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A Review of the Mechanisms of Faecal Coliform Removal from Algal and Duckweed Waste Stabilization Pond Systems

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Abstract: The use of eco-technologies for wastewater treatment such as algal and duckweed-based pond systems is becoming popular in developing countries owing to its affordability and efficiency of pathogen removal in warm climates. The pathogen removal mechanisms of these treatment systems however is still not clearly understood and existing knowledge is also scattered in journals and books of different disciplines. The purpose of this paper is to provide a concise review of knowledge acquired in recent times on faecal coliform removal mechanisms in algal and duckweed ponds in a comparative way while identifying knowledge gaps that still exist. This review pays particular attention to little known removal mechanisms such as the role of algal biomass, attachment and sedimentation of faecal coliforms and the role of predation by macro-invertebrates and protozoans. Recent experiments showed that algal ponds, in comparison with duckweed ponds, are more efficient in faecal coliform removal due to the high pH and oxygenation that occur in the former and the rate of inactivation of faecal coliforms increases with increased algal biomass till a certain optimum concentration after which it decreases. This optimal algal concentration for maximum destruction of faecal coliforms can be affected by the quality and strength of the wastewater. Algae also appeared to have a destructive effect on faecal coliforms even in darkness, a phenomenon that may be the effect of toxic substances from the algae. Results also show that the role of invertebrates, particularly macro-invertebrates may be more important in duckweed pond systems. Removal of faecal coliforms through attachment and sedimentation in both duckweed and algal ponds appear to be dependent largely on concentrations of faecal coliforms present and to some extent on suspended plant and particulate matter concentrations. Wide variations in removal efficiencies were however observed. We conclude that the wide variations in removal efficiencies can be addressed by standardizing operating conditions of treatment systems. Further work is necessary to identify the substances produced by algae which appeared to be toxic to faecal coliforms as well as establishing the relative importance of predation by protozoans and macro-invertebrates in the removal of faecal coliforms.

Keywords: Macro-Invertebrates, Pathogens, Performance, Treatment, Wastewater

Introduction

Waste Stabilization Pond systems (WSPs) may consist of anaerobic, facultative and maturation ponds. The primary objective of maturation ponds in waste stabilization pond systems is the removal of pathogens as indicated by faecal coliform or *Escherichia coli* counts. Faecal coliforms (also known as thermo tolerant coliforms) are special members of the total coliform group of bacteria which originate from the intestinal tract of warm blooded mammals. Total coliforms have characteristic rod-shaped, gram negative and able to ferment lactose with the release of carbon dioxide in 24 h at a temperature of 35-37°C but unlike the rest of their counterpart coliforms, faecal coliforms can also ferment lactose with the release of carbon dioxide in 24-48 h at a temperature of 44.5-48°C. 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, *Escherichia coli* being more representative (Tallon *et al*., 2005). The WHO (2006) guidelines for reuse of wastewater also adopted the use of *E. coli* counts 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).

Maturation ponds of WSPs may consist of algae, duckweeds or others which affect the survival of pathogens in the pond systems. Algal WSPs are more common but the use of duckweed pond systems is increasingly becoming popular due to its ability to curtail mosquito breeding, reduce algal suspended solids in effluents and increase BOD removal efficiency as well as its potential to serve as feed for chicken and pigs (Ansa *et al*., 2012a; Awuah *et al*., 2004; El-Shafai *et al*., 2007; Mo and Zhang, 2013). In spite of the numerous literature available on the performance of various types of WSPs and reviews on mechanisms of faecal coliform removal from algal maturation ponds (Maynard *et al*., 1999; Davies-Colley *et al*., 2000), the mechanisms of removal of faecal coliforms from maturation ponds, particularly duckweed maturation ponds is still not fully understood. The purpose of this review is to present an update of knowledge acquired so far and to identify gaps and areas that need further research. It also seeks to elucidate the similarities and differences in the performance and mechanisms of removal of faecal coliforms in algal and duckweed ponds systems.

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). Table 1 shows the variation in the treatment performance of the various types of pond systems. The algal pond system removal varying from 1-5 log units, duckweed 1-4 log units, while combined systems of algal and duckweed varied from 2-6 log units. This high performance of combined systems, in addition to other benefits such as higher BOD removal (Ansa *et al*., 2012a) may explain its current popularity. The examples in Table 1 were chosen because most of the system types received influent of similar concentration and operated under similar conditions of temperature, depth and total hydraulic retention times. Some of the factors that may account for these differences in performance are discussed below.

Removal Mechanisms

The reviews of Maynard *et al*. (1999; 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 the two maturation pond types.

Temperature

Temperature is one of the drivers for pond mixing (Brissaud *et al*., 2002) and may 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 (Table 1). Some authors have attributed this to better solar irradiation in summer than in winter as increased pH and dissolved oxygen concentration were reported (El-Shafai *et al*., 2007; Zimmo *et al*., 2002). 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 (Maiga *et al*., 2009). This may also explain the higher inactivation of faecal coliforms in duckweed ponds in summer compared to winter (Table 1).

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 as availability of carbon and energy sources for heterotrophic bacteria abound in algal ponds. Addition of glucose prolonged the survival of *E. coli* by ten days in wastewater (Van der Steen *et al*., 2000a). Bouteleux *et al*. (2005) also observed *E. coli* growth in the presence of biodegradable algal organic matter and this growth increased by 4-12 folds in the presence of

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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 5.3 mg L^{-1} 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. The relative importance of starvation in the two pond types need to be investigated as dead organic matter from duckweed fronds can constitute an important source of carbon for the survival of heterotrophic bacteria.

Sunlight, pH and Dissolved Oxygen

Sunlight is a major, if not the most important, factor in pond disinfection (Davies-Colley *et al*., 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*., 1992). 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 (400-700 μm) 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 *et al*., 1992). The effect of sunlight interaction with environmental factors in achieving faecal coliform removal in treatment ponds is summarized in Table 2.

Curtis *et al*. (1992) 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 however is not an important mechanism in duckweed ponds in that duckweed covers the entire surface of the ponds cutting off solar radiation. Ansa *et al*. (2012b) observed that inactivation of faecal coliforms was generally similar in magnitude in duckweed ponds in both morning and afternoon while in the algal ponds, the inactivation rates were higher in the afternoon compared to the morning.

Table 1. Performance of algal, duckweed and hybrid algal and duckweed ponds in the removal of faecal coliforms

		Removal (log units)			
Location	Season/Temp. (°C)	DK	AL	CS	Reference
Accra, Ghana	Wet: 24-29	3.8	4.8	4.3	Ansa *et al*. (2012a)
	Dry: 30-33	3.5	4.6	4.3	
	Year round	3.7	4.7	4.3	
Kumasi, Ghana	Year round: 24-27	4.0	5.0		Awuah (2006)
West Bank, Palestine	Winter: 7-13	1.0	3.1		Zimmo *et al*. (2002)
	Summer: 21-27	2.0	2.3		
Negev, Israel	Winter: 18-15		2.6	2.2	Van der Steen *et al*. (2000b)
	Spring: 18-28		2.7	2.3	
Belo Horizonte, Brazil	Year round: 20			6.4	Von Sperling and Mascarenhas (2005)

DK: Duckweed pond, AL: Algal Pond, CS: Combined System of Duckweed and Algal Pond

Table 2. Combined effect of light, pH and dissolved oxygen

Mechanism	Sensitizer	Conditions
(1) Direct photobiological damage to DNA by solar UV-B (300-320 nm)	Independent of sensitizers	pH 7.5-8.5 Net effect depends on the efficiency of DNA repair mechanism
(2) UV-B (300-320 nm) photo-oxidative damage to DNA and DNA repair mechanisms	Endogenous sensitizers (e.g., cell contents)	Oxygen dependent
(3) Long wavelengths (400-700 nm) and UV (300-400 nm) 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: (Ansa *et al*., 2012c; Awuah, 2006; Davies-Colley *et al*., 2000; Van der Steen *et al*., 2000b)

Algal Toxins

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 20 ng (10^9 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 mycrocystin 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* (Maynard *et al*., 1999). Ansa *et al*. (2012c) observed increased inactivation of faecal coliforms with increased chlorophyll a concentration in darkness and concluded that some substance in the algae may be contributing to the inactivation of the faecal coliforms. 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 demoic acid are responsible for amnesic shellfish poisoning in humans (Litaker *et al*., 2008). There are indications that green algae may release substances that are harmful to faecal coliforms thus contributing to their removal (Ansa *et al*., 2012c). Rapid detection methods being developed to detect cyanobacterial toxins (Litaker *et al*., 2008) 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 coliforms (Maynard *et al*., 1999).

Algal and Duckweed Biomass

Some authors have mentioned that algal biomass has negative influence on faecal coliform survival as increased algal biomass may lead to increased oxygenation and pH (Davies-Colley *et al*., 2000) but Van der Steen *et al*. (2000) 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 hypothesis was tested by an experiment conducted by Ansa *et al*. (2012c) under laboratory conditions using artificial light. They observed that for a range of algal biomass depicted by chlorophyll a concentrations of 0-20 mg L^{-1}, inactivation of faecal coliforms increased with increased chlorophyll a concentration till a certain optimum algal density (10±2 mg L^{-1}) after which faecal coliform inactivation decreased with increased chlorophyll a concentration. This optimum algal density, they observed was affected by the strength of the wastewater. It would be useful to also test this hypothesis under field conditions using solar radiation due to high variability usually encountered under field conditions. Algal and duckweed biomass can also serve as surfaces for faecal coliform attachment and the importance of this in treatment pond systems is discussed in greater detail later in this manuscript. The effect of duckweed biomass on faecal coliform removal may occur through attachment to duckweed roots and its subsequent sedimentation after plant decay. This is discussed under the heading: Attachment and sedimentation.

Predation

Faecal bacteria are fed on by various invertebrates colonizing wastewater treatment ponds. The role of predation of faecal bacteria 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 a 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. In a study conducted by Ansa *et al*. (2012b) in a pilot scale treatment pond system consisting of four algal ponds in series running alongside a series of four duckweed ponds in series, two macro-invertebrate taxons, namely Cladocera and Ostracoda dominated. FC numbers correlated strongly and positively with mean ostracod numbers in the entire duckweed ponds (R^2 = 0.989) while no correlation was observed between ostracods and FC numbers in algal ponds. They argued that as Ostracods feed on bacteria and unicellular algae (Khangarot and Das, 2009), in algal ponds the presence of abundance of algal cells may explain the lack of correlation of ostracod numbers with faecal bacteria numbers. On the contrary the limited amount of algal cells in fully covered duckweed ponds suggests a greater preference for bacteria, hence the high correlation. Further studies however may need to be conducted to ascertain the relative importance of these macro-invertebrates to the overall removal of faecal bacteria from treatment pond systems.

Attachment and Sedimentation

Attachment of FC to suspended matter which eventually settles under the force of gravity could remove FC from the water column resulting in cleaner effluents. Soupir *et al*. (2008) noted that 90% of *E. coli* was associated with particles of sizes <3 µm respectively in storm water. Boutilier *et al*. (2009) noted however that particles and algal cells <80 µm in diameter remained in suspension and although 10-50% of *E. coli* were found to associated 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 and this may facilitate their settling out of the water column faster than those in the free form (Characklis *et al*., 2005). The aggregation of suspended matter such as kaolin and *Chlorella* with a negative surface charge or zeta potential (Table 3) 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 (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 umVs-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). This may explain the formation of the bacteria-algae-biomass flocs observed by Gutzeit *et al*. (2005; Medina and Neis, 2007) which settled at the bottom providing a clear supernatant that was shown to improve the water quality of the pond effluent. 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 duck weed ponds, faecal coliforms may attach to duckweed fronds and would therefore be shielded from the effect of solar radiation (MacIntyre *et al*., 2006) but conditions for settling of suspended solids may be better as the bubbling release of oxygen and the effect of wind action is relatively minimal. Awuah (2006) noted that removal of faecal bacteria through attachment to harvested macrophytes accounted for less than 1% of faecal bacteria removal and similar patterns of attachment of FC to suspended matter were observed in the first two ponds of a pilot scale algal and duckweed pond system receiving domestic wastewater (Ansa *et al*., 2012b), the degree of attachment was also enhanced by high concentrations of faecal coliforms (Ansa, 2013).

Table 3. Characteristics of some common suspended matter used in laboratory sedimentation experiments. Source: Henderson *et al*. (2008)

Particle	Size (µm)	Surface area (µm^2)	Zeta potential (mV)
Chlorella vulgaris	5.3	88	-10.0
Chlorella sp	3.5	38	-10.0
Kaolin	4.3	74	-46

Conclusion

Algal ponds, in comparison with duckweed ponds, are more efficient in FC removal due to the high pH and oxygenation that occur in the former, temperature increases playing a part alongside solar radiation. An observation made above suggests that the relative importance of the mechanisms or factors affecting FC removal in duckweed and algal waste stabilization ponds would differ. Starvation for example seems to be of little importance in both pond systems as both types of WSPs do not completely run out of organic matter.

Attachment and sedimentation of FC occurred in both system types and is more important in duckweed ponds considering the absence solar radiation in the latter. The importance of attachment of FC to suspended matter at various depths to the overall removal of FC from duckweed and algal ponds need to be investigated as attachment is likely to be enhanced by increased FC concentration, which can be expected at deeper layers of the pond particularly in low oxygen conditions.

Recent work suggests that green algae produce substances that are injurious to FC even in darkness. Further work to identify the substances produced by the algae is necessary and the concentration in which the substance or substances become toxic to FC need to be established.

The variation of FC inactivation with algal biomass concentrations has implications for pond design but as experiments were conducted under laboratory conditions using artificial light, similar experiments need to be conducted under field conditions using sunlight.

The relative importance of predation by protozoans and macro-invertebrates in the removal of FC in relation to other removal mechanisms is still unknown. The strong correlation between FC and ostracod numbers observed suggests a feeding role by these macro-invertebrates in FC removal. However more research is necessary to quantify the amount of FC removed by the macro-invertebrates and protozoans in a specified period of time. The interactive effects of removal mechanisms such as the disruption of attachment and sedimentation by the feeding and movement activity of macro-invertebrates which can result in the re-suspension of attached coliforms are also unknown.

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Author's Contributions

E.D.O. Ansa: Made contributions to conception and design, literature search and review as well as acquisition and interpretation of data relating to treatment performance and removal mechanisms. Contributed in drafting the article, reviewing it critically for significant intellectual content and Gave final approval of the version to be submitted in any revised version.

E. Awuah: Made contributions to conception and design, acquisition of data relating to removal mechanisms of faecal coliforms and interpretation of data. Contributed in drafting the article, reviewing it critically for significant intellectual content.

A. Andoh: Made contributions to acquisition of data relating to removal mechanisms of faecal coliform, analysis and interpretation of data. Contributed in reviewing it critically for significant intellectual content.

R. Banu: Made contributions to acquisition of data relating to removal mechanisms of faecal coliform, analysis and interpretation of data. Contributed in reviewing it critically for significant intellectual content.

W.H.K. Dorgbetor: Mode contributions to acquisition of data on particle size and forces of attraction, attachment and sedimentation and analysis and interpretation of data. Contributed in reviewing it critically for significant intellectual content.

H.J. Lubberding: Made contributions to conception and design. Contributed in drafting the article, reviewing it critically for significant intellectual content.

H.J. Gijzen: Made contributions to conception and design and contributed in drafting the article, reviewing it critically for significant intellectual content.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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