

## REVIEW ARTICLE

# Wastewater Treatment Plants As a Source of Microbial Pathogens in Receiving Watersheds

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### ABSTRACT

Wastewater treatment facilities have become necessary in ensuring the discharges of high quality wastewater effluents into receiving water bodies and as consequence, a healthier environment. Due to massive worldwide increases in the human population, water has been predicted to become one of the scarcest resources in the 21<sup>st</sup> century, and despite large advances in water and wastewater treatments, waterborne diseases still pose a major threat to public health worldwide. Several questions have been raised on the capacity of current wastewater treatment regimes to remove pathogens from wastewater with many waterborne diseases linked to supposedly treated water supplies. One of the major gaps in the knowledge of pathogenic microorganisms in wastewater is a thorough understanding of the survival and persistence of the different microbial types in different conditions and environments; this therefore brings to the fore the need for a thorough research into the movement and behavior of these microorganisms in wastewaters. In this review paper we give an overview of wastewater treatment practices with particular emphasis on the removal of microbial pathogens.

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### INTRODUCTION

Adsorbed from the liquid is obtained from the air as a certain area of the disc is rotated out of the liquid. In some designs, air is added to the bottom of the tank to provide oxygen and to rotate the disc when those are provided with air capture cups. It is a very useful system in small communities instead of the conventional secondary treatment, obtaining similar quality in the effluent. RBCs have also been developed for the biological treatment of odours (Smeets *et al.*, 2006). It is flexible enough to undergo fluctuating organic loads, requires little personal attention, cheap to run and does not require too much land. The RBCs have been used in treating winery wastewater and has also been used in the treatment of effluents produced by various industries such as gold mining and domestic sewage treatment (Tawfit *et al.*, 2002).

### Review Literature

#### Activated sludge system

The activated sludge process is the most widely applied biological wastewater treatment process in the world. The primary objective of the activated sludge system is the removal of soluble biodegradable compounds. It also removes pathogenic microorganisms from wastewaters. It is capable of achieving equal reductions in soluble substrate in reactors of much smaller volume while producing an effluent relatively free of suspended solids (Dewil *et al.*, 2006). The removal efficiency of pathogenic and indicator microorganisms in these wastewater treatment plants vary according to the treatment process type, retention time, other biological flora present in activated sludge, oxygen concentration, pH, temperature and the efficiency in removing suspended solids (Doorn *et al.*, 2006).

#### Components of the Activated Sludge Treatment Process

##### 1. Anaerobic zone

The anaerobic zone is considered to be one in which both dissolved oxygen and oxidized nitrogen are absent. In this zone, sludge from the clarifier flows in jointly with the influent wastewater. It has been reported that for this zone to operate efficiently, oxygen and nitrates must be absent. This is responsible for the release of phosphate (Tanaka *et al.*, 2007).

##### 2. Primary anoxic zone

The primary anoxic zone is the main denitrification reactor in the process; it is fed by the effluent from the anaerobic zone and mixed liquor recycled from the aerobic zone. The presence of nitrate or nitrite and absence of oxygen leads to the enrichment of denitrifying bacteria, which reduces nitrate or nitrite to

molecular nitrogen. Thus soluble and colloidal biodegradable matters are readily removed in this zone (Metcalf and Eddy, 2003).

### 3. Primary aerobic zone

The primary aerobic zone functions mainly to oxidize organic material in wastewater, ammonia into nitrate and also provides an environment to take up all the phosphate released in the anaerobic zone (Torpak, 2006). For the removal of ammonia, it must first be oxidized to nitrites by nitrifying bacteria such as *Nitrosomonas*, *Nitrospira* and *Nitrosolobus* spp. Nitrites are then oxidized to nitrates by *Nitrobacter*, *Nitrospira* and *Nitrococcus* spp. These nitrates are then removed in the primary anoxic zone by denitrifying bacteria. Phosphates uptake is based on the enrichment of the activated sludge with bacteria capable of taking orthophosphate and *E. coli* which also have been associated with the enhanced phosphate removal in activated sludge (Sci-Tech. Encyclopedia, 2007).

### 4. Secondary anoxic zone

This zone further converts an excess nitrate which was not removed in the zone preceding it into nitrogen. Because of the very slow denitrification rate in this zone, the quantity of nitrate removed is very small. The retention time in the anoxic zone is relatively long because of the lower chemical oxygen demand (Torpak, 2006).

### 5. Secondary aerobic zone and clarifier

This zone removes additional phosphate, which was not removed in the primary aerobic zone. Residual ammonia is also oxidized in this zone. The secondary aerobic zone increases the level of the dissolved oxygen between 2 and 4 mg-l in the mixed liquor before it enters the clarifier. Aeration should be more to promote phosphate uptake and maintain good aerobic conditions. Phosphorus is retained in the biomass as long as aerobic condition prevails. This zone prevents the development of anaerobic condition in the clarifier and phosphate release before clarification. In the clarifier, treated wastewater, free of organic matter and dissolved solid is released (Smeets et al., 2006).

#### a. Microbiology of activated sludge

The activated sludge process is a biological method of wastewater treatment that is performed by a variable and mixed community of microorganisms in an aerobic aquatic environment (Jenkins *et al.*, 2003). These microorganisms derive energy from carbonaceous organic matter in aerated wastewater for the production of new cells in a process known as synthesis, while simultaneously releasing energy through the conversion of this organic matter into compounds that contain lower energy, such as carbon dioxide and water, in a process called respiration. A variable number of microorganisms in the system also obtain energy by converting ammonia nitrogen to nitrate nitrogen in a process termed nitrification. This consortium of microorganisms, the biological component of the process, is known collectively as activated sludge (Norstrom, 2005). Bacteria, fungi, protozoa, and rotifers constitute the biological mass, of activated sludge. In addition, some metazoa, such as nematode worms, may be present. Cell makeup depends on both the chemical composition of the wastewater and the specific characteristics of the organisms in the biological community. However, the constant agitation in the aeration tanks and sludge recirculation are deterrents to the growth of higher organisms (Lardotter, 2006).

#### b. Stages of treatment of wastewater

##### Preliminary treatment

As wastewater enters a treatment facility, it usually undergoes preliminary treatment. This treatment typically involves screening to remove large floating objects, such as rags, cans, bottles and sticks that may clog pumps, small pipes, and downstream processes (USEPA, 2004). Screens are generally placed in a chamber or channel and inclined towards the flow of the wastewater. The inclined screen allows debris to be caught on the upstream surface of the screen, and allows access for manual or mechanical cleaning. Some plants use devices known as comminutors or barminutors which combine the functions of a screen and a grinder. These devices catch and cut or shred the heavy solid and floating materials. In the process, the pulverized matter remains in the wastewater flow in smaller pieces to be removed later in a primary settling tank (Mara, 2004).

##### Primary treatment

Primary treatment is the second step in wastewater treatment and this step helps to separate suspended solids and grease from wastewater (USEPA, 2004). In some treatment plants, primary and secondary stages may be combined into one basic operation (Environment Canada, 2003). At many wastewater treatment facilities, influent passes through preliminary treatment units before primary and secondary treatments begin. With the screening completed and the grit removed, wastewater still contains dissolved organic and inorganic constituents along with suspended solids. The suspended solids consist of minute particles of matter that can be removed from the wastewater with further treatment such as sedimentation or gravity settling, chemical coagulation, or filtration. Pollutants that are dissolved or are very fine and remain suspended in the wastewater are not removed effectively by gravity settling. When

the wastewater enters a sedimentation tank, it slows down and the suspended solids gradually sink to the bottom, as primary sludge which can then be removed from the tank by various methods (Environment Canada, 2003).

### **Secondary treatment**

This is a biological treatment process that removes dissolved organic matter from wastewater. Ninety percent of the organic matter in wastewater could be removed by this treatment processes. Sewage microorganisms are cultivated and added to the wastewater. The microorganisms absorb organic matter from sewage as their food supply in the process removing such organic matters from circulation (USEPA, 2004). The three most common conventional methods used to achieve secondary treatment are attached growth processes, suspended growth processes and lagoon systems (Upadhyaya *et al.*, 2007). Attached growth processes involve microbial growth in surfaces such as stone or plastic media. Wastewater passes over the media along with air to provide oxygen. Attached growth process units include trickling filter, biotowers and rotating biological contractors. The growth processes are effective at removing biodegradable organic material from the wastewater (Environment Canada, 2003). Suspended growth processes are designed to remove biodegradable organic material and organic nitrogen containing material by converting ammonia nitrogen to nitrate. In this growth processes the microbial growth is suspended in an aerated water mixture where the air is pumped in, or the water is agitated sufficiently to allow oxygen transfer. Suspended growth process unit include variations of activated sludge, oxidation ditches and sequencing batch reactor (Mbwele *et al.*, 2003). A wastewater lagoon or treatment pond is a scientifically constructed pond, three to five feet deep, that allows sunlight, algae, bacteria and oxygen to interact. Biological and physical treatment processes occur in the lagoon to improve water quality. The quality of water leaving the lagoon, when constructed and operated properly, is considered equivalent to the effluent from a conventional secondary treatment system. Lagoons remove biodegradable organic material and some of the nitrogen from wastewater (Larsdotter *et al.*, 2003).

### **Advanced or tertiary treatment**

Tertiary treatment is the term applied to additional treatment that is needed to remove suspended and dissolved substances remaining after conventional secondary treatment. This may be accomplished using a variety of physical, chemical or biological treatment processes to remove the target pollutants (Environment Canada, 2003). Tertiary treatment may include: Filtration, Removal of Ammonia and other specific contaminants and Disinfection to destroy pathogens (Hijnen *et al.*, 2006).

### **Disinfection**

Untreated or inadequately treated wastewaters may contain pathogens. Processes used to kill or deactivate these harmful organisms are called disinfection. Chlorine is the most widely used disinfectant but ozone and ultraviolet radiation are also frequently used for wastewater effluent disinfection (Hijnen *et al.*, 2006). Chlorine kills microorganisms by destroying cellular materials and can be applied to wastewater as a gas, liquid or in a solid form. However, any free (uncombined) chlorine remaining in the water, even at low concentrations, is highly toxic to beneficial aquatic life (Hijnen *et al.*, 2006). Therefore, removal of even trace amounts of free chlorine by dechlorination is often needed to protect fish and aquatic life. Ozone is also used for disinfection, and it is produced from oxygen exposed to a high voltage current. Ozone is very effective at destroying viruses and bacteria and decomposes back to oxygen rapidly without leaving harmful by-products. The setback in the use of ozone however, is its high energy costs (Hijnen *et al.*, 2004). Ultraviolet (UV) radiation disinfection is a physical treatment process that leaves no chemical traces. Organisms can sometimes repair and reverse the destructive effects of UV when applied at low doses. Furthermore, UV can only be applied on small scale basis (Hoyer, 2004).

### **Regulatory standards for wastewater effluent quality**

Wastewater treatment aims at producing effluent suitable for agricultural or aquacultural reuse (or both), or to produce an effluent that can be safely discharged into inland or coastal waters. Effluent quality requirements often termed effluent quality standards are set by regulatory agencies that are empowered by legislation to make such regulations. These agencies have duties, either explicitly defined in the governing legislation or at any rate implicitly, to set sensible regulations. Unfortunately, in many countries not all such regulations are sensible as they should be (Mara, 2004; von Sperling and de Lemos Chernicharo, 2005). Permits for wastewater treatment systems must obtained from appropriate authorities (WHO, 2006). In the US for example, if the discharge from a treatment plant enters a stream, a National Pollutant Discharge Elimination System (NPDES) permit is required. The NPDES permit specifies the maximum allowable level of total suspended solids, biochemical oxygen demand, nutrients and bacteria that can be discharged to a stream as well as the minimum level of dissolved oxygen that must be present in the discharge. The levels specified in the NPDES permit are determined by the condition of the receiving stream. Therefore, NPDES permits are subject to change every 5 years as water quality concerns change throughout (WHO, 2006).

Wastewater poses a significant pollution threat to water-bodies and soil and hence the quality of the effluents must be controlled, especially with regards to the two variables- (i) polluting power (BOD, TOC, suspended solids and COD) (ii) nutrients (phosphate, nitrate, and ammonium). Toxins are also controlled depending on the industry type, and these would include solvents, heavy metals, phenols, chlorinated compounds and such like (WHO, 2006).

#### **Effectiveness of wastewater treatment**

The effectiveness of conventional wastewater treatment processes has become limited over the last two decades because of new challenges (Smeets *et al.*, 2006). Zhou and Smith (2002) observed that increased knowledge about the consequences from water pollution and the public desire for better quality water has promoted the implementation of much stricter regulations by expanding the scope of regulated contaminants and lowering their maximum contaminant levels (MCLs). Another factor is the diminishing water resources and rapid population growth and industrial development (USEPA, 2004). Some of the key challenges faced by the wastewater treatment sector today include: old and worn-out collection facilities requiring further improvement, repair or replacement to maintain their useful life; the character and quantity of contaminants presenting problems today are far more complex than those that presented challenges in the past; population growth is taxing many existing wastewater treatment systems and creating a need for new plants; farm runoff and increasing urbanization provide additional sources of pollution not controlled by conventional wastewater treatment; and one third of new development is served by decentralized systems (e.g., septic systems) as population migrates further from metropolitan areas (Mara, 2004).

Treatment plants remove varying amounts of contaminants from wastewater; depending on the level of treatment they provide (Environment Canada, 2003). Chlorination, UV irradiation and ozonation are three common disinfection techniques among others that have shown various degree of success in the removal of pathogens from wastewater over the years (EPA, 2002). Recent literature however, points to the inadequacies of these techniques in the removal of some pathogens from wastewater. For example, UV and chemical disinfection with chlorine has been reported to be ineffective against some viruses and bacteria spores, *Acanthamoeba*, *Cryptosporidium* and *Giardia* spp. (Tree *et al.*, 2003; Gomez *et al.*, 2006). Ozone applied at low CT (concentration and contact time) values to limit formation of bromate was also reported to have relatively little effect on the infectivity of the protozoan (oo) cysts (Hijnen *et al.*, 2006). Hoch *et al.* (1996) reported that heterotrophic bacterial community was not significantly affected by the input of treated sewage, as faecal contamination was readily detected over a comparatively long stretch of 30 km in the receiving watershed (Danube River, Vienna, Austria) following the point of sewage discharge. Factors that influence microbial sensitivity to disinfection include attachment to surfaces, encapsulation, aggregation and low-nutrient growth (LeChevallier and Au, 2004). Waste-Activated Sludge (WAS) processes which are key technologies to treat wastewater have been shown to also have presence of heavy metals in the excess sludge which are difficult to remove by common sludge treatment methods such as aerobic or anaerobic digestion (Dewil *et al.*, 2006). It was opined that the advancement of wastewater treatment technology notwithstanding, treated sewage may still contain some harmful substances irrespective of thoroughness and sophistication of treatment process, albeit in smaller quantities than in raw sewage (Environment Canada, 2003). The authors further reported that in many cases, the concentrations of the remaining pollutants may still be high enough to cause serious environmental damage.

#### **Consequences of inadequate wastewater treatment**

The consequences of discharging untreated or inadequately treated wastewater into the environment are as diverse as they are many. Municipal wastewater can result in increased nutrient levels (eutrophication), often leading to algal blooms; depleted dissolved oxygen, sometimes resulting in fish kills; destruction of aquatic habitats with sedimentation, debris, and increased water flow; and acute and chronic toxicity to aquatic life from chemical contaminants, as well as bioaccumulation and biomagnification of chemicals in the food chain (Boesch *et al.*, 2001).

The release of untreated or inadequately treated municipal wastewater effluents may put public health at risk from drinking water contaminated with pathogenic bacteria, protozoans (such as *Giardia* and *Cryptosporidium* spp.) and several toxic substances (Paillard *et al.*, 2005). Carcinogenic and endocrine disrupting substances as well as pharmaceuticals can pass through even the most advanced wastewater treatment systems (Heberer, 2002). Endocrine disrupting substances are known to disrupt or mimic naturally occurring hormones and may have an impact on the growth, reproduction, or development of many species of wildlife (Furuichi *et al.*, 2004).

Wastewater pollution also has its socio-economic impacts on the teeming populace. Wastewater pollution negatively affects the ecosystem; with the high rate of wetland destruction, depletion of plant biomass, effects on aquatic wildlife habitat, and the decrease in fresh water access, the ecosystem services

provided by these components will continue to degrade (Boesch *et al.*, 2001). The natural capital of the earth is thus depleted where the ecosystem loses its capacity to provide the usual vital services. The main objective therefore of championing the course of a high wastewater effluent standard, is to maintain the natural capital so as to ensure that adequate resources are available for natural benefits (Smith *et al.*, 2005).

### **Microbial pathogens in wastewater**

Microbial pathogens which can be potentially present in wastewater can be divided into three separate groups: viruses, bacteria, and the protozoans/helminths (LeChevallier and Au, 2004).

#### **Viruses**

Viruses are among the most important and potentially most hazardous pathogens in wastewater (Tree, 2003). Viruses are generally more resistant to treatment, more infectious, more difficult to detect in environmental samples such as wastewater and require smaller doses to cause infection than most of the other pathogens (Gomez *et al.*, 2006). The common viruses found in wastewater enter the environment through faecal contamination from infected host or carriers (Leclerc *et al.*, 2000). Most of the commonly detected pathogenic viruses in wastewater are the enteroviruses; they are small, single-stranded RNA viruses and include the poliovirus types 1 and 2. Others are multiple strains of echovirus, enterovirus and coxsackievirus (Tanji *et al.*, 2002).

#### **Bacteria**

Bacteria are the most common of microbial pathogens found in wastewater. A wide range of bacterial pathogens and opportunistic pathogens associated with wastewater are enteric in origin and have been reported in literature (Simson and Charles, 2000). Gastrointestinal infections are amongst the most common diseases caused by bacterial pathogens in wastewater (LeChevallier and Au, 2004). Wastewater associated infections generally include diarrhoea, dysentery, dysentery-like infections, *Leptospira interrogans* infections, typhoid, human enteritis, legionellosis, melioidosis, stomach ulcer and cancer (Liang *et al.*, 2006). The contamination of food by water containing known toxin producing organisms such as *Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli*, or *Clostridium perferinges* can cause outbreaks of food poisoning (often severe and widespread). One of the emerging wastewater bacterial pathogens of grave public health concern in recent times is *Listeria monocytogenes* otherwise known as invasive Listeria. Several cases of Listeriosis outbreaks associated with wastewater have been reported around the globe (Paillard *et al.*, 2005).

#### **Protozoa**

Pathogenic protozoa are more prevalent in wastewater than any other environmental source. Pathogenic protozoans associated with wastewater include, *Entamoeba histolytica*, *Giardia intestinalis* (formerly *Giardia lamblia*) and *Cryptosporidium parvum*, and these organisms have been frequently isolated from wastewater sources with faecal contamination (Caccio *et al.*, 2003).

#### **Helminths**

Helminths (nematodes and tape worms) are common intestinal parasites which, like the enteric protozoan pathogens, are usually transmitted by faecal route in humans (Feenstra *et al.*, 2000). Helminth parasites commonly detected in wastewaters include the round worm (*Ascaris lumbricoides*), the hook worm (*Ascaris duodenale* or *Nector americanus*), and the whip worm (*Trichuris trichiura*) and *Strongyloides stercoralis* the causative agent of strongyloidiasis (Feenstra *et al.*, 2000). It has been estimated that approximately 25% of the world human population is infected with the round worm, *Ascaris lumbricoides* (WHO, 1989). The prevalence of *Ascaris* infection is influenced by population density, education standards, sanitation levels, degree of agricultural development, and cultural dietary habits (Smith *et al.*, 2001). The World Health Organization lists intestinal nematodes to be of greatest health risk in the use of untreated excreta as well as wastewater for agricultural/aquacultural purposes (WHO, 1989). Children under the age of 19 were reported to be the most affected by nematode infection (Feenstra *et al.*, 2000).

### **Microbial indicators of wastewater pollution**

The detection, isolation and identification of the many different types of microbial pathogens associated with wastewater would be difficult, time consuming and hugely expensive undertaking if attempted on a regular basis. To avoid the necessity of undertaking such huge ventures, indicator microorganisms are used to determine the relative risk of the possible presence of pathogenic microorganisms in a sample (Ashbolt *et al.*, 2001). To function effectively as indicators, such microorganisms should be a member of the intestinal microflora of warm-blooded animals; should be present when pathogens are present, and absent in uncontaminated samples; it should be present in greater numbers than the pathogen(s); should be at least equally resistant as the pathogen to environmental factors and to disinfection in water and wastewater treatment plants; it should not multiply in the environment; It should be detectable by means

of easy, rapid, and inexpensive methods and the indicator organism should be non pathogenic (Bitton, 2005).

*Escherichia coli* have for a very long time been used as indicators of faecal contamination of water sources, and its growth characteristics and behaviour in the environment is relatively well known (Ashbolt *et al.*, 2001). The ability of *E.coli* to be cultured at elevated temperatures (44.5°C) has earned them the name of thermotolerant coliforms (TTC) and they have become the mainstay indicator for the water industry (Leclerc *et al.*, 2000). Thermotolerant coliforms are however disadvantaged in that they are more sensitive to environmental changes and treatment processes than a number of more resistant bacterial pathogens and almost all of the viruses, protozoan cyst and helminth eggs (Ashbolt *et al.*, 2001). Another drawback with the use of TTC as an indicator of faecal pollution is that coliform bacteria reside in the gut of many different warm blooded animals. Thus, the detection of TTC in a water source does not necessarily confirm the contamination of that water body with human excrement or the presence of human pathogens. The inappropriateness of faecal coliforms (or TTC) as indicators of human faecal contamination of water sources and of the effectiveness of treatment processes has led to the search for more appropriate indicator microorganisms. A number of bacteria and bacteriophages have been studied for their suitability as indicators.

*Clostridium perfringens* were most useful as indicators of human faecal pollution and the only reliable indicator for the presence of *Giardia intestinalis* when compared with faecal streptococci and F-RNA bacteriophages. Other potential bacterial indicators for the presence of microbial pathogens in water are the enterococci, bifidobacteria, and bacteroides (Leclerc *et al.*, 2000). Anaerobic indicator bacteria such as bacteroides and bifidobacteria are however difficult to apply as indicators of faecal contamination on a large scale due to handling difficulties associated with strict anaerobes. One of the problems associated with the use of bacteria as indicator for the presence of microbial pathogens in water is the greater resistance of protozoan cysts and viruses to environmental factors and treatment processes (Tree *et al.*, 2003; Hijnen *et al.*, 2006; Gomez *et al.*, 2006). Viruses in particular are difficult to detect in many water sources due to low numbers, and the difficulty and expense of culturing (Tanji *et al.*, 2002). To overcome these problems, bacterial viruses (bacteriophages) have been examined for use in faecal pollution and the effectiveness of treatment processes to remove enteric viruses (Ashbolt *et al.*, 2001). The most common bacteriophage studied is male-specific (F-RNA) bacteriophage (in particular MS2 and PRD-1) which infect gram negative bacteria containing the F+ sex plasmid; somatic coliphages (bacteriophage which infects coliforms); and *Bacteroides fragilis* specific bacteriophage (Leclerc *et al.*, 2000; Hijnen *et al.*, 2006). Somatic coliphage and F-RNA bacteriophage have been shown to survive but not replicate for long periods in tropical pristine rivers, indicating that they could be useful as indicators in environmental waters. One of the main interests in the use of bacteriophage is their potential of indicating the effect treatment processes have on the survival of pathogenic viruses.

All of the potential indicators studied till date has one or more characteristics which prevent their implementation as replacement for faecal coliforms (Ashbolt *et al.*, 2001; Bitton, 2005). Thus, despite their drawbacks, faecal coliforms still remain the major organisms used to indicate faecal pollution and the effectiveness of treatment processes. However, the improvements in the detection of microorganisms by molecular techniques which have occurred in the last 10 years may mean that the use of indicators may no longer be required (Bitton, 2005).

#### **Isolation and detection of wastewater pathogens**

Methods used to identify and quantify microbial populations in wastewater can be divided into three main groups: culture, immunology and nucleic acid-based.

##### **Culture-based methods**

This method employs selective and/or differential media, which provide a „presumptive identification“ and may be followed by a number of other tests. The tests provide confirmation of the identity of isolates by biochemical, immunological or molecular methods. Abundance is either inferred from the number of colony forming units (CFUs) on culture plates or by Most Probable Number (MPN) dilutions of wastewater samples. For accurate quantification, representative presumptively positive strains must be corroborated by more extensive characterization with biochemical tests or molecular assays. The dilution or concentration (by filtration) of samples prior to culture-based enumeration can accommodate a wide dynamic range of wastewater microbial population sizes (Thompson *et al.*, 2004).

One of the disadvantages of culture-based techniques in wastewater sample is that they depend on how reproducibly and quantitatively the target pathogen population will grow on culture media. This is quite limiting as certain pathogens can enter a viable but non-culturable state (VBNC) in response to shifts in environmental conditions possibly complicating interpretation of population dynamics observed in culture-based studies (Besnard *et al.*, 2000). Another disadvantage is that since culture-based techniques inherently rely on growth, they are limited by how fast the target population grows to detectable levels;

otherwise they may be outgrown by no target populations. With notable exceptions, most culture-based identification schemes for specific populations are time and labour-intensive, and may require preliminary enrichment or decontamination steps that confound enumeration (Besnard *et al.*, 2000). Despite the above-mentioned limitations of culture-based methods, significant benefits remain. Most notably, the cost of materials needed for culture-based assays in wastewater are relatively cheap and does not require extensive training, and highly specialized materials and equipment. In addition, cultured isolates allow subsequent investigations into the virulence and/or clinical significance of environmental pathogen populations (Thompson *et al.*, 2004).

### **Immunological methods**

Immunological detection has been used to identify and in some cases, enumerate pathogenic populations in wastewater samples. These methods rely on the inherently high specificity of immune reactions and typically target pathogen-specific antigens such as cell-wall lipopolysaccharides (LPS), membrane and flagellar proteins or toxins. Immuno-assays can be categorized into three main groups: enzyme-linked immunosorbent assay (ELISA), immunofluorescent microscopy, and agglutination assays (Besnard *et al.*, 2000; Bitton, 2005). There are several notable challenges for the implementation of immunological methods to detection of pathogens in wastewater samples, which contain a large diversity of unknown bacteria. First, the sensitivity of many current methods is not high enough for detection of pathogens at low, environmentally relevant, concentrations. Second, false positive results can be generated by cross-reaction of antibodies with antigens of similar but non-target organisms. This is particularly problematic when polyclonal antibodies are used since these are complex mixtures of antibodies against multiple, mostly uncharacterized cell structures (Thompson *et al.*, 2004; Bitton, 2005). Finally, design and production of specific antibodies generally requires growth of target microorganisms, constraining the applicability of the methods to culturable populations (Bitton, 2005). Despite these limitations, immunological methods have many potential applications for detection of pathogens in wastewater environment (Bitton, 2005).

### **Nucleic acid based methods**

Advances in molecular biology have revolutionized wastewater microbiology by facilitating the identification of emerging pathogens, the detection of environmental populations, and the discrimination between closely related pathogenic and non-pathogenic bacteria (Persing *et al.*, 2003). Discrimination of nucleotide variation among genes, whose occurrence is specific to an organism or whose sequence differentiates organisms, is often achieved by nucleic acid hybridization; other methods rely on restriction cutting of the chromosome. Hybridization-based methods include fluorescence in situ hybridization (FISH) (Rompere *et al.*, 2002) and filter hybridization (colony and dot-blot hybridization) (Jiang and Fu, 2001), and the polymerase chain reaction (PCR). The PCR couples hybridization of short DNA molecules (primers) to template molecules followed by amplification with a polymerase. Molecular typing methods have used PCR [multi-locus sequence typing (MLST)] or restriction cutting [pulsed field gel electrophoresis (PFGE)] for analyzing genomic signatures (van Belkum, 2003). The general principles of hybridization-based, PCR-based and molecular typing methods have been reviewed in widely available protocol books (Persing *et al.*, 2003).

Nucleic acid-based detection techniques have the advantages of being very target specific, relatively more sensitive and less time consuming. They also have the advantage of detecting viable but non-culturable organisms. However, due to their sensitivity, nucleic acid-based methods for detecting wastewater pathogens as in other microorganisms are unable to differentiate between viable and nonviable pathogens (or their resting stages). There is also the issue of false positive reactions due to contamination by extraneous nucleic acids, often through contact with laboratory equipment. Further, there is the need to concentrate large volumes of water in order to get a significant amount of total genomic DNA and this might lead to loss of significant population of the target organism(s). Thus, while the use of nucleic acid-based detection techniques show great promise for the detection of pathogens in wastewater, a number of issues need to be resolved before these techniques could be fully deployed as standard detection methods for the wastewater industry.

### **CONCLUSIONS**

The advancement of wastewater treatment technology notwithstanding, treated sewage may still contain some harmful substances (including microbial pathogens) irrespective of thoroughness and sophistication of the treatment process. There is a wide range of microbial pathogen types which can occur in wastewater, with the type and number present being highly dependent on the socioeconomic conditions and customs of the communities creating the wastewater. In order to propose an efficient way of treating wastewater, there is need to understand the negative environmental impacts posed by the untreated or inadequately treated wastewater entering the nearby ecosystems, especially on the lives

that depend on the ecosystem for sustenance. Survival and persistence of such microbial pathogens especially in conventional wastewater treatment facilities is increasingly becoming of interest and is a subject of ongoing investigation in our laboratory.

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