

A DYNAMIC MODEL DESCRIBING HETEROTROPHIC CULTURE OF *CHLORELLA* AND ITS STABILITY ANALYSIS

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ABSTRACT. *Chlorella* is an important species of microorganism, which includes about 10 species. *Chlorella* USTB01 is a strain of microalga which is isolated from Qinghe River in Beijing and has strong ability in the utilization of organic compounds and was identified as *Chlorella* sp. (H. Yan et al, Isolation and heterotrophic culture of *Chlorella* sp., *J. Univ. Sci. Tech. Beijing*, 2005, **27**:408-412). In this paper, based on the standard Chemostat models and the experimental data on the heterotrophic culture of *Chlorella* USTB01, a dynamic model governed by differential equations with three variables (*Chlorella*, carbon source and nitrogen source) is proposed. For the model, there always exists a boundary equilibrium, i.e. *Chlorella*-free equilibrium. Furthermore, under additional conditions, the model also has the positive equilibria, i.e., the equilibria for which *Chlorella*, carbon source and nitrogen source are coexistent. Then, local and global asymptotic stability of the equilibria of the model have been discussed. Finally, the parameters in the model are determined according to the experimental data, and numerical simulations are given. The numerical simulations show that the trajectories of the model fit the trends of the experimental data well.

1. Introduction. *Chlorella* is an important species of microorganism, which includes about 10 species (see, for example, [3]). A single *Chlorella* cell is of sphere with the diameter from $2\mu\text{m}$ to $12\mu\text{m}$. *Chlorella* has the following characteristics. (a) It grows quickly and can efficiently photosynthesize. (b) It includes rich protein, highly unsaturated fatty acids, carotenoid, lutein, astaxanthin and a variety of vitamins (see, for example, [3], [36] - [38] and [40]). In particular, lutein in *Chlorella*

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has the functions of restraining the decreasing of sight and the blindness caused by age and of inhibiting on the growth of tumor etc. (see, for example, [12], [13], [15], [19] and [23]). In addition, *Chlorella* also contains *Chlorella* growth factor which has the effect of increasing immune response of T cells and B cells. *Chlorella* also has the effect of decomposing and eliminating harmful substances in environment (see, for example, [10] and [33]). Furthermore, *Chlorella* has the effect of absorbing the heavy metal copper etc.. Hence, in environmental science field, *Chlorella* is usually used to remove organic pollutants and heavy metals (see, for example, [34] and [35]).

1.1. Culture of *Chlorella*. The culture of *Chlorella* is divided into autotrophic culture and heterotrophic culture. Heterotrophic culture is divided into batch culture, feed-batch culture and continuous culture. Batch culture is extensively used in applications. In batch culture, microorganisms are put into the culture vessel which contains certain nutrients, under appropriate temperature, salinity and pH. Then, after a period of time of culture, the microorganisms in the culture vessel are harvested one-time. Batch culture is simple, but it is usually difficult to achieve higher biomass of microorganisms. Furthermore, the increasing of the initial concentration of nutrients may result in the inhibition on the growth of microorganisms. In feed-batch culture, nutrients are added into the culture vessel for every fixed time. Hence, feed-batch culture can reduce the inhibition on the growth of microorganisms and achieve higher biomass of microorganisms. Continuous culture is divided into semi-continuous culture and continuous culture. Semi-continuous culture is that nutrients are added into the culture vessel and, at the same time, microorganism is harvested for every fixed time. In continuous culture, nutrients are put into the culture vessel continuously and, at the same time, microorganisms flow out continuously. Hence, a balance between the inflow rate and the outflow rate can be maintained. Furthermore, continuous culture can also decrease the inhibition on the growth of microorganism caused by higher concentration of nutrients and accumulation of harmful substances in the culture vessel.

1.2. Heterotrophic culture of *Chlorella* sp. USTB01. It has been found that some species of *Chlorella* can not only be cultured in suitable organisms even in the absence of light (see, for example, [8] and [30]), but also contain richer lutein (see, for example, [8], [21] and [27]). Usually, carbon source and nitrogen source are main nutrients in culture of *Chlorella*. In [25], [26] and [31], the impact of concentration of glucose on the growth of *Chlorella* has been studied. However, till now, there are few species of *Chlorella* which can grow quickly (see, for example, [18], [22], [36] and [38]).

Recently, a species of *Chlorella*, named *Chlorella* sp. USTB01, which can be cultured quickly by the method of heterotrophic culture has been successfully sieved out from Qinghe river in Beijing (see, for example, [36]). Then, the impact of carbon-to-nitrogen mass ratio on the growth of *Chlorella* sp. USTB01 has also been investigated in details in [31], [36] and [37]. In the experiments, nitrogen sources are urea, ammonium chloride and potassium nitrate, and the culture time is limited to 36 hours. The experimental data show that, while carbon-to-nitrogen mass ratio is 20:1 and nitrogen source is ammonium chloride, the biomass of *Chlorella* sp. USTB01 in the culture vessel is higher (see Fig.1), but the protein contained is lower.

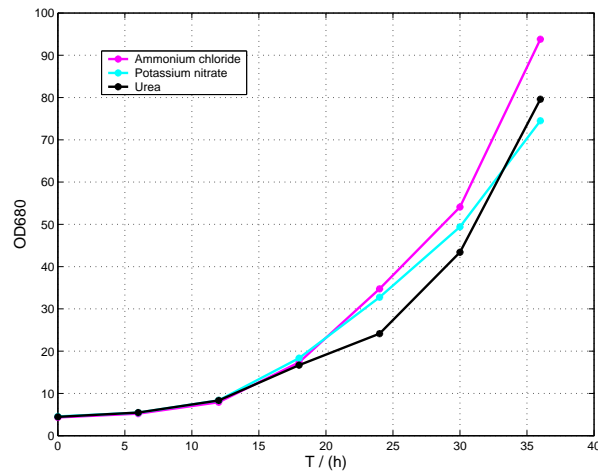


FIGURE 1. The effect of different nitrogen sources on the growth of *Chlorella* USTB01 when carbon-to-nitrogen mass ratio is 20:1

If carbon-to-nitrogen mass ratio is still 20:1, but nitrogen source is replaced by potassium nitrate, then, the biomass of *Chlorella* sp. USTB01 in the culture vessel is not higher (see Fig.1), but the protein contained is higher.

Furthermore, if carbon-to-nitrogen mass ratio is 10:1 and nitrogen source is ammonium chloride, the biomass of *Chlorella* USTB01 in the culture vessel is higher (see Fig.2), and the protein contained is also higher.

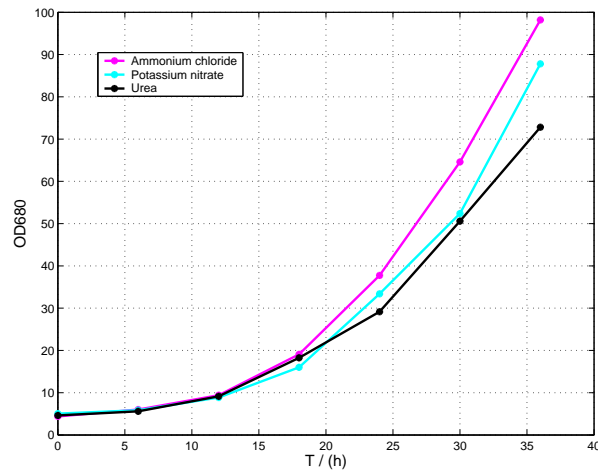


FIGURE 2. The effect of different nitrogen sources on the growth of *Chlorella* USTB01 when carbon-to-nitrogen mass ratio is 10:1

The purpose of the paper is to model the growth of *Chlorella* USTB01 by constructing a dynamic model described by differential equations and to predict the growth status of *Chlorella* sp. USTB01 for longer culture time. The organization of the paper is as follows.

In Section 2, firstly, standard Chemostat models are introduced. Secondly, a dynamic model governed by differential equations is proposed based on standard Chemostat models and the experimental data obtained for heterotrophic culture of *Chlorella* sp. USTB01. Thirdly, for the model, global existence and nonnegativity of the solutions with appropriate initial conditions and classification of the equilibria are discussed. In Section 3, local and global asymptotic stability of two classes of the equilibria (i.e., *Chlorella*-free equilibrium and the equilibria for which *Chlorella*, carbon sources and nitrogen sources are coexistent) of the model in Section 2 are discussed by stability theory of differential equations. In Section 4, the parameters in the model in Section 2 are determined according to the experimental data and then numerical simulations are carried out. The numerical simulations suggest the following interesting facts. (i) The trajectories of the model in Section 2 fit the experimental data very well. (ii) *Chlorella* sp. USTB01 is in the exponential growth phase from 36 hours to 40 hours. (iii) If the culture time is extended to 60 hours, the biomass of *Chlorella* sp. USTB01 in the culture vessel can reach at least 95% of the theoretical biomass. The proofs of the main results in Section 3 are finally given in Appendices A and B.

2. Dynamic model.

2.1. Chemostat models. Dynamical models which describe continuous culture of microorganisms are also called Chemostat models (see, for example, [28]). Chemostat models can be used to the researches on the growth of algae and plankton in lakes and oceans, as well as the growth of endangered species (see, for example, [4] and [28]). The standard Chemostat model with a single limiting resource and a single microorganism is usually described by the following differential equations (see, for example, [11] and [28]),

$$\begin{cases} \dot{S}(t) = D(S_0 - S) - \delta^{-1}\mu(S)X, \\ \dot{X}(t) = (\mu(S) - D)X, \end{cases} \quad (1)$$

where $S(t)$ and $X(t)$ are the concentrations of the limiting resource (substrate) and microorganism at time t , respectively. $S_0 > 0$ denotes the concentration of input limiting resource and is assumed to be constant. The constant $D > 0$ is the dilution rate. The death rate of microorganism is assumed to be insignificant compared to the dilution rate and is ignored. The function $\mu(S)$ denotes how X consumes S and is called the uptake function. In most cases, $\mu(S)$ is chosen as Monod (1950) function, that is $\mu(S) = \mu_m S / (K_m + S)$, where μ_m is the maximum growth rate of microorganism, $K_m > 0$ is the Michaelis-Menten (or half-saturation) constant. In more general case, $\mu(S)$ is assumed to be a monotone function which satisfies the conditions:

$$\mu'(S) > 0 (S > 0), \quad \mu(0) = 0, \quad \lim_{S \rightarrow \infty} \mu(S) = \bar{S} = \text{const.}$$

$\delta = \delta(S)$ is called the yield coefficient with respect to the nutrient S . Usually, $\delta(S)$ is chosen as constant or linear function of the nutrient S : $\delta(S) = AS + B$, where $A \geq 0$ and $B > 0$ are constants (see, for example, [4], [24], [28], [29] and the references therein). Based on different biological meanings, the model (1) has been modified and extended in the following aspects in recent years (see, for example, [1], [2], [5] - [7], [11], [16], [17], [20], [24], [28], [29], [32] and the references therein): (i) The models in which multiple microorganisms compete for single limiting resource, and the dilution rates of microorganisms may be different; (ii) The models in which single

or multiple microorganisms compete for multiple resources, and the resources are perfectly substitutable or perfectly complementary; (iii) The models in which uptake functions are nonmonotone and the inhibiting effects of higher concentrations of substrates to the growth of microorganism are considered; (iv) The models in which there are time delays in the nutrients conversion processes; (v) The models in which the effects of inhibitors (internal or external) on microbial competition (such as plasmid-bearing, plasmid-free competition etc) are considered. (vi) The models in which the input rates of limiting resources are not constants (such as periodic functions of time t etc).

2.2. Dynamical model in the heterotrophic culture of *Chlorella*. *Chlorella* sp. USTB01 (*Chlorella* in short hereafter) has a strong ability in the utilization of organic compounds and includes rich protein (see, for example, [36]). In this subsection, based on the basic Chemostat model (1) and the experimental data in [36] in the heterotrophic culture of *Chlorella*, a dynamical model is proposed.

Let us first look back the experimental procedures in [36]. (i) First, an appropriate amount of *Chlorella* is added into the culture vessel which has definite volume and is fed with an appropriate amount of carbon source and nitrogen source. (ii) Then, after the beginning of the culture, an appropriate amount of carbon source and nitrogen source are added into the culture vessel in batches (every 6 hours), and the quantity of the carbon source and nitrogen source added are dependent on the consumption of the carbon source and nitrogen source and the biomass of *Chlorella* in the culture vessel. Furthermore, the quantity of the carbon source and nitrogen source added every 6 hours is not less than the quantity added at the beginning of the experiment, and proportional to the biomass of *Chlorella* in culture vessel (see Fig 3). (iii) The culture time is limited 36 hours and the outflow of carbon source, nitrogen source and *Chlorella* in the culture vessel are not considered. (iv) The experimental data show that the mortality of *Chlorella* in the culture vessel increases as the biomass of *Chlorella* increases (i.e., product inhibition). Hence, the growth of *Chlorella* in the culture vessel is density-dependent.

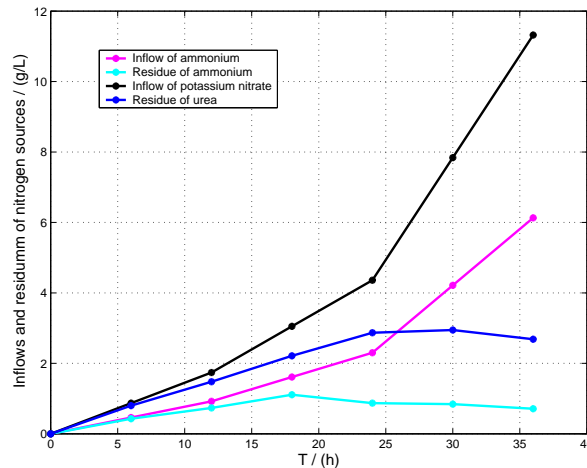


FIGURE 3. Total inflows and residuum of ammonium chloride and potassium nitrate when carbon-to-nitrogen mass ratio is 20:1

Let $X(t)$, $C(t)$ and $N(t)$ denote the mass of *Chlorella*, carbon source and nitrogen source, respectively, in the culture vessel at time t . We would point out here that, for simplicity, it is assumed that input of carbon source and nitrogen source is continuous. Hence, the dynamic model describing the growth of *Chlorella* in the heterotrophic culture can be expressed as following,

$$\begin{cases} \dot{X} = \theta_1(r\mu_1(C)\mu_2(N) - d_1 - fX)X, \\ \dot{C} = \theta_2(\alpha X + a - d_2C - \frac{X}{\delta_1}r_1\mu_1(C)\mu_2(N)), \\ \dot{N} = \theta_3(\beta X + b - d_3N - \frac{X}{\delta_2}r_2\mu_1(C)\mu_2(N)), \end{cases} \quad (2)$$

where the constant $d_1 > 0$ is the mortality rate of *Chlorella*. The constants $d_2 > 0$ and $d_3 > 0$ are the attrition rates of carbon source and nitrogen source, respectively. The term fX^2 in which $f > 0$ is constant indicates that the growth of *Chlorella* in the culture vessel is density-dependent. The terms αX and βX in which $\alpha > 0$ and $\beta > 0$ are constants indicate that the quantity of carbon source and nitrogen source added every 6 hours are proportional to the biomass of *Chlorella* in culture vessel, respectively. The constants $a > 0$ and $b > 0$ represent the constant input of carbon source and nitrogen source, respectively. Since carbon source and nitrogen source are perfectly complementary in the culture of *Chlorella*, the term $r\mu_1(C)\mu_2(N)$ represents the growth rate of *Chlorella*, and the terms $r_1\mu_1(C)\mu_2(N)X$ and $r_2\mu_1(C)\mu_2(N)X$ represent the quantity of decreasing of carbon source and nitrogen source, respectively, where $r > 0$, $r_1 > 0$ and $r_2 > 0$ are constants, and the functions $\mu_1(C)$ and $\mu_2(N)$ are nonnegative and continuous for $C \geq 0$ and $N \geq 0$. δ_i ($i = 1, 2$) are yield coefficients, which are defined as

$$\delta_i = \frac{\text{mass of organism formed}}{\text{mass of substrate consumed}} \quad (i = 1, 2).$$

The constants $\theta_1 > 0$, $\theta_2 > 0$ and $\theta_3 > 0$ are relative growth rates of *Chlorella*, carbon source and nitrogen source, respectively.

For simplicity of theoretical analysis, in this paper, the functions $\mu_1(C)$ and $\mu_2(N)$ are chosen as Monod type functions, i.e.,

$$\mu_1(C) = \frac{C}{k_1 + C}, \quad \mu_2(N) = \frac{N}{k_2 + N},$$

where $k_1 > 0$ and $k_2 > 0$ are the half-saturation constants with respect to carbon source and nitrogen source, respectively. The yield coefficients δ_1 and δ_2 are assumed to be constants. Hence, without loss of generality, r_1/δ_1 and r_2/δ_2 are still denoted by r_1 and r_2 , respectively. Therefore, the dynamic model (2) can be rewritten in the following simpler form,

$$\begin{cases} \dot{X} = \theta_1(\frac{rCN}{(k_1+C)(k_2+N)} - d_1 - fX)X, \\ \dot{C} = \theta_2(\alpha X + a - d_2C - \frac{r_1CNX}{(k_1+C)(k_2+N)}), \\ \dot{N} = \theta_3(\beta X + b - d_3N - \frac{r_2CNX}{(k_1+C)(k_2+N)}). \end{cases} \quad (3)$$

2.3. Existence of equilibria. In this subsection, let us consider nonnegativity of the solutions and existence of the equilibria of (3).

First, according to biological meanings, the initial condition of (3) is given as

$$X(0) = X_0 \geq 0, \quad C(0) = C_0 \geq 0, \quad N(0) = N_0 \geq 0, \quad (4)$$

where the constants X_0 , C_0 and N_0 represent the initial mass of *Chlorella*, carbon source and nitrogen source, respectively. From Lemma 1 in Appendix B and standard theory on existence of solutions of ordinary differential equations (see, for

example, [9]), it can be easily shown that the solution $(X(t), C(t), N(t))$ of (3) with the initial condition (4) is existent, unique and nonnegative for all $t \geq 0$.

Let (X, C, N) be any equilibrium of (3). Then, (X, C, N) satisfies the following nonlinear algebraic equations,

$$\begin{cases} \frac{rCNX}{(k_1+C)(k_2+N)} - d_1X - fX^2 = 0, \\ \alpha X + a - d_2C - \frac{r_1CNX}{(k_1+C)(k_2+N)} = 0, \\ \beta X + b - d_3N - \frac{r_2CNX}{(k_1+C)(k_2+N)} = 0. \end{cases} \tag{5}$$

There are two cases to be discussed.

(i) (3) always has the boundary equilibrium $E_0 = (X, C, N) = (0, a/d_2, b/d_3)$. The existence of E_0 indicates that, if there is no *Chlorella* to be added into the culture vessel at the beginning of the culture, the concentrations of carbon source and nitrogen source in the culture vessel always maintain the constants values a/d_2 and b/d_3 , respectively. The equilibrium E_0 is also called *Chlorella*-free equilibrium.

(ii) Let (X, C, N) be any equilibrium with $X > 0, C > 0$ and $N > 0$. From (5), we have that

$$\begin{cases} (d_1 + fX)(k_1 + C)(k_2 + N) = rCN, \\ C = \frac{1}{d_2r} \{ ar - [(r_1d_1 - \alpha r)X + r_1fX^2] \}, \\ N = \frac{1}{d_3r} \{ br - [(r_2d_1 - \beta r)X + r_2fX^2] \}. \end{cases} \tag{6}$$

Clearly, X should satisfy the conditions

$$ar - [(r_1d_1 - \alpha r)X + r_1fX^2] > 0 \tag{7}$$

and

$$br - [(r_2d_1 - \beta r)X + r_2fX^2] > 0. \tag{8}$$

Substituting the second and the third equations of (6) into the first equation of (6) gives a fifth order algebraic equation. Hence, under suitable conditions, there may be at most five different positive roots for the fifth order algebraic equation. Let $X = X^*$ be any such positive root which also satisfies the conditions (7) and (8). Then, from (6), $C = C^* > 0$ and $N = N^* > 0$ can be obtained. Therefore, the dynamical model (3) at most has five equilibria of the type of $E^* = (X, C, N) = (X^*, C^*, N^*)$ with $X^* > 0, C^* > 0$ and $N^* > 0$. The equilibrium $E^* = (X^*, C^*, N^*)$ is also called positive equilibrium which indicates that *Chlorella*, carbon source and nitrogen source are coexistent for any time $t \geq 0$.

From the first equation in (6), we obtain the function

$$\begin{aligned} F(X) &= (d_1 + fX)(k_1 + C)(k_2 + N) - rCN \\ &= fX(k_1 + C)(k_2 + N) + d_1(k_1k_2 + k_1N + k_2C) + (d_1 - r)CN, \end{aligned}$$

which implies that $d_1 < r$ is a necessary condition for a positive equilibrium to exist. In fact, E_0 is globally asymptotically stable when $d_1 \geq r$ (see Theorem 3.3 in Section 3 below).

General speaking, it is not easy to give a complete theoretical analysis for the existence of all the positive equilibria. But, according to the experiment data provided in [36], the approximate estimations of all the positive equilibria can be obtained by Matlab programming.

3. Stability analysis of equilibria. Stability analysis of the equilibria of the dynamic model (3) is very important for understanding the growth status of *Chlorella*. In this section, some sufficient conditions are given to ensure global asymptotic stability of boundary equilibrium $E_0 = (0, a/d_2, b/d_3)$ and local asymptotic stability of

the positive equilibrium $E^* = (X^*, C^*, N^*)$ by standard stability theory of ordinary differential equations (see, for example, [9], [28]).

First of all, for local asymptotic stability of $E_0 = (0, a/d_2, b/d_3)$, we have the following

Theorem 3.1. (i) If

$$d_1 > \frac{rab}{(k_1d_2 + a)(k_2d_3 + b)} \triangleq \theta, \quad (9)$$

then the boundary equilibrium $E_0 = (0, a/d_2, b/d_3)$ is locally asymptotically stable.

(ii) If $d_1 = \theta$, then the boundary equilibrium $E_0 = (0, a/d_2, b/d_3)$ is linear stable.

(iii) If $d_1 < \theta$, then the boundary equilibrium $E_0 = (0, a/d_2, b/d_3)$ is unstable.

The proof of Theorem 3.1 is given in Appendix A.

Remark 1. In view of the biological meanings of the parameters in (3) and the condition (9), Theorem 3.1 indicates that the biomass of *Chlorella* may tend to zero and the mass of carbon source and nitrogen source may tend to the constant values a/d_2 and b/d_3 , respectively, as time t increases, if one of the following two cases occurs: (a) decreasing the value of the constant input rate a or b ; (b) increasing the value of the attrition rate d_2 or d_3 or the mortality rate d_1 . These cases are reasonable, since they imply the insufficient sources for *Chlorella* to grow.

For local asymptotic stability of the positive equilibrium $E^* = (X^*, C^*, N^*)$ of the dynamic model (3), we have the following

Theorem 3.2. If the positive equilibrium $E^* = (X^*, C^*, N^*)$ exists, and the conditions

$$br + fr_2X^{*2} - rd_3N^* > 0, \quad ar + fr_1X^{*2} - rd_2C^* > 0 \quad (10)$$

hold, then $E^* = (X^*, C^*, N^*)$ is locally asymptotically stable.

The proof of Theorem 3.2 is also given in Appendix A.

Remark 2. Note that sufficient conditions for (10) to hold are $a \geq d_2C^*$ and $b \geq d_3N^*$. Hence, for fixed constants $a > 0$ and $b > 0$, if the attrition rates d_2 and d_3 of carbon source and nitrogen source, respectively, are small enough and the growth rate r of *Chlorella* is large enough, the conditions (7), (8) and (10) can be satisfied. Therefore, it follows from Theorem 3.2 that the positive equilibrium $E^* = (X^*, C^*, N^*)$ is locally asymptotically stable. This implies that *Chlorella*, carbon source and nitrogen source in the culture vessel are coexistent and their masses tend to constant values as time t increases.

Remark 3. There may exist multiple positive equilibria for the dynamical model (3). Since complexity of the expression of the positive equilibrium $E^* = (X^*, C^*, N^*)$, detailed theoretical and numerical analysis on multi stability are omitted. In Section 4 below, according to the experimental data in [36], the parameters in the dynamical model (3) are chosen, and then the value of the positive equilibrium $E^* = (X^*, C^*, N^*)$ is computed numerically. Theorem 3.2 and numerical simulations show that the positive equilibrium $E^* = (X^*, C^*, N^*)$ is asymptotically stable and that the trajectories of the dynamical model (3) fit the trends of the experimental data well.

From the point of view in both mathematics and biology, theoretical analysis on global asymptotic stability of the equilibria of the dynamical model (3) has more important significance. Global asymptotic stability of the equilibria E_0 or E^*

implies that the asymptotic properties of *Chlorella*, carbon source and nitrogen source in culture vessel are not dependent on the initial values X_0 , C_0 and N_0 of *Chlorella*, carbon source and nitrogen source.

For global asymptotically stability of the boundary equilibrium E_0 , we have the following

Theorem 3.3. *If $d_1 \geq r$, or $d_1 < r$ and*

$$d_1 \geq \frac{r[af + \alpha(r - d_1)][bf + \beta(r - d_1)]}{[f(k_1d_2 + a) + \alpha(r - d_1)][f(k_2d_3 + b) + \beta(r - d_1)]} \triangleq \bar{\theta}, \tag{11}$$

then, the boundary equilibrium E_0 is globally asymptotically stable.

The proof of Theorem 3.3 is given in Appendix B.

Remark 4. The condition $d_1 \geq \bar{\theta}$ in Theorem 3.3 implies the condition $d_1 > \theta$ in Theorem 3.1. Furthermore, numerical simulations strongly suggest that the boundary equilibrium E_0 should also be globally asymptotically stable even if the condition $d_1 \geq \theta$ holds. Therefore, the condition (11) in Theorem 3.3 may be further improved.

As for global asymptotic stability of the positive equilibrium E^* , detailed discussions shall be given in other paper.

4. Numerical simulations and discussions.

4.1. Numerical simulations. In this subsection, let us discuss how the trajectories of the dynamical model (3) fit the experimental data in [36] based on Theorem 3.2 and numerical simulations. To observe the growth status of *Chlorella* in the culture vessel, two sorts of nitrogen sources (i.e., ammonium chloride and potassium nitrate) are used. Carbon source and nitrogen source are added into the culture vessel for every 6 hours with two kinds of carbon-to-nitrogen mass ratios 20:1 and 10:1.

Case (I) Nitrogen source is ammonium chloride and carbon-to-nitrogen mass ratio is 20:1. By suitable computations, we have from the experimental data in [36] that the parameters in (3) can be chosen as follows,

$$r = 0.837, k_1 = 5.926, k_2 = 2.377, f = 0.002, \alpha = 0.2703, a = 0.6545, \beta = 0.022, \\ b = 0.0476, r_1 = 1.73, r_2 = 0.14, \theta_1 = 1, \theta_2 = 1, \theta_3 = 1, d_1 = d_2 = d_3 = 0.01.$$

It is easy to check that (3) has the positive equilibrium $E^* = (X^*, C^*, N^*) \approx (62.5998, 8.2038, 0.9162)$, and that the condition (10) holds. Hence, we have from Theorem 3.2 that the positive equilibrium E^* is asymptotically stable. Fig.4 shows that the trajectory of (3) obtained by the numerical simulation with the initial value $(X_0, C_0, N_0) = (1.54, 0, 0)$ fits the experimental data in [36] well.

Case (II) Nitrogen source is potassium nitrate and carbon-to-nitrogen mass ratio is 20:1. By the same computations as Case (I), the parameters in (3) can be chosen as follows,

$$r = 0.21, k_1 = 2.908, k_2 = 0.287, f = 0.003, \alpha = 0.2943, a = 0.5665, \beta = 0.0427, \\ b = 0.0824, r_1 = 0.42, r_2 = 0.06, \theta_1 = 0.9, \theta_2 = 1, \theta_3 = 1, d_1 = d_2 = d_3 = 0.01.$$

(3) has the positive equilibrium $E^* = (X^*, C^*, N^*) \approx (47.3691, 9.6855, 4.6436)$ which is asymptotically stable. Fig.5 shows that the numerical simulation trajectory of (3) with the initial value $(X_0, C_0, N_0) = (1.63, 0, 0)$ fits the experimental data in [36] well.

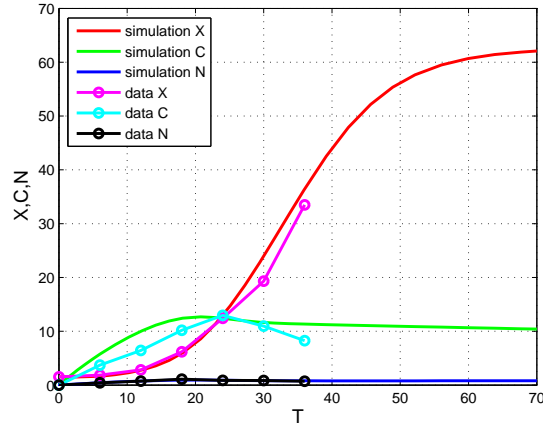


FIGURE 4. The experimental data and the trajectory of (3) when the nitrogen source is ammonium chloride and carbon-to-nitrogen mass ratio is 20:1

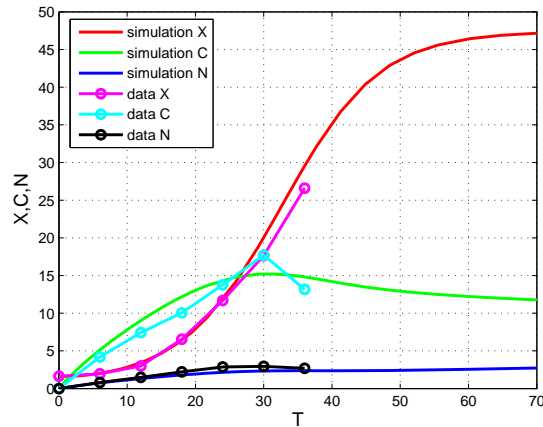


FIGURE 5. The experimental data and the trajectory of (3) when the nitrogen source is potassium nitrate and carbon-to-nitrogen mass ratio is 20:1

Case (III) Nitrogen source is ammonium chloride and carbon-to-nitrogen mass ratio is 10:1. The parameters in (3) can be chosen as follows,

$$r = 0.408, k_1 = 5.168, k_2 = 0.496, f = 0.003, \alpha = 0.2501, a = 0.6324, \beta = 0.0409, b = 0.0891, r_1 = 0.575, r_2 = 0.093, \theta_1 = 0.7, \theta_2 = 1, \theta_3 = 1, d_1 = d_2 = d_3 = 0.01.$$

(3) has the positive equilibrium $E^* = (X^*, C^*, N^*) \approx (58.1376, 6.2921, 2.3099)$ which is asymptotically stable. Fig.6 shows that the numerical simulation trajectory of (3) with the initial value $(X_0, C_0, N_0) = (1.57, 0, 0)$ fits the experimental data in [36] well.

Case (IV) Nitrogen source is potassium nitrate and carbon-to-nitrogen mass ratio is 10:1. The parameters in (3) can be chosen as follows,

$$r = 0.279, k_1 = 6.819, k_2 = 0.145, f = 0.003, \alpha = 0.2925, a = 0.521, \beta = 0.0846,$$

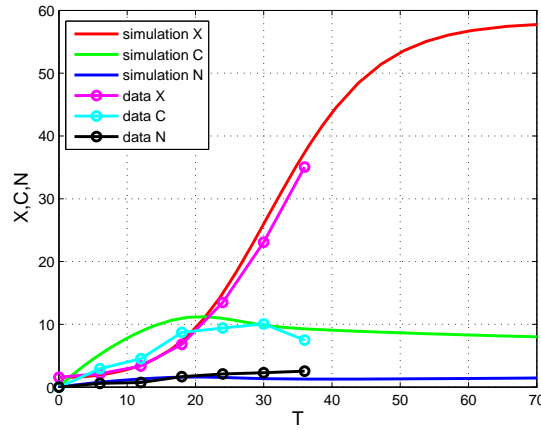


FIGURE 6. The experimental data and the trajectory of (3) when the nitrogen source is ammonium chloride and carbon-to-nitrogen mass ratio is 10:1

$b = 0.1539, r_1 = 0.45, r_2 = 0.13, \theta_1 = 0.8, \theta_2 = 0.6, \theta_3 = 0.8, d_1 = d_2 = d_3 = 0.01$. (3) has the positive equilibrium $E^* = (X^*, C^*, N^*) \approx (58.4398, 14.6835, 5.1652)$ which is asymptotically stable. Fig.7 shows that the numerical simulation trajectory of (3) with the initial value $(X_0, C_0, N_0) = (1.8, 0, 0)$ fits the experimental data in [36] well.

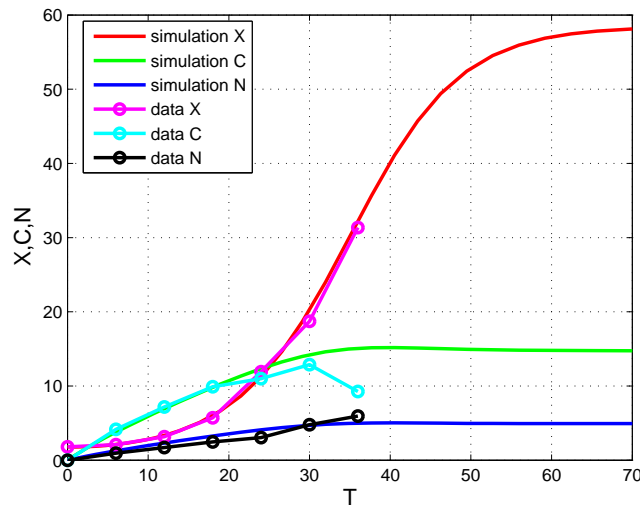


FIGURE 7. The experimental data and the trajectory of (3) when the nitrogen source is potassium nitrate and carbon-to-nitrogen mass ratio is 10:1

4.2. **Discussions.** First, the numerical simulations show that the dynamic model (3) can describe the growth status of *Chlorella* in the culture vessel in [36] very well. The numerical simulations further suggest that, in the culture of *Chlorella*,

the growth of *Chlorella* is in delay phase from 0 hour to 12 hours, in the accelerated phase from 12 hours to 24 hours, in exponential growth phase from 24 hours to 40 hours, and in deceleration phase from 40 hours to 60 hours. Furthermore, we have from Figs 4-7 that, for Cases (I), (II), (III) and (IV) in Subsection 4.1, the saturated biomass of *Chlorella* are about $X^* \approx 62.5998$, $X^* \approx 47.3691$, $X^* \approx 58.1376$ and $X^* \approx 58.4398$, respectively. When the culture time is limited 36 hours (see, for example, [36]), the experimental data shows that the biomass of *Chlorella* in the culture vessel are approximately 53.51% ($33.5/62.5998 \approx 0.5351$), 56.15% ($26.6/47.3691 \approx 0.5615$), 60.31% ($35.06/58.1376 \approx 0.6031$) and 53.65% ($31.35/58.4399 \approx 0.5365$) of the saturated biomass, respectively. If the culture time is extended to 60 hours, the numerical simulations in Subsection 4.1 shows that the biomass of *Chlorella* are approximately 95.93% ($60.05/62.5998 \approx 0.9593$), 97.68% ($46.27/47.3691 \approx 0.9768$), 97.56% ($56.72/58.1376 \approx 0.9756$) and 97.62% ($57.05/58.4399 \approx 0.9762$) of the saturated biomass, respectively.

Remark 5. The purpose of the paper is to model the growth of *Chlorella* USTB01 which have some special characteristics in applications and proposes an ordinary differential equation model (3) according to the experimental procedures in [36]. The model (3) can be revised as the following more general form with time delays,

$$\begin{cases} \dot{X}(t) = \theta_1 \left(\frac{r e^{-d_1 \tau} C(t-\tau) N(t-\tau) X(t-\tau)}{(k_1 + C(t-\tau))(k_2 + N(t-\tau))} - d_1 X(t) - f X^2(t) \right), \\ \dot{C}(t) = \theta_2 \left(\alpha X(t) + a - d_2 C(t) - \frac{r_1 C(t) N(t) X(t)}{(k_1 + C(t))(k_2 + N(t))} + \rho_1 X(t - \sigma) \right), \\ \dot{N}(t) = \theta_3 \left(\beta X(t) + b - d_3 N(t) - \frac{r_2 C(t) N(t) X(t)}{(k_1 + C(t))(k_2 + N(t))} + \rho_2 X(t - \sigma) \right). \end{cases} \quad (12)$$

In (12), the constants $\rho_1 \geq 0$ and $\rho_2 \geq 0$ are the rate constants at which carbon source and nitrogen source are recycled because of the death of *Chlorella*. The constant $\tau \geq 0$ denotes the time delay involved in the conversion of nutrients to *Chlorella*. The constant $\sigma \geq 0$ is a fixed time during which carbon source and nitrogen source are released completely from dead *Chlorella*. The factor $e^{-d_1 \tau}$ represents the approximate proportion of *Chlorella* that remain in the culture vessel during the conversion process.

Theoretical analysis on stability of the equilibria of (12) and its applications in the research of the control strategies of algal toxins of blooms of the local lakes and rivers in Beijing city shall be given in the other papers.

Appendix A. Analysis on local asymptotic stability.

Proof of Theorem 3.1. Without loss of generality, let us assume that $\theta_1 = \theta_2 = \theta_3 = 1$. Let $A = (a_{ij})_{3 \times 3}$ be the corresponding Jacobian matrix at any equilibrium $E(X, C, N)$ of the dynamical model (3). Then we easily have that

$$\begin{aligned} a_{11} &= \frac{rCN}{(k_1 + C)(k_2 + N)} - d_1 - 2fX, & a_{12} &= \frac{rk_1NX}{(k_1 + C)^2(k_2 + N)}, \\ a_{13} &= \frac{rk_2CX}{(k_1 + C)(k_2 + N)^2}, & a_{21} &= \alpha - \frac{r_1CN}{(k_1 + C)(k_2 + N)}, \\ a_{22} &= -\left(d_2 + \frac{r_1k_1NX}{(k_1 + C)^2(k_2 + N)}\right), & a_{23} &= -\frac{r_1k_2CX}{(k_1 + C)(k_2 + N)^2}, \\ a_{31} &= \beta - \frac{r_2CN}{(k_1 + C)(k_2 + N)}, & a_{32} &= -\frac{r_2k_1NX}{(k_1 + C)^2(k_2 + N)}, \end{aligned}$$

$$a_{33} = -\left(d_3 + \frac{r_2 k_2 C X}{(k_1 + C)(k_2 + N)^2}\right).$$

At the boundary equilibrium E_0 , we have that $a_{11} = \theta - d_1$, $a_{22} = -d_2$, $a_{33} = -d_3$, $a_{12} = a_{13} = a_{23} = a_{32} = 0$. Hence, the corresponding characteristic equation is

$$|\lambda E - A| = (\lambda + d_1 - \theta)(\lambda + d_2)(\lambda + d_3) = 0,$$

and the corresponding characteristic roots are $\lambda_1 = -d_1 + \theta < 0$, $\lambda_2 = -d_2 < 0$ and $\lambda_3 = -d_3 < 0$. This completes the proof of Theorem 3.1. \square

Proof of Theorem 3.2. The corresponding characteristic equation at the positive equilibrium $E^* = (X^*, C^*, N^*)$ can be expressed as the following form,

$$|\lambda E - A| = \lambda^3 + a_1 \lambda^2 + a_2 \lambda + a_3 = 0,$$

where $a_1 = -(a_{11} + a_{22} + a_{33})$, $a_2 = a_{11}a_{22} + a_{11}a_{33} + a_{22}a_{33} - a_{12}a_{21} - a_{13}a_{31} - a_{23}a_{32}$, $a_3 = a_{11}a_{23}a_{32} + a_{12}a_{21}a_{33} + a_{13}a_{31}a_{22} - a_{11}a_{22}a_{33} - a_{12}a_{23}a_{31} - a_{13}a_{21}a_{32}$. Here, the definition of a_{ij} is the same as that in the proof of Theorem 3.1, but $E(X, C, N)$ is replaced by $E^*(X^*, C^*, N^*)$. Since

$$\frac{rC^*N^*}{(k_1 + C^*)(k_2 + N^*)} = d_1 + fX^*,$$

we have that

$$a_{11} = \frac{rC^*N^*}{(k_1 + C^*)(k_2 + N^*)} - d_1 - 2fX^* = -fX^*.$$

Hence,

$$a_1 = fX^* + d_2 + d_3 + \frac{r_1 k_1 N^* X^*}{(k_1 + C^*)^2 (k_2 + N^*)} + \frac{r_2 k_2 C^* X^*}{(k_1 + C^*)(k_2 + N^*)^2} > 0,$$

$$a_2 = fX^*d_2 + fX^*d_3 + d_2d_3 + \frac{k_1 N^*}{(k_1 + C^*)^2 (k_2 + N^*)} A + \frac{k_2 C^*}{(k_1 + C^*)(k_2 + N^*)^2} B,$$

where

$$\begin{aligned} A &= \frac{rr_1 N^* C^* X^*}{(k_1 + C^*)(k_2 + N^*)} + fr_1(X^*)^2 + d_3 r_1 X^* - \alpha r X^* \\ &= r(\alpha X^* + a - d_2 C^*) + fr_1(X^*)^2 + d_3 r_1 X^* - \alpha r X^* \\ &= ar + fr_1(X^*)^2 + d_3 r_1 X^* - d_2 r C^* > 0, \end{aligned}$$

$$\begin{aligned} B &= \frac{rr_2 N^* C^* X^*}{(k_1 + C^*)(k_2 + N^*)} + fr_2(X^*)^2 + d_2 r_2 X^* - \beta r X^* \\ &= r(\beta X^* + b - d_3 N^*) + fr_2(X^*)^2 + d_2 r_2 X^* - \beta r X^* \\ &= br + fr_2(X^*)^2 + d_2 r_2 X^* - d_3 r N^* > 0. \end{aligned}$$

Here, the assumptions in Theorem 3.2 and the following equalities

$$\begin{aligned} \frac{r_1 N^* C^* X^*}{(k_1 + C^*)(k_2 + N^*)} &= \alpha X^* + a - d_2 C^*, \\ \frac{r_2 N^* C^* X^*}{(k_1 + C^*)(k_2 + N^*)} &= \beta X^* + b - d_3 N^*, \end{aligned}$$

have been used. Hence, $a_2 > 0$. Furthermore, we have that

$$a_3 = fX^*d_2d_3 + \frac{d_2 k_2 C^*}{(k_1 + C^*)(k_2 + N^*)^2} F + \frac{d_3 k_1 N^*}{(k_1 + C^*)^2 (k_2 + N^*)} H,$$

where

$$\begin{aligned}
 F &= r \frac{r_2 N^* C^* X^*}{(k_1 + C^*)(k_2 + N^*)} + f r_2 X^{*2} - \beta r X^* \\
 &= r(\beta X^* + b - d_3 N^*) + f r_2 X^{*2} - \beta r X^* \\
 &= br + f r_2 X^{*2} - r d_3 N^* > 0, \\
 H &= r \frac{r_1 N^* C^* X^*}{(k_1 + C^*)(k_2 + N^*)} + f r_1 X^{*2} - \alpha r X^* \\
 &= r(\alpha X^* + a - d_2 C^*) + f r_1 X^{*2} - \alpha r X^* \\
 &= ar + f r_1 X^{*2} - r d_2 C^* > 0.
 \end{aligned}$$

Hence, we have $a_3 > 0$. In the following, let us show that $a_1 a_2 - a_3 > 0$. In fact, we have that

$$\begin{aligned}
 &a_1 a_2 - a_3 \\
 &> (f X^* + d_2 + d_3) \left\{ d_2 d_3 + \frac{k_1 N^*}{(k_1 + C^*)^2 (k_2 + N^*)} A + \frac{k_2 C^*}{(k_1 + C^*)(k_2 + N^*)^2} B \right\} \\
 &\quad - \left\{ f X^* d_2 d_3 + \frac{d_2 k_2 C^*}{(k_1 + C^*)(k_2 + N^*)^2} F + \frac{d_3 k_1 N^*}{(k_1 + C^*)^2 (k_2 + N^*)} H \right\} \\
 &> f X^* d_2 d_3 + d_3 \frac{k_1 N^*}{(k_1 + C^*)^2 (k_2 + N^*)} A + d_2 \frac{k_2 C^*}{(k_1 + C^*)(k_2 + N^*)^2} B \\
 &\quad - \left\{ f X^* d_2 d_3 + \frac{d_2 k_2 C^*}{(k_1 + C^*)(k_2 + N^*)^2} F + \frac{d_3 k_1 N^*}{(k_1 + C^*)^2 (k_2 + N^*)} H \right\} \\
 &= d_3 \frac{k_1 N^*}{(k_1 + C^*)^2 (k_2 + N^*)} d_3 r_1 X^* + d_2 \frac{k_2 C^*}{(k_1 + C^*)(k_2 + N^*)^2} d_2 r_2 X^* \\
 &> 0.
 \end{aligned}$$

Hence, we have from Routh-Hurwitz criterion that the positive equilibrium E^* is locally asymptotically stable. This completes the proof of Theorem 3.2. \square

Appendix B. Global asymptotic stability of the equilibrium E_0 .

First of all, let us establish the following two lemmas.

Lemma 4.1. *The solution $(X(t), C(t), N(t))$ of (3) with the initial condition (4) is existent, unique and nonnegative for all $t \geq 0$, and satisfies*

(i) *If $r \leq d_1$, then $\lim_{t \rightarrow +\infty} X(t) = 0$, $\lim_{t \rightarrow +\infty} C(t) = a/d_2$, $\lim_{t \rightarrow +\infty} N(t) = b/d_2$.*

(ii) *If $r > d_1$, then $\lim_{t \rightarrow +\infty} X(t) \leq (r - d_1)/f \triangleq M$, and*

$$\limsup_{t \rightarrow +\infty} C(t) \leq (\alpha M + a)/d_2 \triangleq M_1, \quad \limsup_{t \rightarrow +\infty} N(t) \leq (\beta M + b)/d_3 \triangleq M_2.$$

Proof. Let us assume that the solution $(X(t), C(t), N(t))$ is existent and unique on $[0, \rho]$ for some $\rho > 0$. It can be easily shown that, for any $t \in [0, \rho]$, $(X(t), C(t), N(t))$ is nonnegative. In fact, since the first equation of (3) can be written as the form,

$$\begin{aligned}
 \dot{X}(t) &= X(t) \left(\frac{r C(t) N(t)}{(k_1 + C(t))(k_2 + N(t))} - d_1 - f X(t) \right) \\
 &\triangleq X(t) G(X(t), C(t), N(t)),
 \end{aligned}$$

we have that $X(t) \equiv 0$ for any $t \in [0, \rho]$ if $X_0 = 0$, and that $X(t) > 0$ for any $t \in [0, \rho]$ if $X_0 > 0$. If there is a $t_2 \in [0, \rho]$ such that $C(t_2) = 0$, we have from the second equation of (3) that $\dot{C}(t_2) = \alpha X(t_2) + a > 0$, which clearly implies that $C(t) \geq 0$ for any $t \in [0, \rho]$. Similarly, we have that $N(t) \geq 0$ for any $t \in [0, \rho]$.

For $t \in [0, \rho]$, we have that

$$\dot{X} \leq rX - d_1X - fX^2 = X(r - d_1 - fX).$$

Consider the comparison system,

$$\dot{Z} = Z(r - d_1 - fZ), \quad Z(0) = X_0.$$

Since the solution of the comparison system is existent and nonnegative for any $t \geq 0$, we have from comparison principle that $X(t) \leq Z(t)$ for any $t \in [0, \rho]$. Thus, $X(t) \leq M_0$ for some positive constant M_0 and all $t \in [0, \rho]$. Therefore, for $t \in [0, \rho]$, we have from (3) that

$$\dot{C} \leq \alpha M_0 + a - d_2C, \tag{13}$$

$$\dot{N} \leq \beta M_0 + b - d_3N. \tag{14}$$

Again, we have from (13), (14) and comparison principle that $C(t)$ and $N(t)$ are bounded for $t \in [0, \rho]$. Hence, the solution $(X(t), C(t), N(t))$ can be continued to $+\infty$ and is unique and nonnegative.

Note that $\lim_{t \rightarrow +\infty} Z(t) = 0$ for $r \leq d_1$, and $\lim_{t \rightarrow +\infty} Z(t) = M$ for $r > d_1$. Thus, we have from (13),(14) and comparison principle that, for $r \leq d_1$, $\lim_{t \rightarrow +\infty} X(t) = 0$, $\lim_{t \rightarrow +\infty} C(t) = a/d_2$ and $\lim_{t \rightarrow +\infty} N(t) = b/d_3$; and that, for $r > d_1$, $\lim_{t \rightarrow +\infty} X(t) \leq (r - d_1)/f = M$, $\limsup_{t \rightarrow +\infty} C(t) \leq (\alpha M + a)/d_2 = M_1$ and $\limsup_{t \rightarrow +\infty} N(t) \leq (\beta M + b)/d_3 = M_2$. This completes the proof of Lemma 4.1. □

Lemma 4.2. *The compact set*

$$G = \{(X, C, N) \mid 0 \leq X \leq M, 0 \leq C \leq M_1, 0 \leq N \leq M_2\}$$

attracts all the solutions of (3), and is positively invariant with respect to (3).

Proof. According to Lemma 4.1, it only needs to show that $X(t) \leq M$, $C(t) \leq M_1$ and $N(t) \leq M_2$ for any $t \geq 0$ and $(X(0), C(0), N(0)) \in G$. In fact, if there exists some $t_3 > 0$ such that $X(t_3) > M$, then we have from the Lagrange mean-value theorem that there is some $t_4 \in (0, t_3)$ such that $X(t_4) > M$ and $\dot{X}(t_4) > 0$. On the other hand, we have from (3) that

$$\begin{aligned} \dot{X}(t_4) &= X(t_4) \left(\frac{rC(t_4)N(t_4)}{(k_1 + C(t_4))(k_2 + N(t_4))} - d_1 - fX(t_4) \right) \\ &< X(t_4)(r - d_1 - fM) = 0, \end{aligned}$$

which is a contradiction. Thus, $X(t) \leq M$ for any $t \geq 0$. Therefore, we have from (3) that for any $t \geq 0$,

$$\dot{C} \leq \alpha M + a - d_2C, \quad \dot{N} \leq \beta M + b - d_3N,$$

from which we easily have that $C(t) \leq M_1$ and $N(t) \leq M_2$ for any $t \geq 0$. This completes the proof of Lemma 4.2. □

Proof of Theorem 3.3. If $r \leq d_1$, we have from Lemma 4.1 and Theorem 3.1 that the non-*Chlorella* equilibrium E_0 is globally asymptotically stable. Let us consider the case of $r > d_1$. Since the condition $d_1 \geq \bar{\theta}$ in Theorem 3.3 implies the condition $d_1 > \theta$ in Theorem 3.1, hence, the *Chlorella*-free equilibrium E_0 is locally asymptotically

stable. Consider the nonnegative function $V = x$ on G . It is clear that $V = x$ is continuous on \bar{G} and that the derivative along the solutions of (3) satisfies

$$\begin{aligned}\dot{V} = \dot{X} &= \frac{rCNX}{(k_1 + C)(k_2 + N)} - d_1X - fX^2 \\ &\leq X\left(\frac{rM_1M_2}{(k_1 + M_1)(k_2 + M_2)} - d_1 - fX\right) \\ &= X(\bar{\theta} - d_1 - fX) \leq -fX^2 \leq 0.\end{aligned}$$

Hence, $V = x$ is a Liapunov function of (3) on G .

Define the subset of G as $E = \{(X, C, N) | (X, C, N) \in G, \dot{V} = 0\}$, and let H be the largest invariant set of (3) in E . Since $E_0 \in H$, we have that H is nonempty and that $E \subseteq \{(X, C, N) \in G | X = 0\}$. From the invariance of H and (3), it is easy to show that $H = \{E_0\}$. Therefore, it follows from well known Lyapunov-LaSalle invariance principle that E_0 is globally attractive. This completes the proof of Theorem 3.3. \square

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