Food and Chemical Toxicology 78 (2015) 116–121

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Assessing the combined effects from two kinds of cephalosporins on green alga (*Chlorella pyrenoidosa*) based on response surface methodology

Ruixin Guo, Weishu Xie, Jianqiu Chen *

Department of Environmental Science, China Pharmaceutical University, 210009 Nanjing, China

ARTICLE INFO

Article history:
Received 29 July 2014
Accepted 2 February 2015
Available online 12 February 2015

Keywords:
Chlorella pyrenoidosa
Toxicity
Combined effect
Cefradine
Ceftazidime
RSM

ABSTRACT

The present work evaluated the combined effects of cefradine and ceftazidime on the green alga Chlorella pyrenoidosa using response surface methodologies (RSM). After a 48 h-exposure, the population growth rate (PGR), the chlorophyll-a content and the SOD content of the alga increased with increased concentrations of two antibiotics. However, the three responses did not continue to demonstrate significant increases once antibiotic concentrations exceed a moderate level. Three two order polynomial regression equations were obtained to describe well the relationship between the responses of the alga and the two antibiotics' concentration ($R^2 = 0.9997$, 0.9292 and 0.9039, respectively). Three 3 D-surface graphs and their contour plots showed directly the changing trends of the alga under the combined effects of two antibiotics. This study for the first time employed the RSM in ecotoxicology, which indicated that the RSM should be placed under a feasible and a potential application prospect in toxicity assessment.

1. Introduction

Green alga, like *Chlorella*, as the primary producer in freshwater and marine ecosystem, plays a key role in the substance and energy transportation in the food webs (Jonsson and Aoyama, 2007). *Chlorella pyrenoidosa* has been named a green, healthy food by the Food and Agriculture Organization (FAO) and widely applied in the food industry (Robledo and Freile Pelegrín, 1997). Besides the nutritional value, the green algae can inhibit the relevant enzymes and therefore exert a beneficial anti-diabetic effect as a new generation drug (Sun and Chen, 2012). Additionally, it also has high capacity for photosynthesis and synthesizes neutral lipids for biofuel (Wen et al., 2014). Due to the value of the green algae in industry, agriculture and pharmacy, the environmental safety of the aquatic organism should also attract more attention.

Usually, aquatic ecosystems are influenced by many countless stresses, which are of anthropogenic origin. Pharmaceuticals and personal care products (PPCPs) have therefore caused widespread attention in recent years for their probable threats to the aquatic environment and to human health eventually. China faces serious environmental pollution by PPCPs due to the growing production and consumption of the chemicals, not to mention antibiotic abuse (Liu and Wong, 2013). On average, the use of antibiotics in China has been 10 times more than the usage in the United States. Antibiotics may be distributed in the environment mainly from excretion, medical waste, wastewater treatment facilities, agricultural antibiotic application and overland runoff (Sarmah et al., 2006). Contamination by antibiotics of natural water bodies has been reported in China (Xu et al., 2007). Disseminated antibiotics usually cause ecological problems because of their possible impact on aquatic species as the non-target organisms. Antibiotics are often considered to be "pseudo-persistent" contaminants due to their continued release into the environment and their permanent presence (Hernando et al., 2006). Thus, it is necessary to evaluate and assess the effects of antibiotics on aquatic environment's safety, especially on the algae.

Alga is sensitive to most contaminants, even if at a relatively low concentration (Chen and Jiang, 2011). The effects of the common antibiotics individually on the non-target organisms have been quite well documented recently (Boxall et al., 2003; Halling-Sorensen, 2000; Lutzhoft et al., 1999; Pan et al., 2008; Robinson et al., 2005). However, effects of contaminants on aquatic organisms are usually evaluated by ecotoxicological standard testing, in which the model organisms are exposed to different test concentrations of a given substance. In fact, individual chemicals, which disseminate in aquatic environments, usually occur in mixtures. Thus, contrary to the single toxicity testing, the mixture toxicity assessment may better predict the actual exposures of organisms in the environment (Chen et al., 2014), although recent researches focused on the toxicity of the

* Corresponding author. Department of Environmental Science, China Pharmaceutical University, 210009 Nanjing, China. Tel.: +86 25 86185190; fax: +86 25 86185190.

E-mail address: cjqalga@163.com (J. Chen).

http://dx.doi.org/10.1016/j.fct.2015.02.007
0278-6915/

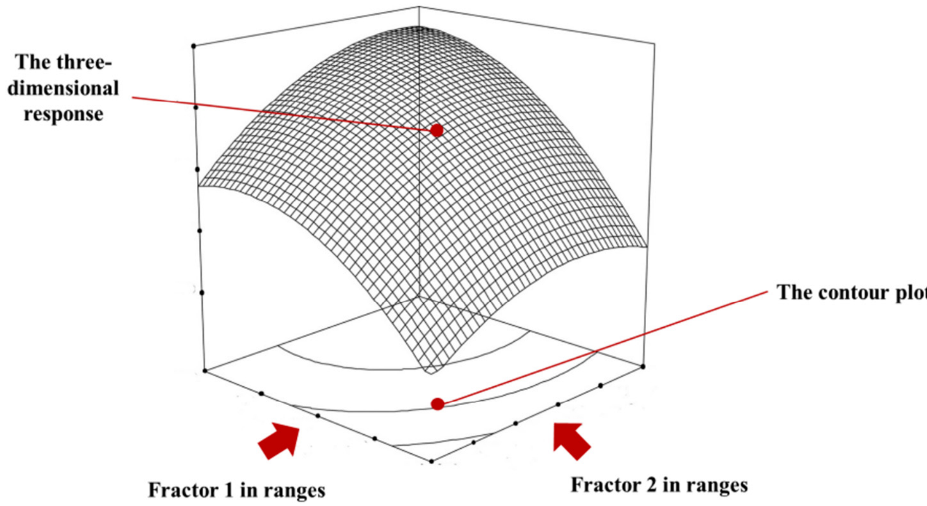

Fig. 1. Reference surface represent graphically the function of the two factors in ranges.

combined antibiotics on diatoms, green algae and cyanobacterium (González-Pleiter et al., 2013; Hagenbuch and Pinckney, 2012). The mixture effects were computed by traditional methods such as the combination index (CI) method, concentration addition (CA) or independent action (IA) method (Altenburger et al., 2004; Chou, 2006). Based on the calculated toxicity data (e.g. EC_{50}, EC_{10}), the mixture effect was denoted as antagonistic, additive or synergistic. These methods, however, less characterize the actual changes of the toxic responses of alga under the given compounds in ranges.

Response surface methodologies (RSM) were developed since the 1950s with the initial aim to optimize the chemical reactions in industry by sequentially involving factors. Thus, the information about the contribution of the testing factors and their interactions was acquired together (De Schamphelaere et al., 2003). In addition, the same methodology also could be applied to model any observed responses that are influenced significantly by the levels of the given factors (Giloni-Lima et al., 2010). The three-dimensional response and its contour plot could represent graphically the function of the two factors in ranges (see Fig. 1). Thus, we could suggest that the methodology could also be employed to describe the toxic response that is influenced by the relevant variables. Our previous study employed the methodology to indicate the relationship between the behavioral response of rotifers and the exposure concentration and time (concentration–time–response analyses) (Guo et al., 2012). Another recent work focused on the neurotoxicity and genotoxicity of the herbicide Roundup on the common carp *Cyprinus carpio* L. (Gholami-Seyedkolaei et al., 2013). The recovery parameters such as temperature, time, etc., and their optimization were calculated by RSM. However, contrary to the wide application in chemical, food and pharmaceutical industry, the employment of RSM in other fields, especially in toxicity evaluation, is limited. Thus, the object of the present work is to expand the application range of the methodology in ecotoxicology. The combined effects of two common antibiotics ceftazidime and cefradine to the freshwater alga *C. pyrenoidosa* have been evaluated by RSM. To the best of our knowledge, this is the first time that the methodology is used in the joint toxicity assessment of algae. Our previous published study indicated that the alga could tolerate cephalosporins at a given concentration and performed a satisfactory removal capacity. The result of the present study could help us to make use of algae to treat wastewater pollution by antibiotics.

2. Materials and methods

2.1. Test organisms

The freshwater green algae *Chlorella pyrenoidosa* were purchased from FACHB-collection, Chinese Academy of Sciences. Cells of the alga were cultivated at 26 ± 1 °C on the photoperiod 12:12 (L:D) with the light at 2000 lx. BG-11 media were invoked as the culture media, whose pH value were adjusted to 8.0. The cells were noted microscopically. Chlorophyll-a and Superoxide dismutase (SOD) were analyzed using a standard method (Zhang and Huang, 1991). The population growth rate (PGR, r) of the alga was calculated as the formula (Levasseur et al., 1993):

$$\boldsymbol{r} = \frac{\ln \boldsymbol{N_t} - \ln \boldsymbol{N}_0}{\boldsymbol{t}}$$

where N_0 and N_t are the algal densities at day 0 and day t respectively; t is time in days when the alga density is maximized. Alga culture without antibiotic was used as a control.

2.2. Experimental design and data analyses

RSM is a collection from the experimental design and the data analyses (Hanrahan and Lu, 2006). Thus, the application of the methodology processes in several key steps are as follows:

(1) According to the aim of the given study, the target factors which might produce major effects should be screened first. In the present study, the possible combined effects of two antibiotics should be assessed. Thus, the concentration of cefradine and ceftazidime was selected as two independent factors respectively. The concentration of the individual antibiotic using in the test was determined by our preliminary study (Guo and Chen, 2015).

(2) An appropriate experimental design model should be chosen. Central composite design (CCD) and Box–Behnken design (BBD) are two common design models in RSM. CCD often produces the amount of information on the direct effects of the tested independent factors and their possible combined effects (Heijerick et al., 2003). Thus, the concentrations of two antibiotics are studied at five levels: $\pm\alpha$, ± 1 and 0; among them, α-values depend on the number of factors. Because of two antibiotics in the present study, α is 1.41 (Bezerra et al., 2008). The factors were coded as follows:

$$x_i = \left(\frac{X_i - X_0}{\Delta X_i}\right), i = 1, 2, 3, \ldots, k$$

where x_i is the coded value and X_0 and X_i are the actual value of the center point and the ith factor, respectively. ΔX_i indicated change value in every step (Bayraktar, 2001). Thus, the range and level of the factors in the coded value and actual value are given in Table 1.

(3) The data obtained were processed statistically to acquire a best fit polynomial model. The model's fitness, the coefficient of determination estimate (R^2) should be evaluated before acceptation.