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Review Article

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Nanotechnology in Wastewater Treatment; Influence of Nanomaterials on Microbial Systems

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During the last few decades there is growing interest for the use of nanoparticles in water and wastewater treatment technology, due to their distinctive physiochemical characteristics. This review outlines the conceivable opportunities regarding the interaction of nanoparticles with the biological systems during water and wastewater treatment processes. An overview on the recent advances in application of nanoparticles in biofouling prevention and disinfection is provided. Recent advances in nanoscience allow for development of nanosensors for biomonitoring and diagnostic purposes. Due to remarkable use of nanoparticles, wastewater treatment plants received considerable amount of NPs such as TiO_2, ZnO, N_2O, AgO, CuO, SiO_2, Al_2O_3 and CeO_2. Toxic effects of nanoparticles on structural and physiological activity of various microbial communities including; nitrification, denitrification and phosphorus removal processes are critically reviewed.

Introduction

Water is the most vital substance in our life. Reliable and sustainable access to clean, safe and affordable water is considered to be one of the most essentials for human being. Approximately, one-sixth of the world's population suffers from access to clean drinking water (WHO, 2004). Nowadays, overpopulation, limited water resources, pollution and lack of water sustainability are considered as the most common difficulties facing human needs.

Therefore, an urgent need is required to develop an innovative technology to provide clean and affordable water to meet human needs.

Over the last few decades, nanotechnology is emerging as a rapidly growing sector of a "knowledge-based economy" due to unique physiochemical properties of nanomaterial. This technology gained a tremendous impetus due to its capability of reformulating the particle of metals into new nano-sized form, with dimension less than 100 nm in size. Hence, it is used in manufacture of a wide range of products and in wastewater treatment (Bora and Dutta, 2014; Wang *et al*., 2014; Qu *et al*., 2013;Yang *et al*., 2013; Yang *et al*., 2012; Guzman *et al*., 2006). Due to remarkable use of nanoparticles, wastewater treatment plants received considerable amount of nanoparticles such as TiO_2, ZnO, N_2O,AgO, CuO, SiO_2, Al_2O_3 and CeO_2, with potential risk to human and environment (Brar *et al*., 2010; Klaine *et al*., 2008; Nel *et al*., 2006).

Recently, implementation of nanotechnology in wastewater treatment enabled high performance, reasonable water and wastewater treatment solutions that less relies on large infrastructures (Qu *et al*., 2013). Wide range of nanomaterials tested regarding resistance of biofouling, elimination of toxic metals, organic and inorganic pollutants, pathogen detection as well as disinfection (Amin *et al*., 2014; Prachi *et al*., 2013; Qu *et al*., 2013; Richards *et al*., 2012; Tiwari *et al*., 2008). From economic point of view, nanotechnology allow for utilization of the most challenging water resources and energy conservation. Unfortunately, costs of this new technology should be properly managed due to competition with traditional wastewater treatment technologies (Crane *et al*., 2012).

This review outlines the conceivable opportunities regarding applications of nanoparticles in biofouling prevention, disinfection and as sensors for pathogen detection (Table 1). An overview on recent advances to discuss the major interaction of nanoparticles with biological systems in water and wastewater treatment is provided. Toxicity effect of nanoparticles on structural and physiological activity of microbial communities as well as nitrification, denitrification and phosphorus removal is critically reviewed.

Biofouling Resistance

Nanocomposite Membranes

Among major challenges of membrane technology in wastewater treatment is the membrane biofouling and virus penetration (Barhate and Ramakrishna, 2007). Recently, incorporation of functional nanomaterials into ultrafilteration membrane systems allow for improvement of membrane properties especially; mechanical and thermal stability, membrane permeability and fouling resistance through increasing membrane hydrophobicity. Nanoparticles used for this purposes are; Al_2O_3, TiO_2, zeolite, nano-Ag and CNTs, bi-metallic nanoparticles and TiO_2 (Bottino *et al*., 2001; Ebert *et al*., 2004; Bae and Tak, 2005; Maximous *et al*., 2010; Pendergast *et al*., 2010).

For example; incorporation of silver nanoparticles into polymeric membranes led to inhibition of bacterial growth, biofilm formation and inactivated viruses (Zodrow *et al*., 2009; De Gusseme *et al*., 2011; Mauter *et al*., 2011). Applications of carbon nanotubes CNTs in inactivation of bacteria was reported (Brady-Estevez *et al*., 2008). Ahmed *et al*. (2012) recorded that more than 90% of bacteria inactivated by using polyvinyl-N-carbazole-SWNT nanocomposite at 3 wt% of SWNT. In addition, hydrophobicity and permeability of the polysulfone membranes were increased by addition of oxidized MWNT in a very low amount (Choi *et al*., 2006b). On the other hand, membranes incorporated with photocatalytic nanoparticles (TiO_2 nanoparticles) were developed to degrade some contaminants (Choi *et al*., 2006a). Interestingly, nanocatalysts based on semiconductor materials, zero-valence metal and bimetallic nanoparticles used in degradation of many environmental contaminants such as PCBs (polychlorinated biphenyls), azo-dyes, halogenated aliphatic, organochlorine pesticides, halogenated herbicides, and nitro aromatics (Xin *et al*., 2011; Wu and Ritchie, 2008; Wu *et al*., 2005).

Thin Film Nanocomposite Membranes (TFN)

Breakthrough in efficiency of thin film nanocomposite membranes (TFN) achieved by the use of nano-zeolites for enhancement of membrane permeability. Jeong *et al*. (2007) reported the increase of salt rejection up to 80% over TFC was with salt rejection of 93.9%. Furthermore, Lind *et al*. (2010) reported that TFC incorporated with 250 nm nano-zeolites (0.2%wt) enhanced permeability and increased salt rejection up to 80% than commercial reverse osmosis membranes. Interestingly, nano-zeolites used as carriers for antimicrobial agents such as Agþ, which incorporate antifouling property to the membrane (Lind *et al*., 2009).

Moreover, Lee *et al*. (2008) revealed that incorporation of nano-TiO_2 into the TFC membrane slightly increased rejection process. Indeed, reduction of biological contaminants, biological fouling and inactivation of microorganisms achieved by photocatalyic activity of TiO_2 upon UV irradiation(Chong *et al*., 2010). However, membrane material may encounter negative effects due to long-term exposure (Chin *et al*., 2006).Carbon nanotubes CNTs applied in TFN membranes due to their antimicrobial activities (Tiraferri *et al*., 2011).Moreover, TiO_2 nanotubes showed great efficiency in decomposition of organic compounds (Macak *et al*., 2007).

Disinfection

It is well recognized that traditional water disinfection methods might lead to adverse effect. Chlorination and ozone may lead to generation of toxic or mutagenic by-products, hence increase risk of cancer (Monarca *et al*. 2004). On the other hand, germicidal activity of ultraviolet (UV) irradiation depends on the total suspended solids that may reduce the activity (Chang *et al*. 1985). Therefore, progressive increase in research focuses on development of a novel water disinfection method to overcome limitations encountered by other methods (Qu *et al*., 2013). Variety of nanoparticles, e.g.; Ag, nano-ZnO, nano-TiO_2 and carbon nanotubes potentially used in disinfection of water and wastewater (Li *et al*. 2008; Vahabi *et al*. 2011; Baruah *et al*.,2012). Elmi *et al*. (2014) synthesized ZnO nanoparticles by mechano-chemical and sol–gel methods. The nanoparticles were successfully used as antimicrobial agent in wastewater treatment, preferably with UV disinfection to improve water quality.

Antimicrobial Activity

Antimicrobial activity of many nano-sized metal ions (nanoparticles) allows for design of nanocatalyts such as; AgCCA catalyst, N-doped TiO_2 and ZrO_2. The high efficiency of nanoparticles led to removal of microbial contaminants from wastewater (Shalini *et al*., 2012). TiO_2-AGS composite is efficient in remediation of toxic Cr (VI) ions from wastewater. Indeed, enhanced biodegradation of halogenated organic compounds; based on specific interaction between halogens and Pd nanoparticles was reported (Zhang *et al*., 2012). Interestingly, the ferromagnetic properties of nanocatalysts are highly beneficial in their recycle and reuse. Also, silver-based nanocatalyst was efficiently used for degradation of organic dyes (Wu *et al*., 2010). Indeed, photacatalytic activity of Pd incorporated ZnO nanparticles allows for removal of *E. coli* from wastewater (Khalil *et al*., 2011). In another approach, palladium nanoparticles (PdNPs) used as catalyst for in situ bioremediation and reduction of highly toxic Cr (VI) to less toxic Cr (III) ions (Omole *et al*., 2009). Interestingly, the reactivity of catalyst can be enhanced by combination with nanosorbents i.e. combining sorption and degradation of contaminants in wastewater.

The possible usage of nanomaterials including; nano-Ag, nano-ZnO, nano-TiO_2, nano-Ce_2O_4, and CNTs in disinfection and pathogens control, with clear explanation for the mechanism, limitations and application in wastewater treatment, has previously revised by Li *et al*., (2008) and updated by Qu *et al*., (2013). Evidently, the drastic effect of nanoparticles on microbial cells ranged from potential damage to proteins and cell membrane, suppression of DNA replication, generation of toxic reactive oxygen species and inactivation of enzymes (Wang *et al*., 2014; Vecitis *et al*., 2010, Xiu *et al*., 2011, Feng *et al*., 2000, Liau *et al*., 1997).

The crucial role of nanomaterials during wastewater treatment relays on their antimicrobial activity during disinfection, membrane biofouling control, as well as control of biofilm formation on various surfaces. Peter-Varbanets *et al*., 2009 reported the possible use of Ag-nanoparticles for disinfection and against waterborne pathogens by incorporation into ceramic microfilters. Indeed, commercial devices based on Ag-nanoparticles are already available e.g. MARATHON_ and Aquapure_ systems. Indeed, novel antimicrobial CNT filter designed for removal of bacteria and viruses (Brady-Estevez *et al*., 2010). The retained microbes are inactivated by CNTs within hours. However, application of small voltage can drastically decrease the time to few seconds (Rahaman *et al*., 2012). Advantageous use of nanomaterials as antimicrobial agent allows for their incorporated into storage tanks and distribution pipes surfaces to prevent microbial contamination, biofilm formation and corrosion (Qu *et al*., 2013). Recently, antimicrobial peptides synthesized as nanoparticles through self-immobilization mediated by biomineralization reactions to increase stability and protection (Eby *et al*., 2008). Brogden (2005) mentioned that the biocidal effect of peptide nanoparticle is due to interaction between the nanoparticles with the bacterial cell membrane.

Sensing and Monitoring

Due to extreme importance of water and wastewater monitoring specially regarding detection of pathogenic microorganisms, there is crucial need for innovative sensors. These sensors should meet high quality standards including; high sensitivity, selectivity, reliability, ease-of-use and quick response in contaminant detection. Advances in nanotechnology are having a significant impact on the field of diagnostics and biomolecular detection (Table 1) (Fortina *et al*., 2005). Interestingly, new synthesis and fabrication of nanomolecules allows for possible tailor of their binding affinities for various biomolecules through surface modification and engineering (Rosi and Mirkin, 2005).

Pathogen Detection

In the meantime, most of conventional indicator systems are insensitive enough regarding diagnosis of many human pathogens in drinking water (Theron *et al*., 2010). However, the development of pathogen sensors could be a possible solution. Advances in sensors technology based on nanomaterial focused on the development of sensitive and fast responsive recognition elements and signal transducer, due to their unique physiochemical characteristics (Vikesland and Wigginton, 2010). Recently, nanosensors developed for detection of whole cells and biomolecules (Theron *et al*., 2010, Vikesland and Wigginton, 2010). Nanomaterials such as magnetic nanoparticles, CNTs, noble metals, Quantum dots (QDs) and dye-doped nanoparticles used in pathogen detection(Yan *et al*., 2007, Sukhanova *et al*.,2004; Petryayeva and Krull, 2011; Jain *et al*., 2006).

Nowadays, CNTs gain tremendous attention in design of electrodes due to ease electrochemical detection through promotion of electron transfer, interactions and high adsorption capacity (McCreery, 2008, Collins *et al*., 2000). CNTs applied as a single nanotube electrode, or incorporated into electrodes via random or aligned coating or used in nanoscale (Yang *et al*., 2010, Heller *et al*., 2008). Unfortunately, heterogeneity and nonspecific binding are the major challenges for application of CNTs nanosensors in water and wastewater treatment.

Table.1 Microbiological Applications of Nanotechnology in Wastewater Treatment

Process	Nanomaterial	Properties	Application
Biofouling resistance and disinfection	Nano-zeolite	Molecular sieve, higher hydrophilicity	Nanocomposite membranes
	Carbon Nanotubes	Antimicrobial activity, high mechanical and chemical activity, large surface area, ease of use	Antibiofouling membranes and water disinfection
	Nano-TiO_2	Photocatalytic activity, low human toxicity, stability, low cost	Reactive membranes and nanocomposite membranes, photocatalytic reactors, disinfection systems
	Nano-Ag	Wide-spectrum antimicrobial activity, low toxicity to human, ease to use	Water disinfection, Antibiofouling surface
Sensing and monitoring	Quantum dots (QDs) and dye-doped nanoparticles	Wide-adsorption spectrum	Optical pathogen detection
	Metal nanoparticles	Higher conductivity	Optical and electrochemical pathogen detection
	Carbon nanotubes	Higher surface area, high stability	Electrochemical pathogen detection

Adopted from Qu *et al*., 2013 and Cloete *et al*., 2010

Toxicity

Due to tremendous use of nanoparticles, wastewater treatment plants (WWTPs) based on activated sludge treatment process received considerable amount of NPs as TiO_2, ZnO, N_2OAgO, CuO, SiO_2, Al_2O_3 and CeO_2 (Chen *et al*., 2012a, 2012b; Choi and Hu, 2008; Hou *et al*., 2012; Zheng *et al*., 2012, 2011a,b). Possible removal of NPs from activated sludge in wastewater treatment via adsorption, aggregation and settling processes was reported (Hou *et al*., 2012; Stark *et al*., 2008). For example, tremendous application of nanoparticles (e.g. AgNPs) led to continuous release to environment and discharge to wastewater treatment facilities (Benn and Westerhoff, 2008; Blaser *et al*., 2008). It is estimated that the concentrations of AgNPs in effluent and sludge samples of WWTPs are 0.021 mg/L and 1.55 mg/kg, respectively (Gottschalk *et al*., 2009). The predicted effluent total silver concentrations in wastewater treatment range from 2 to 18 mg/L (Blaser *et al*., 2008). This value can be increased to 105 mg/L in case of industrial discharges (Shafer *et al*., 2009). Discharge may leads to decrease in the effectiveness of biological wastewater treatment process due to toxicity measures. Recently, Kiser *et al*. (2009) reported that most of NPs released into the environment retained in biological wastewater treatment systems.

Furthermore, widespread application of silver nanoparticles as antimicrobial agent might help in contamination of aquatic ecosystems. Das *et al*. (2012) recorded that bacterial growth and extracellular alkaline phosphatase were significantly reduced in nine Ag NP-exposed samples after 1 hour. After 48 h, bacterial growth was recovered by 40 to 250% at low AgNP concentrations. In contrast, addition of $AgNO_3$(from 0.01 to 2mg Ag/L)led to complete inhibition of bacterial growth over the 48h exposure.

Crucial effect of silica-based nanoparticles (e.g. SiO_2 NPs) on biological removal of nitrogen and phosphorus was discussed (Zheng *et al*., 2012, 2011a,b). As revealed by DGGE and qPCR assays, the structure of microbial community was significantly changed. Denitrifying enzyme activities was decreased due to chronic exposure of activated sludge to 50 mg/L of SiO_2 NPs nanoparticles, while phosphorus removal was not affected (Zheng *et al*., 2012).

Chen *et al*. (2014) indicated that the long term exposure of sludge fermentation system to higher concentration of TiO_2 NPs, exert marginal influence on bacteria and methanogenic archaea, probably due to exopolymers. However, the microbial activities as well as methane production were not affected. In another approach, no significant difference in activated sludge microbial community was detected after long term exposure of membrane bioreactor (MBR) process to higher concentration of silver nanoparticles (0.010 mg Ag/L) (Zhang *et al*., 2014). Although, nitrifying bacterial community as well as membrane fouling rate were not changed whereas, the extracellular polymeric substances (EPS) significantly increased after each nanosilver dosing. Zhang *el al*. (2014) suggested that microbial activities in activated sludge help to reduce nanosilver particles toxicity through adsorption or precipitation.

Analysis of microbial community in activated sludge after exposure to 1 mg/L AgNPs was monitored by 16S rRNA gene analysis through DGGE (Sun *et al*., 2013). No change in heterotrophic plate count was detected; however, specific microbial communities in the intact activated sludge flocs were highly sensitive to Ag NPs. This effect depends on the physical structure of the flocs, spatial distribution of microorganisms, and the community structures in the activated sludge flocs (Sun *et al*., 2013).

Musee *et al*. (2011) revealed that the effect of engineered nanoparticles ENPs on microbial community of WWTPs depends on several factors e.g. type, size, surface area, pH, temperature, composition of organic matter. To depict the potential risk of nanoparticles discharged into the environment, the impact of Ag NPs on the growth of *P. aeruginosa* in presence of humic acid material was investigated (Fabrega *et al*., 2009). It was recorded that the humic material caused a partial disaggregation of Ag NP aggregates by nanoscale film formation. Moreover, dissolved Ag reduced bacterial growth at 19 μM under different environmental conditions.

The short- as well as the long-term toxic effect of alumina nanoparticles (Al_2O_3 NP) on wastewater bacterial community (especially for nitrogen and phosphorus removal) was investigated by Chen *et al*. (2012a). These nanoparticles adsorbed on the activated sludge flocs, without affecting its integrity and viability. However, nitrification, denitrification and P removal were slightly influenced during short-term exposure to 1 and 50 mg/L of Al_2O_3 NPs. Nevertheless, long-term exposureto 50 mg/L Al_2O_3 NPs decrease the denitrifying enzyme activities or denitrification process and total nitrogen removal from 80.4% to 62.5%, although P removal and PHAs as well as glycogen were not affected.

For actual assessment of nanoparticles toxicity, a simulation study was carried out by Chen *et al*. (2014b). In this study, Cu NPs were added to influent before anaerobic treatment of wastewater for production of volatile fatty acids. Interestingly, no significant effect was documented. However, addition of Cu NPs directly to the sludge reactor reduced VFA production. Furthermore, incorporation of Cu NPs into wastewater treatment system improved sludge solubilization due to reduced sludge particle size as well as the link between sludge and microorganisms. Dramatic decrease in hydrolysis, acidification of the sludge and VFA production was recorded due to direct addition of NPs to the fermentation system (Chen *et al*., 2014b).

Conclusion and Perspectives

Although water plays a crucial role in every facet of human activity, it is becoming an increasingly scarce resource in many parts of the world. It is recognized that nanotechnology and their applications play an important role in resolving issues relating to water shortage and water quality. Owing to larger surface areas and size-dependent catalytic properties of nanomaterials, considerable efforts are being done to explore their application especially in wastewater treatment. Moreover, nanomaterials can be ligand to different chemical groups to increase affinity, recyclability, high capacity and selectivity. Although much attention focused on the development and potential benefits of nanomaterials in water and wastewater treatment processes, concerns raised regarding their potential human an environmental toxicity. Generally, a common framework for risk research, risk assessment, and risk management are still lacking. Nevertheless, development of novel nanomaterials will play a key role in ensuring sufficient and good quality water to meet the ever-increasing demand for drinking water. On the other hand, the high availability, low cost and high wide antimicrobial activity of many nanoparticles makes them attractive in water purification. Indeed, due to emergency of many waterborne diseases and limited safe water resources, there is a great demand for improvement of water filtration system. Nanofibers and nanobiocides can be useful solution to ensure safe and easy access drinking water. Due to recent advances in nanotechnology, next generation of diagnostic methods for pathogen detection is developing. However, some technical and practical problems need to be resolved before potential realization. This includes tight control over synthesis and functionalization. Besides, sample processing, detection of multiple agents in a single sample, as well as improving sensitivity and selectivity of the assays for significant application to complex environmental samples is highly recommended.

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