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Sorption Effectiveness of *Nannochloropsis salina* Towards Mn^{2+} Ion in Different Salinity Waters

Y. Hala[a], E. Suryati[b], P. Taba[a], Harjuma, and K. M. Manan[a]

[a]Faculty of Mathematic and Natural Sciences Hasanuddin University, Makassar lndonesia Jl. Perintis Kemerdekaan Km. 10, Kampus Tamalanrea Makassar 90245
[b]Research institute for Coastal Aquaculture, Maros lndonesia Jl. Makmur Dg. Sitakka, Maros 90511
Corresponding author, E-mail address: yusafirhala@gmail.com

ABSTRACT

Research on sorption effectiveness of *Nannochloropsis salina* towards Mn^{2+} ion in different salinity waters has been carried out. This research was conducted to observe the effectiveness of *N. salina* as biosorbent for Mn^{2+} pollutants in waters. Addition of Mn^{2+} ion with concentrations of 2, 4, and 8 ppm respectively, was conducted at the beginning of the *N. salina* growth in Conwy Medium. Those variations were conducted at salinity of 5‰ and 30‰, aeration, continuous illumination, and also at temperature of 20 ^{o}C. The *N. salina* growth after addition of metal ion was observed every day using haemocytometer and the concentration of metal ion after adsorption was determined by atomic absorption spectrophotometer. Results showed that the optimum time for growth of *N. salina* as a control was obtained on day 8th at 30‰ salinity while at 5‰ salinity was obtained on day 15th except for 8 ppm Mn^{2+} was obtained on day 19th. The maximum removal efficiency of Mn^{2+} with the concentrations of 2; 4; and 8 ppm at 30‰ salinity medium was 52.50; 59.25 and 55.38% respectively and for 5‰ salinity, the maximum removal efficiency of Mn^{2+} concentrations of 2, 4 and 8 ppm were 65.50; 64.66 and 67.20% respectively. The optimum time for growth of *N. salina* at 5‰ salinity medium is higher than at 30‰ salinity but the removal efficiency is conversely associated.

Keywords: sorption, nannochloropsis salina, effectiveness, manganese

1. INTRODUCTION

One of the most hazardous heavy metal pollutants is manganese. In 1994 to 2000, Mn^{2+} concentration in Ciliwung river, Jakarta was around 0.03 to 0.7 ppm [1], while in Kahala area, East Kalimantan, it was around 1.4971 ppm [2]; [3]. This concentration exceeded the quality standard in government regulation No. 82 of 2001, which is 0.1 ppm. Another example of the high content of Mn^{2+} ion in the water environment was that in Kuripan river Bandar Lampung, the concentration of Mn^{2+} was 1.31 ppm [4]. Eventhough considered as hazardous, Mn is also essential and give merit in various form of life [5], for example as one of micronutrient playing important role in photosynthesis of plants cyanobacteria [6], helping in chlorophyll production and activating enzymes in Krebs cycle [7]. One of methods to be considered to reduce the concentration of heavy metals in water is biosorption [8], which is relatively cheaper and more effective. Microalgae

Nannochloropsis salina is an example that can be used as biosorbent because of its capability to accumulate heavy metals in its body despite of the high concentration of the metals in the water [9] and wide range of salinity (0 to 25‰) as well [10]. The salinity of different types of water are as follow, 30 to 35 ‰ (seawater), 5 to 25‰ (estuary) and 0.5 to 5 ‰ for freshwater [11].

Alongside with the explanation above, the research on sorption effectiveness of Mn^{2+} with various salinity of culture medium has been conducted using *N. Salina* as biosorbent in the cultivation medium with salinity of 30‰ and 5‰.

2. MATERIAL AND METHODS

A. Materials

Materials used in this research were sterile seawater, sterile natural water, a unialgal strain of *N. Salina*, and Conwy cultivated medium [16]. The unialgal strain of *N. Salina* was obtained from The Research Institute for Coastal Aquaculture, Maros Indonesia; HNO_3 (p.a) solution; double destilled water. Stock solutions of Mn^{2+} (1.000 ppm) were prepared by dissolving 4.5687 g of $Mn(NO_3)_2.4H_2O$ with a few of HNO_3 (p.a) in a 1 L volumetric flask diluted with double destilled water until 1 L. The stock solutions were then diluted to the certain concentration.

B. Apparatus

Apparatus used in this research included haemocytometer Marienfeld LOT-No 4551, hand counter, aerator Amara,

autoclave All American No. 1925X, atomic absorption spectrophotometer (AAS) Buck Scientific model 205 VGP, phase contrast microscope Olympus IX71 magnification 40 times, stirrer Hettich Mikro 22R, and oven Memmert, cellulose nitrate membrane filter Millipore (0.45 µm).

C. Methods

C.1.Optimum time of N. salina growth

Experiment I: Sterile seawater with the salinity of 30‰ was put into a 1 L container, added with 2 mL of Conwy medium and unialgal strain of *N. salina* with the initial populations of about 30 x 10^4 cells/mL. The volume of the mixture was adjusted with sterile sea water to be 1 L. The solution was mixed, connected to an aerator with the culture following conditions: continuous irradiation ($\approx$ 4.000 lux), aeration and room temperature of 20 ℃ [16]. The growth of *N. salina* was observed with a haemocytometer every day until the maximum growth was obtained.

Experiment II: Same as the experiment I procedure, but using sterile natural water with a salinity of 5%.

C.2. Exposure of Mn²⁺ ion into the culture of N. salina

Experiment I: Sterile seawater with salinity 30‰ was put into 4 containers of 1 L, solutions of Mn^{2+} ion with a concentration of 2, 4, and 8 ppm were separately put into 3 containers. Another container was used as a control. A Conwy medium solution (2 mL) and the unialgal strain of *N. salina* (4 mL) with the initial population of 30 x 10^4

cells/mL was added into each container, and the volume was adjusted into 1 L using sterile seawater. The solution was aerated and the growth of *N. salina* was observed every day.

Experiment II: Same as the experiment I procedure, but using sterile natural water with a salinity of 5%.

D. Determination of concentration of metal ion adsorbed by N. Salina.

The solution of metal ion was separated from the residue of *N. salina*, after sorption at the optimum time using a stirer Hettich Mikro 22R for 15 min at a temperature of 4 °C with a rate of 6000 rpm. Then, 2 drops of 2M HNO_3 were added into the solution and its absorbance was measured by AAS.

E. Determination of removal efficiency

The concentration of metal ion adsorbed by *N. salina* (C_s) is the difference between the initial concentration (C_o) and the ion concentration in the solution (C_f), calculated using equation (1). The removal efficiency (R_e) is calculated using equation (2).

$$C_s = C_o - C_f \qquad (1)$$

$$R_e = \frac{C_s}{C_o} \times 100\% \qquad (2)$$

3. RESULTS AND DISCUSSION

A. Optimum time of N. salina growth

In the cultivation medium with salinity of 30‰, the growth of *N. Salina* control seems progressing slowly at first, but then on the 3th to 8th day, the population of *N.*

Salina seems significantly increasing (Fig. 1). This is the result of adaptation of the culture to the medium causing the lag phase, where the activity of cell division is relatively slow but then occuring fast after it has adapted, causing the population doubled rapidly. Growth curve shows that the optimum growth time was achieved on the 8th day with cell population of 136.5×10^4 cells/mL. However after that, the population decreased naturally on the next few days.
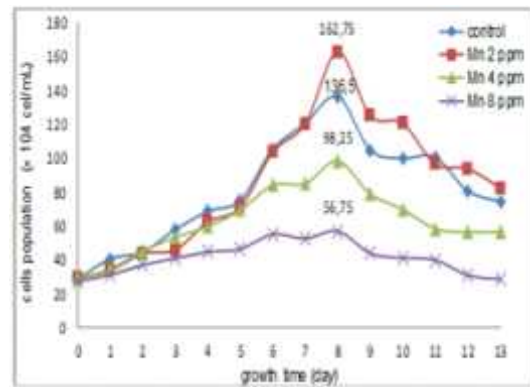

Fig. 1 Population curve of *N. salina* in cultivation medium with salinity 30‰.

Fig. 2 shows the growth of *N. Salina* in cultivation medium with salinity of 5‰. The trend looks similar to the 30‰ medium but with longer optimum time for growth, which is on 15th day and higher population number (471.75×10^4 cells/mL). Research of [10], shows that *N. Salina* grows very well in the salinity of 2.5‰ better than in the salinity of 0; 10 and 25‰ with the optimum growth time of 15th days.
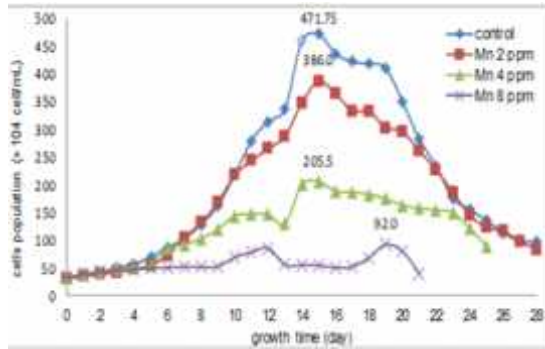
Fig. 2 Population curve of *N. salina* in cultivation medium with salinity 5‰.

Microalgae experiences three stages of growth [12], those are: (1) adaptation stage to the medium, (2) division stage started after the microbe absorbs nutrient from the medium, and (3) growing stage followed by the death stage. The decrease in population number in the medium was caused because of the declination of nutrient in the medium affecting the ability for cell division [13]. The accumulation of organic substances manifested by the death of biomass, becomes the new competitor of *N. salina* in obtaining the dissolved oxygen in growth medium [16]. The growth of *N. salina* exposed to Mn^{2+} observed having the same pattern as the control where the population number of *N. salina* experience increment up to the optimum time and then decrease as the time by.

In the cultivation medium with salinity of 30‰, exposed *N. salina* to Mn^{2+} with concentration of 2 ppm reached highest population at the 8th day, which was 162.75×10^4 cells/mL. This number is higher than in control, as result of low concentration of Mn^{2+} in the medium which is optimum for

the algae's growth as Mn^{2+} is one of essential metal needed in trace amount by the organism. Ion Mn^{2+} plays important role in metabolism process, especially in photosynthesis, activation and cofactor various enzymes, such as Mn-catalase, Carboxylase pyruvate, and phosphoenolpyruvate [14]; [15]. Exposed cultivation medium of *N. salina* with concentration of 4 and 8 ppm reach lower population number, which were 98.25×10^4 cells/mL and 56.75×10^4 cells/mL respectively at the time of optimum growth. Metal concentration linearly correlated with growth impedance. Exposure of 8 ppm of Mn^{2+} shows greater hindrance than those of other concentration. In the culture with salinity of 5‰, optimum growth time of exposed *N. salina* with 2 and 4 ppm concentration are reached after 15th day, with population of 386.0 and 205.5×10^4 cells/mL respectively. While in the culture with 8 ppm of Mn^{2+}, optimum growth time was 92×10^4 cells/mL. [16] explained that the metal ion in the culture can act as hindrance to the growth and reproduction of the organism, as result of metal interaction on the surface of microalgae cells which contain lipid tissue. Microalgae's body surface consisted of cell membrane having potential to interact with metal ion in the water [17], containing functional group such as N-terminal from NH_2, C-terminal from group $-COO^-$, S-terminal from $-SH$ group, and side chain functional group acting as metal binder on the body surface of microalgae cell [18].

B. Removal efficiency of N. salina

Fig. 3 shows that at the culture with salinity of 30‰, sorption of Mn^{2+} by *N. salina* started at the beginning of exposure. The exposure of Mn^{2+} with concentration of 2; 4; and 8 ppm at first day achieve R_e of 43.5; 57.5; and 55.38% respectively. R_e value insignificantly fluctuating from day 1 to day 10, since *N. salina* will try to reach the metal sorption balance during the biosorption process. This is very much affected by the active site of *N. salina* [19]. Numbers of active site binding in the microlgae shows that the steric effect happens on the surface after first sorption mechanism occur, so it is probably taking longer time to reach balance [20].
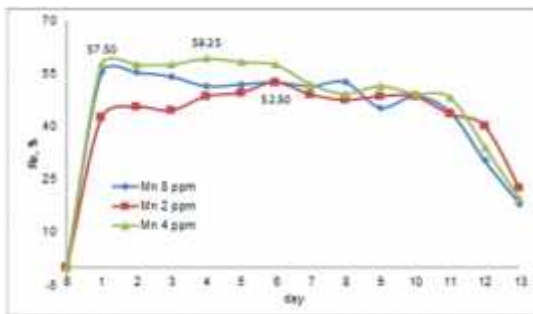

Fig. 3 Trend of R_e curve at cultivation medium with salinity 30‰

Maximum R_e value for exposed *N. salina* with concentration of 2; 4; and 8 ppm were obtained at 6th, 4th, and 2nd day, with value of 52.5, 59.25, and 55.38%. In 2 ppm concentration, *N. salina* is able to tolerate Mn^{2+} presence in the medium. The lowest R_e value for the concentrations achieved on 13th day, which were 22.5; 19.5; 18.25%. These value differ significantly for each variation if

compared at 12th day of observation. This is because of the number of *N. salina* to absorb also decreases in time. Decrease of R_e value also indicates the occurence of release of ion or molecule that have been bonded in the active site of algae. This is considered to be self defense mechanism of the microalgae so that the bonded metal ion could be released and back to the medium.

Fig. 4 shows the maximum sorption of Mn^{2+} by *N. salina* with concentration of 2; 4; and 8 ppm in the cultivation medium with salinity of 5‰ occured on 3rd day, 4th day, and 1st day with R_e of 64.29, 64.19 and 67.26 respectively. This proves that the capability of *N. salina* to adapt and absorb Mn^{2+} with concentration of 8 ppm is higher than those of 2 and 4 ppm. R_e value illustrated that Mn^{2+} absorption by *N. salina* is most likely to happen at the beginning of contact. The order of maximum R_e for each Mn^{2+} concentration with the salinity of 30‰ is 4 ppm>8 ppm>2 ppm, while at 5‰ salinity, it is 8 ppm> 2 ppm> 4 ppm.
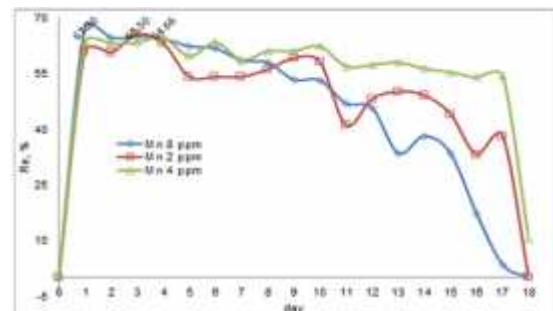

Fig. 4 Trend of R_e curve at cultivation medium with salinity 5‰

4. CONCLUSIONS

The optimum time of *N. salina* growth as a control was obtained on day 8th at 30‰ salinity while at 5‰ salinity was obtained on day 15th except for concentration of 8 ppm Mn^{2+} was obtained on day 19th. The maximum removal efficiency of Mn^{2+} with the concentrations of 2; 4; and 8 ppm at 30‰ salinity medium was 52.50; 59.25 and 55.38% respectively and for 5‰ salinity, the maximum removal efficiency of Mn^{2+} concentrations of 2, 4 and 8 ppm were 65.50; 64.66 and 67.20% respectively. The optimum time for growth of *N. salina* at 5‰ salinity medium is higher than at 30‰ salinity but the removal efficiency is conversely associated.

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