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Sunlight inactivation of faecal coliforms in domestic wastewater

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ABSTRACT

The relationship between sunlight effect, algal biomass and faecal coliform inactivation in wastewater pond treatment systems is still not clearly understood. Increased pH and dissolved oxygen concentration in treatment ponds results in an increased destruction of faecal coliforms. Increased algal growth however results in a decreased destruction of faecal coliforms due to light attenuation. Algae also releases variable amounts and types of organic matter at various rates and quantities depending on environmental conditions and this can either aid or retard faecal bacteria destruction. We investigated the effect of algal density on faecal coliform destruction under field conditions in sunlight and darkness and how this can be affected by light intensity. In darkness, increased inactivation of faecal coliform occurred with increasing algal density. Rates of decay of faecal coliforms were much faster in sunlight than in darkness even in the absence of algae. In sunlight, rates of decay of faecal coliforms increased with increasing algal density up to a chlorophyll-a concentration of 1.3 ± 0.1 mg/L after which rates of decay decreased. Increased decay rates of faecal coliforms occurred with increasing light intensity or light input. With decreased light input of 20% of 213 W/m^2, the optimum algal density for maximum faecal coliform decay decreased to a value which is 6–7 times the value of that under normal insolation of 213 W/m^2. It is recommended that in future studies relating to the assessment of performance and estimation of rate of *Escherichia coli* or faecal coliform inactivation, one of the parameters that need to be reported as well is the insolation.

Keywords: Algae; Insolation; Treatment; Waste stabilization ponds

1. Introduction

Waste stabilization pond systems are amongst the most efficient and ecologically friendly technologies available for the removal of pathogenic bacteria in domestic wastewater. The mechanism of removal of pathogenic bacteria, as indicated by the removal of faecal coliforms however, is still not clearly understood. A better understanding of the removal mechanisms may help in optimizing the removal efficiency.

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The elevation of pH and concentration of dissolved oxygen as a result of algal photosynthetic activity renders the aquatic environment hostile to faecal coliforms. Curtis et al. [1] showed sunlight damaged faecal coliforms (FC) in waste stabilization ponds and that the damage was completely dependent on oxygen, the rate of damage being proportional to the oxygen concentration. Sunlight inactivation of *Escherichia coli* is known to increase strongly with pH greater than 8.5 [2]. pH also acts synergistically with dissolved oxygen in a process known as photo-oxidation to achieve die-off of faecal coliforms [3]. Van der Steen et al. [4] showed that light attenuation occurs at high algal densities and argued that rapid algal growth that occurs in tropical regions may compromise the gains of increased pH and oxygenation. Van der Steen et al. [4] hypothesized that an optimum algal density may exist whereby maximum faecal coliform die-off is achieved. This was however not proven by experimentation. Ansa et al. [5] showed that under laboratory conditions using artificial light with wavelength 380–780 nm, increased algal density leads to increased rate of faecal coliform inactivation till a certain optimum algal density after which the rate of inactivation decreases. It is however not known whether this phenomenon may occur in natural conditions in the field using solar radiation and to what extent.

Part of the objectives of this paper thus is to investigate the occurrence of an optimum algal density for maximum decay of faecal coliforms under batch conditions in the field and how this optimum algal density can be affected by reduced light intensity as indicated by a reduced light input. Increased intensity of sunlight can lead to increased rate of faecal coliform inactivation in waste stabilization ponds [4] but it is not known how this change in light intensity can affect the variation of decay rates of faecal coliforms in varying conditions of algal biomass in the treatment system and consequently the optimum algal density. This is particularly so as the degree of light attenuation may differ with different light intensities.

The aim of this paper therefore is to investigate, in addition to the objectives stated above, how light intensity may influence the faecal coliform die-off rates in a continuous flow system. Curtis et al. [1] and Van der Steen et al. [4] investigated how light intensity may affect faecal coliform inactivation rates in batch reactors but conditions in batch reactors are different from that in continuous flow systems. Under batch conditions, the reactor or vessel in which the processes are taking place do not receive any inflows or experience any outflows. The continuous flow system however experiences an inflow and outflow in contrast with batch conditions. The estimation of the effect of light intensity is necessary to estimate the extent of variation in changes in decay rates with changes in weather conditions as pertains in warm and cold climates.

2. Materials and methods

2.1. Algae culture and preparation

Algae were allowed to grow naturally in 500 mL of equal portions of tap and demineralized water with artificially prepared nutrient solution containing 13.5 mg of nitrogen and 2.2 mg of phosphorus in the form of KNO_3 and KH_2PO_4, respectively. Temperature conditions varied from 26 to 29°C during period of algae culture as well as period of experimentation. Resulting algae were mainly *Chlorella* sp., just as occurred in waste stabilization ponds and these were harvested after 14 d, sieved using 250 and 90 μm mesh nets and concentrated by centrifugation at 1,000 rpm for 30 min into a thick algal paste. Experiments made use of domestic wastewater obtained from the grit chamber of the Kotoka International Airport treatment plant, Accra, Ghana. The characteristics of the domestic wastewater are shown in Table 1, determined according to methods outlined in Ref. [6]. The experimental set-up was situated on the CSIR Water Research Institute premises in Accra, Ghana (5° 44′42″ N, 0° 6′27″ W) in the open and received an average insolation of 213 W/m^2 [7].

2.2. Incubation in batch reactors

In order to investigate the occurrence of an optimum algal density for maximum decay of faecal coliforms under batch conditions and how this optimum algal density can be affected by reduced light intensity under natural field conditions, concentrated algal samples prepared as described above were used to inoculate 400 mL of domestic wastewater in 500-mL plastic containers. Two replicates of set-ups having 0 (control), 0.2, 1.2 and 2.3 mg/L algae were obtained and placed in the open to receive sunlight. This resulted in 2.12×10^7, 2.13×10^7, 2.16×10^7 and 2.20×10^7 cfu 100 m/L of faecal coliforms as starting concentrations of faecal coliforms for the respective incubations. A second set of two replicates of same concentrations of algae in wastewater were covered with dark opaque lids of the plastic containers and placed together with the rest of the set-ups in open sunlight as incubations in darkness. A third set of two replicates of same concentrations of algae in wastewater were covered with opaque lids of the plastic containers with only 20% of its area exposed to sunlight and placed together with

Table 1
Characteristics of raw wastewater and holding tank effluent

	Wastewater[a]	Holding tank effluent[a]
Temperature (°C)	30 ± 2	30 ± 2
pH	7.2–7.4	7.2–7.6
BOD (mg/L)	236 ± 33	190 ± 21
Ammonia (mg/L)	44 ± 31	43 ± 7
Nitrite (mg/L)	BDL	0.2 ± 0.1
Nitrate (mg/L)	BDL	0.2 ± 0.1
Total phosphorus (mg/L)	4.5 ± 0.2	4.1 ± 0.1
FC count (100 m/L)	$2.2 \times 10^7 \pm 2.0 \times 10^6$	$2.12 \times 10^7 \pm 6.0 \times 10^6$

[a]±: standard deviation; $N = 9$; BDL: below detectable limit; FC: faecal coliforms.

the rest of the set-ups in open sunlight. The 500-mL plastic containers were embedded in moistened sand in a wooden tray placed on a table. The incubations of different algal densities in sunlight and darkness were monitored for seven days for changes in faecal coliform numbers, temperature, pH, dissolved oxygen and algal concentrations. Loss of water by evaporation was compensated with dimineralized water.

Fluctuations in pH and dissolved oxygen concentration (DO) were monitored daily at 08.00–09.00 h GMT with a WTW 340 pH meter and WTW 330 Oximeter probes, respectively. "pH and DO was measured during that time to give an idea of how alkaline and oxygenated the system remains after much depletion of oxygen in the night when photosynthetic activity is absent."

Faecal coliform counts were noted at time 0, 1, 3, 5 and 7 d after incubation and this was estimated by plating on chromocult agar medium, incubated at 35–37°C for 24 h [8] using spread plate technique [6]. Algal concentrations were estimated by the use of chlorophyll-a concentration using a spectrophotometer according to Ref. [9]. Algal concentrations are averages of that measured at the beginning and end of the incubation. The decay rates, K_d of the faecal coliforms in the incubations were calculated from the regression line of the first-order decay equation below [10]:

$$\ln N_t = -Kdt + \ln N \quad (1)$$

where N_t is the FC count 100 m/L at a time t; N_o is the FC count per 100 mL at the start of the experiment and t is the time (d) of incubation.

Decay rates were compared statistically using an independent sample t-test of Minitab 15.0 statistical package. Faecal coliform numbers before and after pushing through syringes were statistically compared using paired samples t-test to assess whether faecal coliforms were attaching to each other and thus resulting in underestimation of faecal coliform numbers. No differences in numbers were observed after being pushed through the syringes suggesting that decreases in faecal coliform numbers can be attributed to actual die-off [11].

2.3. Continuous flow experiment

A pilot-scale treatment plant consisting of four ponds in series (Fig. 1) was set up in the open on the CSIR Water Research Institute premises in Accra, Ghana (5° 44′42″ N, 0° 6′27″ W). Each pond has a diameter of 45 cm and a depth of 30 cm and receives domestic wastewater from a holding tank (HT) measuring 100 cm × 100 cm × 100 cm. The wastewater flowed by gravity and was regulated by taps installed between the holding tank and the influent ponds. A hydraulic retention time of 5 d was maintained in each pond and average wastewater flow rate was 6.9×10^{-3} m^3/d. Raw domestic wastewater was obtained from the grit chamber of the Kotoka International Airport treatment plant to feed the holding tank. The characteristics of the raw wastewater

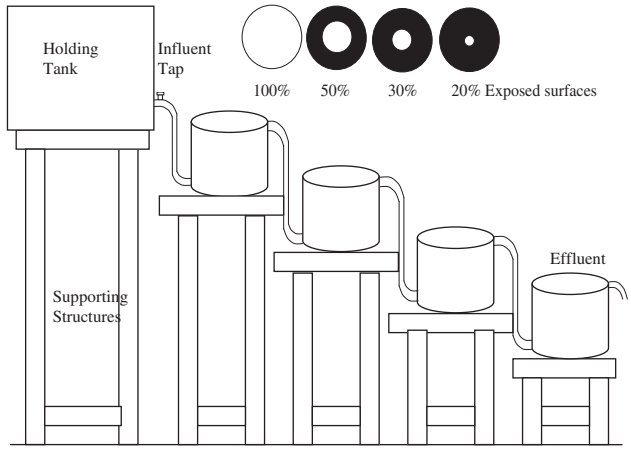

Fig. 1. A sketch of the pilot-scale treatment plant.

and the effluent of the holding tank are shown in Table 1. Algae development in the algal ponds occurred naturally as occurred in waste stabilization ponds. The experiment was conducted after steady-state conditions had been achieved. Under continuous flow conditions, the effect of precipitation and evaporation was assumed to be negligible.

Fluctuations in pH and DO were monitored once a week for five weeks at 08.00–09.00 h GMT. These were measured by WTW 340 pH meter and WTW 330 Oximeter probes placed 10 cm below the pond surface. Effluent samples taken from the outlet of each of the four ponds at 08.00–09.00 h were analysed for FC counts by plating on chromocult agar medium, incubated at 35–37˚C for 24 h [8] using spread plate technique [6]. The series of four pond system were exposed to sunlight with the whole pond surface exposed (100%), and 50, 30 and 20% exposed for a period of five weeks each. FC counts were done weekly using three replicates per sampling time. The decay constant (K_d) was obtained as the gradient of the regression line of Eq. (2) approximating it to be first order [4] and assuming completely mixed conditions in each pond:

$$1 / (1 + K_d * \theta/n)^n = N_t/N_o \quad (2)$$

where n is the number of ponds in series; K_d is the decay constant (d^{-1}); N_t is the effluent FC count at a time t (100 m/L); N_o is the influent FC count (100 m/L) and θ is the total retention time (d).

3. Results

Sunlight had a significant effect on faecal coliform die-off as decay rates were significantly higher in all

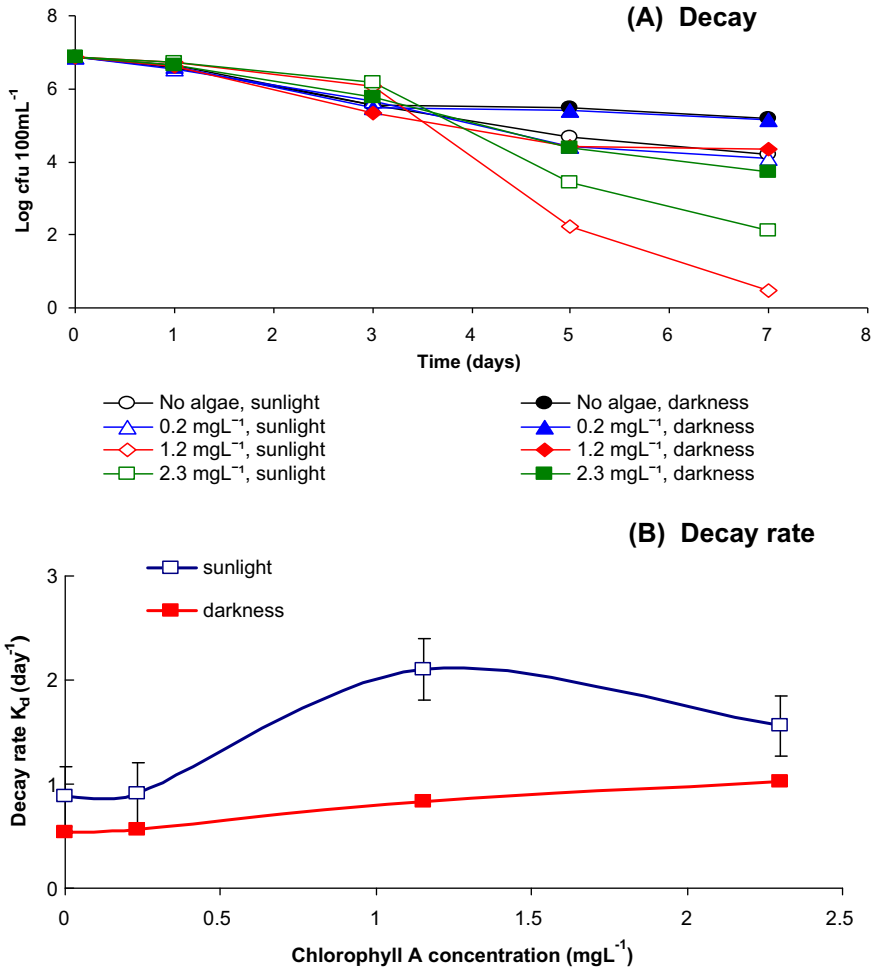

Fig. 2. Faecal coliform decay during incubation (A) and decay rate variation with algal density and (B) in sunlight monitored at 08.00–09.00 am. Point values represent the means of duplicated treatments with three sub-replicates ($N = 6$). Standard deviation of point values of incubation in darkness is <0.1.

incubations in sunlight compared to corresponding incubations of same algal densities in darkness (Fig. 2).

Higher rates of decay of faecal coliforms were observed in incubations under solar radiation compared to control (no algae). The only exception is the 0.2 mg/L incubation which had comparable rate of decay as in the control. Up to an algal density of 1.2 mg/L in sunlight, a strong positive correlation of algal density with decay rate ($r = 0.984$, $N = 9$) was observed. Beyond the algal density of 1.2 mg/L, decay rates decreased with increased algal density (Fig. 2).

3.1. Effect of algal density, pH and DO concentration

Increased pH with time in sunlight positive algal incubations were observed and these pH values were higher than those in darkness. Highest pH observed for control incubations was 8.4. Similarly, dissolved oxygen concentrations in algal incubations increased with time with higher dissolved oxygen concentrations occurring in sunlight positive algal incubations (Table 2). Dissolved oxygen concentration rose above 12.0 mg/L for non-control incubations. Algae still had an effect in darkness. A positive correlation was observed between algal density and decay rates in darkness ($r = 0.97$, $N = 12$, Fig. 1).

3.2. Effect of light input

Reduced input of sunlight, as a measure to simulate reduced sunlight intensity resulted in decreased decay of faecal coliforms in comparable algal density incubations (Fig. 3). Optimum algal densities for maximum faecal coliform removal differed under different sunlight inputs, with highest decay rate of the 20% sunlight input incubation being lower than lowest decay rate in 100% sunlight input incubations.

Table 2
Variation in pH and dissolved oxygen concentration (± standard deviation) during period of incubation in sunlight and darkness

	Light		Darkness	
Concentration[a]	pH	DO (mg/L)	pH	DO (mg/L)
0	8.3–8.4	5.8 ± 5.1	7.7–7.8	1.3 ± 0.6
0.23	8.2–8.8	6.8 ± 5.6	7.8–7.9	1.2 ± 0.6
1.15	8.8–9.8	9.6 ± 7.1	7.8–7.9	1.2 ± 0.7
2.3	8.9–9.9	9.3 ± 6.7	7.7–7.9	1.5 ± 0.8

[a]Chlorophyll a concentration of algae (mg/L).

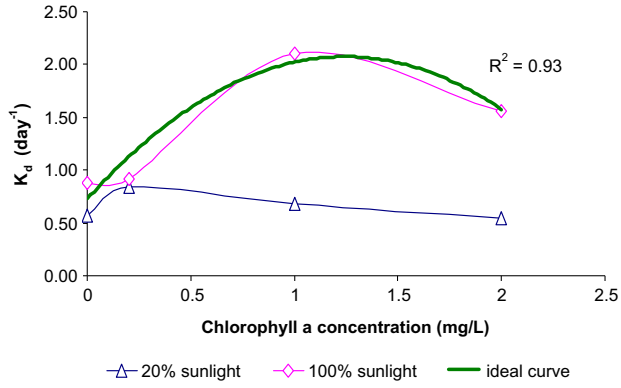

Fig. 3. Faecal coliform decay rates at different algal densities in full and 20% sunlight monitored at 08.00–09.00 am. Point values represent the means of duplicated treatments with three sub-replicates ($N = 6$, standard deviation <0.1 for all point values).

3.3. Continuous flow experiment

The decay rates (d^{-1}) increased with increasing light input. Only the 20% light input curve showed a decay similar to that of a first-order equation although all the other curves were assumed to be first order (Fig. 4(A)). Decay rate in the absence of solar radiation (darkness) was 0.61 d^{-1}. The following relationship was established between the radiation input, r (W/m^2) and the decay rate, K_d (d^{-1}) as (Fig. 4(B)):

$$K_d = 0.011r + 0.61 \quad (3)$$

4. Discussion

4.1. Decay in sunlight

Fig. 2 shows the effect of sunlight on faecal coliform removal. In the past, some studies have disputed the importance of sunlight in the inactivation of faecal coliform [3] but recent work [12,13], including this paper have shown that sunlight plays an important role in the inactivation of faecal coliforms. Differences in the decay rates of faecal coliforms in sunlight can be attributed to the presence of algae (Fig. 2(B)). Decay curves in Fig. 2(A) shows that all incubations showed similar rate of decay in sunlight and darkness within at least 24 h of incubation. A direct effect of sunlight should have resulted in a much faster inactivation of faecal coliforms in sunlight positive setups as compared to those in darkness.

Algal presence leads to pH elevation and increased oxygen concentration, both of which are bactericidal to faecal coliforms [1,2,14]. Rate of decay of faecal

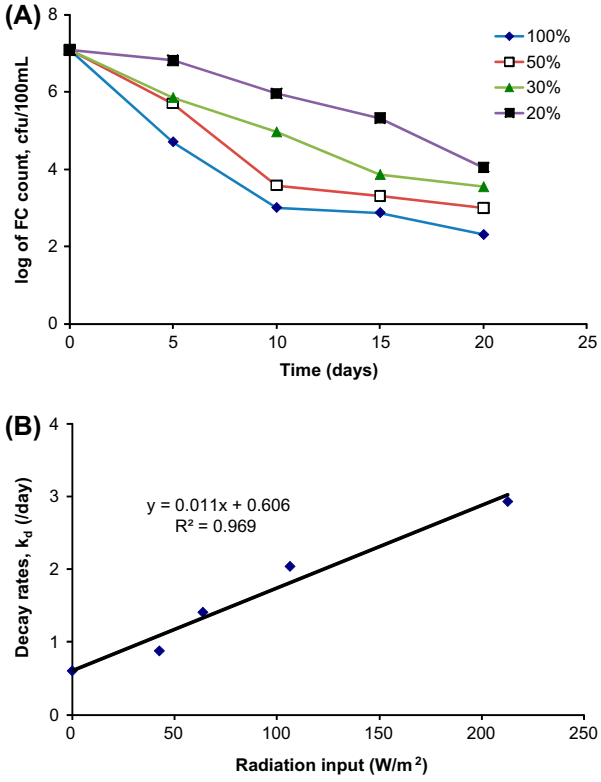

Fig. 4. Faecal coliform decay in varying conditions of sunlight in a pilot scale wastewater treatment system. Point values represent the decay rates of faecal coliforms ($N = 15$) obtained at particular input of sunlight by a linear regression plot of $4(N_o/N_e)^{1/4}$. N_o being the influent faecal coliform count and N_e is the effluent faecal coliform count. (A) Effluent concentration and (B) decay rate variation with light input.

coliforms has been observed to increase with increasing dissolved oxygen concentration [1]. This explains the higher decay rates in sunlight compared to darkness. The non-linear relationship between algal density and decay rates suggests that other factors apart from toxic oxygen molecules are involved in FC inactivation. Lyses of algal cells occur particularly at very high algal densities without any form of induction and this lysis leads to the release of algal organic matter (AOM), some of which may serve as sensitizers by absorbing electromagnetic radiation, transmitting it to faecal bacteria cell membrane leading to its destruction [1,12,15]. Others that are not capable sensitizers may serve as carbon and energy sources for faecal bacteria survival [4].

The ratio of these two categories of dissolved organic matter (DOM) may determine the contribution of DOM in sewage and algae to the destruction of faecal coliforms in natural wastewater treatment systems. This explanation in addition to reduced effect of light when algal densities are high as a result of light attenuation [4], may explain the lower rate of decay at high algal densities. This observation agrees with the findings of [5] which occurred under laboratory conditions. Higher decay rates however were recorded in this experiment compared to that conducted by Ansa et al. [5] under laboratory conditions. For example, under sunlight, a decay rate of 2.0 d^{-1} was recorded at an optimum algal density of 1.3 mg/L while [5] recorded a decay rate of less than 1.3 d^{-1} at the same algal density. This difference can be attributed to higher intensity of sunlight and this is discussed further in this paper.

4.2. Effect of algae in darkness

A strong positive linear correlation between algal density and decay rates in darkness suggests an effect of algae even in darkness. This observation is consistent with similar observations made by Ansa et al. [5] under laboratory conditions. In the absence of light, the role of humic substances in the form of dissolved organic matter may be ineffective [1]. Dissolved organic matter from both algae and wastewater may therefore serve as a carbon and energy source for faecal coliform survival [4]. Since significant die-off was achieved above the control rate of die-off, it suggests that another factor against faecal coliform survival was strong enough to cause significant die-off, nullifying other factors promoting survival. This factor is neither pH nor dissolved oxygen concentration as both were comparable in all incubations in darkness including control. The factor may be an algal toxin [3] that is released during lyses of algal cells, thus increasing its concentration with increased algal density. Further research is necessary to ascertain the nature of the substance causing the inactivation of the faecal coliforms.

4.3. Effect of "light input"

Light intensity correlates positively with "light input" and could therefore be estimated through the use of the latter [4]. Reduced light input resulting in decreased rate of decay of faecal coliforms is yet another example of the sunlight effect in achieving faecal coliform inactivation (Fig. 3). The optimum algal density promoting maximum destruction of faecal coliforms was affected by light input, decreasing with decreased light input (Fig. 3). Decreased sunlight input reduced the amount of algae receiving long wavelengths of electromagnetic radiations, resulting in lesser productivity.

4.4. Continuous flow experiment

Increased decay rate of faecal coliforms with increasing light input is consistent with the findings of similar studies done under batch conditions [1,4,13]. Van der Steen et al. [4] also observed that the decay of FC usually deviates from first order and had to estimate the decay rates from the first half of the decay curve of FC count vs. time. This probably may have led to the higher rates of decay predicted by their model compared to that reported by Curtis et al. [1]. As this experiment was conducted under continuous flow conditions, the decay rate of 2.93+ d^{-1} observed at an insolation of 213 W/m^2 in this study cannot be compared with similar studies on the effect of solar radiation on FC decay rates as these studies [1,4,13], unlike the present one, were conducted under batch conditions. The mathematical expression $K_d = 0.011r + 0.61$, established from this study predicted within a 95% confidence limit, K_d values from a continuous flow treatment system of another study [16] conducted in Kumasi the same country (average insolation 193 W/m^2) using wastewater of similar strength. The accuracy of the model can be attributed to the estimation of the decay rate taking into consideration the FC concentration at the end of the experiment, thus reducing the error introduced by assuming the entire decay process to be first order. As observed by Blaustein et al. [17], *E. coli*, and for that matter faecal coliform decay does not follow a first-order equation and dependence of logarithm of concentration on time is not approximately linear but rather piecewise linear with usually two linearity sections. Few studies had been conducted on the overall decay rate of a continuous flow treatment system in which the magnitude of the insolation was quoted [18]. It is therefore recommended that in studies where rates of decay of treatment systems are reported, one of the parameters that need to be reported as well is the insolation.

5. Conclusions

(1) In sunlight, relatively high pH and dissolve oxygen concentrations in incubations led to high decay rates. In darkness, inactivation of faecal coliforms occurred and this may be a net effect of substances promoting faecal coliform survival and a toxic substance released by algae. Further investigation is necessary to ascertain what substance is causing the inactivation of FC even in darkness.

(2) In sunlight, faecal coliform rate of decay increased with increasing algal density till a certain critical algal concentration (1.3 ± 0.1 mg/L), after which faecal coliform decay decreased suggesting the existence of an optimum algal density where maximum faecal coliform inactivation is achieved.

(3) Increased decay rates of FC occurred with increasing light intensity or light input. With decreased light input of 20% of 213 W/m^2, the optimum algal density for maximum FC decay decreased to a value which is 6–7 times the value of that under normal insolation of 213 W/m^2.

(4) The mathematical expression established from this study predicted the overall K_d value from a continuous flow treatment system of a study by Awuah et al. [16]. As few studies had been conducted on the overall decay rate of a continuous flow treatment system in which the magnitude of the insolation was quoted, it is therefore recommended that in future studies relating to the assessment of performance and estimation of rate of *E. coli* or faecal coliform inactivation in natural waters, one of the parameters that need to be reported as well is the insolation.

List of Symbols

n — number of ponds in series
K_d — decay constant or rate of inactivation of faecal coliforms (d^{-1})
N_t — effluent faecal coliform count at a time t per 100 mL or faecal coliform count per 100 mL at a time t (for batch reactors)
N_o — influent faecal coliform count per 100 mL or faecal coliform count per 100 mL at the start of the experiment (for batch reactors)
Θ — total retention time (d)
r — radiation input
t — time (d) of incubation
N — number of replicates per treatment

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