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EFFECT OF SALINITY AND DENSITY OF *Artemia salina* ON MAGNESIUM AND CALCIUM IMPURITIES LEVEL IN SALT PRODUCTION

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ABSTRACT

Salt is one of the versatile commodities that are used from the household to the chemical industry. The number of national salt production is still not sufficient so far, particularly those which is used for industrial needs. The quality of salt is determined by the concentration of NaCl. The concentration of NaCl in salt for consumption must be higher than 94% while salt for industrial use must contained NaCl higher than 98%. The residual components of salt are impurities, including magnesium and calcium. Artemia is organism that lives in sea water and has ability to intake magnesium and calcium in sea water for biological activity. This study is intended to determine the optimal density of *Artemia salina* and sea water salinity in decreasing concentration of magnesium and calcium in sea water to improve the quality of salt. *Artemia salina* cysts was hatched and reared to reach adult phase. Artemia was used in beaker glass for decreasing magnesium and calcium in sea water. The content of magnesium, calcium, chloride, and sulfate in sea water was analyzed. The result of this study showed that the reduction of ion content in sea water, e.g. magnesium, calcium, sulfate, and chloride content was slightly constant. The effect of salinity in decreasing concentration of magnesium and calcium was not shown as the alteration of salinity did not affect the reduction of magnesium and calcium significantly. The optimum salinity to decrease the concentration of magnesium and calcium is 6.8% while the optimum density of Artemia is 500 individuals per liter.

KEYWORDS: ***Artemia salina, magnesium, calcium, chloride, sulfate***

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INTRODUCTION

The quality of salt that has been produced by farmers in Indonesia is still too far to meet the current standards, both for consumption and industry use. Consequently, the price of salt is so cheap that the salt farmers could not get high income from salt selling. Moreover, the needs of salt for industry were not fulfilled because the quality of salt is only around 80% of NaCl with the residual components are impurities. The impurities contained in salt are magnesium and calcium that will precipitate at same salinity with NaCl. Thus, the salt that are produced will have high magnesium and calcium content. It is required to perform methods for decreasing magnesium and calcium content in sea water that would be used as raw material for salt production. Artemia is classified as crustaceans of the Arthropoda phylum. Artemia is generally used for both aquaculture and marine aquaculture as feed that contains high nutrients for fisheries. Artemia live in the environment of 3-30% salinity. Ion contents in the medium are used by Artemia for the biological activity. The existence of *Artemia salina* in salt ponds has contributed for improving the quality of produced salt. Artemia can absorb magnesium and calcium for its growth, forming an exoskeleton and run the metabolism. With this capability, Artemia can be utilized on salt ponds to reduce the content of magnesium and calcium in sea water. The impurities will then reuse to produce another salt. Salinity is one of the important physical factors in the life of marine organisms, including Artemia[1]. Salinity will also affect egg hatching, Artemia resistance in growth phase (nauplii phase and juvenile phase), and biomass content in Artemia. The effect of salinity on Artemia cyst binding relates to the degree of hydration achieved by Artemia cysts. Salinity also affects the amount of glycerol required to reach the critical state by the osmotic pressure in the outer cuticle membrane of the cyst[2]. At the naplii and adult levels, best physiological performance of Artemia is at a lower salinity (from 32 g /l to 65 g /l) in terms of growth rate and food conversion efficiency.Artemia gets the supply of calcium and magnesium from the environment[3]. The calcium ion is coordinated with Glu protein, while magnesium is coordinated with alanine protein. These ions are also involved in the process of homeostasis, to maintain the concentration of fluids in the body[4]. Magnesium involved in energy formation and DNA multiplication. These minerals, including calcium and magnesium are required for growth, especially in the nauplius conditions. This accumulation is expected to build the exoskeleton nauplius[5-6]. This study was conducted to decrease the impurities in sea water using *Artemia salina* for the salt production. Artemia was cultivated in the reactor. Salinity of the seawater and the density of *Artemia salina* were varied. The variation test of salinity was done by creating artificial sea water according to the desired salinity while the variation test for density of Artemia was done by manually counting the number of *Artemia salina*. These variations were performed to see the effect of sea water salinity and density of Artemia in decreasing magnesium and calcium content in sea water.

METHODS

Materials.

Artemia that were used in this study were obtained from cultivated Artemia cysts. Sea water that was used for cultivating Artemia came from Pari Island with salinity of 3-4%. Artificial sea water will be used in decreasing impurities by diluting 26.85 g of NaCl; 4.57 g of Na_2SO_4; 0.777 g of KCl; 0.196 g of $NaHCO_3$; 1.68 g of $CaCl_2.2H_2O$; 12.32 g of $MgCl_2.6H_2O$ with a litre of aquadest to obtain sea water with salinity 4%. Package of mineral water with volume of five gallons was used for Artemia cultivation.

Artemia salina Culture.

Culture was filled by sea water with 3-4% of salinity and volume of 10-15 liter. Artemia cysts were put in the culture with the weight of 2 gr/L of medium. Culture was aerated, illuminated with 2000 lux of lamp, and fed with 20 g of microalgae medium. Artemia hatched in first until second day of hatching and grew adults in 2-3 weeks of cultivating process. These adult Artemia was ready to use for decreasing magnesium and calcium. Figure 1 shows the illustration of Artemia culture.

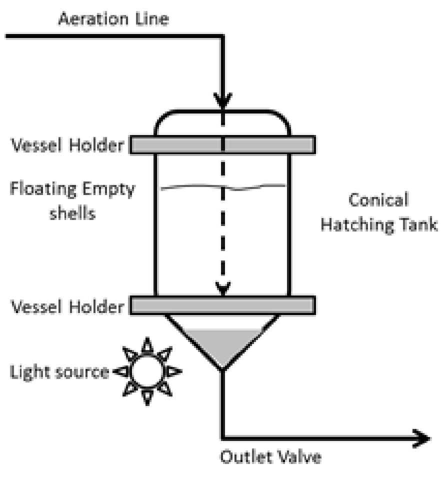

Figure 1
Illustration of conical tank for Artemia culture

Magnesium and Calcium Content Decreasing Test in Sea Water by Artemia.

500 ml of artificial sea water was poured into beaker glass. Artemia were filtered using plankton net from the culture. Prior to the filtering, the number of Artemia was counted to obtain certain number of Artemia. Filtered Artemia was put into beaker glass that contained artificial sea water with certain salinity. Decreasing test of magnesium and calcium content by Artemia was done with aeration for 8 hours. The sample was taken every hour with volume of 15 ml. Variations that were performed were salinity on 5%, 7%, 9%, and 11%; and density of Artemia for 200, 300, 400, and 500 individuals per liter.

Analysis of Magnesium, Calcium, Chloride, and Sulfate Content.

The content of magnesium and calcium in sample were analyzed using Atomic Absorption Spectroscopy (AAS) with wavelength of 282.5 nm for magnesium and 422.7 nm for calcium. On the other hand, the content of chloride was analyzed with argentometry titration method while the content of sulfate was analyzed with turbidimetry method using spectrometer with wavelength of 420 nm.

RESULTS

Artemia Salina Culture.

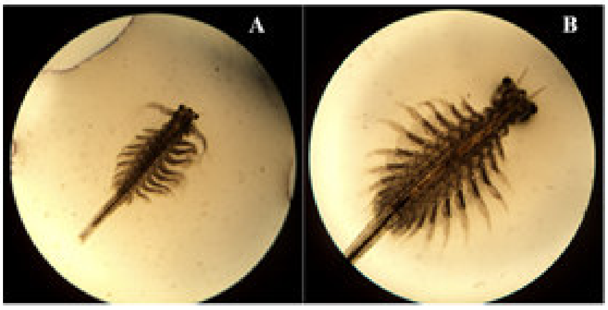

Figure 2
The Artemia salina in the stage: a) female nauplii (40x magnitude); and b) female juvenile (40x magnitude)

The development of *Artemia salina* captured by microscope with various magnification. The dormant cyst was characterized by circular skin covering the egg. After several hours, it can be seen from the microscope with 40 times magnitude that several cysts have hatched. Figure 2 shows the female nauplii and juvenile after nine days from hatching.

Magnesium Concentration.

The analysis of magnesium concentration was performed to see the effect of Artemia addition in magnesium concentration every hour. Figure 3 shows the reduction of magnesium concentration in sea water by various density of Artemia with salinity of 3.18%.

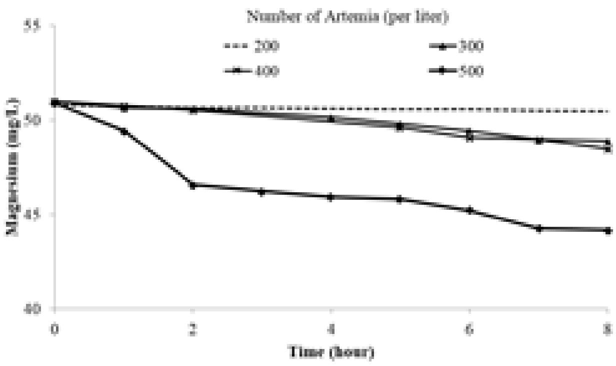

Figure 3.
The reduction of magnesium concentrations in sea water by various density of Artemia

It can be seen from Figure 3 that the decreasing of magnesium concentration occurred in all variations density of Artemia. However, the magnesium concentration were most decreased in density of 500 individuals per liter. By this result, it is concluded that the increasing number of Artemia will decrease the more concentration of magnesium. In density of 300 and 400 animal per liter of Artemia, the value of magnesium decreased was not different significantly in eighth hour. It could occur because of the different ability that possessed by Artemia in 300 individuals per liter density and 400 individuals per liter density. Figure 3 also shows that Artemia could deacrese magnesium concentration in sea water as the effect of the Artemia addition. This proves that Artemia could utilize magnesium from sea water.

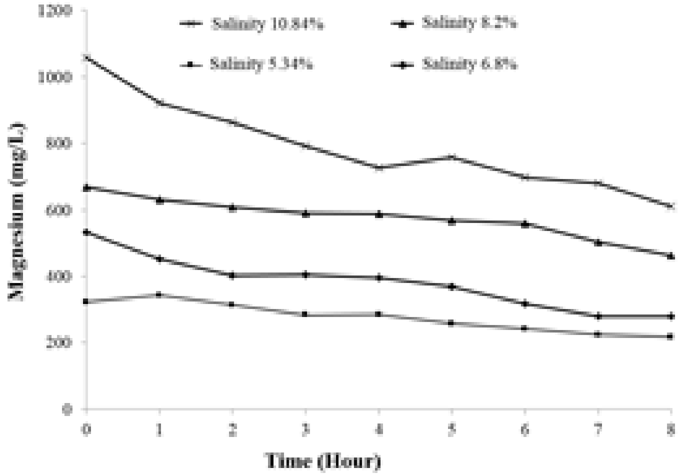

Figure 4.
The reduction of magnesium concentrations in sea water by various salinity

Figure 4 shows the reduction of magnesium concentration in all salinity variation with Artemia density 200 individuals per liter for eight hours. Figure 4 also shows that salinity of 10.84% was occurred as the most decreasing number of magnesium from all salinities.

Calcium Concentration.
Figure 5 shows the calcium concentration in sea water by various density of Artemia with salinity 3.18%.

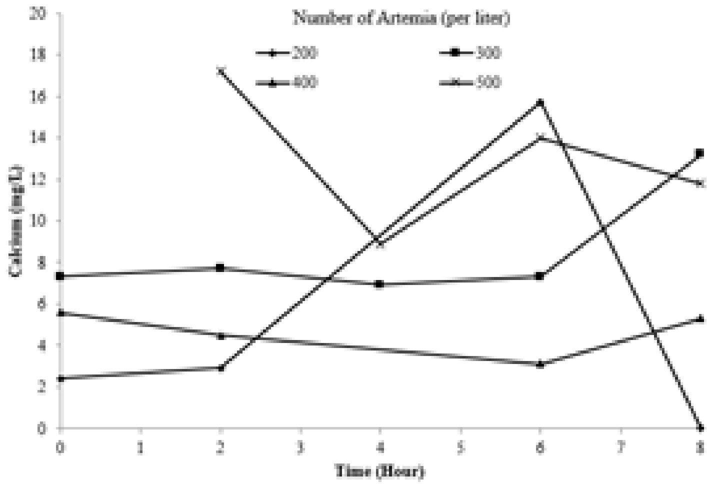

Figure 5
Calcium concentrations in sea water with Artemia density variation

Figure 5 shows that calcium concentration in sea water was fluctuated. Figure 5 also shows there was no relationship between density of Artemia and the reduction of calcium concentration. Figure 6 shows calcium concentration in salinity variation with 200 individuals per liter of sea water.

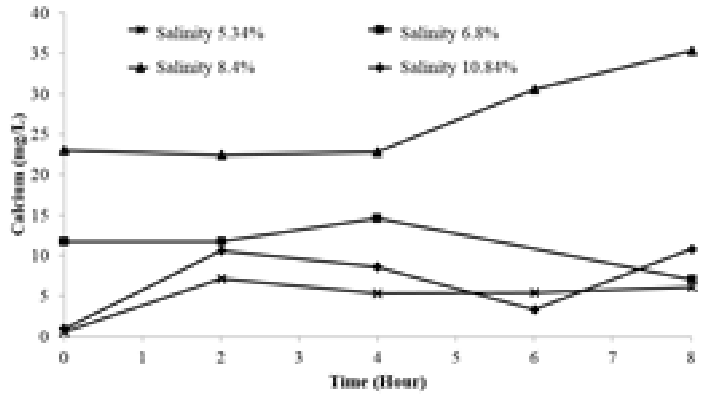

Figure 6
Calcium concentrations in sea water with salinity variation

Figure 6 shows that calcium concentration was fluctuated. This phenomenon was occurred in all variation, except in salinity 6.8%. In salinity 6.8%, calcium concentration was decreased at eighth hour compared to hour to zero.

Chloride Concentration.

Chloride concentration is related to NaCl which is main product of salt production. Figure 7 shows chloride concentration in salinity variation with Artemia density 200 individuals per liter.

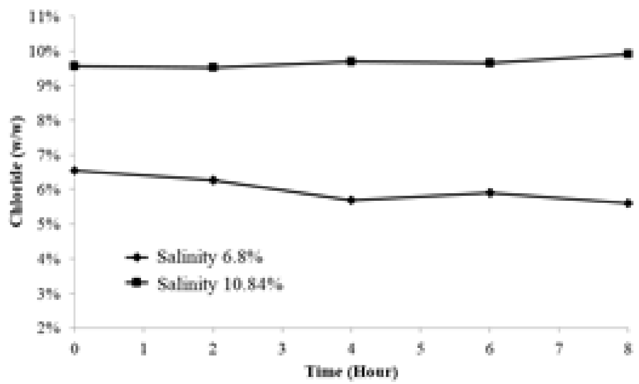

Figure 7
Chloride concentrations in sea water with salinity variation

Figure 7 shows chloride concentration was not significantly changed. Figure 8 shows chloride concentration in Artemia density variations with salinity 3.18%.

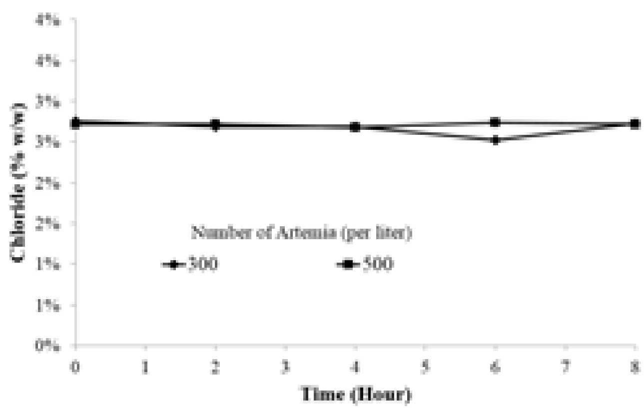

Figure 8
Chloride concentrations in sea water with Artemia density variation

Sulfate Concentration.
Sulfate concentration analysis was performed to see effect of Artemia to sulfate concentration in sea water Figure 9 shows that sulfate concentration in Artemia density in salinity 3.18%.

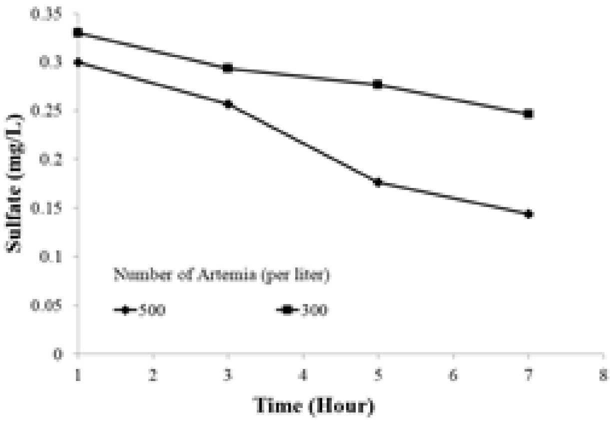

Figure 9
Sulfate concentrations in sea water with Artemia density variation

Figure 9 shows that sulfate concentration decreased. Sulfate reduction value was higher with addition of Artemia density. Sulfate concentration also was analyzed with salinity variation. Figure 10 shows sulfate concentration with salinity variation using Artemia density 200 individuals per liter.

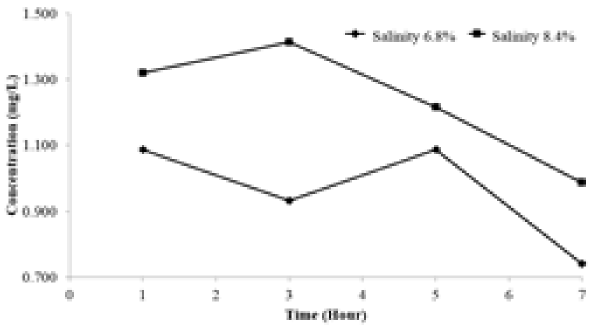

Figure 10
Sulfate concentrations in sea water with salinity variation

Figure 10 shows decreasing of sulfate concentration. This decreasing occurred in all salinity variation. To compare value of reduction of sulfate, graph from Figure 10 was converted into percentage with first hour as a basis. Figure 11 shows sulfate concentration in percentage. It can be seen from Figure 11 that sulfate reduction value in salinity 6.8% with 30% reduction of sulfate concentration. For salinity 8.4%, sulfate concentration decrease 20% from first hour. This phenomenon also occurred in magnesium and calcium analysis that the most sulfate reduction value occurred in salinity 6.8%.

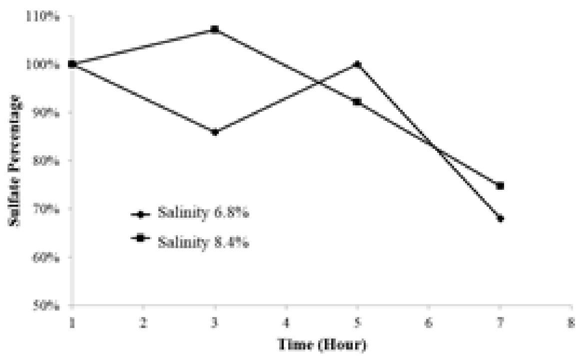

Figure 11
Sulfate percentages in sea water with first hour as basis in salinity variation

DISCUSSION

In this study, the concentration of magnesium decreased during observation. This decreased might happen due to magnesium uptake from sea water by the Artemia related to osmoregulation activity. Magnesium is essential for growth and survival of Artemia. Magnesium also has significant role in lipid, protein, and carbohydrat metabolism as cofactor in enzymatic and metabolic reaction[7]. Low concentration of magnesium ion can cause high respiration rate. High respiration rate in low magnesium concentration lead to stress and decrease of survival. Moreover, magnesium has role to activate energy, by absorbed it into haemolymph and the excreted to activate energy in Artemia[8]. Salinity is one of the important factors in survival and reproduction[9]. It is reported that high number of survival which reached 70-80% in 21 day of Artemia culture was obtained by salinity 6%[1]. On the contrary, lower salinity (below 5%) can decrease the survival number of Artemia due to the existence of predators[9]. Moreover, nutritional deficiencies also affect the survival number of Artemia as the organism undergoes stress in low salinity environment. Stress that triggered by low salinity is related to osmotic pressure in Artemia[10]. However, the salinity higher than 8% can retard growth of Artemia[11]. It can be concluded that both hypertonic and hypotonic conditions can decrease growth efficiency because nutrition is allocated for adapting in pressure osmotic environment[12]. Therefore, salinity 6% is the optimal salinity for Artemia, especially in the decreasing of magnesium and calcium. Magnesium concentration analysis showed that magnesium concentration decreased with the more addition of Artemia. By this analysis, it can be seen that the more Artemia was added to medium, the more value of magnesium reduction in sea water. In salinity variation, the optimal value of magnesium reduction was occurred in salinity 6.8% based on percentage of reduction while the optimal value of magnesium reduction occurred in salinity 10.68%, based on number of reduction. Thus, salinity 10.68% is an alternative salinity in decreasing magnesium and calcium concentration in sea water. In salinity variation, there was no significant relationship between salinity and number of magnesium reduction in sea water. Calcium concentration was fluctuated in calcium analysis. This phenomenon occurred due to the moulting or releasing of Artemia exoskeleton. Exoskeleton and hard tissue that owned by Artemia contain calcium carbonate[13] that can increase the calcium content in the sample. Artemia utilized medium for osmoregulation activity. Some of the ions that contained in the medium, including the calcium absorbed in ephitelium of Artemia[14]. Calcium is essential for animals to build hard tissue structure and as a cofactor for cell enzymatic reactions. The outer shell or exoskeleton of crustacean animals, including Artemia contained calcium. Calcium was absorbed into the body from the environment[15]. The chloride concentration is related to the concentration of NaCl in sea water. The content of NaCl and other ions in sea water associated with Artemia osmoregulation. Artemia absorb water into the body to maintain the steady condition of haemolymph in hypertonic medium. Inspite of the lack of the medium, this activity will be still continued[16]. Water that was absorbed into Artemia body would pass through the epithelium, a simple tube gut that formed by a single layer of epithelial cells. Absorption of NaCl and other ions was occurred in the epithelium into the haemolymph. This was indicated that water which was absorbed by Artemia had higher osmotic pressure than in the epithelium[16]. The concentration of chloride that related to concentration of NaCl did not change significantly. This condition benefits Artemia as NaCl was the main product. Reduction of the sulfate in seawater was higher due to the capability of Artemia to absorb sulfate into its body. The phenomenon occured as the Artemia absorbed the medium that contained sulfate. Sulfate ion will then be absorbed to epithelium. Sulfate was important in metabolism of Artemia, particularly in xenobiotic metabolism that occured in marine invertebrate animals. Sulfate was converted into sulfonate in this metabolism. The sulfonate groups will generate negative ions which initiated ion interactions in cell. Sulfate ion can also stimulate xenobiotic excretion. Therefore, this will affect the sulfate content of the biological activity of the organism[17].

CONCLUSION

The initial salinity did not significantly influenced the reduction rate of magnesium and calcium. However, the Artemia density affected the reduction of magnesium and calcium concentration, as more dense Artemia showed more Mg and Ca uptake. This study showed the optimal salinity for decreasing magnesium and calcium was at 6.8% while the optimal Artemia density for decreasing magnesium and calcium was 500 individuals per liter.

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CONFLICT OF INTEREST

Conflict of interestdeclared none.

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