ELSEVIER

Process Biochemistry 35 (1999) 385–389

PROCESS BIOCHEMISTRY

www.elsevier.com/locate/procbio

# Kinetic models for heterotrophic growth of *Chlamydomonas reinhardtii* in batch and fed-batch cultures

Xue-Wu Zhang [a], Feng Chen [a,*], Michael R. Johns [b]

[a] *Department of Botany, the University of Hong Kong, Pokfulam Road, Hong Kong, Hong Kong*
[b] *Department of Chemical Engineering, The University of Queensland, Brisbane, QLD 4072, Australia*

Received 24 May 1999; accepted 6 June 1999

**Abstract**

Heterotrophic growth of *Chlamydomonas reinhardtii* using acetate as a carbon source and nitrate, ammonium and urea as nitrogen sources in batch cultrues was investigated. These nitrogen sources supported good growth of *C. reinhardtii*, the resulting cell concentrations for nitrate, ammonium chloride and urea were 0.48, 0.44 and 0.61 g $l^{-1}$, respectively. The specific growth rate $\mu_x$ of the urea culture was the highest (0.071 $h^{-1}$), followed by that of the nitrate culture (0.062 $h^{-1}$), and the specific growth rate $\mu_x$ of the ammonium culture was the lowest (0.058 $h^{-1}$). Urea is therefore considered to be the best nitrogen source for the growth of the alga. Based on these results fed-batch cultures were performed to reach the maximum cell concentration of 1.1482 g $l^{-1}$, about 1.9-fold that obtained in batch culture. Finally, a group of kinetic models for describing cell growth, pH variation and acetate consumption were proposed and a satisfactory fit between the experimental results and predicted values was demonstrated. The effects of dilution rate and acetate feed concentration on cell growth were analyzed with those with these models. 

*Keywords:* *Chlamydomonas reinhardtii*; Acetate; Nitrogen sources; Kinetic model

## 1. Introduction

Microalgae have found applications in agriculture, waste treatment and aquaculture [1–3]. Many microalgal products with commercial potential, particularly high-value chemicals and pharmaceuticals have been discovered [4–6]. A significant limit to the widespread use of microalgae is their mass culture in photoautotrophic growth processes, due to the difficulty of controlling important environmental factors [7]. Heterotrophic culture may provide a cost-effective and large scale alternative method of cultivation for some microalgae, since it may overcome the common problems encountered in the photoautotrophic system, and so high cell density and productivity can be achieved [8].

The microalga *Chlamydomonas reinhardtii* is unable to utilise sugars that can be cultured in darkness on acetate as a source of carbon [9]. It was selected for this study since it is one of the few microalgae for which shuttle vectors have been developed to aid genetic manipulation [10,11] and has been used extensively in biochemical and physiological studies [10,12,13].

Acetate is a poor substrate for heterotrophic growth since it inhibits microbial growth at relatively low concentrations, which makes high cell density culture difficult. Inhibition is highly pH dependent [14]. On the other hand, nitrogen sources are important nutritional elements, but little information is available concerning the effects of different nitrogen sources on the growth of *C. reinhardtii* under heterotrophic conditions. The objectives of this study were to investigate the effects of the various nitrogen sources on the growth of *C. reinhardtii*, and to develop a kinetic model incorporating substrate inhibition and pH dependence which would be used to assist the design and scale-up of the cultivation process.

* Corresponding author. Tel.: +852-28591945; fax: +852-28578521.
*E-mail address:* sfchen@hkusua.hku.hk (F. Chen)

0032-9592/99/$ - see front matter 
PII: S0032-9592(99)00082-5

## 2. Materials and methods

### 2.1. Microalga and media

The green microalga, *Chlamydomonas reinhardtii* Dangeard (CS-51), was obtained from the CSIRO Marine Labortories, Hobart, Australia, and maintained axenically at 4°C on nutrient agar slopes. CR-M1 medium [15] and 0.2 g $l^{-1}$ nitrogen source (nitrate, ammonium chloride and urea) was used for batch cultures. For fed-batch culture only one nitrogen source, urea, was used and the feed CR-M1 medium contained 1.7 g $l^{-1}$ acetate and 0.5 g $l^{-1}$ acetate and 0.5 g $l^{-1}$ urea. Antifoam agent (DowCorning) was added at 0.01% v/v.

### 2.2. Cultures

Algal cells were grown in a 1-litre glass fermentor on 700 ml CR-M1 medium containing 0.85 g $l^{-1}$ acetate and 0.2 g $l^{-1}$ nitrogen source in batch cultures. The culture was agitated at 400 rpm with a magnetic stirrer and the temperature was maintained at 35°C. Sterile air was supplied to maintain the dissolved oxygen concentration above 50% saturation during cultivation. For fed-batch culture, in the late exponential phase of growth, the sterile medium was fed by peristaltic pump at a constant flow rate of 3 ml $h^{-1}$.

### 2.3. Analytical methods

Sample were withdrawn from the fermentor every 12 h. The acetate and cell concentrations were determined by HPLC and optical density measurement at 750 nm, respectively, according to [15]. The dissolved oxygen concentration in the samples was determined by a DO meter with a galvanic electrode (Uniprobe Instruments Ltd, South Glamorgan, UK).

## 3. Theoretical models

### 3.1. Dynamic model for cell growth

The most widely used unstructured model for the specific growth rate $\mu$ is the Monod equation:

$$\mu = \frac{\mu_m S}{k_S + S} \tag{1}$$

where $S$ is substrate concentration (g $l^{-1}$) and $\mu_m$ is the maximum specific growth rate ($h^{-1}$). $K_s$ is the Monod saturation constant (g $l^{-1}$) However, the Monod model often fails to account for substrate inhibition of growth at higher substrate concentrations. To overcome the drawback, the Haldane model [16] may be employed:

$$\mu = \frac{\mu_m S}{K_S + S + \frac{S^2}{K_i}} \tag{2}$$

where $K_i$ is the inihibition constant (g $l^{-1}$).

However, the effect of pH on cell growth is not involved in the model. pH dependence of $\mu_m$ was usually expressed as [17,18]:

$$\mu_m = \frac{\mu_m^*}{K_{h1} + \frac{K_{h2}}{H^+} + \frac{H^+}{K_{h3}}} \tag{3}$$

where $H^+ (= 10^{-pH})$ is the hydrogen ion concentration and $\mu_m^*$, $K_{h1}$, $K_{h2}$ and $K_{h3}$ are constants. Substituting Eq. (3) into Eq. (2) gives:

$$\mu_x = \frac{\mu_m^*}{K_{h1} + \frac{K_{h2}}{H^+} + \frac{H^+}{K_{h3}}} \frac{S}{K_S + S + \frac{S^2}{K_i}} \tag{4}$$

### 3.2. Dynamic model for pH changes in the fermentation process

As the culture grows, a decrease in acetate concentrations will cause an increase in pH in the culture broth. For simplicity, it is reasonable to assume that the increasing rate in pH is proportional to the decreasing rate in acetate concentrations, that is:

$$\frac{dH}{dt} = K_h\left(-\frac{dS}{dt}\right) \tag{5}$$

where $H$ means pH value, $S$ is acetate concentration (g $l^{-1}$), and $K_h$ is a constant.

#### 3.2.1. Dynamic model for substrate consumption

The following expression was employed to describe the relationship between substrate consumption and cell growth:

$$-\frac{dS}{dt} = \frac{1}{Y_x}\frac{dX}{dt} + mX \tag{6}$$

where $X$ is cell concentration (g $l^{-1}$), $Y_x$ is the cell yield coefficient on acetate (g g $^{-1}$), $m$ is the maintenance energy coefficient ($h^{-1}$).

Eqs. (4)–(6) are the kinetic models proposed in this study to describe cell growth, pH variation and acetate consumption in batch culture. After slight modifications [19] the kinetic models for fed-batch culture are obtained as shown below:

$$\mu_x = -D + \frac{\mu_m^*}{K_{h1} + \frac{K_{h2}}{H^+} + \frac{H^+}{K_{h3}}} \frac{S}{K_S + S + \frac{S^2}{K_i}} \tag{7}$$

$$\frac{dH}{dT} = -DS + K_h\left(-\frac{dS}{dt}\right) \tag{8}$$

$$-\frac{dS}{dt} = -D(S_f - S) + \frac{1}{Y_x}\frac{dX}{dt} + mX \tag{9}$$

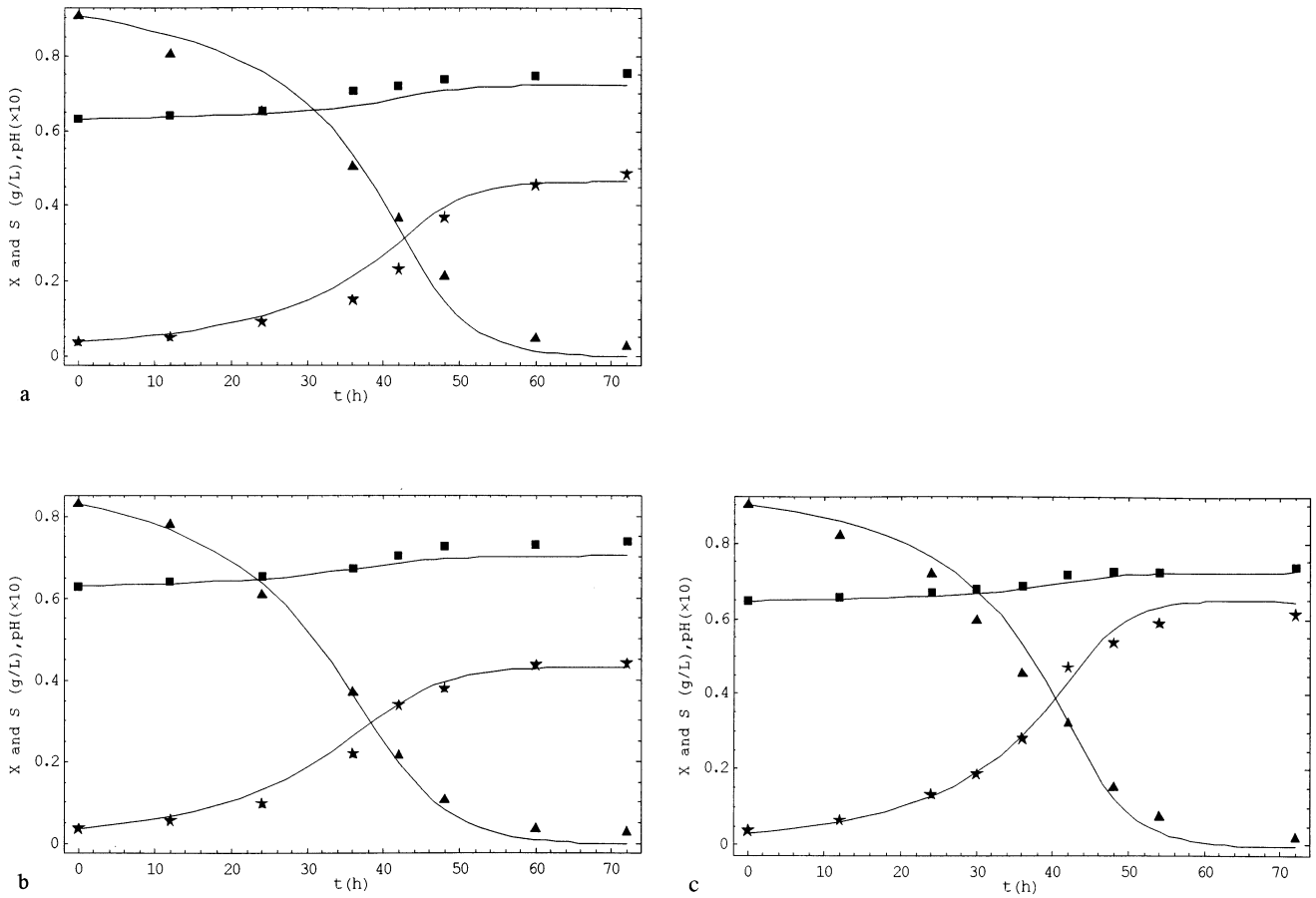


Fig. 1. Time courses of cell dry weight concentration, pH variation, acetate consumption in heterotrophic growth of *C. reinhardtii* on various nitrogen sources in batch cultures. (a) The nitrate culture; (b) the ammonium culture; (c) the urea culture. Solid line for calculated values; star, box, and triangle for experimental data of cell concentrations, pH, and acetate concentrations, respectively.

where $D$ is the dilution rate ($h^{-1}$), $S_f$ is the feed acetate concentration (g $l^{-1}$).

Solutions were obtained by evaluating the derived analytical expressions using standard and readily available software packages: Microsoft EXCEL Ver 5.0 for spreadsheet computations and MATHEMATICA Ver. 3.0 for symbolic calculations [20].

## 4. Results and discussion

Cell growth, pH variation and acetate consumption profiles with time in batch cultures are presented in Fig. 1 for nitrate, ammonium chloride and urea. These nitrogen sources supported good growth of *C. reinhardtii*. In each case, pH varied between 6.3 and 7.5; there was a minimal lag phase and acetate was completely utilised after 70 h. The resulting cell concentrations for nitrate, ammonium chloride and urea were 0.48, 0.44 and 0.61 g $l^{-1}$, respectively. The urea culture produced higher cell concentrations than the other two cultures.

By fitting experimental data from batch fermentations on different nitrogen sources, the kinetic models for cell growth, pH variation, and acetate consumption were given:

For nitrate:

$$\mu_x = \frac{1.4528}{0.7602 + \dfrac{0.401 \times 10^7}{H^+} + \dfrac{H^+}{0.865 \times 10^7}} \frac{S}{2.6085 + S + \dfrac{S^2}{0.1065}} \tag{10}$$

$$\frac{dH}{dt} = 1.0266\left(-\frac{dS}{dt}\right) \tag{11}$$

$$-\frac{dS}{dt} = \frac{1}{0.4803}\frac{dX}{dt} + 0.0010X \tag{12}$$

For ammonium chloride:

$$\mu_x = \frac{1.2942}{1.1835 + \dfrac{0.2826 \times 10^7}{H^+} + \dfrac{H^+}{0.6660 \times 10^7}} \frac{S}{2.2956 + S + \dfrac{S^2}{0.1557}} \tag{13}$$

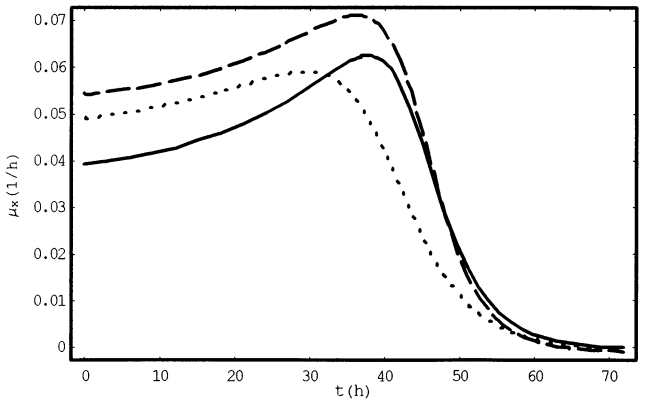


Fig. 2. Time courses of the specific growth rate on various nitrogen sources in batch cultures. (Solid) the nitrate culture; (dotted line) the ammonium culture; (dashed line) the urea culture.

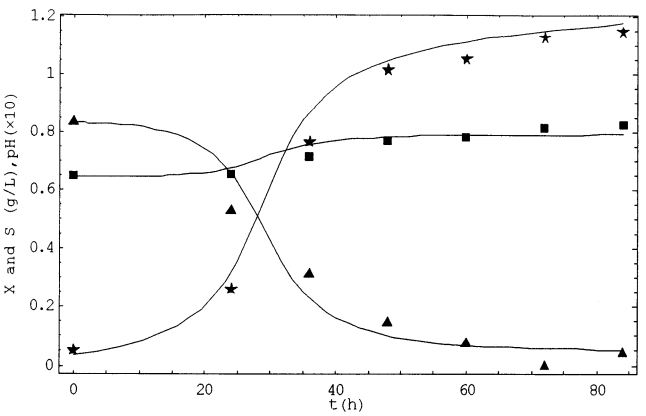


Fig. 3. Time courses of cell dry weight concentrations, growth, pH variation, acetate consumption in heterotrophic growth of *C. reinhardtii* on various nitrogen sources in fed-batch culture. Solid line for calculated values; star, box, and triangle for experimental data of cell concentrations, pH, and acetate concentrations, respectively.

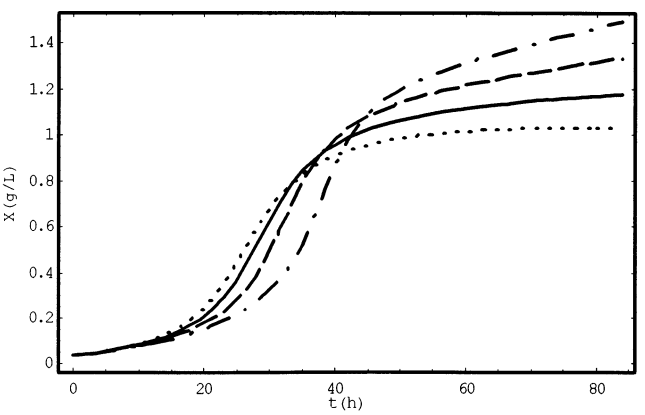


Fig. 4. Effects of dilution rate on cell dry weight concentrations in the urea cultures. Solid line, $D = 0.003$ ($h^{-1}$) (this study); dotted line, $D = 0.0015$ ($h^{-1}$); dashed line, $D = 0.0045$ ($h^{-1}$); dot-dashed line, $D = 0.0060$ ($h^{-1}$).

$$\frac{dH}{dt} = 0.8759\left(-\frac{dS}{dt}\right) \tag{14}$$

$$-\frac{dS}{dt} = \frac{1}{0.4874}\frac{dX}{dt} + 0.0012X \tag{15}$$

For urea:

$$\mu_x = \frac{1.4198}{0.3499 + \dfrac{0.2609 \times 10^7}{H^+} + \dfrac{H^+}{0.8042 \times 10^7}} \frac{S}{2.3978 + S + \dfrac{S^2}{0.0708}} \tag{16}$$

$$\frac{dH}{dt} = 0.8505\left(-\frac{dS}{dt}\right) \tag{17}$$

$$-\frac{dS}{dt} = \frac{1}{0.7104}\frac{dX}{dt} + 0.0016X \tag{18}$$

A satisfactory fit between the experimental data and the predicted values was found in all the cases ($r^2 =$ 0.9831, 0.9926 and 0.9875 for the nitrate culture, ammonium culture and urea culture, respectively) (Fig. 1). Fig. 2 shows the effect of different nitrogen sources on the specific growth rate and demonstrates that $\mu_x$ ($=$ 0.071 $h^{-1}$) in the urea culture is the highest followed by $\mu_x$ ($= 0.062$ $h^{-1}$) in the nitrate culture, and then $\mu_x$ ($= 0.058$ $h^{-1}$) in the ammonium chloride culture. Considering both, the maximum cell concentration and the specific growth rate, it was concluded that urea was the best nitrogen source for the heterotrophic cultivation of *C. reinhardtii*.

Based on the experimental results in batch cultures, fed-batch culture of *C. reinhardtii* using acetate as carbon source and urea as nitrogen source was carried out in a 1-l fermentor. The maximum cell concentration of 1.1482 g $l^{-1}$ was obtained which was about 1.9-fold that in batch culture.

By fitting the experimental data into Eqs. (7)–(9), the following kinetic models were obtained:

$$\mu_x = -D + \frac{0.5990}{0.1421 + \dfrac{0.0185 \times 10^8}{H^+} + \dfrac{H^+}{0.3496 \times 10^8}} \frac{S}{2.5330 + S + \dfrac{S^2}{0.2100}} \tag{19}$$

$$\frac{dH}{dt} = -DS + 1.9963\left(-\frac{dS}{dt}\right) \tag{20}$$

$$-\frac{dS}{dt} = -D(S_f - S) + \frac{1}{1.2226}\frac{dX}{dt} + 0.0030X \tag{21}$$

where $D = 0.003$ $h^{-1}$, and $S_f = 1.7$ g $l^{-1}$.

There was also a good fit between the experimental data and calculated values ($r^2 = 0.9907$); the experimental and calculated results are plotted in Fig. 3.

Figs. 4 and 5 show the dynamic behaviour of cell growth at varying dilution rates and feed acetate con-

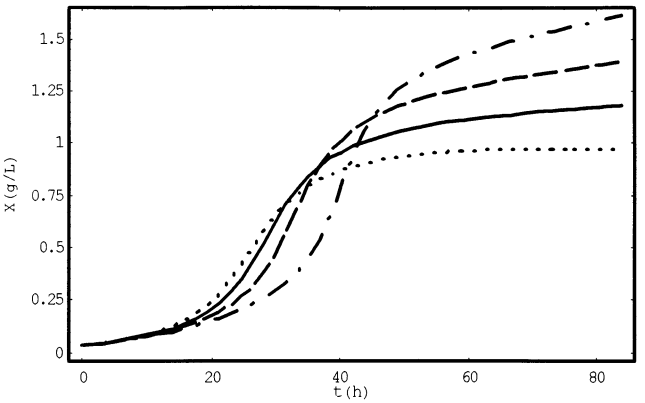


Fig. 5. Effects of feed acetate concentration on cell dry weight concentration in the urea culture. Solid line, $S_f = 1.7$ (g $l^{-1}$) (this study); dotted line, $S_f = 0.85$ (g $l^{-1}$); dashed line, $S_f = 2.55$ (g $l^{-1}$; dot-dashed line, $S_f = 3.4$ (g $l^{-1}$).

centrations, respectively. When the dilution rate $D$ was changed from 0.0015 $h^{-1}$ to 0.006 $h^{-1}$ (four-fold), the maximum cell concentration increased from 1.0116 to 1.4192 g $l^{-1}$ (about 1.4-fold). In contrast, when the feed acetate concentration $S_f$ was changed from 0.85 to 3.4 g $l^{-1}$ (four-fold), the maximum cell concentration increased from 0.9571 g $l^{-1}$ to 1.6898 g $l^{-1}$ (about 1.8-fold). This clearly shows that to achieve high cell densities, a strategy to enhance feed acetate concentrations is superior to the strategy to enhance dilution rates.

## Acknowledgements

This research was supported by Research Grant Council of Hong Kong and the University of Hong Kong Committee on Research and Conference Grants.

## References

[1] De Pauw N, Persoone G. Microalgae for aquaculture. In: Borowitzka MA, Borowitzka LJ, editors. Microalgal Biotechnology. Cambridge: Cambridge University, 1988:197–221.
[2] Meeting B. Microalgae in agriculture. In: Borowitzka MA, Borowitzka LJ, editors. Microalgal Biotechnology. Cambridge: Cambridge University, 1988:288–304.
[3] Oswald WJ. Microalgae and waste-water treatment. In: Borowitzka MA, Borowitzka LJ, editors. Microalgal Biotechnology. Cambridge: Cambridge University, 1988:305–28.
[4] Behrens PW, Delente JJ. Microalgae in the pharmaceutical industry, BioPharm 1991;June:54–58.
[5] Borowitzka LJ, Borowitzka MA. Industrial production: methods and economics. In: Cresswell RC, Rees TAV, Shah H, editors. Algal and Cyanobacterial Biotechnology. Harlow: Longman Scientific and Technical, 1989:294–316.
[6] Metting B, Pyne JW. Biologically active compounds from microalgae. Enzyme Microb Technol 1986;8:386–94.
[7] Richmond AE. Microalgaculture. CRC Crit Rev Biotechnol 1986;4:368–438.
[8] Chen F, Johns MR. A strategy for high cell density culture of heterotrophic microalgae with inhibitory substrates. J Appl Phycol 1995;7:43–6.
[9] Sager R, Granick S. Nutrional studies with *Chlamydomonas reinhardtii*. Ann NY Acad Sci 1953;56:831–8.
[10] Bingham SE, Cox JC, Strem MD. Expression of foreign DNA in *Chlamydomonas reinhardtii*. FEMS Microbiol Lett 1989;65:77–82.
[11] Hasnain SE, Manavathu EK, Leung WC. A method for DNA-induced transformation of *Chlamydomoas reinhardtii*. Biotechniques 1987;5:110–6.
[12] Dagestad D, Lien T, Knutsen G. Degradation and compartmentalisation of urea in *Chlamydomonas reinhardtii*. Arch Microbiol 1981;129:261–4.
[13] Giroud C, Gerber A, Eichenberger W. Lipids of *Chlamydomonas reinhardtii*. Analysis of molecular species and intracellular site of biosynthesis. Plant Cell Physiol 1988;29:587–95.
[14] Maiorella B, Blanch HW, Wilke CR. By-product inihibition effects on ethanolic fermentation by *Saccharomyces cerevisiae*. Biotechnol Bioeng 1983;25:103–21.
[15] Chen F, Johns MR. Substrate inhibition of *Chlamydomonas reinhardtii* by acetate in heterotrophic culture. Process Biochem 1994;29:245–52.
[16] Andrews JF. A mathematical model for the continuous culture of microorganisms utilizing inhibitory substrates. Biotechnol Bioeng 1968;10:707–23.
[17] Sinclair CG. Microbial process kinetics. In: Bu'Lock J, Kristiansen BB, editors. Basic Biotechnology. London: Academic, 1989:102–3.
[18] Tang IC, Okos MR, Yang ST. Effect of pH and acetic acid on homoacetic fermentation of lactate by *Clostricium formicoaceticum*. Biotechnol Bioeng 1989;34:1063–74.
[19] Agarwal P, Koshy G, Ramirez M. An algorithim for operating a fed-batch fermentor at optimum specific growth rate. Biotechnol Bioeng 1989;33:115–25.
[20] Wolfram S. The Mathematica Book. Cambridge, UK: Cambridge University, 1996.