

Electronic Supplementary Information

Development of Light Harvesting Model

Abbreviations

FPR = Flat Panel Reactor

LHM = Light Harvesting Model

OEC = Oxygen Evolving Complex

PSII = Photosystem II

Nomenclature

A= Illuminated surface area of FPR (m²)

A_{max}= Maximum illuminated surface area of FPR(m²)

c = Constant of integration of PSII degradation kinetics

H_c = Heat of combustion of algae (kJ kg⁻¹)

k_a= Molar light absorptivity coefficient of algae (kg⁻¹ m²)

k_p= Rate constant of PSII degradation (s⁻¹)

k_r= Rate constant of PSII repair mechanism (s⁻¹)

K = α - αβ (number of photons⁻¹ m²)

$$K' = \frac{K}{2} \cdot \frac{p_o}{V} \cdot PAR_{i,o}$$

K_c = constant of proportionality for quantum yield (mol cell (mol PSII)⁻¹)

K_{g,o}, constant of proportionality for OEC decay losses due to bulk mixing

K_{g,1} constant of proportionality for OEC decay losses due to intercellular self-shading

K_{g,2} = constant of proportionality for OEC decay losses due to intracellular self-shading

K_{g,3} is the coefficient of physiological maintenance.

K_{light} = Energy content per photon (kJ photon⁻¹)

I_a = Average light intensity absorbed by algae (number of photons m⁻² s⁻¹)

I_m = minimum light intensity required to initiate photosynthesis (number of photons m⁻² s⁻¹)

I_o = Average incident light intensity (number of photons m⁻² s⁻¹)

I_t = Average light intensity transmitted through FPR (number of photons $m^{-2} s^{-1}$)

L:D = Ratio of light cycle to dark cycle

M = Concentration of active PSII in an algal cell (mol PSII mol cell⁻¹)

M* = Concentration of inactive PSII in an algal cell (mol PSII mol cell⁻¹)

M₀ = Total PSII in an algal cell (mol PSII mol cell⁻¹)

p = Average light penetration in FPR (m)

$p_{m,n+1}$ = light penetration (m) in V_i beyond which OEC decay may occur due to insufficient light (local light intensity $\leq I_m$), in the (n+1)th time interval.

$p_o = V/A_{max}$ (m)

PAR_a = Fraction of PAR_i absorbed by algal cells (number of photons)

PAR_c = Fraction of PAR_a used for glucose production (number of photons)

PAR_g = Fraction of PAR_c used for biomass growth (number of photons)

PAR_i = Average amount of photons incident on FPR (number of photons)

PAR_{i,o} = PAR_{i,0.5} in a FPR of known volume, light penetration and biomass density (number of photons)

t = Time (s)

V = Working volume of reactor (m³)

V_i = Illuminated working volume of reactor (m³)

W = Width of reactor (m)

X = Algal biomass density (kg m⁻³)

X_a = Apparent algal biomass density in FPR exposed to light (kg m⁻³)

X_{a,n} = Apparent algal biomass density in the (n+1)th time interval exposed to light (kg m⁻³)

X_f = Algal density in FPR (kg m⁻³) when growth ceases due to light limitation

X_n = Algal biomass density in (n+1)th time interval (kg m⁻³)

Greek Symbols

α = Coefficient of photodamage with respect to I_o (number of photons⁻¹ m²)

β = Coefficient of repair with respect to photodamage

$\eta_{BM,n+1}$ = loss in $\eta_{OEC,n+1}$ due to bulk mixing in the (n+1)th time interval

$\eta_{PSII,n+1}$ = loss in $\eta_{OEC,n+1}$ due to intracellular self-shading of active PSII in the (n+1)th time interval

$\eta_{SS,n+1}$ = loss in $\eta_{OEC,n+1}$ due to intercellular self-shading in the (n+1)th time interval

$\eta_{OEC,n+1}$ = efficiency of photolysis of water at OEC in the (n+1)th time interval.

ρPAR_i = Amount of incident photons per unit working volume per unit time (number of photons $m^{-3} s^{-1}$)

ρPAR_{max} = Value of ρPAR_i at which specific growth rate of algae is maximum (number of photons $m^{-3} s^{-1}$)

ρPAR_0 = Value of ρPAR_i at which 50% of PSII are active

μ = Specific growth rate of algae (h^{-1})

μ_d = Relative death rate of algae (h^{-1})

μ_{max} = Innate maximum cell division rate of the algal species (h^{-1})

μ_{n+1} = Specific growth rate (h^{-1}) in (n+1)th time interval

$\mu_{max,n+1}$ = Maximum specific growth rate (h^{-1}) in (n+1)th time interval

μ_{net} = net specific growth rate of algae (h^{-1})

$\mu_{net,n+1}$ = Net specific growth rate (h^{-1}) in (n+1)th time interval

τ = Average exposure time of algae in FPR to light (s)

τ_a = the amount of the time algae spends in the well-lit (local light intensity > I_m) region of reactor (s)

τ_m = mixing time of reactor (s)

ω = number of cell divisions that occur per unit PAR_i supplied (number of photons)⁻¹

1 Model Development

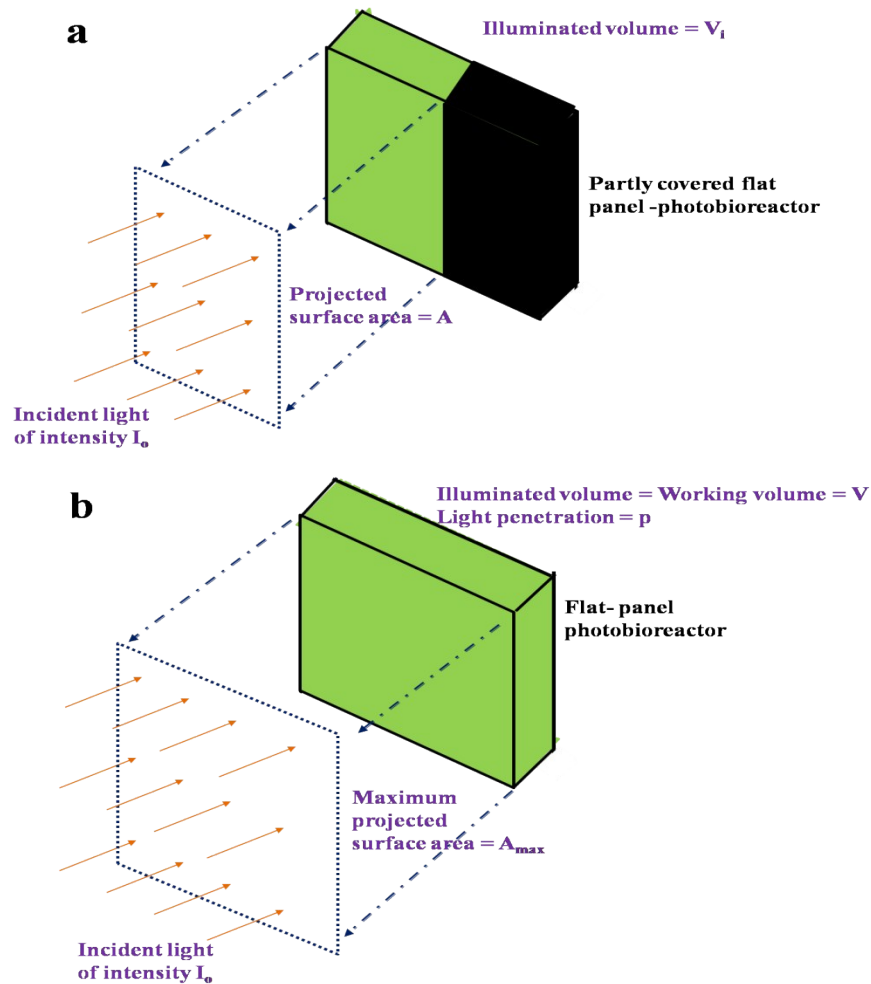


Figure 1. Schematic of a flat panel reactor (a) with part surface covered and (b) completely exposed to light.

In the present study, we attempt to study algal growth in Flat panel reactors (FPRs) by analyzing the spatial distribution and metabolic utilization of light by algae in the same. FPRs were selected for this analysis due to their simplicity in reactor geometry. Figure 1a shows a FPR of working volume V (m^3) and illuminated surface area A (m^2). The incident light is perpendicular to the illuminated surface and an opaque material is used to alter the area exposed to light. The maximum illuminated surface area is denoted as A_{max} (m^2) and is limited by reactor

(PAR_c) is evaluated. Finally, the amount of PAR_c utilized for biomass production (sans cell maintenance) or PAR_g is determined.

1.2 Determination of PAR_a

According to the Beer-Lambert's law ¹

$$I_a = (1 - 10^{-k_a X_{a,n} p}) \cdot I_o \dots\dots\dots(3)$$

where, k_a= molar absorptivity coefficient of algae (kg⁻¹ m²) and is a constant for an algal species, X_{a,n}= apparent density of algal cells in the (n+1)th time interval which are exposed to light (kg m⁻³), I_a= average light intensity absorbed by algae (number of photons m⁻² s⁻¹) and p = average light penetration in the illuminated volume of reactor (m).

Thus the illuminated volume (V_i) = A.p(4)

The amount of algal cells exposed to light are given by V_iX_{a,n} and those experiencing self-shading is calculated by V_i·(X_n - X_{a,n}). Hence, 0 ≤ X_{a,n} ≤ X_n.

Multiplying both sides of Equation 3 by [A · $\frac{L}{L + D}$ · (t_{n+1} - t_n)];

$$PAR_a = (1 - 10^{-k_a X_{a,n} p}) \cdot PAR_i \dots\dots\dots(5)$$

where, $PAR_a = I_a \cdot A \cdot \frac{L}{L + D} \cdot (t_{n+1} - t_n) \dots\dots\dots(6)$

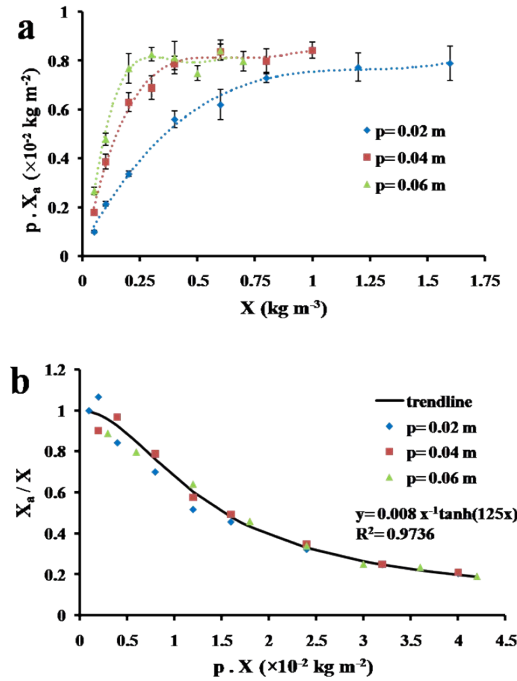


Figure 2. Profile of a) Change in apparent algal density (X_a) with existing algal density (X) at different light penetration distances. b) Variation in X_a / X with the amount of algae exposed to light.

Figures 2a and 2b depict the variation in apparent biomass densities and the self-shading of algae, respectively, at with total biomass densities and light penetration. The empirical relationship between the exposed cells and total cells in the reactor is given by the hyperbolic equation

$$X_a = \frac{(p \cdot X_a)_{max}}{p} \cdot \tanh \left[\frac{p \cdot X}{(p \cdot X_a)_{max}} \right], R^2 = 0.9736 \quad (7)$$

where, $(p \cdot X_a)_{max} = 0.008$ kg m⁻² from Fig. 2a.

1.3 Determination of PAR_c :

The total number of photons absorbed by algae is either used for photosynthesis or lost via photoinhibition and non-photosynthetic quenching including heat losses. Photosystem II (PSII) utilize PAR_a for photosynthesis. The amount of functional PSII in an algal cell is influenced by PSII degradation kinetics.

1.3.1 Development of a PSII degradation kinetics model for an algal cell:

PSII is generally composed of D1 and D2 proteins, chlorophyll a, β -carotene, pheophytin, plastoquinone, Mn_4CaO_5 cluster containing oxygen evolving complex (OEC), lipids and other subunits or cofactors. ²Photoinhibition reduces the number of active PSII in algae, eventually leading to death (by photooxidation) at high light intensities.

The Mn_4CaO_5 cluster containing OEC is the primary electron generation site of photosynthesis. The OEC absorbs photons and splits water to release protons, oxygen and electrons, which are transported further using the electron transport chain. The manganese hypothesis of photoinhibition ³ suggests that the OEC is also degraded by light at all I_o (continuous or flashing) which leads degradation of active PSII. Takahashi and Murata⁴ elaborated the photoinhibition process with a two-step mechanism suggesting that PSII degradation occurs due to loss of Mn from the OEC (rate limiting step) which further leads to PSII degradation. The subsequent repair of PSII occurs by synthesis and insertion of pre-D1 protein and reassembly of PSII (Figure 3a). Campbell and Tysstjärvi⁵ estimated the extent of PSII degradation by

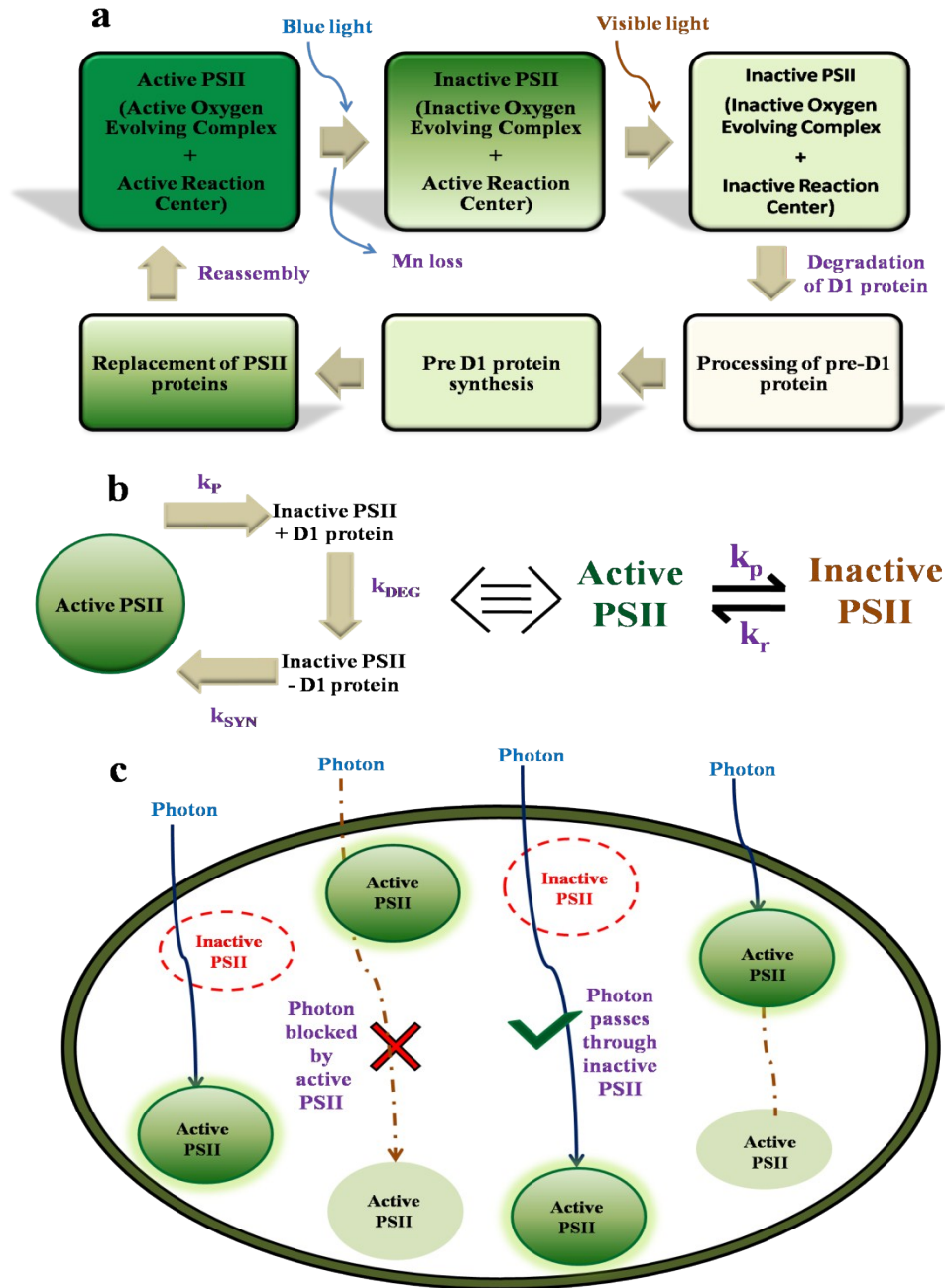


Figure 3. (a) Two step mechanism of PSII degradation and recovery of chlorophyll⁴ (b) Simplification of the two step mechanism⁵ where k_p and k_r are rate constants of PSII degradation and PSII repair, respectively (c) Schematic of intracellular shading by active PSII in an algal cell.

calculating the amount of active PSII via a first order reaction kinetics model of the two-step mechanism (Figure 3b).

However, the first order kinetics model of PSII degradation does not explain the complete loss of photosynthesis at high light intensities, culminating in cell death.^{6,7}Hence, algal growth kinetic models^{8,9} do not predict the death of algae at high light intensities. We attempt to resolve this lacuna by introducing the concept of intracellular PSII shading in an algal cell (Figure 3c). We assume that PSII is uniformly distributed throughout the cell. When an active PSII absorbs a photon, it prevents the photon from reaching another PSII in its shadow. Therefore,

Probability of incident photon colliding with active PSII

$$= \frac{M^*}{M_o} \dots\dots\dots(8)$$

where ,M* = concentration of inactive PSII in an algal cell (mol PSII mol cell⁻¹), M = concentration of active PSII in an algal cell (mol PSII mol cell⁻¹) and M_o = M* + M.

Subsequently,

Rate of PSII degradation

$$= k_p \cdot M \cdot \frac{M^*}{M_o} \dots\dots\dots(9)$$

where, k_p= rate constant of PSII degradation (s⁻¹),

The inactive PSII is renewed using the D1-protein synthesis mechanism which is inhibited by the reactive oxygen species released during the inactivation of OEC.^{10,11}

∴ Probability of reactive oxygen species damaging the repair mechanism

$$= \frac{M^*}{M_o} \dots\dots\dots(10)$$

and

Rate of PSII repair

$$= k_r \cdot M^* \cdot \left(1 - \frac{M^*}{M_o}\right) \dots\dots\dots(11)$$

where, k_r= rate constant of PSII repair mechanism (s⁻¹).

The reaction kinetics for PSII damage and repair can now be written as

$$-\frac{dM}{d\tau} = \text{Rate of PSII degradation} - \text{Rate of PSII repair}$$

where, τ = average exposure time of an algal cell to light.

$$\therefore -\frac{dM}{d\tau} = k_p \cdot M \cdot \frac{M^*}{M_o} - k_r \cdot M^* \cdot \left(1 - \frac{M^*}{M_o}\right)$$

$$\therefore -\frac{dM}{d\tau} = (k_p - k_r) \cdot M \cdot \left(1 - \frac{M}{M_o}\right)$$

$$\therefore \frac{M_o}{M \cdot (M_o - M)} \cdot dM = -(k_p - k_r) \cdot d\tau \dots\dots\dots(12)$$

12)

Equation 12 integrates to

$$\ln(M) - \ln(M_o - M) = -(k_p - k_r)\tau + c$$

where, c = constant of integration.

$$\therefore \frac{M}{M_o - M} = e^{-(k_p - k_r)\tau + c}$$

$$\therefore \frac{M}{M_o} = \frac{e^{-(k_p - k_r)\tau + c}}{1 + e^{-(k_p - k_r)\tau + c}} = \frac{1}{2} \left(1 - \tanh\left(\frac{(k_p - k_r)\tau - c}{2}\right)\right) \dots\dots\dots \text{VVVV}$$

.....(13)

$$\text{because, } \tanh(\theta) = 1 - \frac{2e^{-2\theta}}{1 + e^{-2\theta}}$$

According to Campbell and Tysstjärvi⁵,

$$k_p = \alpha I_o \text{ and } k_r = \beta k_p$$

where, α (number of photons⁻¹ m²) and β are the coefficient of photodamage with respect to I_o and coefficient of repair with respect to photodamage, respectively, and are constant.

$$\therefore \frac{M}{M_o} = \frac{1}{2} \left(1 - \tanh\left(\frac{KI_o\tau - c}{2}\right)\right) \dots\dots\dots(14)$$

4)

$$\frac{k_p - k_r}{I_o}$$

where, $K = \alpha - \alpha\beta = I_o^{-1}$ (number of photons⁻¹ m²).

During the time interval $t_{n+1} - t_n$, the reactor is illuminated for $\left[\frac{L}{L+D}(t_{n+1} - t_n)\right]$. The fraction of time an algal cell is present in the illuminated volume of the reactor (due to circulation between illuminated and dark zones of the reactor) is $\left[\frac{V_i}{V}(t_{n+1} - t_n)\right]$. Furthermore, the

probability of an algal cell in the illuminated volume to be exposed to light is $\left(\frac{X_{a,n}}{X_n}\right)$. Therefore, the exposure time of algal cell is given by

$$\tau = \frac{A \cdot p \cdot X_{a,n}}{V \cdot X_n} \cdot \frac{L}{L+D} \cdot (t_{n+1} - t_n) \dots\dots\dots(1)$$

5)

Equation 14 now becomes

$$\frac{M}{M_o} = \frac{1}{2} \left[1 - \tanh \left(\frac{K \cdot p \cdot X_{a,n}}{2 \cdot V \cdot X_n} \cdot PAR_i - \frac{c}{2} \right) \right] \dots\dots\dots(16)$$

In order to calculate the value of c, we consider the special case where, $\tanh(0) = 0$ causes

$$\frac{M}{M_o} = 0.5$$

$$\therefore \frac{c}{K} = \frac{p \cdot X_{a,n}}{V \cdot X_n} \cdot PAR_o = \text{constant} \dots\dots\dots(17)$$

)

where, PAR_o is the value of PAR_i so that $\frac{M}{M_o} = 0.5$ and is determined in the FPR under

conditions of maximal illumination of algal cells in the reactor $\left(p = p_o = \frac{V}{A_{max}}\right)$ and $\left(\frac{X_{a,n}}{X_n} = 1\right)$.

$PAR_{i,o}$ is constant for an algal species at all biomass densities in the FPR under consideration because the L.H.S. of Equation 17 is independent of reactor configuration and illumination conditions.

Thus, $c = K \cdot \frac{p_o}{V} \cdot PAR_o$ (18)

Combining Equations 16 and 18

$$\frac{M}{M_o} = \frac{1}{2} \left[1 - \tanh \left\{ \left(\frac{K p}{2 \cdot V} \cdot \frac{X_{a,n}}{X_n} \cdot PAR_i - \frac{K p_o}{2 \cdot V} \cdot PAR_o \right) \right\} \right]$$

$$\therefore \frac{M}{M_o} = \frac{1}{2} \left[1 - \tanh \left\{ -K' \left(1 - \frac{X_{a,n} p}{X_n p_o} \cdot \frac{PAR_i}{PAR_o} \right) \right\} \right] \dots\dots\dots(19)$$

where, $K' = \frac{K p_o}{2 \cdot V} \cdot PAR_{i,o} = \frac{c}{2}$.

To the best of the authors' knowledge the PSII degradation kinetics as described by Equation 19 has been developed for the first time. The noteworthy aspect of Equation 19 (Logistic Equation)

lays in the fact that, $\frac{M}{M_o} \in [0,1] \forall PAR_i \in R$ unlike models in literature.^{5,8,9}

1.3.2 Utility of PSII degradation kinetics model for estimation of PAR_c

Photoinhibition reduces the efficiency of photosynthesis¹², which in turn affects the amount of glucose produced. Campbell and Tyystjärvi⁵ define quantum yield of photosynthesis as

$$Quantum\ yield\ of\ photosynthesis = \frac{PAR_c}{PAR_a} = \eta_{OEC,n+1} \cdot K_c \cdot M$$

.....(20)

where, K_c = constant of proportionality for quantum yield [mole cell (mol PSII)⁻¹].

$$\therefore PAR_c = \eta_{OEC,n+1} \cdot K_c \cdot M_o \cdot \frac{1}{2} \left[1 - \tanh \left\{ -K' \left(1 - \frac{X_{a,n} p}{X_n p_o} \cdot \frac{PAR_i}{PAR_o} \right) \right\} \right] \cdot PAR_a \dots\dots\dots(21)$$

where, η_{OEC,n+1} = efficiency of photolysis of water at OEC in the (n+1)th time interval.

1.3.3 Decay of Oxygen Evolving Complex

According to Kok cycle¹³, the OEC is present in four excited states (S₁, S₂, S₃ and S₄) during photolysis and progresses from S₁ to S₄ with each successive photon entrapment. S₁ is stable in dark and S₄ immediately reduces to the ground state resulting in photolysis of water. Conversely, S₂ and S₃ decay into S₁ and S₂, respectively, if the subsequent photon does not

impinge on the same within 0.5 milliseconds of their formation.^{14,15} Thus, “OEC decay losses” are the loss of photons (intended for photosynthesis) which occur because of the decay of the excited states of the OEC.

OEC decay would occur when (a) an OEC in excited state, in the illuminated volume of reactor, is exposed to light intensity lesser than the minimum light intensity required to initiate photosynthesis (I_m), (b) an algal cell moves from illuminated volume of reactor to dark zone due to bulk mixing, (c) an algal cell in the illuminated volume is self-shaded (intercellular) by other algal cells and (d) Intracellular self-shading of active PSII by the same. The four cases are mutually exclusive and exhaustive.

According to the Beer-Lambert’s law ¹

$$I_o - I_m = \left(1 - 10^{-k_a X_{a,n} p_{m,n+1}}\right) \cdot I_o \dots\dots\dots(22)$$

$$\therefore p_{m,n+1} = \begin{cases} \frac{\log_{10}(I_o) - \log_{10}(I_m)}{k_a X_{a,n}}, \frac{\log_{10}(I_o) - \log_{10}(I_m)}{k_a X_{a,n}} < p \\ p, \frac{\log_{10}(I_o) - \log_{10}(I_m)}{k_a X_{a,n}} \geq p \end{cases} \dots\dots\dots$$

...(23)

Where $p_{m,n+1}$ = light penetration (m) in V_i beyond which OEC decay may occur due to insufficient light (local light intensity $\leq I_m$), in the (n+1)th time interval.

Generally during reactor operations mixing time of reactor < duration of one light cycle.

Let, τ_m = mixing time of reactor (s) $\ll \frac{L}{D}(t_{n+1} - t_n)$.

Let $\eta_{BM,n+1}$, $\eta_{SS,n+1}$ and $\eta_{PSII,n+1}$ be the loss in $\eta_{OEC,n+1}$ due to bulk mixing, intercellular self-shading and intracellular self-shading by active PSII in the (n+1)th time interval, respectively. The probability of OEC decay due to bulk mixing depends on the fraction of time for which the algal cell is not exposed to sufficient light. It also depends upon the frequency of cell cycling between dimly-lit or dark and well-lit regions of the reactor, since only excited OEC states may decay.

During time interval τ_m ,

$$= \tau_m \frac{V_i}{V} = \tau_m \frac{A}{A_{max}} \frac{p}{p_o}$$

Time spent by algae in V_i

Let, τ_a = the amount of the time algae spends in the well-lit (local light intensity $> I_m$) region of reactor(s).

$$\therefore \tau_a = \tau_m \frac{A}{A_{max}} \frac{p}{p_o} \frac{p_{m,n+1}}{p} \frac{X_{a,n}}{X_n} = \tau_m \frac{A \cdot p_{m,n+1}}{A_{max} \cdot p_o} \frac{X_{a,n}}{X_n} \dots\dots\dots(2)$$

4)

Frequency of algal cell movement between dimly-lit or dark and well-lit regions of the reactor

$$\text{due to bulk mixing} \propto \frac{\tau_m - \tau_a}{\tau_a} = \frac{A_{max} \cdot p_o}{A \cdot p_{m,n+1}} \frac{X_n}{X_{a,n}} - 1 \dots\dots\dots$$

(25)

$$\begin{aligned} \therefore \eta_{BM,n+1} &\propto \frac{\tau_m - \tau_a}{\tau_m}, \frac{\tau_m - \tau_a}{\tau_a} \\ &= K_{g,o} \left(1 - \frac{A \cdot p_{m,n+1}}{A_{max} \cdot p_o} \frac{X_{a,n}}{X_n} \right) \left(\frac{A_{max} \cdot p_o}{A \cdot p_{m,n+1}} \frac{X_n}{X_{a,n}} - 1 \right) \\ &= K_{g,o} \frac{A \cdot p_{m,n+1}}{A_{max} \cdot p_o} \frac{X_{a,n}}{X_n} \left(\frac{A_{max} \cdot p_o}{A \cdot p_{m,n+1}} \frac{X_n}{X_{a,n}} - 1 \right)^2 \end{aligned}$$

(26)

The probability of OEC decay due to intercellular self-shading depends on the fraction of time for which the algae (and subsequently, OEC) is present in the well-lit region of V_i under intercellular self-shading conditions. It also depends upon the frequency with which an algal cell is alternately exposed to light and darkness attributable to intercellular self-shading.

$$\begin{aligned} \therefore \eta_{SS,n+1} &\propto \frac{\tau_a}{\tau_m} \left(1 - \frac{X_{a,n}}{X_n} \right), \frac{X_n - X_{a,n}}{X_{a,n}} \\ &= K_{g,1} \frac{A \cdot p_{m,n+1}}{A_{max} \cdot p_o} \left(1 - \frac{X_{a,n}}{X_n} \right)^2 \dots\dots\dots \end{aligned}$$

..(27)

Finally, the probability of OEC decay due to intercellular self-shading depends on the fraction of time for which the active PSII in an algal cell exposed to light is self-shaded (intracellular) by

other active PSII. It also depends upon the frequency with which active PSII oscillates between light and shade caused by intracellular self-shading.

$$\begin{aligned} \therefore \eta_{PSII,n+1} &\propto \frac{\tau_a X_{a,n} M}{\tau_m X_n M_o} \cdot \frac{M}{M_o - M} \\ &= \frac{K_{g,2}}{2} \cdot \frac{A \cdot p_{m,n+1}}{A_{max} \cdot p_o} \cdot \left(\frac{X_{a,n}}{X_n}\right)^2 \cdot \frac{\left[1 - \tanh\left\{-K' \left(1 - \frac{p}{p_o} \cdot \frac{X_{a,n}}{X_n} \cdot \frac{PAR_i}{PAR_{i,o}}\right)\right\}\right]^2}{\left[1 + \tanh\left\{-K' \left(1 - \frac{p}{p_o} \cdot \frac{X_{a,n}}{X_n} \cdot \frac{PAR_i}{PAR_{i,o}}\right)\right\}\right]} \end{aligned}$$

(28)

where, $K_{g,o}$, $K_{g,1}$ and $K_{g,2}$ are the constants of proportionality for loss of $\eta_{OEC,n+1}$ due to bulk mixing, intercellular self-shading and intracellular self-shading, respectively.

Thus,

$$\eta_{OEC,n+1} = 1 - (\eta_{BM,n+1} + \eta_{SS,n+1} + \eta_{PSII,n+1})$$

(29)

Thus, from Equations 19,21 and 28, we conclude that the quantum yield of the PSII degradation kinetics model developed in the present study $\in [0,1] \forall PAR_i \in R$.

Photooxidative death of algae occurs at $PAR_i > 0$, $\frac{X_{a,n}}{X_n} \rightarrow 1$ and $M = 0$.

1.4 Determination of PAR_g

1.4.1 Estimation of maintenance losses

The glucose produced during photosynthesis (equivalent to PAR_c) is the energy source for all the metabolic activities in the algal cells. The metabolic activities are categorized into two parts: (a) cell division and (b) cell maintenance. Pirt¹⁶ described maintenance as the energy requirement of all non-growth-associated functions of the cell. Van Bodegom¹⁷ further specified the physiological maintenance as "the energy costs of osmoregulation, cell motility, defense mechanisms, and proofreading and internal turnover of macromolecular compounds" and separated them from the non-growth energy expenditure involved in polymer storage, cell death and extracellular losses. Physiological maintenance is proportional to PAR_c ¹⁸ or $K_{g,3} \cdot PAR_c$

, where $K_{g,3}$ is the coefficient of physiological maintenance. The non-growth energy expenditure is calculated by $\mu_d \cdot H_c \cdot V \cdot X_n \cdot (t_{n+1} - t_n)$ where, μ_d = the relative death rate (h^{-1}) and was verified to be a constant for an algal species under the reactor operation conditions of this study. Thus, the number of photons required for cell division (PAR_g) is given by

$$PAR_g = (1 - K_{g,3}) \cdot PAR_c - \frac{H_c}{K_{light}} \cdot \mu_d \cdot V \cdot X_n \cdot (t_{n+1} - t_n) \quad (30)$$

1.5 Formulation of generic equation for specific growth rate

Combining Equations 2, 5, 21 and 30

$$\begin{aligned} H_c \cdot V \cdot (X_{n+1} - X_n) &= K_{light} \cdot (1 - K_{g,3}) \cdot \eta_{OEC,n+1} \cdot K_c \cdot M_o \cdot \frac{1}{2} \left[1 - \tanh \left\{ -K' \left(1 - \frac{X_{a,n}}{X_n} \cdot \frac{p}{p_o} \cdot \frac{PAR_i}{PAR_o} \right) \right\} \right] \\ &\quad \left(1 - 10^{-k_a X_{a,n}^p} \right) \cdot PAR_i - H_c \cdot \mu_d \cdot V \cdot X_n \cdot (t_{n+1} - t_n) \\ \therefore \frac{1}{X_n} \cdot \frac{X_{n+1} - X_n}{t_{n+1} - t_n} &= \frac{1}{X_n} \cdot \frac{K_{light} \cdot \eta_{OEC,n+1} \cdot K_c \cdot M_o}{2 \cdot H_c} \cdot \left[1 - \tanh \left\{ -K' \left(1 - \frac{X_{a,n}}{X_n} \cdot \frac{p}{p_o} \cdot \frac{PAR_i}{PAR_o} \right) \right\} \right] \cdot \left(1 - 10^{-k_a X_{a,n}^p} \right) \\ &\quad \frac{PAR_i}{V \cdot (t_{n+1} - t_n)} - \mu_d \end{aligned}$$

(31)

$\mu_{net, n+1}$ is the net specific growth rate of algae in the $(n+1)^{th}$ time interval (h^{-1}) and is defined as¹⁹

$$\mu_{net, n+1} = \frac{\ln(X_{n+1}) - \ln(X_n)}{t_{n+1} - t_n} = \frac{1}{X_n} \cdot \frac{X_{n+1} - X_n}{t_{n+1} - t_n} \dots\dots\dots$$

..... (32)

Let, $\mu_{net, n+1} = \mu_{n+1} - \mu_d \dots\dots\dots$

(33)

where, μ_{n+1} = specific growth rate of algae (h^{-1}) in the $(n+1)^{th}$ time interval such that

$$\mu_{n+1} = \frac{K_{light} \cdot \eta_{OEC,n+1} \cdot K_c \cdot M_o}{2 \cdot H_c} \cdot \frac{\left[1 - \tanh \left\{ -K' \left(1 - \frac{X_{a,n}}{X_n} \cdot \frac{p}{p_o} \cdot \frac{PAR_i}{PAR_o} \right) \right\} \right] \cdot \left(1 - 10^{-k_a X_{a,n}^p} \right) \cdot PAR_i}{X_n \cdot V \cdot (t_{n+1} - t_n)}$$

(34)

Let $\rho PAR_i = \frac{PAR_i}{V \cdot (t_{n+1} - t_n)}$ where, ρPAR_i is the incident light energy per unit working volume

per unit time (number of photons $m^{-3} s^{-1}$) and $\frac{\rho PAR_i}{\rho PAR_o} = \frac{PAR_i}{PAR_o}$ where $\rho PAR_{i,o}$ is the ρPAR_i

required so that active PSII fraction is 50%. Also, $Z = \frac{K_{light} \cdot K_c \cdot M_o}{2 \cdot H_c}$. Hence,

$$\mu_{n+1} = Z \cdot (1 - K_{g,3}) \cdot \eta_{OEC,n+1} \cdot \frac{\left[1 - \tanh \left\{ -K \left(1 - \frac{X_{a,n} \cdot p \cdot \rho PAR_i}{X_n \cdot p_o \cdot \rho PAR_o} \right) \right\} \right] \cdot (1 - 10^{-k_a X_{a,n}^p}) \cdot \rho PAR_i}{X_n}$$

(35)

1.5.1 Determination of maximum value of μ

1.5.1.1 On basis of reactor operating conditions

The maximum value of $\mu_{net,n+1}$ is attained when μ_{n+1} is maximum. The theoretical maxima of $\mu_{n+1} = \mu_{max, n+1}$ occurs when all cells in the illuminated volume of the reactor are exposed to light and undergo minimum OEC decay or maintenance losses i.e. $X_n = X_{a,n}, \forall X_n, K_{g,3} \approx 0$ and $\eta_{OEC,n+1} \approx 1$.

$$\therefore \mu_{n+1} = Z \cdot \left[1 - \tanh \left\{ -K \left(1 - \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right) \right\} \right] \cdot (1 - 10^{-k_a X_{a,n}^p}) \cdot \frac{\rho PAR_i}{X_{a,n}} \dots \dots \dots (36)$$

The $\rho PAR_i = \rho PAR_{max}$ at which $\mu_{n+1} = \mu_{max, n+1}$, is the solution of Equation 36 and satisfies Expression 30.

$$\frac{d\mu_{n+1}}{d\rho PAR_i} = 0 \dots \dots \dots$$

(37)

$$\frac{d^2 \mu_{n+1}}{d^2 \rho PAR_i} < 0 \dots \dots \dots (38)$$

8)

$$S = \frac{Z \cdot (1 - 10^{-k a^n p})}{X_{a,n}} > 0$$

Let,

Upon solving Equation 36 we get,

$$\therefore S \cdot \left[1 - \tanh \left\{ -K' \left(1 - \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right) \right\} \right] - S \cdot K' \cdot \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \left[1 - \tanh^2 \left\{ -K' \left(1 - \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right) \right\} \right] = 0$$

Since S is independent of ρPAR_i ,

$$\left[1 - \tanh \left\{ -K' \left(1 - \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right) \right\} \right] \left[1 - K' \cdot \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \left\{ 1 + \tanh \left(-K' + K' \cdot \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right) \right\} \right] = 0 \dots\dots\dots(39)$$

Now, $\left[1 - \tanh \left\{ -K' \left(1 - \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right) \right\} \right] \neq 0$, because the quantum yield of photosynthesis would be zero.

$$\therefore 1 - K' \cdot \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \left[1 + \tanh \left\{ -K' \left(1 - \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right) \right\} \right] = 0 \dots\dots\dots(40)$$

$$\therefore 1 - 2K' \cdot \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \cdot \frac{1}{1 + e^{-2K' \left(1 - \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right)}} = 0$$

$$\therefore e^{-2K'} = \left(2K' \cdot \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} - 1 \right) \cdot e^{\left(-2K' \cdot \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right)}$$

Taking natural log on both sides

$$\therefore \ln \left(2K' \cdot \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} - 1 \right) - 2K' \left(\frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} - 1 \right) = 0 \dots\dots\dots(41)$$

ρPAR_{max} is the solution set of the transcendental Equation 41. Thus, Equations 17 and 41 reveal that K' , ρPAR_{max} and ρPAR_o are experimentally-determined constants for a particular reactor configuration.

From Equation 40, we get the quantum yield of photosynthesis at $\mu_{max,n+1}$ as

$$1 - \tanh \left\{ -K' \left(1 - \frac{p \cdot \rho PAR_{max}}{p_o \cdot \rho PAR_o} \right) \right\} = \left(2 - \frac{p_o \cdot \rho PAR_o}{p \cdot K' \cdot \rho PAR_{max}} \right) \dots\dots\dots(42)$$

In order to verify whether $\rho PAR_i = \rho PAR_{max}$ is a maxima

$$\begin{aligned} \therefore \frac{d^2 \mu_{n+1}}{d^2 \rho PAR_i} &= -S \cdot K' \cdot \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \left[1 - \tanh^2 \left\{ -K' \left(1 - \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right) \right\} \right] \\ &+ 2S \cdot \left(K' \cdot \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right)^2 \cdot \tanh \left\{ -K' \left(1 - \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right) \right\} \cdot \left[1 - \tanh^2 \left\{ -K' \left(1 - \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right) \right\} \right] \\ &= -K' \cdot \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \left[1 - \tanh^2 \left\{ -K' \left(1 - \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right) \right\} \right] \cdot \left[1 - 2K' \cdot \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \cdot \tanh \left\{ -K' \left(1 - \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right) \right\} \right] \cdot S \\ &= -K' \cdot \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \left[1 + \tanh \left\{ -K' \left(1 - \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right) \right\} \right] \cdot \left[1 - \tanh \left\{ -K' \left(1 - \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right) \right\} \right] \\ &\quad \left[1 - 2K' \cdot \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \cdot \tanh \left\{ -K' \left(1 - \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right) \right\} \right] \cdot S \end{aligned}$$

Inserting $\rho PAR_i = \rho PAR_{max}$ and using Equations 40 and 42, we get

$$\frac{d^2 \mu_{n+1}}{d^2 \rho PAR_i} \Big|_{\rho PAR_i = \rho PAR_{max}} = (-1) \cdot \left(2 - \frac{p_o \cdot \rho PAR_o}{p \cdot K' \cdot \rho PAR_{max}} \right) \cdot \left[1 - 2 \left(1 - K' \cdot \frac{p \cdot \rho PAR_{max}}{p_o \cdot \rho PAR_o} \right) \right] \cdot S$$

$$\frac{d^2 \mu_{n+1}}{d^2 \rho PAR_i} \Big|_{\rho PAR_i = \rho PAR_{max}} = - \left(2 - \frac{p_o \cdot \rho PAR_o}{p \cdot K' \cdot \rho PAR_{max}} \right) \cdot \left(2K' \cdot \frac{p \cdot \rho PAR_{max}}{p_o \cdot \rho PAR_o} - 1 \right) \cdot S < 0 \dots\dots\dots$$

(43)

because, $\left(2 - \frac{p_o \cdot \rho PAR_o}{p \cdot K' \cdot \rho PAR_{max}} \right) > 0$ is the quantum yield of photosynthesis of actively growing

algae, $S > 0$ and according to Equation 42, $\left(2K' \cdot \frac{p \cdot \rho PAR_{max}}{p_o \cdot \rho PAR_o} - 1 \right) > 0$, for all real values of ρPAR_{max} .

Combining Equations 36 and 42 we get,

$$\mu_{max, n+1} = Z \cdot \left(2 - \frac{p_o \cdot \rho PAR_o}{p \cdot K' \cdot \rho PAR_{max}} \right) \cdot \left(1 - 10^{-k_a X_{a,n} p} \right) \cdot \frac{\rho PAR_{max}}{X_{a,n}} \dots\dots\dots$$

(44)

1.5.1.2 On basis of innate potential for cell division

According to Equations 32 and 33, the maximum value of $\mu_{net, n+1}$ occurs when $\mu_d = 0$

$$\therefore [\mu_{net,n+1}]_{max} = \mu_{max,n+1} = \frac{1}{X_n} \left[\frac{X_{n+1} - X_n}{t_{n+1} - t_n} \right]_{max}$$

$$\therefore \mu_{max,n+1} = \frac{X_{a,n}}{X_n} \left[\frac{1}{X_{a,n}} \cdot \frac{X_{n+1} - X_n}{t_{n+1} - t_n} \right]_{max}$$

We know that, only cells that are exposed to light undergo cell division to produce additional daughter cells in the time interval $t_{n+1} - t_n$. The maximum cell division rate of the algal cells exposed to light is the innate maximum cell division rate of the algal species, μ_{max} (h^{-1}).

$$\therefore \mu_{max,n+1} = \mu_{max} \cdot \frac{X_{a,n}}{X_n}$$

(45)

where,
$$\mu_{max} = \left[\frac{1}{X_{a,n}} \cdot \frac{X_{n+1} - X_n}{t_{n+1} - t_n} \right]_{max}$$

The maximum potential for innate cell division is only expressed under when all cells in the illuminated volume of the reactor are exposed to light and undergo minimum OEC decay or maintenance losses. Therefore, upon combining Equations 44 and 45 we get

$$\mu_{max} \cdot \frac{X_{a,n}}{X_n} = Z \cdot \left(2 - \frac{p_o}{p} \cdot \frac{\rho PAR_o}{K \cdot \rho PAR_{max}} \right) \cdot (1 - 10^{-k_a X_{a,n}^p}) \cdot \frac{\rho PAR_{max}}{X_{a,n}} \dots \dots$$

(46)

1.5.2 Algal growth kinetics

1.5.2.1 Dynamic growth kinetics

Dividing Equation 35 by Equation 46 we get

$$\mu_{n+1} = \mu_{\max} \cdot \eta_{OEC,n+1} \cdot \frac{\left[1 - \tanh \left\{ -K' \left(1 - \frac{X_{a,n}}{X_n} \cdot \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right) \right\} \right]}{\left(2 - \frac{p_o \cdot \rho PAR_o}{p \cdot K' \cdot \rho PAR_{\max}} \right)} \cdot \frac{\rho PAR_i}{\rho PAR_{\max}} \cdot \left(\frac{X_{a,n}}{X_n} \right)^2 \cdot (1 - K_{g,3})$$

(47)

Thus,

$$\begin{aligned} \mu_{net,n+1} &= \mu_{\max} \cdot \eta_{OEC,n+1} \cdot \frac{\left[1 - \tanh \left\{ -K' \left(1 - \frac{X_{a,n}}{X_n} \cdot \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right) \right\} \right]}{\left(2 - \frac{p_o \cdot \rho PAR_o}{p \cdot K' \cdot \rho PAR_{\max}} \right)} \cdot \frac{\rho PAR_i}{\rho PAR_{\max}} \cdot \left(\frac{X_{a,n}}{X_n} \right)^2 \cdot (1 - K_{g,3}) - \mu_d \end{aligned}$$

(48)

or

$$\mu_{net,n+1} = \mu_{\max} \cdot \eta_{OEC,n+1} \cdot \frac{M}{M_o} \cdot \frac{2p \cdot K' \cdot \rho PAR_{\max}}{2p \cdot K' \cdot \rho PAR_{\max} - p_o \cdot \rho PAR_o} \cdot \frac{\rho PAR_i}{\rho PAR_{\max}} \cdot \left(\frac{X_{a,n}}{X_n} \right)^2 \cdot (1 - K_{g,3}) - \mu_d$$

1.5.2.2 Intrinsic Kinetics

When $\frac{X_{a,n}}{X_n} \approx 1$ and $\eta_{OEC,n+1} \approx 1 \forall n$, Equation 48 reduces into the intrinsic kinetics of algal growth

$$\mu_{net} = \mu_{\max} \cdot \frac{\left[1 - \tanh \left\{ -K' \left(1 - \frac{p \cdot \rho PAR_i}{p_o \cdot \rho PAR_o} \right) \right\} \right]}{\left(2 - \frac{p_o \cdot \rho PAR_o}{p \cdot K' \cdot \rho PAR_{\max}} \right)} \cdot \frac{\rho PAR_i}{\rho PAR_{\max}} \cdot (1 - K_{g,3}) - \mu_d$$

(49)

where, μ_{net} = net specific growth rate of algae (h^{-1}).

1.6 Empirical determination of $K_{g,0}$ and $K_{g,1}$

When $\mu_{net,n+1} = 0$, cell growth stops due to light limitation. Hence,

$$\eta_{OEC,n+1} = \frac{\mu_d}{\mu_{max}} \cdot \frac{\left(2 - \frac{p_o}{p} \cdot \frac{\rho PAR_o}{K' \cdot \rho PAR_{max}}\right)}{\left[1 - \tanh \left\{ -K' \left(1 - \frac{X_{a,n}}{X_n} \cdot \frac{p}{p_o} \cdot \frac{\rho PAR_i}{\rho PAR_o}\right)\right\}\right]} \cdot \frac{\rho PAR_{max}}{\rho PAR_i} \cdot \left(\frac{X_n}{X_{a,n}}\right)^2 \cdot \frac{1}{1 - K_{g,3}} \quad (50)$$

Upon solving Equation 50 for the final algal densities obtained for *Chlorella vulgaris* when cultivated in FPR of W= 0.02 m at $I_o = 66.67, 100$ and $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ when L:D = 24:0 and A= 0.18 m², we get the empirical equations

$$2.929K_{g,0} + 0.109K_{g,1} = 0.82 \quad (51)$$

$$2.805K_{g,0} + 0.166K_{g,1} = 0.84 \quad (52)$$

$$2.39K_{g,0} + 0.253K_{g,1} = 0.89 \quad (53)$$

For $I_o = 66.67, 100$ and $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively.

Solving Equations 51 – 53 simultaneously, we get the solution set $0.2 \leq K_{g,0} \leq 0.25$ and $0.83 \leq K_{g,1} \leq 1.54$. A final tuning gives the value of $K_{g,0}$ and $K_{g,1}$ as 0.2 and 1.5, respectively.

1.7 FAME Chromatogram

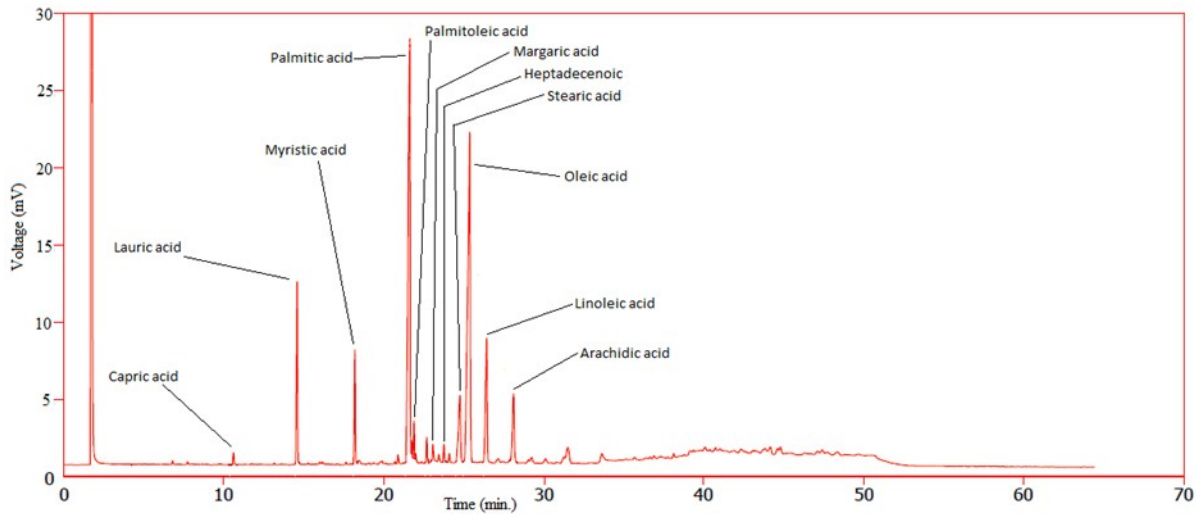


Figure 4. FAME Chromatogram showing saturated and unsaturated fatty acid profiles.

1.8 Photon losses due to absorption by microalgal solutions

The present study did not include light attenuation due to microalgal solutions because of two reasons:

1. Including light attenuation would modify Equation 5 to

$$PAR_a = \left(1 - 10^{-k_a X_{a,n}^p}\right) \cdot 10^{-k_w p} \cdot PAR_i \dots \dots \dots (54)$$

where, k_w = molar absorptivity coefficient of microalgal solution (m^{-1})

This would not influence the final growth kinetics model (Equation 48) as the influence of light absorption (by algae and microalgal solution) is cancelled out in Equation 47.

2. Calculation of the well-lit zone would become complex

$$I_o - I_m = \left(1 - 10^{-k_a X_{a,n}^p m, n + 1}\right) \cdot I_o \cdot 10^{-k_w p m, n + 1} \dots \dots \dots (55)$$

Readers interested in including light attenuation due to microalgal solutions in their work may modify utilize Equation 55 in place of Equation 22. The remaining equations remain constant.

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