Developing a way to select plants for eutrophication eco-remediation by their nutrient uptake and growth kinetics characteristics

To cite this article: J.H. Sun et al 2018 IOP Conf. Ser.: Earth Environ. Sci. 199 022070

View the article online for updates and enhancements.
Developing a way to select plants for eutrophication eco-remediation by their nutrient uptake and growth kinetics characteristics

J.H. Sun1*, Y. Li2, Y. J. Yang1, P. Cui1, Z. Y. Cheng1, X.T. Qiao1, Y. J. Xu3
1Tianjin Key Lab of Aqua-Ecology and Aquaculture, College of Fisheries, Tianjin Agricultural University, Tianjin 300384, China.
2College of Engineering and Technology, Tianjin Agricultural University, Tianjin 300384, China.
3Key Laboratory for Applied Marine Biotechnology of Ministry of Education, School of Marine Sciences, Ningbo University, Ningbo 315211, China.
*Corresponding author: jhsun1008@163.com.(J. H. Sun)

Abstract: In this article, a method of selecting eutrophication-remediated plants is proposed, which established the selecting index that is based on the ability of removing nitrogen (N) and phosphorus (P) rapidly and efficiently. This article combines parameters of absorption kinetics and growth kinetics to reflect their ability of removing nitrogen and phosphorus of the selected plants. The results showed: (1) The optimum growth conditions of 3 species respectively, temperature 31.30, 23.38, 26.10℃; salinity 32.10, 21.10, 22.77; light intensity 287.23, 229.07, 216.65 μmol/(m²·s). (2) Under their optimum growth conditions, the maximum absorption rates (Vmax) respectively were 57.83, 54.26, 52.32 µmolN·dwg⁻¹·h⁻¹, 3.142, 2.802, 2.132 µmolP·dwg⁻¹·h⁻¹. (3) Selecting index Y and Y ' were 13.93, 5.03, 10.84, and 5.99, 1.92, 4.60 for N; 59.40, 58.67, 57.92, and 0.41, 0.23, 0.27 for P, respectively. Both Y and Y ' of G. lichenoides for removing N and P were bigger than those of G. lemaneiformis and G. tenuistipitata var. liui.

1. Introduction
Seaweed is an effective biofilter, which can filter nitrogen and phosphorus pollutants in seawater, and reduce HABs (He et al., 1987; Reddy and DeBusk, 1987; Yang et al., 2005; Zou et al., 2015). Seaweed also has good economic value. Furthermore, they had other merits, such as, asexual reproduction (no worry about seedling), fast-growth, extensive farming and easy harvest. However, seaweed is hard to form and generalize a large-scale ecological-restoration technology because of its environmental adaptability (Qiu et al., 2011). Suitable plant would be produce a satisfactory effect (Tong et al., 2003; Xiang, 2010), because the effect of ecological remediation mainly depends on the absorption rate and absorption quantity to nutrients by the plant. The absorption rate depends on strain of the plant, which is relative with its genes (Xiang, 2010). Now, study of plant’s ability to absorb nutrients is mainly through the absorption kinetics test. ( Ana et al., 2001; Covino et al., 2010)The absorption quantity to nutrients was close relative with growth rate of the plant, rapid growth of plants will absorb more nutrients from the environment to synthesize their own compound. In this process, plants can absorb and assimilate nutrients in their full extent. Therefore, the plant, as suitable remediated plant, has a basic
characteristic, that is abilities of rapid absorption and rapid growth.

The aim in this paper is developing a method to find out a suitable species for increasing the alleviated efficiency to nutrients. Several steps included as follows, (1) Initially selected plant species, near the spot where will be remediated; (2) Analyzed their suitable scope and optimum conditions of growth; (3) Determined growth kinetic parameters and absorption kinetic parameters of plants under optimum conditions; (4) Compared kinetic parameters among remediated plants; (5) Combined other conditions, decided the plant species.

2. Materials and methods

2.1 Plant source and its temporary culture

Suitable macrophytes, which should have economic value and a stable seedling source, were selected around the spot where would be remediated. In our experiment, we selected 3 strains Rhodophyta (G. lichenoides, G. lemaneiformis and G. tenuistipitata var. liui) as the representative to develop the method. Three seaweeds were taken to the lab, cleaned and cultured temporarily in an incubator (Jiangnan Corp., Ningbo, China). Sand-filtered seawater with f/2 culture medium, other conditions were set up according to the live spot where seaweeds were collected (salinity 15-25 ppt, temperature 10-31°C, light intensity 90-285 µmol·m⁻²·s⁻¹, etc.). During the cultivation, nutrition was added every 2 days and the culture media was stirred 4-6 times per day. Several days later, the plant was divided into 0.1-0.2 g plantlets for convenience in the following experiments.

2.2 Optimization of growth conditions

Single factor experiment was conducted to investigate the suitable range of each condition of the seaweed growth (Don’t do it if we could find parameters in references, such as G. tenuistipitata var. liui, temperature, 20-30 °C; salinity, 15-30; light intensity, 100-250 µmol·m⁻²·s⁻¹(Liu et al., 2001). The gradients of G. lichevoides and G. lemaneiformis were respectively, temperature (°C): 8, 12, 16, 20, 24, 28, 32, 36; salinity (ppt): 5, 10, 15, 20, 25, 30, 35, 40; light intensity (µmol·m⁻²·s⁻¹): 40, 80, 120, 160, 200, 250, 300. The specific growth rate (SGR= ((Wt-Wo) 1/t-1)×100%) was treated as the experiment index, where W₀ was the initial fresh weight of seaweed (g), Wₜ was the fresh weight at t time (g), t was the interval (d). Under the suitable conditions measured in the signal factor experiment, uniform design experiment, showed in Table 1 and the experimental period was 2 weeks, was employed to optimize their growth conditions.

2.3 Absorption kinetics test

Under the optimal growth conditions, nutrients (nitrogen and phosphorus) absorption kinetics of 3 seaweeds were tested according to the Michaelis-Menten equation, and the absorption kinetics parameters were analyzed, included the maximum absorption rate (Vmax) and coefficient (Km).

2.3.1 Experimental design

Concentration gradients of nitrogen and phosphorus were respectively, N (µmol·L⁻¹): 4 (background value), 10, 15, 20, 25, 30, 40, 50, 60, 80, 100; P (µmol·L⁻¹): 2 (background value), 5, 10, 15, 20, 25, 30, 40, 50, 60. Each treatment was triplicate. A control treatment without seaweed was set up for adjusting data. Other conditions were set up according to the optimal results.

| Test No. | Salinity (ppt) | Temperature (°C) | Light intensity (µmol/(m²·s)) | SGR (%/d) |
|----------|----------------|------------------|---------------------------|----------|
| n1       | [1] 5          | [5] 21 (18)      | [7] 160                   | 0.002±0.000 (0.052±0.009, 0.117±0.005) |
| n2       | [2] 8          | [10] 35 (33)     | [3] 60                    | 0.005±0.000 (0.115±0.032, 0.073±0.038) |
### 2.3.2 Determination and analysis of absorption rate

Thirty six conical flasks of 500 mL were added 400.0 mL seawater and 2.0 g N- or P- starvation seaweed, each flask mouth was covered with a sheet of aluminum foil, then put them into the incubator. Water samples were collected at 0.5, 1, 2, and 4 h after the beginning of the experiment for analyzing concentrations of nitrogen and phosphorus in each flask. According to the following formula, the absorption rate of nitrogen and phosphorus were calculated. The average value of 4 times was regarded as the absorption rate of the species. \( U = \frac{(C_0 - C_t) \cdot V}{t \cdot G} \). Where \( U \) was the absorption rate (\( \mu \text{mol} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1} \)), \( C_0 \) was the content of N or P in the control (\( \mu \text{mol} \cdot \text{L}^{-1} \)). \( C_t \) was the content of N or P in the cultured treatment at \( t \) time (\( \mu \text{mol} \cdot \text{L}^{-1} \)). \( V \) was the volume in each flask at \( t \) time (L), \( t \) was the test time of duration (h), \( G \) was the dry weight of seaweed (gDW).

### 2.4 Growth kinetics test

According to Monod equation, the growth kinetics of 3 seaweeds, included maximum growth rate (\( I_{\text{max}} \)) and concentration of substrate at \( 1/2 \ I_{\text{max}} \) (\( S_m \)), for nitrogen and phosphorus, would be tested under their optimal conditions.

#### 2.4.1 Experimental design

Concentration gradients of nitrogen and phosphorus were respectively, N (\( \mu \text{mol} \cdot \text{L}^{-1} \)): 4 (background value), 10, 20, 30, 40, 60, 80, 100, 150, 200; P (\( \mu \text{mol} \cdot \text{L}^{-1} \)): 2 (background value), 5, 10, 15, 20, 30, 40, 60, 80, 100. Each treatment was 2 repetitions. Other conditions were set up according to the optimal experimental results.

#### 2.4.2 Determination and analysis of growth rate

Twenty transparent 10 L acrylic tanks were added 8 L seawater and 10.0 g fresh seaweed, put into 2 same incubators. During the experiment, concentrations of N and P were analyzed and the seaweed was weighed in each flask every 3 days, then all conditions were restored to the initial state. Calculated SGR According to the formula in Section 1.2, and compared the SGRs of 2 adjacent measurements in each treatment, if no difference between them, stopped this treatment experiment and began another cycle that was the same as before, the average SGR with 3 cycles was the SGR of the seaweed in the corresponding N or P concentration. Combined the concentration of N or P with the SGR, the \( I_{\text{max}} \) and \( S_m \) value of the seaweed would be attained.

### 2.5 Seaweed selecting equation

Absorption kinetics parameters represent the absorption efficiency to nitrogen and phosphorus, and growth kinetics parameters represent the capacity of rapid growth. Therefore, the remediated plant must have characteristics of quick absorption rate and growth rate. Combined parameters of absorption
kinetics and growth kinetics, the equation of selection was \( Y = \frac{V_{\text{max}}}{K_m} \times \frac{I_{\text{max}}}{S_m} \). The \( Y \) value was larger, means the species had the better remediation effect.

Generally, concentration of N and P has a high level in the eutrophic water, So \( K_m \) can be ignored in the equation; all remediated plants are also opportunity species and have a high growth rate, so \( I_{\text{max}} \) can be ignored too. Therefore, the equation can be simplified as \( Y' = \frac{V_{\text{max}}}{S_m} \). Compared the value of \( Y' \) of different plants, the plant with higher \( Y' \) value would be selected for remediation.

2.6 Statistical analysis
The absorption and growth kinetics parameters of each nutrient were calculated by nonlinear regression. The maximum absorption rate in different interval of time and the growth rate will be compared by \( t \) test.

![Fig.1 Single factor experimental results for G. lichevoides and G. lemaneiformis](image)

3. Results

3.1 Growth conditions optimization
From the Single factor experiment and references, the suitable ranges of growth for 3 strains seaweed were given. In their ranges, the experiment of growth conditions optimization had done by uniform experiment design, results were shown in Table 1. After a stepwise regression analysis, the regression equations were followed.

\[
SGR_{G \text{ lemaneiformis}} = -34.246 +0.143L +1.025S -3.26\times10^4L^2 -3.95\times10^2T^2 -2.38\times10^{-2}S^2 +3.26\times10^{-4}TL -9.09\times10^{-5}LS. \\
(R^2=0.935, \ F=17.068, \ P < 0.01);
\]

\[
SGR_{G \text{ lichenoides}} = -10.902 +0.268T +0.400S -5.787\times10^2L -1.58\times10^4L^2 +6.02\times10^2T^2 -6.23\times10^{-2}S^2 -3.808\times10^{-3}TL. \\
(R^2=0.966, \ F=57.844, \ P < 0.01);
\]

\[
SGR_{G \text{ tenuistipitata var. liui}} = -14.607 +0.176T +0.655S -9.3\times10^2L -2.33\times10^4L^2 +1.87\times10^2T^2 -3.26\times10^2S^2 -1.68\times10^{-3}TL. \\
(R^2=0.948, \ F=32.766, \ P < 0.01);
\]

From the regression equations, we could acquire the optimal growth conditions for 3 seaweeds.

3.2 Absorption kinetics analysis
Under their optimal growth conditions, the absorption kinetics of nitrogen and phosphorus of 3 strains seaweed were shown in Figure 2. The maximum absorption rate of nitrogen of \( G. \) lichevoides, \( G. \) lemaneiformis and \( G. \) tenuistipitata var. liui was 57.83, 54.26 and 52.32 \( \mu \text{mol N}\cdot\text{gDW}^{-1}\cdot\text{h}^{-1} \) respectively. The maximum rate of phosphorus of 3 strains was 3.142, 2.802 and 2.132 \( \mu \text{mol P}\cdot\text{gDW}^{-1}\cdot\text{h}^{-1} \) respectively.

There was no difference among 3 strains with the maximum absorption rate. However, a significant difference can be shown in their curves when they reached the maximum rate. When nitrogen was about 20 \( \mu \text{mol} \cdot \text{L}^{-1} \), the absorption rate of \( G. \) lichevoides and \( G. \) tenuistipitata var. liui reached the maximum, but \( G. \) lemaneiformis had its maximum absorption rate at the N concentration...
of 40 μmol·l⁻¹. Similarly, when phosphorus was about 15 and 20 μmol·l⁻¹, the absorption rate of *G. tenuistipitata* var. *liui* and *G. lichevoides* reached the maximum, and *G. lemaneiformis* had its maximum rate at 30 μmol·l⁻¹. *G. lichevoides* and *G. tenuistipitata* var. *liui* were easier and faster to reach the maximum absorption rate than *G. lemaneiformis*. In other words, *G. lichevoides* and *G. tenuistipitata* var. *liui* absorb N or P in a faster and more efficient way.

![Graph of uptake kinetics curves of N and P of 3 seaweeds](image)

Figure 2. Uptake kinetics curves of N and P of 3 seaweeds

Through the double reciprocal plot, absorption kinetic parameters of 3 strains seaweed can be obtained (Table 2). There were no significantly difference in $V_{max}$ and $K_m$ among 3 strains, but had a significant difference in $V_{max}/K_m$ among them, the $V_{max}/K_m$ value of *G. lemaneiformis* was higher than those of *G. lichevoides* and *G. tenuistipitata* var. *liui*.

| Plant species | $V_{max}$ (μmol·dwg⁻¹·h⁻¹) | $K_m$ (μmol·l⁻¹) | $V_{max}/K_m$ | n | $R^2$ |
|---------------|-----------------|-----------------|---------------|---|-------|
| N *G. lemaneiformis* | 54.26±1.31 | 5.93±0.52 | 9.150±0.336 | 3 | 0.8182 |
| N *G. lichevoides* | 57.83±1.48 | 7.45±1.12 | 7.762±0.565 | 3 | 0.8627 |
| N *G. tenuistipitata* | 52.32±2.08 | 6.73±0.56 | 7.774±0.437 | 3 | 0.8567 |
| P *G. lemaneiformis* | 5.120±0.28 | 0.08±0.01 | 36.70±0.876 | 3 | 0.8239 |
| P *G. lichevoides* | 3.142±0.36 | 0.11±0.05 | 28.56±0.577 | 3 | 0.8458 |
| P *G. tenuistipitata* | 2.132±0.22 | 0.07±0.02 | 30.46±0.637 | 3 | 0.8274 |

### 3.3 Growth kinetics analysis

The growth kinetics curves of 3 seaweeds were shown in Figure 3. Similarly, there also was no difference of $I_{max}$ among them. While growth saturated concentration of nitrogen differed greatly with 60 μmolN·l⁻¹ (*G. lichevoides* and *G. tenuistipitata* var. *liui*) and 100 μmolN·l⁻¹ (*G. lemaneiformis*) respectively. The phosphorus saturated concentration was also different with 30 μmolN·l⁻¹ for *G. lichevoides* and *G. tenuistipitata* var. *liui* and 40 μmolN·l⁻¹ for *G. lemaneiformis* respectively. Growth kinetic parameters were shown in Table 3.
Figure 3 Growth kinetics curves of N and P of 3 seaweeds

Table 3 Comparison of growth kinetics parameters of 3 seaweeds

| Plant species | I max (%·d⁻¹) | S m (µmol·l⁻¹) | I max/S m | n | R² |
|---------------|---------------|----------------|-----------|---|----|
| N G. lemaneiformis | 15.52±1.44 | 28.23±2.14 | 0.5498±0.113 | 2 | 0.9134 |
| N G. lichevoides | 17.32±2.67 | 9.65±1.21 | 1.7948±0.245 | 2 | 0.9067 |
| N G. tenuistipitata | 15.86±2.02 | 11.37±1.65 | 1.3949±0.137 | 2 | 0.9237 |
| P G. lemaneiformis | 16.83±1.228 | 10.53±1.03 | 1.5986±0.165 | 2 | 0.8827 |
| P G. lichevoides | 15.67±1.331 | 7.76±0.963 | 2.0198±0.312 | 2 | 0.8652 |
| P G. tenuistipitata | 15.04±1.56 | 7.91±0.864 | 1.9014±0.233 | 2 | 0.8756 |

3.4 Comparison of selecting index
According to the selecting equation, the selecting index of Y and Y'were shown in Table 4. G. lichevoides was the selected seaweed as eutrophication-remediated plant to purify nitrogen or phosphorus according to the value of Y or Y'.

Table 4 Comparison of the selecting index among 3 seaweeds

| Plant species | V max/K m | I max/S m | Y | Y' |
|---------------|-----------|-----------|---|----|
| N G. lemaneiformis | 9.150 | 0.5498 | 5.031 | 1.922 |
| N G. lichevoides | 7.762 | 1.7948 | 13.931 | 5.993 |
| N G. tenuistipitata | 7.774 | 1.3949 | 10.844 | 4.602 |
| N G. lemaneiformis | 36.70 | 1.5986 | 58.669 | 0.226 |
| P G. lichevoides | 28.56 | 2.0198 | 59.399 | 0.405 |
| P G. tenuistipitata | 30.46 | 1.9014 | 57.917 | 0.270 |

4. Discussion
Eutrophication results from excess nitrogen or phosphorus (Qin et al., 2013; Feng et al., 2012). Phyto-remediation is an effective method and technology to deal with this problem, which required the plants had the characteristics of fast growth and high-efficient absorption to N and P. Compared with single-factor experiment and orthogonal experiment, the obtained results were more precise and creditable, then, the obtained absorption and growth kinetic parameters were more close to the reality. The more effective would be selected.

V max/K m showed the characteristic of nutrient absorption of a plant and I max/S m showed its rapid growth characteristic (Jiang et al., 2006). In this study, the absorptions of nitrogen and phosphorus by the 3 seaweeds were in accordance with the saturated absorption kinetics, consistent with other reports (Xiang, 2010; Tong et al., 2003; Xu et al., 2007), and the related parameters were fit in with the ranges
of $K_m$ and $V_{\text{max}}$ of the absorption rate to N and P by seaweed (Qin et al., 2013; Feng et al., 2012). But more precise, which can differentiate varieties of characteristics of similar seaweeds in the same genus. So, the selecting equation can be used to reflect the characteristics of plants, feasible for plant selection.

In this study, NO$_3$-N was the only source of nitrogen and $V_{\text{max}}$ was maybe slightly lower that of NH$_4$-N or NH$_4$-N+NO$_3$-N as nitrogen source, but the results would be more representative. Different nitrogen forms had little effect on $K_m$ (Tong et al., 2003). Consequently, this selecting method is applicable to remediate the polluted water whether it was new or old. In general, nitrogen and phosphorus has been in a high level in the eutrophic water, which are much higher than $K_m$, can fully meet the rapid growth of the plant. Similarly, as the opportunity organisms, the seaweed had a rapid growth rate ($I_{\text{max}}$). So the selecting equation was simplified as $Y' = V_{\text{max}}/S_m$.

References

[1] Ana, B. A., Jefferson, M., Paulo, C., 2001. Radicular uptake kinetics of $^{15}$NO$_3^-$, CO($^{15}$NH$_2$)$_2$, and $^{15}$NH$_4^+$ in whole rice plants. *Journal of Plant Nutrition*, 24(11):1695-1710.

[2] Covino, T., Meglynn, B., Mcnama ra, R., 2010. Tracer additions for spiraling curve characterization (TASCC): Quantifying stream nutrient uptake kinetics from ambient to saturation. *Limnology & Oceanography Methods*, 8(9):484-498.

[3] Feng, W. U., Zhan, J., Deng, X., 2012. Influencing factors of lake eutrophication in China--A case study in 22 lakes in China. *Ecology & Environmental Sciences*, 21(1):94-100.

[4] He, P. M., Xu, S. N., Zhang, H. Y., 1987. The application of algae in the marine ecological restoration and comprehensive breeding in seawater. *Fishery Modernization*, 19(10): 61-79.

[5] Jiang, Y., Gan, X., Tang, X., Chen, X., Yu, R. C., 2006. Effects of nutrients nitrogen and phosphorus on *Heterosigma akashiwo* growth. *Chinese Journal of Applied Ecology*, 17(3):557-559.

[6] Qin, B. Q., Gao, G., Zhu, G. W., Zhang, Y. L., Song, Y. Z., Tang, X. M., Xu, T. H., Deng, J. M., 2013. Lake eutrophication and its ecosystem response. *Chin Sci Bull*, 58(10): 855-864.

[7] Qiu, D. R., Wu, Z. B., Liu, B. Y., Deng, J. Q., Fu, G., He, F., 2011. The restoration of aquatic macrophytes for improving water quality in a hypertrophic shallow lake in Hubei Provine, China. *Ecol Eng*, 18(2) : 147 – 156.

[8] Reddy, K. R., DeBusk, T. A., 1987. State-of-the-art utilization of aquatic plants in water pollution control. *Water Science & Technology*, 19(10): 61-79.

[9] Tong, C. H., Yang, X. E., Pu, P. M., 2003. Effects and mechanism of hydrophytes on control of release of nutrient salts in lake sediment. *Journal of Agro-environmental Science*, 22(6): 673-676.

[10] Xiang, L. C., 2010. Effectiveness of purification of eutrophic water by three aquatic plants and its influencing factors, Zhejiang. Zhejiang University Ph.D Dissertation.

[11] XU, Y. J., Qian, L. M., Wei, W., Wang, Y. S., 2007. Studies on nutrient kinetics characteristics of two species seaweeds (Rhodophyta) at outdoor natural conditions. *Marine Environmental Science*, 26(2):161-165.

[12] Yang, Y. F., Song, J. M., Lin, X. T., Nie, X. P., 2005. Seaweed cultivation and its ecological roles in coastal waters. *Marine Environmental Science*, 24(3):77-80.

[13] Zou, Y., Hu, Z., Zhang, J., Xie, H., Liang, S., 2015. Investigation and optimization of nitrogen transformations in aquaponics. *Chinese Journal of Environmental Engineering*, 9(9):4211-4216.