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Review

Bioremediation of polluted wastewater influent: Phosphorus and nitrogen removal

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The world is faced with problems related to the management of wastewater due to extensive industrialization, increasing population density and a highly urbanized society. The quality of wastewater effluents is responsible for the degradation of receiving water bodies, such as lakes, rivers, streams. In order to meet Goal 7 of the Millennium Development Goals "ensure environmental sustainability" and maximization of the health and environmental benefits associated with the use and discharge of wastewater, several legislations and guidelines have been developed, both at international and national levels. The two main processes for the removal of impurities from wastewater influents are chemical and biological. Because of the many drawbacks of chemical wastewater treatment, biological treatment is advocated in the last few decades. Biological phosphorus and nitrogen removal systems have been extensively investigated for municipal wastewater treatment over the past decades. Several questions have been raised on the role of the different microbial groups in the removal of nitrogen and phosphorus in activated sludge systems. In this paper, an attempt is made to give an overview of the population dynamics of the activated sludge. The role of the different microbial groups present in the activated sludge systems, with particular reference to bacteria and protozoa in the removal of phosphorus and nitrogen is also reviewed. This will enhance decisions that are science-based with respect to biological wastewater treatment.

Key words: Activated sludge, nitrogen, phosphorus, wastewater, bacteria, protozoa.

INTRODUCTION

The two main treatment processes for the removal of impurities from wastewater are chemical and biological. The main advantages of chemical treatment over biological processes are mineralization of non-biodegradable compounds and smaller reactor volume. Despite the advantages, the disadvantages of chemical treatment (Table 1) are enormous (Josephs and Edwards, 1995; Metcalf and Eddy, 2003).

Because of the above drawbacks of chemical treatment processes, biological treatment of wastewater is advocated in the last few decades. All biological wastewater treatment processes take advantage of the ability of microorganisms to use diverse wastewater constituents to provide the energy for microbial metabolism and the building blocks for cell synthesis (Schultz, 2005). The common wastewater treatment processes include trickling filters, lagoons, stabilization ponds, constructed wetlands and activated sludge processes (EPA, 1993; Gray, 2002).

Presently, the activated sludge system is the most widely used biological treatment process for both domestic and industrial wastewaters. The system is a biological method that is performed by a mixed community of microbes and uses the metabolic reactions of the microbes to produce high-quality effluent in an aquatic environment (Water Environment Association, 1987; Muyima et al., 1995; EPA, 2002). This is achieved by converting and removing substances that have an oxygen demand. In the liquid side of the treatment scheme, it typically follows pretreatment and primary clarification, although, depending on the wastewater

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Table 1. Overview of chemical and biological processes of wastewater treatment .

	Chemical	Biological
Advantages	1. Mineralization of non biodegradable compounds 2. Small reactor volume	1. Low capital and operating cost 2. Reduction of aquatic toxicity 3. Operational flexibility 4. Reduction in sludge production 5. Reduction in filamentous growth 6. Improved sludge settle ability 7. Improved sludge dewatering 8. Reduction in oxygen requirement 9. Suitable for simultaneous nitrogen and phosphorus removal
Disadvantages	1. Increased aquatic toxicity 2. Increased sludge production 3. Increased filamentous growth 4. Decreased sludge settle ability 5. Decreased sludge dewatering characteristics 6. Increased cost	1. Inhibition from non-biodegradable compounds 2. Slow digestion rates 3. Large storage tanks requirement 4. Provision of enabling environment for survival of microorganisms
Common processes	1. Chemical precipitation 2. Coagulation/flocculation 3. Chemical oxidation 4. Ion exchange, 5. Solvent extraction	1. Onsite treatment processes- pit latrine, composting latrines, pour flush toilets, septic tanks 2. Offsite treatment processes- trickling filters, aerated lagoons, waste stabilization ponds, constructed wetlands, activated sludge system

characteristics and plant design, the primary clarifier may be omitted (Water Environment Federation, 1996; Eikelboom and Draaijer, 1999; Gray, 2002; Sci-Tech. Encyclopedia, 2007).

The overall reactions occurring in the activated sludge system are determined by the composite metabolism of all the microorganisms in the activated sludge (Sci-Tech. Encyclopedia, 2007). The metabolic process consists of the separate, yet simultaneously occurring, reactions of synthesis and respiration. Synthesis is the use of a portion of the waste matter (food) for the production of new cells (protoplasm), while respiration is the coupled release of energy through the conversion of food material to lower energy-containing compounds, typically carbon dioxide, water and possibly the various oxidized products forms of nitrogen. The precise nature of the products formed depends to some extent on process design, including reaction time, temperature and process loading of the system (Eikelboom and Draaijer, 1999; Sci-Tech. Encyclopedia, 2007).

In municipal wastewater treatment systems, the common water quality variables of concern are biological oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), suspended solids, nitrate, nitrite and ammonia nitrogen, phosphate, salinity and a range of other nutrients and trace metals (Decicco, 1979; Brooks, 1996). The presence of high concentrations of these pollutants (mostly nitrogen and phosphorus) above the critical values stipulated by national and international regulatory bodies is considered unacceptable in receiving water bodies. This is because, apart from causing a major drawback in wastewater treatment systems, they also lead to eutrophication and various health impacts in humans and animals (EPA, 2000; CDC, 2003; Runion, 2008).

Nutrient-induced production of aquatic plants in receiving water bodies has the following detrimental consequences: (1) Algal clumps, odour and decolouration of the water, thus interfering with recreational and aesthetic water use; (2) Extensive growth of rooted aquatic life interferes with navigation, aeration and channel capacity; (3) Dead macrophytes and phytoplankton settle to the bottom of a water body, stimulating microbial breakdown processes that require oxygen, thus causing oxygen depletion; (4) Extreme oxygen depletion can lead to the death of desirable aquatic life; (5) Siliceous diatoms and filamentous algae may clog water treatment plant filters and result in reduced backwashing, and (6) Algal blooms may shade and submerge aquatic vegetation, thus reducing or eliminating photosynthesis and productivity (Atlas and Batha, 1987; Ratsak et al., 1996; Kurosu, 2001; Alm, 2003; Mbewele, 2006; McCasland et al., 2008).

In this review, the general principle of the biological wastewater treatment is explored with particular reference to the activated sludge treatment system. The exploration is based on the dynamic population and their roles in the removal of phosphorus and nitrogen in the activated sludge system.

DYNAMIC POPULATION OF ACTIVATED SLUDGE SYSTEMS

The major microorganisms found in wastewater influents are viruses, bacteria, fungi, protozoa and nematodes. Although the presence of some of these organisms in water is considered to be critical factors in the spread of diseases, they play beneficial roles in wastewater effluents. Traditionally, these organisms are used in secondary treatment of wastewater in the removal of dissolved organic compounds. Their presence during the different treatment phases can enhance degradation of solids, resulting in less sludge production. Apart from solid retention, they are also involved in nutrient recycling, such as phosphorus, nitrogen and heavy metals. If the nutrients that are trapped in dead materials are not broken down by these microbes, they will never be available to help sustain the life of other organisms (Haas et al., 1996; Kris, 2007).

The constant aeration, agitation and recirculation in an activated sludge system create an ideal environment for the numerous microorganisms present, while inhibiting the growth of larger organisms. Bacteria, fungi, rotifers, viruses, nematodes and protozoa are commonly found in the activated sludge, though all may not exist in any single system.

Despite the presence of other micro-organisms, bacteria are typically considered to be the significant organisms, consuming the organic matter in wastewater. Algae, because of their need for light, rarely exist in mixed liquor (Gray, 2002; Richard et al., 2003).

The cell make-up of the organisms in the activated sludge system depends on both the chemical composition of the wastewater and the specific characteristics of the organisms in the biological community (Water Environment Association, 1987). The constant agitation in the aeration tanks and sludge recirculation are deterrents to the growth of higher organisms, thus the biological mass/component of activated sludge comprises bacteria, fungi, protozoa, rotifers, and, in addition, some metazoan, such as nematode worms (Curds and Cockburn, 1970a, b; Anderson and Griffic, 2001). The species of microorganisms that dominate a system depends on environmental conditions, process design, the mode of plant operation and the characteristics of the secondary influent wastewater.

Bacteria

Bacteria are of the greatest numerical importance in the activated sludge system. The preponderance of bacteria living in activated sludge is facultative, that is, they are able to live in either the presence or absence of oxygen (Spellman, 1997; CDC, 2002; Absar, 2005). Although both heterotrophic and autotrophic bacteria reside in activated sludge, the former predominate.

Heterotrophic bacteria obtain energy from the carbonaceous organic matter in the influent wastewater for the synthesis of new cells and also release energy via the conversion of organic matter compounds such as CO_2 and H_2O. Important heterotrophic bacteria genera in activated sludge are *Achromobacter, Alcaligenes, Arthrobacter, Citromonas, Flavobacterium, Pseudomonas, Zoogloe* and *Acinetobacter* (Water Environment Association, 1987; EPA, 1996, Oehmen et al., 2007).

Bacteria are responsible for the stabilization of wastes coming into a treatment plant. Many of these bacteria form floc particles or clusters of bacteria that break down waste. The floc particles also serve as sites on which waste can be absorbed and broken down later. In addition, filamentous bacteria form trichomes or filaments. These chains of bacteria provide a backbone for the floc particles, allowing the particles to grow in size and withstand the shearing action in the treatment process. When filamentous bacteria are present in excessive numbers or length, they often cause solid/liquid separation or settleability problems (Gray, 2002; Paillard et al., 2005).

Fungi

Fungi are multicellular organisms that are also constituents of the activated sludge. Under certain environmental conditions in a mixed culture, they metabolize organic compounds and can successfully compete with bacteria. Also, a small number of fungi are capable of oxidizing ammonia to nitrite, and fewer still to nitrate. The most common sewage fungus organisms are *Sphaerotilus natans* and *Zoogloea* sp (Painter, 1970; LeChevallier and Au, 2004).

Rotifers

Rotifers are the most abundant macro-invertebrates found in the activated sludge process. They are able to consume both microbes and particulate matter. They are strict aerobes and more sensitive to toxic conditions than bacteria. Rotifers are found only in very stable activated sludge environments (Curds and Cockburn, 1970a; LeChevallier and Au, 2004).

Viruses

Viruses are also found in wastewaters, particularly human viruses that are excreted in large quantities in faeces. Viruses that are native to animals and plants exist in smaller quantities in wastewater, although bacterial viruses may also be present (Grabow, 1968; Toze, 1997; Gomez et al., 2000; Okoh et al., 2007).

Nematodes

Nematodes are aquatic animals present in fresh, brackish and salt waters and soil worldwide (WHO, 1998). Freshwater nematodes can be present in sand filters and aerobic treatment plants. They are present in large numbers in secondary wastewater effluents, biofilters and biological contractors. Freshwater nematodes inhabit freshwater below the water table with species utilizing oxygen dissolved in fresh water. Nematodes are part of the ecosystem, serving as food for small invertebrates (Painter, 1970; WHO, 1998; Metcalf and Eddy, 2003). Nematodes crawl onto floc particles and move in whip-like fashion when in the free-living mode. They secrete a sticky substance to be able to anchor to a substrate (media), so that anchored nematodes can feed without interference from currents or turbulence. A lack of nematode activity can be one of the bio-indicators of a toxic condition that may be developing in the treatment process (Water Environment Association, 1987; EPA, 1996; WHO, 1998).

Protozoa

Protozoa are microscopic, unicellular organisms. They are found in the activated sludge process and perform many beneficial functions in the treatment process, including the clarification of the secondary effluent through the removal of bacteria, flocculation of suspended material and as bio-indicators of the health of the sludge. Protozoa that inhabit the activated sludge process are capable of movement in at least one stage of their development (Amaral et al., 2004; Ingraham and Ingraham, 1995).

Protozoa are useful biological indicators of the condition of the activated sludge (Curds, 1970a). Being strict aerobes, they prove to be excellent indicators of an aerobic environment (though some protozoa are capable of surviving up to 12 h in the absence of oxygen). They also act as indicators of a toxic environment, as they exhibit a greater sensitivity to toxicity than bacteria. A clue that toxicity may be a problem in a system is the absence of or a lack of mobility of these indicators in activated sludge. The existence of large numbers of highly evolved protozoa in the biological mass of an activated sludge system is the hallmark of a well-operated and stable activated sludge system (Fried et al., 2000; Fried and Lemmer, 2003; Metcalf and Eddy, 2003).

They can be placed into one of five groups, according to their means of locomotion. These groups are the free-swimming ciliates, crawling ciliates and stalked/sessile ciliates, flagellates and amoebae (Curds and Cockburn, 1970a; b; Caccio et al., 2003). The three types of ciliates are free-swimming ciliates, crawling ciliates and stalked ciliates. All of these three have short hair-like structures or cilia that beat in unison to produce water current for locomotion and capturing bacteria. The water current moves suspended bacteria into a mouth opening (Bitton, 1999; Ingraham and Ingraham, 1995).

Free-swimming ciliates such as *Litonotus* and *Paramecium* possess cilia on all surfaces of the body and can usually be found suspended or swimming freely in the bulk solution. Crawling ciliates such as *Aspidisca* and *Euplotes* possess cilia only on the ventral or belly surface where the mouth opening is located. Crawling ciliates are usually found on floc particles (Gerardi, 2007). Stalked ciliates, such as *Carchesium* and *Vorticella*, have cilia around the mouth opening only and are attached to floc particles. They have an enlarged anterior portion and a slender posterior portion. The beating of the cilia and the springing action of the stalk produce a water vortex that draws dispersed bacteria into the mouth opening (Bitton, 1999; Gerardi, 2007).

Flagellated protozoa are oval in shape and have one or more whip-like structures or flagella. The whipping action propels the protozoa through the activated sludge in a cork-screw pattern of locomotion. While in motion, flagellates accidentally hit substrate. With decreasing numbers of suspended bacteria, flagellates find it more difficult to find substrate (Sherr et al., 1998; Gerardi, 2007).

Two types of amoebae commonly found in activated sludge processes are the naked amoebae, such as *Actinophyrs, Mayorella* and *Thecamoeba*, and the shelled or testate amoebae, such as *Cyclopyxis* and *Difflugia*. The naked amoeba has no protective covering. The testate amoeba has a protective covering or testate that consists of calcified material (Curds and Cockburn, 1970; Richard et al., 2003, Gerardi, 2007).

In the aeration tank of biological processes, a true trophic web is established. The biological system of these plants consists of populations in continuous competition with each other for food. The growth of decomposers, prevalently heterotrophic bacteria, depends on the quality and quantity of dissolved organic matter in the mixed liquor (Mara and Horan, 2003). For predators, on the other hand, growth depends on the available prey.

Dispersed bacteria are thus food for heterotrophic flagellates and bacterivorous ciliates, which, in turn, become the prey of carnivorous organisms. The relationships of competition and predation create oscillations and successions of populations until dynamic stability is reached. This is strictly dependent on plant management choices based on design characteristics aimed at guaranteeing optimum efficiency (Mara and Horan, 2003; Metcalf and Eddy, 2003).

Some ciliates, however, are predators of other ciliates or are omnivorous, feeding on a variety of organisms including small ciliates, flagellates and dispersed bacteria. All bacterivorous ciliates rely on ciliary currents to force suspended bacteria to the oral region (Curds and Cockburn, 1970b). Ciliated protozoa are numerically the most common species of protozoa in activated sludge, but flagellated protozoa and amoebae may also be present. The species of ciliated protozoa most commonly observed in wastewater treatment processes include *Aspidisca costata, Carchesium polypinum, Chilodonella uncinata, Opercularia coarcta, Opercularia microdiscum, Trachelophyllum pusillum, Vorticella convallaria* and *Vorticella microstoma* (Curds and Cockburn, 1970a; Caccio et al., 2003; Amaral et al., 2004).

NUTRIENT REMOVAL IN THE ACTIVATED SLUDGE SYSTEM

Phosphorus removal

In activated sludge systems, enhanced biological phosphorus removal is widely used to remove phosphorus from wastewater (McMahon et al., 2002). Microorganisms that are largely responsible for phosphorus removal are referred to as polyphosphate accumulating organisms (PAOs). These organisms have the ability to store phosphate as intracellular polyphosphate, leading to phosphate removal from the bulk liquid phase in the waste activated sludge (Ekama et al., 1984; Jeon et al., 2003; Oehmen et al., 2007).

Biological phosphate removal in the activated sludge process was first reported by Levin et al. (1972). The first attempt to identify microorganisms involved in phosphate removal was over 30 years ago (Barker and Dold, 1996). *Acinetobacter*, which was first described by Fuhs and Chen (1975), was the first bacterium that was proposed to be responsible for phosphate removal; hence most subsequent studies were focused on this bacteria genus (Barnard, 1975; Ekama et al., 1984; Beacham et al., 1990; Auling et al., 1991; Kavanaugh, 1991; Wentzel, 1991; Bark et al., 1992). However, through the use of several molecular techniques, such as fluorescence *in situ* hybridization (FISH), 16s rRNA-based clone libraries or denaturing gradient gel electrophoresis, a number of high-diversity phylogenetic groups of bacteria are now known to be present in laboratory and full-scale enhanced biological phosphate removal systems (Hascoet et al., 1985; Carucci et al., 1995; Cloete and Steyn, 1998; Zeng et al., 2003).

Studies have shown that *Acinetobacter* sp. is of little significance in phosphate removal when compared to members of other phylogenetic groups of bacteria (Wagner et al., 1994; Momba and Cloete, 1996; Band et al., 1999; Jeon et al., 2003). *Microlunatus* sp., *Lampropedia* sp. and *Tetraphaera* sp. are other microorganisms that were also hypothesized to play an important role in phosphate removal but were later discovered not to demonstrate characteristics of PAOs (Seviour et al., 2003; Oehmen et al., 2007). Some authors (Toerien et al., 1990; Tian-Ming et al., 2007) have reported the *Proteobacteria* to be the most dominant PAOs in synthetic wastewater and in clarified effluent of a conventional activated sludge system. Other bacteria that have been implicated in dominant phosphate removal include *Aeromonas*, *Vibrio*, *Pseudomonas* and the coliforms (Kavanaugh, 1991; Momba and Cloete, 1996; Snaidr et al., 1997; Seviour et al., 2003).

Although there is a lack of information on the roles protozoa play in nutrient removal in activated sludge systems, their roles in nutrient recycling in aquatic systems have evolved (Andersen et al., 1986). Earlier researchers have shown that high concentrations of ciliates and other protozoa are characteristics of decomposing sewage (Johannes, 1964; 1965; Caron et al., 1985; Andersen et al., 1986). Johannes (1964, 1965) was the first investigator to emphasize the role of protozoa in the regeneration of phosphorus, a role traditionally assigned mainly to bacteria (Fenchel, 1986, 1988). Although Johannes' findings were initially criticized, more studies after two decades were able to make it clear that both ciliates and microflagellates regenerate nutrients in large quantities while grazing (Gast and Horstmann, 1983; Sherr et al., 1983; Andersson et al., 1985, 1986; Caron et al., 1985).

Over the past years, protozoa and phytoplankton, at the base of the food web, have become well established as dominant phosphorus remineralizers in aquatic systems. This can be attributed in part to their high biomass-specific metabolic rates and their lower gross growth efficiencies, relative to bacteria, which were previously assumed to be the sole nutrient remineralizers (Caron and Goldman, 1993; Landry, 1993). Several heterotrophic protozoan species, such as *Euplotes* sp, *Strombidium* sp, *Poterioochromonas* sp, *Paraphysomonas* sp and *Spumella* sp., have been implicated in phosphorus assimilation in both freshwater and marine ecosystems (Johannes, 1965; Dolan et al., 1997).

Nitrogen removal

The biological nitrogen removal process is the most common method for removing nitrogen in the activated sludge system. The process generally results from the combined processes of nitrification and denitrification (Ekama et al., 1984; Wentzel, 1991; Carrera et al., 2003; Oguz, 2005). Nitrification in wastewater treatment is commonly regarded as a two-step process. The first step is the conversion of ammonia to nitrite by *Nitrosomonas* while the second step is the further oxidation of nitrite to nitrate, which is commonly accepted to be carried out by *Nitrobacter* (Antoniou et al., 1990; Sedlak, 1991). Both of these genera are autotrophs, although *Nitrobacter* is not an obligate autotroph and hence can grow using organic carbon.

There are two groups of nitrifiers (autotrophic and heterotrophic). The whole process of nitrification and growth is balanced very delicately, as both groups of nitrifiers are inhibited by high concentrations of their own substrates and have little energy to spare for high-affinity uptake systems. It has been estimated that 80% of the energy generated by autotrophs is used to fix carbon dioxide (Painter, 1970; Prosser, 1989; Sabalowsky, 1999). Unlike autotrophic nitrification where nitrification is required in order to generate energy necessary for growth, it is generally accepted that heterotrophic nitrification is not linked to cellular growth (Prosser, 1989; Pennington and Ellis, 1993).

Biological denitrification enables the transformation of oxidized compounds by a wide spectrum of heterotrophic bacteria that convert nitrate to harmless nitrogen gas (Foglar et al., 2005). The necessary condition for denitrification to take place in activated sludge systems is the presence of a facultative microbial mass. These organisms are characterized by the fact that they can use either oxygen or nitrate as an oxidant for organic matter. Many common denitrifiers found in activated sludge systems appear to be capable of heterotrophic nitrification, which appears to occur simultaneously with denitrification (Robertson et al., 1988; Prosser, 1989; Pel et al., 1997).

Earlier workers (Blasczyk, 1993; Lazarova et al., 1994; Martienssen and Schops, 1999) have reported the effectiveness of several heterotrophic bacteria in denitrification. Common bacteria genera that have been reported to be denitrifiers in activated sludge systems include *Achromobacter, Aerobacter, Alcaligenes, Bacillus, Brevibacterium, Denitrobacillus, Flavobacterium, Lactobacillus, Micrococcus, Brevibacterium, Pseudomonas, Spirillum, Proteus, Xanthomonas, Staphylococcus* and *Paracoccus* (Gray, 1990; Metcalf and Eddy, 1999; Sabalowsky, 1999).

Although there is a lack of data on the role of protozoa in nitrogen removal in activated sludge systems, studies carried out in aquatic ecosystems have shown that ciliates and phagotrophic microflagellates regenerate and mineralize nitrogen in large quantities while grazing (Gast and Horstmann, 1883; Andersen et al., 1986). Other investigators have revealed the fact that flagellated and ciliated protozoa account for a major portion of nitrogen recycling (uptake and excretion) in both marine and freshwater habitats (Caron and Goldman, 1988; Pace and Funke, 1991).

According to some authors (Harrison, 1978; Verity, 1985), the small size and high specific metabolism of protozoa imply that they are primary agents of nitrogen (in the form of ammonia and nitrate) mineralization. Andersen et al. (1986) investigated the recycling of nitrogen by the microflagellate *Paraphysomonas imperforate*.

There is now increasing evidence that although protozoa prey on bacteria, they play a role in recycling nitrogen from all grazed microbes (Caron and Goldman, 1988, 1993; Landry, 1993). Other protozoa that have been reported to have nitrogen assimilation ability in aquatic environments include *Tintinopsis* sp., *Pseudobodo* sp., *Poterioochromonas* sp., *Paramecium* sp., *Paraphysomonas* sp., *Oxyrrhis* sp., *Monas* sp. and *Euplotes* sp. (Johannes, 1965; Dolan, 1997).

CONCLUSION

The two fundamental reasons for treating wastewater are to prevent pollution of water sources and to protect public health by safeguarding water supplies against the spread of diseases. Water quality assurance is an integral part of environmental quality management. Wastewater treatment is one of the strategies for water quality management. In recent years, due to the many drawbacks of chemical treatment, biological treatment had been advocated. This is to avoid unpleasant conditions for natural water resources.

Biological phosphorus and nitrogen removal in activated sludge systems have been extensively investigated. Although, there is a general consensus on the active biomass in the activated sludge system, there are conflicting reports on the roles of bacteria and protozoa in nutrient removal. Most investigations on nutrient removal concentrated on bacteria, and their roles in enhanced removal of phosphorus and nitrogen is well reported.

Despite this, the roles protozoa play in nutrient removal in aquatic systems have also evolved. In the past, the sole role of protozoa has been reported as their effectiveness in the purifying process by feeding on dispersed bacteria, thus eliminating them. It is however also reported in the past years that significant fraction of nutrient mineralization (excretion and uptake) taking place in activated sludge systems is due to protozoan activity.

Although activated sludge systems are composed of large numbers of microorganisms that actively contribute in the removal of nutrient responsible for eutrophication of water resource, there is still the need for more nutrient-related research monitoring in order to achieve unpolluted wastewater discharge. This will help to ensure science-based decisions with respect to effluent standards and limitations, as set by regulatory bodies and a clearer understanding and explanation of observation on microbial life in wastewater treatment systems.

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