

ACI curves

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Abstract

The study of the photosynthetic process frequently involves analysis of net assimilation - intercellular CO_2 concentration (ACI) curves. These are used in the estimation of key parameters associated with the Farquhar-van Caemmerer-Berry (FvCB) [4] model:

- $V_{c_{max}}$: the rate of maximum Rubisco carboxylation;
- J : electron transport rate;
- R_d : daytime respiration; and
- g_m : mesophyll conductance.

Accurate, unbiased estimation of these parameters is a non-trivial exercise with the optimal method still a matter of debate within the field [7], [16], [9]. The problem of selecting suitable starting values for a non-linear model is thus complicated by the lack of unanimity on what is a “good” estimate of these. The core model we are using to estimate the parameters is based on Sharkey et al. [11].

Keywords— ACI curves, parameter estimation, FvCB model

1 FvCB model

The brilliant realisation of Farquhar et al. [4] was that A could be accurately modeled as being in one of two steady-state systems. In one (*Rubisco-limited photosynthesis*), the rate is limited by the properties of the enzyme ribulose 1.5 biphosphate carboxylase/oxygenase (Rubisco) (we assume saturating levels of the substrate, RuBP). In the second we assume that it is the substrate that determines the rate of assimilation; specifically its regeneration rate. Hence the second state is referred to as *RuBP-regeneration-limited photosynthesis* [11].

Aside 1.1

Currently I focus only on the FvCB model, but since the original paper in 1980, many stalwarts have extended the model to specific cases (such as C_4 species) or to encompass more physiologically limiting factors (such as triose phosphate utilization (*TPU*) in the synthesis of starch and sucrose). These will be added as deemed relevant, and for now we only include the *TPU* limiting effect mentioned by Sharkey et al. [11].

1.1 Some assumptions

For parsimony’s sake, we make several simplifying assumptions regarding the homogeneity of conditions within the leaf. We disregard any gradient of temperature (T) or O_2 within the leaf. According

to von Caemmerer [13], these, specifically within C_3 species, are unlikely to be important. Due to the thinness of leaves we also assume an equal distribution of light to all chloroplasts within the leaf; however, it has been shown that under certain conditions these assumptions and their corollary, that photosynthesis itself is homogeneous across the leaf, do not hold. Non-uniform photosynthesis can occur and this will affect the gas-exchange measurements and their interpretation [12].

1.2 Rubisco limited photosynthesis

The photosynthetic carbon reduction (PCR) and photorespiratory carbon oxidation (PCO) cycles are linked by an enzyme common to both, RuBP [4]. In the PCO cycle, 0.5 mol of CO_2 is released, thus:

$$A = V_c - 0.5V_o - R_d \quad (1)$$

Where:

- V_c : the rate of carboxylation; and
- V_o : the rate of oxygenation.

We have from Farquhar [5] (assuming RuBP binding first in the reaction):

$$V_c = V_{c_{max}} \frac{C_c}{C_c + K_c (1 + O/K_o)} \cdot \frac{R}{R + K'_r} \quad (2)$$

$$V_o = V_{o_{max}} \frac{O}{O + K_o (1 + C_c/K_c)} \cdot \frac{R}{R + K'_r} \quad (3)$$

Where:

- $V_{c_{max}}$: the maximum velocity of the carboxylase;
- $V_{o_{max}}$: the maximum velocity of the oxygenase;
- O : the partial pressure of oxygen in the chloroplast;
- K_c : the Michaelis-Menten constant for CO_2 ;
- K_o : the Michaelis-Menten constant for O ;
- R : the concentration of free RuBP; and
- K'_r : the effective Michaelis-Menten constant for RuBP.

This leads to the ratio of oxygenation to carboxylation:

$$\phi = \frac{V_o}{V_c} = \frac{V_{o_{max}}}{V_{c_{max}}} \cdot \frac{O/K_o}{C_c/K_c} \quad (4)$$

We also have that the velocity of carboxylation, at saturating levels of RuBP, (W_c) is given by [6]:

$$W_c = V_{c_{max}} \frac{C_c}{C_c + K_c(1 + O/K_o)} \quad (5)$$

Note that this arises from setting the RuBP component of equation 2 to 1. The compensation point for C_c at which there is no net assimilation, Γ^* , is given by [4]:

$$\Gamma^* = \frac{K_c O}{2K_o} \cdot \frac{V_{o_{max}}}{V_{c_{max}}} \quad (6)$$

Relating equations 1, 4, 5 and 6 we have:

$$A = V_{c_{max}} \frac{C_c - \Gamma^*}{C_c + K_c(1 + O/K_o)} - R_d \quad (7)$$

This is the Rubisco-limited rate of assimilation, assuming saturation of RuBP.

1.3 RuBP regeneration limited photosynthesis

The physiology of this model is somewhat more complex, involving analysis of the cycle for the production of NADPH [4]. A thorough understanding of this is beyond the scope of this project, but we recommend the reader to the original paper [4] and von Caemmerer [13]. We are content to state that when A is limited by RuBP regeneration:

$$A = J \frac{C_c - \Gamma^*}{4C_c + 8\Gamma^*} - R_d \quad (8)$$

Where J is the rate of electron transport. Sharkey et al. [11] recommend the conservative use of 4 and 8 in the denominator based on the number of electrons required for NADP^+ reduction, however this is not always the case; values of 4.5 and 10.5 also occur in the literature.

Aside 1.2

I imagine that the model will use default values of 4 and 8 with an option to use 4.5 and 10.5. It is possible that we will allow any values.

1.4 TPU limited photosynthesis

TPU is required at one third the rate of CO_2 fixation. If this is not acquired, the level of free phosphate declines and the rate of photosynthesis is limited. When this limit is imposed, it can be seen that photosynthesis becomes independent of the ratio of oxygenation to carboxylation [10]. Thus, the rate equation is simply:

$$A = 3TPU - R_d \quad (9)$$

Where TPU is the rate of triose phosphate utilization.

Aside 1.3

From von Caemmerer [13] we know that this model is too simple for the reality, please see section 2.4.3 for an extension as formulated by Harley and Sharkey in 1991. Currently I ignore it as equation 9 is sufficient for now, and may even be unnecessary itself for the scope of this project.

1.5 Summary

For the final model, our actual assimilation rate is calculated by combining these individual curves. The curve fitted is:

$$A = \min \{A_{Rubisco}, A_{RuBP}, A_{TPU}\} \quad (10)$$

Where:

- $A_{Rubisco}$: the rate calculated from equation 7;
- A_{RuBP} : the rate calculated from equation 8; and
- A_{TPU} : the rate calculated from equation 9.

2 Parameters

Several parameters require estimation, and some measurements are not feasible for the majority of experiments. For these we need either a starting value that is our initial best guess (eminently feasible in C_3 species, less so in C_4) or a correction to some related measurement. We show the reasoning behind our choices below.

2.1 Relating C_i to C_c

One of the driving factors in net assimilation rate (A) is the level of CO_2 present at the Rubisco site of carboxylation in the chloroplasts (C_c) [14]. Estimation of the intercellular CO_2 partial pressure (C_i) is based on gas exchange measurements and is not a matter of controversy. Less well established is estimation of C_c [16]. We can relate C_i to C_c using Flick's first law relating the diffusive flux to the concentration under steady-state assumptions. We have:

$$J = -D \frac{d\phi}{dx} \quad (11)$$

Where:

- J is the diffusion flux;
- D is the diffusion coefficient;
- ϕ is the concentration; and
- x is position.

From this we can consider A as our flux, and the difference between C_c and C_i as our gradient in concentration:

$$A = -D(C_c - C_i) \quad (12)$$

The diffusion conductance between the substomatal cavities and the chloroplasts is g_m , the mesophyll conductance [8]. Hence we can write equation 12 as:

$$A = g_m(C_i - C_c) \quad (13)$$

Or equivalently:

$$C_c = C_i - \frac{A}{g_m} \quad (14)$$

Thus we can calculate C_c , the variable relevant to our models, using C_i .

Aside 2.1

This function might be used in an iterative process to calculate the parameters: I imagine a two step method where we initialise C_c to C_i or use the starting value of g_m to calculate C_c and then:

1. Calculate parameters (incl. g_m) using current values of C_c ; and
2. Update C_c based on current value of g_m and equation 14.

Once C_c and g_m converge we stop. Possibly this is too expensive to include, but possibly could be an option in the function.

We should also allow for someone having done the correction to C_c themselves, so a bool instructing use of the correction should be included.

2.2 Light dependence of electron transport rate

We can relate J_{max} to the incident irradiance by empirical equation:

$$\theta J^2 - J(I_2 + J_{max}) + I_2 J_{max} = 0 \quad (15)$$

Where:

- I_2 : the useful light absorbed by PS II;
- J : the electron transport;
- J_{max} : the maximum electron transport; and
- θ : an empirical curvature factor (often around 0.7 [2]).

I_2 is related to total incident irradiance I by:

$$I_2 = I \times \text{abs}(1 - f)/2 \quad (16)$$

Where:

- abs : the absorptance of leaves (commonly around 0.85 [13]);
- f : correction for the spectral quality of light (approximately 0.15 [3]); and
- The 2 in the denominator is due to the split of light between photosystems I and II.

We can thus solve for J in the usual way:

$$J = \frac{I_2 + J_{max} - \sqrt{(I_2 + J_{max})^2 - 4\theta \cdot I_2 \cdot J_{max}}}{2\theta} \quad (17)$$

This is the non-rectangular hyperbola function.

2.3 Model parameters

Many parameters can be assigned *a priori*, leaving only the estimation of a small number of key variables. The kinetic constants of rubisco vary very little among C_3 species such that one can use the same K_c , K_o and Γ^* across all members of this category [13]. This means that the only parameter requiring estimation for Rubisco is the maximal Rubisco activity, $V_{c_{max}}$.

For RuBP-limited photosynthesis, we need to solve for J_{max} as we can relate this to J by equation 17. According to Walcroft et al. [15], the ratio $J_{max} : V_{c_{max}}$ is expected to vary from 2 to 1.4 across the range $[8^\circ\text{C}, 30^\circ\text{C}]$.

Evans and von Caemmerer [1] recommend setting $g_m = 0.0045V_{c_{max}}$. Other values can be seen, with an assumption of $g_m = \infty$ used frequently, but this is controversial and can lead to biased estimates of $V_{c_{max}}$ and J_{max} [16]. Specific plants in specific conditions can see diverging values, but we feel this is a sufficient initial value.

This means that if we have an empirically driven initial value for $V_{c_{max}}$ we can initialise all our parameters to some default temperature, however these relationships vary with temperature, the relationship of which we state below.

2.3.1 Temperature dependency

The dependency of the rate of carboxylation and oxygenation of Rubisco is reflected in the temperature dependency of A . As a rate dependent upon temperature, we will be using Arrhenius functions, equations of the form:

$$x(T) = k \cdot \exp\left(-\frac{E_a}{R \cdot T}\right) \quad (18)$$

Where:

- $x(T)$: the temperature dependent rate we are interested in;
- k : the rate coefficient;
- E_a : the activation energy, the kinetic energy of substrate required for the reaction to occur;
- R : the universal gas constant ($8.314 \text{ J K}^{-1} \text{ mol}^{-1}$); and
- T : temperature (in Kelvin).

As most photosynthesis measurement are recorded in $^{\circ}\text{C}$, and specifically use a default value of 25°C , we transform the equation to this scale. In this case we have:

$$x(T') = k' \cdot \exp \left(-\frac{(25 - T')E_a}{298.15R \cdot (273.15 + T')} \right) \quad (19)$$

Where T', k' are the relevant form of T, k for a scale centred on $T = 25^{\circ}\text{C}$. As we will not be using the Kelvin scale again, we denote $T = T', k = k'$.

Many reactions in photosynthesis are reversible, with differing activation energies depending on the direction of the reaction. Thus the net activation energy may vary depending on the ratio of forward to backward reactions. This means that the Arrhenius function 19 is only semi-empirical, but it does allow easy comparison between studies.

Another frequently used method to describe a dependency on temperature is the Q_{10} temperature coefficient. This is a measure of the rate of change of a system as a result of raising the temperature by 10°C . It is calculated:

$$Q_{10} = \left(\frac{R(T_2)}{R(T_1)} \right)^{10^{\circ}\text{C}/(T_2 - T_1)} \quad (20)$$

Where:

- Q_{10} : the factor by which the reaction rate increases when temperature is raised by 10°C ;
- T_i : the temperature for the i^{th} measurement; and
- $R(T_i)$: the rate of the reaction at temperature T_i .

Alternatively we can write this in the form:

$$R(T_2) = R(T_1)Q_{10}^{(T_2 - T_1)/10^{\circ}\text{C}} \quad (21)$$

Again, this general form is of less interest as we have a specific, default temperature of 25°C for which we are quite well informed. Hence, we use:

$$R(T) = R(25^{\circ}\text{C})Q_{10}^{(T - 25)/10} \quad (22)$$

Aside 2.2

This function allows us to relate the temperature as $T = 25^{\circ}\text{C}$ to a more general temperature; perhaps it is flawed for extremes, but I do not expect that our starting value calculation will work outside a limited range (say 18°C to 30°C) within more user input. This is not the end of the world and we can't solve everyone's problems all at once. This will be used to relate our default 25°C values (given in table 1) to whatever the user has stated for values of T . This might be harder if we have varying temperatures? Hmm ...

2.3.2 Initial values

We use table 1 for our initial values of photosynthetic parameters in our model. We can combine these with equation 22 to calculate initial values for our model across a range of temperatures. Many of the values in table 1 can be found in von Caemmerer [13].

Parameter	unit	Value	E (kJ mol ⁻¹)	Q ₁₀ (T = 25 °C)
K _c	μbar	260 or 404 ^a	59.36 ^b	2.24
K _o	mbar	179 or 248 ^a	35.94	1.63
Γ*	μbar	38.6 or 37 ^a	23.4	1.37
V _{cmax}	μmol m ⁻² s ⁻¹	80	58.52	2.21
V _{omax}	μmol m ⁻² s ⁻¹	0.25 × V _{cmax}	58.52	2.21
R _d	μmol m ⁻² s ⁻¹	0.01 – 0.02 × V _{cmax}	66.4	2.46
J _{max}	μmol m ⁻² s ⁻¹	1.4 – 2.0 × V _{cmax}	37	1.65
g _m	μmol m ⁻² s ⁻¹	0.0045V _{cmax} ^c or ∞		
θ		0.7		

^a The first value is appropriate when an internal diffusion conductance is included; the second value should be used if the internal conductance is not included (i.e. g_m = ∞) and C_c is assumed to equal C_i.

^b From Farquhar et al. [4]

^c From Evans and von Caemmerer [1]

Table 1: Photosynthetic parameters and their activation energy for T = 25 °C

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