Journal of Water Process Engineering 16 (2017) 35–40

Contents lists available at ScienceDirect

Journal of Water Process Engineering

journal homepage: www.elsevier.com/locate/jwpe

Effects of nanosized titanium dioxide (TiO_2) and fullerene (C_{60}) on wastewater microorganisms activity

Liliane C.C. Auwerter [a,*], Sabeha K. Ouki [b], Manocher Asaadi [c], Achame Shana [d]

[a] Department of Civil and Environmental Engineering, Imperial College London, London, SW7 2AZ, United Kingdom
[b] Centre for Environmental Health Engineering, University of Surrey, Guildford, GU2 7XH, United Kingdom
[c] A.D. Technologies, Reading, United Kingdom
[d] Thames Water, Island Rd, Reading, Berkshire, RG2 0RP, United Kingdom

ARTICLE INFO

Article history:
Received 30 August 2016
Accepted 22 December 2016

Keywords:
Titanium dioxide
Fullerene
ENMs
Wastewater microorganisms

ABSTRACT

This paper investigates the impact of nano-TiO_2 and fullerene (C_{60}) on the activity of micro-organisms present in wastewater treatment works (WWTWs). Results showed that low concentration levels of nano-TiO_2 (10 $\mu g\,L^{-1}$) and fullerene (1 $\mu g\,L^{-1}$) has little effect on both gas production and the microorganisms' morphology. However higher concentrations of nano-TiO_2 (100 and 1000 $\mu g\,L^{-1}$) and fullerene (10 and 100 $\mu g\,L^{-1}$) reduced or stopped completely gas production. SEM micrographs showed that addition of nanoparticles reduced the microorganisms count up to 30 min of exposure, followed by an increase of selected micro-organisms after a 360 min exposure to TiO_2 and Fullerene. Overall, this study demonstrates that engineered nanoparticle concentration levels and exposure time play an important role in the toxicity of microbial communities present in WWTWs. It also shows that under stressed conditions, the micro-organisms appear to protect themselves via a spore forming mechanism that allows them to survive the environment.

1. Introduction

The use of Engineered Nanoparticles (ENPs) materials has risen considerably in the last decade. In 2006, 212 manufacturing companies were identified to be producing all types of nanoparticles. In 2009, this number exceeded 1000, with silver being the most common, utilised primarily as an antimicrobial [1]. Nanoparticles are utilised in several other products [2–4] and its increasing use has been investigated by several authors [5–13] to cause pollution in natural ecosystems. They claim those materials are released in aquatic systems and have influence on the microorganisms behaviour and fate.

The toxicity mechanisms of nanoparticles on microorganisms is subjected to size, shape, concentration and surface area, which the latter is the most critical parameter recognized to influence microbial activity [14]. However the pathway of interaction between an ENP and a microorganism requires deeper investigation. [15] and [16] reported the presence of nano-TiO_2 inside microorganisms' cells on their studies. Ref. [15] investigated the interaction between nano-TiO_2 and planktonic bacteria under the influence of extracellular polymeric substances (EPS), which is a natural substance excreted by this type of bacteria. It provides a barrier for ENPs because it limits the interaction with the cell wall besides facilitating the attachment of nano-TiO_2. For instance [17], removed loosely bound EPS from a sample of wastewater biofilm and observed significant increase in the toxicity of nano-Ag compared to regular samples, which did not showed major inhibitory effects when exposed to 200 $mg\,L^{-1}$ of nano-Ag. Ref. [6] have described the effects of EPS in protecting intracellular protein and polysaccharide in conditions where EPS layer is thick and when is thin. Nevertheless EPS decreases the exposure of nano-TiO_2 to Ultra-Violet (UV), decreasing as well oxidation through photo-catalysis, which is the main mechanism for this NP to inhibit bacterial activity. Besides photo-catalysis, nano-sized titanium oxide causes agglomeration inside mussel cell's digestive gland [16] resulting in metabolic inhibition not related to exposure to UV.

Fullerene has been reported to have antimicrobial activity. However [18], reported in their study that fullerene stimulated the growth of a bacillus bacteria that is responsible for denitrification in wastewater treatments, which contrasts to other publications whose results were of decrease in biological activity and inhibition. Polymerase Chain Reaction (PCR) was used to quantify strains of

* Corresponding author.
E-mail addresses: l.auwerter15@imperial.ac.uk (L.C.C. Auwerter), s.ouki@surrey.ac.uk (S.K. Ouki), masaadi@adtechnologies.co.uk (M. Asaadi), achame.shana@thameswater.co.uk (A. Shana).

http://dx.doi.org/10.1016/j.jwpe.2016.12.006
2214-7144/

the microorganisms present in the sample. *B.cereus* was identified as the major strain in the sample.

It is uncertain which nanoparticle is more harmful to the environment; moreover studies have shown all ENPs cause harm to ecosystems at certain degree. In this study the influence of TiO_2 and Fullerene C_{60} on aerobic microorganisms from wastewater treatment samples taken after primary sedimentation were investigated. TiO_2 is one of the most used ENPs and its release is expected to increase in aquatic systems [19] while fullerenes were discovered in 1985 and have been linked to stimulation of growth and inactivation of bacteria. Hence this study aimed to assess the influence of nano-TiO_2 and Fullerene C_{60} on the microbial community in wastewater samples taken from lamellas before aeration lanes from a wastewater treatment works in Reading, United Kingdom.

2. Materials and methods

Influence of nano-TiO_2 (25 nm) and Fullerene was observed and measured through two methods: Automatic Methane Production Testing System (AMPTS) and SEM. The first provided data of biological activity by gas production records and the latter allowed observation of the morphology and changes of the biota due to contact with contaminants. The methods were chosen to evaluate the influence of the two nanoparticles in aerobic wastewater samples under three concentrations. The concentrations were chosen according to the average levels found in wastewaters. Concentrations for nano-TiO_2 (25 nm, Sigma-Aldrich, United States) in solution were 10 $\mu g\,L^{-1}$; 100 $\mu g\,L^{-1}$; and 1000 $\mu g\,L^{-1}$. Concentrations for Fullerene (C_{60}, Sigma-Aldrich, United States) in solution were 1 $\mu g\,L^{-1}$; 10 $\mu g\,L^{-1}$; and 100 $\mu g\,L^{-1}$.

The aerobic wastewater samples were collected from a wastewater treatment works sited in Reading, United Kingdom. Samples were sampled randomly from six aeration lamellas following primary sedimentation. The wastewater physical and chemical parameters were measured at the wastewater works laboratory once the water was sampled from the lamellas. In order to compare the effects of the nanoparticles on the samples, blanks were sampled and analysed through SEM, and gas production. Blank samples consisted of wastewater sampled without nano-TiO_2 or fullerene C_{60}.

2.1. Gas production

Total gas production was measured using an Automatic Methane Production Testing System (AMPTS) equipment. The equipment was connected to a computer and a software recorded the gas produced during the experiment. The bottles were closed with the stir cap; when turned on, it stirred, thus homogenising the sample. Gas flowed in the tubes and was sent to cells. Each cell could hold 11 mL of gas. When 11 mL gas was collected, the cell lifted, released the gas contained in it and recorded a gas production of 11 mL. The analyses were performed in triplicates and in two batches of 10 bottles each. They were composed of samples of 180 mL wastewater and 20 mL stock solution, and a blank of 180 mL wastewater and 20 mL deionised water (DW). Gas production was expected to be presented in four stages, following the pattern of bacterial activity: lag (phase 1), log (phase 2), stationary (phase 3), and decay (phase 4) [20].

2.2. SEM aanalysis

Scanning electron microscope analyses were carried out with an analytical field transmission SEM model JEOL JSM-7100F with a spatial resolution of 1.2 nm at 30 kV. Samples were placed in 5 mm × 7 mm silicon chips and fixed with 2.5% (v/v) glutaraldehyde in a PBS solution for 120 min, dried at ambient temperature, dehydrated with ethanol at three concentrations: 30%, 60% and 100% v/v for 15 min then rinsed with deionised water. SEM migrographs were taken for samples at initial time, 30 and 360 min exposure.

3. Results and discussion

3.1. Effects of time and concentration of nano-TiO_2

The comparison of gas production is presented in Fig. 1. Results showed the total gas production of samples spiked with TiO_2 at concentrations of 10, 100, and 1000 $\mu g\,L^{-1}$ as a function of time compared with blanks (sample with DW only).

There were no records of gas production after 191 min. The Lag phase occurred for a short period of time, mostly less than 10 min. Then, microbial activity became enhanced in the log phase, followed by a stationary phase that lasted between 60–120 min; and finally biological activity stopped after 191 min exposure. Results indicated that the sample with 10 $\mu g\,L^{-1}$ nano-TiO_2 was initially beneficial to gas production. The total volume of gas produced was 0.058 mL against 0.044 mL for the blank. The following concentration of 100 $\mu g\,L^{-1}$ showed that the volume of gas produced was 0.029 mL, which was slightly less than the blank. And finally, the highest concentration of nano-TiO_2 (1000 $\mu g.L^{-1}$) was clearly

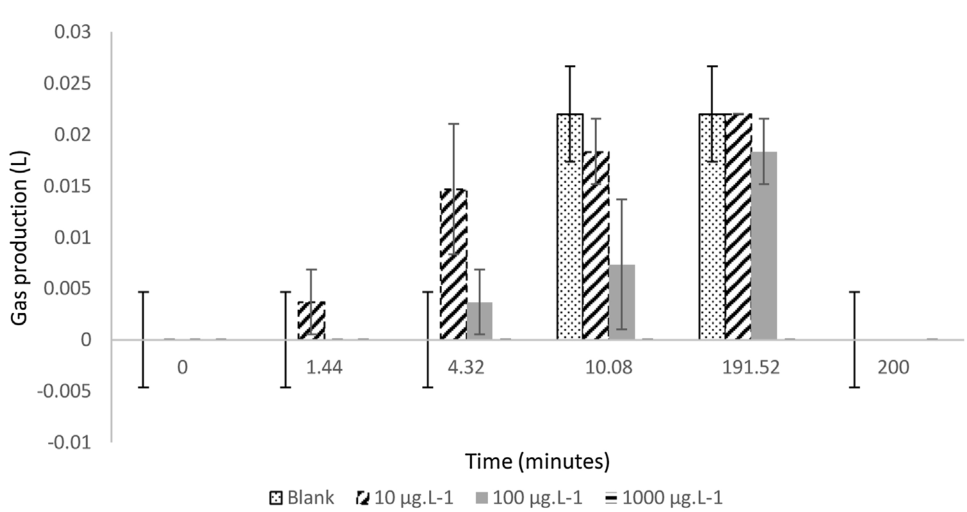

Fig. 1. Effect of nano-TiO_2 concentration on total gas production by microorganisms present in a wastewater sample.