# THE EFFECT OF MAGNESIUM CONCENTRATION ON PROTEIN AND CHOROPHYLL a CONTENT OF *Nostoc* spp. REARED IN TWO CULTURED MEDIA

***Pengaruh Kadar Magnesium Terhadap Kandungan Protein dan Khlorofil a Pada Nostoc spp Yang Dipelihara Dalam Dua Media Kultur***

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## *ABSTRAK*

*Nostoc merupakan jenis blue green alga yang bisa dijadikan sumber protein potensial bagi manusia. Penelitian ini bertujuan untuk mengevaluasi kandungan protein dan klorofil a dari Nostoc yang dikultur di dua media, Modifikasi Bristol dan BG 11 media, dengan perbedaan konsentrasi $MgSO_4.7H_2O$. Di antara kedua media kultur, Nostoc yang dikultur pada modifikasi Bristol media memiliki protein dan klorofil a lebih tinggi dari Nostoc yang dikultur dengan BG 11 media. Berat kering Nostoc dengan Bristol (0.02 - 0.34 gram) lebih tinggi dari BG 11 (0.02 – 0.14 gram). Kandungan protein Nostoc pada Modifikasi Bristol media lebih tinggi (283.58 – 79149.77 µg/gram berat kering) sedangkan yang dikultur dengan BG 11 media (19.31 – 2536.95 µg/gram berat kering). Untuk kandungan klorofil a BG 11 media broth (0.003 – 1.67 mg/gram berat kering) lebih tinggi dari pada Modifikasi Bristol media (0.04 – 1.05 mg/gram berat kering). Perbedaan konsentrasi $MgSO_4.7H_2O$. pada kedua media kultur berpengaruh pada kandungan protein dan berat kering dari Nostoc, akan tetapi tidak berpengaruh pada kandungan klorofil a. Penambahan 0,075 gram/liter konsentrasi $MgSO_4.7H_2O$ selama tiga minggu kultur merupakan kondisi optimal untuk mendapatkan kadar protein dan klorofil a yang optimal untuk modifikasi Bristol media sedangkan pada BG 11 media kandungan protein dan klorofil a yang dihasilkan optimal pada 0,000 gram/liter $MgSO_4.7H_2O$. selama satu minggu kultur.*

*Kata kunci : Cyanobacteria, Nostoc, Protein, klorofil a*

## INTRODUCTION

Prokaryotic blue green algae (cyanobacteria) are amongst the most primitive life forms on the earth. These prokaryotes share structural features with plant, such as having the ability to perform photosynthesis. Moreover, they share structural features with primitive bacteria in that they lack a cell wall. One genus of blue green algae, Nostoc, has been used for food for thousand of years. Indigenous populations in communities as diverse as the people of Japan and Hawaii, and those that occupy terrain where there are large fresh water lakes, have long recognized the value and health benefit of blue green algae in their diets.

Blue green algae is both useful and a nuisance to humans. Some spesies are regionally popular edible delicacies (Emralino & Rudolfo, 1987). Nostoc can be a nuisance on sport turf (Baldwin & Whitton, 1992), can cause unpleasant odours in drinking water and can foul buildings (Wnorowski, 1992). Human pathogens (fungal and bacterial) are inhibited by phenolic extract from Nostoc (De Cano et al., 1990): it is possible that Nostoc may be used by biotechnologist for unique medical compounds (De Mule et al., 1999). The aim of this study was to determine the effect of different concentration of $MgSO_4.7H_2O$ from two kinds of culture media for protein content, and chlorophyll a content of Nostoc.

## METHODOLOGY

**Isolation and Purification Colonies of *Nostoc:*** *Nostoc* spp. isolated from the organic paddy field soil in Udonthani province were incubated at room temperature, light intensity of 1160 lux, and 60% for moisture during one month with blue green algae nitrogen-fixing agar (Atlas, 1993).

**Cultivation of *Nostoc*:** *Nostoc* was cultivated in BG–11 medium and modified Bristol's solution. The micro colonies obtained from the agar plates containing specific medium culture for nitrogen-fixing blue green specific medium culture for blue green algae nitrogen-fixing agar were transferred to both of media. Transfer isolated and purified micro colony of *Nostoc* sp from specific medium culture for nitrogen-fixing blue green into the BG 11 and modified Bristol's solution with varied concentration of $MgSO_4.7H_2O$ from 0.000 to 0.100 gram/litre (0.000, 0.025, 0.050, 0.075, and 0.100 gram/litre). *Nostoc* harvested every week for analyses.

**Determination of Protein Content:** The protein was quantified according to the colorimetric method of Bradford (1976). *Nostoc* crushed with mortar and pestle in NaCl 0.85%, pipeted into a test tube tube and centrifuged at 3000 rpm for 30 min. A 80 µL of supernatant of the crude protein extract was taken and put into a labeled plastic centrifuge tube before diluted in 720 µL NaCl. Drop wise add 200 µL of the Bio-Rad dye solution. The absorbance of the solution was measured at 595 nm. Plot the absorbance versus concentration of protein for each amount of BSA result were calculated and expressed as microgram of Bovine Serum Albumin (BSA) per gram dry weight of *Nostoc*.

**Determination of Chlorophyll a Content:** the chlorophyll a of *Nostoc* was fractionated to 80 % acetone extractable by extraction procedure. The chlorophyll a content of the fraction was evaluated using spectrophotometer. Calculations of Chlorophyll a, and Carotenoid from absorbance to mg Chl $g^{-1}$ dry weight of *Nostoc* following Arnon's formula:

$Chl_a$ (mg $g^{-1}$) = [(12.7 x $A_{663}$) – (2.6 x $A_{645}$) x ml acetone / mg dry weight of *Nostoc*

$Chl_b$ (mg $g^{-1}$) = [(22.9 x $A_{645}$) – (4.68 x $A_{663}$) x ml acetone / mg dry weight of *Nostoc*

Total Chl = $Chl_a + Chl_b$

$C_{x-c}$ = 1000 $A_{470}$ – 1.90$Chl_a$ – 63.14 $Chl_b$/24

[x = xanthophylls and carotenes]

## RESULT AND DISCUSSION

**Culture:** ***Nostoc,*** different treatment concentration of $MgSO_4.7H_2O$ in the culture media affected to the dried weight content of *Nostoc* . The variation of percentage of dried weight content of *Nostoc* ranged from 0.02 – 0.34 gram/gram wet weight for 2 media with different concentration of $MgSO_4.7H_2O$ in the culture media. *Nostoc* cultured in BG 11 medium with the concentration of $MgSO_4.7H_2O$ 0.050 gram/litre at 4th week had much lower percentage of dried weight content than the concentration of $MgSO_4.7H_2O$ 0.050 gram/litre at 1st week. For the *Nostoc* cultured with modified Bristol's solution, the percentage of dried weight content much lower in *Nostoc* cultured in modified Bristol's solution with concentration of $MgSO_4.7H_2O$ 0.100 gram/litre at 1st week than the high concentration of $MgSO_4.7H_2O$ 0.1 00 gram/litre at 3rd week (Figure 1).

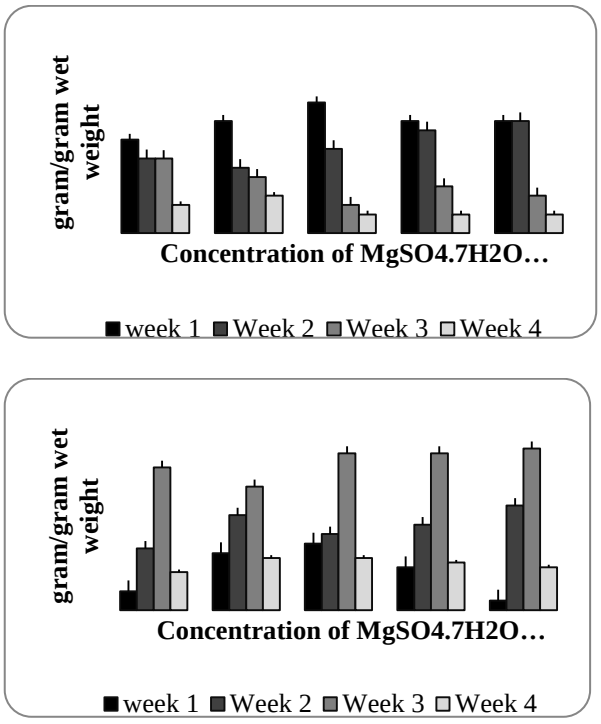


Figure 1. Percentage of Dry weight content of *Nostoc* cultured in BG-11 medium (left) and modified Bristol's solution (right)

This result was expected since *Nostoc* cultured with different media with different concentration of $MgSO_4.7H_2O$ will produce different yield. Both culture media showed increasing wet weight of *Nostoc* yield by the additional concentration of $MgSO_4.7H_2O$ and the differences was statistically significant. Peaks of wet weight yield of $MgSO_4.7H_2O$ treatments were at first week for BG 11 and at third week for Modified Bristol's solution, probably indicated reduction of magnesium content in culture media due to high magnesium requirements during growth period of *Nostoc*. Finkle and Appleman (1953) reported magnesium deficiency has been observed after a period of growth in different culture solutions of *Chlorella vulgaris*. The modified Bristol's solution can support longer growth period of *Nostoc* growth because it contains $K_2HPO_4$, in

which has similar function with $MgSO_4.7H_2O$, that more capable to increase cell growth of Nostoc (Ogawa & Carr, 1969).

**Protein Content:** different treatment $MgSO_4.7H_2O$ in the culture media was also affected to the protein content of *Nostoc*. The variation of protein content of *Nostoc* ranged from 19.31 to 79149.77 µg BSA/gram dry weight for 2 media with different concentration of $MgSO_4.7H_2O$ in the culture media. *Nostoc* cultured in BG 11 medium supplemented with the high concentration of $MgSO_4.7H_2O$ 0.100 gram/litre at 4th week had much lower protein content than low concentration of $MgSO_4.7H_2O$ 0.000 gram/litre at 1st week. For the *Nostoc* cultured with modified Bristol's solution medium, the protein content was much lower in *Nostoc* supplemented with the high concentration of $MgSO_4.7H_2O$ 0.100 gram/litre at 1st week than *Nostoc* supplemented with the concentration of $MgSO_4.7H_2O$ 0.075 gram/litre at 3rd week (Figure 2).

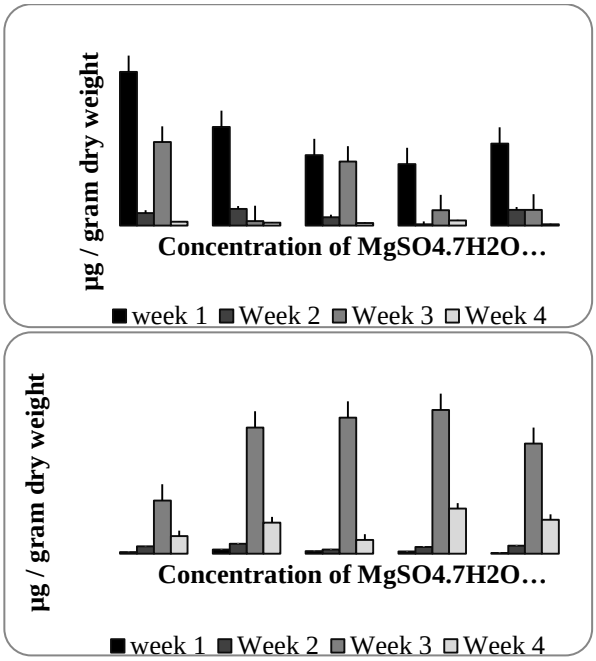


Figure 2. Protein content of *Nostoc* cultured in BG-11 medium (left) and modified Bristol's solution (right)

This result was expected because different concentration of $MgSO_4.7H_2O$ in the culture media affects the protein content of *Nostoc*. Pattern of protein content shown in Figure 2 was consistent with the percentage of dry weight content shown in Figure 1, where peaks of protein content of *Nostoc* cultured in BG 11 and modified Bristol's solution were 1st week for and 3rd, respectively. The difference of $MgSO_4.7H_2O$ supplementation to the protein content of *Nostoc* was statistically significant, particularly in modified Bristol's solution. According to percentage of wet weight data, *Nostoc* cultured in modified Bristol's solution has more number of heterocyst represented by wet weight content. The divalent cation of magnesium ($Mg^{2+}$) has been found to induce of heterocysts formation and more hydrogen formation in *Nostoc*. The increase in frequency of heterocyst occurred only in the active growth phase. Induction of heterocyst could be possibly due to suppression of ammonium level, leading to enchached protein content, in case of

organic substances. Some energy metabolism may be linked with inorganic substances like $Ca^{2+}$ and $Mg^{+}$ responsible for induction of heterocyst in *Nostoc* and $H_2$ metabolism (Dawar et al., 1999).

**Chlorophyll a Content:** different treatment $MgSO_4.7H_2O$ in the culture media did not affected to the Chlorophyll a content of *Nostoc*. The variation of chlorophyll a content of *Nostoc* varied from 0.003 to 1.67 mg/gram dry weight of *Nostoc* for 2 media with different concentration of $MgSO_4.7H_2O$. *Nostoc* cultured with BG 11 medium supplemented with high concentration of $MgSO_4.7H_2O$ 0.100 gram/litre at 4th week had much lower chlorophyll a content than concentration of $MgSO_4.7H_2O$ 0.000 gram/litre at 1st week. For the *Nostoc* cultured with modified Bristol's solution, the chlorophyll a content was much lower in concentration of $MgSO_4.7H_2O$ 0.100 gram/litre at 1st week than concentration of $MgSO_4.7H_2O$ 0.000 gram/litre at 3rd week (Figure 3).

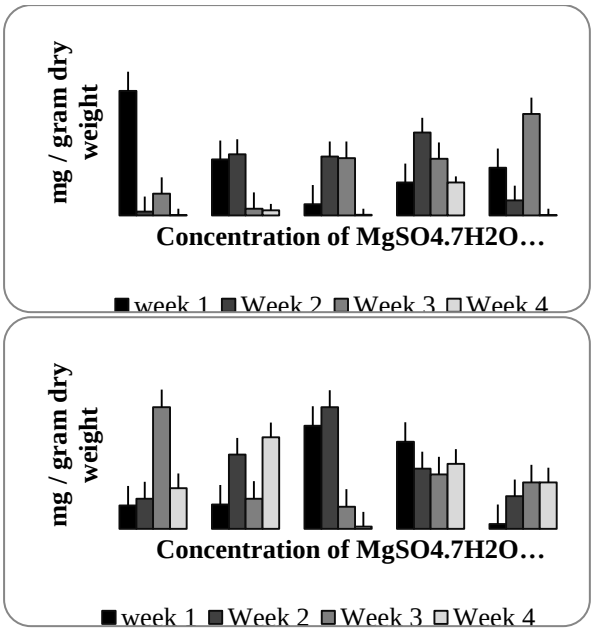


Figure 3. Chlorophyll a of *Nostoc* cultured in BG-11 medium (left) and modified Bristol's solution (right)

The difference of chlorophyll a content of *Nostoc* cultured in both culture media was not statistically significant, indicated that there was no effect on the variations of $MgSO_4.7H_2O$ concentration. The magnesium has important function as *Nostoc* nutrition during photosynthesis, particularly in chlorophyll a formation. Magnesium is a central metal ion, which is bonded to a larger organic molecule called a porphyrin ring (Bohn et al., 2004) and responsible for electron transfer during photosynthesis. In this study, however, magnesium was not directly affected to the chlorophyll a content. It was probably affected by different pH in the culture media. Although we did not observed pH change during *Nostoc* culture and compared them among culture media, different pH during media preparation (7.1 and 6.8 for BG 11 and modified Bristol's solution, respectively) may explain inconsistent pattern of chlorophyll a data. Magnesium ($Mg^{2+}$) is a positively charged ion. When the pH is lowered (increase in hydrogen ion concentration or $H^{+}$), the magnesium is displaced by hydrogen ion and chlorophyll is reduced

because it will be converted into pheophytin which is olive green (Bohn et al., 2004).

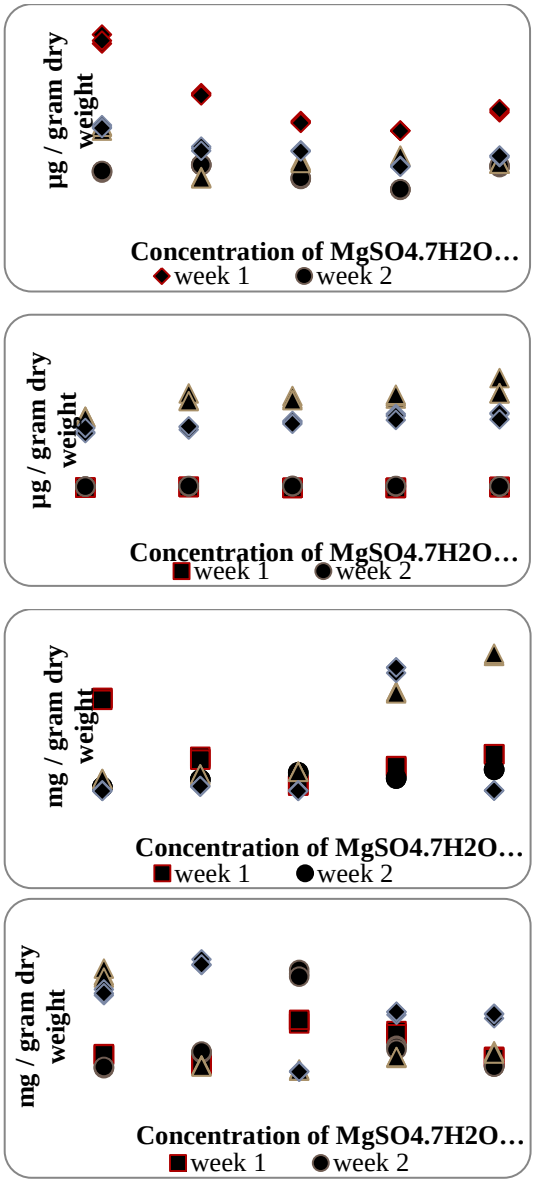


Figure 4. Correlation of $MgSO_4.7H_2O$ to the Protein (above) and Chlorophyll a (below) of *Nostoc* cultured in BG-11 medium (left) and modified Bristol's solution (right)

A linear regression analysis suggested that additional $MgSO_4.7H_2O$ to the BG 11 medium has negative relationship (R = -0.33), but for modified Bristol's solution shows weak positive relationship (R=0.089) to the protein content of *Nostoc*. In contrast, positive relationship to the chlorophyll a content of *Nostoc* has shown at the BG 11 culture medium (R = 0.31) and negative relationship at modified Bristol's solution (R = -0.21). These relationships may describe the characteristic responses of protein and chlorophyll a contents in the two media to the supplementation of $MgSO_4.7H_2O$.

## CONCLUSION

The different concentration of $MgSO_4.7H_2O$ in two cultures media affected to the dried weight and protein content, but did not affected to the chlorophyll a content of *Nostoc*. High content of dry weight, protein and chlorophyll a of *Nostoc* cultured in modified Bristol's solution might affected by $K_2HPO_4$ content that has similar function with MgSO4.7H2O. Supplementation of MgSO4.7H2O with concentration 0.075 gram/litre during three week culture period was the optimum condition for *Nostoc* cultured in the modified Bristol's solution that produces optimum protein and chlorophyll a contents, while BG 11 will produce optimum protein and chlorophyll a contents of *Nostoc* in concentration 0.000 at first week. This study provides baseline data for culture development and nutritional value of *Nostoc* for food supplement use.

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