

THE REMOVAL OF FAECAL COLIFORMS IN WASTE STABILIZATION POND SYSTEMS AND EUTROPHIC LAKES



EBENEZER D.O. ANSA



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Thesis

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by

EBENEZER D.O. ANSA

Born in Swedru, Ghana

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*Dedicated to my children, Mirella and Adriel,
and to
the memory of my mum, Lucy Oye Addo*

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ABSTRACT

The reuse of domestic wastewater presents many challenges including the risk of pathogen infection; hence the removal of pathogens from domestic wastewater is very relevant. It is known that algae play a crucial role in the process of their removal by raising the pH and dissolved oxygen concentration which tend to be injurious to bacteria. It is however not known how algal disinfection ability is affected by biomass changes in sewage of varying strengths and whether algae contribute in sedimenting faecal coliforms (FC) from the water column through attachment to their surfaces. Experiments were conducted to investigate the importance of FC attachment to algae, the effect of varying concentration of algae in sewage of different strengths on FC removal and the effect of algae on FC removal in a tropical eutrophic lake. The effect of reducing algal densities in a pilot-scale hybrid algae-duckweed pond system on FC removal was also investigated with the aim of understanding the importance of FC attachment in such a treatment system in relation to pure algal and duckweed treatment lines. Algae helped in sedimenting FC to the bottom of reactors. It was shown by experimentation under laboratory conditions that in domestic wastewater treatment an optimum algal density exists at which maximum FC removal is achieved. Algae were also important in significantly reducing *Escherichia coli* contamination in a eutrophic lake through increased oxygenation and pH elevation. At algal density $\leq 0.08\text{mgL}^{-1}$ in the Weija Lake, decay rate of *E. coli* was directly proportional to the chlorophyll a concentration of the lake. The strength of domestic wastewater undergoing treatment may also affect the rate of decay of FC, particularly as algal concentration changes. In darkness, higher algal biomass (or chlorophyll a concentration) resulted in higher inactivation of FC although dissolved oxygen concentration and pH were low suggesting a role by another factor in the inactivation of FC. At algal densities $\geq 13.9\text{mg L}^{-1}$ higher removal of FC occurred in MSW (medium strength wastewater) compared with LSW (low strength wastewater) whether in light or in darkness. The highest rate of decay in LSW occurred at 3.2mgL^{-1} chlorophyll a concentration in light while that of the MSW occurred at 20.0mgL^{-1} in light. Addition of raw wastewater to an ongoing wastewater treatment process lowered the rate of FC removal for a wide range of algal densities ($0.6 - 19.6\text{mgL}^{-1}$ chlorophyll a concentration), under light conditions. The hybrid pond system performed well in FC (4.3 log units) and Biochemical Oxygen Demand (BOD) (89%) removal and these parameters in addition to total phosphorus were not affected by seasonal changes. FC attachment to suspended matter was important only in the first two ponds of the duckweed, algal and hybrid pond systems. Little variation of FC decay with depth was observed. FC decay rates in the mornings were usually lower than in the afternoons in algal ponds but not in the duckweed ponds. High densities of macro-invertebrates belonging to the class Ostracoda were associated mainly with the surface and bottom of duckweed ponds and these were much higher than that of algal ponds at similar locations. FC numbers in duckweed ponds correlated strongly and positively with mean ostracod numbers in ponds. FC numbers also correlated well with Shannon-Wiener diversity index of macro-invertebrates in all the three pond systems. Integrating a hybrid pond system such as this for aquaculture would be a big boost economically and health-wise for communities in developing countries with warm tropical conditions.

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1

*Chapter***Introduction****1 BACKGROUND**

Wastewater refers to liquid waste generated from activities emanating from water closets, bathrooms, kitchens and laundry in residences (domestic wastewater) or liquid waste collected from the activities of industries (industrial wastewater). Domestic wastewater may or may not include storm water or run-off rainwater. The composition of domestic wastewater varies depending on the source and life style of the community or country. Metcalf and Eddy (2003) classified domestic wastewater as being typically of three different categories: low strength, medium strength and high strength (Table 1). It is however difficult to have typical values for industrial wastewater as depending on the type of industry, the intensity and duration of its activities, the wastewater constituents and its concentration may vary greatly. For example the composition of wastewater from a textile manufacturing industry would have high colour content such as 2250PCU (Kumar et al, 2008) and this would greatly differ from that of a meat processing factory that would have high organic matter content such as $4,524\text{mgL}^{-1}$ BOD₅ (Chavez et al, 2005).

1.1 The need to treat wastewater

The earth's hydrosphere has 1386million cubic metres of water but only 0.26% of this constitutes renewable freshwater in the form of streams, rivers, reservoirs and lakes (Shiklomanov, 1998).The World Water Council (2000) however maintains that the water crisis today is not about having too little water to satisfy our needs, but rather a problem of 'managing water so badly that billions of people and the environment suffer badly'. While this statement may be true in some regions of the world, in arid and semi-arid places, the freshwater resource is scarce due to high evaporation rates and the difficulty of storage. The natural loss of water through evaporation from the whole Nile basin and the Niger basin for example amounted to $130\text{km}^3/\text{yr}$ and $33\text{km}^3/\text{yr}$ respectively (IHP, 1995).It is also estimated that each year an additional 80 million people will tap the earth's water as a result of rapid population growth (World Bank, 2004). Rapid population growth, coupled with poor management of water resources occurs mainly in developing countries causing large populations in these countries to lack access to good and safe drinking water. In order to achieve the Millennium Development Goal of reducing by half the number of people without sustainable access to safe drinking water and basic sanitation (Johannesburg Summit, 2002; World Bank, 2004), a lot more people would need to be connected to safe drinking water supply. According to United Nations (2011), an estimated 1.1 billion people in urban areas and 723 million people in rural areas gained access to an improved drinking water source over the period 1990-2008. It reported that Africa nearly doubled the number of people using an improved drinking water source from 252 million in 1990 to 492 million in 2008. This means that with increased access to water supply, more wastewater is going to be generated. This needs to be managed well in order to ensure that water resources are protected from rapid water quality deterioration, which in turn would complicate drinking water treatment. It is estimated that currently, over 2.6 billion still lack access to flush

toilets and other forms of improved sanitation, with 1.1 billion people practicing open defaecation (United Nations, 2011). The implication of this is that some of this faecal matter may be washed into water bodies introducing nutrients and pathogens into sources of drinking water supply. The treatment and re-use of wastewater therefore is imperative. Currently in developing countries wastewater is reused mainly indirectly by abstracting discharged wastewater from streams and rivers (unplanned re-use), rather than by deliberately treating the wastewater before reuse (planned reuse). This practice poses a serious challenge to public health due to the risk of infection by water-borne diseases. Common pathogens that can be transmitted by the re-use of untreated wastewater, the diseases they cause and their minimal infective doses are outlined in Table 2.

Table 1. Typical values of domestic wastewater constituents (Metcalf and Eddy, 2003).

<i>Contaminants (mg/L)</i>	<i>Low Strength</i>	<i>Medium Strength</i>	<i>High Strength</i>
Total solids	390	720	1230
Total Dissolved Solids	270	500	860
BOD ₅	110	190	350
Total Organic Carbon	80	140	260
COD	250	430	800
Total Nitrogen	20	40	70
Organic Nitrogen	8	15	25
Free Ammonia	12	25	45
Nitrites	0	0	0
Nitrates	0	0	0
Total Phosphorus	4	7	12
Organic Phosphorus	1	2	4
Inorganic Phosphorus	3	5	8
Chlorides	30	50	90
Sulphate	20	30	50
Oil and grease	50	90	100
Volatile organic compounds (µg/L)	<100	100-400	>400
Total coliform (cfu/100mL)	10 ⁶ -10 ⁸	10 ⁷ -10 ⁹	10 ⁷ -10 ¹⁰
Faecal coliform (cfu/100mL)	10 ³ -10 ⁵	10 ⁴ -10 ⁶	10 ⁵ -10 ⁸
Cryptosporidium oocysts (No./100mL)	10 ⁻¹ -10 ⁰	10 ⁻¹ -10	10 ⁻¹ -10 ²
Giardia lamblia cysts (No./100mL)	10 ⁻¹ -10	10 ⁻¹ -10 ²	10-10 ³

Water supply companies in these developing countries also incur high costs for drinking water treatment due to degradation of the lakes and rivers which are the main sources of water supply for municipalities. Ghana, like most developing countries, uses 70% of its renewable water resource from lakes and rivers for farming (Ministry of Water, Works and Housing, 2011). Re-using wastewater in a planned manner would make high quantities of nutrient rich water available for agricultural purposes (Gijzen, 2001). The World Health Organization minimum guideline for

wastewater reuse requires $\leq 10^3$ *Escherichia coli* per 100mL of effluent for unrestricted agricultural use (WHO, 2006).

Table 2. Common pathogens found in untreated wastewater and their minimal infective doses

Organism	Disease	Concentration in raw wastewater/ <i>Cfu.100mL⁻¹</i>	Minimal infective dose (cells/tissue)
<i>Salmonella spp</i>	Salmonellosis	10^2 - 10^4	10^4 - 10^7
<i>Shigella spp</i>	Shigellosis	10^0 - 10^3	10 - 10^2
<i>Escherichia coli</i>	Gastroenteritis	10^6 - 10^8	10^6 - 10^8
<i>Escherichia coli</i> 0157:H7	Gastroenteritis	-	$<10^2$
<i>Vibrio cholerae</i>	Cholera	-	10^3
<i>Campylobacter jejuni</i>	Gastroenteritis	-	5×10^2
<i>Clostridium perfringens</i>	Gastroenteritis	10^3 - 10^5	1 - 10^{10}
<i>Cryptosporidium</i>	Cryptosporidiosis	10 - 10^3	1 - 10
<i>Giardia lamblia</i>	Giardiasis	10^3 - 10^4	10 - 10^2 cysts
<i>Entamoeba</i>	Amoebiasis	10^{-1} - 10	10 cysts
<i>Ascaris lumbricoides</i>	Ascariasis	10^{-2} - 10^0	1 - 10
Eggs		10 - 10^3	1 - 10
Enteric virus	Gastroenteritis	10^3 - 10^4	1 - 10

Source: Adapted from Bitton (2005) and Metcalf and Eddy, Inc (2003)

1.2 Wastewater treatment options

The treatment of wastewater involves basically four stages. The first stage, preliminary treatment, involves a process of screening to remove debris and coarse materials from the wastewater and a second stage known as primary treatment entails the further removal of settleable solids through a process of screening and sedimentation. The third stage (secondary treatment) is usually a biological unit process which may involve one or more of various types of treatment technologies such as the attached growth processes, suspended growth processes and use of eco-technologies. The fourth stage of wastewater treatment, known as advanced or tertiary treatment aims to further remove BOD, nutrients, pathogens and sometimes toxic substances (Bitton, 2005).

The aim of secondary treatment is to remove BOD, nutrients and pathogens. For biological secondary treatment we can differentiate largely between aerobic treatment processes (which take place in the presence of oxygen) and anaerobic treatment processes. Anaerobic digestion of wastewater consists of a series of the microbiological processes outlined in Figure 1 that convert organic compounds to methane and carbon dioxide, reducing the volatile solids by 35% to 60%, depending on the operating conditions (USEPA, 1992). Aerobic and anaerobic treatment processes

each have their peculiar strengths and weaknesses. Compared with aerobic processes, anaerobic treatment of wastewater is excellent when sewage strength is high. Anaerobic treatment has a lower cost of treatment as aeration cost is absent producing 3-20 times less sludge, it releases methane as a by product which is used for heating digesters or for electricity production. It also possesses a consortium of microorganisms that can degrade xenobiotic compounds like chlorinated aliphatic hydrocarbons and recalcitrant compounds like lignin (Cowan and Burton, 2002; Bitton, 2005). Anaerobic digestion of wastewater however is a slower process, more sensitive to the effect of toxicants with relatively much longer periods for the start-up of the process (Rittman et al, 1988). Thus there is currently a trend towards hybrid systems where aerobic and anaerobic stages are coupled to each other, and/or where anoxic/low oxygen environment is created (e.g. in wetlands, ponds, but lately also in activated sludge systems aiming at nutrient removal). Anaerobic treatment process may involve a suspended growth process as may occur in a septic tank and an upflow anaerobic sludge blanket (UASB) reactor or an attached-growth process as may occur in anaerobic filters and attached-film expanded bed reactors. This thesis however will focus on aerobic treatment of processes.

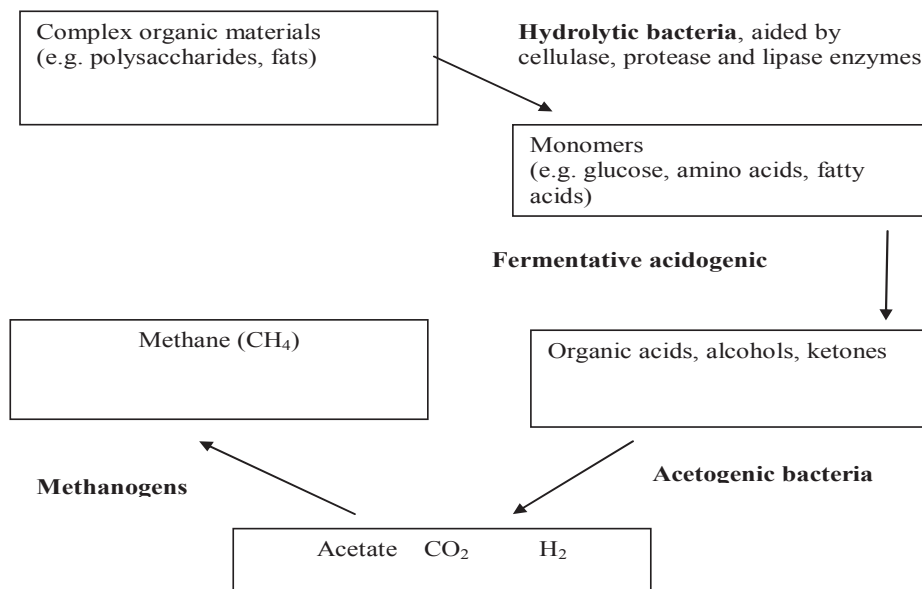


Figure 1. Steps involved in the anaerobic digestion of complex organic compounds (Adapted from Bitton, 2005)

1.2.1 Attached growth processes

The attached growth process, sometimes also known as fixed film process, consists of microorganisms forming biofilms on inert packing material such as rocks, gravel, sand, plastics and synthetic fibres when wastewater flows over these surfaces. The microorganisms grow rapidly (in the case of aerobic processes) by removing organic matter or nutrients from the wastewater.

Common examples of attached growth treatment process are the trickling filter and the rotating biological contactors (RBC). Attached growth processes are used for oxidation of organic matter, nitrification, denitrification and anaerobic digestion of wastewater. Compared to suspended growth processes, attached growth processes are easy to operate and have lower maintenance and upgrading costs. Attached growth processes also have shorter sludge settling period and are able to withstand shock loads of toxic inputs (DelaTolla et al, 2008; 2009). Nitrification rates are less negatively affected by low temperatures (Chia-Yuan et al, 2010). Attached growth processes however experience filter clogging and odour problems when loading rate is high due to excessive growth of slime bacteria in the biofilm. Other drawbacks include low faecal coliform removal and poor effluent quality due to sloughing or sudden and massive detachment of biofilm (Bitton, 2005).

1.2.2 *Suspended growth processes*

In suspended growth biological treatment process, the microorganisms responsible for the treatment are maintained in liquid suspension by appropriate mixing methods. A common example of a suspended growth process is the activated sludge process which involves the production of an activated mass of microorganisms capable of stabilizing a waste by converting organic matter into gases and cell tissues under aerobic conditions (Metcalf and Eddy, 2003). Suspended growth processes can also take place under anaerobic and anoxic conditions. Compared to attached growth processes, advantages of suspended growth process include relatively better faecal coliform removal (Sala-Garrido et al, 2011), shorter residence time in organic matter oxidation, higher operational flexibility with respect to organic load and hydraulic variations and a better efficiency in performance in terms of BOD, nitrogen and phosphorus removal (Hanhan et al, 2011; Sala-Garrido et al, 2011). Limitations of this technology are inability of microorganisms to form flocs that settles, sometimes resulting in turbid effluents (Bitton, 2005; Mungray and Patel, 2011). Denitrification in secondary clarifier of suspended growth processes could release poorly soluble nitrogen gas which attaches itself to activated sludge causing it to float and thereby resulting in scum formation at the surface (Metcalf and Eddy, 2003).

1.2.3 *Eco-technologies*

Eco-technologies for wastewater treatment are sometimes referred to as natural wastewater treatment systems. They are artificially created systems capable of utilizing the ecological, biochemical and physical processes involving wetland flora and fauna (including microorganisms), soils, and their associated macrofauna and microbial assemblages to assist in treating wastewater. Natural wastewater treatment systems differ from conventional processes such as the activated sludge (suspended-growth process) and the rotating biological contactor (attached-growth process) in that they require comparatively little or no energy either in their construction or operation, making them highly sustainable (Muga and Mihelcic, 2008), with a small carbon footprint (Shilton et al., 2008). For example, a population of 20,000 people producing $3600\text{m}^3\text{d}^{-1}$ wastewater requires for treatment, an annual energy of $1000,000\text{ kWhyr}^{-1}$ when using activated sludge, $120,000\text{ kWhyr}^{-1}$ when using rotating biological contactor and 0 kWhyr^{-1} when using a waste stabilization pond (Gray, 2010a). Common examples of treatment systems utilizing a renewable source of energy and having a natural process of wastewater treatment are waste stabilization ponds (WSP) and wetlands. WSPs consists of a set of connected basins in which biological processes break down the organic matter at a natural rate aided by temperature, wind, sunlight and the biological interaction of microorganisms (Mara, 2009). WSPs are cheap, easy to operate and returns nutrients to the surrounding environment (Muga and Mihelcic, 2008), making them a popular choice for developing countries. Table 3 further compares some of the characteristics of natural and conventional wastewater treatment systems. A key advantage of natural wastewater treatment systems is its

ability to efficiently remove pathogens in wastewater (Table 4), a very important requirement in developing countries due to the use of raw water from rivers and lakes by rural communities without any form of treatment. Wetlands may be natural or artificial (also known as constructed wetlands). The use of natural wetlands for domestic wastewater treatment is discouraged although some workers may disagree (USEPA, 2000).

Table 3. Comparison between natural and conventional treatment systems. Adapted from Brissaud (2008), Gray (2010); Mungray and Patel (2011).

	Natural treatment system	Conventional treatment system
Applicability	Rural, small populations	Urban, large populations
<i>Sustainability</i>		
Energy	Renewable	Electricity
Aeration	Diffusion, photosynthesis	Mechanical aeration or diffusers
Chemical usage	None or very low	Moderate to high
Chemical type	Lime	Lime, metal salts, polyacrylamides
Carbon footprint	Low	Very high
Wildlife value	High	Low
<i>Operational factors</i>		
Sludge production	Low, on site disposal	High, off-site disposal
Odour	Low	Moderate to high
Noise	None or very low	Moderate to high
Aerosol formation	None	High
<i>Design and costs</i>		
Design layout	Dispersed	Compact
Land area	High	Low
Capital cost	Low to moderate	High
Operational cost	Low	High

Table 4. Summary of removal efficiencies from selected treatment systems

Treatment technology	Log Removal		
	Bacteria	Helminth eggs	Protozoan cysts
Activated sludge*	0-2	0-2	0-1
Trickling filter*	0-2	0-2	0-1
Waste Stabilization Ponds	1-6	1-3	1-4
Constructed Wetland (SF)	1-4	-	1-2
(SSF)	1-4	-	1-3

*with settling tank. SF: Surface flow constructed wetland; SSF: Sub-surface flow constructed wetland. (Adapted from: Mara and Cairncross, 1989; Quinonez-Diaz *et al.*, 2001; Garcia *et al.*, 2008; Reinoso *et al.*, 2008)

Table 5. Merits and demerits of waste stabilization ponds (WSP) and constructed wetlands (CW).FP refer to facultative pond. FWS: Free Water Surface Wetland, SSF: Sub-Surface Flow Wetland.

Characteristics	WSPs	CW (FWS/SSF)	Literature
Land requirement	With high land costs required by WSPs, it could still the cheapest option	Use of CW cost effective when land availability is not drastically constrained	Arthur (1983) Kadlec (2003)
	Evaporation of water occurs.	Requires 60% more land space than WSP to produce 25mgL^{-1} BOD and $<150\text{mg SSL}^{-1}$	Mara (2000) Mara (2006)
	Can be countered by use of storage reservoirs.		
	Mosquito breeding problems. Can be overcome by use of duckweed	FWS may have similar problems with mosquito breeding	Awuah (2006)
FC removal efficiency	Disinfection is more efficient in maturation pond than in SF-CW	When influent wastewater concentration is high	Ghermandi et al. (2007)
		CW acts as a source of pathogen contamination	Tanner et al. (2005)
BOD removal efficiency	Effluent high in BOD and SS due to algal presence	With low loadings CW are excellent in BOD removal	Kadlec(2003)
Nutrient removal efficiency	Relatively poor, but better when macrophytes are present	Good when loading is low	Kadlec (2003)
Treatment cost (for same effluent quality)	On the basis of land area requirement, performance and capital, O&M costs, WSPs are to be preferred to SSF CW		Mara (2006)

Constructed wetland (CW) may either be a Free Water Surface (FWS) wetland consisting of a pond with emergent, submerged and floating macrophytes or a Sub-Surface Flow (SSF) wetland with no standing water but rather a bed of gravel, stones and sand with planted macrophytes. Based on their land area requirement, ability to remove faecal coliform, organic matter and nutrients, and as well as their total capital costs, the merits and demerits of waste stabilization ponds and constructed wetlands are summarized above in Table 5. In recent times the use of combined systems of CW and WSP with WSP effluent serving as the influent of the CW is fast gaining ground. The use of combined systems of WSPs and CWs, expectedly combines the advantages of both systems while eliminating some of the limitations of either system type. Kadlec (2003) mentioned that the use of

FWS for polishing effluents from ponds is cost effective when land availability is not drastically constrained. Tanner et al. (2005) and (Reinoso et al., 2008) also showed that combined systems of WSPs and CWs may provide more consistent effluent quality than either system alone. Additional benefits of combined systems include reduction in sites for mosquito breeding, greater biological complexity resulting in higher robustness and operational stability (Tanner et al., 2005), better removal of nutrients, BOD, suspended solids and commercial benefits from eco-tourism and aquaculture (Wang et al, 2005; Herrera et al., 2009). Combined systems could further be preceded by an anaerobic treatment step, which could significantly reduce BOD and SS, and would yield renewable energy in the form of biogas.

It has been suggested that in determining the appropriateness or the sustainability of a wastewater treatment technology, the following factors need to be considered:

Its robustness while meeting effluent standards, its generation of wastes such as sludge and by products such as carbon dioxide emissions, its capacity for various re-use options as well as environmental benefits, the extent of chemical usage and the degree of environmental nuisance it poses, its energy source and consumption as well as land and other capital requirements (Brix, 1998; Shutes, 2001). In terms of these factors WSPs compares favourably with other wastewater treatment technologies (Tables 3, 4, 5).

1.3 Domestic wastewater treatment with waste stabilization ponds

1.3.1 Removal of organic matter

The removal of organic matter from wastewater is necessary in order to avoid the creation of anaerobic conditions in waterbodies when the treated wastewater is discharged into these waters. Development of anaerobic conditions in a freshwater body may lead to biodiversity loss, and to development of bad odours and survival of facultative anaerobic bacteria pathogens such as *Enterobacter*, *Vibrio* and *Shigella* (Bitton, 2005). In treatment ponds, the presence of sewage-derived organic matter (Van der Steen, 2000a) and algal organic matter (Bouteleux et al., 2005) could prolong the survival of heterotrophic bacteria as the organic matter provides the carbon and energy source for its survival. Dissolved organic matter however could serve as a sensitizer for faecal coliform destruction depending on the source of the organic matter (Curtis et al., 1992a). The overall effect of dissolved organic matter on faecal coliform removal therefore would be a trade-off between these two effects suggesting that this overall effect could vary with varying algal density. This phenomenon had not yet been investigated.

The removal of organic matter takes place either in anaerobic ponds (APs) or in primary facultative ponds (FPs) which receive wastewater with Biochemical Oxygen Demand (BOD) loadings of more than 100g BOD/m³day. Alternatively a high rate anaerobic reactor such as a UASB could be placed as a pre-treatment before a WSP (Van der Steen et al, 2000; Caicedo et al, 2002). In either APs or FPs, the objective is primarily the removal of BOD and COD (Chemical Oxygen Demand). In APs, BOD is removed by sedimentation of settleable solids which are digested in the accumulating sludge layer in a four stage process of hydrolysis of complex organic compounds, acidogenesis, acetogenesis and methanogenesis (conversion of acetates, hydrogen and carbon dioxide to methane gas), (Mara, 2003). A drawback is that these systems release large amounts of methane gas into the atmosphere, which is a strong greenhouse gas.

BOD and COD removal continues in secondary FPs, usually 1.5-2.5m deep and consisting of three layers: a photic aerobic zone, a mid facultative zone and a bottom anaerobic zone harbouring aerobic, facultative and anaerobic bacteria respectively for degrading the organic matter (Figure 2).

The photic zone of the FPs, is made aerobic by algae usually of 500 to 2000 $\mu\text{g/L}$ chlorophyll a concentration (Mara, 2002), providing the oxygen needed by heterotrophic bacteria for the oxidation of organic matter. The heterotrophs in return provide the algae with micronutrients and carbon dioxide for its photosynthetic activity.

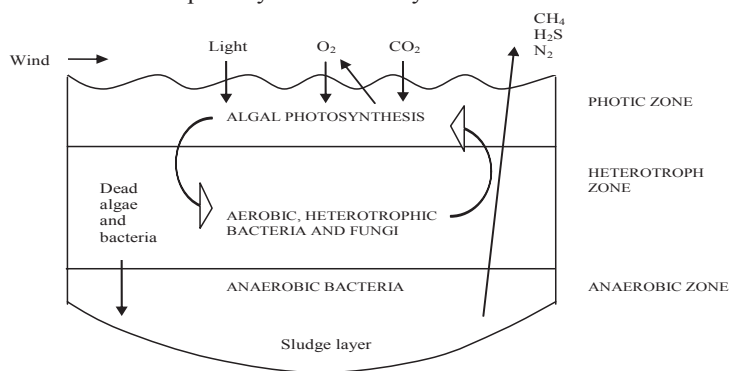


Figure 2. Processes in facultative ponds. Source: Bitton (2005).

Dead algal and bacteria cells in addition to other solids settle at the bottom undergoing anaerobic digestion, releasing gases such as methane, hydrogen sulphide, carbon dioxide and nitrogen gas. Hydrogen sulphide production by sulphate-reducing bacteria encourages the growth of photosynthetic bacteria, namely the purple sulphur bacteria. Photosynthetic bacteria are found below the algal layers in FPs and they help protect pond algae from the deleterious effect of hydrogen sulphide (Bitton, 2005). It had been estimated that 25-50% of removed BOD is through the form of methane escape from the surface of FPs (Arceivala, 1981). Methane can be produced during daytime periods in the anoxic zone at the bottom part of FP. The anoxic conditions in the lower portion of the pond is created by the formation of a thick layer of non-motile algae sinking to portions of pond which do not have access to light and creating an oxygen demand just below the the interphase between a thermally stratified layer of warm and cold water. This is triggered when pond mixing is not occurring and a thick layer of motile algae forms above this interphase, cutting off light penetration to non-motile algae. In maturation ponds (MPs), although some removal of BOD and COD occur, this is not much in comparison with what occurs in APs and FPs as most of the BOD occurs in the APs and FPs.

1.3.2 Removal of nutrients

Removal of nutrients from domestic wastewater entails basically the removal of nitrogen and phosphorus. Removal of nitrogen occurs in all the stages of treatment through the transformation of nitrogen to other forms. The use of anaerobic pre-treatment followed by further treatment in macrophyte-based WSPs is an effective means of nutrient and energy recovery from domestic wastewater (Gijzen, 2002). Under anaerobic conditions, organic nitrogen is transformed to ammonia while in the presence of oxygen ammonia could be oxidized to nitrite and then nitrate by obligately aerobic autotrophs, although ammonia could also be lost to the atmosphere when pH is high (Zimmo et al., 2002; Mara, 2003). Uptake of ammonia by plants in WSPs constitutes a major means of removal of nitrogen (El Shaffai et al., 2007). In situations where treated domestic wastewater is for irrigation of crops, the nutrient rich effluents application can compensate for the use of commercial fertiliser (Gijzen, 2001). At pH greater than 9, phosphorus is removed in FPs and MPs as calcium hydroxyapatite through precipitation (Mara, 2003). The discharge of nutrient rich wastewater into waterbodies makes these waters eutrophic leading to fish kills and death of other

aquatic life. Limited nutrient inflow however may increase the productivity of freshwater bodies and perhaps its self-purification capabilities through the presence of algae. This study investigates the importance of algae in faecal coliform removal in eutrophic waters.

1.3.3 Removal of pathogens

Pathogens in domestic wastewater (Table 2) are generally classified as protozoan parasites, helminth parasites, viral pathogens and bacterial pathogens (Leclerc et al., 2002; Bitton, 2005). The mechanism of removal of protozoan cysts, helminth eggs and viruses is not well understood but it is believed that it is mainly through sedimentation and damage by sunlight (Mara, 2003). A greater portion of cysts and eggs of parasites in wastewater are usually removed in the APs and FPs. *Giardia* cysts were observed to have been completely removed in a WSP after 16 days total retention time (Amahmid et al., 2002) but complete helminth egg removal could be achieved in 5 to 15 days depending on the number of eggs present in the raw wastewater (Mara, 2003). The denser the egg or cyst, the faster the sedimentation and therefore removal are likely to be (Table 6). Data on the removal of viral and bacterial pathogens taken at the same time shows that their removal correlates well with the removal of *Escherichia coli* thus making it a good indicator of pathogenic bacteria and viral contamination (Oragui et al. 1987, Mara, 2003).

Table 6. Settling abilities of protozoan cysts and eggs.

Parasite	Egg/Cyst size (μm)	Relative density	Settling velocity (m/h)
<i>Giardia</i>	14	1.05	0.02
<i>Cryptosporidium</i>	6	1.08	0.004
<i>Ascaris</i>	55 x 40	1.11	0.65
<i>Trichuris</i>	22 x 50	1.15	1.53
Hookworms	60 x 40	1.06	0.39

Adapted from Shuval et al. (1986), Department of Environment and Department of Health (1990) and Mara (2003).

1.4 Faecal or thermotolerant coliforms

Most of the studies on the removal of bacterial pathogens from treatment ponds made use of indicator bacteria as their presence correlates well with the presence of faecal contamination (Leclerc et al., 2001) and therefore the possible presence of pathogens. Coliform bacteria are members of the intestinal microflora, occur abundantly in wastewater, are easy to culture and exhibit the same or greater survival characteristics as the (bacteria) pathogens. Maier et al. (2000) indicated that an ideal indicator must in addition to the characteristics mentioned above be present only when faecal contamination is present and the indicator organism must not reproduce outside of the host organism. Unfortunately no indicator currently has all these characteristics. The faecal coliforms group was selected for its ability to indicate faecal contamination by the culturing of this subgroup of indicators at 44.5 °C instead of the 35°C used for total coliforms. Recent increases in knowledge of the genetic constitution of members of this group has led to the adoption of the name 'thermotolerant' coliforms paving the way for members with similar characteristics to be included in the group (Leclerc et al., 2001; Tallon et al., 2005). Unfortunately, some members of this thermotolerant or faecal coliform group are present in the environment when faecal contamination is absent (Byamukama et al., 2000; Gauthier and Archibald, 2001; McLellan et al., 2001). This shortcoming has led to the adoption of *Escherichia coli* as a most preferred indicator of faecal contamination as it is relatively rare in the environment and is most representative of thermotolerant

coliforms accounting for some 94-96% of thermotolerant coliforms in faeces (Tallon et al, 2005). The WHO (2006) guidelines for reuse of wastewater also adopted the use of *E. coli* as a measure of the microbial quality of the effluent instead of the previous use of faecal coliforms. Recently developed methods utilising the β -glucuronidase enzyme in *E. coli* are also easy to use, fast, specific and more sensitive than those for other thermotolerant coliforms (Tallon et al., 2005). This notwithstanding some *E. coli* survive, grow and establish populations in some natural environments such as algal mats (Olapade et al, 2006), lakes (Power et al., 2005), streams (Byappanahalli et al, 2003) and soils (Byappanahalli and Fujioka, 2004) making it still an imperfect indicator. More research therefore is needed to correlate the presence of *E. coli* with specific pathogens. For the purposes of assessment and comparison of the performance of wastewater treatment systems however, thermotolerant coliforms and *E. coli* may be adequate especially with the use of β -galactosidase and β -glucuronidase sensitive media. Cost and efficiency has been a key factor in the choice of detection methods and so has the selection of the technologies of treatment.

1.5 Treatment performance

Waste stabilization ponds are among the most efficient and low-cost technologies available for the removal of faecal coliforms from domestic wastewater (Mara, 2000). Treatment performance however exhibits wide variations due partly to varying operating conditions of influent concentrations, pond depth, and hydraulic retention times amongst others (Table 7). Faecal coliform log removal can vary from less than one log unit in duckweed pond systems (Falabi et al., 2002) to as high as 6.42 log units in combined algal and duckweed pond systems (Von Sperling and Mascarenhas, 2005) as shown in Table 8. The elevation of pH and dissolved oxygen concentration occurring in algal ponds do not occur in duckweed ponds. Some authors have attributed some *Escherichia coli* decay in algal ponds to the high pH and fluctuating pH that occurs in this pond type. Duckweed ponds therefore are not as efficient in faecal coliforms removal as algal ponds but do very well in nutrient removal (Awuah, 2006). This amongst others explains why combining both pond types is gaining popularity. Table 8 shows a summary of treatment performances of algal ponds, duckweed ponds and combinations of the two.

1.6 Removal mechanisms

The mechanism of faecal coliform removal in eutrophic lakes may resemble that of algal ponds in that the physical, chemical and biological characteristics of MPs that play a dominant role in faecal coliform removal may be comparable to eutrophic lakes (Gray, 2010), although the relative importance of specific mechanisms may differ. In addition, water column processes within Free Water Surface wetlands (FWS) or open waters are nearly identical to ponds with surface autotrophic zones dominated by planktonic or filamentous algae or by floating or submerged aquatic macrophytes (IWA, 2006). The presence of microalgal populations in both eutrophic lakes and maturation ponds of waste stabilization ponds may give rise to high and fluctuating pH and dissolved oxygen concentration. Eutrophic lakes however may have chlorophyll-a concentrations less than 300 μ g/L with deeper photic zones, thermal stratification and stronger wind action supplementing oxygenation by algal presence. The reverse may hold for maturation ponds where chlorophyll-a concentration may typically vary from 500-2000 μ g/L, short photic zones, no thermal stratification and very minimal wind action.

The reviews of Maynard et al. (1999) and Davies-Colley et al. (2000) mentioned temperature, starvation, the interactions of sunlight with pH and oxygen radicals, algal toxins, algal biomass, predation and sedimentation of attached faecal coliforms as key factors affecting the removal of

faecal coliforms from maturation ponds, which are also known as tertiary lagoons. These factors are discussed below in relation to how they may vary in eutrophic lakes and maturation ponds.

Table 7. Performance of algal, duckweed and hybrid algal and duckweed ponds in the removal of faecal coliforms.

<i>System type</i>	<i>Influent conc. (cfu/ 100mL)</i>	<i>Total retention time (days)</i>	<i>Season temp (°C) Location</i>	<i>Removal efficiency (log units)</i>	<i>Literature reference</i>
AP					
D _{alg} : 75cm	6.31 x 10 ⁵	20	winter (7)	2.00	Garcia et al. (2008)
	6.31 x 10 ⁵	20	summer (27)	3.66	
	6.31 x 10 ⁵	20	total (year round) Leon, Spain	3.22	
D _{alg} : 29cm	3.0 x 10 ⁵	5	winter (8-15.4)	2.60	Van der Steen et al. (2000b)
	6.9 x 10 ⁵	5	spring (18.3-28.1) Negev desert, Israel	2.70	
			(25.9-29.1) Akuse, Ghana	5.32	
D _{alg} : NA	5.8 x 10 ⁶	40	(25.9-29.1) Akuse, Ghana	5.32	Hodgson (2000)
D _{alg} : NA	8.5 x 10 ⁷	9-16	(22.9-25.9) Yaounde, Cameroun	3.00	Noumsi et al. (2005)
D _{alg} : 63cm	3.7 x 10 ⁶	28	27 ± 3.4 Kumasi, Ghana	5.00	Awuah (2006)
HRAP					
D _{alg} : 30cm	6.31 x 10 ⁵	10	winter (7)	1.68	Garcia et al. (2008)
	6.31 x 10 ⁵	10	summer (27)	2.35	
	6.31 x 10 ⁵	10	total (year round) Leon, Spain	2.04	
DP					
D _{dw} = 90cm	1.77 x10 ⁶	9	(2-31) year round Arizona, USA	0.47	Falabi et al. (2002)
D _{dw} = 90cm	1.95 x 10 ⁴	28	winter	1.03	Zimmo et al. (2002)
	2.4 x 10 ⁴	28	summer West Bank, Palestine	1.97	
D _{dw} = 63cm	3.7 x 10 ⁶	28	27 ± 3.4 Kumasi, Ghana	4.00	Awuah (2006)
D _{dw} = 48cm	1.1 x 10 ⁹	15	winter (13-18)	2.83	El Shafai et al. (2007)
	2.9 x 10 ⁸	15	summer (25-41) Cairo, Egypt	4.35	
HADP					
D _{alg} : 30cm					
D _{dw} : 160cm	3.0 x 10 ⁵	10.1	winter (8-15.4)	2.2	Van der Steen et al. (2000b)
	6.9 x 10 ⁵	10.1	spring (18.3-28.1) Negev desert, Israel	2.3	
D _{alg} : 60cm			Year round (20) Belo Horizonte, Brazil	6.42	Von Sperling and Mascarenhas (2005)
D _{dw} : 55cm	4.9 x 10 ⁹	7.4			

D_{alg}: Depth of algal pond. D_{dw}: Depth of duckweed pond. NA: Not Available.

AP: Algal Pond; HRAP: High Rate Algal Pond; DP: Duckweed Pond; HADP: Hybrid Algal and Duckweed Pond

1.6.1 Temperature

Temperature is one of the drivers for pond mixing (Brissaud et al., 2002) and may particularly be important in the inactivation of faecal coliforms in darkness in both algal and duckweed ponds (Maynard et al., 1999). Faecal coliform decay in pond systems, both algal and duckweed had been observed to be higher in summer than in winter. Some authors have attributed this to better solar irradiation in summer than in winter as increased pH and dissolved oxygen concentration were reported (Davies-colley, 1999; Zimmo et al, 2002; El-Shaffai et al., 2007; Garcia et al., 2008). This however may not be the case for duckweed ponds as light penetration is poor (Dewedar and Bahgat, 1995). Most inactivation occurring in algal ponds can be attributed to sunlight exposure but higher inactivation of *E. coli* in darkness observed in algal ponds in warm season compared to cold season suggest a kinetic effect of temperature in inactivation (Ynoussa et al., 2009). This may also explain the higher inactivation of faecal coliforms in duckweed ponds in summer compared to winter.

The effect of temperature on faecal coliform removal in eutrophic lakes may be indirect and more complex. Changes in the thermal structure of lakes influence the magnitude and composition of the phytoplankton community (Jones and Elliot, 2007). Generally, numbers and biomass of bacteria increase with increasing lake productivity, concentration of inorganic and organic compounds but very little seasonal variation in bacteria abundance occurs in eutrophic lakes (Wetzel, 2001).

1.6.2 Starvation

Depletion of the carbon sources in ponds could starve faecal bacteria of its carbon and energy sources leading eventually to death (Maynard et al., 1999; Van der Steen et al., 2000a). Starvation is likely to be more important in duckweed ponds than algal ponds or eutrophic lakes as availability of carbon and energy sources for heterotrophic bacteria abound in algal ponds and eutrophic lakes. Addition of glucose prolonged the survival of *E. coli* by ten days in wastewater (Van der Steen et al., 2000a). Bouteleux et al. (2005) observed *E. coli* growth in the presence of biodegradable algal organic matter and this growth increased by 4-12 folds in the presence of biodegradable organic matter from ozonated algae, concluding that the behaviour of *E. coli* may depend on the source of the organic matter. This may explain why starvation may not be important in algal ponds. In the experiment conducted by Bouteleux et al. (2005), the source of the algal organic matter was *Chlorella*, releasing 52% carbohydrates, the rest being other biodegradable fractions when oxidized by 5.3mg/L ozone. The quality of biodegradable algal organic matter can be significantly altered by the type of algae present (Wetzel, 2001) and this phenomenon therefore needs to be investigated further in ponds from different regions. Interaction between pH and starvation may also exist as resisting elevated pH may deplete bacteria energy, accelerating starvation.

1.6.3 Sunlight, pH and dissolved oxygen concentration

Sunlight is a major, if not the most important, factor in pond disinfection (Davies-Colley, 2000) and sunlight effect on faecal coliforms depends on pond depth, with shallower ponds being more efficient in faecal coliform removal (Pearson et al., 2005). Effect of sunlight also decreases with decreased light intensity or increased light attenuation (Van der Steen et al., 2000a). Sunlight interacts synergistically with oxygen and pH using photo-sensitizers in a process known as photo-oxidation (Curtis et al., 1992a). Photo-sensitizers outside the bacterial cell (exogenous sensitizers such as dissolved organic matter) and inside the bacterial cell (endogenous sensitizers such as porphyrins) respectively absorb long (400-700µm) and short light wavelengths (<500 µm), passing this light energy to oxygen and forming singlet oxygen and hydrogen peroxides in the process, which damages cytoplasmic membrane or DNA depending on their location (Curtis, 1990; Curtis et

al., 1992a). The effect of sunlight interaction with environmental factors in achieving faecal coliform removal in treatment ponds and possibly in eutrophic lakes is summarized in Table 8.

Table 8. Combined effect of light, pH and dissolved oxygen.

<i>Mechanism</i>	<i>Sensitizer</i>	<i>Conditions</i>
1) Direct photobiological damage to DNA by solar UV-B (300-320nm)	Independent of sensitizers	pH 7.5-8.5 Net effect depends on the efficiency of DNA repair mechanism
2) UV-B (300-320nm) photo-oxidative damage to DNA and DNA repair mechanisms	Endogenous sensitizers (e.g. cell contents)	Oxygen dependent
3) Long wavelengths (400-700nm) and UV (300-400nm) photo-oxidative damage to cell membrane of bacteria	Exogenous sensitizers (e.g. humic substances)	pH > 8.5 Enhanced by increased oxygen concentration.
4) High pH > 9.5 and fluctuating pH.	Independent of sensitizers	Effective even in darkness

Source: (Davies-Colley et al., 2000; Van der Steen et al., 2000b; Awuah, 2006).

Curtis et al. (1992a, 1992b) found that oxygen alone could not damage faecal coliforms but rather in the presence of sunlight, the rate of damage of faecal coliforms is proportional to the oxygen concentration. Sunlight disinfection is not an important mechanism in duckweed ponds in that duckweed covers the entire surface of the ponds cutting off light.

Light penetration in eutrophic lakes is influenced by the absorption characteristics, suspended and dissolved organic matter as occurs in algal ponds. Whitman et al. (2004) observed that insolation (amount of sunlight incident on a given surface in a given time) is a major factor in the inactivation of *E. coli* in lakes. Lindstrom et al. (2005) also observed that pH together with temperature and retention time explained 64% of the variation in lake bacteria numbers. Ambient conditions such as high pH, dissolved oxygen concentration, temperature, wind speed and direction are also important variables that may act synergistically to affect *E. coli* or faecal coliform survival in eutrophic lakes (Wetzel, 2001; Whitman et al., 2004). The fluctuation in pH and oxygen concentration as a result of the daily cycle of light-dark periods can also result in faecal coliform inactivation as occurs in waste stabilization ponds (Table 8).

1.6.4 Algal toxin

The role of algal toxins in the inactivation of faecal coliforms has been a subject of much debate (Maynard et al., 1999) but some recent publications have come in favour of the possible release of algal toxins in maturation ponds. Oudra et al. (2000) observed that the cyanobacteria (or blue-green algae) *Synechocystis sp* produced 20ng (10⁹ cell)⁻¹ of the toxin microcystin in waste stabilization ponds (WSPs), showing that this toxin can harm faecal bacteria as well as algae communities. Cyanobacteria however are not common in WSPs. Two green algae *Chlorella vulgaris* and

Scenedesmus quadricauda both responded to the toxin microcystin LR by producing large amounts of polysaccharides to protect their cells (Mohamed, 2008). *Chlorella* was observed to have secreted a substance toxic to *Vibrio cholerae* (Mezrioui et al., 1994; Maynard et al., 1999). Most recent work on algal toxin release unfortunately had focused on cyanobacteria and not on green algae which are more common in WSPs. This may be due to the immediate concerns for human health as some cyanobacterial toxins such as domoic acid is responsible for amnesic shellfish poisoning in humans (Litaker et al., 2008). Thus most of these studies had been done in eutrophic lakes and stormwater ponds and the focus had been on possible threat to other beneficial aquatic lives and not on faecal coliforms or pathogenic bacteria (Eynard et al., 2000; Serrano and DeLorenzo, 2008; Gong et al., 2009; Lindon and Heiskary, 2009). Green algae may also release toxins that are harmful to faecal coliforms thus contributing to their removal. Rapid detection methods being developed to detect cyanobacterial toxins (Litaker et al., 2008; Doucette et al., 2009) could be modified in future to detect and quantify possible toxins produced by green algae with the aim of assessing their importance in faecal or pathogenic bacteria inactivation. This would hopefully put to rest the debate on the role of green algal toxins in the inactivation of faecal bacteria or coliforms (Maynard et al., 1999). The role of green algal toxins in the inactivation of faecal coliforms is however outside the scope of the present study.

1.6.5 Algal biomass

Some authors have mentioned that algal biomass has negative influence on faecal coliform survival (Troussellier et al., 1986; Davies-Colley et al., 2000) but Van der Steen et al. (2000a) showed that high algal biomass can indirectly decrease faecal coliform inactivation by weakening the effect of solar radiation. They further argued that an optimum algal biomass may exist where maximum faecal coliform destruction is achieved. This assertion has however not been proved by experimentation. The effect of algal biomass on the rate of inactivation of faecal coliforms in eutrophic lakes and maturation ponds is not reported in literature. It is possible that algal biomass effects may be complicated by the production of possible algal toxins (Oudra et al., 2000) and the release of biodegradable algal organic matter via excretion (Wetzel, 2001) and cell lysis (Wetzel, 2001; Bouteleux et al. 2005). This thesis would investigate the effect of varying algal biomass on faecal coliform inactivation and how significant the effect may be in a tropical eutrophic lake. The thesis would also attempt to address the question of how this effect may vary in wastewater of different strengths considering the variation in faecal bacteria inactivation rates observed by Awuah (2006) in a pilot scale continuous flow wastewater algal pond treatment system.

1.6.6 Predation

Faecal bacteria are fed on by various invertebrates colonizing wastewater treatment ponds. The role of predation in WSPs is one area that had not received much attention in literature since the reviews of Maynard et al. (1999) and Davies-Colley et al. (2000). Awuah (2006) studied the role of protozoans in the removal of *E. coli* and *Salmonella* sp in pilot scale algal, duckweed and water lettuce ponds and concluded that the role of protozoan predation was important only in the water lettuce ponds, although the algal ponds had the highest numbers of protozoans and species diversity. This could be due to the ability of algae to also serve as food sources for many invertebrates (Wetzel, 2001). Invertebrates in algal ponds may therefore have plentiful supply of algae as food in addition to the availability of faecal bacteria. Studies on the role of macro-invertebrates associated with the different pond systems of algae and duckweeds need to be studied to assess their importance in the removal of faecal coliforms as they abound in these pond systems. Such a study in treatment ponds is rare compared to similar studies conducted in eutrophic lakes (Gonzalez, 1999; Wetzel, 2001). Predation by macro-invertebrates ($\geq 1.2\text{mm}$ by size) on bacteria (bacterivory)

is a major and often a dominant mortality factor in eutrophic lakes (Wetzel, 2001). Accurate quantification of bacterivory in eutrophic lakes is made difficult by the fact that among some macro-invertebrate populations, only a fraction may be feeding at any given point in time and some like the bacterivorous cladocerans or water fleas (0.2-3.0mm by size) appear only seasonally in eutrophic lakes (Simon et al., 1998; Gonzalez, 1999).

1.6.7 Attachment and sedimentation

Bacteria attachment in the aquatic environment may vary from quick reversible attachment to surfaces that may typically last for 3 hours (Kiorboe et al, 2002) to longer irreversible adhesions involving biofilm formation due to the secretion of extracellular polymer substances consisting of glucose, galactose, mannose, fructose, rhamnose, N-acetylglucosamine and the production of more cell biomass (Yao et al, 2002). Preliminary reversible adsorption or attachment to surfaces prior to biofilm formation usually involves the use of cellwall of bacteria which have adhesive properties (Costerton and Stewart, 2001). *Vibrio cholera* 0139 for example had been observed to have produced a surface pilus known as the mannose-sensitive haemagglutinin (MSHA) which facilitated attachment of *V. Cholera* to phytoplankton and zooplankton (Chiavelli et al, 2001). Motile forms of bacteria rapidly attach to aggregates while non-motile forms do not (Kiorboe et al, 2002). Attachment to aggregates by *E. coli* may also be facilitated by water flow, presence of enriched organic substrates, surface charge of aggregates, the type and size of the available surface among others (Jamieson et al, 2005; Liu et al, 2009). The size and density of the particles in wastewater and the development of high densities of algae in waste stabilization ponds therefore could influence the rate of removal of faecal coliforms due to settling. Auer and Niehaus (1993) and Soupier et al (2008) noted that 90% of *E. coli* was associated with particles of sizes 0.45-10µm and <3µm respectively in urban lake water and storm water. Boutilier et al (2009) noted however that particles and algal cells <80µm in diameter remained in suspension and although 10-50% of *E. coli* were found to associate with these particles found in domestic wastewater, settling was not observed.

Bacteria typically occur on aggregates in concentrations that are higher than the ambient water environment (Kiorboe et al, 2002) and this may facilitate their settling out of the water column faster than those in the free form (Characklis et al, 2005). This may be as a result of the aggregates having higher densities and hence sinking faster under the force of gravity. Scanning electron microscopy had shown the formation of a slime matrix engulfing both bacteria and some algae (Holmes, 1986). The size of *Chlorella* (Table 10), which is a dominant algal species in waste stabilization ponds and eutrophic lakes, indicates that it is only the aggregation of the cells that could lead to the formation of more dense aggregates capable of sedimenting attached faecal coliforms. The aggregation of suspended matter such as kaolin and *Chlorella* with a negative surface charge or zeta potential (Table 9) would depend on the availability in solution of acid-soluble polysaccharides which protonates, making its amino groups positively charged (Liu et al, 2009). The cationic polymers neutralizes the negative charge on the particulate or algal surfaces leading to inter particle bridging thus incorporating cells into flocs of high density, size and settleability (Jun et al, 2001, Henderson et al, 2008). The surface charge of algae however varies with its growth phase. The surface charge of *Chlorella* varies from -1.6 to -1.4 µmVs⁻¹ on transition from log growth phase to stationary phase due to variation in the quantity and composition of extracellular organic matter (EOM) attached to the cell surface (Henderson et al, 2008). *E. coli* has neutral buoyancy and therefore would have negligible settling rates (Boutilier et al, 2009). The importance of suspended solids and suspended algal matter in the removal of faecal coliforms in waste stabilization ponds and eutrophic lakes therefore would depend on the sizes of particulate

matter present as well as the type and concentration of dissolved organic compounds present. In eutrophic lakes, bacteria settling occur in pulses in response to other changes such as precipitation of calcium causing water whitening, disruption of the lake chemocline, sinking of phytoplankton biomass, among others (Romero et al., 2006). The importance of the possible settling of faecal coliforms at the lake bottom as a result of its attachment to algal biomass however had not been assessed. The present study will investigate the degree of faecal coliform attachment to algae in a tropical eutrophic lake and whether this can contribute to the settling or removal of faecal coliform from the water column.

Table 9. Characteristics of some common suspended matter used in laboratory sedimentation experiments. Source: Henderson et al. (2008)

Particle	Size(μm)/ shape	Surface area (μm^2)	Density (gL^{-1})	Zeta potential (mV)
<i>Chlorella vulgaris</i>	5.3 sphere	88	1070	-10.0
<i>Chlorella sp</i>	3.5 sphere	38	1070	-10.0
Kaolin	4.3 crystalline	74	2670	-46

In waste stabilization ponds, the formation of bacteria-algae-biomass flocs that settle at the bottom provides a clear supernatant that has been shown to improve the water quality of the effluent at least in terms of its algal concentration (Gutzeit et al., 2005, Medina and Neis, 2007). In the bacteria-algae floc, the algae excrete complex organic compounds which are broken down by the bacteria for their carbon and energy source and releasing inorganic carbon for the use of algae in photosynthesis (Wetzel, 2001; Nielsen, 2002).

As the availability of surfaces of attachment is one of the key factors affecting attachment and sedimentation in natural wastewater treatment systems, the importance of faecal coliform attachment and subsequent sedimentation would vary in algal and duckweed treatment pond systems. The phenomenon however is hardly reported in literature. In macrophyte pond systems such as duckweed ponds, faecal coliforms may attach to duckweed fronds and would therefore be shielded from the effect of solar radiation. In duckweed ponds however, conditions for the settling of suspended solids may be better as the bubbling release of oxygen and the effect of wind action is relatively minimal. MacIntyre et al. (2006) noted that *E. coli* numbers decreased when floating plants were removed from a surface flow constructed wetland and suggested that *E. coli* may survive longer because the plants provided sites of attachment and shades from ultra-violet radiation for the *E. coli*. Awuah (2006) also noted that removal of faecal bacteria through attachment to harvested macrophytes accounted for less than 1% of faecal bacteria removal. Algal ponds however have higher suspended matter (in part due to suspended algae) which may provide a much larger surface area for faecal coliform attachment. The process of sedimenting attached faecal coliforms however could be affected by macro-invertebrates whose feeding and movement activity in algal and duckweed pond sediments can result in the re-suspension of attached faecal coliforms. This phenomenon is not reported in literature. Awuah (2006) recommended the need to investigate the importance of attachment and subsequent sedimentation in algal pond systems. This study would compare attachment and sedimentation in algal, duckweed and hybrid algal-duckweed ponds and would investigate the role of macro-invertebrates in this process.

2 MOTIVATION AND FOCUS OF THE STUDY

WSPs exhibit high performance in FC removal when compared with constructed wetlands and conventional wastewater treatment systems. This performance however is less than perfect as the mechanisms by which FC are removed are still not well understood. Over the past years, the role of algae in inactivating FC through fluctuating and elevated pH and dissolved oxygen concentration and its promotion of FC survival via attenuation of solar radiation and supply of carbon has been documented. It is however not known how algal biomass variation affects FC inactivation and how this effect may vary in wastewater of varying strength. It is also not known whether algae contribute to the sedimentation of FC by providing surfaces for attachment and how important this phenomenon is in eutrophic lakes and wastewater treatment pond systems. Questions relating to whether any possible sedimentation of FC by algae could be re-suspended by macro-invertebrates feeding on pond sediments have not been addressed. Understanding better the role of algae in FC inactivation, particularly in relation to algal biomass variation and its sedimentation of FC could lead to the development of better models for predicting FC removal and better designs, operation and maintenance of WSPs for a more efficient removal of pathogens. Higher performance of WSPs in pathogen removal would lead to public health benefits, particularly for developing countries. Developing countries need low cost technologies that are efficient in pathogen removal due to the use of raw water from lakes and rivers by rural communities in these countries.

3 RESEARCH OBJECTIVES

The specific objectives of the study are as follows:

- To investigate the effect of varying concentrations of algal biomass on FC inactivation in an algal wastewater treatment pond system and a tropical eutrophic lake
- To investigate the ability of algae to sediment FC through attachment to its surfaces.
- To assess the performance and the mechanisms of faecal coliform removal of a hybrid system of algal and duckweed ponds.

4 OUTLINE OF THE THESIS

Chapter 1 of the thesis gives a brief background of the need to treat domestic wastewater and identifies some of the knowledge gaps existing with respect to our current knowledge of mechanisms of faecal coliform removal in eutrophic lakes, algal and duckweed ponds. Chapter 2 describes batch experiments in freshwater microcosms investigating the ability of algae and suspended solids to sediment faecal coliforms via attachment to their surfaces. The effect of algal density on faecal coliform removal under conditions of varying sewage strength or dissolved organic matter content is discussed in Chapter 3. The general performance of three parallel lines of algal, duckweed and hybrid algal and duckweed ponds are discussed in Chapter 4 and Chapter 5 discusses the relative importance of faecal coliform attachment to algae and suspended solids, pond depth and macro-invertebrates in the removal of faecal coliforms in these three pond systems. Chapter 6 reports on the role of algae in the removal of *E. coli* from a tropical eutrophic lake while Chapter 7 discusses the conclusions drawn in this thesis and reports on areas that need further research.

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2

Chapter

Escherichia coli* attachment and detachment in algal and kaolin suspensions*ABSTRACT**

The phenomenon of attachment has implications for treatment processes as attached pathogenic bacteria may get sedimented, leading to removal. Attachment of faecal coliforms (FC) may also result in underestimation or inaccurate assessment of the performance of treatment systems for coliform removal, but the importance of this phenomenon has not been studied yet. In order to do so, a clearly defined and efficient procedure of detaching FC from each other and from suspended matter needs to be established. Experiments were conducted to compare the effectiveness of various methods of detaching *Escherichia coli* and also to assess the importance of *E. coli* attachment to suspended solids and algae. The various methods used to detach *E. coli* were Sonication (different equipment types), Vortex-mixing, combination of Vortex-mixing and Sonication, Syringe-needle, and Parozone (a disinfectant). The Syringe-Needle method and the use of 0.11g/L parozone were effective in detaching attached *E. coli*. Algae helped in sedimenting *E. coli* to the bottom of reactors within 24 hours. The mechanism by which this occurred did not involve a process of permanent attachment to algal surfaces, but could be an entrapment by algal jellies which led to the formation of heavier aggregates that sediment faster. *E. coli* did not attach to algae after 24 and 48 hours of incubation.]

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1 INTRODUCTION

In the aquatic environment bacteria may attach to varying kinds of surfaces such as the solid surfaces of a pond (Holmes, 1986), suspended matter (Grossart and Simon, 1998) and even on the bodies of plants and other animals present in the water (McEwen and Leff, 2001, Chiavelli et al, 2001). The flow of nutrient rich water makes these surfaces conducive for bacterial attachment. Bacteria attachment in the aquatic environment may vary from quick reversible colonization of surfaces to longer irreversible adhesions involving biofilm formation. Preliminary reversible adsorption or attachment to surfaces prior to biofilm formation usually involves the use of cellwall of bacteria which have adhesive properties and may take seconds or minutes to occur (Costerton and Stewart, 2001). Persistence of bacteria on this surface may lead to the production of chemicals and structures which include the formation of extracellular polymer substances consisting of glucose, galactose, mannose, fructose, rhamnose, N-acetylglucosamine and others (Costerton and Stewart, 2001; Yao et al, 2002). The entrapment of these growing bacteria cells in this hydrated polymeric matrix leads to the formation of what is known as a biofilm (Davey and O'toole, 2000). Bacteria in biofilm usually are more resistant to stresses such as disinfection and shear stress than their free-living counterparts (Bower and Mitchell, 2001; Moretro et al, 2003) and these bacteria attachments as well as the additional properties they acquire could affect the performance of some wastewater treatment systems. Increased densities of heterotrophic bacteria involved in the degradation of organic matter as a result of biofilm formation is well known and the principle had been utilized in many wastewater treatment technologies such as the rotating biological contactor, trickling filter and the upflow anaerobic sludge blanket reactor (Bal and Dhagat, 2001; Metcalf and Eddy, 2003; Von Sperling, 2007). However very little is known about the importance of the attachment of pathogens to each other and to suspended matter in wastewater treatment systems (Awuah, 2006). The effects of such possible attachment on removal or survival of pathogens is unknown.

The presence of suspended matter in natural wastewater treatment systems in the form of suspended solids and algae may lead to the formation of aggregates which could be colonized reversibly and irreversibly by pathogenic bacteria or faecal coliforms (FC). Such aggregates may sink faster, thus sedimenting attached FC from the water column. FC attachment to particles and to each other in treatment systems could also lead to underestimation of effluent bacteria counts in methods such as the use of plate counts and the most probable number estimates. This could have public health implications for re-use of wastewater. This is because several aggregates of bacteria cells in suspension could appear as just a colony during counting. For example in the enumeration of FC using lactose agar, George et al (2002) had to perform ultrasonication on the wastewater samples to break up aggregates or detach FC that may be attached to suspended solids. Detachment of FC attached to each other ensures that a more accurate assessment of effluent quality and hence treatment systems are made. Inaccurate estimation of FC numbers in effluent could lead to failure to detect malfunctions in wastewater treatment systems and the possible re-use of highly contaminated effluents on crops. The importance of these phenomena is not well documented in literature.

The quantification of attached bacteria presents many challenges due to the limitations of currently available methods which include loss of viability of cells, loss of luminescence of marker-cells among others (Prosser et al, 1996; Turnbull et al, 2001). It is therefore necessary to develop or adapt various methods for detaching bacteria in order to be able to assess accurately, the quantity of faecal coliforms attaching for the purpose of assessing the importance of faecal coliform attachment in wastewater treatment pond systems. This study reports findings from a comparative assessment of various methods of bacteria detachment using plate counts, and investigates the importance of algae and suspended solids as surfaces for bacteria attachment in natural wastewater treatment systems.

This work also served as the basis for further investigations into the role of algae in the removal of faecal coliforms from domestic wastewater.

2 MATERIALS AND METHODS

2.1 Parozone disinfectant

Parozone is a trade name of a disinfectant and bleach produced by Jeyes Group, manufacturers of hygiene products (www.jeyes.co.uk). It contains water, sodium hypochlorite, sodium laureth-8-sulphate, sodium hydroxide, sodium lauroyl sarcosinate, polycarboxylate and sodium periodate. It contains 5.0 % w/w available chlorine and 15% w/w solids. Density of parozone is 1.064g/mL and normal use of parozone for disinfection makes use of 83.0mL of water for every 1.0mL of parozone.

2.2 Determination of best method of detaching *E. coli*

Methods involving the use of sonication (McEwen and Leff, 2001, George et al, 2001; 2002), vortex-mixing (Lipponen et al, 2004; Khamm et al, 2004), parozone detergent (Bast, 2001; Bockelmann et al, 2003) and shear stress application using syringe fitted with needle (Bockelmann et al, 2003; Perni et al, 2008) were used to detach *E. coli* from each other and from solid surfaces.

Laboratory cultures of approximately 10^4 cfumL⁻¹ *E. coli* ATCC 25922 (Strain info, 2010) were obtained by dilution using normal saline solution (prepared by 8.5g NaCl of Oxoid BR0053G dissolved in 1L demineralized water, autoclaved at 121°C for 15 minutes). *E. coli* suspension was forced to 'attach' by centrifugation at 1000rpm for 20 minutes. This process was a practical approach necessary for simulating attachment. Evidence of attachment of *E. coli* to each other using this method was obtained by decrease in *E. coli* count after centrifugation with subsequent increase in count when detachment is done (George et al, 2002; Figure 4C and 4D).

Centrifuged *E. coli* suspension was mixed gently using IKAMAG Color Squid magnetic stirrer (IKA-Werke GmbH & Co. KG, Staufen, Germany). Detachment tests were performed on various *E. coli* suspensions according to the treatment regime in Table 1 using Bransonic 220 water bath sonicator (Branson Ultrasonics Corp., Danbury, Ct., USA), 9 speed of a Heidolph REAX 2000 vortex mixer (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany), parozone detergent (Jeyes Group, Thetford, Norfolk, England) and application of shear stress using syringe fitted with a needle. *E. coli* numbers before and after sonication were determined for various time durations and concentrations using spread plate method (APHA, 2005) on chromocult coliform agar, incubated for 24 hours at 37°C (Finney et al, 2003). *E. coli* numbers before and after detachment were compared using students'T-test.

Table 1. Detachment methods tested and their treatment regimes

<i>Method</i>	<i>No. of samples (n)</i>	<i>Mean Initial conc. (cfu.mL⁻¹)</i>	<i>Treatment regimes</i>
Sonication			
Water bath	10	4.0x10 ³	I: 0, 60, 120, 180, 240, 300 (s)
	10	6.1x10 ³	II: 0, 15, 30, 45, 60 (s)
Detergent			
Parozone	10	2.0x10 ³	*60s: 0, 0.01, 0.11, 1.06, 10.64 (gL ⁻¹)
	10	3.0x10 ³	*30 mins: 0, 0.01, 0.11, 1.06, 10.64 (gL ⁻¹)
Shear-stress			
Syringe-Needle	10		10 ⁴ , 10 ⁵ , 10 ⁷ , 10 ⁹ (cfu.mL ⁻¹)
Vortex-mixing	10	1.5x10 ³	0, 30, 60, 90, 120 (s)

*Detachment treatment period adapted from Rice et al, 1999; Rutala et al, 2000; 2008

2.3 Attachment of *E. coli* to algae and kaolin

The purpose of these experiments was to investigate qualitatively, the possibility of *E. coli* attachment to algae and suspended solids in the presence of high concentrations of these suspended matters and whether this attachment could lead to increased *E. coli* numbers at the bottom of flasks (settling of *E. coli*). This was a preliminary experiment aimed at understanding sedimentation of *E. coli* by suspended matter that is further investigated in this chapter.

Algae (microalgae dominated by *Chlorella* sp) grown by natural colonization using pre-settled domestic wastewater was concentrated by centrifugation at 1000rpm for 20 minutes. *E. coli* (10⁵/mL, 2mL) suspended in normal saline was added to 198mL algal suspension, mixed gently with a IKAMAG Color Squid magnetic stirrer. Fifteen millilitres of this suspension, 1.0 x 10⁹ cells/mL (34.8mg/L chlorophyll a concentration) was then transferred into each of nine Erlenmeyer flasks placed on a GFL 3018 shaker rotating at 100rpm. Mean surface area provided by algal cells is 1.59 x 10¹⁰µm²/mL. After 72 and 120 hours of incubation, 0.01mL of this suspension was immediately taken from the bottom of each flask and inoculated on chromocult coliform agar, incubated at 37°C for 24 hours for a colony count (Finney et al, 2003). For each flask, a replicate of three samples were plated. Five millilitres of sample from each Erlenmeyer flask were then treated with the syringe-needle method.

E. coli suspension in normal saline (10⁵/mL, 10mL) was added to 90mL of kaolin suspension obtained by dissolving 0.75g kaolin in 150mL of demineralized water. Kaolin concentration of 0.75g kaolin in 150mL of demineralised water has average particle counts of 6.72 x 10⁹ particles/mL (1.68 x 10¹¹µm² surface area per millilitre). Fifteen mL of this *E. coli*-kaolin suspension was transferred into each of five Erlenmeyer flasks placed on shaker as mentioned above. Samples from this incubation were then taken after 72 and 120 hours as done above and the detachment procedure above repeated for this *E. coli*-kaolin suspension as well. Similar experiments involving bacteria attachment to suspended solids and algae were conducted but modified by having three incubations consisting of domestic wastewater, domestic wastewater with 0.15g kaolin/50mL (4.03 x 10⁹ particles/mL, 1.0 x 10¹¹µm² surface area/mL) of wastewater and thick algal paste diluted in wastewater. Each setup was duplicated and had a replication of three

samples. In these experiments, sampling for plating before and after detachment tests was done after 24 hours and 48 hours of incubation.

2.4 The settling of algae and suspended solids in wastewater

E. coli (10^6 /mL, 2mL), were added to each of two sets of 198mL algal suspension of concentration 130.6mg/L (3.78×10^9 algal cells/mL; $6.0 \times 10^{10} \mu\text{m}^2$ surface area per millilitre) and thoroughly mixed by the IKA colour squid magnetic stirrer. Two millilitres of one set of the resulting algae-*E. coli* suspension was pipetted off and replaced with 2mL of 10.64g/L parozone solution to prevent possible attachment of *E. coli* cells. This was carefully mixed and 20mL of this algal-*E. coli*-parozone suspension were transferred into each of 10 Erlenmeyer flasks. Twenty millilitres of the algae-*E. coli* suspension (without parozone) was also transferred into each of another set of 10 Erlenmeyer flasks. The whole setup of 20 Erlenmeyer flasks was placed on a IKA HS 260 basic shaker rotating at 100rpm. From all incubations (both algae-*E. coli* and algal-*E. coli*-parozone), 0.01mL were taken from each Erlenmeyer flask immediately and inoculated on chromocult coliform agar plates to determine concentration of *E. coli* in setup at start of experiment for algae-*E. coli* and algal-*E. coli*-parozone incubations respectively. Inoculated chromocult coliform agar plates were incubated in a Gallenkamp size one incubator at 37°C for 24 hours for a plate count. After 24 and 48 hours, 0.01mL of samples from both algae-*E. coli* and algae-*E. coli*-parozone were taken from the top and bottom, inoculated on chromocult coliform agar plates and incubated as done at the start of experiment. *E. coli* counts at the top and bottom of the two incubations were statistically compared using T-test of the SPSS statistical package. Two other setups of 20 Erlenmeyer flasks were prepared as previously done for algae but involved only wastewater and wastewater with parozone in the same concentration. Detachment tests were performed on all samples 24 hours and 48 hours after incubation by taking 5mL of samples, treated with the syringe-needle method. Detached and undetached samples were plated on chromocult agar and statistically compared as done above.

3 RESULTS

3.1 Determination of best method of detachment of *E. coli*

Various methods for detachment of bacteria were investigated. This included the use a water bath sonicator, vortex mixing, parozone, soniprep sonicator and syringe-needle. The use of these methods involved first investigating the optimum time, energy or concentration needed in bacteria detachment with little or no death occurring. The syringe-needle method showed the results in terms of the quantity of *E. coli* that was recovered after detachment. Efficiency of the syringe-needle method was assessed by calculating the number of attached *E. coli* that were detached by this method (Table 2). The student's T-test was used to compare *E. coli* numbers before and after detachments and also to establish any differences in means between two treatments.

The use of sonication (Table 1) did not prove to be an adequate means of detaching bacteria (Figure 1A and 1B). Vortex mixing showed an optimum time of ≥ 60 seconds ≤ 90 seconds, and 60 seconds vortex mixing time was adopted for subsequent experiments (Figure 1C). A parozone concentration of 0.11 gL^{-1} emerged as ideal for detachment of *E. coli* (Figure 1D)

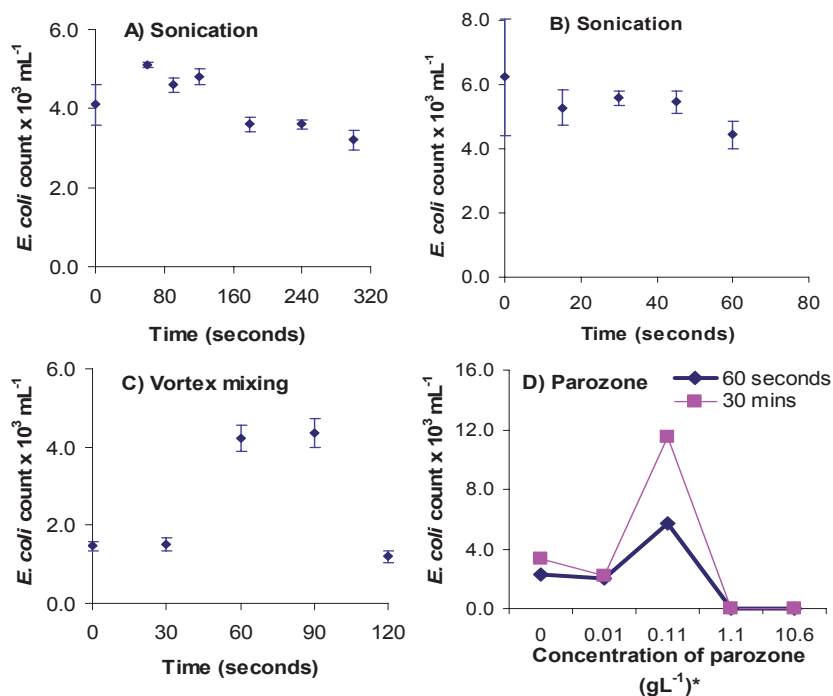


Figure 1. Determination of optimum time of sonication (A and B) and optimum time of vortex mixing (C). Determination of ideal concentration of parozone for detachment of *E. coli*. 60 seconds and 30mins are treatment time with parozone adapted from Rice et al (1999), Rutala et al (2000) and Rutala et al (2008) (D).

The syringe-needle method was adopted for subsequent detachment experiments and in some experiments the use of 0.11 g L^{-1} parozone solution was also employed (Figure 4, 5 and 6). Using *E. coli* concentrations of $2.2 \times 10^9 \text{ mL}^{-1}$, the syringe-needle method was able to recover completely all the attached *E. coli* and can therefore be considered as 100% detachment (Table 2).

Table2. Determination of the efficiency of syringe-needle detachment procedure.

<i>Concentration of E. coli ($\times 10^4$) mL^{-1}</i>				
<i>Initial concentration</i>	<i>Conc. after centrifugation</i>	<i>Conc. after detachment</i>	<i>Attached (%)</i>	<i>p-value</i>
1) 1.8 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	Not sig.	>0.05
2) 63 ± 4.5	54 ± 1.4	71 ± 12	Not sig.	0.154
3) 1800 ± 77	1900 ± 140	2000 ± 81	Not sig.	>0.05
4) $220,000 \pm 1700$	$190,000 \pm 12000$	$270,000 \pm 14000$	39.9*	0.004

* Efficiency of detachment of syringe-needle method using $2.2 \times 10^9 \text{ mL}^{-1}$ *E. coli* suspension was 100%. Number of samples, $n=10$. Concentrations indicated \pm standard error.

3.2 Attachment of *E. coli* to algae and kaolin

The amount of *E. coli* that attaches to algae and kaolin was assessed after incubating algae-*E. coli* and *E. coli*-kaolin suspensions for 3 and 5 days. The purpose of this experiment was to assess the importance or degree of faecal coliform attachment to algae and to suspended solids in freshwater microcosms and by extrapolation, treatment pond systems. Detachment tests performed after 3 days and 5 days of incubation in algae-*E. coli* setup did not reveal significant increases in *E. coli* numbers ($p = 0.081$ for 3 days, and $p = 0.097$ for 5 days (Figure 2). In the case of *E. coli*-kaolin suspension, *E. coli* counts before and after detachment tests for both 3 and 5 days of incubation showed comparable results ($p > 0.05$).

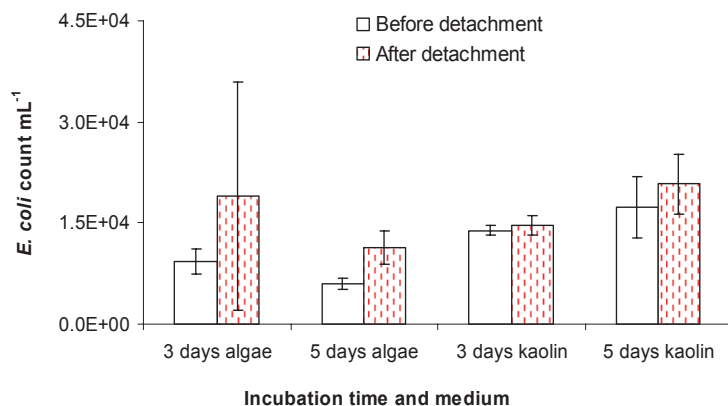


Figure 2. Attachment of *E. coli* to algae and kaolin after 3 and 5 days of incubation.

Wastewater ($p = 0.001$, $t = 4.57$) and kaolin ($p = 0.018$, $t = 3.860$) setups respectively showed significant *E. coli* numbers attached after 24 hours. Detachment test performed on wastewater and kaolin setups after 48 hours did not show significant *E. coli* numbers attached (Figure 3A and B). Algal incubations at 24 and 48 hours did not show significantly attached *E. coli* numbers ($p > 0.05$, Figure 3A and B).

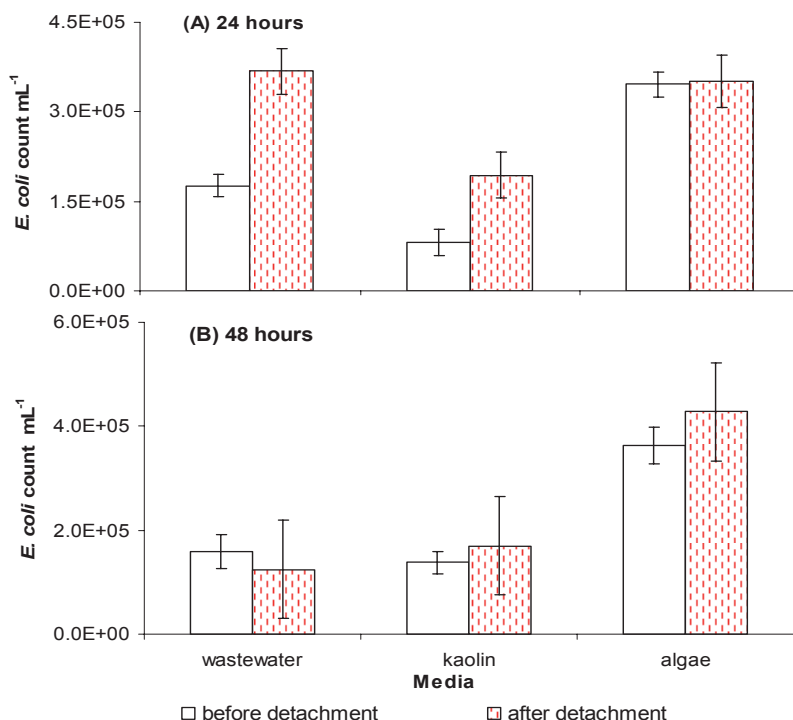


Figure 3. Attachment of *E. coli* to suspended solids in wastewater and attachment to increased suspended matter in wastewater in the form of kaolin and algae after 24 hours (A) and 48 hours (B) of incubation.

3.3 Settling of algae and suspended solids

Algae and suspended solids settle at the bottom of reactors after some few days. The ability of algae and suspended solids to sediment *E. coli* to the bottom of freshwater microcosms or wastewater treatment ponds was investigated. The use of 0.11gL^{-1} parozone prevents the attachment of *E. coli* to each other and to other solid surfaces.

E. coli numbers were comparable for both algae and algae with parozone at the beginning ($p = 0.292$). Day 3 and day 5 of both incubations however showed significantly higher *E. coli* numbers at the bottom ($p < 0.05$, Figure 4A and 4B). No significant differences in *E. coli* numbers at the bottom of both setups were observed on day 3 and 5 respectively ($p > 0.05$, Figure 4C).

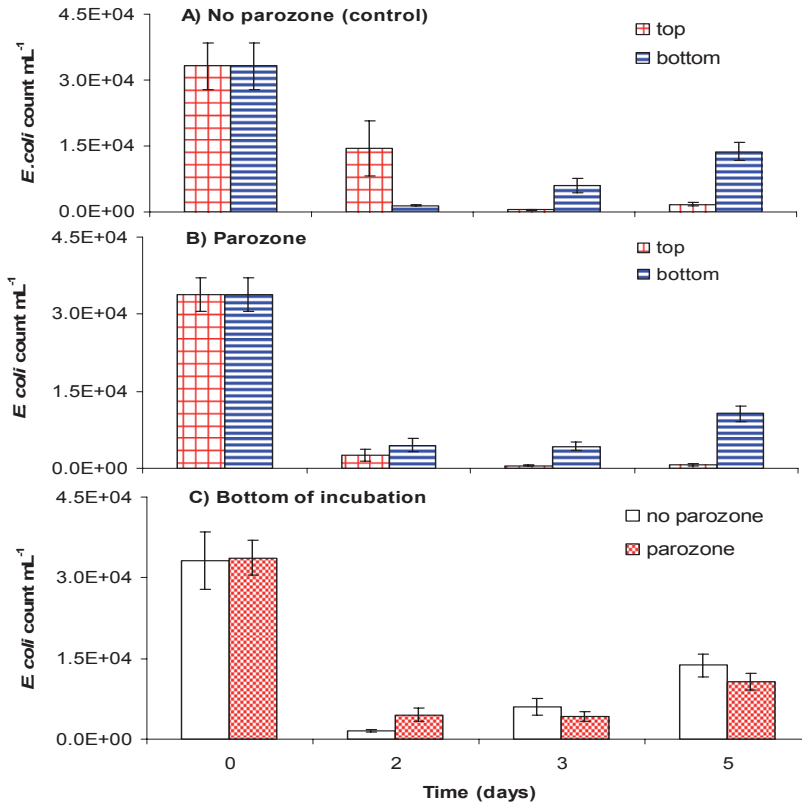


Figure 4. *E. coli* numbers at the top and bottom of no parozone (A) and parozone (B) incubations and a comparison of *E. coli* numbers at the bottom of control and parozone containing incubations (C).

In incubating wastewater with increased suspended matter, it was hypothesized that sedimentation of faecal coliforms in wastewater would increase (at the bottom), particularly for incubations that do not contain parozone as attachment may be minimal in incubations with parozone. Addition of parozone to wastewater did not lead to differences in faecal coliform numbers ($p = 0.134$, Figure 5A). After 24 hours incubation, significantly higher faecal coliform numbers were observed at the bottom of the flask with algae compared to the top portion ($p < 0.001$, $t = 60.29$, Figure 5A and 5B). After 24 hours incubation higher faecal coliform numbers were observed at the bottom of algae with parozone compared to the top ($p = 0.001$, $t = -8.65$). Comparing mean separation (t-value or test statistic) of the top and bottom of algae only incubation and algae-parozone incubation, a much higher t-value for the algae only incubation was observed compared with the algae-parozone incubation (after 24 hours).

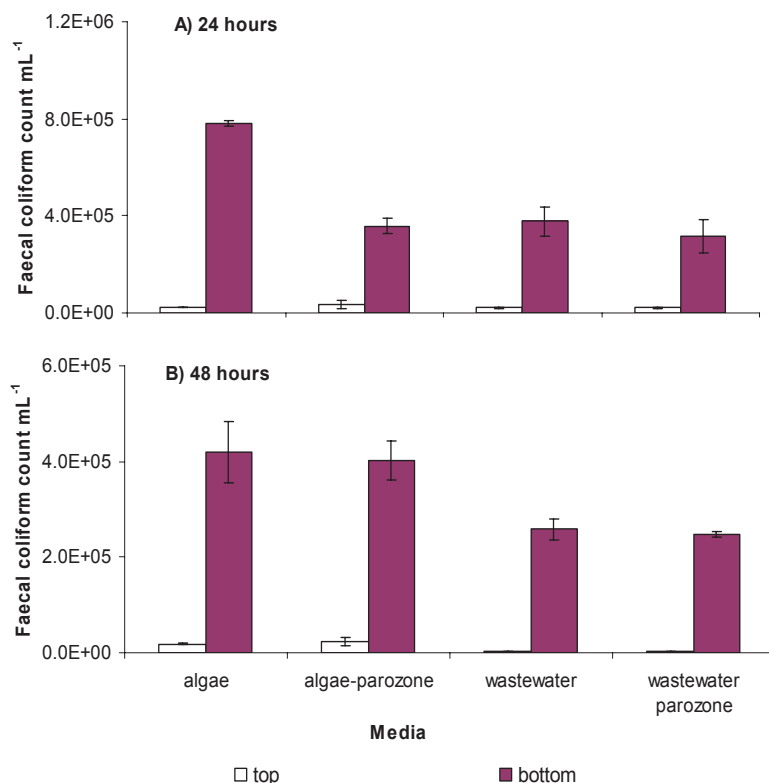


Figure 5. Faecal coliform numbers at the top and bottom after 24 hours (A) and 48 hours (B) of incubations.

In both algae-only and algae-parozone setups, higher faecal coliform numbers were observed after 48 hours at the bottom compared to the top ($p < 0.001$, $t = 19.20$ for algal and $p = 0.001$, $t = -9.11$ for algal-parozone). Significantly higher faecal coliform counts were observed at the bottom of the wastewater only setup compared to the top portion after 24 hours ($p = 0.004$, $t = -5.92$) as well as after 48 hours ($p < 0.001$, $t = -10.96$). Similar observations were made when parozone was added to wastewater after 24 hours ($p = 0.011$, $t = -4.45$) and 48 hours ($p < 0.001$, $t = -27.12$) of incubation (Figure 5A and 5B). Detachment tests did not show significant faecal coliform attachment (Figure 6). Faecal coliform numbers after 24 hours of incubation decreased significantly ($p = 0.008$, $t = 10.83$). Faecal coliform numbers at the bottom of the algal-parozone setup after 24 hours of incubation ($p = 0.529$) and after 48 hours of incubation ($p = 0.634$) were comparable to bottom numbers at the start of the experiment. Algal setup without parozone had significantly greater number of faecal coliforms at the bottom ($p = 0.026$, $t = -6.14$) after 24 hours of incubation compared with faecal coliform numbers at the bottom at the start of the experiment.

After 48 hours of incubation however, the numbers did not differ from the ones at the beginning of the experiment. ($p = 0.694$). The wastewater setup did not show any increase (in faecal coliform numbers) at the bottom (of the setup) after 24 hours ($p = 0.09$) and 48 hours ($p = 0.173$). Wastewater with parozone setup was comparable in faecal coliform count at the start of the experiment and after 24 hours of incubation but significantly decreased in faecal coliform numbers at the bottom after 48 hours of incubation ($p = 0.046$, $t = -4.506$).

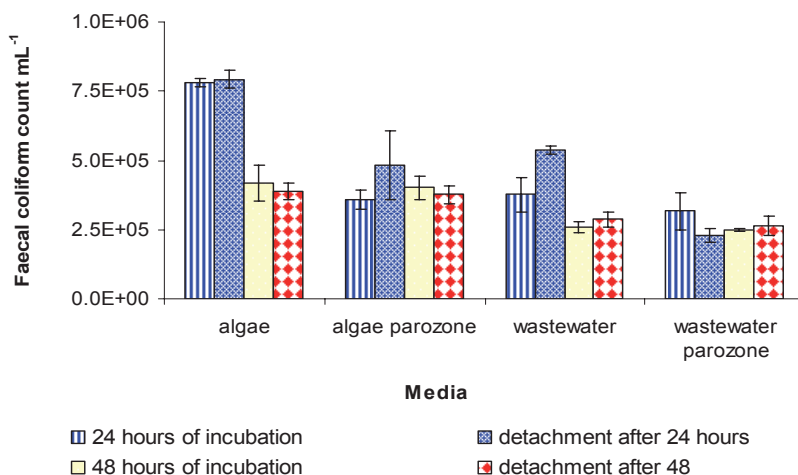


Figure 6. Faecal coliform numbers at the bottom after 24 hours and 48 hours of incubation.

4 DISCUSSION

4.1 Detachment methods

Water bath sonicators were designed for cleaning bacteria-contaminated wares while vortex-mixers and detergents such as parozone were primarily designed for mixing solutions or suspensions and for cleaning bacteria-contaminated surfaces respectively. As a result these methods to detach bacteria may be either too excessive resulting in loss of viability of bacteria cells or too limited resulting in incomplete detachment of attached bacteria (Turnbull et al, 2001). As strength of attachment also vary from one bacterium species to the other (Costerton and Stewart, 2001) it was therefore necessary to determine an optimum force or concentration capable of achieving maximum bacteria detachment without loss of viability or culturability of bacteria when the plate count method is used. Variation in *E. coli* numbers after detachment using varying periods of vortex mixing showed that the use of centrifugation at 1000rpm for 20 minutes was capable of simulating attachment.

The use of sonication was not viable for detachment of *E. coli* as this detachment process resulted in significant loss of viability of bacteria cells. The use of 0.11g/L parozone (Figure 1D) and syringe-needle (Table 2) emerged as viable methods for detaching attached bacteria and these were used in subsequent experiments. When used to detach 10^9 /mL of *Escherichia coli* in saline solution, the syringe-needle method was presumably able to recover all attached bacteria (Table 2). In Table 2 syringe-needle treatment using initial concentrations of (1) 1.8×10^4 cfu/mL and (3) 1.8×10^7 cfu/mL showed comparable values to initial concentration after centrifugation considering the standard error. This suggests that *E. coli* cells did not attach and increased *E. coli* numbers observed after detachment in the case initial concentration (3) was due to chance or error ($p > 0.05$). The syringe-needle method has a limitation of not always having an exact or constant force applied due to the human factor of pushing fluid through the needle. Its ease of use, cost effectiveness and reliability however overshadowed this limitation. Higher *E. coli* numbers observed after detachment using initial concentration of 2.2×10^9 cfu/mL (4) suggests that some *E. coli* cells were already

attached to each other before the centrifugation process. It was obvious that chances of *E. coli* attachment increased with increased bacteria density as was also observed by Turnbull et al (2001). Lower concentration of *E. coli* was used in order to avoid significant loss of *E. coli* to attachment on walls of glassware used in the experiment (Kjelleberg et al, 1983).

4.2 Attachment of *E. coli* to algae and suspended solids

No *E. coli* attachment to algae or kaolin was observed after 3 and 5 days of incubation. It is possible that no attachment took place or a weak reversible attachment took place or *E. coli* attached and died before 3 days (Figure 2). Subsequent experiments with incubation time of 24 hours and 48 hours showed that *E. coli* attached to each other and to suspended solids after 24 hours of incubation (Figure 3A) and either got sedimented or died after that as less *E. coli* numbers were found attached after 48 hours (Figure 3B). *E. coli* attached to suspended solids in wastewater were 1.9×10^5 cfu mL⁻¹ and 1.1×10^5 cfu mL⁻¹ *E. coli* were attached to kaolin after 24 hours. Surprisingly, *E. coli* was not found attached to algae either after 24 hours or 48 hours of incubation (Figure 3). This suggests that either *E. coli* did not attach to algae at all or did so and died in less than 24 hours. It is possible that the attachment of *E. coli* to algal surfaces is weak, temporal and reversible. Awuah (2006) observed that faecal bacteria numbers in an algal pond decreased in suspension but increased on the walls of the pond within 2 days only to decrease on the walls and re-appear in suspension again within the next 2 days. As their sampling took place every two days, it is possible this phenomenon of reversible attachment was quicker than 2 days. It is also possible that *E. coli* were sedimented together with algae or attached to the walls of the reactor. Algae are known to settle at the bottom of beakers or counting chambers after 24 hours of incubation (APHA, 2005).

Figure 4 showed that after 3 and 5 days of incubating *E. coli* suspension with and without (control) parozone, higher *E. coli* numbers were observed at the bottom compared with the top in both control and parozone treatment. As numbers of *E. coli* in both control and parozone treated incubation were comparable at the bottom, it suggests that little or no attachment of *E. coli* to each other may have taken place in the control. Incubating *E. coli* with algae in subsequent experiments (Figure 5) showed that algae helped *E. coli* to settle at the bottom faster within 24 hours but the *E. coli* was not attached to the algae as detachment tests conducted on bottom-settled *E. coli* samples revealed no significant difference before and after (Figure 6). Algae usually form a jelly-like slime around itself and this could trap *E. coli* cells, helping to sediment more *E. coli*. More *E. coli* numbers were observed in the algae-*E. coli* control compared with algae-*E. coli* with parozone treatment. Using electron microscopy, Holmes (1986) showed the presence of a slime matrix engulfing both bacteria and algae in an algae-bacteria suspension. Preliminary experiments involving parozone showed that 0.11g L⁻¹ concentration of this compound prevents *E. coli* from attaching to each other or to other surfaces. As after 48 hours of incubation no real difference in *E. coli* numbers occurred at the bottom of the control and parozone treated algae-*E. coli* incubation, this suggest that it was algae that helped more *E. coli* to accumulate at the bottom of the reactor in 24 hours, the slower parozone rendered-single-celled *E. coli* eventually also settling at the bottom after 48 hours. After 24 hours of incubation, *E. coli* numbers at the bottom of control were significantly higher than at the beginning of the experiment. After 48 hours of incubation however, *E. coli* numbers at the bottom decreased again to numbers comparable to that at the start of the incubation (not shown in Figure) suggesting that *E. coli* settling at the bottom were dying.

In both the wastewater with and without (control) parozone incubations, significantly higher *E. coli* numbers were observed at the bottom compared to the top after 24 and 48 hours. *E. coli* numbers at the bottom of wastewater control and wastewater with parozone were comparable after 24 and 48 hours of incubation. It is obvious that the treatment with parozone had little effect in creating any

differences and it could be speculated that the effect of the parozone (attributed to its sodium salts) may have been neutralized by the buffering capacity of the wastewater.

5 CONCLUSIONS

- Findings from this work shows that the use of syringe fitted with a needle could be an effective means of detaching attached bacteria.
- The use of 0.11g/L parozone was also effective in preventing attachment of *E. coli* to each other or to suspended matter.
- Algae helped in sedimenting faecal coliforms to the bottom of reactors within 24 hours. This work served as the preparatory work for further studies on faecal coliform attachment to suspended matter reported in chapter 5.

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3

*Chapter***The effect of algal biomass on the removal of faecal coliforms from domestic wastewater****ABSTRACT**

The effect of algal density on faecal coliform (FC) decay under conditions of light and darkness were monitored in low and medium strength wastewater and in a 'mixture of treated and raw wastewater depicting conditions of a variety of dissolved organic compounds. Rates of decay of FC varied in darkness with varying chlorophyll-a concentration, supporting the hypothesis that algae may produce substances that are toxic to FC. The first empirical evidence that optimum chlorophyll-a concentration ($10 \pm 2 \text{ mgL}^{-1}$) for maximum FC destruction in wastewater exist is reported. Rate of decay was higher in medium strength wastewater compared with low strength wastewater at higher algal densities of $\geq 13.9 \text{ mgL}^{-1}$ chlorophyll-a both in light and in darkness while addition of fresh wastewater to an ongoing wastewater treatment process may lower the rate of FC decay for a wide range of algal densities ($0.6 - 19.6 \text{ mgL}^{-1}$), under light conditions.

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1 INTRODUCTION

Algal growth in wastewater treatment ponds leads to increases in pH and dissolved oxygen concentration which may render the aquatic environment hostile to faecal coliforms (FC). Curtis et al. (1992) showed that sunlight damaged FC in waste stabilization ponds, 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 (Davies-Colley et al. 1999). In addition, pH also acts synergistically with dissolved oxygen in a process known as photo-oxidation to achieve die-off of FC (Maynard et al. 1999). Van der Steen et al. (2000) showed that light attenuation occurs at high chlorophyll-a concentrations and rapid algal growth that occurs in tropical regions may compromise the gains of increased pH and oxygenation. They hypothesized that optimum chlorophyll-a concentration of algae may exist whereby maximum FC die-off is achieved. This was however not proven by experimentation. Ansa et al. (2011) showed using laboratory simulated lake conditions that an optimum algal density where maximum FC is inactivated could exist in a eutrophic lake but this hypothesis had not been tested in wastewater treatment systems. One of the objectives of this study is to test whether there indeed exist optimum chlorophyll-a concentration for maximum FC decay in domestic wastewater treatment under batch, laboratory conditions.

The role of organic matter in FC removal in domestic wastewater is still a subject that is not well understood. Dissolved organic matter (DOM) in domestic wastewater, depending on its origin, may act as sensitizers, absorbing long wavelengths of electromagnetic radiation and transmitting it to faecal bacteria cell membranes leading to their subsequent destruction (Curtis et al. 1992; Sinton et al. 2002). At the same time some DOM could inhibit short wavelengths in waste stabilization ponds (Curtis et al. 1994, Maynard et al. 1999) and also promote FC survival by providing their carbon and energy needs (Bouteleux et al. 2005). Based on the BOD, Metcalf and Eddy (2003) classified raw domestic wastewater as low strength or weak (110 mgL^{-1}), medium strength (200 mgL^{-1}) and strong (400 mgL^{-1}). As the concentration of sensitizers affects die-off of FC (Curtis et al. 1992), and would vary in wastewater of different strengths, it is hypothesized that the effect of algal biomass on FC die-off would vary in wastewater of different strengths. This phenomenon is not reported in literature. Algal cells release low molecular weight DOM by secretions and autolysis and the rate of release of these DOM vary with algal density and environmental conditions (Wetzel 2001). As algal organic matter (AOM) enhance the survival of FC (Bouteleux et al. 2005), depending on the variety of individual DOM present, DOM may either support the survival or destruction of FC. This is what occurs in full scale stabilisation ponds. In order to simulate real pond conditions in a batch laboratory experiment, a wide variety of DOM is expected to be present in mixing raw and partially decompose wastewater. It is therefore suggested that a 'mixture' of first and second feed of domestic wastewater (consisting of domestic wastewater that had undergone some decomposition or treatment and raw domestic wastewater respectively), would comprise of DOM of varying quality and quantity and as such should affect the rate of inactivation of FC differently from having either of the two wastewater types. This study aims to understand, in addition to the objective mentioned above, the effect of varying concentrations of algal biomass in

low/weak and medium strength wastewater on FC removal under batch laboratory conditions and how this effect is affected by a ‘mixture’ of first and second feed of raw domestic wastewater depicting a variety of organic matter.

2 MATERIALS AND METHODS

2.1 Algae culture and preparation

Algae were grown in the laboratory by inoculation of nutrient solution (APHA 2005) with laboratory stock of *Chlorella sp* obtained from Wilson Group Inc., USA (Wilson Group 2010) under light of wavelength 380-780nm provided by a powerstar HQI-BT 400 lamp. Culture solution contained 13.5mgL^{-1} of nitrogen and 2.2mgL^{-1} of phosphorus in the form of nitrate and phosphate respectively and kept at 20 to 25°C temperature conditions. Resulting algae were harvested after 14 days, sieved using $250\mu\text{m}$ and $90\mu\text{m}$ mesh nets and concentrated by centrifugation at 1000rpm for 30minutes into a thick algal paste. Raw domestic wastewater (characteristics shown in Table 1) from the inlet of a wastewater treatment plant at Hoek van Holland, The Netherlands, was stored in a refrigerator for four hours before using it for the experiments.

Table 1. Physico-chemical characteristics of influent wastewater from January to November, 2009.

Parameter	Value*
TSS (mgL^{-1})	242 ± 53
BOD (mgL^{-1})	163 ± 46
Nitrate Nitrogen(mgL^{-1})	BDL
Total Kjeldahl Nitrogen (mgL^{-1})	36.7 ± 10.8
Total Phosphorus (mgL^{-1})	7.2 ± 2.1

*(Source: HHR 2009) BDL-Below Detectable Limits, n=28

2.2 Optimum algal density determination

Algal paste was used to inoculate twenty Erlenmeyer flasks containing 200mL of raw wastewater. The averages of the initial and final concentrations of algae in the flasks after seven days of incubation were as follows: 0, 1.2, 1.7, 6.7 and 17.5mgL^{-1} of chlorophyll-a, determined using NEN 6520 (1981). Control flasks had no algae. The various algal incubations or treatments had corresponding initial FC concentration of 8.23×10^7 , 8.25×10^7 , 8.27×10^7 , 8.32×10^7 and $8.41 \times 10^7\text{cfu100mL}^{-1}$. Six replicates of each treatment were randomly arranged on a shaker (model GFL 3019) and run at 120 rpm. The six replicates were kept under alternating light and dark conditions. In the light condition, the replicates were kept 0.8m below the HQI-BT 400 lamp for 16 hours per day. In the dark conditions, the replicates were covered with four layers of black polyethylene sheet. After 24 hours of incubation, samples were taken from each flask, and pushed through syringes fitted with needles to detach any attached bacteria (Ansa et al. 2009) and results (not reported here) showed that FC numbers before and after detachment were statistically comparable and were therefore not attaching to algae.

FC numbers, dissolved oxygen concentration and pH were monitored using the spread plate technique (APHA 2005) on chromocult agar medium incubated for 24 hours at 35-37 °C (Finney et al. 2003), portable WTW330 oxygen meter and WTW340 pH meters respectively. Decay rates of FC Kd for the different treatments were determined for the period of incubation and these were statistically compared using independent sample t-test of SPSS 12.0 statistical package. The decay rates, Kd were calculated from the regression line of the first order decay equation (1) (Marais 1974):

$$\ln N_t = -K_d t + \ln N_0 \quad (1)$$

where

N_t = FC count per 100mL at a time t

N_0 = FC count per 100mL at the start of the experiment

t = Time (days) of incubation

2.3 Faecal coliform decay in low and medium strength wastewater

In order to compare how FC decay rates vary with varying algal biomass in wastewater of different strengths, the procedure outlined above was repeated using low strength wastewater ($78 \pm 19 \text{ mgL}^{-1} \text{BOD}_5$) and medium strength wastewater ($162 \pm 46 \text{ mgL}^{-1} \text{BOD}_5$) at the same time and conditions. Algal concentrations were deliberately chosen to differ from concentrations of previous experiment so as to be able to observe the effect of these algal concentrations as well. To obtain low strength wastewater (LSW), raw wastewater, classified as medium strength (MSW) based on the BOD (Metcalf and Eddy 2003), was diluted in the ratio of 1:1 by adding an equal volume of demineralised water (Awuah et al. 2004). After inoculation of wastewater with algal paste, the following algal concentrations: 0, 3.2, 13.9, and 20.0 mgL^{-1} chlorophyll-a were obtained for both LSW and MSW. Control flasks had no algae. Starting FC concentrations of MSW were 7.70×10^7 , 7.76×10^7 , 7.86×10^7 and $7.96 \times 10^7 \text{ cfu100mL}^{-1}$ for the respective algal treatments. Monitoring of dissolved oxygen concentration, pH, FC and determination of Kd and its statistical analysis were done as mentioned in the previous experiment.

2.4 Effect of second feed of raw wastewater on faecal coliform decay

The effect of a mixture of a ten-day treated wastewater and a second feed of raw wastewater on FC decay was investigated to understand how this unique composition of wastewater affects FC decay. The experimental set-up above was repeated except for the range of algal concentrations chosen (0, 0.6, 1.7, and 19.5 mgL^{-1} chlorophyll-a), starting FC concentrations (3.70×10^7 , 3.71×10^7 , 3.72×10^7 and $3.82 \times 10^7 \text{ cfu100mL}^{-1}$ respectively) and period of incubation (10 days). The above algal concentrations were chosen based on results of previous experiments which show that big differences are likely to be revealed by that range of algal density. The ten days period of incubation was chosen in order to observe any possible changes that might occur after seven days of incubation. After ten days incubation (end of phase one), additional 200mL of raw wastewater was added to each flask (as beginning of phase two). Monitoring of dissolved oxygen concentration, pH, FC and determination of Kd for phase one and two and its statistical analysis were done as mentioned in the previous experiments. BOD

concentration of incubations were not monitored as withdrawal of samples for BOD analysis, volume-wise, would interfere with the assessment of FC decay and therefore dissolved oxygen profile was used to explain possible changes in BOD.

3 RESULTS

3.1 Optimum algal density determination

In light algal incubations pH and dissolved oxygen concentrations were higher than those in darkness and exceeded 10.0 and 8.5mgL^{-1} respectively (Figure 1). The rate of FC decay varied with varying chlorophyll-a concentration. An optimum curve ($R^2=0.80$, $n=24$), with maximum decay rates occurring at $10.0 \pm 2\text{mgL}^{-1}$ chlorophyll-a was observed. Lower decay rates were associated with high chlorophyll-a concentrations (Figure 1). Algae still had an effect on FC decay in darkness. A positive linear correlation was observed between chlorophyll-a concentration and decay rates in darkness ($R^2 = 0.77$, $n = 30$), with lower decay rates associated with lower chlorophyll-a concentrations. For all the chlorophyll-a concentrations used, significantly higher die-off rates of FC were observed in light compared to darkness ($p < 0.001$, Figure 1). Variation in the t-value when light and darkness decay rates were compared using the students t-test suggest a complex interaction of light with other factors such as pH and DO to achieve die-off.

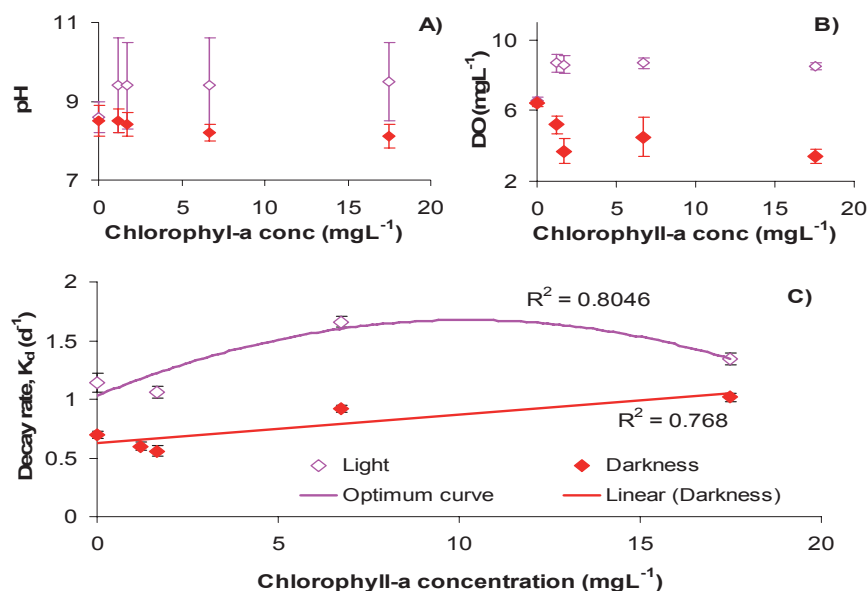


Figure 1. Conditions of pH (A), dissolved oxygen concentration (B) and rates of faecal coliform decay, K_d (C) at different chlorophyll-a concentrations during period of

incubation in light and darkness, taken at 10:00-11:00am. Standard deviation in (A) and (B) represents variations during period of incubation.

3.2 Decay of faecal coliform in low and medium strength wastewater

Mixing of wastewater with demineralised water to prepare low strength wastewater aerated the light and darkness incubations even before the start of the experiment and stirring of incubations also introduced some atmospheric oxygen into the incubations. Comparable values of pH and DO over time were observed in LSW and MSW in light and darkness (Figure 2). Generally, decay rates in light were significantly higher than that in darkness for both LSW and MSW (Figure 3). At higher algal densities $\geq 13.9\text{mgL}^{-1}$ in light, significantly higher decay rates were observed in MSW compared to LSW ($p < 0.05$). Optimum algal densities (algal densities for maximum decay of FC) in light were different for LSW and MSW occurring at 3.2mgL^{-1} and 13.9mgL^{-1} chlorophyll-a concentrations respectively. Decay rates occurring at the optimum algal densities for LSW and MSW were however comparable statistically. At chlorophyll-a concentration of 20.0mgL^{-1} in darkness, decay rates in MSW were higher than in LSW. Below this algal density (20.0mgL^{-1}), comparable decay rates were observed for LSW and MSW in darkness (Figure 3).

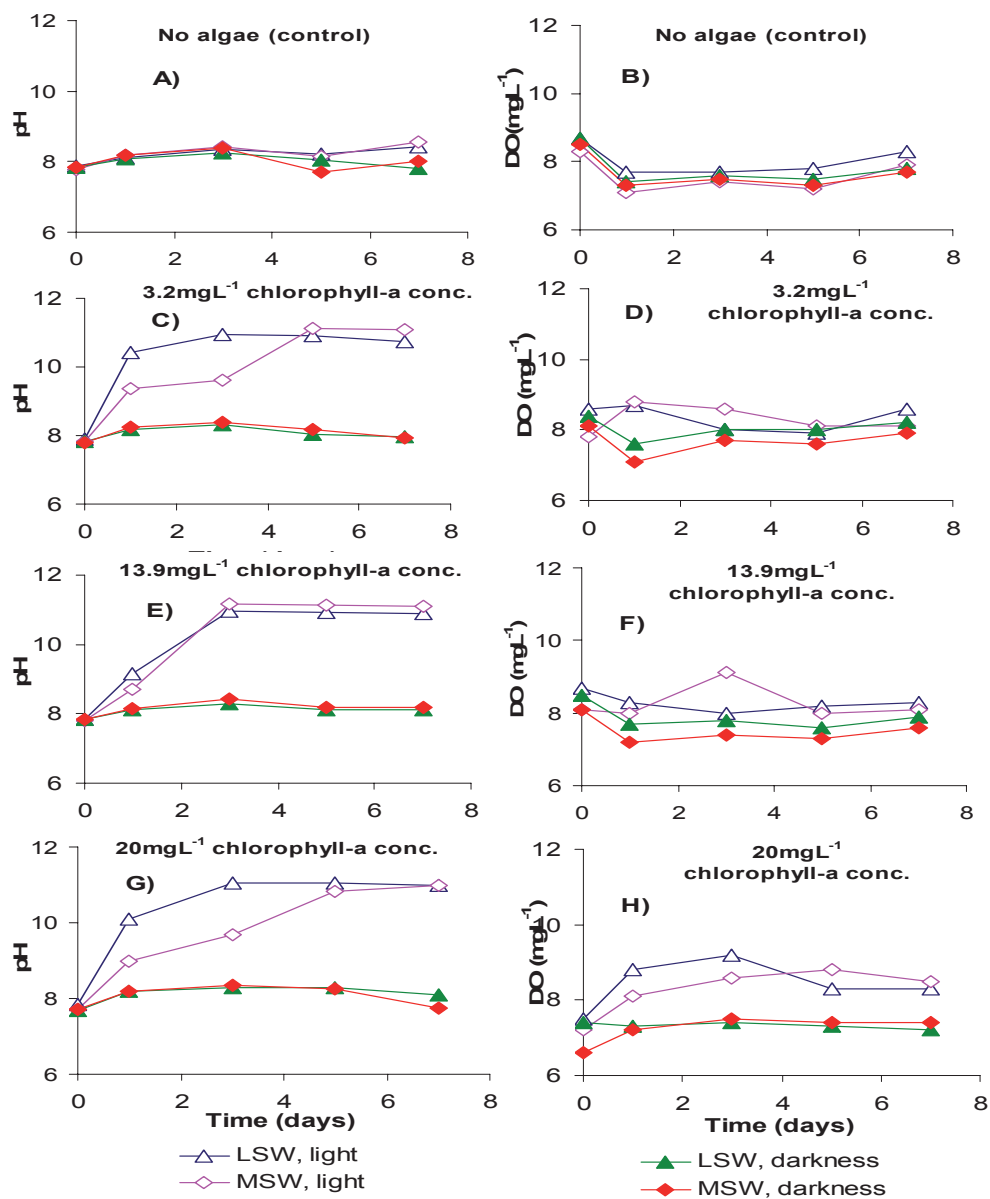


Figure 2. Conditions of pH and dissolved oxygen concentrations in low (LSW) and medium strength wastewater (MSW) inoculated with algae and monitored at 10:00-11:00am.

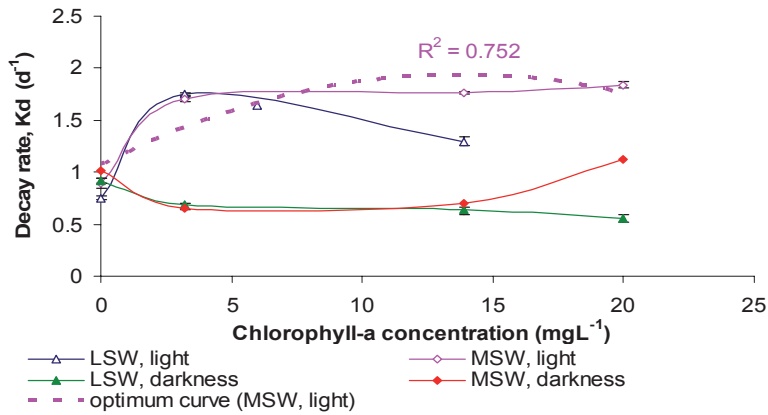


Figure 3. Decay rates of faecal coliforms, K_d in low (LSW) and medium strength wastewater (MSW) inoculated with algae and monitored at 10:00-11:00am.

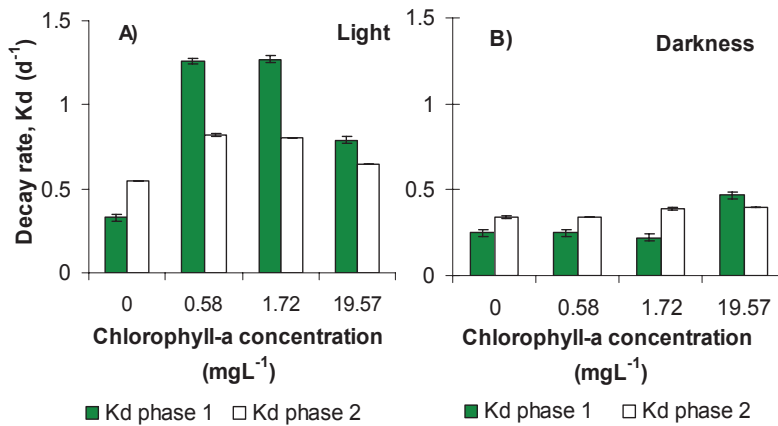


Figure 4. Decay rates, K_d of faecal coliforms before (phase 1) and after (phase 2) second feed of raw wastewater monitored at 10:00-11:00am. Bar values represent duplicated treatments each having 3 sub-replicates.

3.3 Effect of second feed of raw wastewater on faecal coliform decay

Introduction of second feed of wastewater led to lower values of pH and DO in light. In darkness pH and DO levels before and after second feed of wastewater were similar (Figure 5). Decay rates of FC in phase 1 and 2 were compared. Lower decay rates were observed in phase 2 incubations exposed to light (Figure 4). The survival or decay of FC in experiments kept in darkness was not affected by the second feed of wastewater.

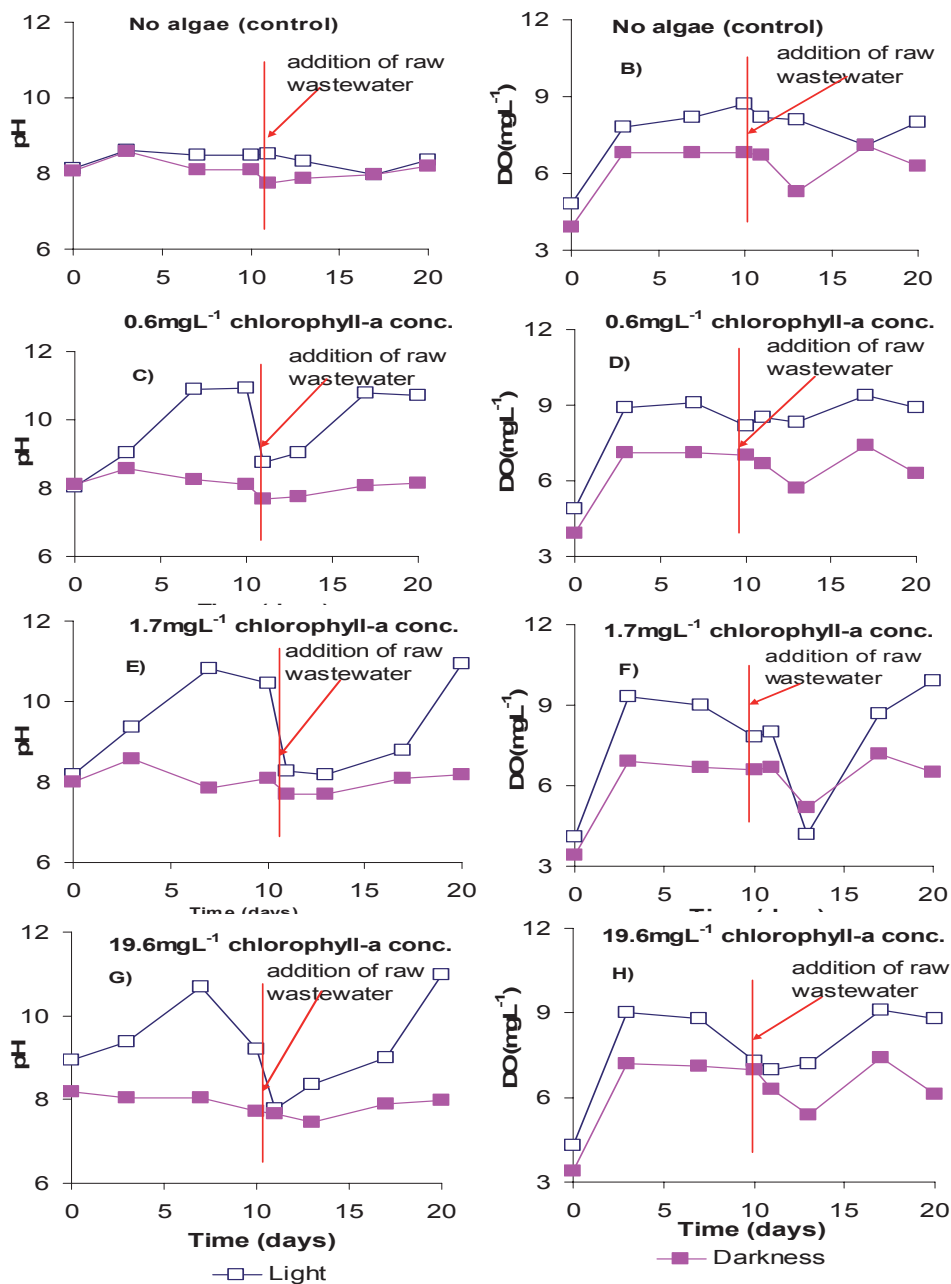


Figure 5. Conditions of pH and dissolved oxygen concentration before and after addition or second feed of raw wastewater monitored at 10:00-11:00am.

4 DISCUSSION

4.1 Optimum algal density

Considering the concentrations of algae used in this experiment, rates of decay of FC were higher in light than in darkness (Figure 1). The present work including some recent work (Shilton et al. 2008), shows that light plays an important role in the inactivation of FC contrary to some earlier work that disputed this (Maynard et al. 1999). Higher decay rates were observed in the presence of algae and the differences in decay rates can be attributed to the differences in chlorophyll-a concentration. Decay rates increased with increased chlorophyll-a concentration till a certain optimum ($10 \pm 2 \text{ mgL}^{-1}$) after which decay rates decreased with increased chlorophyll-a concentration (Figure 1). Algal presence leads to pH elevation and increased oxygen concentration, both of which are bactericidal to FC (Davies-Colley et al. 1999; Awuah et al. 2001). Decay rates of FC have been observed to increase with increased dissolved oxygen concentration (Curtis et al. 1992). Curtis et al. (1992) explained that in the presence of light toxic forms of oxygen molecules are produced (notably peroxides and singlet oxygen) which increase in concentration with increased DO concentration. These toxic forms of oxygen are injurious to bacteria, particularly its cytoplasmic membrane. They also observed that light-mediated damage of FC are highly sensitive to elevated pH values, which enables light of wavelengths $>425\text{nm}$ (in the presence of a sensitizer) to damage the bacteria. This may explain the increases in decay rates with increased pH and DO concentration. At high chlorophyll-a concentrations, algal presence could lead to light attenuation, with highly turbid systems having greater attenuation thus reducing the effect of shorter wavelengths of electromagnetic radiation (Van der Steen et al. 2000), leading to lower decay rate of FC.

4.2 Effect of algae in darkness

Increased FC decay rates in darkness with increased chlorophyll-a concentration suggest an effect of algae even in darkness. As pH and dissolved oxygen concentrations in algal incubations were similar or comparable, a factor such as an algal toxin or inhibitor that is released during lyses of algal cells (Wetzel 2001; Maynard et al. 1999), and whose concentration increases with increased chlorophyll-a concentration may be inactivating FC. The role of algal toxin in the inactivation of FC is still a subject of great debate (Maynard et al. 1999) and this observation gives credence to the existence of such toxins that inactivate FC.

4.3 Decay of faecal coliform in low and medium strength wastewater

At chlorophyll-a concentration $\leq 3.2\text{mgL}^{-1}$, decay rates in LSW and MSW were comparable (Figure 3). At higher chlorophyll-a concentrations ($\geq 13.9\text{mgL}^{-1}$), decay rates of FC were higher in MSW than in the LSW. At chlorophyll-a concentration of $\leq 3.2\text{mgL}^{-1}$, both LSW and MSW had pH increasing up to a maximum of 10.7 and 11.1 respectively (Figure 2). These were higher than the critical pH of 9-9.5 that is bactericidal to FC (Maynard et al. 1999). The oxygen concentration ranges of $7.9\text{-}8.6\text{mgL}^{-1}$ and $7.8\text{-}8.8\text{mgL}^{-1}$ for LSW and MSW respectively were also comparable. The higher decay rates of FC in

MSW can therefore be attributed to the higher organic matter present in the MSW. Some wastewater-derived dissolved organic matter acts as sensitizers absorbing light energy and passing on this energy to oxygen radicals, resulting in the damage of FC (Maynard et al. 1999) and some sensitizers can themselves injure the cytoplasmic membrane of FC directly (Sinton et al. 2002). Some dissolved organic matter such as algal organic matter may however promote FC survival by supplying its carbon and energy needs (Bouteleux et al. 2005) and this may explain why the optimum algal densities (algal densities for maximum decay of FC) in light were different for LSW and MSW occurring at 3.2mgL^{-1} and 13.9mgL^{-1} respectively. Algal cells release DOM by secretions and autolysis and the rate of release of these DOM increases with increased chlorophyll-a concentration (Wetzel 2001). Thus the optimum algal densities may occur at concentrations of algae which may have a net higher concentration of sensitizers as wastewater-derived organic matter as opposed to concentration of growth-promoting organic matter.

4.4 Effect of second feed of raw wastewater on faecal coliform decay

The effect of a mixture of dissolved organic compounds (created by mixing a ten-day treated wastewater with raw wastewater) on FC rate of decay was investigated. Increased pH and oxygenation led to higher rates of FC decay during phase one light-incubations containing algae (Figure 5) as was also reported earlier in this paper and also by Awuah (2006). The buffering capacity of the raw wastewater lowered the pH during phase two and increased organic matter content depleted quickly the dissolved oxygen concentration leading to lower pH and dissolved oxygen concentration and consequently lower decay rates of FC (Figure 4). Addition of raw wastewater also introduced additional nutrients and organic substrates and therefore extra carbon and energy sources which may enhance the survival of FC (Van der Steen et al. 2000). Expectedly, decay rates in darkness before and after second feed of wastewater did not vary much as sufficient carbon sources may be present in the wastewater for the use of FC.

5 CONCLUSIONS

- In light FC rate of decay increased with increased chlorophyll-a concentration till a certain optimum chlorophyll-a concentration ($10 \pm 2 \text{ mgL}^{-1}$), after which FC decay rates decreased. The optimum chlorophyll-a concentration is affected by wastewater strength and the quality of dissolved organic compounds present in the raw wastewater. Increase in the quality of dissolved organic compounds through a second feed of raw wastewater led to lower rates of FC inactivation.
- At algal densities $\geq 13.9\text{mgL}^{-1}$ in light, rate of decay of FC are higher in MSW than in LSW. The highest rate of decay in LSW occurred at 3.2mgL^{-1} in light while that of the MSW occurred at 13.9mgL^{-1} chlorophyll-a concentration respectively. These have implications for pond designs as ponds with optimal algal densities would tend to be more efficient in FC removal.
- In darkness, algae produced a substance which inactivated FC. Further investigations are needed to ascertain the kind of substance produced.

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Performance of a hybrid algal and duckweed pond system treating raw domestic wastewater

ABSTRACT

The performance of a hybrid algal and duckweed pond system in removing BOD and faecal coliform was compared to solely algal or duckweed pond systems under tropical (West African) climatic conditions. Each pond system type is a pilot scale continuous flow system consisting of four ponds in series receiving domestic wastewater from a holding tank by gravity. The hybrid pond system consisted of two duckweed ponds sandwiching two algal ponds. The treatment plant with a hydraulic retention time of five days per pond and a flow rate of $6.9 \times 10^{-3} \text{ m}^3 \text{ d}^{-1}$, was operated for 14 months to capture the performance in the wet season (April to September) and the dry season (December to March). Final BOD₅ concentrations of hybrid pond system ($\leq 20 \text{ mgL}^{-1}$) were similar to that of the duckweed pond system and lower than algal pond system effluent. Removal of BOD, total phosphorus and faecal coliforms were not affected by seasonal changes in all the three pond system types. Ammonia removal efficiency was affected by seasonal changes in the duckweed pond system with wet and dry seasons having removal efficiencies of 68% and 93% respectively. Faecal coliforms (FC) removal in the wet season were 3.8, 4.8 and 4.3 log units respectively for the duckweed, algal and hybrid pond systems while log removals of 3.5, 4.6 and 4.3 were observed during the dry season, respectively. Chlorophyll-a concentration in the effluents of the duckweed, algal and hybrid ponds were 39 ± 8 , 383 ± 52 and $76 \pm 23 \mu\text{gL}^{-1}$ respectively. With significantly reduced algal concentration of hybrid pond system effluent, the use of the hybrid algal-duckweed pond systems effluent as the influent of a constructed wetland for further polishing without the problem of algal matter clogging could be feasible. Hybrid systems are therefore recommended for optimal performance in BOD and faecal coliform removal.

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1 INTRODUCTION

The performance of wastewater treatment ponds vary widely in literature due to differences in pond types (i.e., algal-based or macrophyte-based), pond depth, total retention times, climatic conditions amongst others (Awuah 2006; Maynard et al. 1999). Solar radiation plays a key role in algal pond disinfection (Davies-Colley et al. 2000) and the purpose of shallow ponds designed as maturation ponds is to assist primarily in the removal of pathogens as shown by indicator bacteria and secondarily for further removal of BOD (including the settling of algae). Algal ponds appear to be more efficient in the removal of pathogens than duckweed ponds (Awuah et al. 2004), perhaps due to the elevated pH and dissolved oxygen concentration created in algal ponds during day time. Duckweed ponds on the other hand perform better in BOD removal than algal ponds (Awuah et al. 2004; Caicedo 2005; Zimmo et al. 2002). Waste Stabilisation Ponds (WSPs) in tropical regions experience high solar radiation intensity leading to rapid growth of algae. The development of high algal biomass in algal treatment ponds leads to solar radiation attenuation, weakening the disinfection effect of solar radiation in the deeper part of the ponds and thereby resulting in lower removal of faecal coliforms (Van der Steen et al. 2000a). It has been shown that inserting duckweed ponds in between a series of algal ponds could reduce the rate of algal growth and hence promotes better solar radiation penetration in the algal ponds, creating conditions for improved faecal coliform decay (Van der Steen et al. 2000b). Additionally, the use of duckweed may have economic benefits such as the generation of biomass for the manufacture of fertilizer, animal and fish feed (El Shafai et al. 2007; Gijzen and Ikramulah 1999; Oron 1994). The economic value of duckweed is of significant importance considering the suitability of WSPs for the treatment of domestic wastewater in rural communities as occurs in Africa. WSPs apart from its requirement for large land space are very efficient in pathogen removal, with low capital and maintenance costs (Mara 2000). The presence of pathogens in discharged effluents is a key concern in many African countries as rural communities tend to use raw water from freshwater bodies without any form of treatment. Employing a hybrid algal-duckweed approach therefore could make this treatment system better adapted for African climatic conditions, yielding maximum treatment efficiencies and economic incentives. Zimmo (2003) noted higher nitrification-denitrification rates in algal pond systems than in duckweed pond systems and introducing aerobic zones in between duckweed ponds could lead to higher removal of nitrogen at a shorter retention time (Benjarano 2005).

Von Sperling and Mascarenhas (2005) showed under Brazilian tropical conditions (21-25°C), that algal ponds having a duckweed effluent pond could meet the WHO guidelines for unrestricted irrigation even with a short total retention time of 7.4 days. Noumsi et al. (2005) investigated the comparative performance of a hybrid algal and *Pistia stratiotes* pond system and a fully algal system under African climatic conditions but their pond systems did not operate simultaneously but rather was operated one after the other. This approach may not be adequate for comparison as changes in season could influence performance. The comparative performance of a hybrid treatment system consisting of duckweed and algal ponds has not been assessed at the same time alongside parallel lines of full algal and duckweed pond systems under tropical conditions. Variations in

performance may also occur even in tropical regions due to differences in climatic conditions such as rainfall patterns, solar radiation intensity and temperature. Caicedo (2005) under Colombian tropical conditions operated a hybrid algal and duckweed pond system but the pond system was run in phases, was compared with only a duckweed pond system and assessed only nitrogen removal. Van der Steen et al (2000b) operated a hybrid algal and duckweed pond system and assessed its performance in faecal coliform removal in the Negev desert of Israel but their pond system received partially treated wastewater from an Upflow Anaerobic Sludge Blanket (UASB) treatment system unlike this study which sought to treat raw wastewater with a hybrid pond system. The purpose of this study is to assess the suitability and efficiency of a hybrid algal and duckweed system in producing an effluent with low suspended algal matter, BOD, nutrient and faecal coliform which is suitable for further polishing in a constructed wetland and subsequent discharge into water bodies used in its raw form by villagers. It also sought to either imitate or improve the operational conditions adopted by Awuah et al (2004) in operating a fully algal and fully macrophyte treatment system in the same country but 300km apart. It is anticipated that this study would eventually lead to the creation of an integrated pond-constructed wetland resource recovery treatment system where duckweed produced by treatment system is utilised by a fishpond occurring as an integral part of the treatment plant. This would be the first of its kind in sub-Saharan Africa. This paper investigates the comparative performance of three treatment systems consisting of a series of algal ponds, duckweed ponds and a hybrid system of algal and duckweed ponds under tropical (West African) conditions. Differences in performance in the wet season (April to September) and the dry season (December to March) are also assessed.

2 MATERIALS AND METHODS

2.1 Description of setup

A pilot-scale treatment plant was setup outdoor on the Council for Scientific and Industrial Research (CSIR)-Water Research Institute premises in Accra, Ghana (5° 44'42''N, 0° 6' 27''W). The treatment plant consisted of three parallel pond systems of duckweed (*Spirodela polyrhiza*) (D), algae (A) and hybrid ponds of duckweed and algae (H) with two duckweed ponds sandwiching two algal ponds (Figure 1). Each of the three pond system types consisted of four ponds in series. Duckweed, algal and hybrid ponds are identical in dimensions, each pond having an average diameter of 0.382m and a depth of 0.30m (Figure 1). The three pond systems receive influent domestic wastewater from a holding tank (HT) measuring 1.00m x 1.00m x 1.00m. The holding tank is continuously supplied with wastewater from the grit chamber of the Kotoka International Airport wastewater treatment plant, and wastewater stays in the holding tank for an average period of 1 day. The wastewater flow was by gravity and was regulated by taps installed between the holding tank and the inlet of ponds.

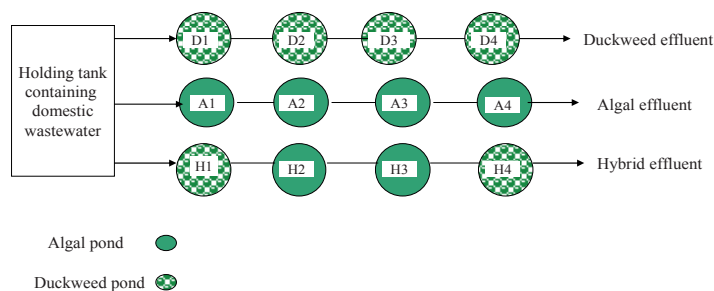


Figure1. Schematic diagram of the treatment systems

2.2 Operation and monitoring of the treatment plant

The system was allowed to run as a continuous flow system for two months before monitoring began in April. The treatment plant was operated for 14 months to capture the performance in the wet and dry seasons. The characteristics of the raw wastewater and the effluent of the holding tank are shown in Table 1. The duckweed treatment lines (D) as well as the duckweed ponds of the hybrid lines (H) were inoculated with duckweed fronds (*Spirodela polyrrhiza*) from the beginning of the experiment and allowed to grow naturally to form a dense cover, before harvesting and monitoring started. Duckweed in these ponds was harvested once every two weeks to sustain growth of duckweed in these ponds (Awuah et al. 2004). Average duckweed production rate by the duckweed pond system was $135 \pm 22 \text{ gm}^{-2} \text{ d}^{-1}$ fresh weight ($6.48 \text{ gm}^{-2} \text{ d}^{-1}$ dry weight). Algal development in the algal ponds also occurred naturally. A hydraulic retention time of five days was maintained in each pond of the three pond system types and average wastewater flow rate was $6.9 \times 10^{-3} \text{ m}^3 \text{ d}^{-1}$.

Monitoring of the treatment performance was conducted from April to September for the wet season and from December to March for the dry season. In the wet season, BOD_5 , ammonia nitrogen, nitrite nitrogen, nitrate nitrogen and total phosphorus concentrations were measured only for the final effluent ponds (pond 4), the holding tank and the raw wastewater. The samples from the treatment ponds were collected in duplicate at a depth 10cm below the pond surface every other week for laboratory analysis using standard methods (APHA 2005). Physico-chemical conditions of pH, temperature and dissolved oxygen concentration were measured in situ once a week during the wet season in all ponds using a WTW 340 pH meter, thermometer and a WTW 330 Oximeter, respectively at 8:00-10:00am. Faecal coliform count measurement in the wet season was done once a week for the raw wastewater, holding tank effluent and from the outlet of the effluent pond (pond 4) only, using spread plate technique on chromocult agar, incubating at 35-37°C for 24 hours for a colony count (Finney et al. 2003).

During the dry season, water samples for BOD_5 , ammonia nitrogen, nitrite nitrogen, nitrate nitrogen, total phosphorus, and faecal coliform analysis were collected and

physico-chemical conditions of pH, temperature, dissolved oxygen concentration were measured in situ at 8:00-10:00am, once a week for raw wastewater, holding tank and all ponds 10cm below the water surface. Samples were analysed using methods indicated earlier. Seasonal differences in means were compared using paired sample t test of Minitab 15.0 while different treatment systems were compared with independent sample t-test and analysis of variance (ANOVA). Due to logistical limitations, it was not possible to measure organic nitrogen concentration and total Kjeldahl nitrogen in order to perform a mass balance for nitrogen. Average annual chlorophyll-a concentration in the effluent pond (pond 4) of the duckweed, algal and hybrid ponds were 39 ± 8 , 383 ± 52 and $76 \pm 23 \mu\text{gL}^{-1}$ respectively. Chlorophyll-a concentrations were determined using NEN 6520 (1981). The average daily solar irradiation in Accra ranged between 4.5 to 5.7kWh/m²/day (values obtained from Ministry of Energy, Ghana).

3 RESULTS

3.1 Characteristics of influent wastewater and holding tank effluent

Temperature of the raw wastewater ranged from 24.5 to 26.9°C and 29.0 to 31.8°C for the wet and dry seasons respectively while that of the holding tank was 24.4 to 26.6 °C and 28.8 to 31.4 °C, respectively. Wet and dry season's pH varied from 7.0 to 7.4 and 7.1 to 7.5 respectively for raw wastewater. That of holding tank (pH) was 7.0 to 7.6 and 7.1 to 7.3 for wet and dry seasons respectively (Figure 3). No real differences existed in the pH, BOD, ammonia and total phosphorus of the raw wastewater in the wet and dry seasons ($p > 0.05$). Faecal coliform count the in raw wastewater in the wet season however were significantly lower than that in the dry season ($p = 0.005$). Raw wastewater and holding tank effluent pH, BOD, ammonia and total phosphorus concentrations were similar both in the wet and dry seasons ($p > 0.05$). Higher faecal coliform counts were observed in the raw wastewater compared to the holding tank effluent year round and in the dry season. In the wet season however faecal coliform counts were comparable in the raw wastewater and in holding tank effluent.

3.2 Environmental conditions in ponds

Figure 2 shows that temperature variations in all treatment pond systems were similar. The temperature range was 24.0°C to 28.5°C in the wet season and 29.5-33.0°C in the dry season. Seasonally, there was little variation in the pH values for the duckweed treatment line for the wet season (6.5-6.9) and the dry season (6.4-7.0) as well as the duckweed ponds of the hybrid line wet season (6.2-7.0) and dry season (6.8-7.0) (Figure 3). The algal ponds treatment line showed high variation in the pH values during the wet (7.1-7.9) and the dry (9.1-9.5) seasons. The pH values in the algal ponds were significantly higher than that of the duckweed ($p < 0.05$) and the hybrid lines while the last two were comparable. Fluctuations in DO values were higher in the algal ponds than in the duckweed ponds during the period of monitoring (Figure 4). Diurnal variation in DO showed a similar variation of high DO in algal ponds and low DO in duckweed ponds. These are reported in Ansa et al (2012). The duckweed and algal pond effluents showed little difference seasonally while the hybrid pond effluent had higher DO values in the wet season compared with the dry season ($p=0.037$). Higher DO values were observed in

the algal pond effluents compared with the duckweed effluents year round ($p<0.001$) and in the dry season ($p<0.001$) but these differences were not significant in the wet season ($p=0.093$). Algal effluent DO was higher than that of the hybrid pond for both the wet ($p=0.005$) and dry seasons ($p<0.001$).

BOD decreased steadily from pond 1 to 4 for each of the three pond systems in the dry season (Table 2). BOD removal efficiencies were similar in the wet and dry seasons for all the treatment lines (Table 3). The treatment line of duckweed ponds had the highest BOD removal efficiency both in the wet (94%) and the dry seasons (91%) compared to the algal ponds (75%, 71%) and the treatment line of the hybrid ponds (89%, 89%) pond systems in the wet and dry seasons respectively (Table 3). BOD removal efficiency in the hybrid pond system was higher than that in the algal system in the wet season but comparable in the dry season. The BOD in the effluent of duckweed and hybrid pond systems did not differ significantly in the wet and dry seasons ($p < 0.05$).

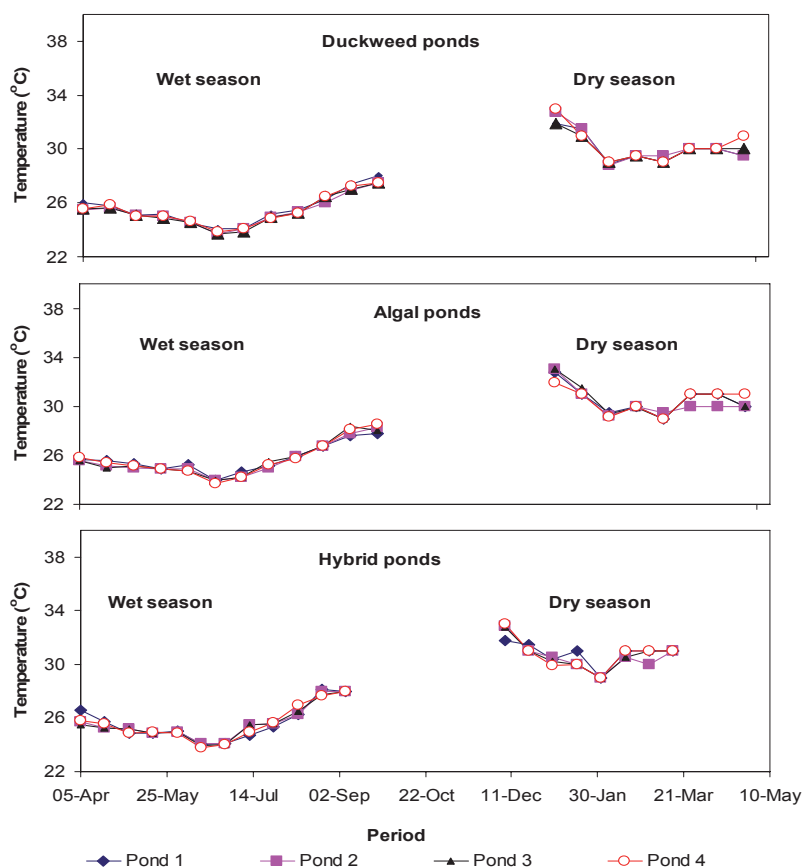


Figure 2. Temperature during the Wet and Dry seasons at 8:00-10:00am.

Table 1. Physico-chemical and microbiological characteristics of raw wastewater and holding tank effluent and that of final effluent of duckweed, algal and hybrid ponds.

	Raw wastewater		Holding tank		Duckweed pond		Algal pond		Hybrid pond	
	Wet season	Dry season	Wet season	Dry season	Wet season	Wet season	Wet season	Wet season	Wet season	Wet season
BOD ₅ (mgL ⁻¹)	200±29	217±47	156±9	179±23	10±2	39±8	17±4			
NH ₃ -N (mgL ⁻¹)	68.6±8.8	91.1±20.2	52.2±12.6	86.5±11.7	16.7±3.3	18.0±6.7	9.8±0.6			
NO ₂ -N (mgL ⁻¹)	0.12±0.01	0.10±0.10	0.10±0.01	0.13±0.05	0.14±0.03	0.12±0.03	0.13±0.02			
NO ₃ -N (mgL ⁻¹)	0.23±0.05	0.05±0.06	0.33±0.28	0.09±0.07	0.22±0.11	0.64±0.03	0.23±0.08			
Total Phosphorus (mgL ⁻¹)	5.56±2.22	6.69±1.66	6.06±2.43	6.64±1.54	2.09±0.61	3.06±1.22	2.23±0.46			
Faecal coliforms (cfu 100mL ⁻¹)	2.1x10 ⁷ ± 4.0x10 ⁶	7.1x10 ⁷ ± 9.8x10 ⁶	1.5x10 ⁷ ± 3.1x10 ⁶	2.1x10 ⁷ ± 3.0x10 ⁶	2.5x10 ³ ± 8.3x10 ²	2.3x10 ² ± 9.0x10	7.2x10 ² ± 3.0x10 ²			

(± standard deviation) n = 20

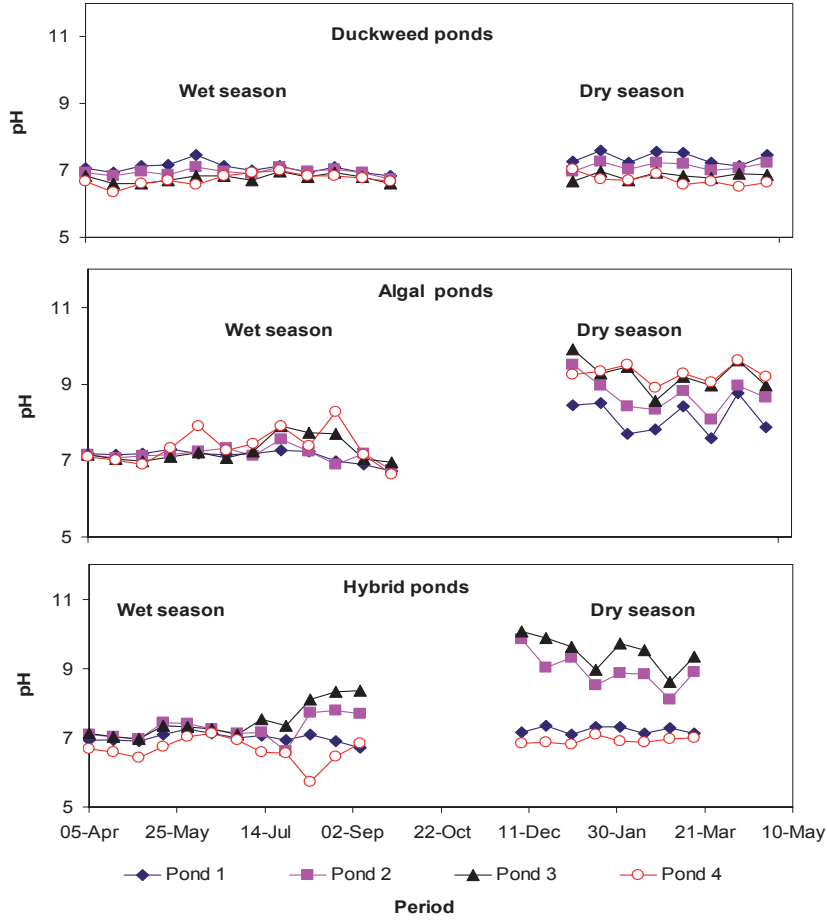


Figure 3. pH levels in the Wet and Dry seasons at 8:00-10:00am.

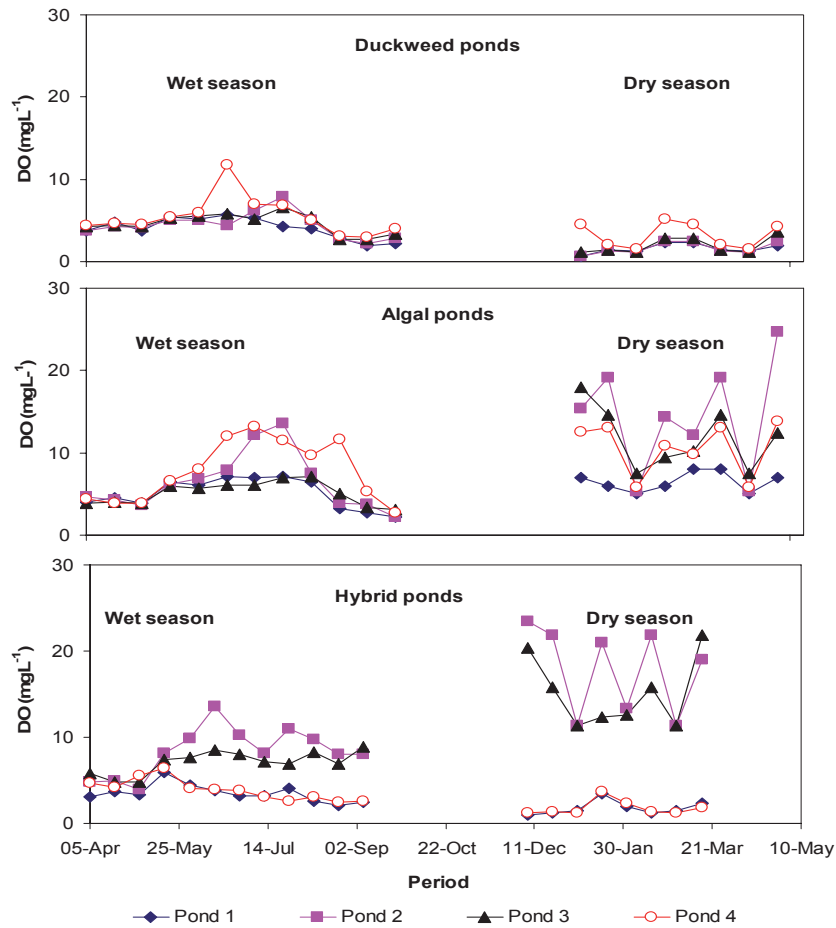


Figure 4. Dissolved oxygen levels in the Wet and Dry season at 8:00-10:00am. Organic matter removal

3.3 Nutrient removal

Nutrient removal was indirectly estimated through the removal of ammonia and total phosphorus. Comparable seasonal removal efficiencies of ammonia were observed in the algal pond system ($73\pm13\%$, $98\pm2\%$) for the wet and dry seasons respectively ($p=0.076$, test statistic, $t=3.41$), Table 3, ‘comparable’ meaning no statistical difference existing between the two. In the duckweed system, however, removal efficiency was lower in the wet season ($76\pm2\%$) compared to the dry season ($92\pm7\%$). The hybrid pond system also showed lower ammonia removal efficiency in the wet ($86\pm1\%$) compared with the dry season ($96\pm3\%$). Removal efficiencies of algal and hybrid systems did not show

significant differences in both the wet and the dry seasons. The hybrid system again showed comparable removal efficiency in the wet and dry seasons to the removal efficiency in the duckweed system. Overall, all three systems showed comparable removal efficiency for ammonia (84%, 86% and 91% for duckweed, algal and hybrid systems, respectively).

Removal efficiency of total phosphorus in the hybrid pond system was higher in the dry season (71%) than the wet season (56%) and the effluents had 1.64 and 2.23mgL⁻¹ total phosphate, respectively. Comparable removal efficiencies were observed in the wet and dry seasons for duckweed ($p < 0.05$) and algal pond systems ($p < 0.05$). Overall removal efficiencies of total phosphorus in all three systems were comparable.

Table 2. Chemical and microbiological characteristics of individual ponds in D, A and H treatment lines during the dry season.

	Duckweed ponds				Algal ponds				Hybrid ponds			
	D1	D 2	D 3	D 4	A1	A 2	A3	A4	H1	H 2	H 3	H4
BOD ₅ (mgL ⁻¹)	84±6	27±5	23±8	17±5	119±15	81±16	64±10	52±14	87±6	57±9	33±18	20±6
NH ₃ -N (mgL ⁻¹)	48.4±8.1	31.1±5.3	12.7±2.9	6.4±4.4	46.5±7.3	26.3±3.2	10.2±1.0	2.0±1.8	39.7±5.6	18.4±2.6	10.1±3.3	3.3±2.8
NO ₂ -N (mgL ⁻¹)	0.33±0.10	0.42±0.11	0.22±0.10	0.08±0.03	0.46±0.24	0.56±0.18	0.03±0.01	0.06±0.05	0.64±0.15	0.32±0.11	0.66±0.26	0.19±0.13
NO ₃ -N (mgL ⁻¹)	0.49±0.31	0.82±0.03	0.28±0.10	0.13±0.12	0.68±0.21	0.67±0.04	0.59±0.09	0.51±0.31	0.44±0.37	0.80±0.00	0.38±0.35	0.22±0.11
Total Phosphorus (mgL ⁻¹)	3.84±0.67	3.77±01.56	1.86±0.70	1.19±0.94	3.81±0.25	3.82±0.56	2.34±0.35	2.38±0.29	4.17±0.18	3.89±0.89	2.11±0.25	1.64±0.49
Faecal coliforms (cfu/100mL ⁻¹)	3.7x10 ⁵ ± 8.7x10 ⁴	1.1x10 ⁵ ±6.2x10 ⁴	6.0x10 ³ ±2.6x10 ³	5.9x10 ³ ±7.0x10 ²	8.9x10 ⁴ ±6.3x10 ³	1.8x10 ⁴ ±8.3x10 ³	3.0x10 ³ ±2.3x10 ²	4.8x10 ² ±1.2x10 ²	3.4x10 ⁵ ±1.0x10 ⁴	8.9x10 ⁴ ±6.1x10 ³	3.3x10 ³ ±1.5x10 ²	1.1x10 ³ ±6.2x10 ²

* ± Standard deviation, n= 20

Table 3. Treatment efficiencies of the duckweed, algal and hybrid pond systems. Overall treatment efficiency represents the average of both the wet and dry seasons.

	Duckweed pond system				Algal pond system				Hybrid pond system			
	Wet season	Dry season	Overall		Wet season	Dry season	Overall		Wet season	Dry season	Overall	
BOD ₅ (%)	93.7±1.6	90.7±2.8	92.2±2.6		75.1±5.9	70.8±7.7	72.9±6.6		89.8±3.4	89.0±3.1	89.2±2.9	
NH ₃ -N (%)	75.9±2.4	92.1±7.1	84.0±10.0		73.2±13.2	98.0±1.7	85.6±16.0		85.6±1.3	96.4±2.8	91.4±6.22	
Total Phosphorus (%)	58.7±12.1	78.6±16.9	68.6±17.1		39.5 ±24.1	57.4±5.2	48.5±18.4		56.0±9.0	70.6±8.7	63.3±11.3	
Faecal coliforms (Log removal)	3.8	3.5	3.7		4.8	4.6	4.7		4.3	4.3	4.3	

* ± Standard deviation

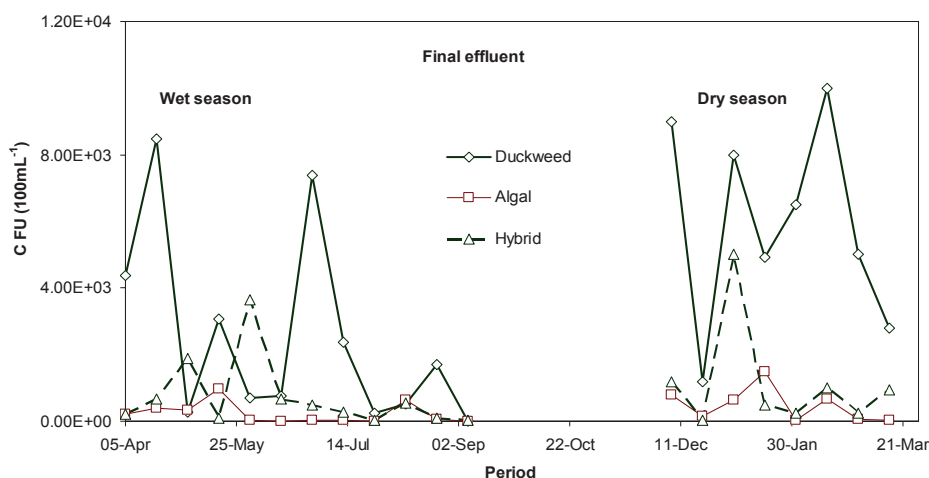


Figure 5. Faecal coliform concentrations in final effluent of duckweed, algal and hybrid ponds.

3.4 Faecal coliforms removal

In the wet season average faecal coliform counts in the final effluents of duckweed, algal and hybrid pond systems were 2500, 230 and 720cfu100mL⁻¹ (Table 1) with corresponding FC removal efficiencies from holding tank effluent of 3.8, 4.8 and 4.3 log units respectively (Table 3). Algal effluent had comparable FC counts to that of the hybrid effluents ($p=0.147$) but higher removal efficiency to that of duckweed pond effluent ($p<0.05$) in the wet season (Figure 5, Table 3).

Removal of FC was not affected by seasonal changes in the duckweed, algal and hybrid pond systems ($p>0.05$). In the dry season, FC removal efficiencies were 3.5, 4.6 and 4.3 for duckweed, algal and hybrid pond systems (Table 3) with corresponding average effluent FC concentrations of 5900, 480 and 1100cfu100mL⁻¹ respectively (Table 2). For the dry season, duckweed effluent FC counts were higher than that of algal and hybrid effluent FC counts ($p<0.05$). Hybrid effluent count was not significantly different from that of the algal effluent ($p=0.113$).

4 DISCUSSION

4.1 Raw sewage and holding tank effluent

Over the period of monitoring not much difference was observed in the pH, BOD, ammonia and total phosphorus of the raw sewage in the dry and wet seasons. The average BOD of 209 mgL⁻¹ obtained during the period of monitoring was lower than the 264 mgL⁻¹ reported by Awuah (2006), but much higher than the 69 mgL⁻¹ by Hodgson (2000) from different cities in Ghana. However, the ammonia to BOD ratio observed (0.38) was comparable to the 0.39 by Hodgson (2000), but higher than 0.22 reported by Awuah

(2006) and 0.13 by Metcalf and Eddy (2003). The high ammonia to BOD ratio reported in this study and that of Hodgson (2000) may be due the freshness of the sewage at the time of analysis. The high ammonia concentration in the wastewater could be explained by the release of ammonia from accumulated sludge in the grit chamber from where the wastewater was obtained. Emptying of grit chamber is usually not done at regular time intervals and prolonged storage of wastewater may result in such a release. Under anaerobic conditions organic nitrogen in sludge could be hydrolysed to ammonia. Higher faecal coliform counts in the dry season ($7.1 \times 10^7 \text{cfu100mL}^{-1}$) compared to the wet season ($2.1 \times 10^7 \text{cfu100mL}^{-1}$) may be attributed to higher dilution with rain water during the rainy season.

Comparable levels of BOD, ammonia and total phosphorus in raw sewage and holding tank in both the dry and wet seasons were observed. Caicedo et al. (2002) observed that anaerobic pre-treatment of raw sewage did not affect the removal efficiency of nutrients. Overall faecal coliform concentrations however, were lower in the holding tank ($1.8 \times 10^7 \text{cfu100mL}^{-1}$) compared to the raw sewage ($4.6 \times 10^7 \text{cfu100mL}^{-1}$) as a result of sedimentation and natural die-off. An overall faecal coliforms removal of 0.4 log units occurred in the holding tank during the whole period of monitoring. This is low compared to 1.0 log units of removal by anaerobic pond recorded by Awuah (2006).

4.2 Organic matter removal

BOD removal efficiency from holding tank effluent did not show any seasonal differences in all the three treatment systems. Temperature increase of 4°C in the dry season was not enough to enhance BOD removal even in the duckweed system that is more temperature dependent (Awuah 2006). El Shafai et al. (2007) observed a BOD removal efficiency of 73% in a duckweed treatment system under a temperature condition of $24\text{--}34^\circ\text{C}$ and noted that effect of temperature on BOD removal efficiency was minimal perhaps due to a low total retention time of 15 days and also the presence of high populations of invertebrates in the pond whose death increased the organic matter content of the effluent pond. The 92% BOD removal efficiency in our duckweed pond system was similar to the 95% recorded by Awuah (2006) under similar climatic conditions with a total retention time of 28 days and an anaerobic pond effluent BOD concentration of 343mgL^{-1} .

Although overall BOD removal efficiency was higher in the duckweed pond (92%) than in the hybrid pond (89%), overall effluent BOD concentrations were comparable ($p < 0.05$) and met the Environmental Protection Agency Ghana guideline of 50mgL^{-1} (EPA Ghana 2010). Better removal of BOD in the hybrid pond systems compared to the algal pond system may be due to the suppression of algal development in the final effluent pond, which like the other duckweed ponds was fully covered with duckweed. The removal efficiency of BOD in the hybrid system was better than the 62% removal reported by Van der Steen et al. (2000b) but had higher overall effluent BOD of 24.5mgL^{-1} compared with 11mgL^{-1} reported by Van der Steen et al. (2000b). Lower influent BOD concentration of 29mgL^{-1} was reported by Van der Steen et al. (2000b) and that may explain their low removal efficiency. The rate of BOD removal at any given

time is proportional to the amount of BOD present in the system at that time (Mara 2003). Higher removal of BOD could be achieved with a higher initial BOD concentration.

4.3 Nutrient removal

The use of ammonia for estimating the removal efficiency of nitrogen in treatment systems is limited by the transformations of nitrogen into various forms under various environmental conditions of temperature, pH and oxygen concentration. Generally, depending on the freshness of the sewage, ammonia may form more than 50% of nitrogen in influent sewage (Metcalf and Eddy 2003). The overall ammonia removal efficiencies of the duckweed (84%), algal (86%) and hybrid (91%) treatment systems were comparable. Removal however was lower than the 98% in summer duckweed system reported by El Shafai et al. (2007). It was also lower than the 93% and 99.7% reported by Awuah (2006) for duckweed and algal systems respectively. The relatively lower ammonia removal efficiency observed in this experiment might be due to the relatively shorter total retention time of 20 days compared to 28 days reported by Awuah (2006) under similar climatic conditions. Major mechanisms of ammonia removal in the duckweed, algal and hybrid treatment involve plant uptake and incorporation into algal cells (Mara 2003). The gains of ammonia volatilisation in the algal ponds during the dry season (pH=9.3) may have been compensated for in the duckweed ponds by rapid plant uptake and subsequent harvesting. The duckweed production rate of $135\text{gm}^{-2}\text{d}^{-1}$ fresh weight ($6.48\text{gm}^{-2}\text{d}^{-1}$ dry weight) observed in this study was greater than the $79.8\text{gm}^{-2}\text{d}^{-1}$ fresh weight reported by Awuah (2006) but much lower than $821.8\text{gm}^{-2}\text{d}^{-1}$ reported by El Shafai et al. (2007). With a consumption of nitrogen and phosphorus per gram of duckweed per day being 0.535 and $6.1 \times 10^{-5}\text{g}$ fresh weights respectively (El Shafai et al. 2007), nitrogen and phosphorus uptake by duckweed in this study is estimated at $0.1\text{gm}^{-2}\text{d}^{-1}$ and $0.008\text{gm}^{-2}\text{d}^{-1}$ respectively and this was lower than the $0.44\text{gm}^{-2}\text{d}^{-1}$ and $0.05\text{gm}^{-2}\text{d}^{-1}$ reported by El Shafai et al. (2007). The removal of ammonium through nitrification according to Mara (2003) is almost negligible in warm climates (temperatures above 25°C) due to short total retention times of less than 40 days. Hurse and Connor (1999) observed significant removal via nitrification but their WSP had an overall retention time of 80 days and temperature conditions were $8\text{-}23^{\circ}\text{C}$. Nitrification is also affected by pH and oxygen concentration prevailing in the pond system.

A major means of phosphorus removal is its precipitation and subsequent sedimentation from the water column (Mara 2003) and this may explain the similar removal efficiencies of total phosphorus observed in the duckweed, algal and hybrid pond systems. Plant uptake of phosphates does not constitute a major means of phosphorus removal (Mara 2003) as was observed in this study. A major contribution by plant uptake in this study would have been characterized by higher removal of phosphates in the duckweed ponds as a result of frequent harvesting. Reported overall removal efficiency of total phosphorus in this study (69%, 49%, and 63%) for duckweed, algal and hybrid systems agrees with values quoted by Mara (2003) but are lower than the 83%, 77% reported by Zimmo et al. (2002) for duckweed and algal systems and 73% reported by El Shafai et al. (2007) for a duckweed system. This study's removal efficiency was however higher than the $<30\%$ for both duckweed and algal systems reported by Awuah (2006) in the same country.

Better removal in this study as compared to Awuah (2006) may be due to the shallower nature of the ponds which aided faster sedimentation and pH elevation.

4.4 Faecal coliform removal

Expectedly faecal coliform removal was better in the algal treatment systems as algal ponds experience better solar radiation penetration and algal photosynthetic activity leads to dissolved oxygen and pH increases, both of which tend to inactivate faecal coliforms (Awuah 2006; Davies-Colley et al. 2000; Maynard et al. 1999). Duckweed ponds had lower oxygen and pH values compared to algal ponds and solar radiation penetration would be relatively poor due to the duckweed cover. Removal of FC by pH and toxic oxygen effect in the form of oxygen radical or singlet oxygen (Davies-Colley et al. 2000) may therefore be of minimal importance in the duckweed ponds. Sedimentation of FC from the water column may constitute an important mechanism of removal as Awuah (2006) observed that sedimentation accounted for more than 90% of FC removal in a duckweed pond system. Increased suspended matter (as occurring in algal ponds of the hybrid pond system) may also enhance FC removal via sedimentation by entrapment of FC in algal biomass. This may explain why the hybrid effluent pond had comparable concentrations of FC count as the algal pond. Okurut (2000), using alternating ponds of planted and unplanted free water surface (FWS) constructed wetlands, observed that FC were abundant in the open water free flow zone but significantly reduced in the planted root-mat zone suggesting a filtering or entrapment of FC effect by the plants. Water column processes within FWS constructed wetlands are nearly identical to ponds with surface autotrophic zones dominated by planktonic or filamentous algae or by floating or submerged aquatic macrophytes (IWA 2006).

Removal of FC was not affected by seasonal changes in the duckweed, algal and hybrid pond systems. Overall faecal coliform log removals of 3.7, 4.7 and 4.3 were observed for duckweed, algal and hybrid systems respectively, which were similar to observations made by Awuah (2006) in fully duckweed and fully algal experimental treatment plants. The hybrid pond system in this study however performed better than the 2.2 log removal reported by Van der Steen et al. (2000b) in a hybrid algal and duckweed system with comparable depth. The lower log removal may be due to the shorter retention time per pond of 2 days employed in their experiment. A WHO (1989, 2006) guideline for unrestricted reuse of wastewater is $\geq 1000 \text{ cfu (100mL)}^{-1}$ of *Escherichia coli*. This guideline was met by the hybrid pond system only in the wet season (720cfu) but not in the dry season (1100). The reuse guideline could be met by increasing the total retention time either through the use of one more pond or addition of a constructed wetland.

5 CONCLUSIONS

- The hybrid pond system (combinations of algal and duckweed ponds) performed well in BOD and faecal coliform removal and is therefore recommended for optimized BOD and FC removal.
- Final FC concentrations of hybrid pond system were similar to that of the algal pond system and final BOD concentration similar to that of the duckweed pond

system and met the EPA, Ghana BOD guideline of 50mgL^{-1} for discharge into freshwater bodies.

- Removal of BOD, total phosphorus and faecal coliform were not affected by seasonal changes in all the three pond system types.
- The use of hybrid algal-duckweed pond systems effluent as the influent of constructed wetlands is therefore recommended for optimal performance in BOD and faecal coliform removal.

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5

Chapter

Removal of faecal coliforms from duckweed, algal and hybrid algal-duckweed pond systems**ABSTRACT**

The importance of macro-invertebrates and bacteria attachment to suspended matter in the removal of faecal coliforms (FC) in pilot scale algal, duckweed and hybrid algal and duckweed wastewater treatment ponds were investigated. FC attachment to suspended solids was important only in the first two ponds of the three four-pond systems studied, and usually was more pronounced at the bottom. The shallow nature of the pond systems led to less prominence in the variation of FC decay with depth. Greater FC decay rates in algal ponds were observed in the afternoons compared with the mornings. The two most dominant macro-invertebrate groups in the ponds were ostracods and cladocerans. High densities of ostracods were associated with the surface and bottom of duckweed ponds and these were significantly higher than that of algal ponds at similar locations. FC numbers in the duckweed pond systems correlated strongly and positively with mean ostracod numbers in pond ($R^2=0.989$) and at the surface ($R^2=0.996$) with decreased numbers of FC associated with decreased numbers of ostracods. There was no correlation of FC numbers in the duckweed pond with ostracod numbers at the bottom of ponds. This suggests that macro-invertebrate feeding and movement activity could be an important contributor to the removal of FC in duckweed ponds. FC numbers also correlated well with Shannon-Wiener diversity index of macro-invertebrates in the duckweed ($R^2=0.714$), algal ($R^2=0.803$) and hybrid ponds ($R^2=0.664$), giving an indication of the pond water quality.

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1 INTRODUCTION

In algal ponds, the synergistic effect of solar radiation, elevated pH and dissolved oxygen concentration results in significant die-off of faecal coliforms (FC) (Curtis et al, 1992; Maynard et al, 1999; Davies-Colley et al, 2000). It is also known that algal pond disinfection is greatly reduced with increased depth as a result of light attenuation by algal matter (Van der Steen et al, 2000a). In comparison with duckweed ponds however, the thick layer of duckweed leaves cuts off light rays resulting in an insignificant effect of light in the inactivation of FC (Dewedar and Bahgat, 1995; Van der Steen et al, 2000b) making decay of FC in duckweed ponds likely to be unaffected by pond depth. Sludge accumulation in duckweed ponds is much lesser than in algal ponds (Awuah, 2006). This suggests that suspended matter may be higher in algal ponds than in duckweed ponds bringing to the fore the hypothesis that greater surfaces for possible faecal coliform attachment may exist in algal ponds compared to duckweed ponds and faecal coliform may eventually get sedimented to the bottom after attaching or getting adsorbed to these surfaces. If this phenomenon occurs, faecal coliform attachment to suspended matter should increase with depth as the disinfection effect of light decreases with depth. However, it is also not known whether this phenomenon may differ in a hybrid treatment system of algal and duckweed ponds as suppression of algal development by duckweed in this system decreases the amount of suspended matter in the pond system.

The importance of invertebrates in the removal of FC in algal and duckweed treatment ponds is barely reported in literature. Awuah (2006) observed that protozoans contribute to the removal of FC in duckweed and algal ponds and El-Shafai et al (2007) noted that macro-invertebrates contributed to the removal of COD and TSS in duckweed ponds but the importance of macro-invertebrates in the removal of FC in either duckweed or algal ponds is not documented in literature. Feeding and physical activity of macro-invertebrates (Leff and Leff, 2000; McEwen and Leff, 2001) as well as environmental conditions such as temperature and dissolved oxygen concentration (Duchet et al, 2010) can affect the distribution and abundance of bacteria in freshwater ecosystems. Physical activity of macro-invertebrates in duckweed and algal ponds may thus affect FC removal through either disruption or re-suspension of attached and sedimented FC or simply reducing FC abundance through feeding. This study investigates the relationship between faecal coliform attachment to suspended matter and depth in algal and duckweed ponds, and explores the possible effect of macro-invertebrates on the removal of FC in duckweed and algal ponds.

2 MATERIALS AND METHODS

2.1 Description of experimental setup

A pilot-scale treatment plant was setup in the open on the CSIR Water Research Institute premises in Accra, Ghana (5° 44'42"N, 0° 6' 27"W). The treatment plant consisted of three parallel lines of pond systems, one using a cover of *Spirodela polyrhiza* commonly known as duckweed (D), another using alga (A) and the third being a hybrid of duckweed and algal ponds (H) with two algal ponds sandwiched between two duckweed ponds (Figure 1). Each pond system consisted of four ponds in series, each pond having a diameter of 45cm and a depth of 30cm. The three pond systems receive raw domestic wastewater from a holding tank (HT) measuring 100cm x 100cm x 100cm. The wastewater flowed by gravity and was regulated by taps installed between the holding tank and the influent ponds. A hydraulic retention time of five days was maintained in each pond and average wastewater flow rate was $6.9 \times 10^{-3} \text{ m}^3 \text{ d}^{-1}$. 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 and the effluent of the holding tank are shown in Table 1. The duckweed treatment line (D) as well as the duckweed ponds of the hybrid line (H) were inoculated with duckweed from the beginning of the experiment and allowed to grow naturally. Duckweed in these ponds was harvested once every two weeks to maintain a dense cover in these ponds. Algal development in the algal and hybrid ponds occurred naturally. The treatment plant started running in February, 2006 and was monitored from April, 2006 to September, 2006. The experiment was conducted between April to June, 2007.

Samples were taken from 10cm below the surface of effluent ponds once a month for three months at 08:00-09:00 hours GMT for chlorophyll a analysis using NEN 6520 (1981) using three sub-replicate per sampling time. Daily fluctuations in pH and DO were monitored once every two weeks for ten weeks by taking readings in the morning (08:00-09:00 hours GMT), afternoon (13:00-14:00 hours GMT) and evening (17:00-18:00 hours GMT). These were measured by WTW 340 pH meter and WTW 330 Oximeter probes placed 10cm below the pond surface. Diurnal FC counts and decay rates were obtained by taking morning and afternoon samples at the times mentioned above, 10cm below the pond surface alongside the measurement of pH and DO using three sub-replicates per sampling time of once every two weeks for ten weeks. FC decay rates at different depths, top (just below pond surface), midpoint (15cm below pond surface) and bottom (30cm below pond surface) were also investigated by taking samples at these depths using the same sampling frequency. For each sample taken, detachment tests were performed to detach any attached FC using syringe fitted with a needle (Ansa et al, 2009, Chapter 2). FC counts before and after detachment were determined by plating on chromocult agar medium, incubated at 35-37°C for 24 hours (Finney et al, 2003) using spread plate technique (APHA, 2005). The decay rate (K_d) at each depth, was calculated assuming completely mixed conditions in each pond and using the Marais (1974) equation:

$$N_e = N_0 / (1 + K_d \theta)$$

Where,

N_e = Effluent FC count at a particular depth and time (100mL^{-1})
 N_o = Influent FC count at that same depth and time (100mL^{-1})
 θ = Retention time per pond (days)

Mean FC counts of detached and undetached samples were compared using t-test of Minitab 15.0. Macro-invertebrate sampling of the ponds was done by taking 10mL samples from the top, midpoint and bottom as defined earlier, using a broad-tip pipette, using 4 sub-replicates per sampling time of three. Samples were sieved through a $295\mu\text{m}$ mesh sieve and preserved in 50% alcohol for identification using Dejoux et al (1982) identification key. Relationship between FC and macro-invertebrate numbers and species diversity (Begon et al, 1996) were explored using regression analysis of MS Excel and PRIMER 5 (Plymouth Routines In Multivariate Ecological Research) software.

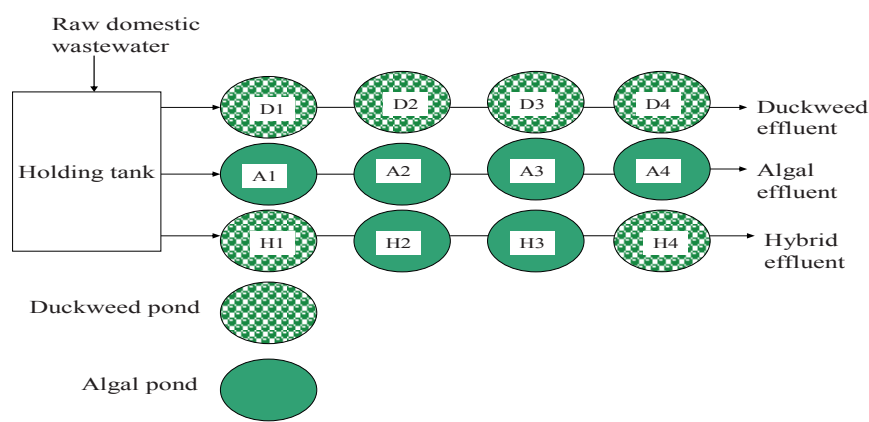


Figure 1. Schematic diagram of the pond systems

3 RESULTS

Table 1 shows the characteristics of the raw wastewater used and that of the effluent of the holding tank.

Table 1. Physico-chemical and microbiological characteristics of raw wastewater and effluent of holding tank

	*Wastewater	* Holding tank effluent
Temperature (°C)	29.8±1.3	30.2±1.1
pH	7.4±0.2	7.2±0.1
BOD (mgL ⁻¹)	240±38	190±11
Ammonia (mgL ⁻¹)	74±31	83±7
Nitrite (mgL ⁻¹)	0.10±0.09	0.31±0.36
Nitrate (mgL ⁻¹)	0.10±0.07	0.32±0.45
Total Phosphorus (mgL ⁻¹)	5.08±0.15	4.88±0.86
FC count (100mL) ⁻¹	6.6x10 ⁷ ±2.0x10 ⁶	3.5x10 ⁷ ±6.0x10 ⁶

*: ±standard deviation, n =9.

3.1 Environmental conditions in pond systems

Temperatures in all ponds were identical; the highest temperatures were reached in the middle of the day (Table 2). The pH in the duckweed ponds (Table 2) was neutral and decreased slightly from pond D1 to D4 (from 7.6 to 6.5), while the pH in the algal ponds was alkaline with an increase towards the final pond (from 7.8 up to 10.2 for 13:00-14:00hrs). Very little diurnal variation in pH was observed in the duckweed ponds of the duckweed and hybrid pond systems. In the algal ponds of both algal and hybrid pond systems however, pH increased from morning (08:00 hrs) to afternoon (13:00 hrs) and decreased again towards the evening (19:00hrs). Modest increases in oxygen concentration were observed from ponds D1 to D4 of the duckweed pond system. Dissolved oxygen concentrations were highest at noon in the algal ponds (Table 2). Insertion of two algal ponds in between two duckweed ponds H1 and H4 did not lead to any real increases in oxygen concentration of pond H4 compared to D4.

3.2 Removal of faecal coliforms in pond systems

3.2.1 Attachment to suspended matter

In the duckweed pond system, no particular trend in FC numbers was observed with depth, and also the decay rates at various depths within the duckweed pond system were comparable (Table 3). Pond D2 of duckweed pond system however showed significantly higher decay rates of FC at the midpoint compared to the surface and bottom. Ponds D1 and D3 showed a similar trend although the differences were not statistically significant. FC attachments to each other and to suspended matter were important only at the bottom of duckweed ponds D1 and D2 and the midpoint of duckweed pond D2. The algal ponds generally had higher decay rates compared with the duckweed ponds (Table 3). This includes the algal and the duckweed ponds of the hybrid pond system as well. In the algal pond system decay rates at the surface or midpoint were higher compared to the bottom,

but surface and midpoint decay rates did not differ significantly. FC attachment to each other and to suspended matter was important at the bottom of algal ponds A1 and A2, and the midpoint of algal pond A1. A similar trend of higher decay rate at the surface or midpoint in comparison to the bottom was also observed in the algal ponds of the hybrid pond system. In pond H2 however, decay rate at the surface was comparable to that at the bottom ($p > 0.05$). FC attachment to each other and to suspended matter was important at the surface of the first hybrid pond (H1) and the bottom of the second hybrid pond (H2).

Table 2. Environmental conditions of duckweed, algal and hybrid pond systems and algal biomass of effluent ponds.

Pond	Time (GMT)	pH				Dissolved Oxygen concentration (mgL ⁻¹)				Temperature (°C)	Chlorophyll a conc. (µg L ⁻¹)
		Pond 1	Pond 2	Pond 3	Pond 4	Pond 1	Pond 2	Pond 3	Pond 4		
Duckweed	08:00-09:00	7.1-7.6	7.0-7.2	6.8-6.9	6.5-6.9	1.8±0.5	2.0±0.7	2.4±1.1	3.5±1.6	29.0-31.0	39±8
	13:00-14:00	7.0-7.4	7.0-7.1	6.7-6.8	6.4-6.9	3.1±1.0	3.5±1.2	3.9±0.9	4.5±1.0	35.0-37.0	
	19:00-20:00	7.3-7.5	7.1-7.3	6.8-7.0	6.7-6.9	2.4±0.7	2.7±0.5	2.9±0.4	3.8±0.3	30.5-32.5	
Algal	08:00-09:00	7.6-7.9	8.1-9.0	8.6-9.6	8.9-9.6	8.9±0.5	10.3±3.5	10.8±2.7	10.7±3.1	29.9-31.0	383±52
	13:00-14:00	7.8-8.5	8.6-9.5	9.5-10.0	9.9-10.2	12.6±3.2	13.9±4.5	16.8±1.8	14.5±3.3	36.0-37.0	
	19:00-20:00	7.7-7.9	8.3-8.6	9.3-9.7	9.7-9.9	10.4±1.5	10.5±2.1	12.6±2.9	11.4±2.9	30.5-32.0	
Hybrid	08:00-09:00	7.1-7.3	8.1-9.0	8.6-9.7	6.9-7.1	2.1±0.9	8.1±2.4	10.4±1.7	4.1±1.0	29.0-31.0	76±23
	13:00-14:00	7.1-7.2	8.3-9.6	9.2-10.1	6.9-7.4	3.0±0.5	10.2±1.5	16.5±3.9	4.0±0.2	35.0-38.0	
	19:00-20:00	7.2-7.3	8.3-8.7	9.8-9.9	7.1-7.4	2.2±0.5	9.8±3.6	12.4±4.2	4.2±0.5	30.0-32.5	

Table 3. Decay rates and FC attachment at various depths in pond systems.

Ponds	Duckweed			Algal			Hybrid		
	K _d (d ⁻¹)	Undetached	Detached (%)	K _d (d ⁻¹)	Undetached	Detached (%)	K _d (d ⁻¹)	Undetached	Detached (%)
1 Surface Midpoint Bottom	0.6±0	880.0±45.0	537.8±128.7	0	3.3±0.4	2225.0±278.8	2287.0±529.5	28	93*
	2.0±0.6	433.8±60.6	330.0±55.2	0	6.0±0.8	1350.0±229.1	2912.5±591.4	116*	7
	1.1±0.4	898.8±152.7	3200.0±682.3	256*	1.8±0.2	4062.5±503.0	42500.0±407.9	946*	2
2 Surface Midpoint Bottom	1.1±0.1	140.4±9.7	101.1±10.7	0	7.2±1.2	70.6±8.8	82.0±33.5	17	17
	4.5±1.0	25.9±4.9	60.0±10.6	47*	9.2±2.3	39.3±6.9	44.9±7.4	14	0
	0.7±0.1	211.0±16.8	420.0±54.6	99*	0.9±0.3	932.5±107.6	1633.9±204.4	75*	71*
3 Surface Midpoint Bottom	2.8±0.2	9.8±0.7	4.2±2.1	0	7.4±1.3	2.6±0.5	6.5±0.6	15	0
	3.0±0.7	3.5±0.7	2.9±0.6	0	4.8±1.1	2.1±0.4	0.2±0.1	0	0
	2.1±0.5	52.4±24.1	55.1±15.4	5	2.4±0.4	85.3±12.4	109.2±34.1	28	1
4 Surface Midpoint Bottom	4.2±1.6	0.8±0.1	0.9±1.2	8	1.7±0.4	0.5±0.1	0.54±0.10	9	0
	2.7±0.6	0.3±0.1	0.31±0.10	0	2.1±0.4	0.2±0.1	0.30±0.03	18	0
	1.6±0.3	2.7±0.3	1.3±0.14	0	0.5±0.8	28.0±3.1	2.8±0.6	0	13

(*) represents increase in FC count after partial release of attached FC, n=15.

3.2.2 Diurnal influence

In all the ponds of the duckweed systems, FC counts (sampled at 10cm below the pond surface) in the morning did not show any significant difference from that of the afternoon ($p > 0.05$) and their decay rates, Kd (calculated as outlined above) were also comparable (Table 4). The algal ponds had lower FC counts in the afternoon compared to the morning ($p \leq 0.05$) and decay rates were generally higher in the afternoon than in the morning. The third pond of the algal system A3 however showed lower decay rates in the afternoon ($p \leq 0.05$). Ponds H1, H2 and H3 of the hybrid pond system showed lower FC numbers in the afternoon compared to the morning ($p \leq 0.05$) while the fourth pond H4 had comparable FC numbers in the morning and afternoon ($p > 0.05$). In general, decay rates in the hybrid pond systems did not differ significantly.

Table 4. Diurnal counts and decay rates of faecal coliforms.

Ponds	Duckweed FC count $\times 10^3$ (100mL) ⁻¹		Algal FC count $\times 10^3$ (100mL) ⁻¹		Hybrid FC count $\times 10^3$ (100mL) ⁻¹	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
1	4475.0 \pm 626.2 (1.3)	3837.5 \pm 584.7 (1.7)	2075.0 \pm 495.5 (7.1)	522.5 \pm 79.5 (12.6)	8300.0 \pm 1082.8 (0.6)	2270.8 \pm 1030.6 (6.2)
2	712.5 \pm 102.4 (1.3)	727.5 \pm 91.1 (1.2)	276.3 \pm 66.5 (1.7)	12.0 \pm 1.2 (10.0)	646.3 \pm 125.6 (3.1)	198.8 \pm 27.8 (2.2)
3	81.9 \pm 8.9 (1.8)	75.0 \pm 11.1 (2.1)	7.4 \pm 0.9 (8.0)	0.7 \pm 0.1 (3.5)	22.4 \pm 4.3 (8.2)	6.6 \pm 1.2 (9.0)
4	7.5 \pm 0.8 (2.0)	6.6 \pm 1.0 (2.6)	0.6 \pm 0 (2.7)	0.1 \pm 0 (3.6)	2.8 \pm 0.5 (1.3)	2.0 \pm 0.5 (0.9)

\pm Standard error.

3.2.3 Effect of macro-invertebrates

The two most dominant groups present in the ponds were the class ostracoda and the order cladocera. High densities of ostracods were associated with the surface and bottom of duckweed ponds and these were significantly higher than that of algal ponds at similar locations (Table 5). FC numbers correlated strongly and positively with mean ostracod numbers in the entire duckweed ponds ($R^2=0.989$) and at the surface of duckweed ponds ($R^2=0.996$) with decreased number of FC associated with decreased numbers of ostracods. There was no correlation of FC numbers in the duckweed pond with ostracod numbers at the bottom of ponds. FC numbers also correlated well with Shannon-Wiener diversity index (Begon et al, 1996) of macro-invertebrates in the duckweed ($R^2=0.714$), algal ($R^2=0.803$) and hybrid ponds ($R^2=0.664$). No correlation was found between FC numbers and cladoceran numbers in either algal or duckweed ponds.

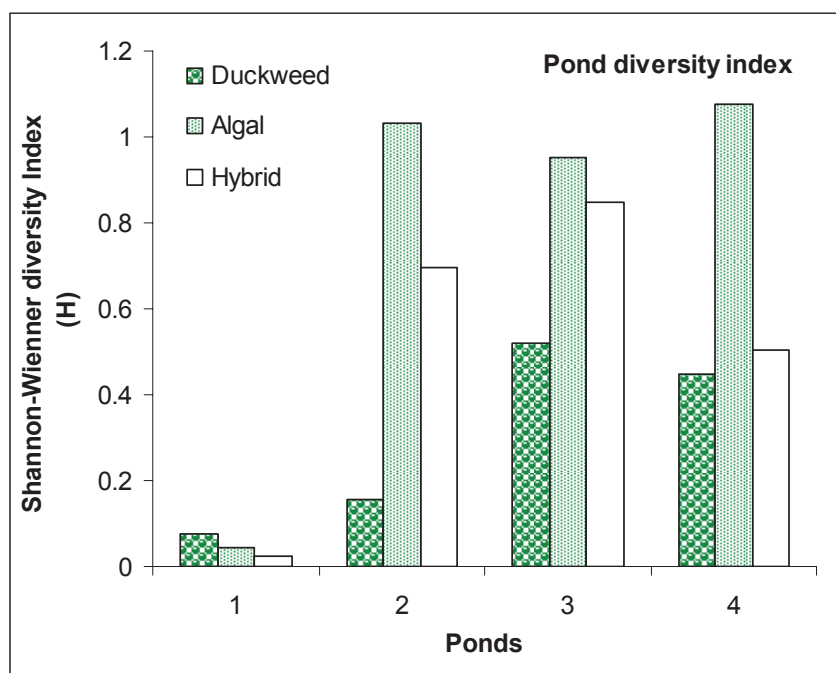


Figure 2. Diversity of macro-invertebrates in the pond systems.

Table 5. Distribution of *Ostracoda* and *Cladocera* in duckweed and algal ponds

Ponds	<i>Ostracoda</i>				<i>Cladocera</i>			
	Surface	Mid-point	Bottom	Total	Surface	Mid-point	Bottom	Total
Duckweed								
D1	29867±143.6	13±2.1	74±5.5	29954	20±15.6	3±1.5	0	23
D2	6151±426.3	62±8.5	200±18.0	6413	64±4.7	0	0	64
D3	934±258.8	62±6.0	241±2.1	1237	60±9	0	0	60
D4	594±16.3	50±5.5	1105±16.7	1749	0	0	0	0
Algal								
A1	0	0	0	0	418±14.8	3±2.0	296±15.8	717
A2	2±2	23±3.1	237±25.2	262	442±41.4	13±6.1	271±18.1	726
A3	0	4±3.5	278±16.8	282	441±11.1	14±1.7	310±7.1	765
A4	0	0	287±30.6	287	569±9.8	65±11.1	300±10.5	934
Hybrid								
H1	1563±470.4	28±2.0	7163±186.0	8754	0	0	0	0
H2	33±6.0	4±1.7	417±17.0	454	525±4.4	43±7.5	0	568
H3	44±9.3	0	97±29.4	141	16±5.1	12±0.6	0	28
H4	4641±52.4	15±5.5	18309±23092.0	22965	31±4.0	0	0	31

(Numbers expressed per 100mL, ± standard deviation, n =12).

4 DISCUSSION

4.1 FC attachment and die-off

The variation in FC decay rates with depth in conventional treatment pond systems (Table 3) is due mainly to the effect of solar radiation (Davies-Colley et al, 2000). In duckweed ponds the dense cover of duckweed fronds prevents or minimizes the effect of solar radiation. Solar radiation achieves die-off of bacteria by disruption of bacteria cell division through the breakage of thiamine-adenine bonds of DNA and RNA, forming a double bond which renders them incapable of cell division and consequently death (Berson, 1996). Light in conjunction with sensitizers such as dissolved organic matter and photosynthetic pigments may also damage bacteria cellwall exposing the cell to harsh environmental conditions such as high pH and toxic oxygen radicals (Curtis et al, 1992; Maynard et al, 1999; Davies-Colley et al, 2000). Dark conditions in the duckweed ponds may explain the low and comparable rates of decay and FC numbers observed. The effect of sunlight is more pronounced in algal ponds in that algae utilise solar radiation (400-700nm) for photosynthetic activity increasing the dissolved oxygen concentration of the pond, utilising dissolved carbon dioxide and thereby causing the formation of hydroxyl ions, rendering the pond medium alkaline. Solar radiation penetration in algal ponds however decreases with increased algal biomass concentration of the pond (Van der Steen et al, 2000a) and this may lead to a decreased decay rate of FC in the deeper part of the water column. In the algal ponds of the algal and hybrid pond systems however, decay rates at the surface and midpoint were comparable suggesting that light penetration at depths 15cm below the pond surface may be comparable to that at the pond surface. Depending on the chlorophyll a concentration of algal ponds, the euphotic depth, which is the depth at which visible light (400-700nm) is reduced to 1% of its value at the water surface, may vary from 11 to 35cm (Davies-Colley et al, 1995; Sukias et al, 2001; Davies-Colley et al, 2005). Lower decay rates however were observed at the bottom of the algal pond systems suggesting that either light penetration to the bottom was poor or FC settling was occurring at the bottom.

Greater decay rates observed at the midpoint of duckweed pond 2 could be due to FC attachment to each other and to suspended solids. Detachment tests yielded an increase of 47% in FC counts. It was also obvious that FC were settling at the bottom of the ponds. Awuah et al (2004) observed that 99% of FC removal by algal and macrophyte ponds was due to sedimentation and subsequent die-off. Attachment was important at the bottom of both algal and duckweed ponds, due perhaps to greater settling of heavier aggregates of suspended matter and FC at the bottom. Laboratory experiments conducted earlier (chapter 2) showed that attachment is enhanced by increased FC concentration. Attachment was important in only the first two ponds and this may be attributed to decreased numbers of FC in the last two ponds as a result of die-off enhanced by longer retention time. Comparable decay rates at the surface and bottom of algal hybrid pond H2 could be explained by the 71% increase in bacteria numbers after detachment. This suggests that decreased FC numbers at the bottom of pond H2 was due to FC attachment to each other and to suspended solids and not die-off.

4.2 Diurnal effects on removal of FC

The inability of solar radiation to penetrate the dense layer of duckweed resulted in the comparable numbers of FC in the morning and in the afternoon. The average diurnal temperature increase of 6°C in the duckweed ponds from morning to afternoon (Table 2) was not sufficient to cause any significant die-off of faecal bacteria (Table 4). El Shafai et al (2007) also observed minimal temperature effect on FC removal in duckweed ponds. FC counts in the afternoon were lower than in the morning in the algal ponds and also decay rates were greater in the afternoon than in the morning in this pond system. As temperature conditions in the algal ponds were similar to that in the duckweed ponds, the differences between afternoon and morning results cannot be attributed to temperature increases. Intensity of solar radiation increases from morning and peaks around midday and this increased intensity may have resulted in the increased inactivation of FC (Liltved and Landfald, 2000). Liltved and Landfald (2000) observed that bacteria decay increased with increased light intensity. This trend of lower FC numbers in the afternoon in algal ponds and comparable morning and afternoon numbers in duckweed ponds occurred also in the algal and duckweed ponds of the hybrid pond system. There were exceptions to this phenomenon however. The high decay rate observed in the first hybrid pond H1 in the afternoon (Table 4) may be due to development of algae in the pond as a slight increase in pH of that pond was observed as well (Table 2). This may have been caused by (temporary) open spots in the duckweed cover, allowing penetration of sunlight.

4.3 Effect of macro-invertebrates on FC removal

The assessment of water quality by means of the study of invertebrate communities is a tool in the management of water resources (Mezquita et al, 1999). Aquatic invertebrates have different degrees of resilience in tolerating environmental stresses such as low oxygen concentration or high BOD. A gradient of invertebrate fauna consisting of more pollution tolerant but less diverse ones to less tolerant but more diverse ones occurs from more polluted water to cleaner ones. This explains the increasing diversity index from ponds 1 to 4 in all three pond systems (Figure 2) and the correlation of Shannon-Wiener diversity index with FC numbers. The algal ponds showed greater diversity than duckweed ponds as algae serve as food for many invertebrates and algal surfaces also harbour many protozoans fed on by grazers. Ponds with greater species diversity therefore are indicative of a better water quality. Under stressful conditions, the diversity of macro-invertebrates decreases and at the same time one or two species could reach much higher densities owing to the elimination of sensitive competitors (Jak et al, 1998). In all three pond systems, two groups of invertebrates, ostracods and cladocerans dominated.

Ostracoda is a class of the subphylum Crustacea and are commonly known as seed shrimps (Brusca and Brusca, 2002). They have been of recent interest as a test model organism for environmental and toxic stress studies (Holmes and Chivas, 2002; Pascual et al, 2002) including toxicity of sewage sludge (Oleszczuk, 2008). As small crustacean grazers (0.5-2mm long) and filter feeders, they graze along roots and leaves of aquatic plants and filaments of algae (Khangarot and Das, 2009) and they may also feed on bacteria and unicellular algae (Grant et al, 1983). This may explain why they were mainly

found at the surface and bottom of duckweed ponds and not at the midpoint (Table 5). At the surface they may graze on bacteria and protozoans attached to duckweed fronds and seem to show a preference for fauna associated with decayed duckweed fronds as they are present more abundantly in the duckweed ponds and their numbers decrease at the surface from ponds D1 to D4 as bacteria numbers decrease (Table 4). Ostracoda numbers also increase at the bottom from ponds D1 to D4 due perhaps to increased settling of decayed duckweed fronds and its associated bacteria. Ostracod distribution is influenced by dissolved oxygen concentration (DO) (Ali et al, 2007), temperature and pH (Mezquita et al, 1999) but differences in numbers of ostracods at different depths in duckweed ponds compared with algal ponds cannot be attributed to differences in DO, temperature or pH. This is because firstly, temperatures in the algal and duckweed ponds are similar. Secondly, despite the low pH (6.8-7.2) and DO ($2.0-2.4\text{mgL}^{-1}$) in ponds D2 and D3 compared with the high pH (8.1-9.6) and DO ($10.3-10.8\text{mgL}^{-1}$) in ponds A2 and A3 in the morning (08:00-09:00) when the macro-invertebrate sampling was done, ponds A2, A3 and D2, D3 showed comparable ostracod numbers at the bottom suggesting that ostracods can tolerate such pH and DO ranges. A strong positive correlation of FC numbers with mean ostracod numbers in the entire duckweed pond ($R^2=0.989$) and at the surface of duckweed ponds ($R^2=0.996$) but not at its bottom ($R^2=0.2918$) suggests an influence of ostracods on FC numbers. As ostracod food preferences are mainly bacteria and algae and limited amounts of algae are found in duckweed ponds, the main available food in the duckweed ponds which are bacteria would include faecal coliforms, hence their decreased number with decreasing bacteria numbers. True predator-prey pattern of abundance shows increased predator population with increased prey numbers and decrease predator numbers as numbers of prey decreases (Begon et al, 2006; Townsend et al, 2008). Ostracods may thus constitute an important means of removal of faecal coliforms in duckweed ponds.

The order Cladocera are also small crustaceans (0.2-3.0mm long) belonging to the class Phyllopoda (Dumont and Negrea, 2002). Cladocerans feed on algae, protozoans, bacteria and decaying organic matter, (Keating, 1985; Abrantes and Concalves, 2003). It is therefore not surprising that they abound in algal ponds more than in duckweed ponds at the pond surfaces. Cladocerans were present at the bottom of the algal ponds only and not at the bottom of the other pond types, suggesting a difference in the ecology of the pond types. Decaying organic matter from algal cells and sludge occur more at the bottom of algal ponds and attached bacteria may also be present at the bottom, mid portion as well as at pond surfaces. Cladoceran numbers may be positively affected by increases in DO as limited presence at the surface of the duckweed pond may have been encouraged by atmospheric oxygen dissolving in pond water (Table 4). The absence of a correlation between FC numbers and cladoceran numbers in either algal or duckweed ponds could be due to their wider range of food preferences and the unlimited availability of other food types other than bacteria in both duckweed and algal ponds (Begon et al, 1996). Cladocerans may thus have little effect on faecal coliform numbers in ponds.

5 CONCLUSIONS

- Faecal coliforms attachment to suspended matter was important only in the first two ponds of all the pond system types and usually was more pronounced at the bottom.
- Attachment of faecal coliforms to suspended matter was not more prominent in any particular pond system type. Sedimentation of FC appears to have been aided by attachment of FC to suspended matter.
- In the algal ponds, rates of decay were lower in the morning than in the afternoon in contrast with duckweed ponds which did not show any differences in rates of decay in the morning and afternoon. This seems to be an effect of light intensity.
- High densities of ostracods are associated with the surface and bottom of duckweed ponds and these are much higher than in algal ponds at similar locations.
- FC numbers in duckweed ponds correlated strongly and positively with mean ostracod numbers in ponds. FC numbers also correlated well with Shannon-Wiener diversity index of macro-invertebrates in all the three pond systems.

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The role of algae in the removal of *Escherichia coli* in a tropical eutrophic lake

ABSTRACT

Eutrophication and its accompanying algal development in lakes is a nuisance and may be problematic for aquatic life but limited algal development may have some beneficial consequences. Dissolved oxygen concentration and pH increases attributed to algae in algal-based treatment ponds may occur in eutrophic lakes and can result in the inactivation of faecal coliforms in eutrophic lakes. We investigated the die-off of *E. coli* placed in dialysis tubes in a eutrophic lake at different depths and locations. The importance of *E. coli* attachment to algae and suspended matter was also assessed. Algal presence in the lake resulted in significant decay of *E. coli*. At chlorophyll a concentration $\leq 0.08\text{mgL}^{-1}$ in the Weiya Lake, decay rate of *E. coli* is directly proportional to the chlorophyll a concentration of the lake. Under laboratory conditions, as chlorophyll a concentration increases in light however, an optimum chlorophyll a concentration (0.24mg/L) is reached after which the rate of decay of *E. coli* decreases. These results show that limited algal presence representing optimum chlorophyll a concentration in restored ecosystems may have public health benefits for rural communities in developing countries that depend on raw water for domestic activities.

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1 INTRODUCTION

Nutrient enrichment of lakes and its accompanying algal development is a nuisance for water supply and recreational uses. Excessive development of algae may result in death of aquatic life but limited algal presence may have beneficial consequences that may not have been previously reported in literature. Wang et al (2009) showed that excessive algal presence can be controlled by the introduction of a mosaic community of macrophytes.

The effect of algae on the removal of faecal bacteria in wastewater treatment ponds and Free Water Surface Wetlands (FWS) are well documented in literature (Curtis, 1990; Curtis et al, 1992; Van der Steen et al, 2000a, 2000b; Awuah, 2006; Garcia et al, 2008) and algal treatment ponds appear to be more effective in the removal of faecal bacteria than macrophyte-based ponds (Awuah, 2006). Eutrophic lakes may show a comparable effect in the removal of faecal bacteria considering the similarity in the biological and biochemical processes in these two aquatic environments. Pu et al (1998) observed that attached algae, submerged and dying macrophytes assisted in the removal of pollutants. Increased oxygenation in treatment ponds or lagoons attributed to algal presence has been observed to affect faecal bacteria die-off due to the production of toxic forms of oxygen (Curtis et al, 1992). Maturation ponds of waste stabilization ponds usually have chlorophyll a concentrations of between 500 to 2000 μgL^{-1} (Feachem et al, 1983). Lakes with chlorophyll a concentrations above 13 $\mu\text{g/L}$ are considered eutrophic and these algal concentrations are enough to increase oxygen concentrations of lakes to levels that may be harmful to faecal bacteria. Oxygen concentrations above 0.5 mgL^{-1} have been shown to contribute to the removal of faecal bacteria (Van Buuren and Hobma, 1991). Algal presence also leads to high pH levels that tend to be bactericidal even in the absence of high oxygen concentrations (Maynard et al, 1999). Eutrophic lakes on the other hand may not have such high pH values as occur in maturation ponds but may experience modest fluctuations in pH due to diurnal variation in carbon dioxide concentration as a result of photosynthesis. Fluctuations in pH are known to negatively affect survival of *Escherichia coli* (Awuah, 2006) and could therefore result in significant removal of faecal bacteria in eutrophic lakes.

Bacteria in the aqueous medium attaches itself to solid surfaces by secreting extracellular polysaccharides and the quantity and composition of this extracellular polysaccharides may affect the attachment properties of the bacteria (Sanin et al, 2003). Algal presence in eutrophic lakes as suspended matter, may also serve as surfaces for bacterial attachment resulting in the formation of aggregates which may eventually get sedimented from the water column to the lake bottom, thus improving the water quality of the lake. A high degree of faecal bacteria attachment to algae and suspended solids in eutrophic lakes could therefore constitute a major natural means of removal of bacteria pathogens from eutrophic lakes. The importance of such a phenomenon in eutrophic lakes has not been reported in literature.

Some studies, however, reported that the release of algal organic matter as a result of algal cell lysis can enhance coliform survival and even growth (Lake et al, 2001;

Bouteleux et al, 2005) because algal degradation provides the carbon and energy sources for the survival and growth of coliforms (Van der Steen et al, 2000a). This suggests that degradation of algal cells may undermine any disinfection activity that living algae may have accomplished.

This study aims to understand the importance of algae in the removal of *Escherichia coli* from eutrophic lakes and to specifically underscore the effect of varying algal concentrations on *E. coli* removal. In addition, the importance of *E. coli* attachment to algae in a eutrophic lake is assessed in relation to its removal from the lake. Survival experiments in a eutrophic lake involving *E. coli* in dialysis tubes immersed at different depths and locations were therefore expected to unmask some of the possible effects of algae in eutrophic lakes. Since the variation in chlorophyll a concentration provided by field conditions were minimal and do not therefore explore possible scenarios that may exist in other eutrophic lakes, additional batch laboratory experiments using *E. coli* and laboratory cultured algae were carried out.

2 MATERIALS AND METHODS

2.1 Study area

The Weija Lake, formed by the damming of the Densu River, is located 40km west of Accra, Ghana (West Africa) and together with the Densu River drains a total area of 2072km². The lake, located within latitude 5° 30'N - 6° 20'N and longitude 0° 10'W - 0° 35'W is polymictic and is mainly a source of drinking water for over 2.6 million people in the western part of Accra as well as irrigation for some 200 hectares of farmland. It has an estimated fish yield of 380 metric tons per year (Ministry of Works and Housing, 1998). Algal sampling of the lake prior to the lake experiment showed *Chlorella* sp and *Anabaena* sp as the most dominant algal species at the experiment sites. Other algae in minimal quantities included *Oscillatoria* sp, *Euastrum* sp and *Synedra* sp.

Table 1. Characteristics of the Weija Lake (unfiltered) over the period 2005 - 2008 (Darko and Ansa-Asare, 2009).

Characteristics			
pH	Dissolved Oxygen (mgL ⁻¹)	Conductivity (µScm ⁻¹)	Turbidity (NTU)
6.5–8.7	3.5–10.5	247–527	2.8–15.9
Biochemical Oxygen Demand BOD ₅ (mgL ⁻¹)	Nitrate Concentration (mgL ⁻¹)	Phosphate Concentration (mgL ⁻¹)	Chlorophyll a (µgL ⁻¹)
1.7–10.1	0.07–2.43	0.001–0.178	1.0–114.0

2.2 *E. coli* decay experiment in the lake

E. coli ATCC 25922 (Strain info, 2010) suspension of concentration (1-3) x 10⁷ mL⁻¹ and henceforth referred to as *E. coli* was washed twice in saline solution (0.85% saline) to

remove nutrients and 15mL of this suspension was placed in Spectra/Por1 dialysis tubes in groups of 10 tubes. Saline solution (0.85% saline) was prepared by dissolving 1 tablet of Oxoid BR0053G saline tablet in 500mL of distilled water, autoclaved at 121°C for 15 minutes. Spectra/Por 1 dialysis tubes are regenerated cellulose and molecular porous dialysis tubes of molecular weight cut off (MWCO) of 6000-8000 dalton. Analysis of the spectra characteristics of the dialysis tubing in a Uvicon 930 UV/Visible spectrophotometer, Kontron instruments, Switzerland, showed that only light of wavelengths $>350\text{nm}$ are transmitted by the dialysis tubes (Bergstein-Ben et al, 1997). The suspension was constantly stirred during distribution into dialysis tubes to avoid settling. Dialysis tubes were suspended 0.20m below the lake surface at two locations (1 and 2), separated by a distance of 5km. Sites were selected based on visual inspection of algal abundance exhibited by green suspended cells of algae and it was assumed that chlorophyll a concentration will differ at these two locations. Additional dialysis tubes were suspended at a depth of 20m below the lake surface at location 2 only. Two control setups were suspended 0.20m below the lake surface at location 2 only, both consisting of filtered lake water containing dialysis tubes of *E. coli* (Figure 1). Filtration of the lake water for the two controls entails pouring lake water through an eight-layered cotton cloth to filter off algal cells. Control A was filtered once (at the start of the experiment) allowing growth of algal spores. Control B was filtered every other day during the period of the experiment.

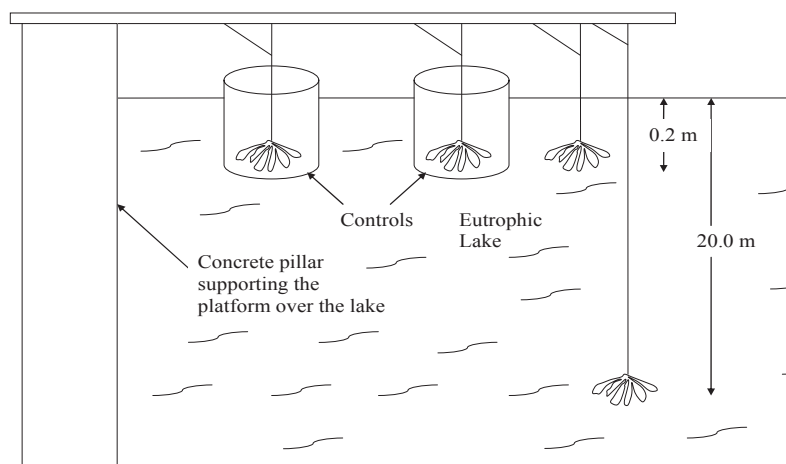


Figure 1. Set-up of lake experiment.

One hour after the immersion of the dialysis tubes into the lake, temperature, dissolved oxygen concentration, pH and conductivity inside and outside of the tubes were determined and monitored during the period of the experiment between the hours of 7:00 am-8:00 am at a depth of 0.2m below the lake surface. Dissolved oxygen concentration, pH and conductivity measurements were done with a WTW Oxi 330, WTW pH 340 and

WTW LF 340 meters respectively. *E. coli* numbers were assessed 0, 1, 3, 5 and 7 days after incubation in the lake. The experiment lasted for 7 days and made use of two duplicates, each having three subreplicates making a total of 6 replicates per treatment. *E. coli* counts inside dialysis tubes were measured using the spread plate technique (APHA, 2005) on chromocult agar plates incubated at 37 °C for 24 hours (Byamukama et al, 2000; Wang and Fiessel, 2008). The decay rates of the *E. coli* in the incubations, K_d were calculated as a gradient of the regression line of the first order decay equation (Marais, 1974; Dewedar and Bahgat, 1995):

$$\ln N_t = -K_d t + \ln N_0 \quad (1)$$

where

N_t = *E. coli* count per 100mL at a time t

N_0 = *E. coli* count per 100mL at the start of the experiment

t = Time (days) of incubation

On the same days (0, 1, 3, 5 and 7 days of incubation in the lake) chlorophyll *a* concentrations were determined at various locations except at 20m depth by taking water samples for chlorophyll *a* analysis. Light sensor readings at depth 20m using LI-COR UWQ 4683 underwater light sensor (LI-COR, Inc, Nebraska, USA) indicated absence of solar radiation at that depth. Chlorophyll *a* concentrations were determined according to NEN 6520 (1981) using four replicates per treatment. Chlorophyll *a* concentration, dissolved oxygen concentration and pH measurements were not done at 20m depth due purely to logistical limitations. These parameters were inferred from absence of solar radiation at this depth. The diurnal variation of pH, temperature and dissolved oxygen concentration of the lake were monitored from 6:00 am to 6:00 pm at a depth of 0.2m on day 0, 1 and 3 ($n=3$). All experiments were carried out in the months of February and March, 2006 when algal blooms are common in the lake. Maximum and minimum day and night ambient temperatures were 33 °C and 19 °C respectively. Decay rates of different treatments were compared statistically using an independent sample t-test of Minitab 15.0 statistical package.

2.3 Attachment of *E. coli* to algae in lake water

Sampling for *E. coli* in the lake was done by collecting 30 sub replicates per sampling location, for three different locations in the lake using 600mL containers. Locations within 4-8m of shoreline were selected as sampling was based on the assumption that activities of neighbouring communities in the water may render these locations high in *E. coli* numbers and that *E. coli* may be attaching to each other and to suspended matter. Sampling was conducted once per week for five weeks at a depth of 0.2m for *E. coli* presence. Water samples were pushed through a 10mL syringe fitted with a needle to detach any attached bacteria. The syringe-needle method is able to recover a high percentage of attached bacteria, sometimes as high as 100% (Ansa et al, 2009). *E. coli* numbers were determined before and after syringe treatment by membrane filtration technique (APHA, 2005) on chromocult agar plates incubated at 37 °C for 24 hours. *E. coli* numbers before and after pushing through syringes was statistically compared using paired samples t-test of Statistical Packages for Social Scientists (SPSS version 12.0).

Data of wind speed on sampling days were obtained from the Surface Water Division of the CSIR Water Research Institute, Accra, Ghana.

2.4 Laboratory experiments

Algae were grown by inoculation of nutrient solution with laboratory stock of *Chlorella* sp obtained from Wilson Group Inc., USA (Wilson Group, 2010) under light of wavelength 380-780nm provided by a powerstar HQI-BT 400 lamp. Culture solution contained 13.5mgL^{-1} nitrogen and 2.2mgL^{-1} phosphorus in the form of nitrate and phosphate respectively. Resulting algae were harvested after 14 days, sieved using $250\mu\text{m}$ and $90\mu\text{m}$ mesh nets and concentrated by centrifugation at 1000 rpm for 30 minutes into a thick algal paste. Into each of four sets of sterile Erlenmeyer flasks, 250mL of thoroughly mixed *E. coli* (ATCC 25922) suspensions of concentration $(1.3\text{-}3.1) \times 10^8\text{mL}^{-1}$ were introduced. Each set consisted of six (6) flasks. *E. coli* suspensions were prepared by a ratio of 1mL stock *E. coli* suspension for 50mL of sterile Oxoid CM0001 nutrient broth solution, maintained at $35\text{-}37^\circ\text{C}$ for 24 hours. The *E. coli* suspensions were washed twice by concentrating and re-suspending in saline solution prepared as mentioned above. *E. coli* suspensions were vortex-mixed at optimum energy and time to avoid clustering of bacteria cells.

Harvested algae from culture setup were used to inoculate five (5) of each of six (6) flasks belonging to each set, one flask was maintained as control, having no algae. Each set of flasks was inoculated with different amount of algae. The following mean chlorophyll a concentrations were obtained for each set: 0, 0.051, 0.24, 2.25, 5.02 and 10.5mgL^{-1} . Two sets of flasks (serving as duplicates of each treatment), each consisting of six flasks were covered with dark polythene sheets and placed on a GFL 3019 orbital shaker together with the other two uncovered sets (also duplicates of each treatment) in a randomised block design such that each treatment of particular chlorophyll a concentration had a subreplicate of 3 samples. Each treatment of a particular chlorophyll a concentration therefore had a total of six replicates per treatment. The GFL 3019 shaker was placed 0.8m below the HQI-BT 400 lamp in a regime of 16hours light and 8 hours darkness rotating at 100rpm. Temperature of setup varied from $20 - 25^\circ\text{C}$. Temperature dropped to 20°C in the night when lamp went off. *E. coli* was monitored at time $t = 0, 0.25, 1, 3, 5$ and 7 days using the spread plate technique (APHA, 2005) on chromocult agar plates incubated at 37°C for 24 hours (Byamukama et al, 2000; Wang and Fiessel, 2008). Chlorophyll a concentration was measured at the beginning and end of the experiment according to NEN 6520 (1981) using four replicates per treatment. Dissolved oxygen concentration and pH of reactors were monitored at 10:00-11:00hrs GMT using WTW 330 Oximeter and WTW 340 pH meter respectively.

In order to determine that decrease in *E. coli* numbers is a result of actual die-off and not *E. coli* attaching to each other and to algae, samples were taken after 1 and 3 days of incubation and samples were pushed through 10mL clinical syringes fitted with needles to detach any attached bacteria (Ansa et al, 2009). *E. coli* numbers before and after pushing through syringes was statistically compared using paired samples t-test of Statistical Packages for Social Scientists (SPSS version 12.0). The decay rates of the *E. coli* in the incubations, K_d were calculated as a gradient of the regression line of the first

order decay equation (1) mentioned above (Marais, 1974; Dewedar and Bahgat, 1995). Decay rates of different treatments were compared statistically using an independent sample t-test of Minitab 15.0 statistical package.

3 RESULTS

3.1 *E. coli* decay experiment in the lake

3.1.1 Physico-chemical characteristics of the lake

Slightly higher pH and dissolved oxygen concentrations were observed in lake dialysis tubes compared to control A and B (Table 2). Physico-chemical conditions of temperature, pH and DO inside the dialysis tubes were comparable to conditions in the lake. pH during the day was the highest at 14:00 hours GMT (8.7) and lowest at 06:00 hours GMT (8.0), while dissolved oxygen concentration peaked at 08:00 hours GMT (8.8mgL^{-1}) and reduced to the lowest value of 5.6mgL^{-1} by 18:00 hours GMT (Figure 2). Figure 3 shows that there was exchange of fluid between the contents of the dialysis tubes and the lake at all locations. Diurnal variation in conductivity was fairly constant with minimal fluctuation from 420.7 to $422.3\mu\text{Scm}^{-1}$. Lower conductivity was observed in the lake compared to the two controls.

Table 2. Physico-chemical conditions inside and outside dialysis tubes in controls and in the lake and *E. coli* decay rates K_d at these locations. Temperature, pH and DO are averages of 10 values \pm standard deviation of measurements taken 0.2m above the lake Surface. Chlorophyll a concentrations represents averages of 20 values \pm standard deviation taken at 0.2m below the lake surface. Control A: algae filtered only at the start of experiment, Control B: algae filtered every other day.

Dialysis tubes	Temp. (°C)	pH	DO (mgL ⁻¹)	^b Chlorophyll a (mgL ⁻¹)	K _d (day ⁻¹)		
					Location 1 (0.2m)	Location 2 (0.2m)	Location 2 (20.0m)
Control A							
Inside	28.8±0.9	7.8±0.1	4.4±0.5	0.041±0.01e		0.85±0.08h	
Outside	29.3±0.6	7.6±0.4	3.8±0.4				
Control B							
Inside	28.8±0.8	7.7±0.3	3.6±1.3	0.011±0.02f		0.55±0.07h	
Outside	29.4±0.7	7.7±0.3	3.0±1.7				
Lake							
Inside	28.5±0.7	8.1±0.1	5.1±0.8	K _{0.047} ±0.01e	1.20±0.07g	1.23±0.03g	0.63±0.07h
Outside	30.0±0.7	8.2±0.1	4.3±1.0	L _{0.048} ±0.01e			

^DMean chlorophyll a concentration \pm standard deviation at location 1, 0.2m deep (K) and location 2, 0.2m deep (L). Same small letters refers to statistically comparable values while different letters are significantly different.

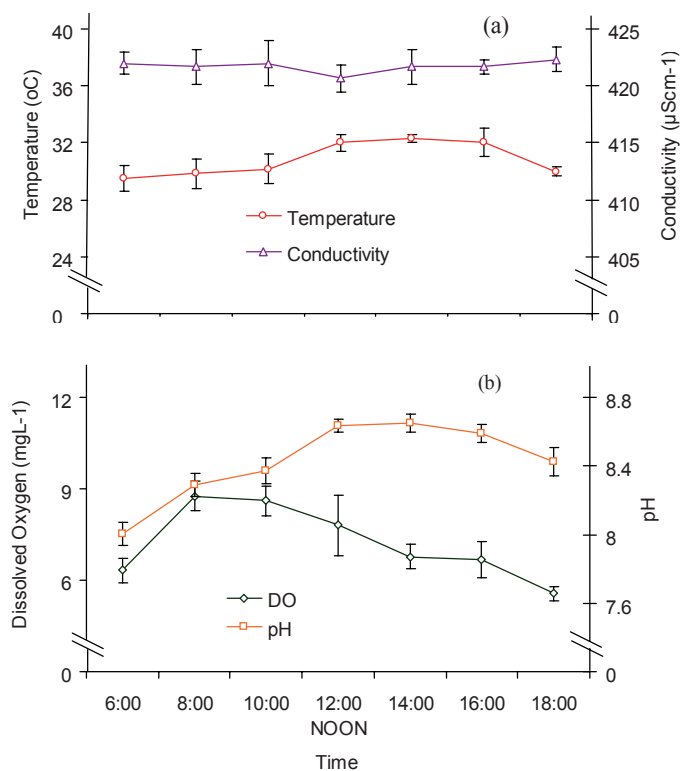


Figure 2. Diurnal variation in (a) temperature and conductivity as well as (b) dissolved oxygen concentration and pH in the Weiya Lake. Point values are means of 5 readings (\pm standard deviation) taken after 0, 1, and 3 days of incubating dialysis tubes in the lake ($n = 3$).

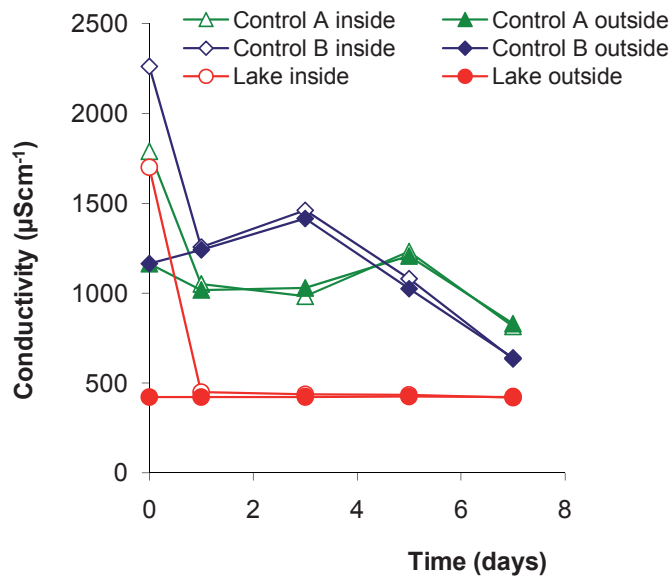


Figure 3. Conductivity ($\mu\text{S cm}^{-1}$) inside and outside dialysis tubes in controls and the lake. Point values represent means of readings of duplicated treatments taken over the duration of the experiment ($n = 2$). Control A represents setup with algae filtered only at start of experiment and Control B represents setup with algae filtered every other day.

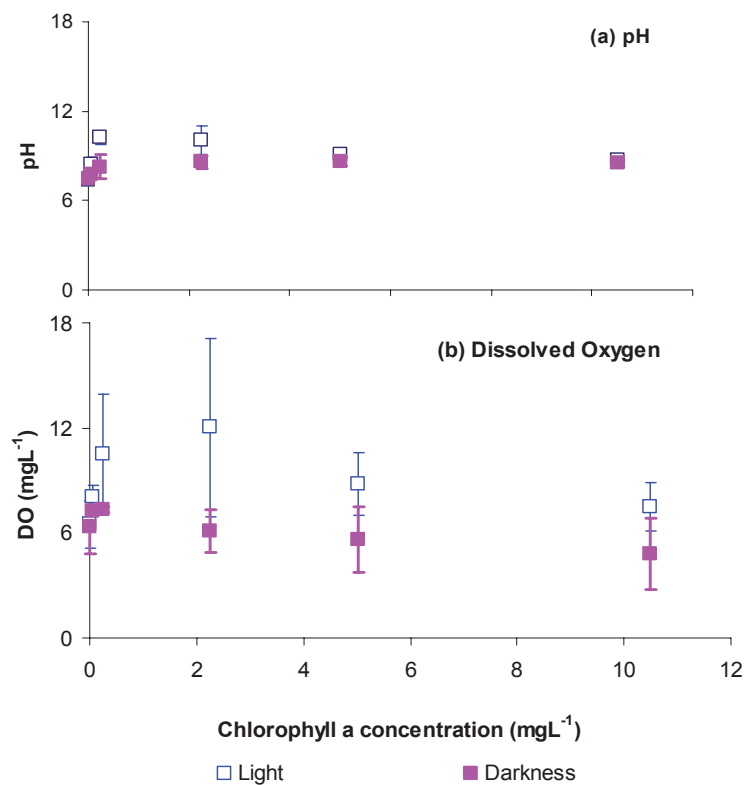


Figure 4. pH (a) and dissolved oxygen (b) variation with chlorophyll a concentration under laboratory conditions. Standard deviation bars represents variation during period of incubation for duplicated treatment (n = 10).

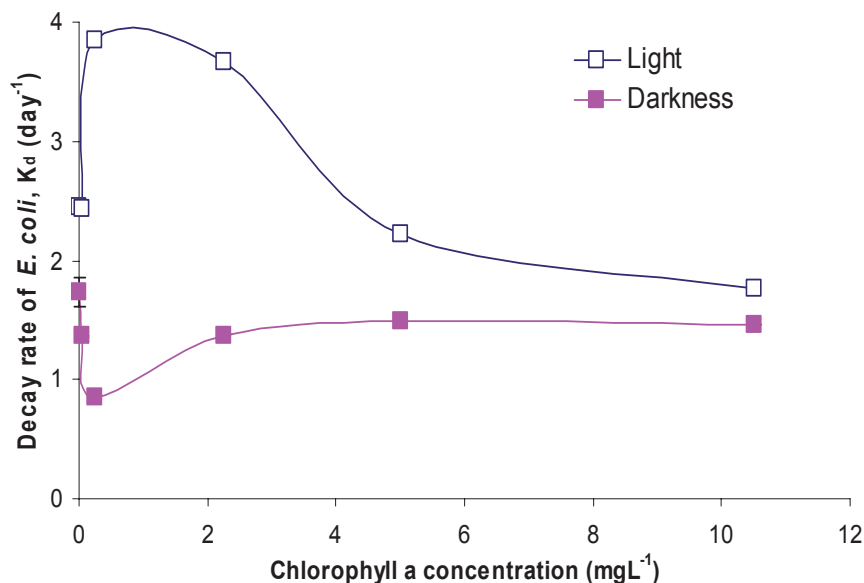


Figure 5. Effect of algae on the decay rate, K_d of *E. coli* in light and darkness under laboratory conditions. Point values represents means of duplicated treatments each having 3 sub-replicates ($n = 6$, standard deviation < 0.1 for all point values).

3.1.2 *E. coli* decay in the absence of algae

At 20.0m deep light penetration was absent and therefore K_d measured at that depth could represent rate of decay of *E. coli* in darkness. Rates of decay of *E. coli* at 20.0m deep (0.63 ± 0.07) did not differ significantly from rate of decay at control B (0.55 ± 0.07) where algae was filtered off every other day.

3.1.3 Effect of algae in light

Rate of *E. coli* decay, K_d in the lake ($1.23 \pm 0.03 \text{ day}^{-1}$) and in control A ($0.85 \pm 0.08 \text{ day}^{-1}$), (where algae was filtered only at the start of the experiment) were both significantly higher ($p < 0.05$) than K_d in control B ($0.55 \pm 0.07 \text{ day}^{-1}$) with no algae (Table 2). Locations 1 and 2 at 0.2m below the lake surface had comparable rates of decay. A positive linear correlation was seen between decay rates and chlorophyll a concentration ($R^2 = 0.67$, $p < 0.01$; $n = 10$) observed in the lake and the two controls, with lower algal densities associated with lower rates of decay. Chlorophyll a concentration in the lake, however, did not exceed 0.08 mgL^{-1} of chlorophyll a throughout the period of the experiment.

3.1.4 Attachment of *E. coli* in lake water

Sampling in the lake showed wide variations in faecal bacteria levels on the different days of sampling and so was the degree of attached bacteria (Table 3). Sampling in week 1, 3 and 5 showed significant bacteria numbers attached ($p < 0.05$) while the rest did not.

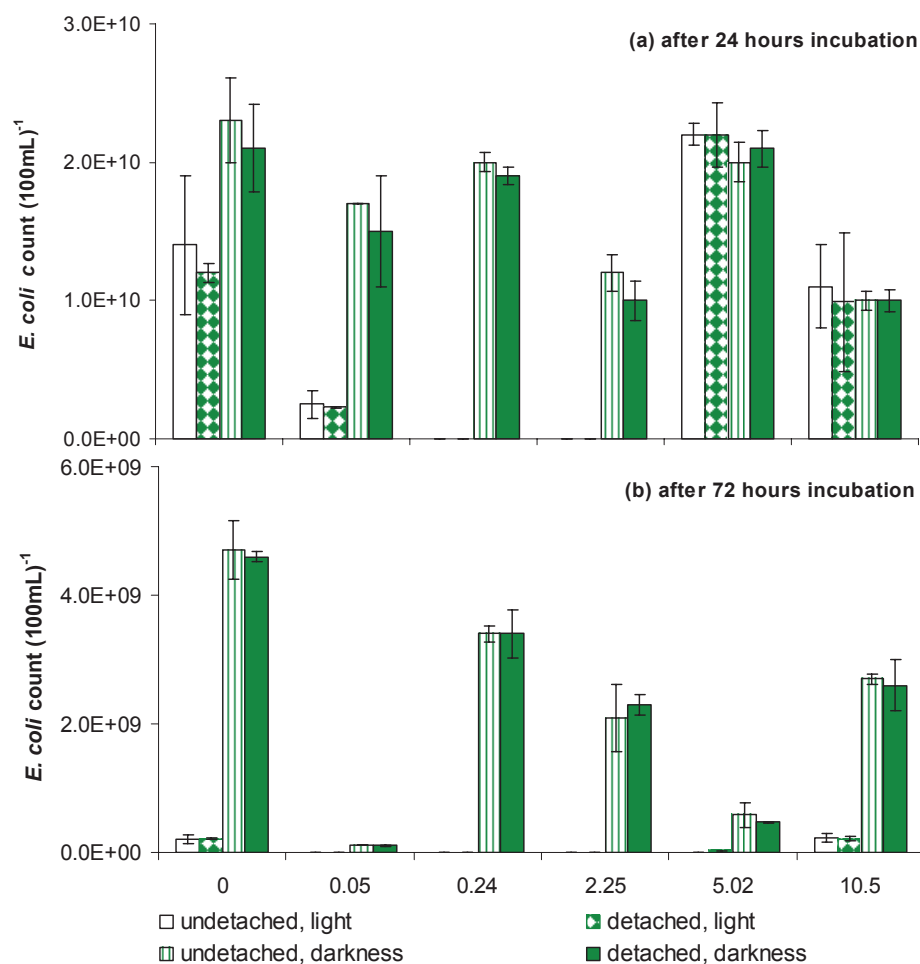


Figure 6. *E. coli* detachment after (a) 24 and (b) 72 hours of incubation. Point values represents means of duplicated treatments each having 3 sub-replicates (\pm standard error, $n = 6$). Missing bars represents *E. coli* counts below detectable limits.

Table 3. Faecal bacteria attachment in the Weija Lake.

Week	^e Wind speed (ms ⁻¹)	<i>E. coli</i> count 100mL ⁻¹		Attached (%)	t-value	P value
		Before detachment	After detachment			
1	2.2	84.9±6.1	251.6±26.1	196.3	6.13	< 0.05
2	1.4	69.1±5.0	64.1±3.9	insignificant	0.74	0.476
3	2.2	933.6±27.4	1170.9±71.4	25.4	3.35	< 0.05
4	1.6	180.8±7.7	209.6±13.4	insignificant	1.64	0.135
5	2.1	272.8±15.0	357.2±24.4	30.9	2.80	< 0.05

^eData obtained from Surface Water Division, CSIR Water Research Institute. Number of samples, n = 30.

3.2 Laboratory experiment

Physico-chemical conditions

pH and dissolved oxygen concentration monitored under laboratory conditions show that pH and DO increased with increased chlorophyll a concentration till 2.25mgL⁻¹, after which it decreased (Figure 4).

3.2.1 Attachment or decay of *E. coli*

Detachment tests carried out on samples taken after 1 day and 3 days of incubation did not show significant increases in *E. coli* numbers after detachment except the 0.05mgL⁻¹ incubation kept in darkness at day 1 ($t=12.1$, $p<0.05$)(Figure 6).

3.2.2 Effect of algae in light under laboratory conditions

Similar observations were made under laboratory conditions, where decay rates increased with increasing chlorophyll a concentration but only up to 0.24mgL⁻¹ chlorophyll a concentration. Beyond this chlorophyll a concentration, decay rates decreased with increasing chlorophyll a concentration. Comparable algal densities had higher rates of decay under laboratory conditions than in the lake. Significantly higher decay rates were observed in algae exposed to light, compared to its corresponding algal densities kept in darkness ($p<0.001$, Figure 5). The extent of the difference (mean separation t), varied as follows: $t=119.1$, 196.8, 130.4, 65.9 and 67.3 respectively for setups with algal densities 0.05, 0.24, 2.25, 5.02 and 10.50mgL⁻¹. The mean difference increased with increasing chlorophyll a concentration till the optimum chlorophyll a concentration of 0.24mgL⁻¹, after which the mean separation begins to decrease. The chlorophyll a concentration with the highest decay rates had the highest survival in darkness.

3.2.3 Effect of algae in darkness under laboratory conditions

The effect of algae on *E. coli* decay rate in darkness was assessed by comparing the control in darkness with all the other algal densities in darkness. Significantly higher decay rates were observed in the dark control compared to all the other algal densities. Survival of *E. coli* increased with increased chlorophyll a concentration till a certain optimum chlorophyll a concentration (0.24mgL⁻¹), after which survival decreased.

4 DISCUSSION

4.1 Decay of *E. coli* in the lake in the absence of algae

There was development of algae over time in control A, where algae were filtered off only at the start of the experiment. This explains why chlorophyll a concentration in this incubation was four times higher than in control B, where algae were filtered off every other day (Table 2). Control with algae filtered off every other day therefore represents oligotrophic conditions with negligible algal productivity as waters with chlorophyll a concentration below 0.013mgL^{-1} are considered oligotrophic. Rates of decay of *E. coli* in control with algae filtered every other day (0.2m below the lake surface) were comparable with rates of decay at 20.0m deep in the lake. At both locations algal presence was negligible with sunlight occurring at the depth of 0.2m but not at 20.0m. Absence of light at 20.0m below the lake surface suggests an absence or limited presence of primary producers which cause elevation of pH and DO concentration. This may explain the much lower decay rates (0.63 d^{-1}) at 20.0m deep. In addition, as lake is polymictic and therefore not thermally stratified, temperatures at 0.2m and 20m deep would not differ much. Light was present in control B and major mechanisms of *E. coli* destruction therefore did not include a direct effect of light. Spectral characteristics of dialysis tubes show that only solar radiation wavelengths $>350\text{nm}$ were transmitted by the dialysis tubes (Bergstein-Ben et al, 1997). At wavelengths $>329\text{nm}$, important mechanisms of faecal bacteria destruction are those that act through photo-sensitizers such as dissolved organic matter and photosynthetic pigments such as algae (Curtis, 1990; Sinton et al, 2002). Low and comparable K_d observed in control with algae filtered every other day and 20.0m deep in the lake could be due to the low concentration of dissolved organic matter and photosynthetic pigments at both locations. The lake BOD usually varies between $2\text{-}10\text{mgL}^{-1}$ (Table 1).

4.2 Effect of algae in light under field and laboratory conditions

4.2.1 Dissolved oxygen and pH effect

Algae affected the decay of *E. coli* through altering of the chemistry of the water. Figure 3 shows that osmotic equilibrium was established by the movement of water into the dialysis tubes as solute concentration inside the dialysis tubes were higher initially. Chemical conditions inside dialysis tubes were therefore comparable to that outside the dialysis tubes. Greater conductivity values observed in controls compared to the lake may be due to the effect of evaporation in a smaller volume of lake water in the control basins and perhaps movement of ions across the dialysis tube membranes into the surrounding water. In control with algae filtered only at the start of the experiment pH and dissolved oxygen concentrations (7.8 ± 0.1 , $4.4\pm0.5\text{mgL}^{-1}$) were comparable to that observed in the lake dialysis tubes (8.1 ± 0.1 , $5.1\pm0.8\text{mgL}^{-1}$). The lake pH and dissolved oxygen concentration however were higher than that of control with algae filtered every other day (7.7 ± 0.3 , $3.6\pm1.3\text{mgL}^{-1}$), Table 2. This may have resulted in the higher rate of decay of *E. coli* in the lake as greater concentration of toxic oxygen radicals may have been produced (Curtis et al, 1992). Algal photosynthetic activity results in pH elevation and increased oxygenation but respiration and organic matter oxidation in the lake may also affect the dissolved oxygen concentration, hence its diurnal variation (Figure 2). Maximum pH

occurring in the lake was 8.7 at 2:00 pm. In the lake, pH therefore did not reach the critical 9.5 level where pH has shown to inactivate faecal bacteria single-handedly (Parhad and Rao, 1974, Awuah, 2006). Long wavelengths such as that transmitted by the dialysis tubes are not able to damage *E. coli* at pH values below 8 (Curtis, 1990) and are highly sensitive to and completely dependent on the oxygen concentration, the rate of damage being proportional to the oxygen radicals' concentration (Curtis et al, 1992). Craggs et al (2004) noted that in the presence of sunlight and oxygen concentration range of 0-22mgL⁻¹, pH range of 8.0-9.2, pH appeared to have little influence on the inactivation of *E. coli*.

Under laboratory conditions, glassware, particularly Pyrex glassware filter off UV light and visible light below 500nm (Thermo Fisher Scientific, 2010). Lights of wavelengths greater than 500nm inactivates *E. coli* mainly through photo-oxidation which is entirely oxygen dependent (Curtis et al, 1992) and possible effects of UVA and UVB lights may either be absent or minimal. Oxygen concentration increased with increased chlorophyll a concentration till a certain optimum (2.3mgL⁻¹) after which oxygen concentration decreased with increased chlorophyll a concentration (Figure 4). Decreased oxygen concentration may be due to oxidation of dissolved organic matter released by lysis of algal cells that occurs at higher algal concentrations (Wetzel, 2001). A similar trend as dissolved oxygen concentration was shown by pH changes though not as pronounced.

4.2.2 Chlorophyll a concentration of algae

Chlorophyll a concentration of the lake was directly proportional to the rate of decay of *E. coli* in the lake, suggesting that algal presence in eutrophic lakes can be an important means of *E. coli* removal. Chlorophyll a concentration however did not exceed 0.08mgL⁻¹. Increased chlorophyll a or algal concentration leads to increased oxygen and pH elevation leading to increased rate of decay of *E. coli*. Expectedly locations 1 and 2 had comparable decay rates as their chlorophyll a concentration of 0.0471±0.01 and 0.0481±0.01mgL⁻¹ respectively were comparable (Table 2). Laboratory experiments showed that very high algal densities depicted by high chlorophyll a concentrations may be a limiting factor in the removal of *E. coli*. Beyond chlorophyll a concentration of 0.24mgL⁻¹ decay rate of *E. coli* decreases (Figure 5). Increased chlorophyll a concentration results in increased disintegration of algal cells releasing dissolved organic matter from its cytoplasm (Bouteleux et al, 2005). Oxidation of this organic matter may lower oxygen concentration (Figure 4). Long wavelengths of light inactivate *E. coli* by creating toxic forms of oxygen molecules which are injurious to bacteria cells (Curtis et al, 1992). Increased chlorophyll a concentration also increasingly filters off the effect of short wavelengths of light that can directly damage *E. coli* (Van der Steen et al, 2000a).

Most eutrophic lakes do not have algal densities exceeding 0.3mgL⁻¹ and this makes eutrophic lakes particularly important as capable systems of self purification. The Weija Lake is a reservoir for supplying drinking water to the Western part of Accra and 20m from the banks of this lake are settlement communities who depend on the raw water of the lake for domestic as well as recreational activities. Natural disinfection of this water body may therefore have significant public health benefits to these communities. Highest *E. coli* numbers observed in this lake was 1200cfu100mL⁻¹ (Table 3).

4.2.3 Effect of algae in darkness under laboratory conditions

Survival of *E. coli* in darkness increased with increased chlorophyll a concentration till a certain optimum (0.24mgL^{-1}), after which survival decreased (Figure 5). As pH and dissolved oxygen values were comparable in all incubations in darkness, and differences in decay rates cannot be attributed to natural decay, inactivation of *E. coli* in darkness may be due to another factor. Klein and Alexander (1986) attributed the inactivation of bacteria in freshwater to the presence of inhibitory substances. Others have also suggested the production of algal toxins that are detrimental to the survival of *E. coli* in darkness (Maynard et al, 1999). Our work supports this later assertion as the variation in decay rate in darkness could be explained by a counteracting effect of both increasing algal organic matter and algal toxin. It is possible that enough quantities of algal toxins capable of inactivating *E. coli* are probably released beyond certain concentrations of algae. Further investigations are needed to ascertain this. *Chlorella sp.*, a common alga in wastewater treatment systems was reported to produce substances toxic to *Vibrio cholerae* (Maynard et al, 1999). Our experiment made use of algae grown by natural colonization, having *Chlorella* as the dominant algal species and therefore may have produced some toxic substances. Not much however, is known about the kind of toxin produced by these algae (Maynard et al, 1999).

4.2.4 Attachment of *E. coli* in lake water

The importance of *E. coli* attachment to algae and suspended particles in the Weija Lake as a possible mechanism of bacteria pathogen removal in the lake was assessed with the assumption that *E. coli* may be attached to each other, algae and suspended particles at the water contact sites of the lake where human activity is high. Electron microscopy of a colonized algae-bacteria mixture had shown that a slime matrix engulfs both the bacteria and the algae during attachment (Holmes, 1986), forming fast sinking aggregates (Grossart and Simon, 1998) which may eventually get sedimented faster to the lake bottom. Sampling in the lake showed wide variations in *E. coli* numbers on the different times of sampling and so was the degree of attached *E. coli* (Table 3). Variations in *E. coli* numbers could be attributed to inflow of water from upstream and also to defaecation along the banks by settlers or free range cattle or both. Sampling on week 1, 3 and 5 showed significant *E. coli* attached ($p < 0.05$) while week 2 and 4 did not. This suggests that bacteria pathogen attachment to algae and suspended solids could be an important mechanism of pathogen removal in eutrophic lakes as attached *E. coli* could eventually be removed from the water column through sedimentation. Wind speed and direction can affect *E. coli* distribution in a lake (Whitman et al, 2004) by re-suspending the *E. coli* in the lake (Table 3). Increased suspended matter could thus constitute an increased surface area for *E. coli* attachment.

4.2.5 Attachment of *E. coli* in laboratory incubations

The purpose of performing detachment tests on day 1 and 3 laboratory incubations was to ensure that *E. coli* attached to each other and to suspended particles and algae is detached in order to avoid underestimation of *E. coli* numbers. Decreased numbers of *E. coli* resulting from *E. coli* attachment could be wrongly interpreted as decay. Bacteria cells sometimes attach to each other, appearing as a single colony on agar plates, a phenomenon that can lead to severe underestimation of the actual bacteria numbers

present. In washing *E. coli* suspension in normal saline, the process of centrifugation is employed and this can lead to attachment of *E. coli* to each other. These attached *E. coli* could subsequently detach and the increased *E. coli* number could be erroneously interpreted as growth (Dewedar and Bahgat, 1995; George et al, 2002). The use of the syringe and needle method can completely recover all attached bacteria by the application of shear stress on the attached bacteria, thus dislodging it (Ansa et al, 2009). This method is however limited by the subjective nature of the force applied on the syringe which could introduce a wide variation in performance. Attachment of bacteria to objects may occur within 24 hours (Awuah, 2006) to 3 days (Leff and Leff, 2000). Detachment tests done on samples taken after 1 day and 3 days of incubation did not show significant increases in *E. coli* numbers after detachment except the 0.05 mg L⁻¹ incubation kept in darkness at day 1. This suggests that the *E. coli* counts were not underestimated as a result of attachment to each other and to algal cells (Figure 5) and that decreased *E. coli* numbers is as a result of decay.

5 CONCLUSIONS

- Algae significantly reduced *E. coli* contamination in the eutrophic lake through increased oxygenation and pH elevation.
- At chlorophyll a concentration $\leq 0.08 \text{ mg L}^{-1}$ in the Weiya Lake, decay rate of *E. coli* is directly proportional to chlorophyll a concentration of the lake. Under laboratory conditions, as chlorophyll a concentration increases in light, an optimum chlorophyll a concentration of 0.24 mg/L is reached after which rate of decay of *E. coli* decreases.
- *E. coli* decay in darkness was affected by chlorophyll a concentration. Further investigations are necessary to ascertain whether other factors such as algal toxins are controlling the decay rates of *E. coli* through algal density.
- Limited algal development representing optimum chlorophyll a concentration for maximum *E. coli* decay can be encouraged in restored ecosystems or wetlands in order to achieve significant reductions in *E. coli* numbers. This may have huge public health benefits for communities in developing countries in particular who use raw untreated water from lakes and other freshwater sources. As this study was done with indicator bacteria (*E. coli*), any parallel conclusions for pathogens need to be verified by further experiments.

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7

Chapter

Conclusions and outlook

1 PROBLEM AND OBJECTIVE STATEMENTS

1.1 Problem statement

Attachment of FC to algal matter and suspended solids in microcosms

The attachment of faecal coliforms (FC) to algal cells and suspended particles could be important in the removal of faecal coliforms in natural wastewater treatment systems (NWTs). Suspended particles and particulate plant matter could serve as surfaces of attachment for FC resulting in their sedimentation. If this process is important, altering NWTs to stimulate attachment of FC to suspended particles and algal cells could enhance the removal of FC in NWTs. The ability of algal cells to serve as surfaces for sedimenting FC however is not known. The quantification of attached FC also presents many challenges due to the limitations of currently available methods for detaching FC. It is therefore necessary to develop or adapt various methods for detaching FC in order to be able to assess accurately, the quantity of FC attaching. This would enable the assessment of the importance of FC attachment in wastewater treatment pond systems.

Algal biomass effect on FC die-off in wastewater

Over the past years, the role of algae in inactivating FC through fluctuating and elevated pH and dissolved oxygen concentration and algal promotion of FC survival via attenuation of solar radiation had been documented. It is however not known how algal biomass variation affects FC inactivation and how this effect varies in wastewater of varying strengths, given the role of dissolved organic matter in FC inactivation. Understanding better the role of algae in FC inactivation, particularly in relation to algal biomass variation and sedimentation of FC could lead to the development of better models for predicting FC removal and better designs, operation and maintenance of waste stabilization ponds (WSPs) for a more efficient removal of pathogens.

Performance of a hybrid algal-duckweed pond system

Algal ponds appear to be more efficient in the removal of pathogens than duckweed ponds, perhaps due to the elevated pH and dissolved oxygen concentration created in algal ponds during day time. Duckweed ponds on the other hand perform better in BOD

removal than algal ponds. The development of high algal biomass in algal treatment ponds leads to solar radiation attenuation, weakening the disinfection effect of solar radiation in the deeper part of the ponds and thereby resulting in lower removal of faecal coliforms. It has been shown that inserting duckweed ponds in between a series of algal ponds could reduce the rate of algal growth and hence promotes better solar radiation penetration in the algal ponds, creating conditions for improved faecal coliform decay. The performance of such a hybrid pond treatment systems relative to full algal and full duckweed pond systems had not been investigated under sub-Saharan African tropical conditions. It is also not known whether such a hybrid treatment pond system would be able to meet guidelines for discharge into freshwater bodies used by rural communities in its raw form without any form of treatment.

Attachment in wastewater treatment pond systems

One of the expected benefits of a hybrid algal and duckweed wastewater treatment pond system is the reduction of algal cell concentration before final effluent discharge. Reduced algal cell concentrations in algal ponds receiving effluent of a duckweed pond could however result in decreased importance of FC attachment to algal cells in hybrid pond systems due to reduction in the available surface area. The importance of this phenomenon however is not known.

Solar radiation penetration in duckweed ponds is lower than in algal ponds. The intensity of light penetration in algal ponds of hybrid pond systems may also vary with depth. Light intensity may be an important factor in the inactivation of FC and in their removal. As a result, FC inactivation or removal may vary with pond depth, with perhaps less inactivation rates and subsequently higher FC numbers in the deeper parts of the pond. As attachment could be enhanced with increased FC numbers, the importance of attachment may vary with depth in such a hybrid pond system of algal and duckweed ponds. The rapid decay and subsequent sedimentation of algal cells in duckweed ponds as a result of poor penetration of solar radiation could also accelerate the sedimentation of FC attached to dead or dying algal cells. Sedimented faecal coliforms however could be re-suspended into the water column by macro-invertebrates and attached FC could be detached in the process. If this phenomenon occurs in a final effluent pond it could result in the deterioration of the effluent water quality. The importance of macro-invertebrates in faecal coliform removal in such ponds systems however is not known. Attached FC cells in final effluent can result in the underestimation of FC count as several FC cells attached to one particle could be counted as one colony. This can lead to failure to detect under performance of a treatment system, something that can have serious implications for public health.

Algal biomass effect in a eutrophic lake

It is not known whether limited algal development as occurs in eutrophic lakes could result in the reduction of FC numbers and the extent to which such reduction would occur, given the documented role of algae in FC inactivation in wastewater treatment pond systems. The importance of FC attachment to suspended matter in eutrophic lakes is also not documented in literature. Significant disinfection effect of algae in eutrophic

lakes could result in public health benefits for rural communities that use freshwater bodies in its raw form.

1.2 Objective statement

The overall objective of this thesis was to investigate the role of algae and other suspended matter in the removal of faecal coliforms in duckweed ponds, algal ponds and eutrophic lakes. A better understanding of the role of algae and suspended matter would assist in optimizing the removal of faecal coliforms from these treatment systems. In this regard, this thesis investigated:

- The importance of algae and suspended solids as surfaces for faecal coliform attachment in algal-based freshwater and wastewater microcosms and the suitability of various methods of detaching faecal coliforms.
- The effect of varying algal biomass on FC die-off in domestic wastewater of weak and medium strengths.
- The performance of a hybrid algal-duckweed pond system in producing an effluent with low suspended algal matter, BOD, nutrient and faecal coliform (suitable for discharge into water bodies used in its raw form by villagers), relative to that produced by full algal and full duckweed pond systems.
- FC attachment in a hybrid algal-duckweed pond system treating raw domestic wastewater and the possible effect of macro-invertebrates on the removal of FC in duckweed and algal ponds.
- The importance of algae in the removal of *Escherichia coli* from a tropical eutrophic lake and to specifically underscore the effect of varying algal concentrations and attachment to algae and suspended particles on *E. coli* removal in such as system.

2 CONCLUSIONS

2.1 Attachment of FC to algal matter and suspended solids in microcosms

Algae assisted FC to settle to the bottom of reactors faster and this occurred within 24 hours. The mechanism by which this occurred however did not involve a process of permanent attachment to algal surfaces. To be able to assess the importance of attachment of FC to suspended solids and algae, it was necessary to devise a method capable of assessing quantities of FC attached and detached. It was also necessary to devise a means of preventing attachment from taking place in some incubation for the purpose of comparison with incubations where attachment was taking place. Existing conventional methods capable of detaching bacteria such as the use of parozone, sonicators and vortex mixers were not developed with the above objective in mind. It was therefore necessary to adapt these methods by finding the optimum energy and time capable of detaching FC without killing them. An improvised method involving the use of a syringe fitted with a needle was effective in detaching attached FC and this was used in subsequent

experiments in chapter 5. The use of 0.11gL^{-1} parozone was also effective in preventing attachment of FC to each other or to suspended matter.

2.2 Algal biomass effect on FC die-off in wastewater

Under laboratory conditions in domestic wastewater decay rates of faecal coliforms increased with increased algal density (measured by the chlorophyll a concentration) till a certain algal density after which decay rates decreased with increased algal density. This supported the hypothesis that a certain algal density exists at which maximum decay rate of faecal coliform is achieved. Increased algal density is accompanied by increased pH and dissolved oxygen concentration which leads to increased inactivation of FC. At high algal densities however, light attenuation occurs leading to lower inactivation rates of FC. At high algal densities also, increased lyses of algal cells occur leading to greater release of algal organic matter, which may promote FC survival by providing the carbon and energy source. The optimum algal density for FC removal was 1.2mgL^{-1} chlorophyll a concentration. It was hypothesized that this optimum algal density would be affected by the wastewater strength and observations from trials with different wastewater strength supported this hypothesis. The highest rate of decay in low strength wastewater (LSW) occurred at 3.2mgL^{-1} chlorophyll a concentration in light while that of the medium strength wastewater (MSW) occurred at 20.0mgL^{-1} chlorophyll a concentration in light. This suggests that the dissolved organic matter concentration of wastewater as well as the algal density of the pond may play a significant role in the inactivation of FC in pond systems. Higher removal rates of FC however occurred in MSW compared with LSW at higher algal densities ($\geq 13.9\text{mgL}^{-1}$) whether in light or in darkness. It was also observed that addition of fresh raw wastewater to an ongoing wastewater treatment process may lower the rate of FC inactivation for a wide range of algal densities ($0.6 - 19.6\text{mgL}^{-1}$), under light conditions. In darkness however, addition of raw wastewater may lead to increased rate of FC inactivation for algal densities $\leq 1.72\text{mgL}^{-1}$. Raw wastewater addition also lowered the elevated pH and dissolved oxygen concentrations in the light incubations. In darkness, higher algal biomass (expressed as chlorophyll a concentration) resulted in higher inactivation of FC although pH and dissolved oxygen concentrations were low suggesting a role by another factor in the inactivation of FC.

2.3 Performance of a hybrid algal-duckweed pond system

This thesis also reported findings on the general performance of a pilot-scale hybrid duckweed and algal pond system under tropical African climatic conditions and the role of macro-invertebrate activity in the removal of FC from this pond system. The hybrid pond system performed well in FC removal having similar effluent FC concentration as an algal pond system. Removal of BOD, total phosphates and FC were not affected by seasonal changes in the hybrid pond system as well as the algal and duckweed pond system operated alongside the hybrid pond system. Unfiltered hybrid pond system effluent met the EPA, Ghana BOD guideline of 50mgL^{-1} in both the wet and dry seasons and removed BOD better than the algal pond system. Ammonia removal efficiency was affected by seasonal changes in the duckweed pond system.

2.4 Attachment in wastewater treatment pond systems

Attachment of FC to suspended matter was important only in the first two ponds of the duckweed, algal and hybrid pond systems and usually was more pronounced at the bottom. In the algal ponds, rates of decay of FC were lower in the morning than in the afternoon in contrast with duckweed ponds which did not show any differences in rates of decay in the morning and afternoon. This seems to be an effect of light intensity. Little variation of FC decay with depth was observed in all the three pond system types. High densities of ostracods were associated with the surface and bottom of duckweed ponds and these were much higher than in algal ponds at similar locations. FC numbers in duckweed ponds correlated strongly and positively with mean ostracod numbers in ponds. FC numbers also correlated well with Shannon-Wiener diversity index of macro-invertebrates in all the three pond system types.

2.5 Algal biomass effect in a eutrophic lake

Under freshwater laboratory conditions, decay rates of *Escherichia coli* increased with increased algal density (measured by the chlorophyll a concentration) till a certain algal density (optimum algal density) after which decay rates decreased with further increase of algal density, as was also observed in domestic wastewater. The optimum algal density in the freshwater microcosm was 0.24mgL^{-1} chlorophyll a concentration. In an experiment conducted in a tropical eutrophic lake, algae were important in significantly reducing *Escherichia coli* contamination through probably increased oxygenation and pH elevation. At algal densities less than 0.08mgL^{-1} chlorophyll a concentration in the Weija Lake, decay rate of *E. coli* was directly proportional to the chlorophyll a concentration of the lake.

3 THE OUTLOOK

3.1 Attachment of faecal coliform to algae and suspended solids

For studies on FC attachment, having a simple, fast but effective method of detaching attached FC is still a challenge. The best method detaching bacteria, the syringe-needle method had a limitation. The limitation stems from the variability in the force applied to detach the bacteria. This applied force is variable from person to person and even for the same person. This limitation could be overcome by the development of an automated syringe that applies a standard (adjustable) force.

Variation of attachment of FC to suspended solids or algae with depth should also be studied further using pond systems that are 1-1.5m deep. Increased depth of pond systems could reveal any stratification that may exist in algal ponds. This is because other workers have shown that sunlight inactivation of FC decreases with depth in algal ponds and it is therefore expected that attachment of FC to suspended solids might increase with depth. This is because attachment is likely to be enhanced by increased FC concentration, which can be expected at deeper layers of the pond if light and oxygen is lower. The absence of

a clear pattern of attachment observed in this work may be due to the shallow nature (0.3m) of the ponds used, and additional experiments at deeper pond depth should be developed.

Observations under a compound microscope suggested algal entrapment of bacteria cells but it would be necessary to investigate the nature of this algae-bacteria interaction (particularly in the presence and absence of 0.11gL^{-1} parozone) further using an electron microscope. Other studies have suggested that the availability of large surface areas in algal ponds may play a significant role in FC removal. While this work agrees with this assertion in respect of suspended solids, this study however shows that algal surfaces may not be the factor promoting the removal of FC through attachment and sedimentation but rather, the formation of exopolysaccharide slime by algae which entraps the FC cells. It may therefore be possible to optimize algal sedimentation of FC in waste stabilization ponds by promoting the growth of slime producing green algae in the pond system. This can be achieved through the regulation of the BOD loading rates so as to obtain a particular range of nitrogen to phosphorus ratio.

3.2 Algal biomass effect on FC die-off in wastewater

The effect of algal density on FC removal in conditions of varying wastewater strength had been reported and additional observations were made which was not part of the research objectives. Inactivation of FC in darkness and its variation in darkness with variation in algal density pointed to an effect of algae in darkness that can be attributed to substance(s) from algae which inhibit(s) or are toxic to FC and which increase(s) with increased algal density. It would be interesting to investigate if these substances or inhibitors exist, especially as the role of algal toxins in the inactivation of FC is still a subject of debate. Questions as to whether this substance has any interactive effect with pH or organic matter would also have to be looked at. Rapid detection methods developed for detecting the presence of toxins produced by cyanobacteria could be modified and used for possible green-algae toxins occurring in waste stabilization ponds and eutrophic lakes. This work contradicts assertions by other workers that high algal density should lead to high pH and dissolved oxygen (DO) concentrations. This is because although pH and DO increases occur with increased algal density, beyond a certain critical algal density (which depends also on the wastewater strength), pH and DO decreased due perhaps to the release of algal organic matter via excretion and algal cell lyses. The decrease in pH and DO could also be due to the inability of some algal cells to photosynthesize as they get shielded from solar radiation, thus confining the photosynthetic effect to top few centimetres. It seems from the foregoing that wastewater strength interaction with algal organic matter release can play a key role in faecal coliform inactivation. It is however too early to suggest major changes in the design or operation of waste stabilization ponds. This is because conclusions were drawn from experiments conducted under laboratory conditions and further investigations to be conducted under field conditions and on a pilot scale are necessary.

3.3 Faecal coliform removal in a hybrid duckweed and algal pond system

The pilot-scale hybrid algal and duckweed pond studied performed well in FC and BOD removal and showed good promise for warm tropical conditions although its FC removal of 4.3 was less than the 6.4 log removal reported by Von Sperling and Mascarenhas (2005) under tropical Brazilian climatic conditions. Recent trends in ecotechnologies for domestic wastewater treatment advocate a combined system of waste stabilization ponds with constructed wetlands (CW), the effluent of the WSP serving as the influent of the CW. Further research involving the use of this hybrid pond system in combination with a CW may be useful in order to address health and socio-cultural concerns bordering on the reuse of domestic wastewater. Addition of a constructed wetland could be useful in adding an aesthetic value to the treatment system and can help dispel the stigmatisation of the water source, not to mention the additional benefits accruing from further polishing or removal of pathogens, and possible production of wetland biomass for reuse. Integrating this pond system with aquaculture technology could further help generate economic incentives for communities in developing countries with warm tropical conditions.

3.4 Algal biomass effect in a eutrophic lake

The effect of algae on *E. coli* die-off in eutrophic lakes has implications for disinfection of water bodies and restored aquatic ecosystems, particularly in communities that use raw water in developing countries without any form of purification. Limited algal development in restored ecosystems may assist in pathogen removal. However, as this study was conducted with indicator bacteria, any parallel conclusions for actual pathogens should be made with caution. Further investigations that monitor the behaviour of real pathogens would be useful.

SAMENVATTING (SUMMARY IN DUTCH)

Voordat huishoudelijk afvalwater hergebruikt kan gaan worden, moeten er vele obstakels overwonnen worden, waarbij het risico van het verspreiden van infectieziekten een van de belangrijkste is. Dus moeten pathogene micro-organismen verwijderd worden uit het afvalwater. Van algen is bekend dat zij een grote rol kunnen spelen in dit verwijderingsproces, omdat ze de pH verhogen en zuurstof in het water brengen. Onbekend is echter nog of verandering in de algenconcentraties in afvalwater de verwijdering van pathogenen beïnvloedt; ook is niet bekend of en hoe algen een bijdrage leveren aan het sedimentatieproces van pathogene micro-organismen. Er werden experimenten uitgevoerd om te onderzoeken of faecale coliformen (FC) hechten aan algen, wat het effect is van veranderende algenconcentraties in afvalwater op de verwijdering van FC en om het effect van algen op de FC verwijdering in een tropisch, voedselrijk meer te testen. Verschillende algenconcentraties werden ook getest in een hybride reactor (algen en eendenkroos) om de FC hechting te kunnen vergelijken met systemen met alleen algen of alleen eendenkroos. Algen droegen bij aan het laten bezinken van FC, waarschijnlijk niet zozeer door hechting aan algen, maar doordat de gelatineuze buitenlaag van algen en FC verstrengeld raken en ze daardoor zwaardere aggregaten vormen. Onder laboratoriumcondities bleek dat er in huishoudelijk afvalwater een optimum algenconcentratie is om FC te verwijderen. Algen droegen in hoge mate bij aan de afname van *Escherichia coli* in een voedselrijk meer door de verhoging van de zuurstof en van de pH. In het Weija meer was beneden een algendichtheid van 0.08mg/L de afsterving van *Escherichia coli* direct gecorreleerd met de chlorophyll a concentratie. De sterkte van het afvalwater kan ook de afstervingssnelheid bepalen van FC, speciaal bij variërende algenconcentraties. De snelste afsterving in laag-geconcentreerd afvalwater (LSW) vond plaats bij 3.2mg/L chlorophyll a in het licht, terwijl in "medium-strength" afvalwater (MSW) dit optimum bij 20mg/L chlorophyll a lag. Ook in het donker resulteerde een hogere algenconcentratie in een snellere afsterving, hetgeen suggereert dat er ook andere factoren dan pH en zuurstof betrokken zijn bij de verwijdering van FC. Toevoeging van vers afvalwater aan een draaiende afvalwaterzuivering verminderde de snelheid waarmee FC verwijderd werden; dat gold voor uiteenlopende algendichtheden (0.6 – 19.6mg/L chlorophyll a) in het licht. Het hybride systeem werkte goed in de verwijdering van FC (4.3 log) en BOD (89%), onafhankelijk van het seizoen. Alleen in de eerste twee vijvers van alle drie systemen (algen, eendenkroos en hybride) speelde hechting aan gesuspenderde deeltjes een belangrijke rol. In algenvijvers was de verwijdering van FC 's ochtends trager, maar in eendenkroos maakte de tijd van de dag niet uit. Zowel aan het oppervlak als op de bodem van eendenkroosvijvers kwamen veel hogere concentraties macro-invertebraten (klasse Ostracoden) voor dan in algenvijvers. Het aantal FC in eendenkroosvijvers was sterk positief gecorreleerd met deze aantallen ostracoden. In alle drie vijversystemen correleerde het aantal FC goed met de Shannon-Wiener diversiteitsindex voor macro-invertebraten. Voor lokale gemeenschappen in ontwikkelingslanden met een tropisch klimaat zou het hybride systeem uitstekend toegepast kunnen worden in de aquacultuur, hetgeen zowel economisch als gezondheidstechnisch positieve gevolgen zou hebben.

AUTHOR'S RESUME



Ebenezer D.O. Ansa graduated with a BSc (Honours) degree from the University of Ghana in Zoology with Botany in 1994. After working with the Department of Zoology, University of Ghana as a Teaching Assistant for two years, he enrolled in the MPhil, Applied Parasitology programme of the University of Ghana and completed in December 1999. His MPhil research focused on the health impact assessment of the damming of the Volta River at Akosombo, thirty years after impoundment.

He worked with the African Environmental Research and Consulting Company (AERC) where he developed an interest in wastewater treatment. At AERC the author was involved in conducting water audits and development of Environmental Management Plans for companies after which he joined the CSIR Water Research Institute, Ghana as a Research Scientist and has been working there since. He has been involved in the development of the Ghana Raw Water Quality Criteria and Guidelines for various water uses and in Environmental Impact Assessments on some streams and rivers of Ghana before enrolling at UNESCO-IHE Institute for Water Education, The Netherlands as a PhD Research Fellow.

Ebenezer Ansa is a recipient of several awards, a member of the Universities Council on Water Resources (UCOWR), Society for Freshwater Science (SFS) and the International Water Association (IWA). His diverse background in Parasitology, Hydrobiology and recently in Environmental Biotechnology has enabled him to be author and co-author of publications on water-related diseases control, environmental impact assessments and pathogen removal mechanisms in eco-technologies in refereed journals.

Ebenezer's research interests include eco-technologies for pollution control and bio-indicators for pollution monitoring.

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The reuse of domestic wastewater presents many challenges, including the risk of pathogen infection and algae is known to play a crucial role in the process of bacteria removal by raising the pH and dissolved oxygen concentration. However the role of algae is still not fully understood. The importance of faecal coliform (FC) attachment to algae in a hybrid pond system of algae and duckweed treating raw domestic wastewater, the effect of varying algal concentration on FC removal in wastewater and a eutrophic lake were investigated.

Algae helped in sedimenting FC to the bottom of reactors. It was shown by experimentation that an optimum algal density exists at which maximum FC removal is achieved. FC numbers in duckweed ponds correlated strongly with ostracod numbers in ponds. FC attachment to suspended matter was important only in the first two ponds of the hybrid pond system and system performed well in FC (4.3 log units) and BOD (89%) removal, making it ideal for integration with aquaculture in developing countries.

Algae achieved significant reduction in *Escherichia coli* contamination in the eutrophic lake. At chlorophyll a concentrations of less than 0.08mgL^{-1} , decay rate of *E. coli* was directly proportional to the chlorophyll a concentration of the lake..

