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Degradation of Norovirus in Sewage Treatment Water by Photocatalytic Ultraviolet Disinfection

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Abstract

Noroviruses (NVs) are known to be one of the major pathogenic viruses implicated in outbreaks of gastroenteritis caused by feeding of seafood. It is supposed that NVs would be emitted into water environment through sewage and the shellfish growing in polluted water environment would enrich with contaminated virus particles in their body. Conventional disinfection processes such as chlorination are not effective in inactivating viruses. Ultraviolet irradiation is an attractive disinfection method for inactivating viruses. However, virus particles remain in sewage effluent even after UV irradiation, and although the viruses are inactivated, they are detected by genetic analysis in shellfish bodies living in waters to which the sewage effluent flows. In the study herein described, we elucidated the procedures for quantitatively determining NVs in sewage effluent and the possibility of a virus-degrading disinfection method. As a result, we found that the real-time PCR would make a powerful tool for determining the NV concentration in water samples. We also made it clear that a combined photocatalytic/UV disinfection (TiO_2/UV) system was effective in decomposing virus particles and reducing the concentration of NVs in sewage effluent.

1. Introduction

There have been epidemics of waterborne infectious diseases, such as when cholera occurred in Japan various times in the middle of the 19th century when the country abandoned its national seclusion policy and actively embarked upon overseas trade. This urged Japan to establish modern sanitary systems. However, it was only in the late 1960s when water supply and sewerage systems covered a considerable portion of the population that the waterborne infectious diseases markedly decreased (see **Fig. 1**)[1]. This fact indicates that water supply and sewerage systems are essential for public health.

Disinfection treatment is applied at the final stage of water purification or sewerage treatment, and chlorination is generally applied for that purpose. However, chlorination entails the production of hazardous disinfection by-products such as trihalomethane. Furthermore, chlorine-resistant pathogens such as Cryptosporidium and Legionella bacteria have been recently discovered, or resuscitated. In consideration of such problems that cannot be solved through chlorination, there is now a search for new disinfection methods that can replace chlorination.

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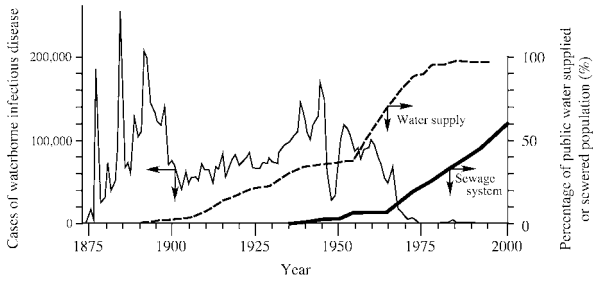


Fig. 1 Waterborne Infectious disease cases and percentage of water supplied or sewered population in Japan

Ultraviolet disinfection has been widely applied in Western countries as a disinfection process to substitute chlorination; Nippon Steel Corporation has applied the process mainly to the sterilization of sewage. We have studied measures to enhance the effect of ultraviolet disinfection focusing attention on photocatalysts[2], and as a result, discovered a highly active photocatalytic material and developed a TiO_2/UV system. The present paper reports the results of the disinfection verification tests of the developed system regarding pathogenic viruses in sewage.

2. TiO_2/UV System as Advanced Disinfection Apparatus

2.1 Principles of disinfection

Ultraviolet lights have an effect of degrading the genes of microorganisms, and microorganisms exposed to ultraviolet irradiation lose the ability to proliferate. However, some microorganisms are known to repair genes damaged by ultraviolet irradiation and recover their activity. Since the gene recovery results from irradiation of visible lights, it is called photoreactivation. Living organisms have been exposed to the solar ultraviolet irradiation for billions of years, and they have presumably developed protective mechanisms of photoreactivation against the ultraviolet irradiation through the process of evolution. In view of the above, some people point out that it is necessary, in the study of the application of ultraviolet disinfection, to study the measures to prevent photoreactivation, as well[3].

Crystals of some semiconductor materials such as titanium dioxide polarize when exposed to ultraviolet irradiation, causing active oxidation-reduction reactions, and as a result, highly oxidizing hydroxyl radicals (OH radicals) form through the oxidation decomposition of water molecules. The hydroxyl radicals degrade microorganisms to the extent that they lose the ability of photoreactivation. As shown in **Fig. 2**, the TiO_2/UV system is an advanced disinfection apparatus wherein the direct virus-gene decomposing effect of ultraviolet irradiation is combined with the indirect virus-gene oxidizing/decomposing effect of the hydroxyl radicals formed by the photocatalytic effect.

2.2 Newly developed photocatalyst

As the photocatalytic material that supports the TiO_2/UV system, films of titanium dioxide were formed by the ion plating method on the surfaces of corrugated foils commonly used as metal catalyst carriers[4]. We formed the TiO_2 films under different conditions, and through performance evaluation and screening in terms of photocatalytic activity, obtained a highly active photocatalyst. The developed photocatalyst was given a trade name of Rose Titania after its characteristic surface microstructure reminiscent of rose petals (see **Fig. 3**).

Fig. 4 schematically shows the structure of a TiO_2/UV irradiation apparatus. An ultraviolet lamp provided on the centerline of a tubular duct irradiates ultraviolet rays, and a cylindrical photocatalyst module of corrugated foil is provided on the inner wall of the

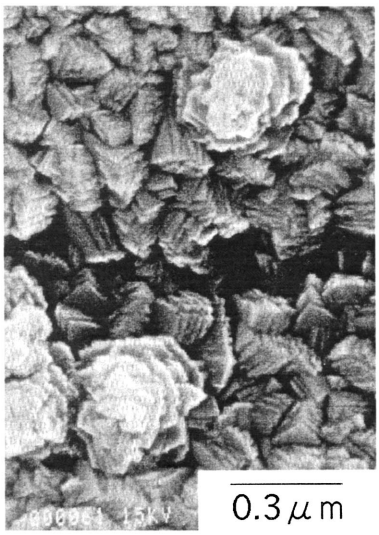


Fig. 3 Scanning electron microscopic photograph of the newly developed photocatalyst

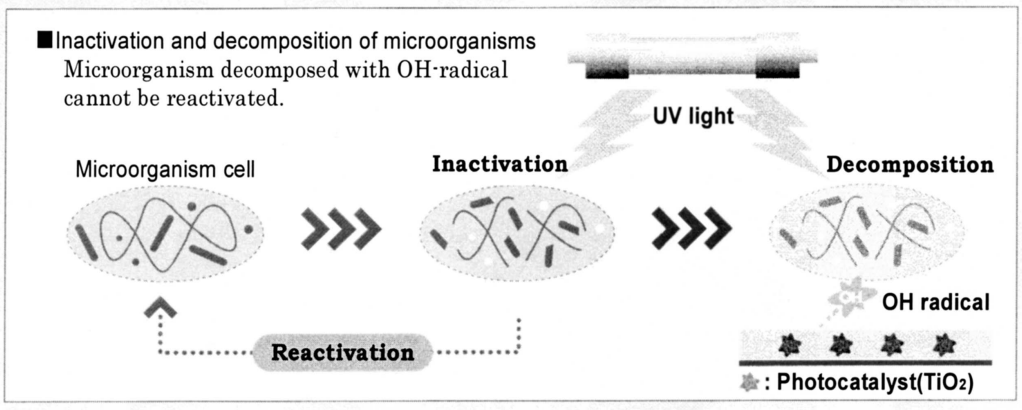


Fig. 2 Schematic presentation of the disinfection mechanisms by TiO_2/UV system

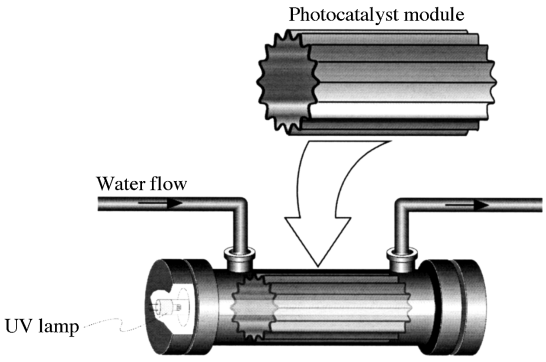


Fig. 4 Example of TiO_2/UV irradiation apparatus

duct. Water is exposed to the ultraviolet irradiation during the passage from an end of the unit to the other, and treated by the hydroxyl radicals forming through the photocatalytic reaction.

3. Verification of Norovirus Decomposition Performance Using Sewage

3.1 Need for countermeasures against noroviruses

Noroviruses are attracting attention recently as the principal causative factor of the intoxication of seafood or drinking water. According to a statistic report of the Ministry of Health, Labor and Welfare, of 29,355 cases of food intoxication in 2003, noroviruses account for no less than 10,702 cases, more than one-third of all the food intoxication cases.

Noroviruses do not proliferate in the organisms of fish and shellfish, and thus the fact that they are found in fish and shellfish means that the viruses come from sewage systems and other sources to the waters where the fish and shellfish live and accumulate and concentrate in their bodies. For this reason, measures against viruses in sewerage effluent water are required.

While most of noroviruses in sewage are removed during the treatment through absorption to activated sludge, some may remain in treated water, not being deactivated by the final treatment of chlorination, and be discharged to environmental waters. Viruses are generally difficult to deactivate by chlorination; reports state that noroviruses are little deactivated when the chlorine concentration is 10 mg/L or so. In contrast, ultraviolet disinfection is considered extremely effective because it destroys the genes of the viruses photochemically.

However, even when noroviruses are deactivated by ultraviolet disinfection and their toxicity is lost, virus particles remain, and after the treated water is discharged to environmental waters, they are accumulated in the bodies of fish and shellfish living there. For this reason, some seafood is classified as “norovirus positive” at the genetic analysis of food hygiene inspection even though there is no danger of food intoxication, and the commercial value of the seafood for eating raw is lost; this causes considerable economic damage to the fishing industry.

Presently, no methods are available for easily confirming the infectiousness and toxicity of noroviruses, and therefore high-sensitivity detection methods for virus genes are inevitably employed in food sanitation tests. Therefore, establishment of a disinfection technology capable not only of deactivating noroviruses but also of decomposing the very virus particles is strongly demanded as a fundamental countermeasure against noroviruses in sewage treatment.

3.2 Experimental

3.2.1 Disinfection test method

The authors carried out disinfection tests of sewage after secondary treatment at a commercial sewage plant operated in a fishery region using tubular ultraviolet disinfection apparatuses as the one shown in Fig. 4. **Figs. 5** and **6** show the flow diagram and appearance, respectively, of the test plant. Two different units of the ultraviolet disinfection apparatuses were used, one with the photocatalyst (System I) and the other without (System II). They were installed parallel to each other to compare them in terms of virus decomposing performance. The System I unit was equipped with a 400-W medium-pressure mercury lamp, and the System II unit with a 65-W low-pressure mercury lamp. The design flow rates of Systems I and II were 16 and 6 m^3/h, respectively.

The sewage after secondary treatment used for the test had the following properties and microorganism concentrations: a pH of 7.4; a SS of 14 mg/L; an ultraviolet absorption of 0.12/cm; a coliform bacteria concentration of 23 MPN/mL; and a general bacteria concentration of 7700 cfu/mL.

3.2.2 Virus analysis method

In verifying the effectiveness of a disinfection apparatus as a countermeasure against noroviruses, a method for quantifying waterborne noroviruses is indispensable. Although the standardization of the methods for detecting norovirus genes is being studied in the food and related industries, in order to quantify the concentration of waterborne noroviruses, which is expected to be very low in any cases, it is necessary to concentrate the norovirus particles prior to the determination. While various methods have been proposed to condense and separate low-concentration waterborne viruses such as membrane filtration[5], flocculation[6] and column absorption[7], the authors

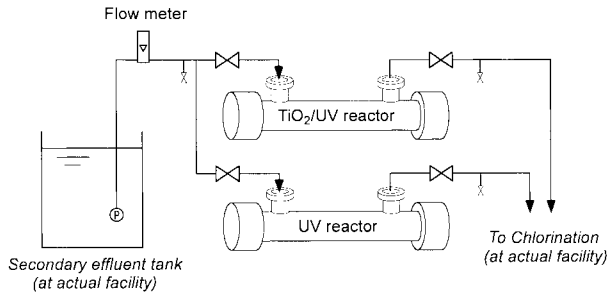


Fig. 5 Experimental flow diagram

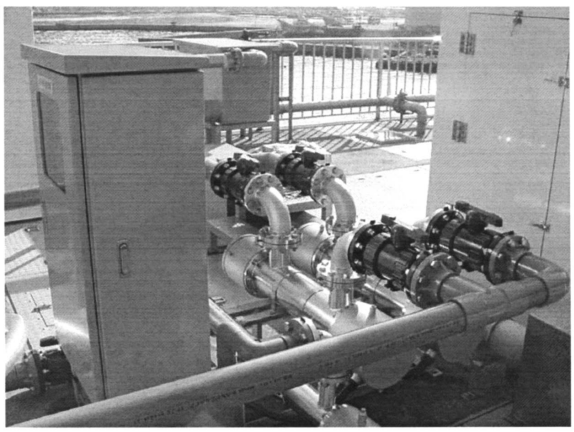


Fig. 6 Photograph of the experimental apparatus

contrived a new column absorption method by combining conventional techniques. The outline of the method is explained below (see reference 8 for details).

Five to ten liters of sample water were flowed through a glass column filled with negative-ion-exchanging DEAE cellulose powder to have virus particles in the water absorbed by the cellulose. The absorbed virus particles were eluted using 30 mL of an alkaline solution (50 mM glycine + 0.5% beef extract, pH 9.5), re-condensed by polyethylene glycol, precipitation and the sediment thus obtained was turned into a condensed virus suspension using 1 mL of Tris buffer solution.

Then, virus RNA was extracted from the condensed virus suspension and subjected to a gene test, that is, the PCR products of the extracted RNA solution were taken, and after amplifying a part of the norovirus genes, DNA fragments amplified by PCR was observed by agarose gel electrophoresis. In addition, to estimate the norovirus concentration in the condensed norovirus suspension, the authors carried out real-time reverse transcription PCR.

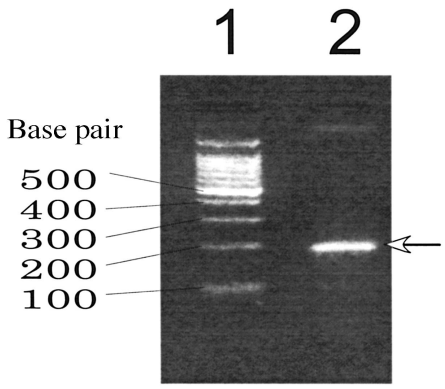
3.3 Results

3.3.1 Verification of virus analysis method

Fig. 7 shows the result of agarose gel electrophoresis of a part of a norovirus gene (about 200 bp long) that was contained in a condensed virus suspension obtained through the above procedures from sewage after secondary treatment and amplified by reverse transcription PCR. As seen in the figure, PCR amplification products about 200 bp long were found in the condensed specimen. Thus, we confirmed that the developed method could condense and separate noroviruses contained in sewage after secondary treatment, and makes it possible to detect the norovirus genes.

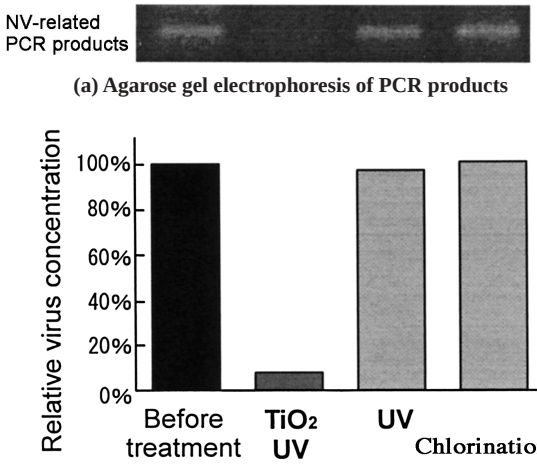
3.3.2 Results of decomposition and disinfection

The authors carried out reverse transcription PCR of the sewage specimens before and after the disinfection treatment using the ultraviolet disinfection apparatuses. As seen in **Fig. 8**(a), where each of the four image portions corresponds to each of the bars of Fig. 8(b), the agarose gel electrophoresis image of norovirus genes disappeared only in the case where both the ultraviolet disinfection and the photocatalyst were applied in combination. The above result indicates to the possibility that the combined effect of the ultraviolet disinfection and the photocatilyst decomposed noroviruses. What is more, the quantification by the real-time reverse transcription PCR of the sewage specimens showed that the concentration of the noroviruses existent in the water before the treatment did not decrease after either the ultraviolet disinfection or chlorination alone, but the concentration decreased markedly after the combined treatment using the ultraviolet disinfection and the photocatalyst (see Fig. 8(b)). This seems to indicate that the combined treatment is capable of decomposing virus particles.



Lane 1: molecular weight maker; lane 2: product from concentrated water sample. The arrow indicates NV-related PCR product.

Fig. 7 Agarose gel electrophoresis of PCR products with NV primers



(a) Agarose gel electrophoresis of PCR products

(b) Relative concentration of NVs

Fig. 8 Comparison of decomposing efficacy of TiO_2/UV, UV or chlorination

4. Summary and Future Prospect

It is presumed that norovirus genes are damaged and the toxicity of the viruses is lost after conventional ultraviolet disinfection treatment. Nevertheless, norovirus genes are detected in specimens by gene testing methods such as the reverse transcription PCR even after the treatment. On the other hand, the combined treatment that uses ultraviolet disinfection and a photocatalyst decomposes norovirus genes to the extent that they are not detected by genetic analysis. For this reason, the developed combined disinfection treatment method is expected to be effective as a countermeasure against noroviruses applicable to sewage treatment plants in regions near fishery waters.

The behavior of noroviruses in sewage has not been altogether clear, and the study and definition of disinfection target figures that indicate the level to which the norovirus concentration of sewerage discharge water must be decreased are awaited.

The authors studied the application of the TiO_2/UV system to the disinfection of sewage, and as a result, clarified the possibility of the system to decompose and disinfect noroviruses as herein described. Based on the above results and under a joint study framework with the government of Mie prefecture, studies are under way for the application of the developed system to an advanced disinfection facility for treating seawater used for the final cleaning of cultured oysters before shipment.

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