Effects of different calcium concentrations on the outdoor growth of *Nostoc sphaeroids* Kütz

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Abstract. The technology of indoor cultivation of *Nostoc sphaeroids* Kütz is relatively mature, but for industrial cultivation, indoor cultivation has the disadvantages of high energy consumption and high cost. In order to find the appropriate amount of calcium concentration required for outdoor cultivation, and to pave the way for mass production of *Nostoc sphaeroids* Kütz, in this study, the *Nostoc sphaeroids* Kütz was cultivated. The pH, chlorophyll content, polysaccharide content, protein content and phytolithoprotein content of *Nostoc sphaeroids* Kütz were measured in different concentrations of calcium nutrient solution. The result showed that, with the increase of calcium concentration in outdoor cultivation, the growth of *Nostoc sphaeroids* Kütz was firstly inhibited and then promoted.

1. Introduction

The ancient name of *Nostoc sphaeroids* Kütz is Tianxian vegetable, Tianxian rice, pearl vegetable, the common name of *Nostoc sphaeroids* Kütz is water fungus [1], the scientific name of *Nostoc sphaeroids* Kütz is nostoc orbicularis. It is a rare edible cyanobacteria, which contains rich amino acids and proteins. It also contains a variety of bioactive extracellular polysaccharide and abundant mineral elements [2]. It is widely distributed in the karst landform, loess regions and other calcium-rich environments. But with the popularization of modern agricultural production technology, most of the environments suitable for *Nostoc sphaeroids* Kütz’s growth have been destroyed [3]. Today's annual production declined to 0.5 x 10³ kg. Pesticides have been found in butachlor is the important reason for the *Nostoc sphaeroids* Kütz output drop [4].

There was a positive correlation between the concentration of calcium and the growth of *Nostoc sphaeroids* Kütz [5]. The calcium may have important influence on the distribution of *Nostoc sphaeroids* Kütz. When the concentration of calcium is high, it satisfies the growth of *Nostoc sphaeroids* Kütz. At the same time, it can also satisfy the growth of impurities algae [6]. But when calcium ion concentration is low, commune will give priority to use calcium to limit impurities algae growth.

However, most studies related to the concentration of calcium are confined to the laboratory environment, and the data are not suitable for Large-scale industrial production. The growth of *Nostoc sphaeroids* Kütz prefers low temperatures (10°C ~ 20°C) [7,8]. The optimum pH for the growth of *Nostoc sphaeroids* Kütz is 6.0 ~ 7.5, and when the pH of 7.0, the concentration of chlorophyll and the various nutrients (soluble sugar and soluble protein) in the algae reached the highest level.

According to the above, the experiment is mainly to explore the growth of Gexianmi under outdoor conditions [9]. The purpose is to find out the optimal calcium concentration suitable for the growth of...
Nostoc sphaeroids Kütz in outside, so that it can be industrialized and greatly develop its economic benefits [10].

2. Materials and methods

2.1. Materials
The experimental material was Nostoc sphaeroids Kütz (1-2 mm in diameter), which was provided by the Institute of Hydrobiology, Chinese Academy of Sciences.

2.2. Seaweed species expand cultivation.
Fresh seaweed seeds were taken and cultivated in 20 L glass incubator at a temperature of 10-20 °C and a pH of 7. The medium was BG-110 Liquid culture medium for 15 days, when the growth of Pueraria is in logarithmic growth stage, it is used for experimental seaweed seed [11].

2.3. Setting and cultivating of calcium concentration.
The experimental group was divided into 5 groups (2 parallel samples in each group), respectively represented by the experimental group 1-5. The experimental seaweed seeds were taken from each group and cultivated in the medium with a calcium ion concentration of 1, 5 and 8 times (the calcium ion concentration used in the experiment is 36g/L CaCl$_2$·2H$_2$O, twice the concentration of calcium ion at 1 ml/L of the solution). Next, 20L bucket was used to cultivate outdoors in the same nutritional pattern for 3 cycles.

| Group  | The addition mode of nutrient solution                  |
|--------|--------------------------------------------------------|
| No. 1  | BG - 110 without calcium                               |
| No. 2  | BG - 110+ 1 times calcium concentration                |
| No. 3  | BG - 110+ 5 times calcium concentration                |
| No. 4  | BG - 110 + 8 times calcium concentration               |
| No. 5  | BG - 110 without calcium + XAC (20ml/20L)              |

2.4. Determination of the pH value
30 mL of algal is taken from each bucket of Nostoc sphaeroids Kütz and the pH value is measured every day.

2.5. Determination of the ratio of dry and fresh weight
Three samples were taken from each bucket at the end of each cycle of Nostoc sphaeroids Kütz. The samples are baked to a constant weight at 80°C, taken out and put into a drying oven, and weighed with an analytical balance. The ratio of dry and fresh weight is obtained by analyzing and calculating this experiment.

2.6. Determination of chlorophyll
At the end of each cycle, 30mL of algal liquid is taken from each bucket of Nostoc sphaeroids Kütz, and the chlorophyll concentration was calculated by measuring the absorbance value at 665nm and by methanol extraction method.

2.7. Determination of polysaccharides
30 mL of alga solution is taken from each bucket at the end of each cycle of Nostoc sphaeroids Kütz. Then phenol sulfuric acid method was used to calculate the polysaccharide content by measuring the absorbance value at 485nm.
2.8. Determination of phycobiliprotein
The absorbance of supernatant at wavelengths of 562, 615 and 652 nm is determined by spectrophotometer. The contents of phycocyanin, allophycocyanin and phycoglobin were calculated according to the formula of Siegelman & Kysia.

3. Results and discussion

3.1. Effects of different calcium ion concentration on the pH of the samples
It is found that different calcium ion concentrations had no significant influence on pH (Fig. 1). At the low calcium concentration, the pH of No. 2 and No. 3 increase in the middle and later stages of each cycle, but the pH of No. 1, No. 4 and No. 5 are generally stable from 7.5 to 8. Overall, the effect of calcium ions on pH is limited, with only a difference of about 1.

![Figure 1. pH of Nostoc sphaeroides Kütz at different calcium concentrations at the end of four weeks. Note: No. 1 is the control group and No. 5 is the group without calcium additives. The pH of No. 2 and No. 3 increases in the middle and later stages of each cycle, while the pH of No. 1, No. 4 and No. 5 is basically stable.](image1)

3.2. Effects of different calcium ion concentration on dry-fresh-weight ratio of samples
It is found that different calcium ion concentrations have an effect on the dry-fresh-weight ratio (Figure 2). With the increase of calcium ion concentration, the dry-fresh-weight ratio of No. 2, No. 3 and No. 4 also increase, and the increasing trend is same but has different amplitude. Compared with the control group, the dry-fresh-weight ratio of No. 2 was lower than that of No. 1 without calcium ion addition, indicating that the growth of Nostoc sphaeroides Kütz is inhibited when the calcium ion concentration is low.

![Figure 2. Dry-fresh-weight ratio of Nostoc sphaeroides Kütz at different calcium concentrations at the end of four weeks. Note: No. 1 is the control group and No. 5 is the group without calcium additives. With the increase of calcium concentration, the dry-fresh-weight ratio of No. 2, No. 3 and No. 4 also increase. And the dry-fresh-weight ratio of No. 4 is the most obvious one.](image2)
3.3. Effects of different calcium ion concentrations on chlorophyll content of Nostoc sphaeroids Kütz

It is found that different calcium ion concentrations have an effect on the chlorophyll content of *Nostoc sphaeroids* Kütz (Fig. 3). By comparing the chlorophyll content, it can be obtained that the chlorophyll content increases with the increase of calcium ion concentration. However, compared with the control group, the chlorophyll content in the medium without calcium ion is similar to that in the medium with high calcium ion.

![Figure 3](image)

*Figure 3.* Chlorophyll content of *Nostoc sphaeroids* Kütz at different calcium concentrations at the end of four weeks. No. 1 is the control group and No. 5 is the group without calcium additives. With the increase of calcium concentration, the chlorophyll content of No. 2, No. 3 and No. 4 also increase. Among these, the chlorophyll content of No. 4 increased most significantly.

3.4. The influence of different calcium ion concentration to *Nostoc sphaeroids* Kütz polysaccharide content

The study found that different influential to commune polysaccharide content of calcium ion concentration (Fig. 4). It can be directly seen that the periodic change of polysaccharides in each group with the increase of calcium ion concentration, the first three cycles of polysaccharide content are increased slightly, but a very small increase in the fourth cycle. Among these, the increase of the polysaccharide content of No. 3 is the most obvious. Compared with the control group, the polysaccharide content is higher when the calcium ion concentration is more appropriate than when no calcium ion was present, but too high concentration can also inhibit the synthesis of polysaccharide, and the polysaccharide content can reduce.

![Figure 4](image)

*Figure 4.* Effects of different calcium concentrations on polysaccharide content after 4 weeks. Note: No. 1 is the control group and No. 5 is the group without calcium additives. With the increase of calcium concentration, the polysaccharide content of No. 2, No. 3 and No. 4 also increase. Among these, the polysaccharide content of No. 3 increase most significantly.
3.5. The influence of different calcium ion concentration on Nostoc sphaeroids Kütz phycobiliprotein

The result found that different calcium ion concentration on the Nostoc sphaeroids Kütz phycobiliprotein content (Fig. 5). Compared with the control group without calcium medium BG - 110, calcium ion concentration of three kinds of algae has little effect on bile protein, both without calcium, low calcium and high calcium bile protein concentration is stable, only small changes. But still with low calcium, calcium concentration increased protein content with increasing trend.

![Graph showing protein concentration across different calcium concentrations](image)

**Figure 5.** Different concentrations of Nostoc sphaeroids each plant spore element content in the end. Note: No. 1 was the control group and No. 5 was the group without calcium additives. With the increase of calcium concentration, the contents of C- phytolouins, Gallbladder proteins and C- phytolouins in No. 2, no. 3 and No. 5 also increase slightly.

The presence of calcium ions will be according to the experimental data may be slightly inhibiting the production of proteins, but still positively correlated relationship between the presence of calcium.

4. Discussion

Dry fresh weight is the most direct reflection Nostoc sphaeroids Kütz growth data, with the increase of calcium ion concentration dry fresh weight than significantly increase. Chlorophyll photosynthesis is an important factor affecting the growth of Nostoc sphaeroids Kütz. Obtained without calcium Nostoc sphaeroids Kütz chlorophyll content is higher, low calcium can reduce chlorophyll content, but with the increase of calcium concentration chlorophyll content will increase. Polysaccharide content and phycobiliprotein are important components of Nostoc sphaeroids Kütz. Their influence trend is that low calcium ion concentration inhibits polysaccharide content and phycobiliprotein content. Then, with the increase of calcium ion concentration, polysaccharide content and phycobiliprotein content also increase.

Nostoc sphaeroids Kütz artificial breeding technology in recent years has made some progress, but there is far from large-scale breeding. Previous researchers have explored the calcium in indoor conditions on the growth of the Nostoc sphaeroids Kütz, but mass production is outside, so the purpose of this research is to find out the optimal calcium concentration, the growth of the Nostoc sphaeroids Kütz outside to industrialization, sharp play its economic benefits.

5. Conclusion

This experiment is mainly to explore the influence of outdoor calcium concentration on Nostoc sphaeroids Kütz growth, chose 5 kinds of data to reflect the growth situation of Nostoc sphaeroids Kütz. pH of various nutrient solution to add mode and has no obvious change, when low calcium, pH will later period in each cycle, otherwise pH was basically stable. In a word, both chlorophyll content and
polysaccharide content were in line with the low concentration inhibition, and then the increase of content was promoted with the increase of calcium ions. The polysaccharides will decrease after reaching a higher value at the appropriate concentration, while the dry weight will reach a lower value at this time. The results show that in outdoor cultivate, with the increase of calcium concentration, to restrain the growth of the *Nostoc sphaeroids* Kütz, and then to promote the growth of *Nostoc sphaeroids* Kütz, and has an extreme value.

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