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A Mathematical Approach For The Evaluation Of Critical Concentration/ Critical Time For Inactivation Of Bacteria Systems With Special Reference To Ozone/*Escherichia Coli* System In Water

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ABSTRACT

Many waters and wastewaters require disinfection of microorganisms which involves transfer of an active disinfectant component into individual bacteria sites or colonies and thereafter making them to become inactive individually and collectively. Here, the active disinfectant component reacts with bacteria at rates determined by the concentrations of the disinfectant and bacteria and the respective rate constants leading to decay/death of bacteria and shrinkage of colonies. Design of disinfection processes require the disinfection to be achieved within a determined time. The time required for a desired degree of disinfection is a function of the colony strength and the initial disinfectant concentration. This paper develops and presents general mathematical co-relations for the determination of the initial concentration of the disinfectant(termed critical concentration c*) required to achieve a given degree of inactivation of a colony of a given initial size, at the end of a specified time(termed as critical time t*). A case study based on experimental data already available for the Ozone/*Escherichia coli* system in water is used to develop a simplified equation for the critical concentration for the system. Application of the data in the co-relations demonstrates satisfactory applicability of the co-relations.

KEYWORDS

Bacteria;
Critical concentration;
Critical time;
Disinfection;
Escherichia coli;
Inactivation;
Ozone.

INTRODUCTION

Bacteria are microorganisms commonly dwelling in many waters and wastewaters[1] and also used in many industrial biological processes. Disinfection of bacteria is required to reduce or eliminate the bacteria content for use or prior to recycle of discharge of waters to the environment[2]. Disinfection can be described as the complete or virtual elimination of enteropathogenic bacteria and is required to protect consumers from associated waterborne illnesses[3-7].

Halogens[7,9], chlorine dioxide, chloramines[10-12] hydrogen peroxide[2], potassium permanganate, potassium ferrate[13,14] are some disinfectants used in practice. Ozone has been a popular as a disinfectant in water treatment[9]. Akbas and Ozdemir[15] have studied the effectiveness of ozone in gas phase inactivation of *Escherichia coli* and bacillus cereus in shelled pistachios.

Ozone is reported to be more effective at low temperatures and higher pH[16]. In general, it has been observed that parameters such as pH and temperature are reported to influence inactivation rates of bacteria[17].

In all inactivation systems, it is essential to estimate the initial disinfectant concentrations required to achieve a specified degree of inactivation of bacteria within a desired time. This becomes important in the design of reactors and processes of inactivation[18]. In practice, the degree of disinfection is typically estimated using as a factor 'CT' which in fact the product of the time of disinfection and the residual concentration of the disinfectant at the time t[3,4,19-21].

The concept of CT has been based on the simplest model for the kinetics of disinfection of bacteria is the model of Chick-Watson[21, 22] as given below.

$$dN/dt = -k_i \cdot c^n \cdot N \quad (1)$$

It has been common to integrate a re-arranged form of the equation (1) as follows.

$$\ln\left(N/N_o\right) = -k_i \int c^n.dt \quad (2)$$

The value of n has been reported to be approximately equal to 1.0 for many systems representing a first-order process[23,24]. It has also been found that the equation (2) generally holds for many inactivation systems[25,26].

The concentration of the disinfectant has been found to be dependant on time and the initial colony strength of bacteria[16,27]. Thus the concentration of disinfectant c at a time t could be expressed as

$$c = f(t, N_o) \quad (3)$$

Therefore, for a given system, the factor $\int c^n dt$ has been calibrated as a function of c.t leading to the 'CT' concept of estimation of the degree of inactivation of bacteria.

The model proposed by Collins-Selleck[28,29] presents a relationship between N/No vs $\int_0^t c.dt$ when n=1 as,

$$N/N_0 = 1 \text{ for } t \le \tau, \text{ and } N/N_0 = (\tau/ct)^z \text{ for } t \ge \tau \quad (4)$$

Both the methods presented above enable ways for the estimation of the value of c.t of(CT) required for a desired inactivation N/No of a bacteria borne sample. Estimating the CT for a given log inactivation is typically a calibration problem[30].

Clark et al.[31,32] have developed a CT equation in order to predict mean inactivation levels. They have also presented a statistically conservative upper bound CT value for the inactivation of cryptosporidium oocysts with ozone and chlorine dioxide respectively. Sivaganesan et al.[30] have presented a Bayesian method in order to estimate the minimum CT requirement for 99% inactivation of *C.parvum oocysts* with chlorine dioxide and ozone. Sivaganesan and Marinas[33] have developed a 'CT' equation for the inactivation of *cryptosporidium parvum* oocysts with ozone.

However, studies on inactivation kinetics of C. *parvum oocysts* with ozone[24,34-38] have shown that the lag phase plays an important role in estimation of inactivation data for the system. The inactivation has been shown to be consistent with a pseudo-first-order delayed Chick-Watson kinetic model[39]. The importance of lag phase is implied only for cases for microorganisms which are more difficult to inactivate(e.g.*Bacillus subtilis* spores(*B.subtilis*), cryptosporidium parvum oocysts(*C.parvum*))[40] and may not be applicable to *Escherichia coil*/ozone system for which inactivation is fast and lag period is negligible.

Tables of 'CT' values based on experimentally evaluated 'CT' data are presented in the form of tables[3,4,20,21] for a desired disinfection The 'CT' concept and the tables used in the estimation of inactivation have been based on results of extensive studies for different bacteria under different pH and temperature conditions.

Estimations of the factor 'CT' could lead to significant deviations from of the true integral value $\int_0^t c^n.dt$ [40].

This usually leads to overdoses of the disinfectant which is not much appealing[41-43]. It is therefore essential to estimate the initial disinfectant concentration for the desired time of exposure which allows a more precise assessment of the disinfection process. It would be very convenient if the factor N/No was theoretically coupled to the initial concentration(c_o) and time(t) rather than coupling to a factor $\int_0^t c^n.dt$ or 'CT' values from tables based on experimental data[3,4,8,21].

A simpler approach where the inactivation time(t^*) could be estimated from the input data of the initial concentration(c_o^*) of the disinfectant and the initial and the desired colony sizes(N_o and N respectively)and other operating conditions such as pH and temperature could make the estimation process and inactivation practice very much easier. Alternatively, a method of estimation of the initial concentration(c_o^*) of the disinfectant in order to achieve a desired disinfection at the end of a time(t^*)for initial and the desired colony sizes(N_o and N respectively) would become very attractive. Here the terms c_o^* and t^* could be termed the critical concentration and the critical time in order to achieve a desired disinfection.

The concept of critical concentration has already been used in inactivation technology in many instances. A study of solvent toxicity in photoautotrophic unicellular microorganisms has employed a critical solvent concentration where the aqueous solvent concentration at which the photosynthetic activity becomes nearly zero[38]. Osborne and coworkers[44, 45] have used a critical concentration in their studies for complete loss of biocatalytic activity. Caravelli et al.[46] have described a critical concentration as the lowest initial dose of the disinfectant that leads to a virtual inhibition of the microorganism respiratory activity. A critical concentration(c_o*) for inactivation of bacteria can therefore be defined as the initial concentration of the disinfectant required to treat an initial count N_o of bacteria to obtain a value of $N/N_o(=f^*)$ which could be considered to indicate a desired level of disinfection.

Theoretical estimations of critical concentrations require in-depth study of kinetics of decay of bacteria. This paper attempts to present a theoretical analysis for the inactivation of bacteria whereby solutions of the equations are used to present expressions co-relating the critical concentration(c_o^*) and the critical time(t^*) for a given inactivation of bacteria colony of strength No to a value N.

MATERIALS AND METHODS

The expectation of the current research has been to develop a co-relation for evaluation of the initial concentration(c_o^*) of disinfectant to achieve a desired inactivation($f^*=N/N_o$) of bacteria at a given time t^* experimental data already available for the inactivation of *Escherichia coil* with ozone in water were applied in the co-relations for validation.

Experimental data and kinetics

Experimental investigations for evaluation of kinetics of *Escherichia coil* inactivation with ozone have been carried out by Hunt and Marinas[23] in semi-batch and continuous-flow tubular reactors with values of pH ranging from 6 to 8 and temperatures ranging from 5°C to 25°C. Ozone concentrations and contact times investigated have ranged from 6 to 41μg/1, and 1.8 to 33s, respectively. The chick watson model has been used for a first order rate with respect to each of N and c according to the expression in the form:

$$\frac{dN}{dt} = -k_i cN \tag{6}$$

Ozone decomposition has been represented by Botznhart et al.[16], and Hassen et al.[27] as:

$$\frac{dc}{dt} = -k_x c.x \tag{7}$$

$$x = c - c_0 + x_0 \tag{8}$$

Here, x_o has been expressed as $x_o = \alpha_o N_o$ in which α_o is expressed in mg of ozone demand per colony forming unit(mg O_3/CFU) or the ozone demand per *Escherichia coli* cell.

Hunt and Marinas[47] have conducted further experiments with varying initial concentrations of ozone and bacteria in order to investigate the parameters $k_{i,}$ k_x as defined by equations(6) and (7). Inactivation kinetics was found to be of first-order with respect to each of the ozone concentration and active microorganism density. The rate constant k_i has been determined to be 130 1/(mg s) at 20^0C, and the corresponding activation energy was 37,100 J/mol. The *Escherichia coli* inactivation reaction is reported to be fast compared with the disinfectant reaction with $k_i > k_x$. The value of ki/k_x is reported to be approximately 80[22]. The average value of a_0 evaluated from the above experiments for the *Escherichia coli*/ozone system is reported to be 1.29×10^{-11} mg/CFU.

The dependence of the inactivation rate constant on temperature for the inactivation of *Escherichia coli* with ozone has been analyzed[23,41] using the classical Arrhenius expression[equation(7)] in order to obtain Ei=37,100J/mol and A_i=the frequency factor for the inactivation=5.37´10^8 L/(mg.s) or ln(A_i)=8.13

$$k_i = A_i \exp\left(-\frac{E_i}{RT}\right) \tag{9}$$

Equations (7) and (8) have been used by Hunt and Marinas[23] in order to obtain the following expression.

$$c = \frac{c_0\,(c_0 - \alpha_0 N_0)}{c - \alpha_0 N_0 \exp(-k_x\,(c_0 - \alpha_0 N_0)t)} \tag{10}$$

All the above studies have presented expressions for variation of concentration c of the disinfectant with time(t) and the variation of N/No in terms of a factor $\int_0^t c.dt$

The following section presents an extended and generalized theoretical analysis of inactivation kinetics in order to co-relate the value of N/N_o with t and initial disinfectant and bacteria concentrations c_o and N_o respectively.

The model and solutions

In the present study, an analogue of the Chick Watson model[22,48] is assumed in a general form as:

$$dN/dt = -k_i \cdot c^n \cdot N^m \tag{11}$$

The reaction model[16,27] presented in equation (8) is also assumed in a general form

$$\frac{dc}{dt} = -k_x c^u . (c - c_0 + a_0 . N_0)^v \tag{12}$$

Both the constants k_i and k_x are assumed to be temperature and pH dependant.

Equations (11) and (12) were combined, re-arranged and integrated in order to obtain two relationships for the microorganism survival fraction f^* and the disinfection contact time t* in terms of c^*_o, α_o, N_o, k_i and k_x as follows.

$$\ln[f^*] = \left(\frac{k_i}{k_x}\right)\int_{c^*_o}^{c}\left(\frac{dc}{c^{(u-n)}(c-\theta)^{(v)}}\right)$$

for n=1

$$(f^*)^{(1-m)} = 1 + \left(\frac{k_i}{k_x}.N_o^{(m-1)}\right)\int_{c^*_o}^{c}\left(\frac{dc}{c^{(u-n)}(c-\theta)^{(v)}}\right)$$

for n^{1}1 **(13)**

And

$$t^* = \frac{1}{k_x}\int_{c^*_o}^{c}\frac{dc}{c^u.(c-\theta)^v} \tag{14}$$

where, $\theta = c^*_o - a_o N_o$

It can be seen that equations (13) and (14) can be regarded as two simultaneous integral equations co-relating t^* and c_o^* for a given system and values of f* and N_o with an intermediate solution for c for each case.

Elaborate solutions of the integral equations are readily available in the literature[49]. However solution of equations (13) and (14) for the estimation of c_o^* for a given t*(or vice versa) can be seen very feasible using modern computer techniques. The next step is to make use of equations (13) and (14) for a selected inactivation system for the purpose of validation.

CALCULATIONS AND RESULTS

The system for the case study

Ozone has been a disinfectant used for water for almost a century[50] with 90% of the water works making use of ozone for disinfection purposes[51]. Particularly in the case of water, a spectrum of pathogenic microorganisms could be present prior to disinfection. As monitoring for every pathogenic microorganism is not feasible, the concept of indicator microorganisms has been adopted[52]. For this purpose, fecal coliforms and *Escherichia coli* have been selected as indicators because their presence indicates that water could be regarded as contaminated with human and/or animal wastes. Using indicator microorganisms to assess disinfection processes has shown to work well as long as the inactivation of the undesired pathogens is at least as efficient as the inactivation of the chosen indicator micro-organisms. Due to the importance of ozone and *Escherichia coli*, this system has therefore been selected for the case study in this paper.

Variation of f^* with disinfection time t* for a given c^*_o

It was seen earlier that all the values of n, m. u and v in equations (13) and (14) are found to be 1.0 for the system of bacteria *Escherichia coli*/ozone/water. The equations (13) and (14) could therefore be solved in order to obtain:

$$t^*.k_x c_o^*(1-\beta) = \ln\left[(1-\beta)(f)^{\left(-\frac{k_x}{k_i}\right)} + \beta\right] \tag{15}$$

Where $\beta . c^*_o = \alpha_o N_o$

The term β can be seen to represent the ratio of the disinfectant demand($\alpha_o N_o$) for complete disinfection to the actual availability of the disinfectant (c_o) in the initial mixture. β could therefore be regarded as the inverse of the fractional availability for disinfection at the onset of the disinfection process(i.e. at t=0). The value of f * can be regarded as the degree of disinfection desired.

Equation (15) is seen to present the critical concentration c_o* in order to achieve a ratio of $N/N_o = f^*$ at a time t*. Here the knowledge of b and k_x/k_i is required.

Data presented in the study mentioned earlier[23,47] for the ozone/*Escherichia coli* in water system were used in this analysis. Figure 1 shows the variation of f^* with t^* for different values of c^*_o as evaluated using equation(15). The required disinfection time is seen to decrease as the initial ozone concentration increases. The lag time in inactivation of *Escherichia coli* by ozone is taken as negligible as reported[23].

The data for Hunt and Marinas[47] presented a value of $k_i/k_x \approx 80$ at 20^0C. In this study, this ratio of k_i/k_x was assumed not to vary significantly over the experimental range of 5^0C to 25^0C and pH of 6.0.

This assumption can be justified by an analysis of the Arrhenius equations for inactivation as well as the ozone reactions where in general k_i/k_x could be expressed as

$$\frac{k_i}{k_x} = \left(\frac{A_i}{A_x}\right).\exp\left(\frac{-\{E_i - E_x\}}{RT}\right) \tag{16}$$

Equation (16) could be simplified to

$$d\ln\left(\frac{k_i}{k_x}\right) = -\frac{\Delta E_{ix}}{R.T^2}.dT \tag{17}$$

Where $\Delta E_{ix} = E_i - E_x$ (18)

The activation energies E_x for most chemical reactions are reported(Von gunten, 2003) to be in the range 35000 to 50000J/mole which are very much comparable with E_i(=37,100). Thus, the factor[$\Delta E_{ix}/RT^2$] is assumed to be small making dln(k_i/k_x)small over the ranges of T and dT considered in this study justifying the assumption of a constant value of (k_i/k_x)=80 over the range of temperatures 5^0C to 25^0C.

The critical concentration c^*_o

Experimental data of Hunt and Marinas[47] presented N/N_o (i.e. f*) and $\int$cdt values for various initial ozone concentrations(c_o^*). The $\int$cdt values en-

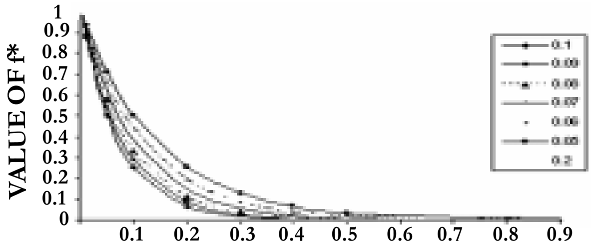

Figure 1: Variation of f^* with time for inactivation t^* for *Escherichia coli* with ozone at 20^0C for different initial ozone concentrations of 0.05 to 0.1mg/L and $N_o = 3.60\times10^9$ CFU/L

abled to estimate the time t* for different f^* with the knowledge of respective k_x values using equation(10). Data points were obtained for temperatures of 5^0C, 10^0C, 25^0C, 20^0C and 25^0C respectively under the condition of pH=6. Data obtained consisted of t^* Vs c^*_o for various values of f^* and β.

The above data for different sets of temperatures were used to estimate the optimum values of A_i and E_i for the best linear fit of the graph of $t^*.k_x c^*_o(1-\beta)$ vs. $\ln[(1-\beta).f^{(-kx/ki)}+\beta]$ for a straight line passing through the origin. The ratio k_i/k_x as assumed to be constant 80.0 for all the temperatures as discussed earlier. Figure 2 shows the optimized plot of $t^*.k_x c^*_o(1-\beta)$ vs. $\ln[(1-\beta).f^{(-kx/ki)}+\beta]$. The optimized plot yielded $\log(A_i)$ and E_i values as 8.584 and 33333 respectively. This can be compared with the respective reported values of 8.13 and 37100 respectively[47]. It can be seen that the theoretical line [equation(15)] fits with experimental points giving a R^2 value of 0.9685 which is reasonably satisfactory. The analysis shows that the equation(15) can be used for co-relating c^*_o with different t^* for given f^* if other system data α_0, k_i, k_x and k_i/k_x are known.

Figure 3 shows a comparison of the time t^* obtained from experimental data with the respective t^* estimated using equation(15) for randomly selected values of f* and c_o^*. The straight-line fit passing through the origin with a slope of unity can be seen to be having a R^2 value of 0.9467. This shows that the predicted t* using the equation fits reasonably with the experimental data.

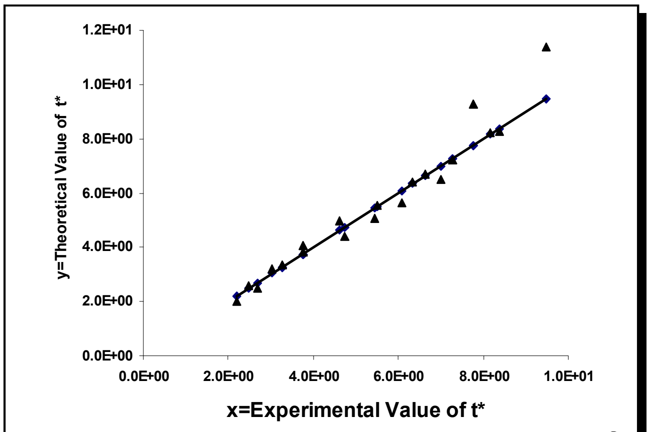

Figure 2: Optimized linear plot of t*.kxc*o(1-β) vs. ln[(1-β)·f(-kx/ki)+β]

DISCUSSION

The inactivation technology for bacteria makes use of 'CT' data based on tables derived experimentally in order to achieve a required inactivation. The tables are basically a set of calibration data[30] based on statistically verified experimental information for each type bacteria, disinfectant and the inactivation conditions. The objective of the present study was to develop a mathematical model to co-relate the critical concentration(c^*_o) required to achieve a specified level($f^*=N/N_o$) of disinfection within a desired time t^* in order to present a mathematical basis as an alternative to the concept of 'CT', for the determination of inactivation doses of disinfectants. Typical kinetics of inactivation presented by Botznhart et al.[15] and Hassen et al.[27] have been generalized in this paper in order to present co-relations(13) and (14) for the estimation of t^* for given c^*_o with a knowledge of three system parameters a_o, k_i, k_x in order to attain a desired inactivation f^*. The solution of the co-relations(13) and (14) could be obtained either through mathematical solution or by use of simple differential equation solvers in a handy computer packages.

However, for many systems where the orders of the inactivation and reaction are unity with respect to each component, a simplified expression(15) can be very conveniently derived. Equation(15) was tested for the system ozone/*Escherichia coli* in water as a case study where inactivation data of Hunt and Marinas[47] for the system have been employed. The analysis showed a linear fit(Figure 2) with R^2 value of 0.9685 for a line with zero intercept, as expected by equation(15), showing satisfactory compatibility

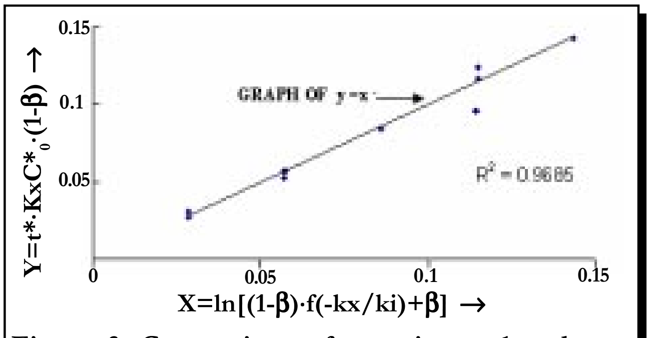

Figure 3: Comparison of experimental and predicted t^* values for randomly selected values of f* and c_o^*.

of experimental data with the equation(15). However, the fitting values of log(A_i) and E_i differed slightly from values of Hunt and Marinas (1999). Minor errors may have crept into calculations because of the assumption of constant k_i/k_x over the temperature range of 5^0C and 25^0C. This would have attributed to a lowering the R^2 value and for the difference between the evaluated values and previously published data for log(A_i) and E_i. However, the deviations could be treated as of minor nature, and therefore equation(15) may be considered as validated for the system considered. The expression(15) could therefore be regarded as a useful expression co-relating the critical concentration c^*_o of the disinfectant in order to achieve a desired disinfection level of bacteria within a specified time t* for a disinfection system and vice versa.

The fit of experimental and predicted t*(Figure 3) with R^2 value of 0.9467 shows the reasonable applicability of the equation(15). Equations(15) can therefore be made use of as an expression for he determination of c* for a desired t* or vice versa for a required inactivation of the *Escherichia coli* using ozone as the disinfectant under the experimental conditions.

The case study also demonstrates that the method proposed makes it possible to estimate the values of c* for a desired t* (or vice versa) for a determined inactivation of bacteria using a disinfectant with the knowledge of six parameters k_i, k_x, m, u, n and v. The equation could replace the use of 'CT' Tables which require use of extensive use of data tables, preformulated using numerous experimental calibrations. The method presented herein therefore seems to be a direct method of estimation requiring knowledge of the six factors k_i, k_x, m, u, n and v for respective inactivation calculations as an alternative to the use of 'CT' Tables.

CONCLUSIONS

A set of general equations for the estimation of the critical concentration of disinfectant/respective critical time for inactivation of bacteria were proposed by equations(13) and (14) based on respective kinetic data.

An equation(equation 15) was presented for the estimation of initial critical disinfectant concentrations (c^*_o) required to attain a desired degree of inactivation(f^*) within a given critical time(t*) based on a first order kinetic system with respect to each component.

Equation (15) was validated for disinfection of *Escherichia coli*/ozone system in water based on inactivation data[47], thus showing the applicability of equation (15) for inactivation of *Escherichia coli* with ozone in water.

The kinetic data log(A_i) and E_i. For the inactivation reactions were evaluated for the *Escherichia coli*/ ozone system in water 8.584 and 33333 respectively and are seen to be comparable with the values 8.13 and 37100 obtained by Hunt and Marinas[47].

The general equations (13) and (14) may be used for estimation of the critical concentration/critical time for other bacteria systems, as an alternative to the use of 'CT' tables.

NOTATION

A_x = the frequency factor in Equation (9)
c = concentration of disinfectant (mg/litre)
c_o = iinitial concentration of disinfectant (mg/litre)
c_o^* = critical concentration of disinfectant (mg/litre) (defined as the initial concentration required to achieve $N/N_o=f^*$ at a time t^*)
CFU = colony forming unit
E_i = the activation energy in Equation (9).
f = N/N_o = degree of inactivation after a time t
f^* = N^*/N_o = degree of inactivation after a time t*
k_i = inactivation rate constant in equations (6),(9) and (11)
k_x = reaction rate constant in litres/(mg.s) in equations(7) and(12)
n = exponent in equation(11)
N = density of viable micro-organisms(CFU/litre)
N_o = initial density of viable microorganisms(CFU/litre).
N* = density of viable micro-organisms at the end of desired degree inactivation(CFU/litre)
m = exponent in equation (11)
t = inactivation time = contact time -τ (s)
t^* = critical inactivation time (s)
u = exponent in equation (12)
v = exponent in equation (12)
x = disinfectant demand of micro-organisms(mg/l of disinfectant.)
x_o = initial disinfectant demand of microorganisms(mg/l of disinfectant.).
z = exponent in equation (5)
α_o = x_o/N_o
τ = lag time

REFERENCES

[1] M.Henze, P.Harremoes, J.L.Jansen, E.Arvin; Springer, 65-76, **(2002)**.

[2] K.Ksibi; Chemical Engineering Journal, **119**, 161-165 **(2006)**.

[3] U.S.Environmental Protection Agency, National Primary Drinking Water Regulations:Total Coliforms (Including Fecal Coliforms and E.coli); final rule, Fed Register, **54(124)**, 27544 **(1989)**.

[4] U.S.Environmental Protection Agency, National Primary Drinking Water Regulations: Filtration, Disinfection, turbidity, Giardia lamblia, viruses, Legionella, and heterotrophic bacteria; Final Rule, Fed Register, **54(124)**, 27486 **(1989)**.

[5] U.S.Environmental Protection Agency, Office of DrinkingWater, Criteria and Standards Division Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources, **(1991)**.

[6] U.S.Environmental Protection Agency, National Primary Drinking Water Regulations: Disinfectants and disinfection byproducts; Final Rule, Fed Register, **63(241)**, 69389-69476 **(1998)**.

[7] C.N.Haas, J.Joff; Environ.Sci.Technol., **28(7)**, 1367-9 **(1994)**.

[8] U.S.Environmental Protection Agency, National Primary Drinking Water Regulations: Interim Enhanced Surface Water Treatment; Final Rule. Fed Regulation, **63(241)**, 68478 **(1998)**.

[9] E.Hamilton, D.Seal, J.Hay; J.Hosp.Infect., **156**, 32 **(1996)**.

[10] J.L.Kool, J.C.Carpenter, B.S.Field; JAWWA, **92**, 88-96 **(2000)**.

[11] J.L.Kool, J.C.Carpenter, B.S.Field; Lancet, **353**, 272-277 **(1999)**.

[12] G.C.White; 'Handbook of Chlorination for Potable Water, Waste Water, Cooling Water, Industrial Processes and Swimming Pools, New York: Van Nostrand Reinhold, **(1972)**.

[13] M.T.Yahya, L.K.Landeen, C.P.Gerba; Environ. Sci.Technol., **11**, 657-62 **(1990)**.

[14] J.Jiang, S.Wang, A.Panagoulopoulos; Chemosphere, **63**, 212-219, **(2006)**.

[15] M.Y.Akbas, M.Ozdemir; International Journal of Food Science and Technology, **41(5)**, 513-519 **(2006)**.

[16] G.M.Botznhart, G.M.Tarcson, M.Ostruschka; J.Chin. Inst.Chem.Eng., **33(3)**, 253-65 **(2002)**.

[17] S.M.Dow, B.Barbeau, U.Von Gunten, M.Chandrakanth, G.Amy, M.Hernandez; Water Res., **40**, 373-382 **(2006)**.

[18] Xu Pei, Marie-Laure Janex, Philippe Savoye, Arnaud Cock, Valentina lazarov; Water Res., **36**, 1043-1055 **(2002)**.

[19] J.A.Thurston-Enriquez, C.N.Haas, J.Jacangelo, C.P.Gerba; Water Res., **39**, 3650-3656 **(2005)**.

[20] US EPA Guidance Manual, 'Disinfection Profiling and Benchmarking', Creating a profile: data requirements and calculations, **3**, 3.1-3.52 **(1999)**.

[21] B.Langlais, D.A.Reckhow, D.R.Brink; Chelsea, Lewis, **(1991)**.

[22] F.Watson; J.Hyg., **8**, 536-542 **(1908)**.

[23] N.K.Hunt, B.J.Marinas; Water Res., **31**, 1355-62 **(1997)**.

[24] A.Driedger, E.Staub, U.Pinkernell, B.Marinas, K.Oster, U.Gunten; Water Res., **35**, 2950-60 **(2001)**.

[25] C.N.Haas; 'A Handbook for Community Water Supplies', New York: McGraw-Hill, 14.11-14.60, **(1999)**.

[26] C.N.Haas; Control of Microorganisms in Drinking Water, 183-188, **(2002)**.

[27] A.Hassen, A.Heyouni, H.Shayeb et al.; Bioresource Technology, **72**, 85-93 **(2000)**.

[28] R.G.Qualls, J.D.Johnson; Water Res., **19**, 1039-1046 **(1985)**.

[29] J.M.Montgommery; Wiley, Washington, DC., 62-279 **(1985)**.

[30] M.Sivaganesan, E.W.Rice, B.J.Marin; Water Res., **37**, 4533-4543 **(2003)**.

[31] R.M.Clark, M.Sivagensan, E.W.Rice, J.Chen; Water Res., **36**, 3141-9 **(2002)**.

[32] R.M.Clark, M.Sivaganesan, E.W.Rice, J.Chen; Water Res., **37**, 2773-2783 **(2003)**.

[33] M.Sivaganesan, B.J.Marinas; Water Res., **39**, 2429-2437, **(2005)**.

[34] J.L.Rennecker, B.Marinas, J.H.Owens, E.Rice; Water Res., **33(11)**, 2481-2488 **(1999)**.

[35] J.L.Rennecker, A.Driedger, S.Rubin, B.Marinas; Water Res., **34(17)**, 4121-4130 **(2000)**.

[36] T.Hirata, A.Shimura, S.Morita, M.Suzuki, N.Motoyama, H.Hoshikawa, T.Moniwa, M.Kaneko; Water Sci. Technol., **43(12)**, 163-166, **(2001)**.

[37] B.Corona-Vasquez, A.Samuelson, J.L.Rennecker, B.J.Marinas; Water Res., **36**, 4053-4063 **(2002)**.

[38] P.A.Rochelle, M.M.Marshall, R.De Leon; Proceedings of the 2002 American Water Works Association Water Quality Technology Conference, Seattle, Washington, Nov., 10-14 **(2002)**.

[39] G.B.Wickramanayake, O.J.Sproul; Ozone Sci.Eng., **10**, 125-135 **(1988)**.

[40] A.M.Driedger, J.L.Rennecker, B.Marinas; Water Res., **34**, 3591-7 **(2000)**.

[41] U.von Gunten, M.S.Elovitz, H.P.Kaiser; J.Water Supply Aqua, **48**, 250-6 **(1999)**.

[42] Z.Do-Quang, M.Roustan, J.P.Duguet; Ozone Sci.Eng., **22**, 99-111 **(2000)**.

[43] H.P.Kaiser, U.Von Gunten; Gas Wasser Abwasser, **80**, 50-61 **(2000)**.

[44] S.J.Osborne, J.Leaver, M.K.Turner, P.Dunnill; Enzyme Microb.Technol., **12**, 281-291 **(1990)**.

[45] S.J.Osborne, J.Leaver, M.K.Turner, J.Tramper, M.H.Vermue, H.H.Beeftink, **(1992)**.

[46] Caravelli Alejandro, E.M.Contreras, L.Giannuzzi, N.Zaritzky; Water Res., **37**, 2097-2105 **(2003)**.

[47] N.K.Hunt, B.J.Marinas; Water Res., **33(11)**, 2633-2641 **(1999)**.

[48] H.Chick; J.Hyg., **8**, 92-158 **(1908)**.

[49] G.P.Bois; Dover Publications Inc., 77-89 **(1961)**.

[50] U.Von Gunten; Water Res., **37**, 1469-1487 **(2003)**.

[51] R.Courbat, S.Ramseier, J.L.Walther, P.Gaille, R.Jordan, H.P.Kaiser, P.Revelly, R.Stettler, U.Von Gunten; Gas Wasser Abwasser, **79**, 843-52 **(1999)**.

[52] EU.Official Journal of The European Community L 330 Directive 98/83/EG, **(1998)**.