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Application of Chemostat culture to Nutrient uptake rate measurements by the
macroalgae *Saccharina japonica* var. *religiosa* (Phaeophyceae) and *Ulva*
australis (Ulvophyceae)

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1 SUMMARY

2 In this study, we applied a chemostat culture method, for the first time, to measure the
3 nutrient uptake rate of macroalgae. We examined two methods of measuring the nutrient
4 uptake rate of two macroalgae, *Saccharina japonica* var. *religiosa* and *Ulva australis*, by
5 comparing nutrient uptake kinetics between the chemostat culture and batch culture. In
6 the chemostat culture, the nutrient concentration was kept constant by monitoring the
7 change in nutrient concentration using an Auto Analyzer in real time and adding nutrients

to compensate for the macroalgae's nutrient consumption. The nutrient uptake in the chemostat culture could be best fitted to the Michaelis-Menten saturation kinetics. In the batch culture, the nutrient concentration decreased with time, either constantly or exponentially due to a rapid uptake of nutrients by the macroalgae. The nutrient uptake rate in the batch culture generally showed a scattered relationship with nutrient concentration, with a weak fitting to the Michaelis-Menten saturation kinetics. This discrepancy seemed to be partly because the change in nutrient concentration was large between the sampling intervals in the batch culture. Determining an appropriate sampling interval for detectable concentration change is difficult unless the nutrient concentration is measured in real time. Therefore, the application of the chemostat culture method to the measurement of the uptake rate by macroalgae could greatly improve our understanding of nutrient uptake kinetics.

Key index words: batch culture; chemostat culture; macroalgae; Michaelis-Menten curve; nutrient uptake kinetics

INTRODUCTION

The batch culture method has generally been used to measure the nutrient uptake rate of macroalgae (D'Elia & DeBoer 1978; Haines & Wheeler 1978; Harlin & Craigie 1978;

Wallentinus 1984; Harrison *et al.* 1986; Pedersen 1994; Pedersen & Borum 1997; Martinez *et al.* 2012). In this method, the uptake rate by macroalgae is measured by incubating a thallus sample in a tank and observing the decrease in nutrient concentration in the surrounding seawater. The uptake rate is calculated by dividing the amount of decrease in concentration by the sampling interval. This rate is further normalized by algal biomass (dry weight) or surface area. This method is convenient, but when a sample has a high nutrient uptake rate, the large drawdown of nutrients within the tank becomes an issue. Generally, the nutrient uptake rate is expressed as a function of the nutrient concentration according to the Michaelis-Menten equation, $V = V_{\max} S / (K_s + S)$, where V is the uptake rate ($\mu\text{mol g dry wt}^{-1} \text{ h}^{-1}$), $V_{\max}$ is the maximal uptake rate, S is the concentration of limiting nutrient (μM), and K_s is the half-saturation constant representing the value of S , where $V = V_{\max}/2$. The species-specific nutrient uptake kinetic parameters, $V_{\max}$ and K_s , have been used to explain species competition for a limiting nutrient (Dugdale 1967; Tilman 1977; Button 1985).

In a batch culture, nutrient concentration within the tank decreases with time due to nutrient uptake, resulting in incorrect evaluation of the relationship between nutrient concentration and uptake rate. A larger tank or shorter sampling interval could be used to reduce the effects of the decreasing nutrient concentration. However, too small of a

change in nutrient concentration could result in larger errors in calculating the uptake rates. Therefore, to ensure precise uptake rate measurement, it is necessary to monitor the nutrient concentration in the incubation tank in real time and keep the concentration at a designated value by compensating for the consumption by algae.

The use of a chemostat culture can allow for the measurement of the nutrient uptake rate while keeping the nutrient concentration constant in the incubation tank. Chemostat is one of the continuous culture methods for keeping the concentration of chemicals at a constant level by monitoring the concentration in real time and supplying chemicals to compensate for their decrease (consumption). The continuous culture generally supplies fresh medium to the culture tank and removes the effluent from the tank at a constant flow rate (Tilman & Kilham 1976). This method can control the growth rate of microalgae (μ) by changing the dilution rate (D , d^{-1}). D is defined as f/v , where f is the volume (L) replaced daily by the fresh medium and v is the volume (L) of the culture. In this method, the limiting nutrient concentration in the culture tank is depleted at a steady state. Thus, the continuous culture cannot control the nutrient concentration at a designated level in the culture tank. In the chemostat culture, a target nutrient concentration in the incubation tank is monitored in real time, and the nutrient decrease rate is calculated to recover the nutrient losses with an appropriate nutrient addition. Generally, an Auto Analyzer is used

to measure nutrient concentrations in seawater (Grasshoff *et al.* 1999). This instrument has the high analytical sensitivity and accuracy required to measure the nutrient concentration in seawater. However, the instrument takes 5-10 minutes to display the results after introducing the sample. This time lag in measurement has to be considered to keep the nutrient concentration at a certain level in the chemostat culture. The objectives of this study were to develop a method of measuring the nutrient uptake rate of macroalgae in a chemostat culture by using an Auto Analyzer in real time and to compare methods to verify the effectiveness of the chemostat culture.

MATERIALS AND METHODS

Sample collection

Samples of *Ulva australis* were collected in August 2015 and those of *Saccharina japonica* var. *religiosa* in April 2019 from the shore of Oshoro Bay, Hokkaido (43°12'28" N; 140°51'53" E). The weight of *Ulva* ranged from 8.5 to 11.2 g wet wt. The length and weight of *Saccharina* (only 1st age) without sorus ranged from 170 to 250 mm and 13.3 to 31.9 g wet wt, respectively. Macroalgae thalli were sampled from the rocky substrate without damaging the holdfasts and were transported to the laboratory under dark and cool conditions. Epiphytes were removed by gentle brushing under running seawater. The

macroalgae were maintained for at least 24 h in the incubator at the in situ temperature in Oshoro Bay (August 2015: $24.0 \pm 0.5^{\circ}\text{C}$, April 2019: $7.0 \pm 0.5^{\circ}\text{C}$) and at $120 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ using fluorescent lamps with daylight spectrum.

Chemostat culture experiment

Three liters of prescreened ($100 \mu\text{m}$ mesh) seawater collected from Oshoro Bay were poured into an incubation tank and stirred constantly with a magnetic stirrer in a temperature-controlled incubator. The stirrer equipped with guard frame was used and a stainless mesh was placed in the incubation tank to prevent possible damage to thallus.

Rotation rate was set at 600 rpm, generating water motion at approximately 3.4 cm s^{-1} . In the experiment using *Saccharina*, the uptake rates of either $\text{NH}_4\text{-N}$ or $\text{NO}_3\text{-N}$ were measured. The $\text{PO}_4\text{-P}$ concentration was adjusted to a sufficient level ($>4.0 \mu\text{M}$). At the beginning of each uptake experiment, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and $\text{PO}_4\text{-P}$ concentrations in the tank were the sum of those contained in the original seawater and the spiked amounts of nutrient stock solution. In contrast, in the experiment using *Ulva*, the uptake rates of $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ were measured. In the $\text{NO}_3\text{-N}$ uptake experiment, the $\text{NO}_3\text{-N}$ concentration was adjusted to $0.5\text{-}20 \mu\text{M}$, while the $\text{PO}_4\text{-P}$ concentration was adjusted to a sufficient level ($>4.0 \mu\text{M}$). In the $\text{PO}_4\text{-P}$ uptake experiment, the $\text{PO}_4\text{-P}$ concentration was adjusted to $0.1\text{-}4.0 \mu\text{M}$, while the $\text{NO}_3\text{-N}$ concentration was adjusted to a sufficient level ($>20 \mu\text{M}$).

The NH₄-N, NO₃-N, and PO₄-P concentrations in the original seawater were < 0.1 μM in August 2015 and April 2019 (except NO₃-N at 5 μM in April 2019). The nutrient stock solutions were prepared from special reagent grade chemicals such as KNO₃, (NH₄)₂SO₄, and KH₂PO₄ at 10,000, 5,000, and 5,000 μM, respectively.

A macroalgal thallus was placed in the tank. The density of macroalgae varied from 1.5 to 5.3 g wet wt L⁻¹ depending on species. Every 10 minutes, an aliquot (10 mL) of seawater was sampled from the tank and filtered using a GF/F filter (pore size 0.7 μm). The filtered sample was placed to the sample tray manually. Nutrient concentrations (NH₄-N, NO₃-N, and PO₄-P) were then quickly measured with an Auto Analyzer (QuAatro, BRAN+LUEBBE, Tokyo, Japan) (Grasshoff *et al.* 1999). The Auto Analyzer took 5-8 minutes to obtain the analytical results. The schematic time course change of concentration and procedures in the chemostat culture are shown in Fig. 1.

The nutrient decrease rate (μmol L⁻¹ min⁻¹), R₀₋₁₀, was calculated from the change in nutrient concentration between t = 0 and t = 10 minutes divided by 10 (min). The amount of the first nutrient addition at t = 20 was calculated to recover the nutrient loss, assuming that R₀₋₁₀ would continue until t = 20 because the result sampled at t = 10 was obtained at t = 15-18. The amount of nutrient addition at t = 20, A₂₀ (μL), was calculated as follows:

$$A_{20} = \frac{\{(C_T - C_{10}) + R_{0-10}\} \times W}{N \times 10^{-6}} \quad (1)$$

where C_T is the target nutrient concentration (μM); C_{10} is the concentration at $t = 10$; N is the nutrient concentration in the nutrient stock solution (μM); and W is the seawater volume (L) in the tank.

The nutrient decrease rate after $t = 30$ was calculated from the change in concentration and the added amount of nutrient in each tank, as follows:

$$R_{(t-10)-t} = \frac{(C_t - C_{t-10}) + C_{A(t-10)}}{10} \quad (2)$$

where C_t is the nutrient concentration (μM) at time t and $C_{A(t-10)}$ is the increase in nutrient concentration by adding nutrient at time $= t-10$, calculated as

$$C_{A(t-10)} = \frac{A_{t-10} \times N \times 10^{-6}}{W} \quad (3)$$

where A_{t-10} is the amount of added nutrient (μL) at time $= t-10$.

Because there were biological fluctuations in the nutrient uptake by macroalgae, the average value of the two most recently calculated nutrient decrease rates, $\overline{R}_t$, was used for the calculation of A_t after $t = 30$, as follows:

$$\overline{R}_t = \frac{R_{(t-20)-(t-10)} + R_{(t-10)-t}}{2} \quad (4)$$

A_t was calculated as

$$A_t = \frac{\{(C_T - C_{t-10}) + \overline{R}_{t-10}\} \times W}{N \times 10^{-6}} \quad (5)$$

This operation was continued for 60-90 minutes until nutrient concentrations and nutrient

uptake rates were stabilized. Four settings could be simultaneously run at one time, with a 5-minute delay for two of four settings. The calculation for the chemostat operation was conducted with an Excel (Microsoft, Redmond, USA) worksheet. This worksheet will be provided upon request by the corresponding author.

Nutrient uptake rates (R) were converted to those based on dry weight as follows:

$$V_{t-(t+10)} = \frac{\{(C_t - C_{t+10}) + C_{A(t)}\} \times W \times 60}{\Delta t \times B} \quad (6)$$

where $V_{t-(t+10)}$ is the nutrient uptake rate between time t to $t+10$ ($\mu\text{mol g dry wt}^{-1} \text{ h}^{-1}$); B is the dry weight of the sample (g dry wt) and Δt is the sampling interval (min).

The thallus was dried at 55 °C on aluminum foil until a constant dry weight was achieved, typically for 4 to 5 days.

The average and standard deviation (SD) of the uptake rate for each concentration setting were calculated from the five to eight uptake rate data from a single experiment. The average and SD of the nutrient concentration were also calculated. The results between $t = 0$ and $t = 20$ were not included in the calculation for the average nutrient uptake rate and concentration. Four to five settings of different concentrations were applied to fit the Michaelis-Menten curve.

The precision of analysis, expressed as the coefficient of variation (CV) on the Auto Analyzer, was about 1%, and the detection limit (c.a., 0.01 μM) was defined as three times

the CV of the lowest standard solution.

The experimental reproducibility (CV%) using different strands varied from 3% to 20%.

The control experiment was conducted in the same way without macroalgae. The effect

of microalgae (phytoplankton) in the 100 μm mesh screened seawater on the measured

uptake rates was negligible because no change in the nutrient concentrations was

observed in the control experiment. Thus, the obtained uptake rates were judged to be

only due to the uptake by macroalgae.

Batch culture experiment

Nutrient concentrations in the tanks and experimental conditions were the same as in the

chemostat culture for the measurement of nutrient uptake rates in the batch culture. An

aliquot of seawater (10 mL) was sampled from each tank every 10 minutes for 60-120

minutes. Duplicate tanks were set up for *Ulva*, and $\text{NO}_3\text{-N}$ concentration was adjusted to

20 μM in the N uptake experiment. The $\text{PO}_4\text{-P}$ concentration was adjusted to 3 μM . Ten

tanks were set up for *Saccharina*, and either $\text{NO}_3\text{-N}$ or $\text{NH}_4\text{-N}$ concentration was adjusted

to ranging from 15 μM to 40 μM in the N uptake experiment. Nutrient concentrations in

these samples were measured discretely with an Auto Analyzer. Nutrient uptake rates

were calculated from the decrease in nutrient for each sampling interval, and the average

nutrient concentration in each interval was used as the substrate concentration for plotting.

The nutrient uptake rate was assumed to change depending on the nutrient concentration, so each uptake rate was plotted as an individual value.

Michaelis-Menten curve

The uptake rate, V , was plotted against the nutrient concentration, S , in each treatment and best fitted to the Michaelis-Menten curve according to the following equation:

$$V = \frac{V_{\max} \cdot S}{K_s + S} \quad (8)$$

where $V_{\max}$ is the maximum uptake rate ($\mu\text{mol g dry wt}^{-1} \text{ h}^{-1}$); K_s is the half-saturation constant (μM) when $S = K_s$; V is half of the $V_{\max}$; and S is the nutrient concentration (μM).

The experimental results were best fitted to the Michaelis-Menten curve using a Kaleida Graph (Synergy Software, Reading, USA). In the fitting to the curve, V at $S = 0$ was assumed to be zero.

Furthermore, an indicator of nutrient uptake efficiency at low nutrient concentration, α (Healey, 1980), was calculated from $V_{\max}$ divided by K_s ($\alpha = V_{\max} / K_s$).

RESULTS

Chemostat culture

Time course changes in $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ in the chemostat cultures using *Saccharina* are shown in Fig. 2. The concentration of $\text{NO}_3\text{-N}$ or $\text{NH}_4\text{-N}$ continued to decrease until $t = 30$ minutes, when the effect of the nutrient addition appeared. After controlling the nutrient concentration through nutrient addition, each nutrient concentration was maintained at a constant level during the experiment. The CV ranges for the concentrations of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ in each tank were 4.8-11.9 and 5.0-18.9%, respectively. In the experiment using *Ulva*, the ranges for $\text{PO}_4\text{-P}$ and $\text{NO}_3\text{-N}$ were 5-13% and 6-24%, respectively (Fig. 3). The averaged nutrient concentration and uptake rates for *Saccharina* (Fig. 4) and *Ulva* (Fig. 5) were best fitted to the Michaelis-Menten equation. $V_{\max}$ and K_s were determined from these curves (Table 1). In the experiment using *Ulva*, K_s values for $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ were 2.39 and 1.26 μM , whereas $V_{\max}$ values were 6.51 and 0.16 $\mu\text{mol g dry wt}^{-1} \text{ h}^{-1}$, respectively. In the experiment using *Saccharina*, K_s values for $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ were 5.35 and 9.29 μM , whereas $V_{\max}$ values were 22.3 and 23.2 $\mu\text{mol g dry wt}^{-1} \text{ h}^{-1}$, respectively.

Batch culture

$\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations in the batch culture using *Saccharina* decreased constantly (Fig. 6). Whereas the $\text{PO}_4\text{-P}$ concentration decreased constantly in the batch

culture using *Ulva*, the NO₃-N concentration decreased exponentially and was depleted by $t = 80$ minutes (Fig. 7). The nutrient decrease rates $((C_{t-10} - C_t)/C_t \times 100, (\%))$ for each sampling interval in the experiment using *Saccharina* and in the PO₄-P uptake experiment using *Ulva* were lower than 10%. In contrast, the rates in the NO₃-N uptake experiment using *Ulva* were higher than 30%. The nutrient uptake rates by *Saccharina* in the batch culture did not become saturated with increasing nutrient concentration but rather showed a linear relationship with nutrient concentration (Fig. 8). The NO₃-N uptake rate for *Ulva* showed a fit to the Michaelis-Menten curve, but no data were available at higher concentrations (Fig. 9a). K_S and V_{max} for NO₃-N were 53 μM and 13.7 $\mu\text{mol g dry wt}^{-1} \text{ h}^{-1}$, respectively. Those for PO₄-P were 1.45 μM and 0.16 $\mu\text{mol g dry wt}^{-1} \text{ h}^{-1}$, respectively (Fig. 9b).

The α value was calculated for the comparison between the results in the chemostat culture and the batch culture. In the experiment using *Saccharina* in the chemostat culture, the α values for NO₃-N and NH₄-N were 4.33 and 2.40, respectively (Table 1). The α values in the batch culture for NO₃-N and NH₄-N were 0.83 and 1.00, respectively. The α values for NO₃-N and PO₄-P in the experiment using *Ulva* in the chemostat were 66.5 and 3.09, respectively. Those values for the batch culture were 7.05 and 2.07, respectively. The α values in the batch culture were lower than those in the chemostat culture for both

species.

DISCUSSION

It is important for the nutrient concentration in the tank to be maintained at a certain level for the uptake rate calculation. However, in the batch culture, the nutrient concentration decreased constantly (Figs 6 and 7), whereas the $\text{NO}_3\text{-N}$ concentration in the *Ulva* experiment decreased exponentially until it was depleted (Fig. 7a). Previous research indicates that *Ulva* grows rapidly in the summer (Ono 1988), so the $\text{NO}_3\text{-N}$ requirement for *Ulva* would be very high in the summer. Harrison et al. (1989) examined several batch culture methods to obtain nutrient uptake kinetic parameters. They conducted batch culture experiments preparing multiple flasks with different nutrient concentrations and varying incubation times (0.05, 1 and 2 hr) and a short incubation time (5 min). Furthermore, they conducted a batch culture experiment with multiple sequential nutrient additions. Batch culture methods provided highly variable uptake rate values with nutrient concentration. This was explained by the feedback inhibition that occurs on a time scale of seconds (McCarthy & Goldman 1979; Goldman & Glibert 1982).

On the other hand, the variation in the nutrient concentration was small in the chemostat culture. The nutrient concentration in the tank decreased from the start of the experiment

until the effect of nutrient addition appeared after $t = 30$ minutes but was maintained at
 the target concentration after the nutrient addition. In the chemostat culture using *S.*
religiosa, the CV in the nutrient concentration was lower than 10% (Fig. 2). The CV in
 the nutrient uptake rate for each sampling interval was 10-50%. The nutrient addition to
 maintain the nutrient concentration at the target concentration was successful, but the
 nutrient uptake rate fluctuated to some extent. This variation in the uptake rate might be
 due to a biological fluctuation, which seemed to be larger than the analytical error.
 The nutrient uptake rates in the chemostat culture became saturated with increasing
 nutrient concentration (Figs. 4 and 5). Even though there were fewer plots than in the
 batch culture, each plot in the chemostat culture was averaged in value from more than
 five measurements and had an error bar.
 No studies have compared the characteristics of nutrient uptake kinetics between a batch
 culture and chemostat culture using macroalgae. For chemostat cultures of *Saccharina*,
 $V_{\max}$ values for $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ were almost the same at 23.2 and 22.3 $\mu\text{mol g dry wt}^{-1}$
 h^{-1} , respectively (Table 1). The K_s for $\text{NO}_3\text{-N}$ was 5.35 μM , which was lower than that
 for $\text{NH}_4\text{-N}$ at 9.29 μM . On the contrary, for batch cultures of *Saccharina*, $V_{\max}$ values
 were 401 and 42.7 $\mu\text{mol g dry wt}^{-1} \text{h}^{-1}$ for $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$, respectively. K_s values
 were 481 and 40.4 μM for $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$, respectively. However, those obtained by

the batch culture showed an order of difference between $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ with substantially large errors. The $V_{\max}$ and K_s for $\text{PO}_4\text{-P}$ of *Ulva* obtained by the batch culture were not significantly different from those by the chemostat culture (t-test, $p>0.05$). This is attributed to the scattering plots and lack of saturated values at a higher nutrient concentration range in the batch culture. The reason why these parameters for $\text{PO}_4\text{-P}$ were not different was not clear in this study.

Harrison et al. (1986) and Subandar et al. (1993) reported $V_{\max}$ of Laminariales for $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ ranging 10-20 $\mu\text{mol g dry wt}^{-1} \text{ h}^{-1}$. This range was similar to the values obtained by the chemostat culture in this study.

O'Brien and Wheeler (1987) reported the in situ uptake rate of $\text{NO}_3\text{-N}$ by *Ulva* using a bell jar technique. The average of 10.8 $\mu\text{mol g dry wt}^{-1} \text{ h}^{-1}$ was similar to values in this study.

However, the nutrient uptake rate of $\text{NO}_3\text{-N}$ by *Ulva* in the batch culture was less than half of the chemostat culture at lower concentrations. (Figs 5 and 9). The reason for this difference was not clear, but one explanation may be less data for the batch culture experiment. Along the Michaelis-Menten curve, the nutrient uptake rate changes linearly at lower concentrations. The nutrient concentration was kept constant at a low concentration in the chemostat culture. Both methods were conducted with the same

experimental conditions, such as water temperature, light intensity, water mixing, and amount of cultured seawater. The uptake rates in the chemostat culture were more precise than in the batch culture method in the lower concentration region. The correlation coefficients of the best-fitted Michaelis-Menten curves in the chemostat culture were high in both samples (for *Saccharina*, NO₃-N: $r = 0.99$, NH₄-N: $r = 0.99$; for *Ulva*, NO₃-N: $r = 0.95$, PO₄-P: $r = 0.93$). The nutrient uptake rates in the batch culture showed a fitting to the Michaelis-Menten curve but with a lower correlation (for *Saccharina*, NO₃-N: $r = 0.63$, NH₄-N: $r = 0.94$; for *Ulva*, NO₃-N: $r = 0.98$, PO₄-P: $r = 0.81$). Some plots did not become saturated with increasing nutrients but rather showed a scattered linear relationship with the nutrient concentration (Fig. 8a). This tendency was reported in a previous study that measured the nutrient uptake rate of *Saccharina latissima* using a batch culture (as *Laminaria groenlandica*, Harrison *et al.* 1986). Linear relationships between the NH₄-N uptake rate and the NH₄-N concentration were reported in previous studies using *Macrocystis sp.* (Haines & Wheeler 1978) and *Gracilaria foliifera* (D'Elia & DeBoer 1978). The reason the chemostat culture method in the present study could be best fitted to the Michaelis-Menten curve seemed to be that an equilibrium is attained between substrate concentration and uptake rate over a longer period (~ 1 h). Thomas and Harrison (1987) conducted real-time monitoring of substrate concentration

in seaweed incubation using an Auto Analyzer. However, they observed a time course change in nutrients but did not control the substrate concentration by adding the nutrient stock solution.

Stable isotope ^{15}N has been widely used for the uptake rate measurement of phytoplankton (Glibert *et al.* 1982; Glibert & McCarthy 1984; Dugdale & Wilkerson 1986; Kudo *et al.* 2015). N uptake rates and uptake kinetics for seaweed and seagrass have been reported using stable isotope ^{15}N (Williams & Fisher 1985; O'Brien & Wheeler 1987; Alexandre *et al.* 2011; Alexandre & Santos 2020). However, the application of this method for macroalgae and seagrass involves the concern of a change in nutrient concentration during the incubation when the uptake rate of the sample tissue is large because the incubation is conducted in the batch culture. To avoid this, a preincubation experiment is necessary to optimize incubation volume and time duration depending on plant biomass. Additionally, the sample for stable isotope measurement must be dried and pulverized; a large thallus presents difficulty for sample preparation.

Macroalgae and phytoplankton would suddenly show a high uptake rate to fulfill their limiting nutrient pools responding to a sudden increase in nutrients (Surge uptake, Lapointe 1985; Thomas & Harrison 1987; Lubsch & Timmermans 2019). Furthermore, in the real ocean, nutrient concentrations remain more or less stable for different periods

of time, and are not immediately affected by the seaweed activity due to the large water volume to biomass ratio. It is ideal to apply the chemostat culture method to the nutrient uptake kinetics study. To the best of our knowledge, the method developed in this study is the first to apply a chemostat culture to measure the uptake rate of macroalgae.

The chemostat culture method is applicable to examine the response to nutrient perturbation, as well as to species-specific nutrient uptake kinetics studies. Another application of the chemostat culture method in the present study are IMTA (Integrated multi-trophic aquaculture) systems, in which species from two or more trophic levels grow in one farm and where the waste of one feeds another (Buschmann 1996; Neori et al. 2004; Cruz-Suárez et al. 2010). Fast-growing seaweed, such as *Ulva prolifera*, has been used in this system (Cruz-Suárez et al. 2010). The system has a constant flow of seawater and nutrients uptake rate may be high in the system. So there may be difficulties in measuring the uptake rate of the seaweeds correctly in IMTA systems when using the batch culture method. In contrast, the chemostat culture method would better simulate conditions and more accurately measure the uptake rate in IMTA systems.

CONCLUSIONS

In this study, we developed a new method to measure the nutrient uptake rate of

macroalgae while maintaining a constant nutrient concentration. These results demonstrate the applicability and the accuracy of measuring nutrient uptake rates. Applying this method to other macroalgal species could therefore deepen our understanding of macroalgal uptake kinetics.

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425

Figure legends

Figure 1 Schematic diagram of temporal change in nutrient concentration in the chemostat culture. S_x and C_x denote the timing of sampling and concentration reading at time x , respectively. A_x denotes the amount of nutrient addition at time x .

Figure 2 The time course changes in $\text{NO}_3\text{-N}$ (a) and $\text{NH}_4\text{-N}$ (b) concentration in each run of the chemostat culture of *Saccharina*. Horizontal lines indicate the duration of the nutrient concentration control and the average value for each run.

Figure 3 The time course changes in $\text{NO}_3\text{-N}$ (a) and $\text{PO}_4\text{-P}$ (b) concentration in each run of the chemostat culture of *Ulva*. Horizontal lines indicate the duration of the nutrient concentration control and the average value for each run.

Figure 4 The best-fitted Michaelis-Menten curves for $\text{NO}_3\text{-N}$ (a) and $\text{NH}_4\text{-N}$ (b) in the chemostat culture of *Saccharina*. Error bars indicate standard deviations (SD) of the nutrient uptake rates and nutrient concentration.

Figure 5 The best-fitted Michaelis-Menten curves for $\text{NO}_3\text{-N}$ (a) and $\text{PO}_4\text{-P}$ (b) in the chemostat culture of *Ulva*. Error bars indicate standard deviations (SD) of the nutrient uptake rates and nutrient concentration.

Figure 6 The time course changes in $\text{NO}_3\text{-N}$ (a) and $\text{NH}_4\text{-N}$ (b) in each run of the batch culture using *Saccharina*.

Figure 7 The time course change in $\text{NO}_3\text{-N}$ (a) and $\text{PO}_4\text{-P}$ (b) in the batch culture using *Ulva*.

Figure 8 The relationship between the uptake rate and the nutrient concentration for $\text{NO}_3\text{-N}$ (a) and $\text{NH}_4\text{-N}$ (b) in the batch culture using *Saccharina*. The line indicates the best-fitted line to Michaelis-Menten curve.

Figure 9 The relationship between the uptake rate and the nutrient concentration for $\text{NO}_3\text{-N}$ (a) and $\text{PO}_4\text{-P}$ (b) in the batch culture using *Ulva*. The line indicates the best-fitted line to Michaelis-Menten curve

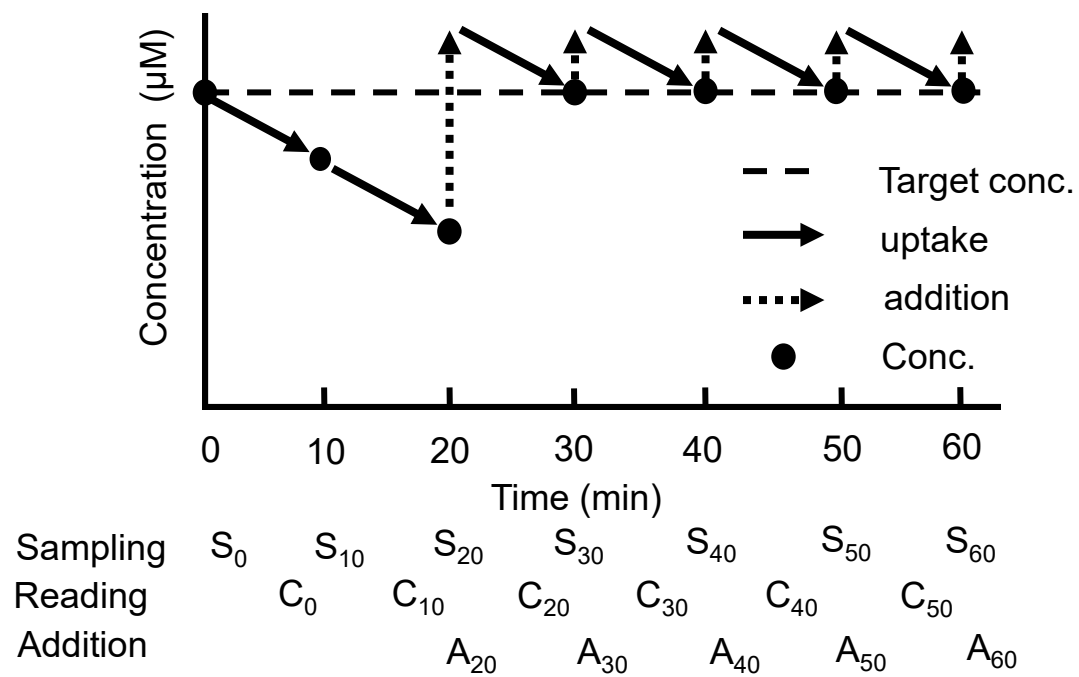


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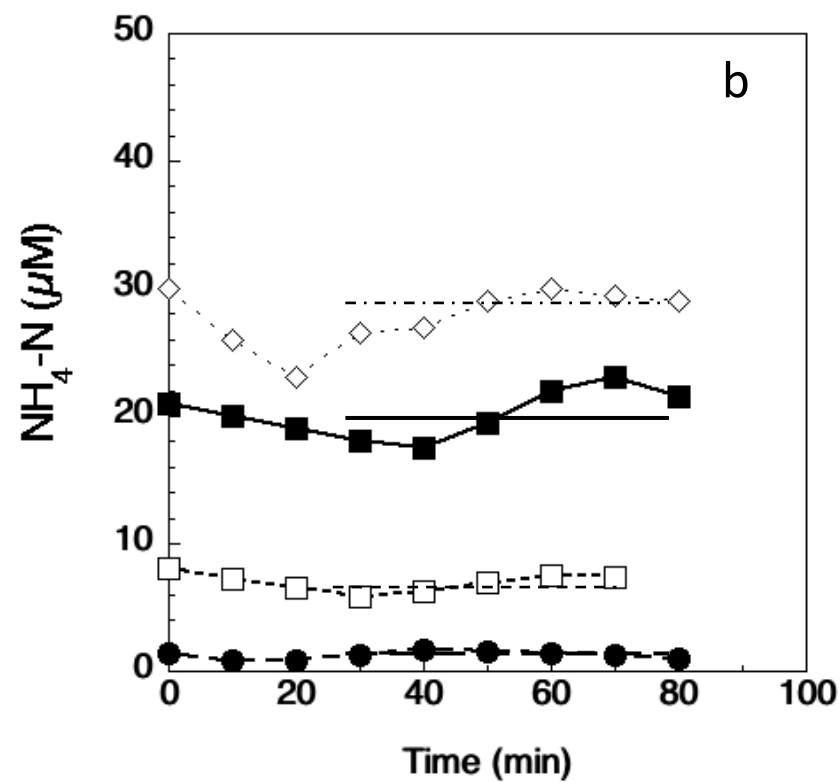
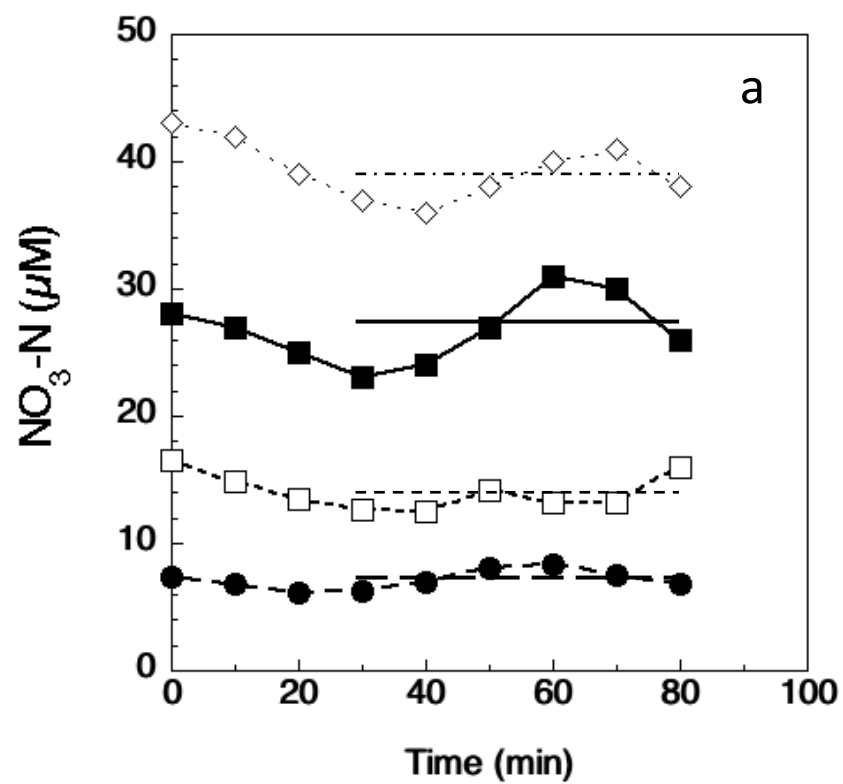


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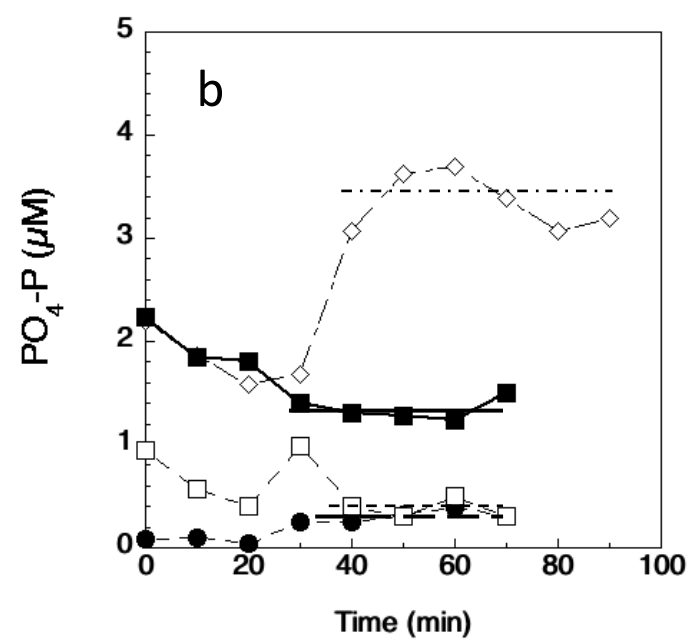
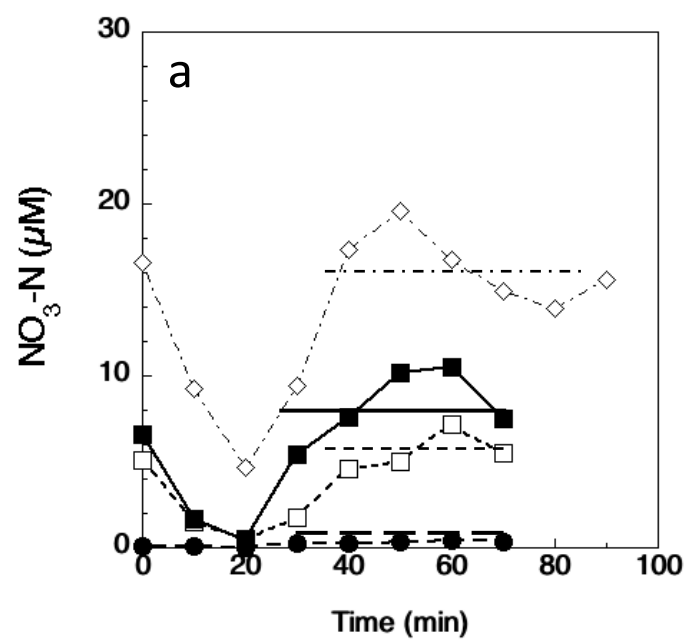


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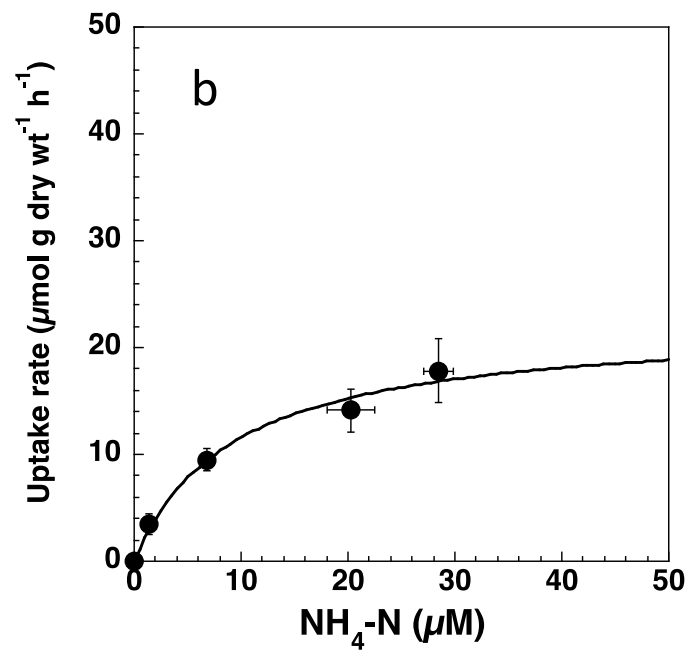
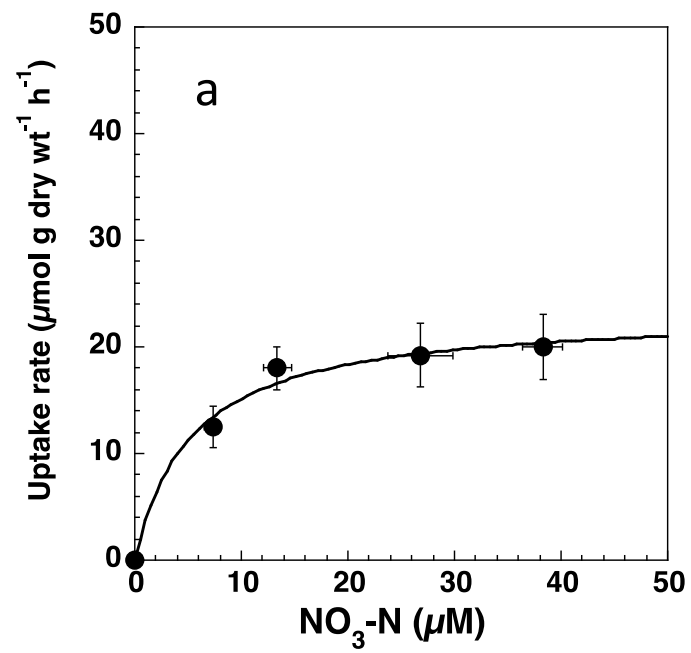


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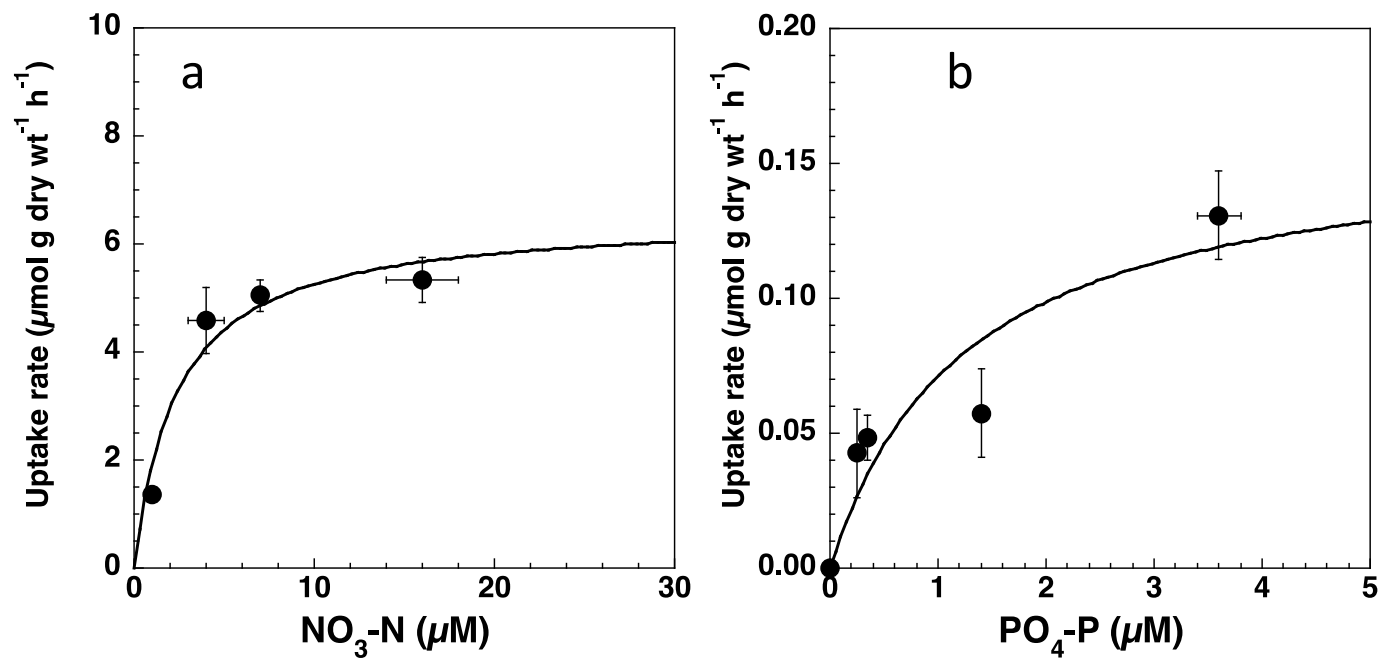


Fig. 5

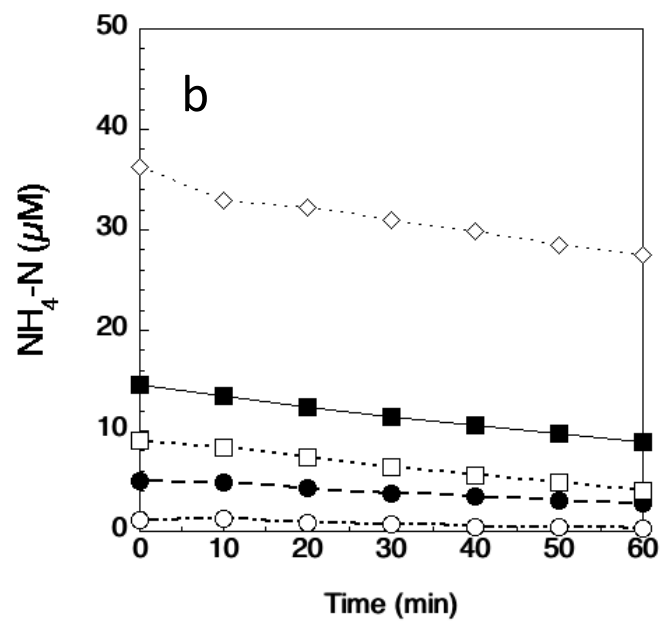
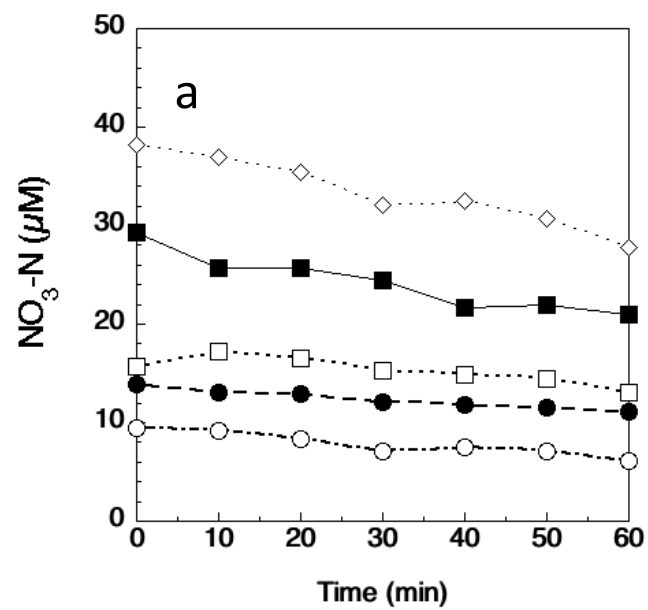


Fig. 6

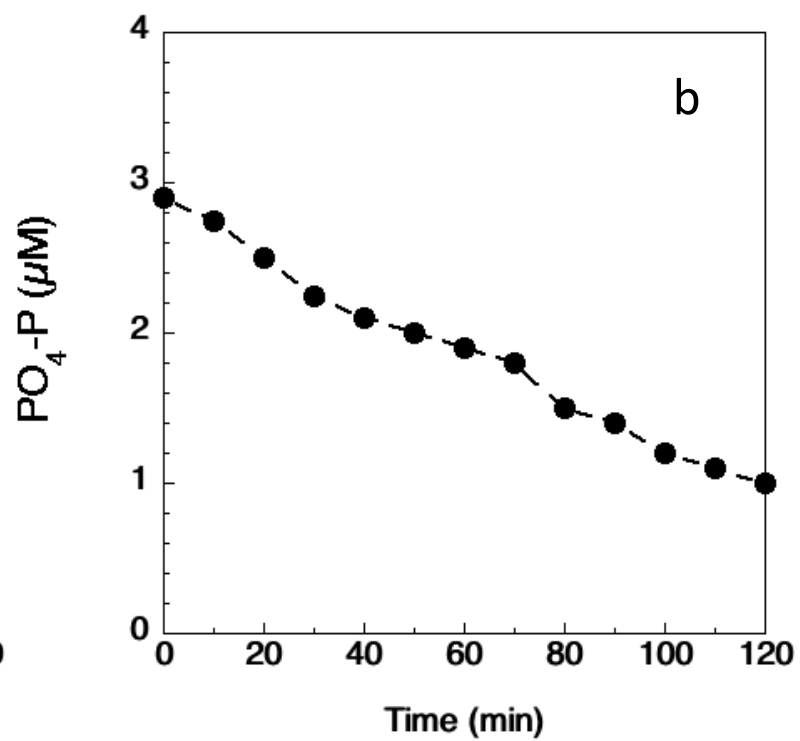
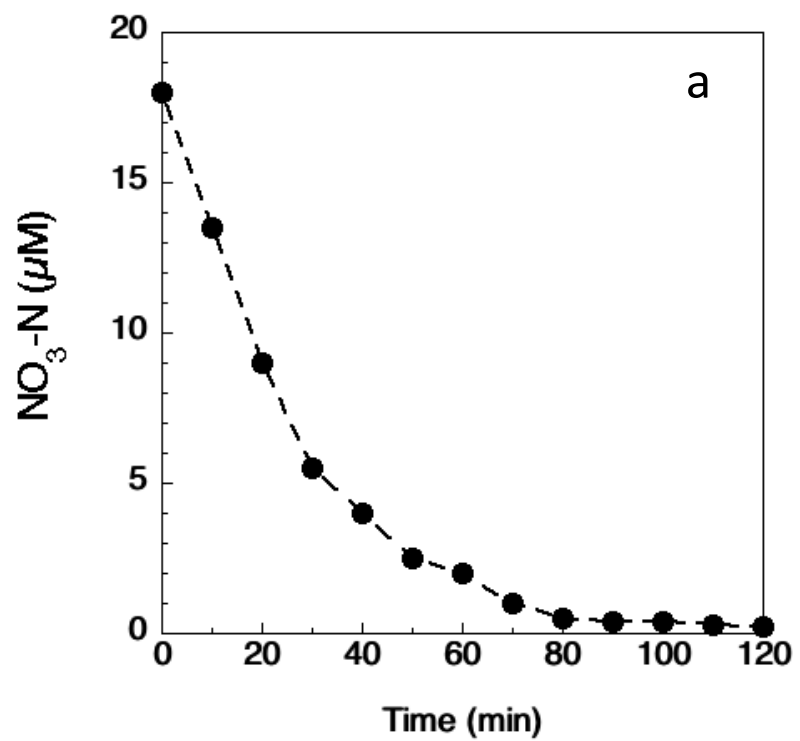


Fig. 7

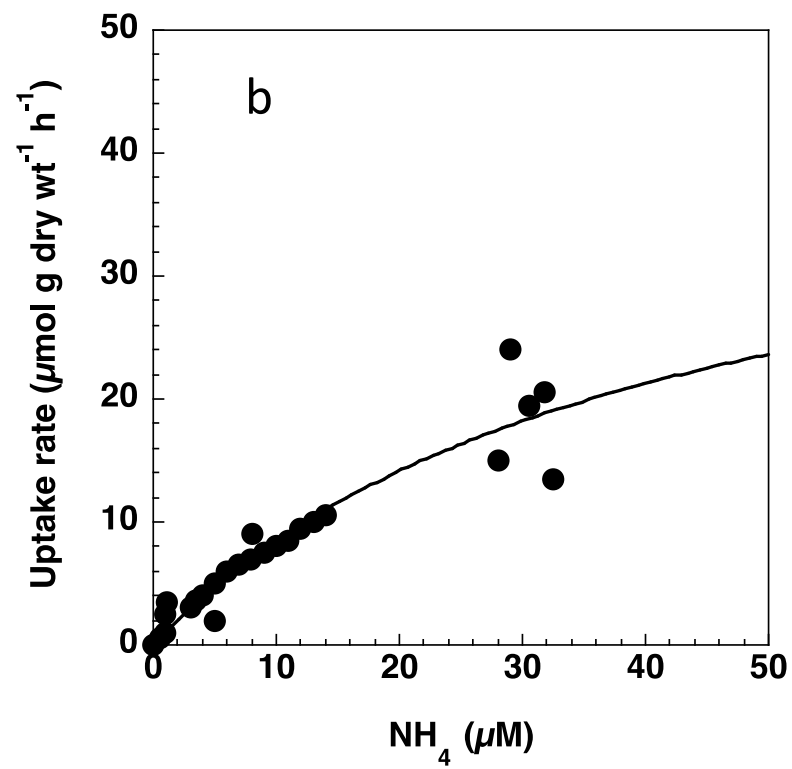
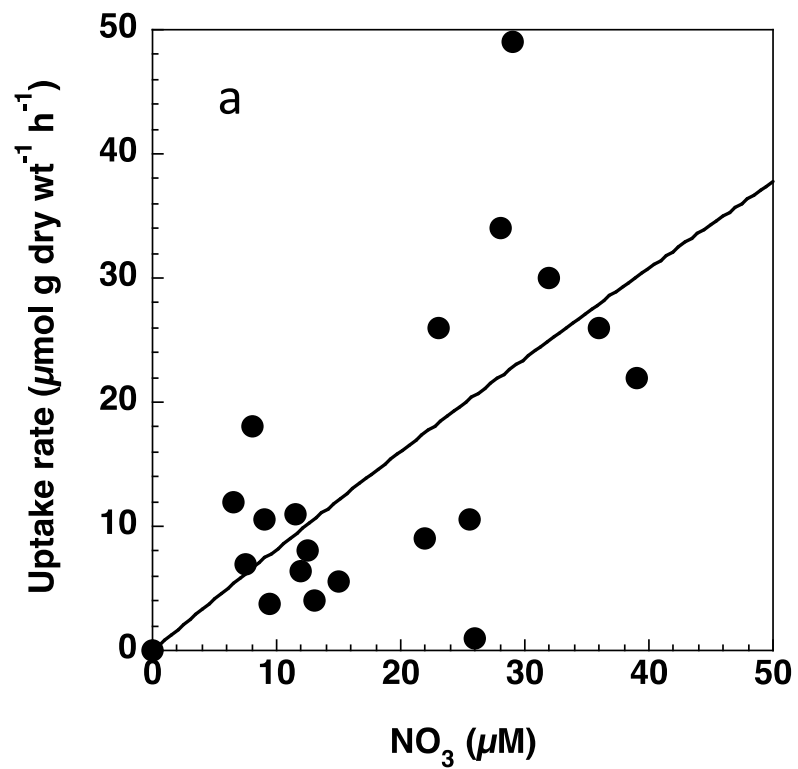


Fig. 8

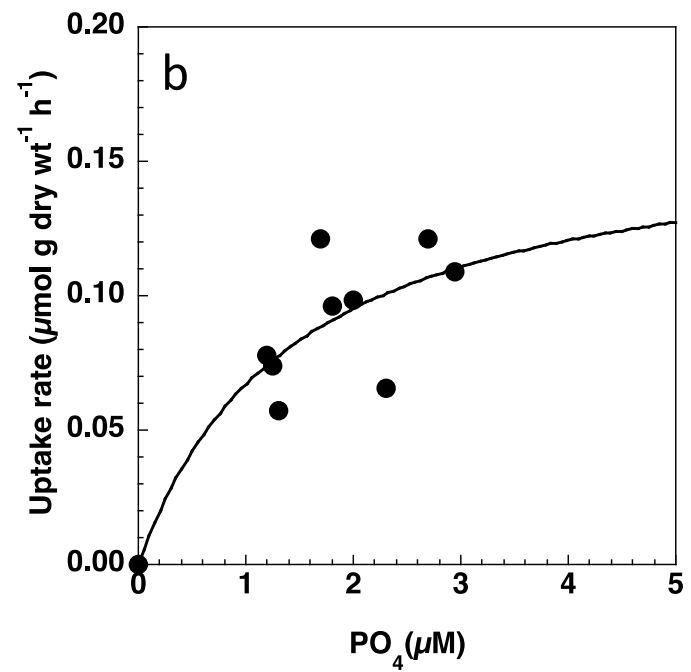
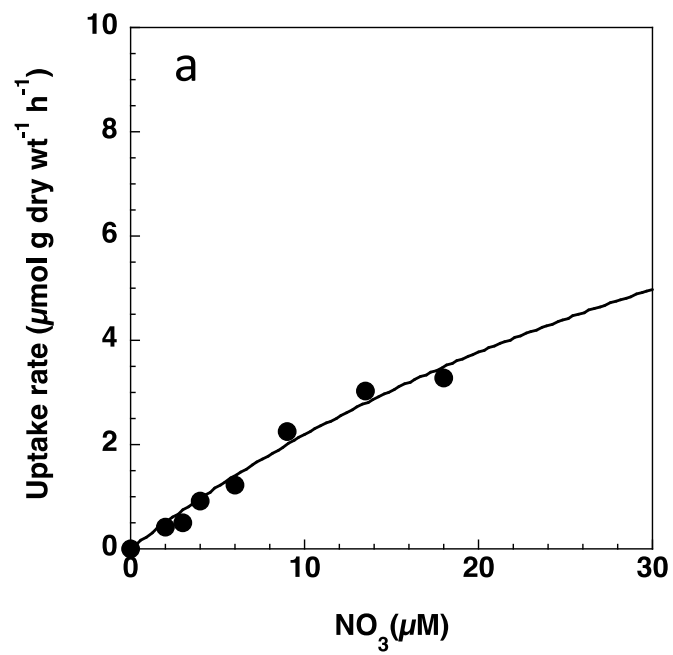


Fig. 9

Table 1 Comparison of Michaelis-Menten parameters between chemostat and batch culture.
Standard errors were given in parenthesis.

		Chemostat				Batch			
		$V_{\max}$	K_s	α	r	$V_{\max}$	K_s	α	r
		($\mu\text{mol g dwt}^{-1} \text{h}^{-1}$)	(μM)			($\mu\text{mol g dwt}^{-1} \text{h}^{-1}$)	(μM)		
<i>S. religiosa</i>									
NH_4		22.3	9.29	2.40	0.99	42.7	40.4	1.00	0.94
		(2.3)	(2.81)			(10.3)	(15.0)		
NO_3		23.2	5.35	4.33	0.99	401	481	0.83	0.63
		(1.5)	(1.44)			(3222)	(4091)		
<i>U. australis</i>									
		$V_{\max}$	K_s	α	r	$V_{\max}$	K_s	α	r
		($\mu\text{mol g dwt}^{-1} \text{h}^{-1}$)	(μM)			($\mu\text{mol g dwt}^{-1} \text{h}^{-1}$)	(μM)		
NO_3		6.51	2.39	66.5	0.98	13.7	53.0	7.05	0.98
		(0.93)	(1.19)			(7.4)	(35.6)		
PO_4		0.16	1.26	3.09	0.92	0.16	1.45	2.07	0.81
		(0.06)	(1.07)			(0.07)	(1.35)		