[PROC. OF JSCE No.375/II-6 (Hydraulic and Sanitary Eng.) November 1986]

DIE-OFF PROCESS OF COLIFORM GROUP IN OXIDATION PONDS

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The die-off process of coliform group was investigated in AIT-oxidation ponds in parallel with the laboratory tests. More than 99 % of coliform group was removed in AIT-oxidation ponds. A major type of coliform group in the influent was E. coli., while Klebsiella spp. took place of E. coli. in the effluent. E. coli. was more sensitive to the environmental conditions in AIT-oxidation ponds than Klebsiella spp. and C. freundii.

The model for predicting the coliform group density in the facultative pond was proposed and the die-off rate coefficient was estimated. In case that a factor affecting the die-off process of coliform group was expressed by the sum of the sunlight effect, the dissolved oxygen effect and the pH effect, a better estimatin of the coliform group density was obtained. And then the estimated die-off rate coefficient was 2.56 (1/day) around 30°C.

Keywords: *coliform group, oxidation ponds, die-off process, estimation of die-off rate coefficient*

1. INTRODUCTION

The oxidation pond is the most common type of wastewater treatment being installed in developing countries. The principal advantages of oxidation ponds consist in their low cost of construction, simplicity in operation and high removal efficiencies of organic matters and pathogenic microorganisms. Especially, the drastical reduction of pathogenic microorganisms in oxidation ponds will bring a big advantage for the public health in developing countries.

In this study, the die-off process of coliform group in stead of pathogenic microorganisms was investigated in AIT-oxidation ponds because of coliform group commonly being used as an indicator of the pathogenic microorganisms. Many researchers have already reported about several environmental factors which affected the die-off of coliform group in oxidation ponds. According to Rarhad (1974)[1], high pH level were primarily responsible for high die-off rate of coliform group in waste stabilization ponds. Merz et al. (1962)[2] attributed high die-off rate of coliform group to toxic extracellular products by algae, but this hypothesis was not experimentally proved. Mara (1975)[3] and Watkings (1973)[4] concluded that high dissolved oxygen levels established conditions which favour antagonists or predators for coliform group. Moreover, other factors such as the bacteriophages, sunlight and so on will be considered responsible for the die-off of coliform group in oxidation ponds. However, in this study, the effects of two factors of sunlight and pH on the die-off process of coliform group were investigated in the laboratory in the parallel with the field survey in AIT-oxidation ponds. And using the results of these investigations, a model for predicting the coliform group density in oxidation ponds was proposed and the die-off rate coefficient was determined by using the sensitivity equation.

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2. PROCEDURES OF FIELD INVESTIGATION AND EXPERIMENTS IN THE LABORATORY

(1) Field investigation

Field investigation was conducted at AIT-oxidation ponds which has recieved the wastewater concerned with the academic and human activities of the faculty members and about six hundreds students. AIT-oxidation ponds comprise a series of a facultative pond and a maturation pond which have hydraulic retention times of 8 days and 20 days, respectively. AIT-oxidation ponds are illustrated with sampling points in Fig. 1. Samples were collected at 10 a. m. every morning for a week. Immediately after sampling, the coliform group density and the coliphage density were measured together with other important parameters of water quality. The diurnal variations of these bacterial and water quality parameters were also investigated.

Standard plate count by Standard Method (1981)[5] was applied to enumerate the coliform group density, using a desoxycholate agar medium (difco). And coliform group occurred on the plate was classified into three distinct species (Escherichia coli., Citrobacter freundii and Klebsiella spp.) on the basis of the results of IMViC tests (indole, methyl red, Voges-Proskauer and sodium citrate) and motility test as shown in Table 1. The percentages of occurrence for each species of coliform group made it possible to obtain the density of each species by means of multiplying the coliform group density by the percentages.

The coliphage analysis was carried out by MPN method. Samples for the coliphage analysis were pretreated by the centrifugation and millipore filter method. 1 ml of appropriate diluted sample, 0.5 ml of young E coli. B broth and two drops of $CaCl_2$-solution were added into 10 ml of phage broth. After mixed well, the mixtures were incubated at 37°C for 24 hours. With a haematocrit tube, the incubated sample was inoculated on the phage agar plate, which was prepared by solidifying two drops $CaCl_2$, 0.5 ml of young E

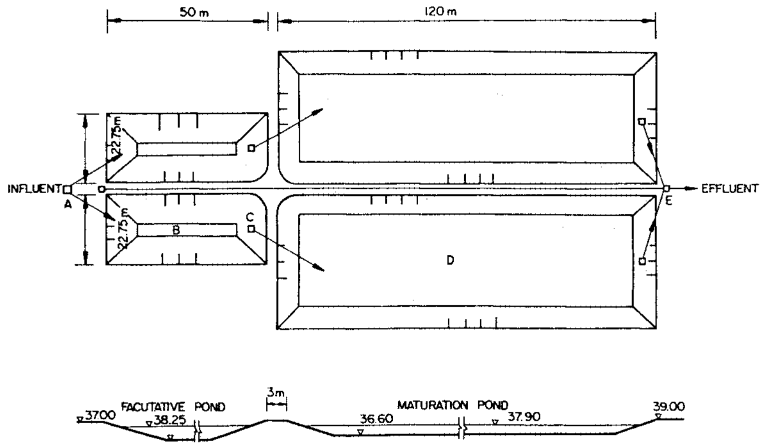

Fig. 1 Layout of AIT-oxydation ponds. A, B, C, D and E indicate sampling points :
A-influent ; B-facultative pond ; C-between faculative and maturation pond ; D-maturation pond ; E-effluent

Table 1 Classification of coliform group by IMViC and motility teste.

Type of coliform group	Indole	Methyl red	Voges-Proskauer	Sodium citrate	Motility
Escherichia coli.	+ or -	+	-	-	+
C. freundii	-	+	-	+	+
Klebsiella spp.	+ or -	-	+	+	-

coli. B broth and 3 ml of melted top agar. Then after incubation at 37°C for 24 hours, the result was positive if a clear zone was noticed around spot made, while it was negative if noclear zone was noticed around spot made. The compositions of media used for coliphage analysis were shown in Table 2, 3, 4 and 5.

(2) Experiments in the laboratory

Laboratory experiments were carried out in order to investigate influences of pH and sunlight on the die-off of each species of coliform group which was isolated from a sewage introduced into AIT-oxidation ponds. These experiments were conducted in 300 ml-BOD testing bottles under controlled conditions, employing pure culture of each species. pH values in BOD-testing bottles were adjusted with 1 N sulfuric acid or 1 N sodium hydroxide solutin to be 3, 5, 7, 9 and 11, respectively. In case of investigating the effect of sunlight, BOD-testing bottles were covered with aliminum foil to a suitable extent, that is, no cover, one third cover, two thirds cover and full cover. And then, these bottles were put into the maturation pond just below the water surface. In all laboratory experiments, BOD-testing bottles were filled with the sewage filtered through non-absorbent cotton and sterilized by the autoclave. Then each species of coliform group was inoculated into BOD-testing bottles so that its density might be the order of 10^6 cell/100 ml which was approximately equivalent to the coliform group density in a sewage.

3. RESULTS AND DISCUSSION OF FIELD INVESTIGATION AND EXPERIMENTS IN THE LABORATORY

(1) Field investigatin

The mean values of water quality parameters in AIT-oxidation ponds were shown in Table 6. The temperature was around 30°C, while the pH value ranged from 7.5 to 8.9. The high pH value in the maturation pond was attributed to the shortage of CO_2 used by algae in the photosynthesis. Corresponding to this shortage of CO_2, no acidity was observed in the maturation pond. Removals of organic materials were 55 percent in the filtered COD, 69 percent in the unfiltered COD and 75 percent in BOD_5, respectively.

Table 2 Composition of phage broth.

Nutrient broth	8.0 g
NaCl	5.0 g
$MgSO_4$	0.2 g
$MnSO_4$	0.05g
with distilled water	1.0 l

Table 3 Composition of phage agar.

Bacto agar	20 g
Phage broth	1 l

Table 4 Composition of top agar.

Bacto agar	8 g
Phage broth	1 l

Table 5 CaCl2 - solution.

$CaCl_2$	15 g
with distilled water	1 l

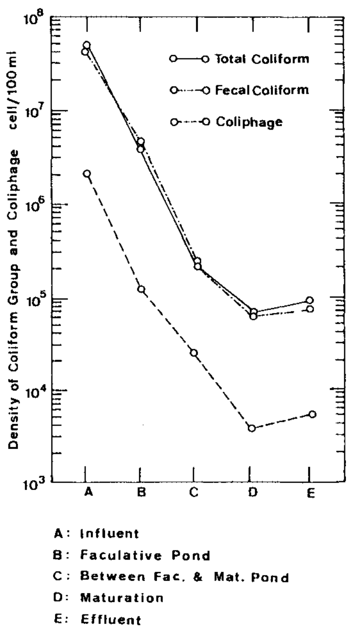

Fig. 2 Total and fecal coliform group densities and coliphage density in AIT-oxidation ponds.

Table 6 Physical and chemical characteristics of AIT-oxidation ponds.

Source	Physical & Chemical Characteristics								
	Temp (°C)	pH (average)	Acidity (average) (mg/ℓ as $CaCO_3$)	Alkalinity (average) (mg/ℓ as $CaCO_3$)	Chloride (average) (mg/ℓ)	COD (average) (mg/ℓ) Filtered	COD (average) (mg/ℓ) Un filtered	BOD (average) (mg/ℓ)	SS (ave.) (mg/ℓ)
Influent (A)	31	7.6	42.6	433	212.0	58.0	201.0	92	111
Facultative Pond (B)	32	8.0	33.7	400	177.4	27.2	107.7	36	62
Between Fac & Mat Pond (C)	31	8.0	40.3	401	187.4	25.7	117.7	31	46
Maturation Pond (d)	29	8.8	-	355	167.3	23.2	69.4	21	43
Effluent (E)	32	8.3	22.0	387	169.1	26.3	63.1	23	31

*Samples were collected and analysed from 15th September 1982 to 23rd September 1982.

**All samples were collected at 10 a.m. everyday.

Mean values of coliform group densities for a week in AIT-oxidation ponds were shown in Fig. 2. More than 99 % of total coliform and fecal coliform were removed in AIT-oxidation ponds. Similar result for removal efficiency of coliform group was reported by Mohammad (1979)[6]. Higher removal of coliform group (99.5 % of total coliform group) was obtained in the facultative pond, while lower removal of coliform group was observed in the matuation pond. The most of species of coliform group detected in AIT-oxidation ponds were E. coli. and Klebsiella spp. as shown in Fig. 3. Especially the major species of coliform group in the influent was E. coli., but Klebsiella spp. took place of E. coli. in the effluent. The percentage of occurrence for E. coli. decreased from the influent towards the effluent. This fact might indicate that Klebsiella spp. was more resistant to the environmental conditions in AIT-oxidation ponds than E. coli. The density of C. freundii was much lower than those of E. coli. and Klebsiella spp.. Especially no C. freundii was observed in the maturation ponds.

The density of coliphage was also shown in Fig. 2. More than 99 % of coliphage was inactivated in AIT-oxidation ponds in the same manner as the behaviour of coliform group. That is, a high positive correlation was found between the density of coliform group and that of coliphage. The inactivative efficiency of coliphage in the facultative pond is much higher than that in the maturation pond. Such a

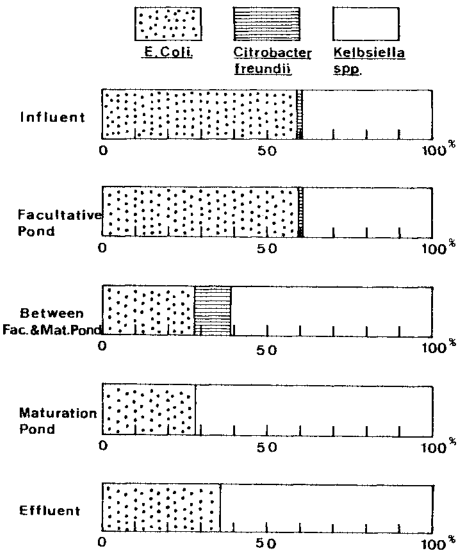

Fig. 3 Percentage of occurrence of coliform group in AIT-oxidation ponds.

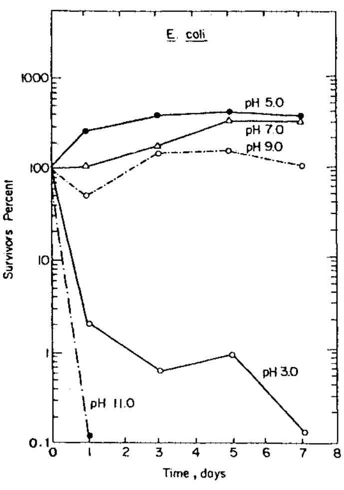

Fig. 4 Effect of pH on die-off of E. coli..

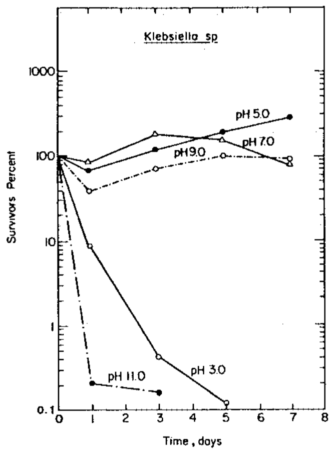

Fig. 5 Effect of pH on die-off of Klebsiella spp..

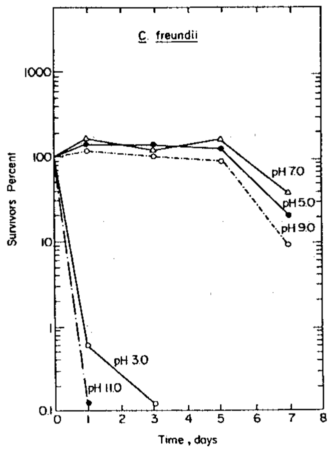

Fig. 6 Effect of pH on die-off of C. freundii.

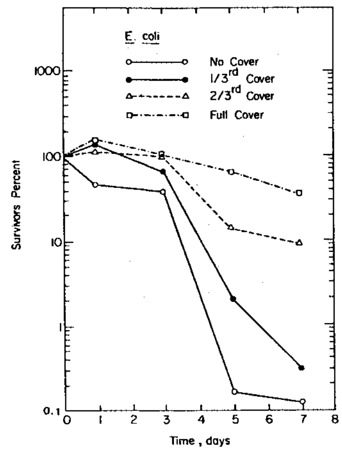

Fig. 7 Effect of sunlight on die-off of E. coli..

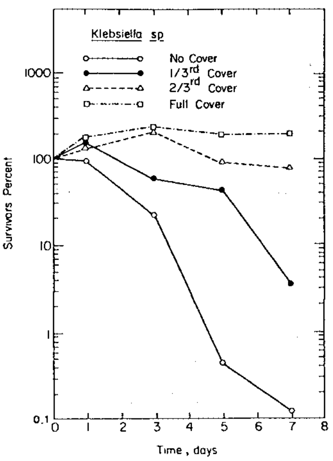

Fig. 8 Effect of sunlight on die-off of Klebsiella spp..

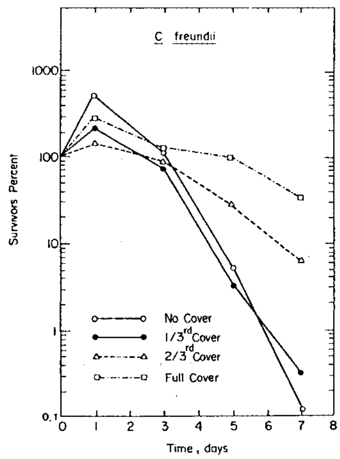

Fig. 9 Effect of sunlight on die-off of C. freundii.

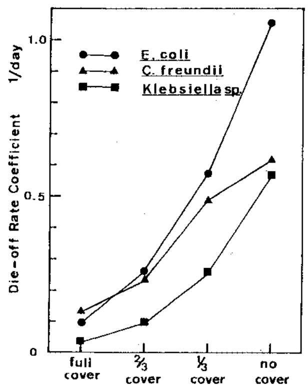

Fig. 10 Changes of die-off rate coeff. with sunlight intensity.

behaviour of coliphage in AIT-oxidation ponds may suggest that coliphage will play an important role in the die-off of coliform group.

(2) Experiments in the laboratory

The results given in Figs. 4, 5 and 6 show the influence of pH on the survivals of E. coli., Klebsiella spp. and C. freundii, respectively. All species of coliform group could multiply or maintain their initial densities during the experiments in the neutral range from pH 5.0 to pH 9.0. However, they are considerably sensitive to high or low pH level, especially to high pH level. As the diurnal variation of pH in the facultative pond ranged from 7.6 to 8.9, the change of pH may not significantly influence the die-off of coliform group.

The effects of sunlight on the die-off of E. coli., Klebsiella spp. and C. freundii were shown in Figs. 7, 8 and 9, respectively. These figures indicate a rapid increase in the die-off rate of coliform group as the increase of exposure to sunlight. That is, sunlight significantly affected the die-off of coliform group in AIT-oxidation ponds. The die-off rate coefficients which were obtained from the slopes of linear parts of die-off curves for coliform group were summerized in Fig. 10. They were calculated by means of least square method. From these results, Klebsiella spp. was most resistant to sunlight, while E. coli. was most sensitive. C. freundii took a middle position between Klebsiella spp. and E. coli.. Taking it into account that Klebsiella spp. was most resistant to the environmental conditions in AIT-oxidation ponds, it is reasonable that Klebsiella spp. was most resistant to sunlight.

4. MODEL FOR PREDICTING COLIFORM GROUP DENSITY IN THE FACULTATIVE POND

As previously mentioned, most of coliform group in the influent were eliminated in the facultative pond, so a model was proposed for predicting coliform group density in the faculative pond. In order to make a model, the diurnal variations of coliform group density, pH and dissolved oxygen in the facultative pond were measured. These data of diurnal variations were applied to the harmonic analysis before being used in modeling.

(1) Proposed model

The facultative pond was assumed as a continuous-flow stirred tank reactor. The model equation was proposed as follows:

$$\frac{dN}{dt}=(N_i-N)/\theta-k\cdot f\cdot N \qquad (1)$$

$f=f_1\cdot f_2\cdot f_3$ or $f_1+f_2+f_3$

where, N : Coliform group density in the facultative pond (cell/100 ml)

N_i : Coliform group density in the influent (cell/100 ml)

θ : Hydraulic retention time of the facultative pond=8 days

k : Die-off rate coefficient of coliform group (1/day)

f : Factor affecting the die-off process of coliform group in the facultative pond (—)

f_1 : Factor of the sunlight effect (—)

f_2 : Factor of the dissolved oxygen effect (—)

f_3 : Factor of the pH effect (—)

Facotr (f) which consisted of the sunlight effect (f_1), the dissolved oxygen effect (f_2) and the pH effect (f_3) was described by two type expressions. That is, one type was expressed by the product of f_1, f_2 and f_3, taking the independency of those effects into account while another one was done by the sum of f_1, f_2 and f_3 considering that they could be looked upon as the exclusive events one another. f_1, f_2 and f_3 were given by the following equations, respectively.

$$f_1=\begin{cases} 0.0 & (0\leqq t<0.25 \text{ or } 0.75\leqq t\leqq 1.0) \\ 0.5+0.5\cos 4\pi t & (0.25\leqq t<0.75) \end{cases} \qquad (2)$$

$$f_2=O_2/O_{2S}/2.0 \qquad (3)$$

$$f_3=22.73(\text{pH}/7.6)^2-45.57(\text{pH}/7.6)+22.84 \qquad (4)$$

where, t : Time (day)

O_2 : Dissolved oxygen concentration in the facultative pond (mg/l)

O_{2S} : Saturation concentration of dissolved oxygen (mg/l)

pH : pH value in the facultative pond

As f_1 means a factor of the sunlight effect, f_1 is zero during the night time ($0\leqq t<0.25$ or $0.75\leqq t\leqq 1.0$), while f_1 is given by the cosine curve during the day time ($0.25\leqq t<0.75$) as shown in eq. 2. Therefore the value of f_1 falls in the range between zero and a unity. f_2 means a factor of the dissolved oxygen effect, so f_2 is expressed as the function of the dissolved oxygen concentration (O_2) as shown in eq. 3. Since the temperature did not almost fluctuate in the facultative pond, the saturation concentration of the dissolved oxygen (7.53 mg/l) at 30°C was given a value of O_{2S}. The reason why the dissolved oxygen effect is added in a model equation is in that many researchers have pointed out the role of the dissolved oxygen in the die-off process of coliform group. f_3 indicates a factor of the pH effect. The results of the laboratory experiment suggested that pH effect on the die-off of coliform group could be nearly neglected. However, as some of researchers have described the high pH level is responsible for the die-off of coliform group in the field and the pH values in the facultative pond range in an alkali side, the pH effect was involved in a model equation. Then f_3 is expressed by the prabolic curve to be zero at pH 7.6 and a unity at pH 6.0 and pH 9.6. It is also unity out of range between pH 6.0 and pH 9.6.

(2) Harmonic analysis

The diurnal variations of the coliform group density, the pH value and the dissolved oxygen concentration in the faculative pond were developed by the harmonic series as follows;

$$O_2=\begin{cases}5.38+0.61\sin(2\pi t-\pi/6)-6.70\cos(2\pi t-\pi/6)-0.42\sin(4\pi t-\pi/3) \\ \quad +1.30\cos(4\pi t-\pi/3)-0.88\cos(6\pi t-\pi/2)+0.03\cos(6\pi t-\pi/2) & (O_2>0.0) \\ 0.0 & (O_2\leqq 0.0)\end{cases} \quad \cdots\cdots (5)$$

$$pH=8.70-0.14\sin(2\pi t-\pi/6)-0.55\cos(2\pi t-\pi/6)+0.06\sin(4\pi t-\pi/3) +0.13\cos(4\pi t-\pi/3)-0.01\sin(6\pi t-\pi/2) \cdots\cdots (6)$$

$$N=10^6\cdot[5.57+1.99\sin(2\pi t-\pi/6)+1.23\cos(2\pi t-\pi/6)+0.14\sin(4\pi t-\pi/3) +0.52\cos(4\pi t-\pi/3)-0.25\sin(6\pi t-\pi/2)-0.33\cos(6\pi t-\pi/2) \cdots\cdots (7)$$

These results were used in estimating the die-off rate coefficient.

(3) Method of optimization

Assuming that the die-off rate coefficient of coliform group is independent of time and differentiating equation 1 with respect to k, the following equation is obtained.

$$\frac{d}{dk}\left(\frac{dN}{dt}\right)=\frac{d}{dt}\left(\frac{dN}{dk}\right)=-(1/\theta+k\cdot f)\frac{dN}{dk}-f\cdot N \cdots\cdots (8)$$

Expressing dN/dk in terms of U which is called the sensitivity with respect to k, equation 8 will be arranged as follows;

$$\frac{dU}{dt}=-(1/\theta+k\cdot f)U-f\cdot N \cdots\cdots (9)$$

Equation 9 is first order ordinary differential equation which is called the sensitivity equation. Since values of f_1, f_2 and f_3 are given by equations 2, 3 and 4, respectively, if a value of the die-off rate coefficient (k) is assumed and initial values of the coliform group density (N) and the sensitivity (U) are given, equations 1 and 9 will be numerically integrated by means of Runge-Kutta-Gill method. $U=0\ (t=0)$ was adopted for the initial value of the sensitivity. Then the coliform group density and the sensitivity can be calulated based on the assumed die-off rate coefficient. Now, if N_{obs} represents the observed value of the coliform group density which was expressed by equation 7, the following equation is derived by Taylor series approximation.

$$N_{obs}-N=\frac{dN}{dk}\cdot\Delta k=U\cdot\Delta k \cdots\cdots (10)$$

where Δk is the correction value on the assumed k. That is, k is corrected so that the difference between N_{obs} and the calculated N can be reduced. This correction is obtained by a least-squares method since values of N_{obs}, N and U have been already known.

$$\Delta k=\sum_{j=1}^{n} U_j(N_{obs}-N)j/\sum_{j=1}^{n} U_j^2 \cdots\cdots (11)$$

This procedure is repeated until Δk is less than 0.01. The die-off rate coefficient obtained by this method is considered a value around 30°C because the water temperature hardly fluctuated in the facultative pond.

(4) Results and discussion

Using the described method, the die-off rate coefficient of coliform group was estimated. At first, the assumed die-off rate coefficient of 1.0 (1/day) was established. And the coliform group density in the influent and the initial coliform group density in the facultative pond had 8.0×10^7 (cell/100 ml) and 6.02×10^6 (cell/100 ml), respectively. Values of f_1, f_2 and f_3 used in the model are shown in **Fig. 11**. In case that

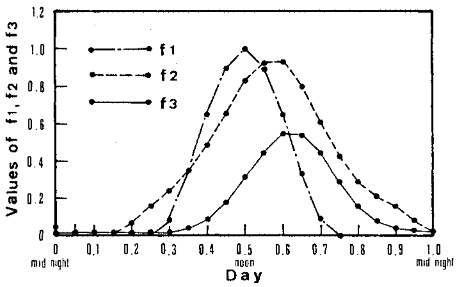

Fig. 11 Values of f_1, f_2 and f_3.

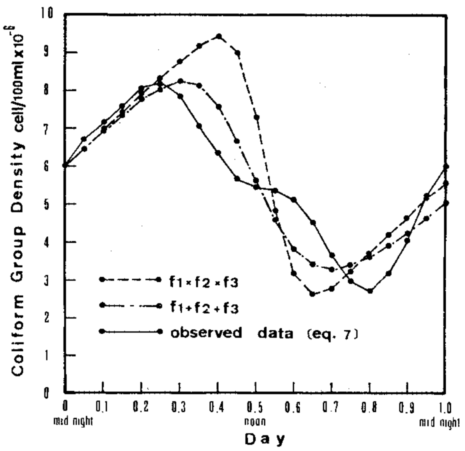

Fig. 12 Results of the estimation fo the coliform group density in the facultative pond.

a factor (f) was expressed by the sum of f_1, f_2 and f_3, after five iterations, the die-off rate coefficient was converged to 2.56 (1/day). On the other hand, in case that a factor (f) was described by the product of f_1, f_2 and f_3, the die-off rate coefficient was converged to 29.1 (1/day) after eight iterations. The results of the integration of equation 1 which were obtained by employing these estimated die-off rate coefficient were illustrated in Fig. 12. From this figure, the model in which a factor (f) was expressed by the sum of f_1, f_2 and f_3 had a better estimation than that by the product of f_1, f_2 and f_3. That is, the die-off process of coliform group in the facultative pond may be dominated by the exclusive relationships among the environmental factors affecting it rather than the independent relationships. Although the simultaneous equations describing the ecosystem in the oxidation ponds should be actually solved in predicting the coliform group density, it is very difficult to get the solution to meet the observed values because of unknown enormous kinetic paramters. Since the method described in this paer is very simple and feasible, this method will be useful for estimating the die-off rate coefficient and predicting the coliform group density in the oxidation ponds.

5. CONCLUSIONS

From this study, the following conclusions may be drawn:

(1) More than 99% of coliform group were removed in AIT-oxidation ponds. Higher removal of coliform group was obtained in the facultative pond.

(2) The percentages of occurrence of E. coli., C. freundii and Klebsiella spp. were changed from place to place in AIT-oxidation ponds. E. coli. was a major type in the influent, while Klebsiella spp. took place of E. coli. in the effluent.

(3) E. coli. was more sensitive to the environmental conditions in AIT-oxidation ponds than others.

(4) Since the positive correlation between the coliform group density and the coliphage density was observed, the coliphage may be one of important factors which affect the die-off of coliform group in AIT-oxidation ponds.

(5) A useful model for predicitng the coliform group density in the facultative pond was proposed. when a factor (f) affecting the die-off process of coliform group was expressed by the sum of the sunlight effect (f_1), the dissolved oxygen effect (f_2) and the pH effect (f_3), a better estimation of the coliform group density was obtained rather than by the product of f_1, f_2 and f_3.

(6) The die-off rate coefficients of coliform group obtained by the model were 2.56 (1/day) around 30°C in case that a factor (f) was expressed by the sum of f_1, f_2 and f_3, and 29.1 (1/day) around 30°C in

case that a factor(f) was described by the product of f_1, f_2 and f_3.

6. ACKNOWLEDGEMENT

Grateful acknowledgement is expressed to the Japanese International Co-operation Agency which provided the funds for this study at the Asian Institute of Technology. The authors would like to express their thanks to Messrs. J. Aizawa, M. Ohtsuburai and S. Chida who assisted in preparing this paper.

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(Received December 13 1985)