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Study on *Microcystis aeruginosa* growth in incubator experiments by combination of Logistic and Monod functions

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ABSTRACT

A combination of Logistic and Monod functions was proposed in this paper to study *Microcystis aeruginosa* growth in incubator experiments. This enables the *Microcystis aeruginosa* growth dynamics to be better described in incubator experiments and its parameters to be calculated more accurately. This method was justified by the data from the experiment and applied to study the effect of prometryn on *Microcystis aeruginosa* growth. In the experiment, a different concentrations of prometryn (0, 50, 100 and 200 $\mu g \cdot L^{-1}$) were added to the culture medium; the algal cell density, concentrations of orthophosphate (PO_4^{3-}-P) and ammonia nitrogen (NH_4^{+}-N) were measured. The results show that *Microcystis aeruginosa* growth with time can be well described using the Logistic function. The maximum algae densities of *Microcystis aeruginosa* corresponding to the four prometryn concentrations are 11.7×10^6, 8.1×10^6, 5.6×10^6 and 3.0×10^6 cells·mL^{-1}, respectively. The derived formula for the specific growth rate, growth rate and inhibition rate using Logistic function agreed reasonably well with the measured data. It was found that variations of consumed nutrients concentrations (PO_4^{3-}-P and NH_4^{+}-N) can also be well described by the Logistic function. A function that describes the relationship between algal densities and consumed nutrient (PO_4^{3-}-P and NH_4^{+}-N) concentrations is also derived from the Logistic function. Combination of Monod and Logistic functions can better describe relationship between specific growth rates and nutrients concentrations compared to the use of Monod function alone. In general, the half saturation coefficient, K_c for PO_4^{3-}-P (4.74×10^{-4}, 1.99×10^{-3}, 5.54×10^{-3} and 3.87×10^{-2} $mg \cdot L^{-1}$) and K_c for NH_4^{+}-N (1.80×10^{-3}, 5.84×10^{-3}, 5.23×10^{-3} and 1.06×10^{-2} $mg \cdot L^{-1}$) in Monod function increase with increasing prometryn concentrations, which indicates that the affinity of algae growth to PO_4^{3-}-P and NH_4^{+}-N decrease with increasing prometryn concentrations. In addition, relationships between nutrients concentrations and time can be derived by combining of Monod and Logistic functions, which agree well with the measured data. It is concluded that the combined application of Monod and Logistic functions provides a promising and more robust method of studying algal growth in incubator experiments.

1. Introduction

With the development of modern agriculture, the amount of herbicides used in agricultural production increases year after year, and herbicides exposed to the environment can enter water bodies though spray drift, soil leaching, rainfall erosion, surface runoff and so on [1]. Prometryn (CAS-No. 7287-19-6) is a persistent methylthiotriazine herbicide [2] acting as a PSII inhibitor, which is frequently found in aquatic ecosystems mainly through terrestrial runoffs or land drainage. Prometryn in water are quite stable with long half-life time and difficult to degrade. It is toxic to higher plants, algae and a lesser extent to other non-photosynthetic organisms. Although prometryn has been banned in Europe since 2004 [3], it is still being widely used in China [3] and can be found in surface water and groundwater. Xu et al. [4] analyzed the concentrations of herbicide in surface water of major basins in China, and prometryn was detected in Taihu Lake, Yangtze River, Heilongjiang River, Songhuajiang River and the eastern and middle canals of the South-North Water Transfer Project. The average concentration in the aforementioned basins was 42.6 $ng \cdot L^{-1}$, 24.8 $ng \cdot L^{-1}$, 19.3 $ng \cdot L^{-1}$, 18.9 $ng \cdot L^{-1}$ and 16.4 $ng \cdot L^{-1}$ respectively. Ma et al. [5,6] analyzed the toxicity of forty herbicides including prometryn to different kinds of green algae and found that different algae have different sensitivities to

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prometryn. In Wilkinson et al.'s [7] study, effective photosynthetic quantum yield of *Halophila ovalis* exposing 11 μg·L^{-1} prometryn decreases by 50% during 24 h, and in the study of *Scenedesmus vacuolatus*, effective photosynthetic quantum yield was reduced by 50% when the concentration of prometryn was 12.5 μg·L^{-1} [8].

Worldwide, not only herbicides but also nutrients are common components of agricultural runoff [9]. As well known, nutrients are necessary conditions for algal growth. Wu et al. [10] believed orthophosphate phosphorus (PO_4^{3-}-P) and ammonia nitrogen (NH_4^+-N) were the most available form of phosphorus and nitrogen, respectively, which can be taken up by algae directly. The preference for ammonia nitrogen is considered to be due, at least in part, to lower energy requirements for the cell, and NH_4^+-N is more easily transported across the cell membrane than NO_3^--N under balanced growth and N limited conditions [11]. García-Fernández and Diez [12] summarized researches on the composition of nitrogen and carbon by *Prochlorococcus* and also found that forms of nitrogen *Prochlorococcus* used depended on the availability of different forms of nitrogen and the energy *Prochlorococcus* dissipated when utilizing nutrients.

Microcystis aeruginosa (*M. aeruginosa*) belonging to cyanobacteria is prokaryotic single-celled organism, which is often found in natural water bodies. It is widely used as a functioning organism in biological experiments [13]. Over the past several years in China, *M. aeruginosa* has become one of the predominant species involved in water blooms around the entire country [14]. *M. aeruginosa* was used in Bittencourt-Oliveira's [15] study to test the effect of saxitoxins on algae, and saxitoxins shows inhibition effect on *M. aeruginosa*. In Wu et al.'s [16] study, glyphosate concentrations of 2, 5 and 10 mg·L^{-1} also significantly inhibited the cell density production of *M. aeruginosa*.

To study microorganism growth, it is easier to apply kinetic models in controlling the microbial processes and predicting the behavior of the processes compared to laboratory experiments [17,18]. In the past, many mathematical models were proposed for microalgal growth, e.g., Logistic function [18–24], Monod function [21,25] and many others [21,24,26,27]. Logistic function describes the dynamics of population that are affected by density-dependence [20], and it is used for a variety of unitary species of algae [18,20,28]. However, Logistic function describes only the number of organisms without the consumption of substrate [18,27]. Monod function considers both the biomass and the rate limiting substrate concentrations in growth rate expression and is the most widely used kinetic equation for batch microbial growth [29–31]. Kargi [29] believed Monod function is mechanistic with meaningful constants, and Logistic function is a mathematical approximation with a rather meaningless rate constant. Thus, both Logistic and Monod functions were considered to describe algal growth in the present study.

In light of the above background, prometryn and *M. aeruginosa* are selected as target herbicide and algae respectively to validate models for algae growth in the absence and presence of prometryn. In the present study, an indoor batch experiment under semi-controlled environmental conditions is carried out to study the effects of immediate exposure of prometryn on the growth process of *M. aeruginosa*, and to test whether and how Monod and Logistic Functions can be used to describe experimental data and determine parameters for algal growth dynamics.

2. Materials and methods

2.1. Experimental materials

Microcystis aeruginosa was obtained from the Freshwater Algae Culture Collection of the Institution of Hydrobiology (FACHB-905), Chinese Academy of Sciences. The algae were cultivated in an illumination. Prometryn [$C_{10}H_{19}N_5S$, 2,4-bis (isopropylamino)-6-(methyl-thio)-*s*-triazine] (purity ≥ 99%) was purchased from Shanghai Aladdin Biochemical Technology Company Limited. Stock solution of prometryn was prepared by distilled water and the concentration was 33 mg·L^{-1}.

2.2. Algal cultures

Before the experiment, the algae *M. aeruginosa* was cultured in M-II culture medium for 15 days. The M-II culture medium was prepared in deionized water with 100 mg·L^{-1} $NaNO_3$, 10 mg·L^{-1} K_2HPO_4, 75 mg·L^{-1} $MgSO_4 \times 7H_2O$, 40 mg·L^{-1} $CaCl_2 \times 2H_2O$, 20 mg·L^{-1} Na_2CO_3, 6 mg·L^{-1} Fe·citrate × H_2O and 1 mg·L^{-1} $Na_2EDTA \times 2H_2O$. The initial pH value was adjusted to approximately 8.0 with 0.5 mol·L^{-1} HCl and 0.5 mol·L^{-1} NaOH. The operational temperature and light intensity were 28 °C and 3000 lx respectively, for the experiment undertaken in the case of illumination. In comparison, the corresponding values for the dark period were 20 °C and 0 lx. The cycle of light and darkness comprised 12 h of illumination and 12 h of darkness.

The medium containing algae was collected and then centrifuged for 15 min at a speed of 3000 r·min^{-1}. After removal of the supernatant, the algae were rinsed with 15 mg·L^{-1} $NaHCO_3$ solution and then centrifuged. After repeating the above procedure twice, the algae obtained via this procedure were cultured in M-II medium without nitrogen or phosphorus. Three days later, the algae would deplete the intracellular polyphosphate stores [10].

2.3. Microcystis aeruginosa growth with prometryn addition

Algae growth characteristics in prometryn media were assessed using batch incubation experiments. The flasks named CK with culture medium and without inoculating algae were used as the control groups. The algae were inoculated in the flasks named M0, M50, M100, M200 containing the same media as those in CK. To test the effect of prometryn on *M. aeruginosa*, low and high concentrations of 0, 50, 100, and 200 μg·L^{-1} of prometryn solution were added to M0, M50, M100 and M200 respectively. Duplicates were prepared. Flasks were shaken and their positions were randomly changed for three times a day. The initial algae density was 5.0 × 10^4 cells·mL^{-1}.

Experiments lasted for 43 days, during which algal cell density was counted every two days using a haemacytometer and a microscope [32]. Counting was performed three times per sample. Water sampling started 1 day after algae addition, and PO_4^{3-}-P and NH_4^+-N were also measured every two days. We did not shake the samples before sampling. NH_4^+-N was analyzed using the phenol-hypochlorite method [33]. Total nitrogen was analyzed using the method of alkaline potassium persulfate digestion with ultra-violet light spectroscopy. Concentrations of PO_4^{3-}-P and TP were determined via the persulfate digestion and ammonium molybdate spectrophotometric method [33].

2.4. Statistical analysis

In this study, several models were examined for their applicability to the experimental data, using Origin 8.6 or SPSS 19.0 to determine the correlation coefficients between the measured and predicted variables.

3. Theoretical background

In this section, equations based on Logistic function for the growth rate, inhabitation rate and specific growth rate are formulated. Logistic function for the consumed nutrients concentrations and combination of Logistic and Monod functions that describe algae growth is introduced, and equations for demarcation times of algae growth process are thus determined.

3.1. Usage of logistic function

It has been well documented that algal growth can be described by Logistic function as shown in Eq. (1) [18,20,21,28,34,35].