Graphitic Carbon Nitride as A Promising Antimicrobial Photocatalyst

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Background & Introduction

• Drinking water remains a potential source of epidemic outbreaks, and each year hundreds of thousands deaths worldwide are resulted from pathogen contamination of drinking water;

• Bacteria, protozoa, helminths and virus are main waterborne pathogens (Figure 1);

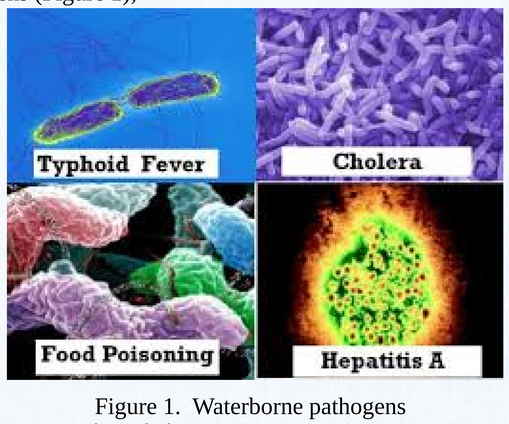


Figure 1. Waterborne pathogens
http://wikieducator.org/User:Pratap/My_ICT_Project

• Photocatalysts harvest photons the energy of which is equal to or greater than the band gap to produce excited electrons and electron vacancies (also known as holes);

• A series of reactive oxygen species (ROS), such as hydroxyl radicals, superoxide anion radicals, and hydrogen peroxide are generated during photocatalysis process;

• Waterborne pathogens will be oxidized and inactivated by these ROS;

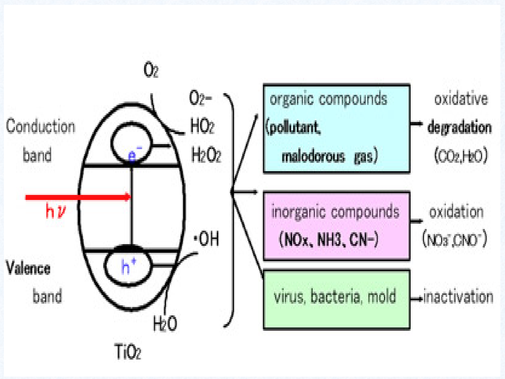


Figure 2. Mechanism of a photocatalyst-TiO_2
https://www.iskweb.co.jp/eng/products/functional05.html

• Compared with traditional photocatalyst, graphitic carbon nitride (g-C_3N_4) can harvest visible sunlight or artificial light;

• g-C_3N_4 is also able to avoid the production of disinfection byproducts (DBPs), which are commonly present in current chemical disinfection strategies and are harmful to human health as well as environment;

• In addition, the raw materials for the production of g-C_3N_4 photocatalysts are the earth abundant, inexpensive C- and N-containing precursors;

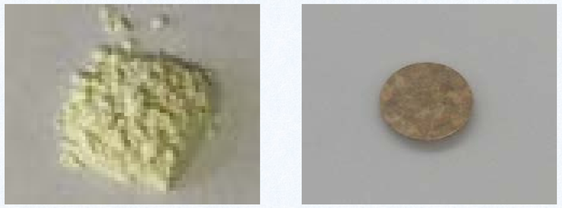
Objective

The objective of our study is first to develop a new g-C_3N_4 photocatalytic material for antimicrobial applications. The inactivation of planktonic bacteria and biofilms is investigated under indoor lighting. The study will shed light on the development of reactive antimicrobial materials for pathogen transmission control and public health protection.

Methods-Material Preparation

• In this study, g-C_3N_4 powder was first prepared via thermal polycondensation of melamine, cyanuric acid, and barbituric acid (Figure 3.a);

• g-C_3N_4 coupons were fabricated from the powder via a hydraulic press (Figure 3.b);



(a) (b)

Figure 3. g-C_3N_4 powder and coupons

Methods-Bacterial Strains

• *Staphylococcus epidermidis (S. epidermidis)* is a Gram-positive bacterium and it is a pathogen which causes hospital acquired infections. *Escherichia coli (E. coli)* is a Gram-negative bacterium and it is commonly found in the lower intestine of warm-blooded organisms (endotherms).

• *Staphylococcus epidermidis* (*S. epidermidis*) and *Escherichia coli (E. coli)* were selected as target microorganisms in this study;

Methods-Disinfection

• *S. epidermidis* and *Escherichia coli* were cultured in Luria-Bertani broth (LB) or tryptic soy broth (TSB) at 37 °C with mixing (120 rpm), respectively;

• Both strains were harvested during their late-exponential phase by centrifugation and diluted in a phosphate-buffered saline (PBS) buffer to prepare bacterial suspension (OD_{600} = 0.5);

• 25 ml of bacterial suspension was mixed with 0.001 g of g-C3N4 powder in a sterile glass beaker. The beaker was placed under a white light emitting diode (LED) lamp (7 W) for bacterial inactivation;

• The distance between the surface of the bacterial suspension and the LED lamp was maintained at 15 cm. Bacterial suspension samples were withdrawn from the beaker with pipette every half an hour. The samples were duplicated, diluted in series with the PBS buffer, and plate counting was conducted to determine bacterial viability;

Methods-Biofilm

• *S. epidermidis* bacterial suspension was prepared as described in disinfection experiment;

• g-C_3N_4 coupons were placed into a sterile six-well plate, and completely submerged by the bacterial suspension (2 ml for each coupon in each well);

• The system was first incubated at 37 °C for 24 h without light and mixing to ensure effective bacterial attachment on coupon surface;

• Next, the coupons were transferred to a new sterile six-well plate, and 2 ml of 10 fold diluted TSB was added to submerge the coupons. The system was incubated at 37 °C with a mixing rate of 80 rpm under LED irradiation and in the dark (control experiment). The experimental setup is shown in figure 4;

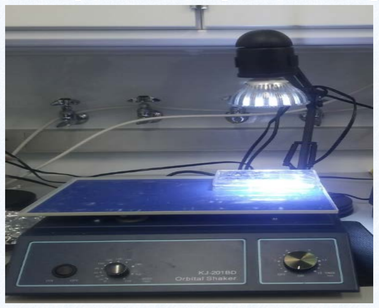


Figure 4. Experimental setup for biofilm development

• TSB was replenished every 24 h. At the end of the experiments, coupons were taken out and gently rinsed with the PBS buffer for three times. The Film tracer LIVE/DEAD Biofilm Viability Kit was used to stain the biofilms on the coupons, and biofilms were imaged by a confocal microscope;

Result

• 2.24 log inactivation of *E. coli* with the presence of g-C3N4 powder under the irradiation of LEDs for 2 h;

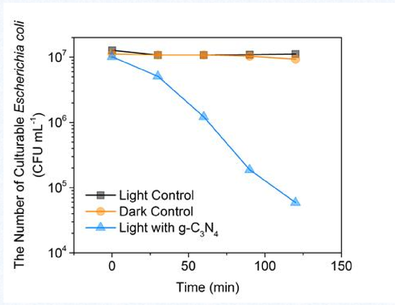


Figure 5. Disinfection experiment result for *E.coli*

Result

• Confocal microscope images of biofilms developed on g-C_3N_4 coupons in the dark condition and under LED irradiation are illustrated in Figure 6. The green spots in the figure illustrates live cells, while the red ones represent dead cells;

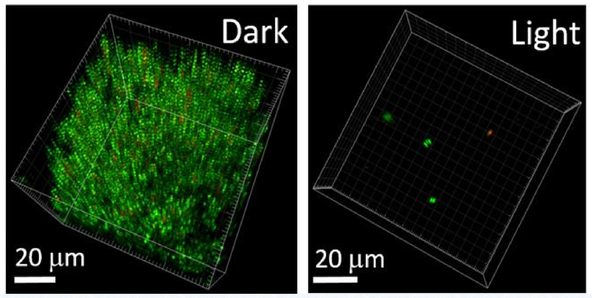
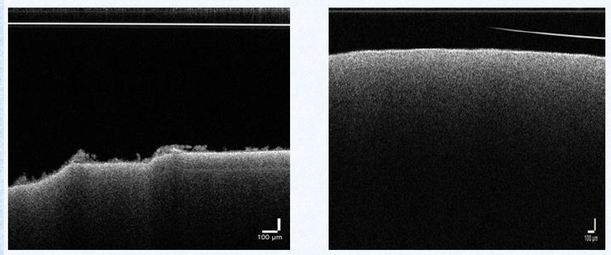


Figure 6. *S. epidermidis* biofilms on g-C_3N_4 coupons

• *S. epidermidis* developed a dense and live biofilm with a thickness of 40-80 um in three days in the dark condition, and the number of dead cells was limited;

• While very limited cells, dead or live, was observed on the coupon surface under white LED irradiation;



(Dark) (Light)

Figure 7. *S. epidermidis* biofilms analyzed by OCT

• *S. epidermidis* Biofilms developed in the dark and under white LED irradiation were also analyzed by the optical coherence tomography (OCT) (Figure 7);

• In addition, biofilms developed in the dark were eliminated after white LED irradiation (Figure 8);

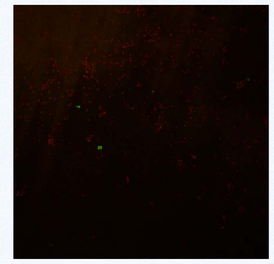


Figure 8. A biofilm eliminated after white LED irradiation

Conclusion

g-C_3N_4 is a promising antimicrobial material under visible light irradiation. Future research will focus on understanding the mechanism of photocatalytic disinfection of g-C3N4.