Potential and Risk of the Visible Light Assisted Photocatalytical Treatment of PRD1 and T4 Bacteriophage Mixtures

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Abstract – In current study UV light and visible light activated photocatalytic inactivation treatment was applied to the less commonly studied subjects, namely bacteriophages PRD1, T4 and their mixture. By using UV light irradiation and high efficiency P25 TiO₂ photocatalyst powders it was demonstrated that individually and in mixture PRD1 bacteriophage is particularly vulnerable to the photocatalytic inactivation and in just approximately 20 min its infectivity is reduced by 100 %. As for the T4 bacteriophage, it has been reported that under UV irradiation T4 triggers self-repair and replication mechanisms therefore under same photocatalytic inactivation conditions infectivity reduction reaches just 60 %. Surprisingly, by studying visible light photocatalytic treatment efficiency of PRD1 and T4 bacteriophage mixture we identified that T4 bacteriophage potentially triggers the same self-repair and replication mechanism as it does under UV light. Moreover, by using two different types of visible light activated photocatalysts we determined that when efficiency of the used photocatalyst is too small the overall infectivity of the T4 bacteriophage can significantly surpass the corresponding property of the untreated control group.

Keywords – Bacteriophage; infectivity; inactivation; photocatalysis; PRD1; T4; TiO₂; ZnO

Nomenclature

LB	Luria Bertani
PBS	Phosphate buffered saline
PFU	Plaque-forming unit
WFD	Water Framework Directive

1. INTRODUCTION

Owing to increasing concern of global climate-change, clean air, fresh water, reuse of waste material etc. attract a great deal of attention from the scientists of various fields [1], [3]. Clean fresh water for humans is one of the key resources which is widely used both at small domestic and at large industrial (including agriculture sector) scales. The central element of European

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water policy is a Directive for ‘Community action in the field of water policy’ (2000/60/EC) – often referred to as the Water Framework Directive (WFD) – which aims to achieve a good ecological and chemical status of European waters [4]. The directive states that “Water is not a commercial product like any other but, rather, a heritage which must be protected, defended and treated as such”. In 2006 WFD was complemented by the Groundwater Directive 2006/118/EC [5] and they both collectively dictate that quality of clean fresh water has to be maintained at each stage of its usage and special attention has to be paid for the physical, chemical cleaning and biological disinfection (decontamination) of wastewater. Cleaning and decontamination of wastewater are critical and essential elements for the preservation of the quality level of the surface and groundwater sources. Failing to preserve (at least) the currently available water quality level would result in the inevitable need of the more complicated and more expensive water pre-treatment technologies [6], [7].

One of the most widely investigated groups of modern sustainable water cleaning technologies are such called Advanced Oxidation processes which have a huge potential to efficiently remove both biological (virus, bacteria and other microorganisms) and industrial (dyes, pesticides, pharmaceutical etc.) contaminants [7]. Amongst them special interest is dedicated for the photocatalytic water treatment by using UV or visible light activated photocatalysts. During such processes photocatalyst absorbs radiation energy and uses it to generate reactive oxygen species which are capable to oxidise various pollutants to harmless constituents [8].

Up to now, scientists have investigated a lot of various photocatalysts (TiO_2 [9], ZnO [10], Bi_2O_3 [11], Fe_2O_3 [12], etc.) which were applied for a wide variety of pollutants including inorganic dyes, bacteria, viruses and other contaminants [4], [9]–[12]. Recognising the universality of the advanced oxidation processes [8], there is a general view that photocatalytic generation of the reactive oxygen species and their application for the inactivation of bacteria and viruses can be the all-round solution for the sustainable waste water disinfection. At the same time, it is assumed that if it is efficient for the treatment of one type of bacteria or virus it should be efficient for the treatment of other species as well. The only question is how much more or less of treatment time is required to achieve the desired effect. Accordingly, when studying photocatalytic inactivation efficiency of biological species researchers usually rely on just a very few types of bacteria (most often choosing *E. coli* or *S. enterica* [4], [13], [14]) which are the most convenient for their studies. Viruses, on the other hand, are not as convenient for the studies as bacteria because they require specific hosts for their replication. Investigations of photocatalytic inactivation of virus mixtures are even more inconvenient and rare because in addition to the set of the chosen viruses they also require corresponding number of the specific host cells [15]. Nevertheless, real life examples of simultaneous wastewater contamination by several types of pathogens indicate that investigations of disinfection efficiency of bacteria and virus mixtures are as important as the investigations dealing with only one type of the pathogen.

Considering the scarcity of the research studies which would be focused on the photocatalytic treatment of viruses and especially their mixtures in current study we decided to investigate UV and visible light induced photocatalytic inactivation of PRD1 and T4 bacteriophage mixtures. Bacteriophages PRD1 and T4 selected for this study are the model viruses which infect *S. enterica* and *E. coli* bacteria, respectively. PRD1 is a lipid envelope containing phage, whereas T4 is a relatively large and stable bacteriophage, able to reactivate under UV irradiation [16]. In order to compare how later property manifests itself for the bacteriophage T4 being in a mixture with and without UV radiation we performed measurements with both UV light and daylight activated photocatalysts.

2. METHODOLOGY

2.1. Photocatalyst and Irradiation Source Selection

Photocatalytic inactivation of PRD1 and T4 bacteriophages was performed using P25 TiO₂ photocatalyst powders (99.5 % purity, obtained from Sigma-Aldrich) activated with UV light. Concentration of TiO₂ powders was 1 mg/ml. P25 TiO₂ powders were chosen for their well-documented physical and photocatalytic properties and credible comparison to the related studies [17]. 365 nm LED UV light source (M365LP1-C1, Thor labs) was used for the irradiation with an approximate irradiation intensity on the surface of the solution of 2 mW/cm².

For the visible light activated photocatalysis there are no commonly accepted commercially available standard photocatalyst material. Therefore, we used two types of the visible light active photocatalysts which were synthesised in our laboratory. The first one was carbon doped TiO₂ (approx. thickness ~2000 nm, band gap ~2.5 eV, amount of doped carbon ~2 atom. %, structure – mix of anatase and rutile) and the second one was metallic Zn rich ZnO (approx. thickness ~2000 nm, band gap ~3.1 eV, structure – Zn rich ZnO). Conditions of the formation (magnetron sputter deposition on 30 mm diameter borosilicate glass discs) of these photocatalyst thin films as well as their structural and photocatalytic properties were described in detail in our previous studies [18] and [19], respectively. Both types of photocatalysts were proven to have significant activity under the visible light irradiation, which was explained by carbon dopant and zinc/oxygen defects in TiO₂ and ZnO oxides respectively (efficiency of carbon doped TiO₂ was higher in comparison to ZnO based photocatalyst). Carbon incorporated into TiO₂ bulk can improve conductivity by facilitating the charge transfer from TiO₂ lattice structure to the surface [20]. Moreover, it can reduce TiO₂ band gap by introduction of localized electronics states [21]. Song *et al.* showed that defects including oxygen and zinc vacancies/interstitials as well as metal-semiconductor Schottky junction are formed during the synthesis of Zn rich ZnO [22]. They create new intermediate energy levels and consequently reduce the band gap by enhancing ROS generation and photocatalysis performance [23]. For the visible light radiation SOLIS-3C (Thorlabs Inc.) white day light LED source was used. Daylight irradiation intensity on the surface of the samples was approximately 40 mW/cm².

2.2. Experimental Parameters of Photocatalytic Treatment

During the experiments of photocatalytic treatment of bacteriophages, the initial concentration of T4 and PRD1 virus particles in the test vessel was 1×10^4 pfu/mL. The experiments were performed using 15 mL volume of magnetically stirred suspension in PBS buffer (pH 7.4) incubated at 22 °C in thermostated vessel with the suspension layer thickness of 1.5 cm for P25 TiO₂ powder and 1 cm for both types of visible light photocatalysts. With UV light phages were irradiated for 40 min and samples were taken after 10, 20 and 40 min of treatment, whereas under day light treatment samples were collected after 30 and 60 min. Samples of irradiated bacteriophage suspensions were serially diluted. 100 µL of phage suspension (about 400 phages) were poured into 4.5 mL of LB medium with 0.5 % agar with 300 µL of night bacteria culture and plated on agar Petri dish. *E. coli* DH5α cells were infected by bacteriophage T4 and *S. enterica* DS88 – by PRD1. After 18–22 h of incubation at 37 °C phage-formed sterile spots were counted (plaque-forming unit (PFU) method). Control measurements were performed using untreated negative control samples, and samples separately affected by UV irradiation and TiO₂ powder.

3. RESULTS AND DISCUSSION

3.1. Bacteriophage Treatment by the UV Light Photocatalysis

Infectivity measurements of T4 phage (Fig. 1) revealed that after 40 min of the combined treatment by UV and TiO_2 the ability of the phage to infect bacteria was reduced by 60 %. Also, it can be noted that when treated separately the phage was slightly more sensitive to the presence of UV radiation (30 % reduction) than to the presence of TiO_2 powders (20 % reduction).

Fig. 1. Effects of treatment on infectivity of bacteriophage T4. The experiments were performed at 22 °C, the initial number of T4 was 1×10^4 pfu/mL, 1 mg/mL of TiO_2 and intensity of UV irradiation 2 mW/cm² were applied.

Similar testing of the PRD1 phage (Fig. 2), revealed that this phage was far more sensitive (100 % infectivity reduction in 20 min) to the combined UV and TiO_2 photocatalytic treatment than bacteriophage T4. In particular it can be noted that PRD1 was more sensitive to UV irradiation. On the other hand, PRD1 phage showed only very minor sensitivity to the presence of TiO_2 powders.

Fig. 2. Infectivity of bacteriophage PRD1. The experiments performed at 22 °C, the initial number of infective PRD1 particles was 1×10^4 pfu/mL, 1 mg/mL of TiO_2 and the intensity of UV irradiation 2 mW/cm² were applied.

For experiments with bacteriophage mixtures, initial concentrations of PRD1 and T4 phages were the same – 1×10^4 pfu/mL. These phages have different hosts, and two different bacterial cultures were infected after the treatment: *E. coli* DH5 α for determination of T4 infectivity, and *S. enterica* DS88 for determination of infectivity of PRD1. The maximum treatment time was 60 minutes.

The obtained results (Fig. 3 and Fig. 4) showed that for both bacteriophages treatment by presence of TiO_2 powders or UV irradiation alone has nearly similar but limited effect – after

40 min of treatment infectivity of T4 phage is reduced by approximately 15 %, whereas infectivity of PRD1 is reduced by just short of 20 %. The combination of TiO_2 and UV irradiation had significantly stronger effect: after 40 min of treatment infectivity of T4 decreased by 60 % and corresponding value for PRD1 was reduced by 98 %. Interestingly, in comparison to the individual treatment which was described above, when being treated in mixture infectivity of T4 bacteriophage has been reduced to nearly the same value of 40 %. But at the same infectivity reduction rate of bacteriophage PRD1 was significantly smaller and even after 40 min of treatment some phage units were still able to infect host bacteria cells (in comparison when treated individually infectivity fell to 0 % already after 20 min of treatment).

Fig. 3. Infectivity of bacteriophages T4: a) and PRD1, b) after the treatment in the mixture. The experiments were performed at 22 °C, the initial number of T4 and PRD1 was 1×10^4 pfu/mL, 1 mg/mL of TiO_2 and intensity of UV irradiation 2 mW/cm^2 were applied.

Fig. 4. Formation of plaques by bacteriophages T4 (a) and PRD1 (b) treated in mixture. Photocatalytic treatment was performed using UV light and P25 TiO_2 powders.

3.2. Bacteriophage Mixture Treatment by the Visible Light Photocatalysis

Results of bacteriophages T4 and PRD1 infectivity after their treatment by the visible light photocatalysis using carbon doped TiO₂ (Fig. 5) and metallic zinc rich ZnO (Fig. 6) thin films are summarised in Fig. 7. As expected, overall efficiency of these photocatalytic disinfection processes was lower than previously described case with UV light and remarkably large surface area P25 powder (average specific surface area of P25 TiO₂ is 50 m²/g which means that surface area of 15 mg of TiO₂ is 0.75 m² [24]. Similarly, there was no surprise that photocatalytic treatment of phages using visible light and TiO₂ based photocatalyst was more efficient than the ones with ZnO based alternative [8], [25], [26].

Looking at the quantitative data (Fig. 7), it should be noted that in 60 min both TiO₂ and ZnO based photocatalysts were capable to significantly reduce infectivity of PRD1 phage down to approximately 50 % and 70 %, respectively. At the same time under tested condition their effect on the T4 phage infectivity diverged and ultimately provided opposite results. After 30 min of treatment under visible light C doped TiO₂ lowered infectivity of T4 phage down to approximately 80 %. During the next 30 min it was reduced even further and after 60 min of treatment infectivity was weakened down to approximately 70 %.

Fig. 5. Formation of plaques by treated bacteriophages T4: a) and PRD1 b). Photocatalytic treatment was performed using visible light and carbon doped TiO₂ thin film.

As for the visible light photocatalytic treatment using the less efficient ZnO based photocatalyst it was observed that in the first 30 min infectivity of T4 phage was reduced by nearly 10 %, but after 60 min of treatment the infectivity of T4 phage was considerably higher and reached almost 140 %. This result shows that visible light photocatalytic treatment of T4 bacteriophage could have triggered some sort of self-repair and/or replication mechanism which was counterbalanced by higher photocatalytic activity of C doped TiO₂ but prevailed when less efficient ZnO based photocatalyst was used. We suppose that this mechanism can be similar to the one reported by Restifo *et al.* [16] who described host-mediated repair

synthesis, in which excision of UV-damaged areas is followed by initiation of replication, strand displacement, and a considerable amount of DNA replication.

Fig. 6. Formation of plaques by treated bacteriophages T4: a) and PRD1 b). Photocatalytic treatment was performed using visible light and metallic Zn rich ZnO thin film.

Fig. 7. Infectivity of bacteriophages T4 and PRD1 after photocatalytic treatment of their mixtures. Treatment was performed using visible light and corresponding photocatalyst thin film.

On the other hand, it is known that zinc is an essential metal for microorganisms. At small concentrations it is a component of enzymes, essential for bacteria growth [27] or for virus infection [28]. Also, it was determined [28] that baseplate proteins of phage T4 contains Zn^{2+} ions. These ions are required for protein gp12 which is important for phage irreversible adsorption to the bacterial cell. During the photocatalytic treatment process fraction of ZnO

films can be dissolved [29] and Zn^{2+} ions can enter into solution. Consequently, Zn^{2+} cations can improve the phage entry into the host cell and increase the infectivity of the bacteriophage.

4. CONCLUSIONS

The obtained results showed that lipid envelope containing phage PRD1 in all cases was much more sensitive to the combined TiO_2 and UV photocatalytic inactivation than only the protein capsid containing bacteriophage T4. The same difference also remained for the bacteriophage mixture treatment by the visible light assisted photocatalysis. These data promise strong effect of the combine treatment on the lipid envelope-containing human viruses, such as Influenza virus, 2019-nCoV or Hiv1. However, it should be noted that when it was present in the mixture after 20 min of combined TiO_2 and UV photocatalytic treatment infectivity of PRD1 phage was reduced to 20 % whereas when treated separately after the same treatment time it was completely non-infective. This means that when these types of viruses are present in the mixtures special attention has to be paid to the reasonable extension of their treatment time in comparison to the individually treated pathogens.

Up to now it was known that after mild irradiation by UV rays bacteriophage T4 activates its host-mediated repair synthesis and initiates replication (including considerable amount of DNA replication) [16]. Current study revealed that there is also potentially similar self-defence mechanism which is activated when bacteriophage T4 is being damaged by the visible light photocatalytic treatment. This finding is of great importance for the visible light assisted photocatalytic inactivation of T4 and some other special types of viruses because if oxidation power and concentration of the photocatalytically generated oxygen reactive species is too low the overall effect of photocatalytic treatment could be negative. The situation can become even more critical if not sufficiently efficient photocatalyst (like small surface area ZnO based films) has potential to dissolve into solution and its ions can reinforce infectivity of the specific viruses like T4. Altogether, current study has experimentally demonstrated that proper evaluation of the photocatalyst efficiency and concentration of the replicating pathogen is especially important for the visible light photocatalysts because typically they are less efficient than most of the traditional UV activated materials such as P25 TiO_2 powders.

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