Lecture 8b: Nutrient Uptake

The Michaelis-Menten Model in Detail

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This Lecture Is Accompanied by the Following Lab

- Lab 4: Uptake Kinetics — Michaelis-Menten

Additional Reading

- **Lecture 8a: Nutrient Uptake**
- **Paper:** Smit (2002)

1 Algal Nutrient Uptake Experiments

The uptake process of nitrogen by algae can be measured *in situ* or under controlled conditions in the laboratory. Nutrient uptake is often determined by measuring the disappearance of a nutrient from the culture medium over a time interval after the addition of the alga. These experiments allow the calculation of *depletion curves*, and from the depletion curve the *uptake kinetics* can be determined.

To do this, we may us two types of experiments: **multiple flask** experiments and **perturbation** experiments. The fundamental concept underlying both methods is to introduce a known quantity of nutrients (termed the substrate) into a flask or a series of flasks and then measure the rate of nutrient uptake (V) at different substrate concentrations ($[S]$). We calculate the nutrient uptake rate as the change in nutrient concentration in the flask over a predefined time interval ($V = \Delta[S]/\Delta t$). Consequently, both experiments generate data that relate the nutrient uptake rate to the corresponding substrate concentration. The primary difference between the two methods lies in the experimental setup and the data analysis.

2 Multiple Flask Method

The multiple flask method is a widely used experimental technique for measuring the nutrient uptake kinetics of macroalgae. This method is used to determine how macroalgae absorb nutrients (such as nitrogen or phosphorus) from their environment under different substrate concentrations. The goal is to generate a relationship between the rate of nutrient uptake and the nutrient concentration available to the macroalgae. The experimental data can then be used to characterise the nutrient uptake kinetics, such as maximum uptake rate (V_{max}) and half-saturation constant (K_m). These model parameters tell us about the mechanisms that algae use to remove nitrogen or other nutrients from their environmental to be incorporated into biomass.

2.1 Experimental setup

In the multiple flask method, a series of flasks are prepared, each containing a different initial concentration of the nutrient (substrate) to span the range of nutrient levels typically encountered by the macroalgae in its natural habitat. This allows for measurements of nutrient uptake rates across a spectrum of substrate concentrations, from low to high.

2.2 Steps of the multiple flask experiment

1. **Substrate Preparation:** Prepare several flasks, each with a known initial concentration of the nutrient (*e.g.*, nitrogen) in solution. These concentrations should cover a range of interest, often from nutrient-limiting to saturating levels.
2. **Algal Introduction:** Introduce a known biomass of macroalgae into each flask. The biomass should be standardised across all flasks (*e.g.*, 4.5 g of fresh macroalgal tissue per flask).
3. **Incubation:** The flasks are incubated for a defined time period, typically 20–30 minutes, under controlled environmental conditions such as light and temperature.
4. **Sampling:** At the beginning of the incubation ($t = 0$) and at the end of the incubation period (*e.g.*, $t =$ 30 minutes), water samples are taken from each flask to measure the concentration of the nutrient in the water.

5. **Nutrient Analysis:** The concentration of the nutrient in each water sample is analysed using chemical methods (*e.g.*, colorimetric analysis or ion chromatography).

The difference in nutrient concentration between the start and end of the incubation reflects the amount of nutrient taken up by the macroalgae during the experiment.

2.3 Data collected

- **Initial substrate concentrations** ($[S_{\text{initial}}]$) in each flask.
- **Final substrate concentrations** ($[S_{\text{final}}]$) after the incubation period.
- **Time** of incubation (Δ_t).
- **Algal biomass** in each flask (usually standardised, *e.g.*, 4.5 g fresh mass).

2.4 Calculations for determining nutrient uptake rate (V)

The following steps outline how to calculate the nutrient uptake rate (V) from the experimental data obtained using the multiple flask method (apply to data obtained from each flask). These steps convert changes in nutrient concentration into actual uptake rates, adjusted for algal biomass and incubation time.

2.4.1 Step 1: calculate the change in nutrient concentration ($\Delta[S]$)

To determine how much nutrient was taken up during the incubation, subtract the final nutrient concentration from the initial nutrient concentration:

$$\Delta[S] = [S_{\text{initial}}] - [S_{\text{final}}]$$

For example:

$$\Delta[S] = 25\,\mu M - 9.9\,\mu M = 15.1\,\mu M$$

This gives the reduction in nutrient concentration over the time period but does not yet account for the volume of the flask or the biomass of algae.

2.4.2 Step 2: convert concentrations to mass of nutrient present per flask

Convert the concentration of the nutrient (in μmol.L^{-1}) into the actual mass of nutrient (in μg) present in the flask. To do this, use the molecular mass (MM) of the nutrient (*e.g.*, nitrogen), which is 14.0067 g.mol^{-1} for N.

For example:

$$25\,\mu M = 25\,\mu mol/L \times 14.0067\,\frac{g}{mol} = 350.17\,\mu g\,N\ (\text{per liter})$$

$$9.9\,\mu M = 9.9\,\mu mol/L \times 14.0067\,\frac{g}{mol} = 138.67\,\mu g\,N\ (\text{per liter})$$

Next, account for the volume of the flask (*e.g.*, 500 mL). Since the above values are for 1 liter, divide by 2 to find the mass in 500 mL:

$$\text{Mass of N at the start} = 350.17\,\mu g/2 = 175.09\,\mu g$$

$$\text{Mass of N at the end} = 138.67\,\mu g/2 = 69.34\,\mu g$$

2.4.3 Step 3: calculate the amount of nutrient taken up by the alga

Now, calculate how much nutrient was taken up by the algae during the incubation:

$$\Delta\ \text{Mass of N} = 175.09\,\mu g - 69.34\,\mu g = 105.75\,\mu g\,N$$

This represents the total amount of nitrogen removed from the water by the algal biomass during the 20-minute incubation.

2.4.4 Step 4: normalise nutrient uptake by algal biomass

To determine how much nutrient was taken up per unit mass of algae, divide the total nutrient uptake by the biomass of algae in the flask (*e.g.*, 4.5 g):

$$\text{Nutrient uptake rate} = \frac{105.75\,\mu g\,N}{4.5\,g} = 23.5\,\mu g\,N/g$$

This gives the nutrient uptake rate in terms of µg of nutrient per gram of algal biomass over the incubation period of 20 minutes.

2.4.5 Step 5: calculate the nutrient uptake rate per hour

If the experiment lasted 20 minutes, but the uptake rate needs to be expressed on an hourly basis, multiply the rate by 3 (since there are three 20-minute intervals in an hour):

$$\text{Nutrient uptake rate per hour} = 23.5\,\mu g\,N/g \times 3 = 70.50\,\mu g\,N/g/hr$$

2.5 Final workflow for calculating nutrient uptake rate

1. **Determine the change in nutrient concentration** between the start and end of the experiment for each flask:

$$\Delta[S] = [S_{\text{initial}}] - [S_{\text{final}}]$$

2. **Convert concentrations to mass of nutrient** (*e.g.*, µg N) using the molecular mass and flask volume:

$$\text{Mass of nutrient} = [S] \times \text{MM of nutrient}$$

3. **Calculate the amount of nutrient taken up** by the algae:

$$\Delta \text{ Mass of nutrient} = \text{Mass of nutrient (initial)} - \text{Mass of nutrient (final)}$$

4. **Normalise the nutrient uptake by the algal biomass**:

$$\text{Nutrient uptake rate} = \frac{\Delta \text{ Mass of nutrient}}{\text{Algal biomass}}$$

5. **Convert the nutrient uptake rate to an hourly rate**, if necessary:

$$\text{Nutrient uptake rate (hourly)} = \text{Nutrient uptake rate} \times \frac{60}{\Delta t}$$

3 Perturbation Method

The perturbation method is an alternative approach for measuring nutrient uptake kinetics in macroalgae. This method involves a single flask where a relatively high initial concentration of a substrate nutrient is introduced, typically set at a level that is ecologically relevant to the system being studied. Rather than using multiple flasks with different initial nutrient concentrations, the perturbation method measures the decrease in nutrient concentration over time within the same flask. By sampling the substrate concentration at regular time intervals (*e.g.*, every 10 or 20 minutes), we can calculate nutrient uptake rates for each time period.

3.1 Experimental setup

In the perturbation method, a single flask is prepared with a high initial concentration of the nutrient (*e.g.*, nitrogen), and a known amount of macroalgal biomass is introduced. The flask is incubated, and water samples

are taken at regular intervals to track the decrease in nutrient concentration. The resultant data allow us to calculate the nutrient uptake rate for each time interval.

3.1.1 Steps of the perturbation experiment

1. **Substrate Preparation:** Add a known and high concentration of the nutrient (*e.g.*, 25 µM nitrogen) to the flask. The concentration should be high enough to ensure measurable changes over the course of the experiment but ecologically relevant.
2. **Algal Introduction:** Introduce a known biomass of a macroalga into the flask (*e.g.*, 4.5 g of fresh macroalgal tissue).
3. **Incubation and Sampling:** The flask is incubated, and water samples are taken at regular intervals (*e.g.*, every 10 or 20 minutes) to measure the nutrient concentration at each time point.
4. **Nutrient Analysis:** The concentration of the nutrient in each water sample is analysed to determine how much nutrient remains at each time point.
5. **Data Collection:** The change in nutrient concentration between each successive time point is used to calculate the nutrient uptake rate over the interval.

The resulting data from the perturbation method consist of a time series of substrate concentrations paired with calculated nutrient uptake rates over specific time intervals.

3.2 Data collected

- **Initial substrate concentration** ($[S_{initial}]$) and substrate concentrations at subsequent time points ($[S_{t1}]$, $[S_{t2}]$, …).
- **Time intervals** (Δ_t, *e.g.*, every 5 or 10 minutes).
- **Algal biomass** in the flask (*e.g.*, 4.5 g of fresh mass).

By plotting the remaining substrate concentration against each time point at which we sampled the water for nutrient measurement, we can construct a **nutrient depletion curve**. From this, we can observe how the nutrient is taken up by the macroalga and calculate the nutrient uptake rate at different stages of the experiment.

3.3 Calculations for determining nutrient uptake rate (V)

Once the experimental data have been collected, the next step is to calculate the nutrient uptake rate for each time interval based on the reduction in nutrient concentration between successive time points. The following steps outline how to perform these calculations.

3.3.1 Step 1: calculate the change in nutrient concentration ($\delta[S]$)

To determine how much nutrient has been taken up during a specific time interval (*e.g.*, the first 5 minutes of the experiment, *i.e.*, $\Delta_t = 0$), subtract the nutrient concentration at the end of the interval from the concentration at the start:

$$\Delta[S] = [S_{\text{start}}] - [S_{\text{end}}]$$

For example:

$$\Delta[S] = 25\,\mu M - 21.3\,\mu M = 3.7\,\mu M$$

This gives the reduction in nutrient concentration over the 5-minute interval.

3.3.2 Step 2: convert concentrations to mass of nutrient present per flask

Convert the nutrient concentration (in µmol.L^{-1}) into the mass of nutrient (in µg) present in the flask. To do this, use the molecular mass (MM) of the nutrient, which is 14.0067 g.mol^{-1} for nitrogen.

For example:

$$25\,\mu M = 25\,\mu mol/L \times 14.0067\,\frac{g}{mol} = 350.17\,\mu g\,N\,(\text{per liter})$$

$$21.3\,\mu M = 21.3\,\mu mol/L \times 14.0067\,\frac{g}{mol} = 298.34\,\mu g\,N\,(\text{per liter})$$

Since the flask contains 500 mL (0.5 L) of solution, divide the values by 2 to get the mass of nitrogen in the 500 mL flask:

$$\text{Mass of N at the start} = 350.17\,\mu g/2 = 175.09\,\mu g$$

$$\text{Mass of N at the end} = 298.34\,\mu g/2 = 149.17\,\mu g$$

3.3.3 Step 3: calculate the amount of nutrient taken up by the alga

Next, calculate the amount of nitrogen taken up by the algae during the 5-minute interval:

$$\Delta\ \text{Mass of N} = 175.09\,\mu g - 149.17\,\mu g = 25.92\,\mu g\,N$$

This represents the total amount of nitrogen removed from the water by the algal biomass in the 5-minute period.

3.3.4 Step 4: normalise nutrient uptake by algal biomass

To determine how much nitrogen was taken up per unit mass of algae, divide the total nitrogen uptake by the algal biomass (*e.g.*, 4.5 g):

$$\text{Nutrient uptake rate} = \frac{25.92\,\mu g\,N}{4.5\,g} = 5.76\,\mu g\,N/g$$

This gives the nitrogen uptake rate in terms of µg of nitrogen per gram of algal biomass over the 5-minute interval.

3.3.5 Step 5: calculate the nutrient uptake rate per hour

Since the uptake was allowed to proceed for 5 minutes over the first interval, but you may want to express the uptake rate per hour, multiply the uptake rate by 12 (since there are twelve 5-minute intervals in one hour):

$$\text{Nutrient uptake rate per hour} = 5.76\,\mu g\,N/g \times 12 = 69.12\,\mu g\,N/g/hr$$

This uptake rate relates to the specific time interval and can be used to track changes in $[V]$ over time. In this example, this uptake rate relates to the first 5 minutes of the experiment. The average $[S]$ during this intervals was $(25\,\mu M + 21.3\,\mu M)/2 = 23.15\,\mu M$.

Repeat these steps for each remaining intervals and express $[V]$ relative the the mean $[S]$ for each interval (some authors use the $[S]$ at the start of the interval instead of the mean for the interval).

3.4 Final workflow for calculating nutrient uptake rate

1. **Determine the change in nutrient concentration** between successive time points:

$$\Delta[S] = [S_{\text{start}}] - [S_{\text{end}}]$$

2. **Convert concentrations to mass of nutrient** using the molecular mass and flask volume:

$$\text{Mass of nutrient} = [S] \times \text{MM of nutrient}$$

3. **Calculate the amount of nutrient taken up** by the algae during the time interval:

$$\Delta \text{ Mass of nutrient} = \text{Mass of nutrient (start)} - \text{Mass of nutrient (end)}$$

4. **Normalise the nutrient uptake by the algal biomass**:

$$\text{Nutrient uptake rate} = \frac{\Delta \text{ Mass of nutrient}}{\text{Algal biomass}}$$

5. **Convert the nutrient uptake rate to an hourly rate**, if necessary:

$$\text{Nutrient uptake rate (hourly)} = \text{Nutrient uptake rate} \times \frac{60}{\Delta t}$$

The important differences between the multiple flask and perturbation experiments are summarised in Table 1.

Table 1: Key differences between multiple flask and perturbation experiments.

Feature	Multiple Flask Experiments	Perturbation Experiments
Experimental Setup	Multiple flasks, each with different $[S]$	Single flask with initial high $[S]$
Data Independence	Data points are independent	Data points are correlated (repeated measures)
Analysis	Nonlinear least squares regression (NLS)	Nonlinear mixed model (NLMM)
R Function	`nls()`	`nlme::nlme()`

Our choice between multiple flask and perturbation experiments depends on our research questions and experimental constraints. In both methods, we must consider all sources of error and variability, such as measurement error, the type of nutrient, the physiological state of the alga, the light intensity, the experimental temperature, and other variables that might affect the uptake response.

4 The Michaelis-Menten Model

We apply the Michaelis-Menten model (Equation 1) to data from multiple flask and perturbation experiments to characterise nutrient uptake. Applied to algae, this model assumes an irreversible uptake process that saturates at high substrate concentrations. It effectively quantifies key characteristics of the nutrient uptake system, including the maximum uptake rate and the algae's affinity for the nutrient.

The Michaelis-Menten equation is given by:

$$V_i = \frac{V_{max} \cdot [S_i]}{K_m + [S_i]} + \epsilon_i \tag{1}$$

Where:

- V_i is the uptake rate at the i-th observation,
- V_{max} is the maximum nutrient uptake rate achieved,
- $[S_i]$ is the substrate concentration at the i-th observation,
- K_m is the Michaelis constant, which represents the substrate concentration at which the uptake rate is half of V_{max}, and
- ϵ_i is the error term at the i-th observation. and

The two parameters of the Michaelis-Menten model are rooted in theory and have ecophysiological interpretations. K_m is a measure of the alga's affinity for the nutrient and is determined by the kinetic constants

governing the formation and dissociation of the enzyme-substrate complex responsible for taking up the nutrient; lower values indicate a higher affinity. V_{max} represents the maximum capacity of the alga to utilise the nutrient.

5 Data Analysis: Fitting the Michaelis-Menten Model with Nonlinear Regression

Advanced Curve Fitting

- Biostatistics: The Book — Chapter 7: Nonlinear Models

I provide this information only as a matter of interest to BDC223 students, as this is an advanced topic that will only be covered in your BSc (Hons) degrees. It is intended to provide a glimpse into the type of analysis that can be performed on nutrient uptake data. For the purpose of this course, you will not be required to perform this analysis and can instead rely on fitting the V vs $[S]$ curve by hand.

To formally model the Michaelis-Menten relationship, we use the `nls()` function in R to apply a non-linear regression the data from multiple flask experiments. For the perturbation experiment, things are a bit more complicated. This method includes dependent data points because the measurements are taken from the same flask at different times, introducing a correlation between observations. This violates the independence assumption required for standard regression models. To accurately analyse these data, I recommend a *nonlinear mixed-effects model* implemented in the `nlme()` function. Mixed-effects models account for fixed effects (overall trends across all observations) and random effects (variations specific to individual experimental units, in this case, time points within the same flask). This helps handle the correlation between repeated measures and produces reliable estimates of the uptake dynamics within the flask.

Bibliography