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Introduction

In algal-based ponds, algae may directly or indirectly play a key role in the modes of removal of fecal bacteria from domestic wastewater. Some indirect modes of removal that may be related to algal presence include starvation, invertebrate grazing, sedimentation and photo-oxidation . Algal growth utilises nutrients and carbon sources that bacteria may need for its survival and may occur in pond systems with long retention times. Grazing and sedimentation may depend primarily on bacterial attachment to algae. Bacterial attachment to living organisms has been observed in freshwater ecosystems (Kansiime and Nalubega,1999), but the importance of this phenomenon in wastewater treatment systems is not well documented. Bacteria may form biofilms in aquatic ecosystems on surfaces of suspended matter including algae. Rotifers and macro-invertebrate scrapers like the larvae and nymphal stages of some aquatic insects ingest algal cells and hence some bacteria associated with these algae. This may however occur only in algal ponds with relatively better effluents quality, where insects and other invertebrates may colonise at various depths. Algal cells may also sink under their weight with attached fecal bacteria thus removing them from the water column. Photo-oxidation, the absorbtion of solar radiation by sensitizer molecules leading to the formation of oxygen radicals that are toxic to fecal bacteria has been observed to increase with increasing oxygen concentration of the system as a result of the presence of algae (Curtis et al. 1992).

In maturation ponds of domestic wastewater treatment systems however, the rapid development of algae may reduce the removal efficiency of these ponds in that short wavelengths of solar radiation are unable to penetrate such highly turbid systems (Van der Steen, 2000 and Davies-Colley et al. 1993). In such systems only long wavelengths may be useful in helping to achieve fecal bacteria die-off (Sinton et al, 2002). Increased turbidity of algal systems may result in increased pH which could kill fecal bacteria even in the absence of sunlight (Parhad and Rao, 1974). The extents of pH increases in these systems however depend on the buffering capacity of the wastewater.

The presence of organic matter in wastewater in algal systems provides ample source of carbon and energy for bacterial survival (Servais et al. 1987, Salonen et al. 1992; Servais et al. 1992). Organic matter in wastewater are humic substances and could serve as sensitizer molecules that can be toxic to fecal bacteria (Curtis et al. 1992). Their role therefore may be a net effect of the sum total of the two processes and this is hardly reported in literature. Lysis of algal cells increases with increased algal density. Algal cells secrete organic compounds and their lysis may lead to the release of significant amounts of algal organic matter (Plummer et al. 2000). Algal organic matter were

observed to have assisted in maintaining the *E. coli* cultivability (Bouteleux et al. 2005). We hypothesize that the net effect of organic matter (from all sources) in domestic wastewater treatment ponds may tend to promote fecal bacteria survival at higher algal cell concentrations. The aim of this study is to understand better, the role of algae in the removal of fecal bacteria from algal based ponds and to specifically investigate the effect of varying algal concentrations on fecal bacteria removal in algal based ponds. Batch laboratory experiments were conducted using light of wavelengths, 380-780nm. Algae were grown by natural colonization and used for inoculating the setup.

Materials and Methods

Algae were grown in the laboratory by natural colonization in artificially prepared nutrient solution containing 13.5mg/L nitrogen and 2.2mg/L phosphorus in the form of nitrates and phosphates respectively. The algal culture was allowed to stand under light of wavelength 380-780nm provided by a powerstar HQI-BT 400 metal halide lamp for 14 days.

250mL of raw domestic wastewater obtained from a treatment plant were introduced into each of four sets of sterile Erlenmeyer flasks. Each set consist of six (6) flasks. Harvested algae from culture setup were used to inoculate five (5) of each of six (6) flasks belonging to each set, one flask maintained as control, having no algae. The following mean chlorophyll a concentrations were obtained for each set: 0, 1,199, 1,653, 6,734, 17,464 and 33,892μg/L. Two sets of flasks were covered with dark polythene sheets and placed on a GFL 3019 orbital shaker together with the other two uncovered sets in a randomised block design. The GFL 3019 shaker was placed under the HQI-BT 400 lamp in a regime of 16hours light and 8 hours darkness rotating at 100rpm. Temperature of setup varied from 20 ^{o}C to 25^{o}C.

Fecal coliform die-off or culturability in setups were monitored at time t = 0, 0.25, 1, 3, 5 and 7 days by plating samples on chromocult medium, incubating at 35-37 ^{o}C for 24 hours for colony counts. Loss of fecal coliform culturability was assumed to be die-off and therefore used in assessing the rate of decay of fecal coliforms in the treatment systems. Algal concentration of setups is obtained by finding the mean concentrations of that at the beginning and end of experiment. Dissolved oxygen concentration and pH of reactors were also monitored using WTW 330 Oximeter and WTW 340 pH meter respectively. The decay rates in treatment systems were compared statistically using an independent sample t-test. Samples taken at T=1 day were pushed through syringes fitted with needles to detach any attached bacteria. Faecal coliform numbers before and after pushing through syringes were statistically compared using paired samples t-test of SPSS.

Results/Discussion

Effect of light

Fecal bacteria die-off rate, Kd was calculated using the first order decay equation

Kd = (lnNo-ln Nt)1/t.

No and Nt being fecal bacteria numbers at start of experiment and at time t, respectively. The effect of light on faecal coliform decay was investigated by comparing treatment systems in darkness with treatment systems in light. In all the treatment systems significantly higher die-off of faecal coliforms were observed in treatment systems in light compared to treatment systems in darkness ($p<0.001$) showing that light played an important role in achieving die-off (Fig.1). Significant die-off in control system with light compared with control in darkness with no algae suggests that the effect of light on faecal coliform die-off in the treatment systems included a direct effect.

Rate of die-off of faecal coliforms varied with varying algal densities (Fig.2).Variation in t-values from control, through lowest algal density to highest algal density suggest a complex interaction of light with other factors to achieve die-off. At a lower algal density (1199μg/L), differences in means of dark and light treatment systems were much higher than in control setups and this gradually decreased with increasing algal density (Fig.2). This decrease in the differences in means with increased algal density could be attributed to the higher turbidity resulting in light attenuation.Differences however began to increase again at even higher algal density and this could be explained by the increased pH and DO observed in this (higher algal density) treatment systems (Fig.3 and 4).

Effect of algal density in light, pH and DO concentration

The effect of algae is revealed by comparison of treatment systems with and without algae in light and in darkness, as well as treatment systems with varying densities of algae.A comparison of control in light with other treatment systems in light showed significantly higher die-off in all treatment systems except the 1653μg/L treatment system which showed comparable die-off as in control setup. With the exception of the 1653μg/L system in light which had significantly lower decay rate in light than all the other treatment systems in light (excluding control setup), die-off rates decreased with increasing algal density.It was anticipated that other treatment systems with higher algal densities would show a lower linear rates die-off but this was not so probably due to rapid increases in DO in the higher treatment systems towards the last two days of the experiment. It can however be seen that optimum decay rates as far as this experiment is concerned is found in the 1199μg/L treatment system in light. It is possible that higher die-off would have been observed in treatment systems with a lesser concentration of algal density. Mean DO concentration in other treatment systems were less than the 8.1mg/L observed in the 1199ug/L system placed in light. pH variation showed a similar trend as DO suggesting that die-off may have been enhanced by increased pH. Curtis (1990) noted that more penetrating, longer wavelengths of >440nm could not damage

faecal coliforms at pH values of less than 8.Even the control setup had pH that was slightly higher than 8 (Fig.4). From day 0 to 3 of experiment, very low oxygen concentrations with correspondingly low pH levels were observed in very high algal density treatment systems probably due to an increased breakdown of algal cells leading to increased amount of organic matter. With increased exposure to sunlight however, rapid increases were recorded as seen in the standard deviation bars of Fig.3 and 4.

Effect of algae in darkness

Interestingly, apart from the 1199μg/L algal system which had lower die-off rate in darkness compared to the control kept in darkness, all the other treatment systems kept in darkness had significantly higher die-off rate than the control setup. This observation seems to be consistent with some observations and deductions made in the previous experiment, suggesting that a certain amount of nutrient or perhaps organic matter or both is capable of enhancing the survival of faecal coliforms in algal treatment systems. In that experiment also, the optimum algal density in light for FC destruction was the same density promoting the highest survival of FC in darkness. A similar observation is made here for the 1199μg/L treatment system. Perhaps at such algal densities, enough organic matter from both wastewater and algal cells balances any toxic effects that may exist alongside. Significantly higher die-off rates were observed in treatment systems with algal densities higher than 1653μg/L placed in darkness. Here also pH and DO levels were comparable in these treatment systems suggesting that other factors or factor may be present that is toxic to faecal bacteria presence. As systems are kept in darkness, this toxic factor cannot be oxygen radicals as sunlight is needed to send these radicals into an excited state, capable of damaging bacteria cells and hence exposing them to harsh environmental conditions. This observation which has been consistent in previous experiments gives credence to the assertion that some algae may produce toxins that are detrimental to faecal bacteria (Maynard et al, 1999).

Conclusion

The experiment supports the assertion that long wavelengths in the range of 380-780nm are capable of damaging faecal coliforms in the presence of humic substances found in domestic wastewater. In light, faecal coliform die-off increases with increasing algal density till a certain critical concentration, after which faecal coliform decay decreases. The optimum algal concentration for faecal coliform die-off in this experiment was 1199μg/L but given that this was the least concentration used in this experiment, a less algal density could yield even higher rate of decay of faecal coliform. The optimum algal density in light for FC destruction was the same density promoting the highest survival of FC in darkness. Expectedly very high algal densities had lower die-off rates. In algal systems where high decay rates were observed, the synergistic effect of light, oxygen and high pH may have played a crucial role. Observed decay rates increased with increased oxygen concentration and pH in light. Presence of algae in darkness seem to enhance the survival of faecal coliforms at certain algal density. The survival levels in these conditions however appear to depend on algal density levels and other counteracting effect in these treatment systems which may be toxic to the faecal coliform.

Higher algal densities in darkness, giving higher rate of die-off even with comparatively similar pH and DO concentration were observed. This observation, which has been consistent in previous experiments, gives credence to the assertion that some algae may produce toxins that are detrimental to faecal bacteria.

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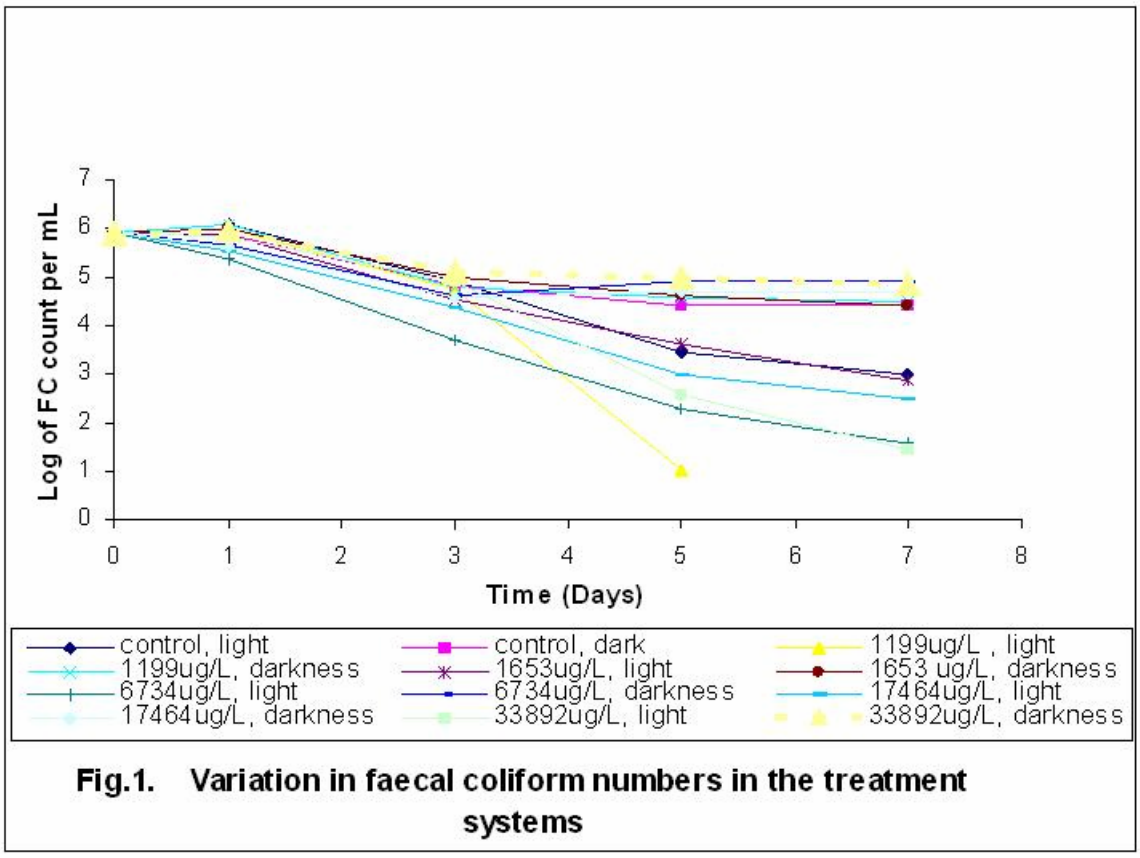

Fig.1. Variation in faecal coliform numbers in the treatment systems

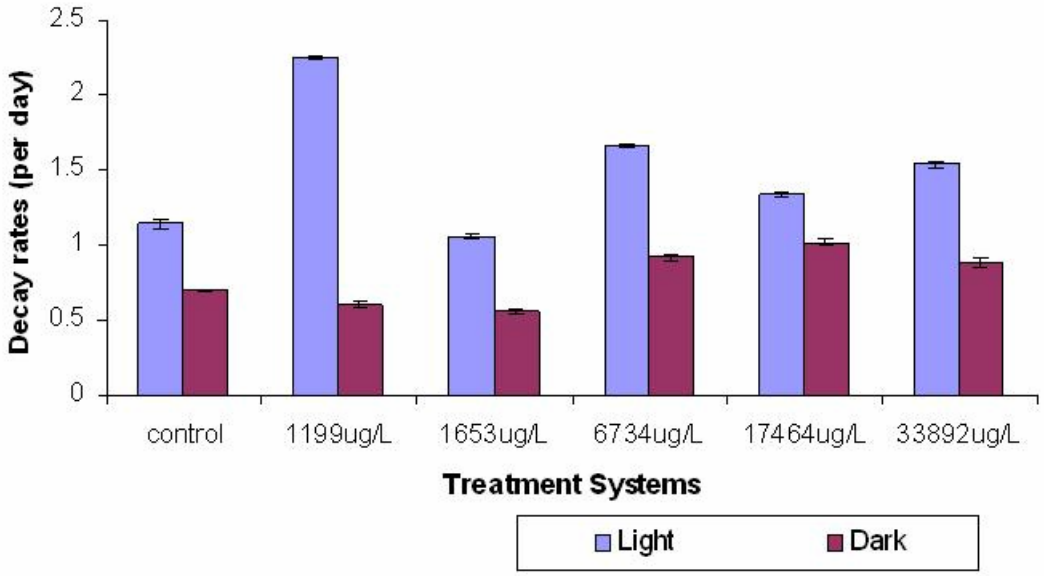

Fig.2. Decay rates in treatment systems under light and dark conditions

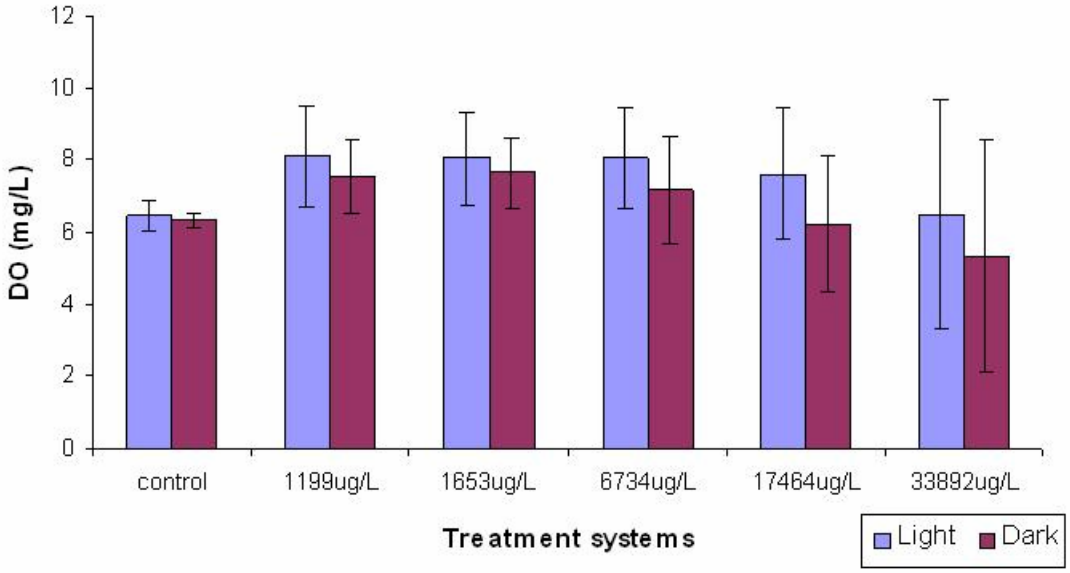

Fig.3. Dissolved Oxygen variation in light and dark treatment systems

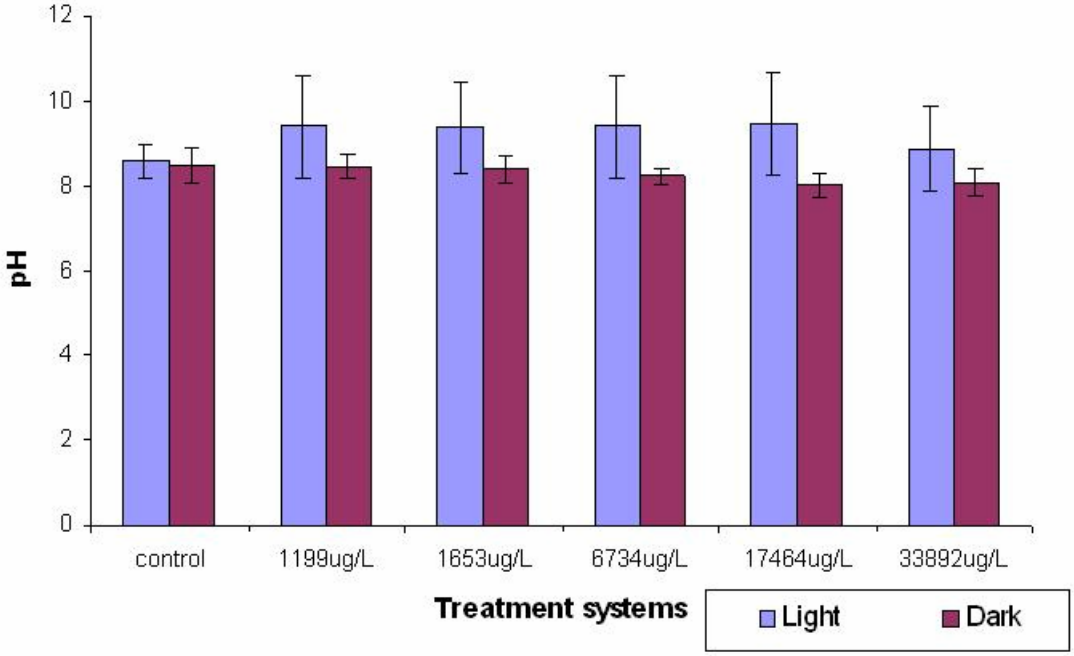

Fig.4. pH variation in light and dark treatment systems