PATHOGEN REMOVAL MECHANISMS IN ANOXIC WASTEWATER STABILIZATION PONDS

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Abstract

Anoxic wastewater stabilization ponds can reduce land requirements by as much as two-thirds of that for facultative ponds and can avoid the environmental nuisances of odour and corrosion, compared with anaerobic ponds. When effluent reuse is to be considered, the aim of wastewater treatment is not only to reduce degradable organic matter but also to remove pathogenic microorganisms. There are many pathogenic agents in domestic sewage and determination of all of them is not practically feasible, so, faecal coliform organisms are normally estimated as indicators of health risk.

It has been found that the volumetric organic loading on ponds affects pathogen removal inversely and temperature has a positive effect. This was confirmed in the present studies on "anoxic" ponds and, in addition, other environmental factors (light, pH, DO and ORP) were found to have influenced pathogen removal in these ponds, which were loaded in the range between conventional loadings for anaerobic and facultative stabilization ponds. However, pathogen removal in anoxic ponds was not found to be significantly better or worse than in anaerobic or primary facultative ponds and, therefore, maturation ponds would be required following anoxic ponds to achieve an effluent quality suitable for unrestricted irrigation.

Keywords :
Anoxic Stabilization Ponds; volumetric organic loading; pH; ORP; Temperature; DO; Algae; *E.coli* and *Streptococcus faecalis*.

INTRODUCTION

The removal of faecal bacteria in ponds depends on a variety of mechanisms regulated by both time and environmental conditions, which may in some circumstances act together. The main factors which have significant effects on faecal bacterial die-off are as follows:

I) Organic loading

For primary facultative ponds, Silva(1982) used Marais' presumptions and determined that increased K_b (2.7-10.4d^{-1}) as the volumetric organic loading decreased 57.7-16.2 g BOD$_5$/m^3.d.

Saqqar and Pescod (1992) proved that the rate of faecal coliform die-off (k_b) increased as sewage passed through the Al Samra stabilization ponds in Amman, Jordan, with the lowest k_b in the first anaerobic ponds and the highest in the final ponds.

II) Solar radiation

Solar ultraviolet light, particularly its germicidal component u.v-β (wavelengths in the range 280-320 mm), can play an important role in faecal coliform die-off, if it can penetrate the water. Moeller and Calkins (1980) determined that the survival of faecal bacteria can be less than 0.00002% within the first 10 cm of the surface in a particular maturation pond, 5% within the second 10 cm layer and 100% below 30 cm.

III) pH

The combination of solar radiation ,algal activity and pH (up to 9.8) affect bacterial die-off in wastewater stabilization ponds (Mayo, 1989). In fact, high values of pH resulted from the activities of algae in the presence of sunlight. Pearson, *et al.* (1987) found, both in their laboratory and in situ studies at Loures, Portugal, that pH was a significant factor bringing about coliform reduction in ponds. They proposed that pH values of 9 or above act as a critical factor in faecal coliform die-off in wastewater stabilization pond systems.

IV) Dissolved oxygen

Marais (1974) and Feachem *et al.* (1983) reported that under aerobic conditions, especially at supersaturation levels of DO, faecal coliform die-off is accelerated. In contrast to this report, Pearson *et al.* (1987) reported on laboratory studies at a pH of 9 which showed that little effect on the rate of faecal coliform die-off could be attributed to high dissolved oxygen concentration.

V) Sedimentation

Most bacterial cells may settle as a result of coagulation, which is caused by physico - chemical reactions within pond systems. Therefore, sedimentation is another removal factor which may play an important role in faecal coliform removal, particularly in primary ponds. It has been proposed that primary ponds, particularly anaerobic ponds, reduce faecal bacteria by about 50% as a result of sedimentation (James, 1987). A bacterial removal of about 90% has been reported using sedimentation ponds with hydraulic retention time of 2 - 3 days in treating domestic wastewater (Soares, 1985).

VI) Oxidation -reduction potential

Klock (1974) suggested the effect of oxidation potential as being inversely related to the die-off of faecal coliforms in stabilization ponds.

VII) Starvation induced mortality of faecal bacteria

The effect of starvation on bacterial die-off has been reported by investigators (Gameson & Gould,1985) and is another factor inducing mortality of bacteria in both fresh and sea water. James (1987) proposed the preservation of low organic concentrations (BOD$_5$ <20 mg/l) in stabilization ponds if starvation is to cause a rapid die-off of FC, but maintenance of this level of organic matter is impossible because organic substrate may be provided by algae. Sinclair and Alexander (1984) reported that the death of *Escherichia coli* in a low organic **nutrient** environment was due to a reduction metabolism of *E.coli.*

Oliveira (1990) noted that the low levels of BOD$_5$, (16, 9, 7 mg/l) in the effluent from a series of maturation ponds(No.7,8,9,respectively).

He suggested that the starving conditions (BOD<20 mg/l, after James, 1987) which occurred in these ponds might in itself have favoured the rapid die-off of faecal bacteria as well as exposing them to other stressing factors, such as high temperature, light intensity and pH.

Curtis *et al.*(1992) concluded that environmental factors such as pH, dissolved oxygen, light penetration affect FC as the photooxidative effects.

VIII) Phytoplankton and zooplankton antagonisms with faecal bacteria

According to Legender *et al.* (1984) competition between bacterial species, inhibition of faecal bacteria by phyto-plankton species, probably by absorbtion and predation of these bacteria by zooplankton are forms of antagonism which can act on the regulation of the indicator bacterial populations in aquatic environments and, in particular, in ponds.

IX) Temperature induced faecal bacterial mortality

Rhodes and Kator (1988) attributed the death of *E.coli* and *Salmonella* species in estuarine environments primarily to predation at warmer temperatures but not at temperatures below 10° C, indicating that antagonism may be affected by external seasonal factors which determine the development of phytoplankton and zooplankton, as described by Legendre *et al.* (1984).

Wastewater treatment mechanisms and feacal coliform die-off in anoxic ponds may be expected to follow those in either under-loaded anaerobic ponds or in heavily loaded facultative ponds.

EXPERIMENTAL PROCEDURE

The study reported was carried out by Almasi (1994) in two laboratory scale anoxic ponds having 1m × 0.2m surface area with a useful depth of 0.95m, and maximum volume of 0.19m^3 . An additional anoxic pond with depth of 75cm was used to study the effect of depth. Each pond was lit for 12 hours per day by six fluorescent tubes and was fed continuously with settled sewage from Morpeth Sewage Treatment Works, Northumberland at the required concentration of organic matter in terms of mg/l BOD_5, adjusted by adding a small portion (1 to 5 mg/l) of wastebeer to the collected settled sewage. The settled municipal wastewater used to feed the anoxic ponds had a mean BOD_5 of 300 mg/l and COD of 625 mg/l. Additional sulphate concentration was supplied by adding 1% (w/v) of magnesium sulphate solution. The operating conditions have been provided in Almasi and Pescod (1995).

Pathogen indicators in terms of *E.coli* and *Streptococcus faecalis*, were determined as follows:

E.coli by the membrane filtration technique with Millipore type HAWG 04751 filters and membrane lauryl sulphate broth incubated at 44.5° C for 18-24 hours; *faecal streptococci* were determined using Salnetz & Bartley medium with the plate count incubated at 44° C for 48 hours. The membrane technique was abandoned after six months experimental work because of the high cost involved and the plate count was adopted for both *E.coli* and *Streptococcus faecalis* determinations. Eosin methylene blue medium (Oxoid) was used for *E.coli,* incubated at 37°C for 18-24 hours. Colonies with a greenish metallic sheen by reflected light and dark purple centres by transmitted light were counted as *E.Coli.* Salnetz & Bartley media was used for *Streptococcus faecalis*, incubated at 44°C for 48 hours.A statistical comparison of the two methods for estimation of *E.coli* in the influent was carried out and, although the geometric means produced by the two techinques were very close, the membrane filtration results were found to be more precise.

PATHOGEN REMOVAL RESULTS

Physico- chemical performance data, to be considered alongside the microbiological and biological results reported herein, are given in the companion paper by Almasi and Pescod(1995) . Pathogen removal was studied using *Escherichia coli* and *Streptococcus faecalis* as the pathogen indicators. The number of these microorganisms varied widely in the model pond. *E.coli* were observed throughout the 24-hour period at volumetric organic loadings(VOLs) of 30, 65 and 100g $BOD_5/m^3.d$ and influent sulphate concentration of 325 mg/l, under warm (25°C) conditions. The performance data are presented in Table1.

The results obtained indicate that, in most of the experiments, an *E.coli* removal of 1.5 to 1.9 $\log_{10}$ numbers was achieved, particularly at a volumetric organic loading of 30g $BOD_5/m^3.d$ under both warm(25° C) and cool (10 ° C) conditions. The same rate of *E.coli* removal was observed at a volumetric organic loading of 65 g $BOD_5/m^3.d$, under warm conditions, whereas under cool conditions the removal rate was significantly less. By comparison, the decrease of *E.coli* removal was significant at a volumetric organic loading of 100 g $BOD_5/m^3.d$, particularly under cool conditions.

The removal rates for *Streptococcus faecalis* were approximately the same as for *E.coli*, mean values being 92 and 92.6% at the significance level of 95% ($p \leq 0.05$) for *E.coli* and *Streptococcus faecalis,* respectively. The removal rates of *E.coli* and *Streptococcus faecalis* were significantly affected by volumetric organic loading and pond temperature. Under warm conditions, *E.coli* reduction was 95, 92 and 84% at volumetric organic loadings of 30, 65 and 100 g $BOD_5/m^3.d.$, respectively, while they were 95, 92 and 82% for *Streptococcus faecalis* at a significance level of 95%. These rates were 92 , 86, 91 and 88% for *E.coli* and *Streptococcus faecalis*, respectively, under warm and cool conditions.

Influent sulphate concentration had an insignificant effect on *E.coli* removal.The produced equations gave a correlation coefficient of 0.90 and 0.665 between the percentages of *E.coli* and *Streptococcus* removal rates and the applied parameters. The produced equations apply at a significance level of 95% with $p \leq 0.001$.

Results from in-pond studies indicated that stratification of *E.coli* occurred. These studies were carried out during three experimental runs under different volumetric organic loadings,(30, 65 and 100 g $BOD_5/m^3.d$) and an influent sulphate concentration of 325 mg/l under warm conditions. Stratification was established in the surface layers in the presence of light, whereas it was negligible under dark conditions. These phenomena were more significant in the experiments at volumetric organic loadings of 30 and 65 g $BOD_5/m^3.d$ (Fig.1 and 2), but stratification was also obtained at a volumetric organic loading of 100 g $BOD_5/m^3.d$

Table 1. Mean log Numbers and Removal Rates (%) of *Escherichia coli* and *streptococcus faecalis* in Anoxic Ponds.

E.coli log numbers/100 ml and removal percentage				Streptococcus feacalis log numbers/100 ml and(%)		
RUN No.(*)	Inf.E.coli	Eff.E.coli	Removal (%)	Inf.FS	Eff.F.S.	Removal (%)
1W	7.6231	5.9232	98	6.5507	4.991	97.0
2W	7.3845	5.6611	98.08	6.0423	4.356	97.9
3w	7.2711	5.4917	98.37	6.3849	4.756	97.7
4c	7.1678	5.4523	97.94	6.0445	4.325	98.1
5c	7.0823	5.4121	97.8	6.2203	4.767	96.5
6c	6.9412	5.0123	98.96	6.1344	4.367	98.3
7w	7.2745	5.5524	98.11	6.5402	4.744	98.0
8w	7.0367	5.5921	96.46	6.3563	4.653	98.1
9w	7.0652	5.8632	93.96	6.6205	5.467	92.9
10c	7.3019	6.0634	92.5	6.5318	5.219	95.2
11c	7.2118	6.0815	92.5	6.5645	5.189	95.4
12c	6.9498	6.0317	87.68	6.3891	5.445	88.6
13W	7.8500	6.8511	90	6.8521	6.112	81.4
14W	7.5603	6.4354	92.5	6.5105	5.501	89.9
15W	7.5212	6.8812	77.09	6.6145	5.589	88.2
16C	6.2719	5.4204	84.7	5.5409	4.745	84.0
17C	7.2913	6.6518	76.3	5.9781	5.267	80.6
18C	7.5204	6.8446	79.1	6.7023	5.602	91.6

*W=warm condition(25°C) C=cool condition(10°C) Volumetric organic loading:Runs 1-6 30g $BOD_5/m^3.d$; Runs 7-12 65g $BOD_5/m^3.d$; Runs 13-18 100g $BOD_5/m^3.d$. Inf. = Influent; Eff. = Effluent; SF=Streptococcus faecalis

The derived empirical equations for percentage of *E.coli* removal is as follows:

$$\eta(E.\ coli\ \%) = 95.58 - 0.0021\ \lambda_V^2 + 0.0069\ \lambda_V\ T$$

(r=0.90) (Eq.1)

where,

η=*E.coli* removal, (%)

T=In-pond temperature (° C)

λ_V=Volumetric organic loading(g $BOD_5/m^3.d$)

The effect of temperature on S. feacalis removal was very similar but the influence of sulphure is given in Equ.2:

$$\zeta\ (S.F.\%) = 95.57 - 0.0014\ \lambda_V^2 + 0.0007\ \lambda S$$

(r=0.664) (Eq.2)

where,

S= The influent sulphur (sulphate + sulphide) concentrations (mg/l)

ζ= *Streptococcus faecalis* removal (%)

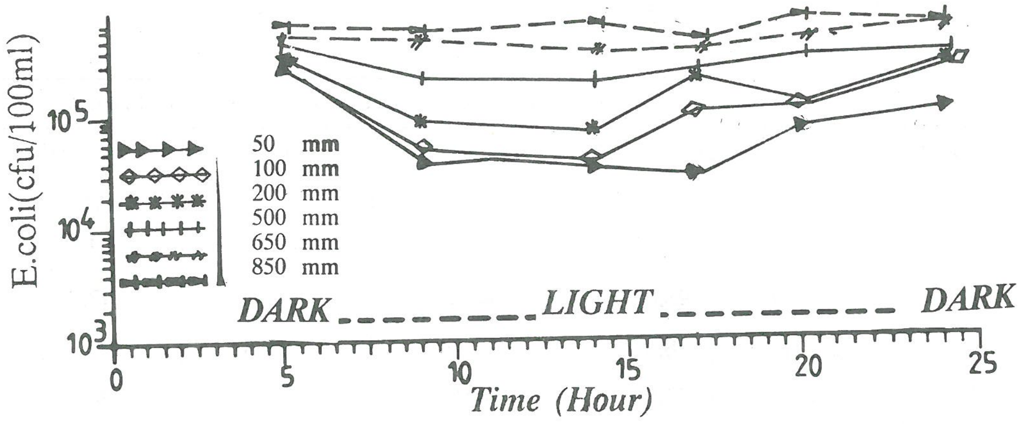

Figure 1 Variation of E.coli during daylight at different depths, volumetric organic loadin of 30 g BoD_5/m^3.d and influent sulphate concentration of 325mg/l under warm condition

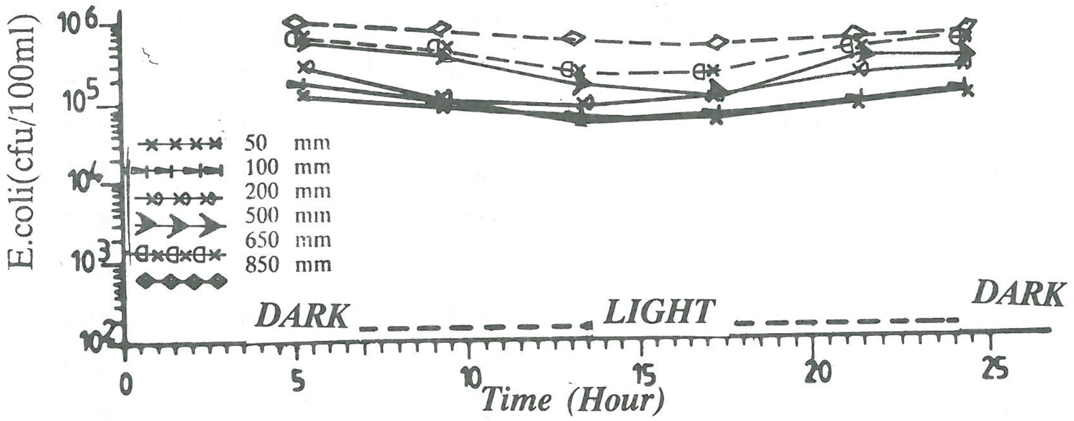

Figure 2 Variation of E.coli during daylight at different depths, volumetric organic loading of 65 g BoD_5/m^3.d and influent sulphate concentration of 325 mg/l under warm conditions.

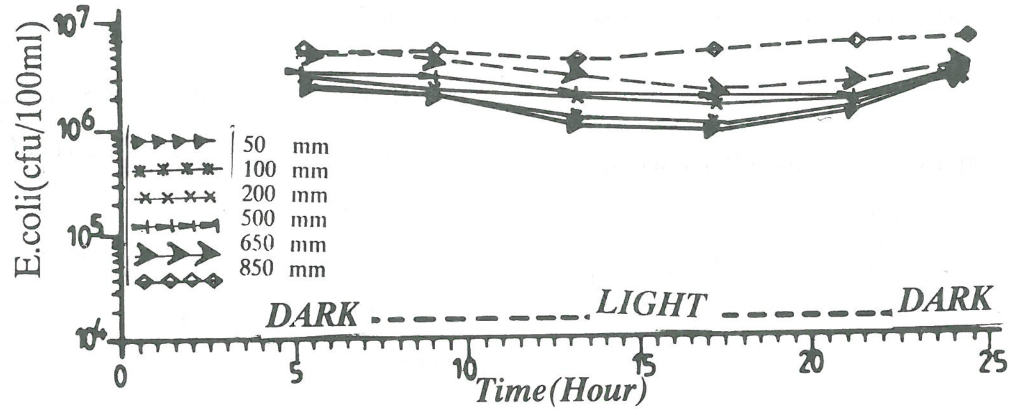

Figure 3 Variation of E.coli during daylight at different depths, volumetric organic loading of 100g BoD_5/m^3.d and influent sulphate concentration of 325 mg/l under warm conditions.

(Fig. 3).Figures 4 to 6 show that *E.coli* concentrations slightly increased with depth in the model anoxic ponds, from the surface to lower layers. Statistical analysis of *E.coli* numbers in the pond contents related to environmental factors (temperature, pH and dissolved oxygen) and depth, revealed, that *E.coli* concentrations were significantly dependent on pH and dissolved oxygen in the surface layer ($r= 0.999$ and $p\leq 0.003$).

Discussion of pathogen removal

The results show that the anoxic waste stabilization ponds performed well in terms of pathogen removal. Pathogen removal is likely to be due to factors which affect bacterial die-off, such as pH , dissolved oxygen, oxidation - reduction potential, temperature, food requirements, sedimentation, predatory action, effect of light and hydraulic retention time. High removal rates for *E.coli* and *Streptococcus faecalis* in all the experiments conducted at a volumetric organic loading of 30 g BOD_5/m^3.d could have been due to most of the above factors, which were all possible influencing parameters under anoxic conditions.

In most of the experimental runs at volumetric organic loadings of 30 and 65g BOD_5/m^3.d, the ponds contained swimming protozoa, such as Paramecium, Cheopeda and Vorticella. Microscopic investigations showed that they harvested algae and, presumably, predated other microbial populations. In this study, pH values (6.8 to 8.2) did not reach critical levels (9 and above) expected to contribute to pathogen die-off,but acceleration of pathogen removal could have been due to the gradual increase in pH during daylight periods, as a result of algal activities.

The results obtained in these studies indicated that temperature had an significant effect on pathogen removal, measured in terms of *E.coli* and *Streptococcus faecalis* numbers. Mean values of *E.coli* removal were 92.4 and 86.8% under warm (25°C) and cool (10°C) conditions, respectively, while they were 91.6 and 88.5% for *Streptococcus faecalis* removal under warm and cool conditions. The confidence level was 95 percent for all cases. In addition, multiple regression analysis of the data showed that temperature interacted with volumetric organic loading and affected *E.coli* removal positively. These effects are **indicated by the** coefficients of the associated parameter in the deduced regression equations (1&2). Temperature will possibly accelerate *E.coli* and *Streptococcus faecalis* die-off as a result of increased metabolic activity and greater susceptibility to toxicants, although this effect was not significant for *Streptococcus faecalis* ($p\geq 0.16$). An increased rate of substrate utilization, which possibly influenced bacterial die-off by starvation, can be attributed to the increase in temperature. The effect of influent sulphur (sulphate and sulphide) concentration on *E.coli* removal was not significant ($p\geq 0.23$) but associated with volumetric organic loading it influenced *Streptococcus faecalis* removal positively. Probably the effect of the influent sulphur (sulphate and sulphide) concentration on *Streptococcus faecalis* removal was due to toxicity of H_2S on this indicator.

The numbers of *E.coli* and *Streptococc* increased in the pond surface layers and, consequently, in the effluent under dark conditions, compared with the results under light conditions. The results of studies on *E. coli* throughout the pond depth and over 24-hour periods are presented in Figures 1 to 6. These figures show that bacterial stratification was established during the light period. The numbers of *E.coli* were low throughout the first 20 centimetres below the surface of the anoxic pond during the light period, whereas the contents of the anoxic ponds were much more homogeneous in the absence of light and there were no significant differences in *E.coli* numbers between the surface and lower layers. Consequently, the low numbers of *E.coli* in the surface layers of anoxic ponds during periods of light might have been due primarily to the effect of photooxidation (pH, dissolved oxygen and light) on bacterial die-off. Figures 1 to 3 show that pathogen indicator concentrations increased from the surface to lower layers in the presence of the light. These could be referred to the indirect effect of phytoplankton, which bring about increase in pH and provide dissolved oxygen in anoxic stabilization ponds. Algae can absorb faecal bacteria and act as a coagulant in pathogen removal. Figures 1 to 3 show the *E.coli* numbers during light periods compared with numbers during dark periods.

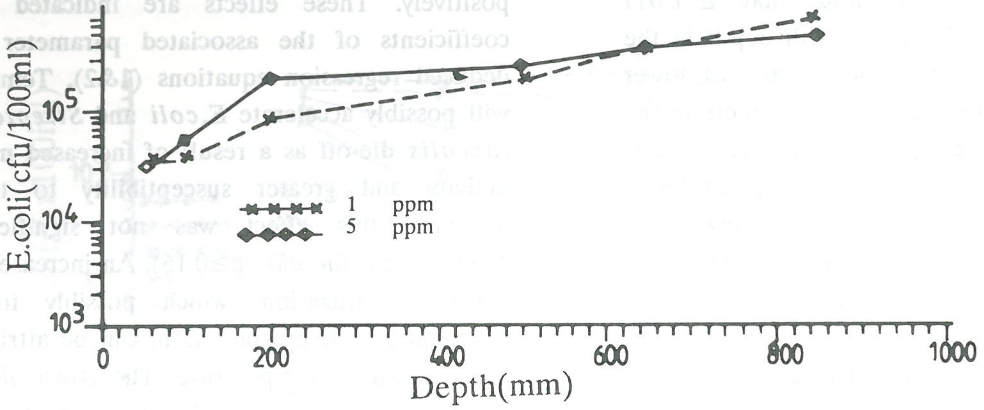

Figure 4 Variation of E. coli throughout anoxic pond depth at volumetric organic loading of 30g BoD$_5$/m^3.d and influent sulphate concentration of 325 mg/l under warm conditions.

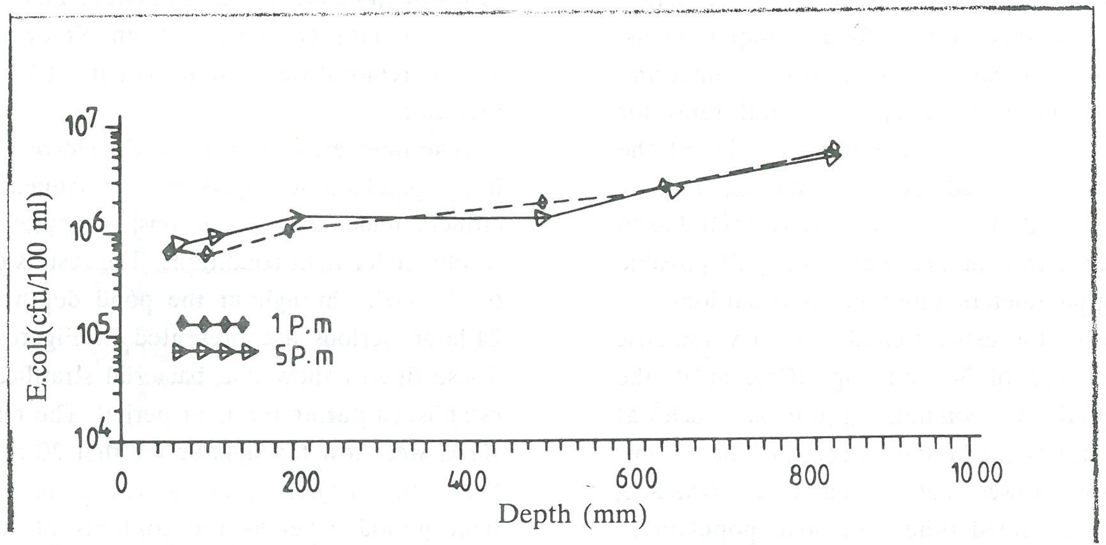

Figure 5 Variation of E. coli throughout anoxic pond depth at volumetric organic loading of 65 g BoD$_5$/m^3.d and influent sulphate concentration of 325 mg/l under warm conditions.

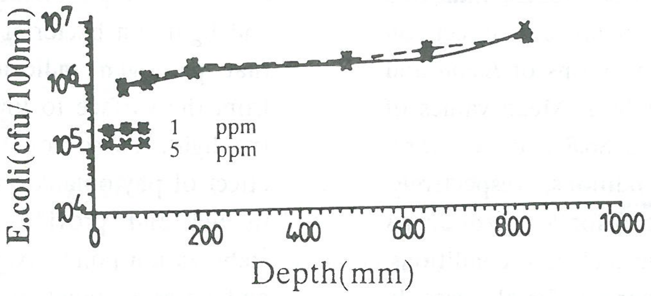

Figure 6 Variation of E. coli throughout anoxic depth at volumetric organic loading of 100 g BoD$_5$/m^3.d and influent sulphate concentration of 325 mg/l under warm conditions.

CONCLUSIONS

The studies reported allow the following conclusions to be drawn:

I) Bacterial die-off was high at a volumetric organic loading of 30 g BOD$_5$/m^3.d, most probably due to a series of factors which occur under anoxic pond operating and environmental conditions, such as light penetration, pH value (7.5 to 8.2), dissolved oxygen, relatively high oxidation - reduction potential, predators and long hydraulic retention time.

II) Under all volumetric organic loadings, bacterial die-off was lower under cool conditions by comparison with warm conditions. This may be due to the effect of temperature on factors which affect bacterial die-off;

III) *E.coli* and *Streptococci* removal decreased from the surface to the bottom of the anoxic ponds. Presumably, this is due to the combination of factors mentioned above (light, pH, dissolved oxygen, precipitation, predators and sensitizers). The effects of these factors are reported by Curtis *et al*, (1992). The surface layer was not a suitable habitat for survival of these pathogenic bacteria. Homogeneous concentrations of *E.coli* were observed in the pond contents in the absence of light, which indicates the effects of light, pH, dissolved oxygen in the euphotic zone during light periods;

IV) *E.coli* and *Streptococcus faecalis* removals decreased as volumetric organic loading increased from 30 g BOD$_5$/m^3.d to 100 g BOD$_5$/m^3.d. This may have been due to the change in pond operating conditions (environmental conditions, such as pH, DO, ORP and predators) representing the change from anoxic to anaerobic loading. Anaerobic conditions are more suitable for the survival of *E.coli* and *Streptococcus faecalis*, as a result of the less hostile environmental conditions;

V) Mean log$_{10}$ numbers of 5 and 4 for *E.coli* and *Streptococcus faecalis*, respectively, remained in the anoxic pond effluent during most of the experiments;

IX) The empirical equations produced, based on percentage of *E.coli* and *Streptococcus* removals, indicate that the removal rates depended on volumetric organic loading, temperature and, partially, on influent sulphur concentration. The effect of influent sulphate and sulphide sulphur concentrations on E.coli removal was not significant, whereas *Streptococcus* removal was positively influenced by an interaction of influent sulphur and volumetric organic loading. The removal rates depended on these factors with correlation coefficient of 0.90 and 0.664 for *E.coli* and *Streptococcus faecalis*, respectively, at significance levels of 95% and p≤0.001.

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