PERGAMON

Water Research 37 (2003) 2937–2943

WATER RESEARCH

www.elsevier.com/locate/watres

Effects of substrate concentrations on the growth of heterotrophic bacteria and algae in secondary facultative ponds

S. Kayombo[a,*], T.S.A. Mbwette[a], J.H.Y. Katima[a], S.E Jorgensen[b]

[a] *Faculty of Engineering, University of Dar es Salaam, P.O. Box 35131, Dar es Salaam, Tanzania*
[b] *Department of General Chemistry, Environmental Section, The Royal Danish School of Pharmacy, Universitetsparken 2, Copenhagen DK-2100, Denmark*

Received 13 April 2001; received in revised form 15 November 2002; accepted 13 December 2002

Abstract

This paper presents the effect of substrate concentration on the growth of a mixed culture of algae and heterotrophic bacteria in secondary facultative ponds (SFPs) utilizing settled domestic sewage as a sole source of organic carbon. The growth of the mixed culture was studied at the concentrations ranging between 200 and 800 mg COD/l in a series of batch chemostat reactors. From the laboratory data, the specific growth rate (μ) was determined using the modified Gompertz model. The maximum specific growth rate (μ_{max}) and half saturation coefficients (K_s) were calculated using the Monod kinetic equation. The maximum observed growth rate (μ_{max}) for heterotrophic bacteria was 3.8 day^{-1} with K_s of 200 mg COD/l. The μ_{max} for algal biomass based on suspended volatile solids was 2.7 day^{-1} with K_s of 110 mg COD/l. The μ_{max} of algae based on the chlorophyll-*a* was 3.5 day^{-1} at K_s of 50 mg COD/l. The observed specific substrate removal by heterotrophic bacteria varied between the concentrations of substrate used and the average value was 0.82 (mg COD/mg biomass). The specific substrate utilization rate in the bioreactors was direct proportional to the specific growth rate. Hence, the determined Monod kinetic parameters are useful for the definition of the operation of SFPs.
© 2003 Published by Elsevier Science Ltd.

Keywords: Heterotrophic bacteria; Half saturation coefficient; Maximum growth rate; Secondary facultative pond; Specific substrate removal

1. Introduction

Waste stabilization ponds (WSPs) are large, shallow basins in which raw sewage is treated by entirely natural processes involving both algae and bacteria. The secondary facultative ponds (SFPs) are those which receive settled sewage from anaerobic ponds or septic tanks and are normally the second in the series of pond systems. The treatment effected by the FPs results from the complex symbiosis of bacteria and algae existing in several living species that bring about an ecological pattern different from that of pure culture behaviour in which the respiratory oxygen requirements of the pond is met by photosynthetic oxygen [1–9]. Obviously, in this natural systems the growth rate and other microbial activities are affected by diurnal variation of pH, light intensity, DO and temperature. Diurnal variation of organic loading to the ponds may influence the performance. Formulation of substrate utilization by microorganisms in SFP based on Monod formulation is scarce in the literature.

Monod [10] equation is the most commonly used model to relate the microbial growth with substrate utilization. Monod found that in pure cultures and continuous reactor systems, the relationship between the bacterial growth and the substrate availability in the

*Corresponding author. Tel.: +255-022-2410500/8; fax: +255-022-2410029/2410514.
E-mail address: kayombo@hotmail.com (S. Kayombo).

0043-1354/03/$ - see front matter © 2003 Published by Elsevier Science Ltd.
doi:10.1016/S0043-1354(03)00014-9

system with a single growth-limiting substrate could be expressed empirically as

$$\mu = \frac{\mu_{max} S}{K_s + S}, \quad (1)$$

where μ is a specific growth rate (day^{-1}), μ_{max} is the maximum growth rate (day^{-1}), S is the substrate concentration (mg/l), and K_s is the half saturation coefficient (mg/l).

The specific growth rate depends on the concentration of the limiting nutrient, which can be a carbon source or other factors needed by the microorganisms for growth [11]. There are several growth models in literature used for evaluation of growth parameters, for example, the models by Gompertz [12], Richards et al. [13], Stannard et al. [14], Schnute [15], the logistic models, etc. Most of these models describe the number of microorganisms without taking into account the consumption of substrate. Zwietering et al. [16] derived a modified mathematical relationship based on the Gompertz model for the increase of the biomass over time, that relates population size over the time, to specific growth rate, lag time, and asymptotic level of organisms as

$$y = \exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t)\right] + 1\right\}, \quad (2)$$

where μ_m is a specific growth rate (day^{-1}), A is a maximum asymptotic value, and λ is the lag time (days).

The re-parameterized Gompertz equation by Zwietering et al. [16] was rewritten by Wijtzes et al. [17] so as to determine the maximum biomass (X_{max}) as

$$\ln(X_t) = \ln(X_0) + \{\ln(X_{max}) - \ln(X_0)\} \exp\left\{-\exp\left[\frac{\mu_m e}{\{\ln(X_{max}) - \ln(X_0)\}}(\lambda - t) + 1\right]\right\}, \quad (3)$$

where t is the time (hours or days), X_t is the biomass at time, t (mg/l), X_0 is the asymptotic biomass at time zero, X_{max} is the maximum biomass (mg/l).

The natural logarithm of the maximum biomass concentration (X_{max}) is defined as a sum of natural logarithms of the estimated initial biomass (X_0) and the estimated asymptotic value (A) from the Gompertz equation. The models given in Eqs. (2) and (3) may be used to determine the biological parameters, μ_m, λ and A from a set of experimental data. The specific growth rate is evaluated as a slope of the tangential line. The point where the tangential line crosses the t-axis is considered as a lag time (λ) and the maximum asymptotic value (A) is obtained at an infinity substrate concentration. The determination of the biological parameters contained in Gompertz's model for SFPs to reflect the processes carried by the two organisms may be complicated by many varying parameters like temperature, pH, DO and the concentration of nutrient and organic matter.

The objective of the bio-kinetic experiment was therefore to quantify the specific growth rate (μ) of the biomass in response to the substrate concentration and to obtain the Monod parameters that describe this response. The parameters obtained apart from expanding the knowledge of the WSP also give a new approach towards the design of such natural systems based on the biological processes.

2. Materials and methods

2.1. Materials

The experimental work in this research was aimed at developing testing procedures for evaluating the biomass activity relating coefficients for SFPs. The biomass activity coefficients determined were μ_{max}, K_s and specific substrate removal rate (q). Initially, the mixed culture containing heterotrophic bacteria and algae was collected from the SFP at the University of Dar es Salaam pond systems. The mixed culture was first grown at a light intensity of 6000 Lux, in the laboratory condition by continuously pumping settled sewage with a peristaltic pump (Lsmate Rego). At steady state of growth, the mixed culture was removed from the continuous growth experiment and used in the batch growth experiments, which were done using a 10 l Bellco culture bottle (Bellco Glass, Inc., USA).

Settled domestic sewage was used in this experiment as a sole source of carbon. It was collected from the inlet works to the WSPs located at the University of Dar es Salaam. The grab samples of raw sewage was collected at interval of 3 h and was left to settle for 24 h. The supernatant was drawn and was analysed for COD. To obtain the required concentration, sometimes dilution with low COD settled sewage was done.

Half a litre of the active microorganisms from the continuous culture was inoculated in each batch reactor containing settled sewage and nutrient thus making a

Table 1
Nutrient composition

Chemical		Amount (mg/l)	Trace metals
$CaCl_2$	50.0	Chemical	Amount (mg/l)
$FeCl_3\ 6H_2O$	100.0	$CuSO_4.5H_2O$	0.05
$MgSO_4.7H_2O$	200.0	$Na_2MoO_4.2H_2O$	0.2
$(NH_4)_2\ SO_4$	500.0	$ZnSO_4.7H_20$	0.22
KNO_3	500.0	H_3BO_3	2.86
KH_2PO_4	100.0	$MnSO_4\ H_2O$	10
Distilled water	998	Na-EDTA	2 ml
Trace minerals	2.0 ml	Distilled water	100 ml

total volume of 9 l. Nutrients with composition shown in Table 1 were added to the cultures in order to supplement nutrient available in the settled sewage. The batch growth was at COD concentrations ranging between 200 and 800 mg/l COD.

The culture bottles were then placed in an incubator which operated at an average temperature of 30°C. The light intensity was measured using light meter (Mavolax digital) and was fixed at 6000 Lux for 24 h. Magnetic stirrer (Stuart Scientific SM1, UK) facilitated the slow mixing.

3. Methods

3.1. Physical, chemical and biological analyses

Samples were collected daily for 15 d for analysis of total and soluble chemical oxygen demand (TCOD, SCOD), total and suspended volatile solids, chlorophyll-*a*, dissolved oxygen and pH. The pH and temperature were measured by Metrohm pH meter model 704, DO was measured using YSI DO meter model 95. TCOD and SCOD were analysed in accordance with Standard Method [18]. Total biomass was obtained by drying an 80 ml sample in a pre-weighed crucible (75 mm internal diameter (ID) and a depth of 36 mm) for 24 h in an oven at 105°C [18]. The algal biomass was obtained by filtering a sample through a pre-dried 0.45 μm Whatman filter paper followed by drying in an oven for 24 h at 105°C. Suspended volatile solids were obtained by incinerating the dried solids at 550°C in a furnace [1]. Chlorophyll-*a* was analysed in accordance with the Standard Method [18]. The suspended volatile solids were used as a measure of algal biomass while the difference between the total and suspended volatile solids was used as a measure of heterotrophic bacterial biomass.

4. Results and discussion

The data obtained from the laboratory at a concentration of 200–800 mg COD/l were used to determine the specific growth rate using the modified Gompertz represented by model Eq. (2). During data collection from the batch bioreactors, the pH and DO values were also monitored and the average values, standard deviation and the range of each parameter are as shown in Table 2. Change of pH was associated with high algal activities in the bioreactors. DO increased in the bioreactors with increase of chlorophyll-*a*. This indicates that the photosynthetic process produced much of the DO in the bioreactors. Increase of algal activities was associated with increase of pH in the reactors.

The natural logarithmic increase of biomass for heterotrophic bacteria, algae and chlorophyll-*a* over the time is shown in Figs. 1–3. Both algae and heterotrophic bacteria show a phase in which the specific growth rate starts at a minimum value and then accelerates to a maximum value in a certain period of time, resulting in lag phase (λ) as also suggested by Zwietering et al. [16].

In order to determine the biological parameters in the Gompertz model, the simulation was done using Stella II® software which integrated the model using the built in fourth-order Runge–Kutta. The specific growth rate and lag time (λ) determined at each concentration of organic carbon using Gompertz model is shown in Table 3. The values in the brackets give the correlation coefficients (R^2) between the measured and simulated results for specific growth rate of heterotrophic bacteria and algae biomass.

The specific growth rate of algae and heterotrophic bacteria obtained from the modified Gompertz equation was then fitted in the Monod model i.e. Eq. (1) to determine the μ_{max} and K_s. Figs. 4 and 5 show the relationship between the actual and the predicted specific growth rate of heterotrophic bacteria and algae based on the Monod kinetic equation, while Fig. 6 shows the relationship between actual and predicted specific growth rate of algae based on chlorophyll-*a*.

The linear regression analysis between the observed and Monod predicted specific growth rate was done in order to determine their agreement. The Monod parameters i.e. μ_{max} and K_s were obtained when the model prediction showed a good regression coefficient as

Table 2
Average and range value of pH, DO and substrate utilization

Concentration (mg/l)	Average pH	pH range	Average DO (mg/l)	DO range (mg/l)
200	7.04±0.84	7.08–8.24	5.50±2.09	3.00–9.67
300	6.94±0.94	5.90–8.31	5.32±1.92	2.9 -9.11
400	6.93±0.91	7.04–8.24	4.38±1.36	3.02–7.00
500	6.98±0.89	7.05–8.24	3.36±1.74	3.02–7.34
600	7.40±0.99	7.14–8.77	7.10–4.18	3.01–16.42
700	8.63±1.58	7.01–10.69	7.99±4.8	3.20–16.97
800	7.84±1.05	6.99–8.78	4.30±3.79	2.89–11.60

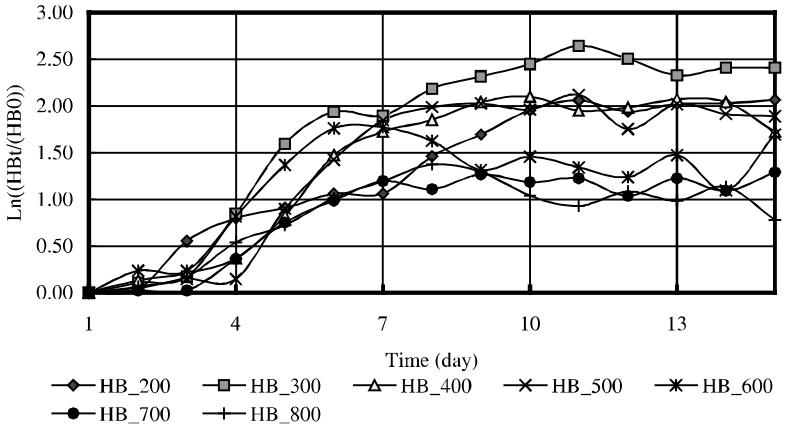


Fig. 1. Logarithmic growth rate of heterotrophic bacteria at various concentration of the substrate.

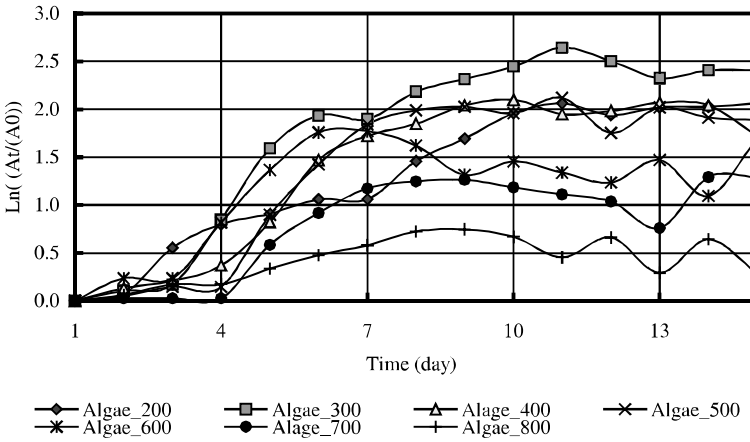


Fig. 2. Logarithmic growth rate of algae.

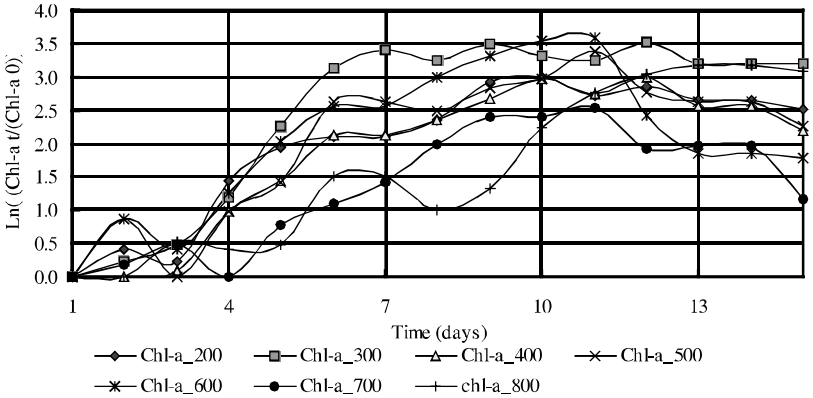


Fig. 3. Logarithmic increase of chlorophyll-*a* at various concentrations of substrate.

shown in Table 4. The K_s for heterotrophic bacteria and algal biomass was 200 and 110 mg COD/l, respectively. This indicates that algae easily accelerate to μ_{max} at low concentrations of organic carbon than heterotrophic bacteria. The observed high values of K_s for heterotrophic bacteria is the reflection of the complexity of organic carbon in the settled sewage as it contains a heterogeneous mixture of compounds. However, the K_s for algae based on chlorophyll-*a* was 50 mg COD/l, indicating that the value of K_s to be used for algae also depend on the method used for the determination of the biomass. Other researchers have reported values of

Table 3
Specific growth rate of biomass

Substrate (mg COD/l)	μ_A (day^{-1})	λ (day)	μ_A chlorophyll-*a* (day^{-1})	λ (day)	μ_{HB} (day^{-1})	λ (day)
0.00	0.000	0	0.000	0.00	0.000	0.0
200	0.345 (R^2: 0.86)	3.5	0.484 (R^2: 0.97)	3.5	0.360 (R^2:0.93)	5.0
300	0.420 (R^2: 0.98)	4.0	0.555 (R^2: 0.97)	3.4	0.460 (R^2:0.98)	4.0
400	0.453 (R^2: 0.98)	4.5	0.568 (R^2: 0.97)	4.0	0.517 (R^2: 0.95)	4.5
500	0.473 (R^2: 0.98)	4.8	0.568 (R^2:0.96)	4.5	0.563 (R^2:0.94)	4.8
600	0.466 (R^2: 0.84)	3.8	0.500 (R^2: 0.84)	4.0	0.560 (R^2: 0.88)	3.8
700	0.474 (R^2: 0.93)	4.3	0.470 (R^2: 0.94)	5.3	0.560 (R^2: 0.95)	4.3
800	0.470 (R^2: 0.86)	4.0	0.405 (R^2: 0.68)	4.5	0.555 (R^2: 0.89)	3.9

μ_A and μ_{HB} is specific growth rate of algae and heterotrophic bacteria, μ_A chlorophyll-*a* is specific growth of algae based on chlorophyll-*a*.

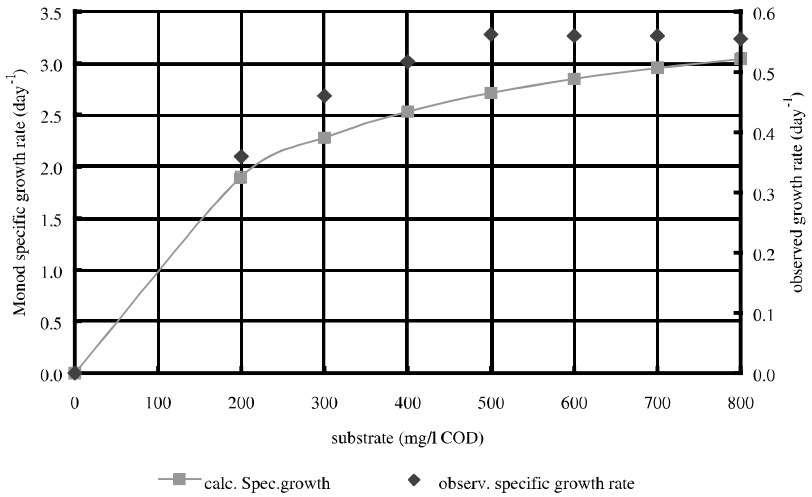


Fig. 4. Observed and calculated specific growth rate of heterotrophic bacterial biomass.

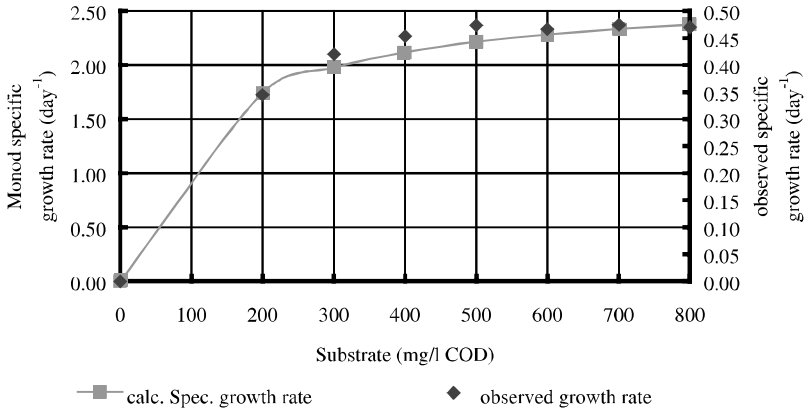


Fig. 5. Observed and calculated specific growth rate of algae biomass.

K_s up to 500 mg/l for poultry wastewater [19–21] reported K_s values ranging between 10 and 180 mg/l for domestic wastewater. However, these values are applicable for sludge systems where mechanical aeration is applied.

The maximum specific growth rate (μ_{max}) and the half saturation coefficient (K_s) are important parameters required for characterization of biomass growth. The specific growth rate is a rate coefficient and as such its value increases with increasing temperature. The effect

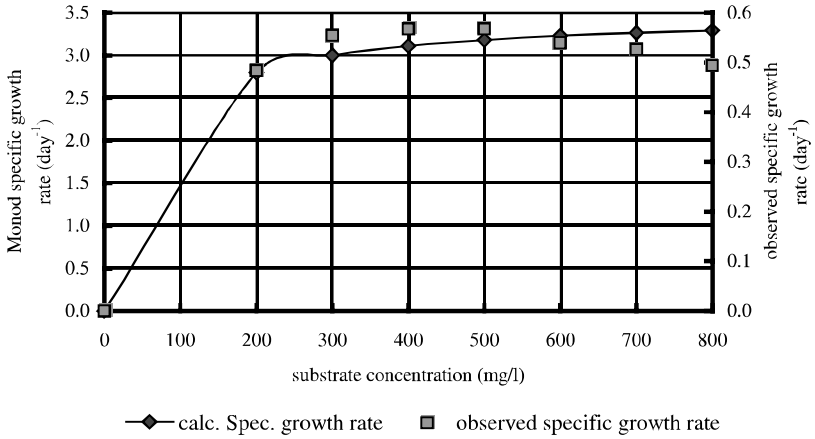


Fig. 6. Relationship between the calculated and observed specific growth rate of algae based on chlorophyll-*a*.

Table 4
Maximum and half saturation coefficients

Organisms	μ_{max} (day^{-1})	K_s (mg COD/l)	Regression coefficient (R^2)
Heterotrophic bacteria	3.8	200	0.99
Algae based on volatile solids	2.7	110	0.99
Algae based on chlorophyll-*a*	3.5	50	0.96

of temperature on the reaction rate of a biological process was determined using Eq. (4), presented by Metcalf and Eddy [22]:

$$r_T = r_{20}\theta^{(T-20)}, \quad (4)$$

where r_T is the reaction rate at T°C (day^{-1}), r_{20} is the reaction rate at 20°C (day^{-1}), θ is the temperature activity coefficient and T is the temperature (°C). For suspended growth systems the average value of θ is 1.04 [22]. Using Eq. (4), the calculated maximum specific growth rate of algae and heterotrophic bacteria at temperature of 20°C was 1.82 and 2.56 day^{-1}, respectively. The calculated maximum specific growth rate for algae based on chlorophyll-*a* at the temperature of 20°C was 2.36 day^{-1}. However, the manner at which the temperature influences on μ_{max} of the biomass in the SFP might be different from the other suspended growth systems.

4.1. Specific substrate removal rate (q)

The substrate utilization rate was determined at a steady state of growth for each batch reactor. The observed substrate utilization rate was between 0.68 and 0.97 (mg COD/mg biomass). The average substrate removal rate was 0.82 (mg COD/mg biomass). The maximum substrate utilization rate of 0.97 was achieved at substrate concentration of 400 mg/l. Hence, the yield in the bioreactors was 1.03–1.47 mg biomass/mg COD. The values obtained compares well with the trend of the specific growth rate obtained from the bioreactors. The substrate utilization rate was directly related to specific growth rate of heterotrophic bacteria as also shown by Papkov et al. [23].

5. Conclusions

Although the Monod equation was developed for the pure culture of bacteria growing in a single organic substrate, the results shown from this work indicate that the model may be used correctly for a mixed culture of algae and heterotrophic bacteria growing in a heterogeneous mixed organic carbon. The (K_s) obtained are the characteristic of the wastewaters treated by SFPs which contains a complex mixture of organic carbon. Furthermore, it appears that the Monod constants depend on the nature of the operation of the system. This is due to the fact that the bacteria growth rate in the activated sludge was reported to range between 2.88 and 13.2 day^{-1}, while for the wastewater treated by WSPs was only 3.8 day^{-1}. The parameters obtained during this experimental work are applicable for the SFPs where algae and bacteria grow symbiotically. The Monod constants for algae depend on the method used on the determination of the biomass. In this research the reported values are based on the chlorophyll-*a* and volatile solids. It was also observed that the specific growth rate was directly related to the specific substrate removal rate.

Acknowledgements

The Authors wish to acknowledge DANIDA ENRECA for the financial support to the Faculty of

Engineering, University of Dar es Salaam, for hosting the research programme on Waste Stabilization Ponds. The experiments were carried out as part of the collaboration research programme between the University of Dar es Salaam, The Royal Danish School of Pharmacy, Technical University of Denmark and The University of Copenhagen.

References

[1] Polprasert C, Dissanayake MG, Thanh NC. Bacteria die off kinetics in waste stabilization ponds. J Water Pollut Control Fed 1983;55(3):285–96.
[2] El-Baroud HM, Moawad SK. Rate of BOD reduction by oxidation ponds. J Water Pollut Control Fed. 1967;39(10, Part 1):1626–46.
[3] Kuentzel LE. Bacteria, carbon dioxide and algal bloom. J Water Pollut Control Fed 1969;41(10):1737–45.
[4] Bartsch AF. Algae as a source of oxygen in wastewater treatment. J Water Pollut Control Fed 1961;33(3):239–49.
[5] Ludwig HF, Oswald WJ, Gotaas HB, Lynch V. Algal symbiosis in oxidation ponds. 1. Growth characteristics of *Euglena Gracilis* cultured in sewage. Sewage Ind Waste 1951;23(11):1337–54.
[6] Oswald WJ, Gotaas HB. Photosynthesis in sewage treatment. J Sanit Eng Div ASCE 1955;81:686–92.
[7] Oswald WJ, Gotaas HB, Ludwig HF, Lynch V. Algal symbiosis in oxidation ponds. II. Growth characteristics of *Chlorella Pyrenoidosa* cultured in sewage. Sewage Ind Waste 1953;25(1):26–37.
[8] Oswald WJ, Gotaas HB, Ludwig HF, Lynch V. Algal symbiosis in oxidation ponds. III. Photosynthetic oxygenation. Sewage Ind Waste 1953b;692–705.
[9] Mackenthum KM, McNabb CD. Stabilization pond studies in Wisconsin. J Water Pollut Control Fed 1961; 33(12):1234–50.
[10] Monod J. The Growth of Bacterial Cultures. Annu Rev Microbiol 1949;3:371–93.
[11] Grady CPL, Daigger GT, Lim HC. Biological wastewater treatment, 2nd ed. New York: Marcel Dekker, 1999. p. 78–93.
[12] Gompertz B. On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. Philos Trans R Soc London 1825;115:513–85.
[13] Richards FJ. A flexible growth function for empirical use. J Exp Bot 1959;10:290–300.
[14] Stannard CJ, Williams AP, Gibbs PA. Temperature growth relationship for psychotrophic food spoilage bacteria. Food Microbiol 1985;2:115–22.
[15] Schnute J. A versatile growth model with statistically stable parameters. J Aquat Sci 1981;38:1128–40.
[16] Zwietering MH, Jongenburger I, Rombouts, Riet K van't. Modelling of the bacterial curve. J Appl Environ Microbiol 1990;56(6):1875–81.
[17] Wijtzes T, De Wit JC, Huis In't Veld JHJ, Van't Riet K, Zwietering MH. Modelling bacterial growth of *Lactobacillus Curvatus* as function of acidity and temperature. Appl Environ Microbiol 1995;61(7):2533–9.
[18] APHA, AWWA, WEF. Standard methods for the examination of water and wastewater, 18th ed. Washington DC: American Public Health Association, 1992.
[19] Jorden WL, Pohland FG, Kornegay BH. Evaluating treatability of selected industrial wastes. Proceeding of the 26th Industrial Waste Conference, Purdue University, Engineering Extension Series No. 140, 1971. p. 514–29.
[20] Henze M, Grady CPL, Gujer W, Marais GVR, Matsuo. A general model for single sludge wastewater treatment systems. Water Res 1987;20:505–15.
[21] Henze M, Grady CPL, Gujer W, Marais GVR, Matsuo. Activated Sludge Model No. 1. IAWPRC Scientific and Technical Reports, No. 1, 1987.
[22] Metcalf and Eddy Inc. Wastewater engineering: treatment, disposal and reuse, 3rd ed. New York: McGraw-Hill, 1991. p. 372–3.
[23] Papkov G, Libman V, Argaman V. Microbial activity: mathematical expression and direct determination. J Environ Eng 2000;126(1):80–7.