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Research Article

# The Effect of N:P:K Ratio, Concentrations, and Adding Frequency of Media on the Growth of Three Marine Microalgae for Mass-Production

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## ABSTRACT

Commercial media from the Hyponex series were shown to contribute different benefits to terrestrial plants with their differing N:P:K ratios. This study investigated the effects of different media N:P:K ratio, media concentrations, and re-supplementation frequencies on the growth of *Isochrysis galbana*, *Nannochloropsis oculata*, and *Tetraselmis chui*. Of the five types of media, H5 (N:P:K=3:1:1) performed the best when each medium was added at 10 mg $l^{-1}$. H5 medium provided at 50 mg $l^{-1}$, 100 mg $l^{-1}$, and 100 mg $l^{-1}$ allowed for the optimal growth of *I. galbana*, *N. oculata*, and *T. chui*, respectively. When there were re-supplementations of 50 mg $l^{-1}$ H5 medium during a 14-day cultivation period, re-supplementation only on day 8 was optimal for *I. galbana*, while re-supplementation every four days was optimal for *N. oculata* and *T. chui*. In conclusion, when provided at a suitable concentration and frequency, H5 (N:P:K=3:1:1) is a promising medium for the mass cultivation of all three species.

**Keywords:** Culture strategy; *Isochrysis galbana*; *Nannochloropsis oculata*; *Tetraselmis chui*; Supplementation

## INTRODUCTION

With the development of large scale farming systems for aquaculture, the productions of farmed fish have increased dramatically [1]. A diversity of fish and shellfish are hatched and bred entirely in cultivation. Moreover, excellent breeding techniques can ensure the quality of aquatic products and provide a stable source of the products for commercial needs.

Production of live feed is important in aquaculture. The quality of the cultivated live feed is affected by the duration and method of cultivation and the source of nutrients. Consequently, the live feed quality affects the health and viability of larvae. High quality live feed should have a size compatible with the mouth size of their predators, move in water with suitable speed, and possess simple structures, so that they can be easily fed on and digested by their predators. They should also be highly nutritional and their metabolites should be non-toxic. For mass-production purposes, live feed should be easy to acquire, cultivate, and maintain; grow and reproduce quickly (short life cycles); and require low cultivation cost [2].

Phytoplankton is an important source of food for the larvae of fish, shellfish, and echinoderms. They are rich in Docosahexaenoic Acid (DHA) and Eicosapentaenoic Acid (EPA), and can be used to enrich the zooplankton used as live feed in aquaculture [3]. In addition to acting as a source of food, specific nutrients in microalgae can add value to aquatic animals, such as the skin color enhancement generated by carotenoids and astaxanthin [4]. Microalga genera commonly used in aquaculture include *Isochrysis*, *Tetraselmis*, and *Nannochloropsis* [5].

In this study, we selected five commercial culture media (H1-H5) with different Nitrogen: Phosphorus: Potassium (N:P:K) ratios (% w/w) in H1 (7:6:19), H2 (1:1:1), H3 (1:3:2), H4 (5:1:4), and H5 (3:1:1). Those N:P:K ratios above have been illustrated to have a series of functions in terrestrial plants such as improving house plant growth, universal fertilizer, blossom, landscape plants growth, and seedling growth, respectively. Interestingly, compared to the f/2 (N:P:K=11:1:0) or Walne (N:P:K=9:2:0) media, the commercial culture media not only have different N:P:K composition but also have more economic efficiency.

In addition, because algae are without organizational differentiation, the concrete effectiveness of N:P:K ratio in algae is unclear but can be evaluated through biomass variation. Basically, N, P, and K are the main elements to sustain the growth of either

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marine or terrestrial plants. So, it is necessary to clarify the effect of medium N:P:K ratio, concentrations, and adding frequency on algae growth to mass-produce the high-quality microalgae needed as feedstuff by the aquaculture industry. Thus, the objective of this study was to determine the optimal medium for the three microalgal strain *I. galbana*, *N. oculata*, and *T. chui* during culture period.

# MATERIALS AND METHODS

## Algal strain and culture maintenance

The microalgae used in this study (*I. galbana*, *N. oculata*, and *T. chui*) were purchased from Full-Algae Biotechnology Co., Ltd. (Yunlin, Taiwan), and were maintained in f/2 medium in 250 ml flasks. The stock cultures were maintained monthly by inoculating 5 ml of microalgae into 100 ml of sterilized seawater (salinity at 35%) enriched with f/2 medium. For motile microalgae, such as *I. galban*a and *T. chui*, inocula were taken from the upper layers of the stock cultures to ensure their quality. The culture media were then maintained at 20°C-23°C and a light intensity of 30 μmol-45 μmol photons $m^{-2}$ $s^{-1}$ without further manipulation between the scheduled maintenance periods.

## Calibration curve of OD values against cell concentrations

The microalgae were scanned with a spectrophotometer (Biochrom Ultrospec 8000, Biochrom Ltd., Cambridge, UK) to determine the wavelength with the maximum absorbance ($\lambda_{max}$) between 400 nm-700 nm for each microalga. When in the stationary phase, cultures were diluted and absorbances measured at the respective $\lambda_{max}$ to obtain Optical Density (OD) values. In addition, the cell concentration of each sample was counted using a hemocytometer. A standard curve of OD value against cell concentration was plotted.

## Experimental design

The three species were cultivated in 250 ml flasks, with a 100 ml working volume containing autoclaved seawater, medium (as specified in section 2.4, 2.5 and 2.6), and the algal mass. The microalgae were cultivated at 25°C in a plant incubator (SS-980, Tominaga, Taiwan) for 14 days. Light was supplied at an intensity of 70 μmol photons $m^{-2}$ $s^{-1}$ and with a photoperiod of 12 h:12 h (L:D). The flasks were shaken manually at 9 a.m. and 5 p.m. daily. All experiments were carried out in triplicate.

Every two days, 1 ml of each culture was taken and measured to obtain OD values. The OD values were substituted into the standard curves to obtain cell concentrations, and specific growth rates were calculated using the equation below:

Specific growth rate, μ ($d^{-1}$)=(ln(final cell concentration)-ln(initial cell concentration))/(final time-initial time) (1)

## Effect of different N:P:K ratio of culture media on growth

Each strain was cultivated in seven groups with different media. Control groups were cultured in f/2 or Walne media. Treatment groups were cultured with the five N:P:K ratio of culture media including H1(7:6:19); H2(1:1:1); H3(1:3:2); H4(5:1:4); and H5(3:1:1), which were supplemented at 10 mg $l^{-1}$. The detailed composition of media is as specified in Table 1.

**Table 1:** The nutrient compositions of H1 to H5 media, as specified by supplier.

| Media series | | H1 | H2 | H3 | H4 | H5 |
|---|---|---|---|---|---|---|
| N:P:K ratio (% w/w) | | 07:06:19 | 01:01:01 | 01:03:02 | 05:01:04 | 03:01:01 |
| Main composition (% w/w) | Total N: | 7 | 20 | 10 | 25 | 30 |
| | $NH_4^+$-N (contained): | 1.2 | 4 | 5 | - | - |
| | $NO_3^-$-N (contained): | 5.8 | 4 | 5 | 4.5 | 1 |
| | Water-soluble P: | 6 | 20 | 30 | 5 | 10 |
| | Water-soluble K: | 19 | 20 | 20 | 20 | 10 |

## Effect of medium concentration on growth

Using the previous results, the optimal medium for the growth of all three strains was selected. N:P:K ratio of 3:1:1 (H5 medium) at 10, 50, or 100 mg $l^{-1}$ was used to cultivate the three strains. Control groups were the same as above (section 2.4).

## Effect of medium concentration and adding frequency on growth

The effects on growth were investigated using a two-factor experimental design. N:P:K ratio of 3:1:1 (H5 medium), the optimal medium for growth of *I. galbana*, *N. oculata*, and *T. chui*, was supplied at different concentrations and frequencies. The control group was H5 medium added on the initial day (day 0)

only. The treatment groups were H5 medium added on the initial day with re-supplementations every four or six days, or a re-supplementation only on day 8. *I. galbana* was supplied with 50 mg $l^{-1}$ H5 medium, while *N. oculata* and *T. chui* were supplied with 100 mg $l^{-1}$ H5 medium.

### Statistical analysis

All statistical analyses were conducted using the Statistical Analysis System (SAS-PC). Two-way ANOVAs were used to test for significant interaction effects of medium concentrations and re-supplementation frequencies on cell concentration and specific growth rate. One-way ANOVAs were used to analyze the cell concentration and specific growth rate in the experiments described in sections 2.4 and 2.5, and Tukey's studentized range test was used to test for significant differences among groups, with significance level set as $\alpha<0.05$. All data are expressed as mean ± standard deviation. The optimal conditions for producing cell concentrations were estimated through Three-Dimensional (3-D) response surface plots.

## RESULTS

### Calibration curve of OD values against cell concentrations

Within the range 400 nm-700 nm, the $\lambda_{max}$ for *I. galbana*, *N. oculata*, and *T. chui* were 688, 684, and 680 nm, respectively. By measuring the optical densities of diluted samples using their respective $\lambda_{max}$ (OD688, OD684, and OD680), the following equations for the calibration curves were obtained:

*I. galbana*:

$y=101.21x-0.9962$ ($R^2=1$) (2)

*N. oculata*:

$y=183.86x+0.3159$ ($R^2=0.9998$) (3)

*T. chui*:

$y=24.308x-0.0105$ ($R^2=0.9997$) (4)

Where;

x=Represents the $OD_{xxx}$ value

y=Represents the cell concentration

### Effect of N:P:K ratio of culture media on growth

Based on the results, the optimal medium for cultivating *I. galbana*, *N. oculata*, and *T. chui* was H5 (N:P:K=3:1:1) medium. Specifically, the performance of each medium (N:P:K) on growth, from the highest cell concentration to the lowest, was H5 (3:1:1)>H4 (5:1:4)>H2 (1:1:1)>H3 (1:3:2)>H1 (7:6:19) for *I. galbana*; H5 (3:1:1)>H2 (1:1:1)>H3 (1:3:2)>H1 (7:6:19)>H4 (5:1:4) for *N. oculata*; and H2 (1:1:1)>H5 (3:1:1)>H3 (1:3:2)>H1 (7:6:19)>H4 (5:1:4) for *T. chui*. H5 medium was either the first or second most effective medium for these strains.

H5 (N:P:K=3:1:1) produced the highest concentrations of *I. galbana* and *N. oculata* (4.84 ± 0.06 and 8.48 ± 0.10 × $10^6$ cells $ml^{-1}$, respectively), with the highest specific growth rates (0.26 ± 0.00 and 0.29 ± 0.00 $d^{-1}$ respectively) after 14 days of cultivation. For *T. chui*, use of H2 (N:P:K=1:1:1) resulted in the highest cell concentration and specific growth rate (6.18 ± 0.13 × $10^5$ cells $ml^{-1}$, 0.13 ± 0.00 $d^{-1}$) among treatment groups after 14 days. Statistical analysis showed no significant differences in either cell concentration or specific growth rate when H5 (N:P:K=3:1:1) was used compared with H4 (N:P:K=5:1:4) for *I. galbana*, when compared with H2 (N:P:K=1:1:1) for *N. oculata*, or compared with H2 (N:P:K=1:1:1) for *T. chui*. However, for all three strains, the control groups had significantly higher cell concentrations and specific growth rates when compared with any of the treatment groups.

### Effect of H5 (N:P:K=3:1:1) medium concentration on growth

I. galbana grew best in 50 mg $l^{-1}$ H5 medium. *N. oculata* and *T. chui* produced optimal cell concentrations in 100 mg $l^{-1}$ H5 medium. When used at an optimized concentration, H5 medium performed better than f/2 and Walne media in promoting growth of the microalgae, with the exception of *I. galbana*.

For I. galbana, 50 mg $l^{-1}$ H5 medium produced the highest cell concentration and the highest specific growth rate (8.46 ± 0.39 × $10^6$ cells $ml^{-1}$, 0.31 ± 0.00 $d^{-1}$) after 14 days, and for *N. oculata* and *T. chui*, 100 mg $l^{-1}$ produced the highest cell concentrations (2.04 ± 0.07 × $10^7$ and 2.10 ± 0.16 × $10^6$ cells $ml^{-1}$, respectively) and the highest specific growth rates (0.35 ± 0.00 and 0.21 ± 0.01 $d^{-1}$, respectively). When cell concentrations were considered, for *I. galbana*, 50 mg $l^{-1}$ of H5 resulted in cell concentrations that were not significantly different from those of the f/2 and Walne control groups; for *N. oculata*, 100 mg $l^{-1}$ of H5 was not significantly different from Walne medium supplementation; and for *T. chui*, 100 mg $l^{-1}$ produced the highest cell concentration, a statistically significant increase. Moreover, none of these three groups showed a significant difference in specific growth rate compared with the Walne culture of their respective strains.

### Effect of H5 (N:P:K=3:1:1) medium concentration and addition frequency on growth

50 mg $l^{-1}$ H5 medium produced the highest concentration of *I. galbana* when re-supplemented on day 8 and the highest concentrations of *N. oculata* and *T. chui* when re-supplemented every four days. To summarize, 50 mg $l^{-1}$ H5 medium at an optimized addition frequency performed better than a single dose of H5 medium in an optimized concentration. Re-supplementation with 50 mg $l^{-1}$ on day 8 for *I. galbana* cultures, and every four days for *N. oculata* and *T. chui* cultures, produced the highest cell concentrations (9.47 ± 0.58 × $10^6$, 2.89 ± 0.02 × $10^7$, and 3.02 ± 0.03 × $10^6$ cells $ml^{-1}$ for *I. galbana*, *N. oculata*, and *T. chui*, respectively) and specific growth rates (0.30 ± 0.00, 0.36 ± 0.00, and 0.24 ± 0.00 $d^{-1}$ for *I. galbana*, *N. oculata*, and *T. chui* respectively).

Two-way ANOVA analyses revealed that the medium concentration and the addition frequency had interaction

effects on both cell concentrations and specific growth rates for the three microalgal strains. The effects on the cell concentrations of *I. galbana* were present on days 2, 12, and 14 ($p<0.0001$), and on the specific growth rates from day 6 to day 14 ($p<0.05$). For *N. oculata*, the interaction effects on both cell concentrations and specific growth rates were present throughout the experiment ($p<0.05$). For *T. chui*, the effects on cell concentrations were present on day 2, and from day 8 to day 14 ($p<0.05$); the effects on specific growth rates were present on day 2, and from day 6 to day 14 ($p<0.05$).

The interaction effects of medium concentrations and addition frequencies on cell growth were further analyzed with Response Surface Methodology (RSM). The 3-D plots (response surface plots) for the cell concentrations of *I. galbana*, *N. oculata*, and *T. chui*. The effects were not apparent for *I. galbana*. However, increasing medium concentrations up to 90 mg $l^{-1}$ and increasing addition frequencies (decreasing interval days) up to re-supplementations every two days would produce higher cell concentrations of *N. oculata* and *T. chui*. As suggested by the 3-D plots, the highest cell concentrations of *N. oculata* could be produced by adding 50 mg $l^{-1}$ to 70 mg $l^{-1}$ H5 medium only on the initial day of 14-day cultivation. On the other hand, the highest cell concentrations of *T. chui* could be produced by adding 70 mg $l^{-1}$ to 80 mg $l^{-1}$ H5 medium every three to five days.

## DISCUSSION

Along with carbon and oxygen, nitrogen is one of the three main elements constituting proteins, amino acids, coenzymes, enzymes, chlorophylls, and genetic material. The type of nitrogen source affects the growth and biochemical composition of microalgae [6]. Microalgae can utilize the following nitrogen sources: Inorganic Nitrate ($NO_3^-$), Nitrite ($NO_2^-$), and Ammonium ($NH_4^+$) salts; and organic urea [7]. Among these, nitrates, ammonium salts, and urea are usually used as nitrogen sources in commercial media for aquaculture purposes. When nitrogen levels are sufficient, the synthesis of carbohydrates can promote protein production. Conversely, when nitrogen is insufficient and carbohydrates accumulate, protein production will decrease and the amount of lipids will increase [8].

Lourenco, et al., cultivated *I. galbana*, *N. oculata*, and *T. gracilis* with different nitrogen sources and found that *I. galbana* and *N. oculata* had optimal growth rates when using $NO_3^-$, and *T. gracilis* when using $NH_4^+$. In another study, *N. oculata* and *I. galbana* were cultivated with different nitrogen sources ($NO_3^-$ and $NH_4^+$) in continuous systems; the use of $NO_3^-$ produced optimal cell concentrations, while $NH_4$+ yielded lower cell concentrations and even inhibited their growth [9]. According to other studies, ammonium salts have negative effects on the growth of microalgae (*Prorocentrum minimum* and *Hillea* sp.), and high concentrations of ammonium salts may be toxic to *N. oculata*. On the other hand, nitrates may maintain higher cell concentrations [10]. In the present study, the first experiment showed that *I. galbana* and *N. oculata* had optimal algal cell concentrations and specific growth rates in H5 (N:P:K=3:1:1) medium, which lacks $NH_4^+$. Conversely, *T. chui* had an optimal algal cell concentration and specific growth rate in H2 (N:P:K=1:1:1) medium, which contains 0.4 mg $l^{-1}$ $NH_4^+$ (Table 1). This is in accordance with the aforementioned research, and supports the conclusion that different types of nitrogen source have different effects on the growth of microalgae.

A study by Fabregas, Herrero, Cabezas, Abalde showed that nitrogen depletion reduces growth rate and metabolic activity, which subsequently decreases photosynthesis. This validates the results of our first experiment. When *I. galbana*, *N. oculata*, and *T. chui* were cultivated with 10 mg $l^{-1}$ media, the available nitrogen was insufficient, so their cell concentrations and specific growth rates were significantly lower than algae cultivated in f/2 or Walne media. The algal cell concentration and specific growth rate of the three strains in groups supplemented with H1 (total N: 0.7 mg $l^{-1}$) or H3 (total N: 1.0 mg $l^{-1}$) were the poorest out of all groups, because these groups had the lowest total nitrogen compared to groups H2 (total N: 2.0 mg $l^{-1}$), H4 (total N: 2.5 mg $l^{-1}$), and H5 (total N: 3.0 mg $l^{-1}$) (Table 1). Though H2 (N:P:K=1:1:1) and H4 (N:P:K=5:1:4) media are lower in total nitrogen compared to H5 (N:P:K=3:1:1) medium, they promoted the growth of I. galbana (H2, H4) and N. oculata (H2). As a matter of fact, H2 (N:P:K=1:1:1) and H4 (N:P:K=5:1:4) media contain a higher concentration of potassium (water-soluble $K_2O$: 2.0 mg $l^{-1}$) compared to H5 (N:P:K=3:1:1) medium (water-soluble $K_2O$: 1.0 mg $l^{-1}$). In a previous study by Arumugam, Agarwal, Arya, Ahmed, that provided different nitrogen sources, the cell concentration of *Scenedesmus bijugatus* was the highest when $KNO_3$ was used, because both nitrogen and potassium are important elements for algal growth [11,12].

Gris, Paim, Farenzena, Trierweiler found that *N. oculata* cultivated with f/2 medium containing 165 mg $l^{-1}$ $NaNO_3$ (27.2 mg $l^{-1}$ $NO_3$-N) achieved the highest cell concentration. Other past research has revealed that *Tetraselmis* sp. grows well in nitrate concentrations up to 40.6 mg $l^{-1}$ $NO_3$-N (22, 23). Song, Zhang, Li, discovered that *I. galbana* cultivated with 24.6 mg $l^{-1}$ $NO_3$-N could achieve the highest cell concentration, but a concentration of 49.3 mg $l^{-1}$ inhibited cell growth. As shown in our second experiment, *I. galbana* achieved the highest cell concentration ($8.456 \pm 0.390 \times 10^6$ cells $ml^{-1}$) when cultivated with 50 mg $l^{-1}$ H5 (N:P:K=3:1:1) medium (0.5 mg $l^{-1}$ $NO_3$-N), but the cell concentration was significantly lower when cultivated with 100 mg $l^{-1}$ H5 (N:P:K=3:1:1) medium (1.0 mg $l^{-1}$ $NO_3$-N). According to Table 1 (section 2.4), H5 (N:P:K=3:1:1) medium contains 30% total nitrogen, which means 100 mg $l^{-1}$ H5 medium contains 30 mg $l^{-1}$ total nitrogen. This could be the cause of the poorer results, since in previous research cell growth was inhibited at nitrogen concentrations higher than 24.6 mg $l^{-1}$ [13]. *N. oculata* and *T. chui* cultivated with 100 mg $l^{-1}$ H5 (N:P:K=3:1:1) medium (1.0 mg $l^{-1}$ $NO_3$-N) achieved the highest cell concentrations ($2.0391 \pm 0.0728 \times 10^7$ and $2.099 \pm 0.161 \times 10^6$ cells $ml^{-1}$, respectively), which could be supported by those findings. For the microalgae in the present study, cell growth was limited when only 10 mg $l^{-1}$ H5 (N:P:K=3:1:1) medium was used; it can be inferred that the nitrogen supply was insufficient to promote cell growth.

Nitrogen concentration is a major factor affecting the growth and photosynthesis of algae. An insufficiency of nitrogen will

limit algal cell growth, as will an oversupply of nitrogen. Two example microalgal strains that have shown this relationship are *Chlorella vulgaris* and *Tisochrysis lutea* [14]. When a high concentration of $NO_3^-$ accumulates in cells, it will inhibit the growth of cells in both batch cultures and continuous cultures. This may be related to the regulation of the nitrate assimilation pathway, where assimilation increases with the concentration of $NO_3^-$ [15]. After $NO_3^-$ is taken up by cells, it will be reduced to $NO_2^-$ by nitrate reductase, then transported to the chloroplast and further reduced to $NH_4^+$ by nitrite reductase [16]. An increase in $NO_3^-$ concentration in culture media will stimulate the activity of nitrate reductase, promoting the reduction of $NO_3^-$. Eventually, when more $NO_2^-$ is available than can be reduced to $NH_4^+$, accumulation of $NO_2^-$ occurs in cells [17]. $NO_2^-$ inhibits photosynthetic electron transport and damages cell membranes, and subsequently inhibits cell growth [18]. Similarly, if the reduction of $NO_3^-$ and $NO_2^-$ is faster than the assimilation of $NH_4^+$, an increase in $NO_3^-$ concentration in culture media will result in the production and accumulation of $NH_4^+$ in cells, which negatively affects the growth of microalgae. From the perspective of energy demand, $NH_4^+$ is taken up before $NO_3^-$ and urea, because less energy is needed for its uptake and it can be directly assimilated into amino acids.

In the present study, when 50 mg $l^{-1}$ H5 (N:P:K=3:1:1) medium was re-supplemented every four or six days, *I. galbana* showed signs of inhibited cell growth, potentially due to excessive $NO_3^-$ content in the environment. On the other hand, algal cell growth was not affected negatively when these nutrients were re-supplemented eight days after the initial culture set up, or when H5 (N:P:K=3:1:1) medium was supplemented at a concentration of 10 mg $l^{-1}$. No signs of inhibition were seen in the *N. oculata* or *T. chui* cultures, even when 100 mg $l^{-1}$ H5 (N:P:K=3:1:1) medium was re-supplemented every four days, implying that these two strains can tolerate much higher concentrations of $NO_3^-$.

According to past studies using continuous systems with a dilution rate of 0.3 $d^{-1}$, *N. gaditana* can achieve its highest cell concentration when cultivated with 158.2 mg $l^{-1}$ $NO_3$-N. In contrast, concentrations lower than 158.2 mg $l^{-1}$ significantly decreased cell concentration. *I. galbana* achieved the highest cell concentration when cultivated with 56 mg $l^{-1}$ $NO_3$-N. However, cell growth was limited in 28 mg $l^{-1}$ and inhibited in concentrations above 56 mg $l^{-1}$. As discovered in the present study, for both *N. oculata* and *T. chui*, re-supplementation of low concentration medium at a high frequency can achieve the highest cell concentrations and specific growth rates. This trend was similar to what was discovered by Huang, Huang, Liao, Fu, Xia, Zhu.

When considering costs for cultivating *I. galbana*, H5 (N:P:K=3:1:1) medium at 50 mg $l^{-1}$ could save about 10- and 43-fold of costs compared to f/2 and Walne media, respectively, allowing the production of similar algal cell concentrations but with a higher specific growth rate. When considering algal cell concentration, supplementing 50 mg $l^{-1}$ H5 (N:P:K=3:1:1) medium every eight days could achieve the highest algal cell concentration and specific growth rate, saving about 5 and 21fold of costs when compared to the use of f/2 and Walne media, respectively. For *N. oculata* and *T. chui*, cultivation with 100 mg $l^{-1}$ H5 (N:P:K=3:1:1) medium could save about 5 and 21-fold the costs of f/2 and Walne media, respectively, while achieving the highest cell concentration and specific growth rate. When considering cell concentration, supplementation of 50 mg $l^{-1}$ H5 (N:P:K=3:1:1) medium every four days could achieve the highest cell concentration and specific growth rate, while saving about 3- and 11-fold of the cost of using f/2 and Walne media, respectively.

## CONCLUSION

In conclusion, H5 (N:P:K=3:1:1) medium is promising for the cultivation of *I. galbana*, *N. oculata*, and *T. chui*, because it has a lower cost, but can also produce high concentrations of algal cells depending on its concentration and frequency of supplementation. As a side note, when the medium concentration was 10 mg $l^{-1}$, *I. galbana* and *N. oculata* produced higher cell concentrations in H5 (N:P:K=3:1:1) medium than in H2 (N:P:K=1:1:1) medium, but the opposite applied to *T. chui*. Thus, further investigation of how cultivation with a mixture of H2 (N:P:K=1:1:1) and H5 (N:P:K=3:1:1) media affects the growth of the three strains is recommended.

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