each PBR. Control is given to the media without the addition of AMD and produces media with a pH of 7.2. The experiment was carried out for 14 days where everyday samples were taken to analyze optical density, pH, and Fe and Mn concentration.

Table 1 Initial solution characterization of each PBR

PBR	AMD addition (*v/v*)	Fe (mg. L^{-1})	Mn (mg. L^{-1})	pH
A	0%	1.86	0.37	7.2
B	2.5%	2.15	0.61	5.6
C	3%	2.47	0.67	5.1
D	3.5%	3.26	0.8	4.8
E	4%	3.82	0.92	4.3

III. RESULT

Microalgae cultivation was conducted in 5 PBRs with AMD concentration variation, as shown in table (1) to understand its effect on *Botryococcus braunii*. As shown in table (2), *Botryococcus braunii* shows optimum growth rate in PBR B and C with maximum biomass of 1.352 $g.L^{-1}$ and μ of 0.143 d^{-1}. In control, PBR microalgae showed lower growth, which is 0.0862 d^{-1}. But at higher concentrations, as shown in PBR D and E, microalgae growth occurs more slowly and has lower productivity.

As shown in fig. 1(a), in PBR B and C the growth lag period of *Botryococcus braunii* is shorter compared to other PBRs, where an increase in growth occurs

on days 6-7. Meanwhile, microalgae on PBR D and E were inhibited, and there was a decrease in the biomass concentration on days 6-10 before having growth

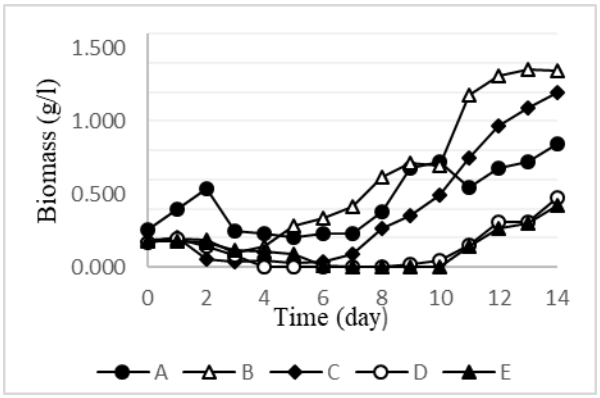
increase on day 11. Changes in pH, as shown in fig. 1 (b), increases to neutral on PBR B and C occur less than 48 hours, while changes in pH on PBR D and E occur more slowly at the start, but increase on day 4 and reach neutral pH on day five.

Fe and Mn were found present on all PBR, this is due to the presence of these two elements in the N: P: K fertilizer used as a source of nutrition. Each PBR showed differences in the removal efficiency of Fe

content, with the highest removal efficiency of Fe at 84.29% in PBR E and the lowest at 6.92% in PBR B. There was a decrease in Mn concentrations in all batches up to below the measurement limit with removal efficiencies above 90% for all PBRs. This shows that Mn is an element essential for *Botryococcus braunii* to grow.

The specific growth rate calculated using equation (3) shows the best *Botryococcus braunii* growth possessed by *Botryococcus braunii* in PBR B, followed by PBR C, A, D and E.

Mn. *Botryococcus braunii* showed different growth rate sequentially from the PBR with the highest rate of PBR B, C, A, D, E with μvalue of 1.403 d^{-1}, 1.374 d^{-1}, 0.0862 d^{-1}, 0.0738 d^{-1}, and 0.0616 d^{-1}, respectively. This indicates concentration of this element affecting its growth rate. There is a tolerance limit of the metal content of Fe and Mn which can stimulate the growth of microalgae, and if it exceeds that value it can limit its growth. Decrease in Fe and Mn concentrations shows that Fe and Mn are essential elements for microalgae growth [16]. In this study, it was found that maximum



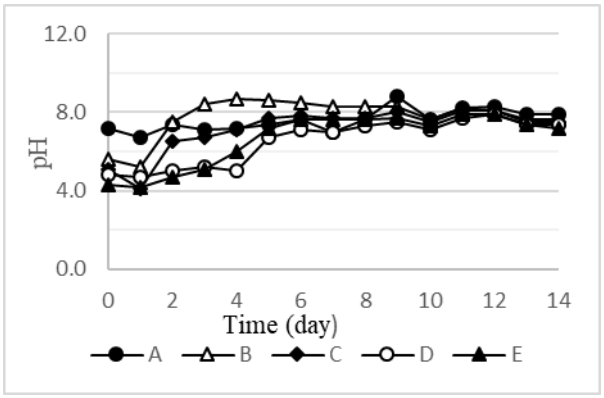


Figure 1. Changes in biomass concentration (a) and pH (b) in Botryococcus braunii culture with variations in AMD concentration

Table 2. Changes in pH, Fe, Mn concentration and specific growth rate

PBR	pH		Fe (mg.L^{-1})		Removal efficiency	Mn (mg.L^{-1})		Removal efficiency (%)	μ (d^{-1})
	t_1	t_{14}	t_1	t14		t_1	t_{14}		
A	7.2	7.9	1.86	1.34	27.96	0.37	<0.023	>94	0.0862
B	5.6	7.6	2.15	2	6.98	0.61	<0.023	>96	0.1403
C	5.1	7.5	2.47	1.2	51.42	0.67	<0.023	>97	0.1374
D	4.8	7.4	3.26	0.67	79.45	0.8	<0.023	>97	0.0738
E	4.3	7.2	3.82	0.6	84.29	0.92	<0.023	>98	0.0616

IV. DISCUSSIONS

Microalgae show the ability to adapt to acidic conditions with different metal contents and this
affects its metabolism. Previous study showed microalgae had a capacity for pH-buffering. This is known to happen in conjunction with its photosynthesis cycle [10], [12]. This pH-buffering ability varied across various microalgae species. In addition, it is also known that Fe and Mn are essential elements for microalgae growth. In this study, it was found that higher concentrations of AMD resulted in a longer lag period in microalgae growth. The increase in microalgae growth occurs at pH 7.0, where the pH supports the growth of microalgae. Based on these observations, it can be concluded that the growth of microalgae occurs along with its ability to neutralize acids. The increase in pH caused by photosynthesis activity causes changes in media conditions to be more suitable for microalgae growth [10], [12].

Aside from being influenced by pH, the growth of microalgae is also influenced by the concentration of Fe and growth was obtained in microalgae with an initial pH of 5.6, and Fe and Mn concentrations of 2.15 and 0.62, respectively.

At the end of the observation, it was found that a higher decrease in Fe concentration in PBR D and E. The difference in Fe uptake was alleged as a result of the acclimatization of *Botryococcus braunii* in batches D and E so that it has a higher removal efficiency. This is possible due to the metabolism of *Botryococcus braunii* in high metal conditions so that *Botryococcus braunii* produces extracellular polymeric substances (EPS) which can absorb metals in the environment and also produce metal precipitate [6], [17], however, this needs to be proven in further study.

V. CONCLUSION

This study was conducted to determine the ability of *Botryococcus braunii* to grow on media with different AMD concentrations. The highest growth of *Botryococcus braunii* was found in media with an initial pH of 5.6, and Fe and Mn concentrations of 2.15 mg.L^{-1} and 0.62 mg.L^{-1}, respectively.

Also known is the adaptability of *Botryococcus braunii* under acidic conditions and high Fe and Mn metal content, where *Botryococcus braunii* plays a role in increasing the pH of the media and is able to absorb Fe and Mn so that it can make AMD as a growing medium for *Botryococcus braunii*.

ACKNOWLEDGMENT

PITTA University of Indonesia financially supported this research with contract number NKB-0784/UN2.R3.1/HKP.05.00/2019.

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