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Effect of photocatalysis on the transfer of antibiotic resistance genes in urban wastewater

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ABSTRACT

The widespread use and abuse of antibiotics in human and animal medicine has produced a reservoir of antibiotic resistant bacteria (ARB) which persist and spread within many environments including natural and wastewater systems. This paper reports the first investigation into the effect of advanced oxidation technologies, photocatalysis, on the potential to induce antibiotic resistant gene transfer within sub-lethally injured ARB. The impact of photocatalytic disinfection (PCD) treatments on three strains of *Escherichia coli*, an antibiotic sensitive strain (K12) and two antibiotic resistant strains (J-53R (rifampicin resistant) and HT-99 (chloramphenicol resistant), within an immobilised titanium dioxide stirred tank reactor. When suspended in distilled water, viable cell numbers (CFU mL^{-1}) of both ARB declined from 3 $\log_{10}$ to 0.5 $\log_{10}$ with 180 min PCD treatment. However, subsequent recovery to 3 $\log_{10}$ of both ARB was observed during post treatment incubation at 37 °C for 24 h. No *E. coli* K12 were recovered immediately after 150 min treatment, or after post treatment incubation. These observations suggest that the ARB are less sensitive to the oxidative stresses involved in PCD treatment than the antibiotic sensitive strain.

Gene pair conjugant numbers in PCD treated mixtures of J-53R and HT-99 cells (a 9:1 ratio) were calculated to be four-fold greater than in the (no treatment) control experiments. Both surviving bacterial cell numbers and conjugant pair numbers were lower when ARB were PCD treated in final effluent from an urban wastewater treatment plant.

In conclusion, the results of this study confirm the efficacy of PCD, but also highlight the importance of applying PCD treatment "long enough" to avoid post treatment recovery from sub-lethal injury and the highly undesirable transfer of antibiotic resistant genes amongst bacteria during wastewater treatment.

1. Introduction

The widespread use and abuse of antibiotics in human and animal medicine has produced a reservoir of antibiotic resistant bacteria (ARB) which persist and spread within many environments including natural water systems, agricultural waste streams, within urban wastewater treatment plants (UWWTPs), rivers, lakes and drinking water supplies [1]. Antibiotic resistance is conferred through acquisition and expression of antibiotic resistance genes (ARGs) within an organism's genome. Due to the frequency of cell division in microbial communities, the transfer of genetic material – including ARGs via horizontal gene transfer such as conjugation, transduction and natural transformation processes – encourages the widespread development of ARB within aquatic ecosystems [2]. This topic, and the mechanisms involved are extensively reviewed by Dodd [2].

Conventional UWWTPs, based upon a series of biological remediation processes, provide a unique microbial ecosystem permitting the exchange of ARGs among a wide range of bacteria strains and therefore significantly contributing to the development and spread of ARB [3]. Indeed, UWWTPs have been described as "hotspots" for the development of antibiotic resistance and the spread of ARB to the environment [4]. For this reason, tertiary disinfection technologies will play an important role in the prevention of ARB being released to the wider environment. Conventional water disinfection process, e.g. chlorine, ozone and UVC treatment, have been shown to be effective for the inactivation of a wide range of pathogens, however, the production of hazardous disinfection by-products [5], the identification of resistant pathogens, such as *Cryptosporidium*, and the detection of

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ARB and ARGs in treated water [2] have been reported. Rizzo et al. recently described the inactivation of a range of antibiotic resistant *Escherichia coli* (*E. coli*) isolated from urban wastewater using UV radiation (UVC) and chlorination [6]. UVC treatment resulted in a total inactivation of *E. coli* strains resistance to amoxicillin (minimum inhibiting concentration (MIC) > 256 mg L^{-1}) and sulfamethoxazole (MIC > 1024 mg L^{-1}), whilst the treatment affected organisms resistance to ciprofloxacin (MIC decreased by 33% and 50% after 60 and 120 min treatment, respectively). On the contrary, chlorination did not affect antibiotic resistance *antibiotic sensitive (ABS) E. coli* strains investigated. This work suggests that further research and new disinfection technologies are required to prevent the spread of ARB from UWWTPs using conventional tertiary disinfection technologies.

Due to the issues described above, there is increasing interest in the area of advanced oxidation processes (AOPs) for both water and wastewater treatment and disinfection. UV–titanium dioxide (TiO_2) photocatalysis has been reported to inactivate a wide range of bacteria, viruses and protozoa commonly found in water and wastewater [7]. When TiO_2 particles are illuminated with near UV radiation, electron–hole pairs (e^-/h^+) are generated. These charge carriers can migrate to the surface of semiconductor particles where they react with hydroxyl groups and dissolved oxygen to form reactive oxygen species, such as hydroxyl and superoxide radicals and peroxides [7]. Due to the availability, affordability, stability and low energy band-gap of TiO_2, it is widely accepted as the most suitable semiconductor material for heterogeneous photocatalysis.

Although a large volume of research is available on the ability of photocatalysis to inactivate a wide range of organisms in water, to date there have been very few reports of the ability of AOP's to inactivate waterborne ARB [8–10]. More recently, work investigating the disinfection of indigenous ARB isolated from wastewater has been reported [11]. To the best of our knowledge, an investigation of the effect of photocatalysis on the transfer of ARGs has not been previously reported.

In this paper we report a preliminary study examining photocatalytic disinfection (PCD) treatment of antibiotic sensitive (K12) and antibiotic resistant *E. coli* strains (J-53R conferring resistance to rifampicin, and HT-99, harbouring a plasmid conferring resistance to chloramphenicol). The aims of this work were to assess the efficacy of PCD on ARB, to determine the potential for conjugative gene transfer between ARB as a result of the oxidative stress exhibited during PCD treatment, and to investigate if conjugative gene transfer during PCD could potentially happen in the environment, i.e. within wastewater.

2. Methods and materials

2.1. Photocatalytic reactor

PCD experiments were conducted in a stirred tank reactor (STR) as previously described [12,13]. TiO_2 (Evonik Aeroxide P25) was immobilised onto borosilicate plates by spray coating (0.5 mg cm^{-2}) and annealed at 450 °C for 1 h in air. Coated plated were back face illuminated using two UVA lamps (Philips PL-S 9 W/10-peak emission 370 nm with a cumulative output between 320 and 400 nm of 80 W m^{-2}). The reactor volume (200 mL) was stirred from above at 2000 rpm and all experiments were air sparged (900 mL min^{-1}) throughout using a small aquarium pump. To ensure efficient mixing of the suspension of bacteria and a steady photon flux from the UVA source the reactor was allowed to equilibrate for 15 min in the dark prior to application of UVA irradiation (T_0). Control experiments (no PCD) included use of uncoated borosilicate glass plates (no TiO_2) in the absence of both UVA light and air sparging.

2.2. Water sources

Disinfection experiments were undertaken with organisms suspended in distilled water (DW) and in autoclaved secondary effluent from a Northern Irish UWWTP. Physicochemical parameters of the sterilised UWWTP effluent were determined as follows: pH 6.8, BOD_5 10 mg L^{-1}, COD 90 mg L^{-1}, TSS 14 mg L^{-1}, TOC 9.02 mg L^{-1}, trace metals were present at ppb levels with Fe at 0.101 mg L^{-1}. Microbial analysis demonstrated that the wastewater used in all experiments was sterile prior to addition of the organisms under study.

2.3. Bacterial stock

E. coli K-12 (ATCC 23631) was cultured by removing a single colony from refrigerated stock plates, sub-culturing in 10 mL of Luria–Bertani (LB) broth, and incubating at 24 h at 37 °C under constant agitation using a rotary shaker (100 rpm) to produce stationary phase cells at a concentration of approximately 9 $\log_{10}$ CFU mL^{-1}. The overnight culture of cells was centrifuged at 3000 rpm for 10 min, the supernatant decanted and bacterial pellet resuspended in 10 mL 1/4 strength Ringer's solution (Oxoid). Two genetically modified strains of *E. coli* paired for conjugation, J-53R conferring resistance to rifampicin, and HT-99, harbouring a plasmid conferring resistance to chloramphenicol, were obtained from National Centre for Biotechnology Education at the University of Reading, UK. Individual liquid cultures of both organisms were prepared in LB broth by overnight incubation at 37 °C as described above.

2.4. Photocatalytic disinfection experiments

PCD using single suspensions of *E. coli* K12, J-53R and HT-99 was conducted with an initial bacterial loading of approximately 3 $\log_{10}$ CFU mL^{-1}. Samples of treated bacteria were removed from the STR at regular intervals and serially diluted in ¼ strength Ringer's solution. Surviving bacteria were enumerated by plating aliquots (100 μL) in triplicate on the following agars: *E. coli* K12, LB agar; J-53R, LB agar containing 100 μL mL^{-1} rifampicin (Sigma, UK); HT-99, LB agar containing 25 μL mL^{-1} chloramphenicol (Sigma, UK). All plates were incubated overnight at 37 °C prior to manual colony identification and enumeration. Experiments examining the effect of UV only were conducted in the STR using an uncoated borosilicate glass plate. No treatment control experiments were also conducted in the STR, where the bacterial suspension was stirred slowly in the dark using a blank borosilicate plate (no TiO_2 present).

2.5. Bacterial re-growth and recovery analysis

To examine the potential for PCD to induce sub-lethal stress leading to reversible injury, samples (1 mL) were removed from the STR and appropriately diluted prior to plating onto non-selective LB agar (as described above) and selective m-Endo agar (Fluka). All agar plates were incubated at 37 °C for 24 h prior to analysis. The following equation was used to quantify the degree of injury [14,15]:

$$Injury\,[\%] = \frac{LB - mEndo}{LB} \times 100 \quad (1)$$

where LB and m-Endo represent the concentration of injured and non injured bacteria, respectively, (measured in CFU mL^{-1}).