



Effects of thermodynamically coupled reaction diffusion in microalgae growth and lipid accumulation: Model development and stability analysis



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ABSTRACT

This study investigates and presents the effects of thermodynamically coupled nonisothermal reaction-diffusion processes on microalgae growth, substrate consumption and neutral lipid production in a pond or wastewater treatment plant. The non-stirred chemostat hypothesis and linear nonequilibrium thermodynamics theory are applied to formulate the model equations that account the bulk phase compositions and temperature, resistances to the heat and mass transfers, and cross effects due to the thermodynamic coupling of heat and mass flow in the presence of chemical reaction. Nondimensional forms of the model equations are numerically solved. Bulk phase concentrations and temperatures, external resistances to heat and substrate transfers, and thermodynamic coupling may generate substantial number of new parameters that control the evolution and stability in microalgal growth and lipid production that are important for biofuels. Instabilities due to perturbations in nutrient concentrations may lead to spatial structures where the wavenumber plays important role in reaction diffusion systems.

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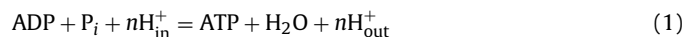
1. Introduction

The population metabolic rate model for the biological systems is focused on the flow of nutrients for metabolism, relevant variables, and the rate at which the metabolic rate model operates (Vershubskii et al., 2004; Virgo et al., 2006). The metabolism is a combined effect of many functional, synchronized, and thermodynamically coupled reaction-diffusion (RD) processes. Thermodynamic coupling refers to a substrate flow, heat flow, or reaction velocity that occurs with, without, or opposite to the direction imposed by its primary thermodynamic driving force, and hence may create the cross (induced) effects (Demirel, 2014). This is consistent with the statement of second law that states that a finite amount of organization may be obtained at the expense of a greater amount of disorganization in a series of coupled spontaneous processes. Turing (1952) demonstrated that a reaction-diffusion system with appropriate nonlinear kinetics can cause instability in a homogeneous steady state and lead to pattern formations.

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Lateral gradients of ions, molecules, and macromolecules may occur between mitochondrial membranes (Frey et al., 2002), which may influence the level of local ion gradients generated by the respiration, the internal diffusion of adenine nucleotides, and other substances. The respiratory electron transport chain in the inner membrane of mitochondria creates a proton motive force across the membrane that is used in synthesizing adenosine triphosphate (ATP). Consequently, the hydrolysis of ATP is coupled in transporting substrates leading to osmotic work of active transport, and other mechanical work (Stucki et al., 1983; Caplan and Essig, 1999; Demirel and Sandler, 2002; Demirel, 2010). The ATP synthesis, in turn, is matched and synchronized to cellular ATP utilization, such as molecular pumps, according to the chemiosmotic theory



where 'in' and 'out' denote two phases separated by a membrane, and n is the ratio H^+/ATP , showing the level of transmembrane proton transport for each ATP to be synthesized (Turina et al., 2003). Such systems represent some examples of how nonspontaneous processes (driven) are being made possible by the virtue of being thermodynamically coupled with other spontaneous processes (driving) with a coupling mechanism (Cortassa et al., 1991; Rothschild et al., 1980). One of the approaches that describe these thermodynamically coupled complex RD systems, is the

nonequilibrium thermodynamic model that does not require the detailed mechanism of coupling phenomena (Caplan and Essig, 1999; Demirel and Sandler, 2001, 2002; Jin and Bethke, 2002).

Microalgae are photosynthetic eukaryotic cells that have been extensively studied for production of food, feed, additive products, and as a potential source of biofuel (Chisti and Yan, 2011). Most of kinetic models on microalgal growth are based on time-variant differential equations (Bordel et al., 2009; Tevatia et al., 2012, 2014a; Bechet et al., 2013). In this study, thermodynamically coupled non-isothermal RD model for microalgal growth, substrate consumption and lipid production is investigated using numerical simulation. The RD model is based on the linear nonequilibrium thermodynamics formulations assuming that the system is in the vicinity of global equilibrium (Cukrowski and Kolbus, 2005; Demirel, 2006, 2014).

Logistic equation and Monod's kinetics are most widely known models for the growth of microalgae (Bechet et al., 2013). The simplicity of logistic equation is in its sole dependence on biomass concentration, without considering substrate utilization, and representing the sigmoidal growth curve. This approach is used to fit photoautotrophic growth of *Chlamydomonas reinhardtii* with the identified biologically significant parameters in terms of initial concentration of the substrate (Tevatia et al., 2012). Since logistic model demands an indirect implementation of the substrate kinetics, more direct approach would be to consider the substrate utilization in the model, which is the basis of Monod's model. Monod's kinetics relates limiting substrate to the microalgae growth, and therefore it follows more mechanistic approach. Most of the algal growth research has been driven to determine the suitability and authenticity of either of these models, and obtain some useful biologically and geometrically significant parameters (Bordel et al., 2009; Tevatia et al., 2012, 2014a; Bechet et al., 2013; Huerlimann et al., 2010). Consequently, there is a need for development of a model, which incorporates the effects of thermodynamic coupling and sub-environmental conditions in the growth and lipid accumulation of microalgae.

Here the behavior of chemostat is analyzed by removing "well stirred" hypothesis, corresponding to actual scenario like growing algae in a pond or wastewater treatment plant (Nie and Wu, 2007, 2009; Wang et al., 2010). In the resulting RD model, effect of temperature, limiting substrate concentration (carbon source under photoautotrophic condition, nitrogen source, or any other component related to growth and lipid accumulation), intracellular nitrogen to carbon ratio, and death rate are incorporated. Logistic and Monod's kinetics are combined in the overall growth model. Lipid production kinetics is given by Leudking–Piret equation, which includes lipid production rate dependency on both growth rate as well as biomass concentration (Tevatia et al., 2012). Besides, the linear stability analysis for the thermodynamically coupled RD is discussed. Incorporation of thermodynamically coupled RD processes and sub-environmental conditions may lead to a more realistic modeling in understanding the growth microalgae and neutral lipid accumulation in an open system like pond or wastewater treatment plant.

2. Model development

2.1. Modified specific microalgal growth rate

In microalgae, a number of responses, such as simultaneous light intensity (Bechet et al., 2013), temperature (Converti et al., 2009), and nutrient limitation (Tevatia et al., 2012, 2014a) can influence the overall growth and product formation. Here, we present a combined approach where Monod's and logistic equations are interactively related to represent the comprehensive microalgal

growth model:

$$\mu(X, S_i, T) = \mu_m \left(1 - \frac{X}{X_m}\right) \prod_{i=1}^n \left(\frac{S_i}{K_{S_i} + S_i}\right) \quad (2)$$

for all values of $0 < X < X_m$; $0 < S_i < S_{0i}$

where X is the microalgal biomass at given time t (g/l), $\mu(X, S, T)$ is the specific growth rate, μ_m is the maximum specific growth rate, X_m is the maximum microalgal biomass, S is the substrate concentration, and K_S is Monod's half saturation constant.

Eq. (2) considers multiple substrates limitations (nutrient stress). For simplification of our simulations and presentation of representative results, we assume that there is only one substrate limitation, and thus Eq. (2) is reduced to:

$$\mu(X, S, T) = \mu_m \left(1 - \frac{X}{X_m}\right) \left(\frac{S}{K_S + S}\right) \quad (3)$$

for all values of $0 < X < X_m$; $0 < S < S_0$

The modified growth model shown in Eq. (3) can be reduced to Monod form, logistic approach or none of these two model equations based on the environmental conditions as shown in Appendix A.

Further, temperature also influences the growth and lipid synthesis in microalgae (Sandnes et al., 2005; Roleda et al., 2013). Coupled and uncoupled models for specific growth of microalgae as a function of temperature and light are already studied (Bechet et al., 2013; Bernard and Rémond, 2012). The coupled models are more focused on the interdependence of light and temperature on specific growth rate of microalgae. The effect of temperature on the microalgae growth can be described by applying uncoupled model and incorporating Droop model (used intercellular quota of nitrogen: carbon ratio for defining growth rate) with temperature response function (Geider et al., 1998). However, this model describes the microalgae growth response at low temperatures (Bechet et al., 2013). The modified equation for μ_m , which considers the enzyme deactivation due to high temperature, can be expressed as:

$$\mu_m = \mu_o \left(\frac{Q - Q_{min}}{Q_{max} - Q_{min}}\right) \left[\frac{\text{Exp}(-E_a/RT)}{1 + K \text{Exp}(-E_a^*/RT)}\right] \quad \text{or}$$

$$\left(\frac{\mu_m}{\mu_o}\right) = \left(\frac{Q^* - Q_{min}^*}{1 - Q_{min}^*}\right) \left[\frac{\text{Exp}(-\gamma/\phi)}{1 + K \text{Exp}(-\gamma^*/\phi)}\right] \quad (4)$$

(Dimensionless form)

where μ_o is the optimal specific growth rate for a given microalgae, T is the temperature, E_a is the overall activation energy required by enzymes to support metabolism of a microalgal cell, E_a^* is the activation energy for enzyme denaturation, R is the universal gas constant, K is the dimensionless inactivation constant, Q is the nitrogen to carbon ratio quota, Q_{min} is the minimum value of Q required to sustain a cell, Q_{max} is the maximum value of Q , and

$$\phi = \frac{T}{T_s}, \gamma = \frac{E_a}{RT}, \gamma^* = \frac{E_a^*}{RT_s}, Q^* = \frac{Q}{Q_{max}}, \text{ and } Q_{min}^* = \frac{Q_{min}}{Q_{max}}.$$

2.2. Balance equations

A chemostat is characterized by constant input and output to keep the constant volume of a vessel. It is widely used for studying microalgal growth rate and lipid production or other microalgal bioproducts (Bordel et al., 2009; Tevatia et al., 2014a; Carvalho et al., 2006; Bumbak et al., 2011; Chen et al., 2011). Here, we consider the unstirred chemostat model (Nie and Wu, 2007, 2009; Wang et al.,

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