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Advanced Water Treatment Technology for Oxidation and Disinfection by Ultraviolet Irradiation

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Abstract

Advanced oxidation processes (AOP) were expected to be powerful water treatment techniques. An aim is to develop an AOP that mainly uses ultraviolet irradiation for treatment of the refractory organics and the disinfecting of pathogenic microbes. It is presumed that the generation of hydroxyl radical is the key factor for the treatment performance of AOP. In this research, a method to quantify the hydroxyl radical generation was first examined, then the obvious generation of hydroxyl radical was observed in a photocatalyst/ultraviolet irradiation. Secondly, a method to detect biological activity of the pathogenic protozoan Cryptosporidium, that caused serious outbreaks in the worldwide was studied. It was revealed that Cryptosporidium could be inactivated by the ultraviolet irradiation.

1. Introduction

Backed by growing global awareness of the environment and human health, there is a growing demand for better quality water, regardless of whether it is wastewater or drinking water. As the generalized indices of organic materials considered chiefly responsible for water pollution, BOD (Biochemical Oxygen Demand) and COD (Chemical Oxygen Demand) have been employed. Beginning with the control and management of water quality by the application of those indices, nutrient salts, such as nitrogen and phosphorus, have come to be controlled as the measures against eutrophication in the field of water research. At present, out of consideration for the influences on the ecosystem and human health, targets for which water treatment technology is required are greatly increasing as seen in the regulations for detecting trace amounts of toxic chemical substances, such as agricultural chemicals and dioxins, or in the measures take to eliminate pathogenic microbes that often lead to large-scale water-related infections.

As a highly advanced technology in which chemical oxidizers, such as ozone and hydrogen peroxide, and photochemical reactions using ultraviolet rays and photocatalysts are used jointly to treat a variety of complex pollutants, AOP (Advanced Oxidation Process) has been attracting public attention. AOP is a process in which a hydroxyl radical, a strong oxidizer, is effectively generated in water where it plays an important role in its purification. However, the mechanism of water purification by AOP has not been clarified because of the lack of a simple method of measuring a hydroxyl radical.

Moreover, large-scale water-born infections caused by pathogenic protozoa are increasing at an alarming rate worldwide. Due to the difficulty in controlling such infectins using conventional chlorine disinfecting agents, there has been urgent calls to establish technologies that prevent the spread of infections, particularly relating to water supply and drainage. However, there remain major problems, as experienced in the difficulty of establishing countermeasure technologies because of the lack of techniques to determine whether or not protozoa and parasites on animals, are dead or alive. Such techniques are indispensable for evaluating the efficacy of disinfecting agents.

Aiming at the treatment of a trace amount of toxic chemical sub-

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stances in water and the disinfecting of pathogenic microbes, the authors et al. are developing a highly advanced technology of treatment by UV rays. In this paper, research examples will be discussed with emphasis placed on the technologies of evaluating the performance of purification (technologies of measuring hydroxyl radicals and inspection of protozoa) for the optimization of AOP.

2. Measurement of the amount of hydroxyl radicals generated and a proposal of highly efficient disinfecting technology[1)]

2.1 Disinfecting by UV rays and photocatalyst/UV irradiation

As an effective technology of disinfecting without using chemicals, such as chlorine, disinfecting using UV rays is being commercialized, particularly in Europe and in the U.S. UV-ray disinfecting is based on the principle that UV rays prevent the proliferation of microbes by damaging their genes. In some cases, however, organisms that have been exposed to the sun's UV raysduring evolution have developed defense mechanisms against it. For example there is a phenomenon wherein the part of microbes exposed to visible light after UV irradiation is reactivated with the enzymes induced to repair the damages (called photo-reactivation). It is pointed out in UV-ray disinfecting that further study is necessary to eliminate the phenomenon of photo-reacivation[2)].

This has led us to jointly use a photocatalyst as a technology that can be expected to disinfect based on a mechanism different from the direct effects of UV radiation. A photocatalyst, such as titanium dioxide, polarizes in its crystal by UV radiation to induce the oxidative decomposition of water, and the resulting hydroxyl radicals decompose microbes by oxidation using a mechanism different from that of UV radiation.

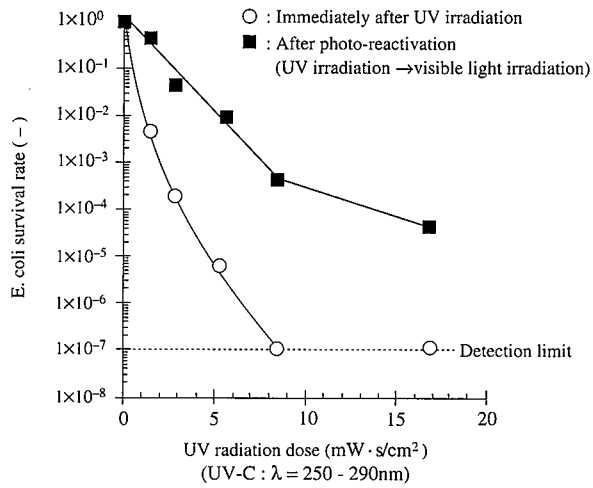
2.2 Grasping of photo-reactivation phenomenon[3)]

2.2.1 Experimental methods

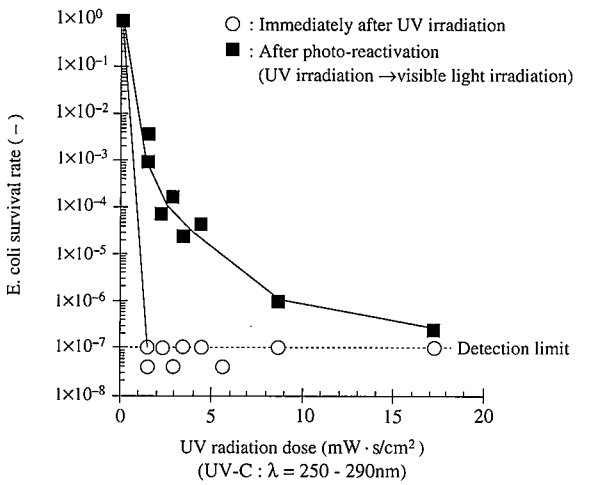
E. coli suspension (E. coli JM 109 strain: about 10^6/mL) was placed in a Petri dish, UV rays were irradiated from above, then the number of E.coli were counted. Visible light was further irradiated to the already UV-irradiated Petri dish for 60 minutes under the 20-W fluorescent lamp. The number of sample E.coli that photo-reactivated were counted. Then, the rate of survival of E.coli was calculated by dividing the number of sample E. coli after the application of UV rays and photo-reactivation by the number of sample E. coli before the irradiation of UV rays. Two kinds of mercury lamps now widely used for water disinfecting were the UV ray sources. A low-pressure mercury lamp and a medium-pressure lamp were compared relative to the difference in disinfecting efficacy. It is known that UV radiation wavelengths differ according to the difference in the pressure when charging mercury in a lamp. For low-pressure charging, UV-C is the main form radiation with a wavelength of 254 nm, while medium-pressure charging exhibits UV radiation over a wider range of wavelengths, including a longer wavelength of UV rays.

2.2.2 Experiment results

Fig. 1(a) and **Fig. 1(b)** respectively show the experiments' results using low-pressure and medium-pressure mercury lamps as the light sources. In the Figures, the doses of UV radiation (doses measured of UV-C using a UV sensor) and the then rate of survival were plotted on the x axis and the y axis, respectively. The rate of survival (marked ○ in the Figure) immediately after UV radiation decreased rapidly to below the detection limit in proportion to an increase in radiation dose. By contrast, the rate of survival increased from 2-digit to 4-digit when photo-reactivated by visible light radiation after UV radiation.



(a) Low-pressure mercury lamp



(b) Medium-pressure mercury lamp

Fig. 1 Effect of sterilization by UV radiation disinfecting

2.3 Confirmation of photo-catalyst/UV radiation disinfecting effects

2.3.1 Experiment methods

The rate of survival of E.coli was measured by the same method as in the foregoing sub-section using a Petri dish covered therein with titanium dioxide usinga sol-gel method.

2.3.2 Experiment results

Fig. 2 shows the results relative to the rate of survival after photo-reactivation when the subjects were treated by photocatalyst/UV irradiation together with those when the subjects were treated by UV irradiation only. From the Figure, it is evident that the rate of survival after photo-reactivation becomes lower when the subjects were treated by low- and medium-pressure mercury lamps together with photo-catalyst/UV irradiation than when the subjects were treated by UV irradiation alone, and that photo-reactivation can be reduced when a photo-catalyst is used in combination. However, a comparison of the two kinds of mercury lamps used as the sources of UV radiation shows that the medium-pressure lamp performs better in the reduction of photo-reactivation. Accordingly, it can be assumed

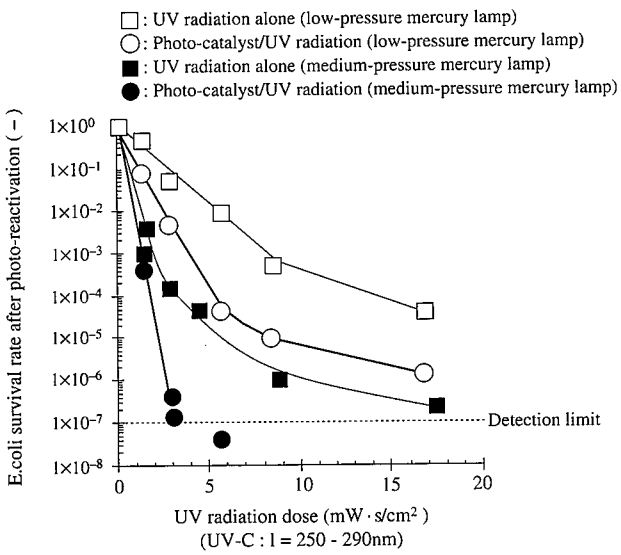


Fig. 2 Comparison of sterilizing effect between photo-catalyst / UV radiation disinfecting and UV radiation disinfecting

that, as a whole, medium-pressure lamps exceeds low-pressure lamps in UV radiation dose.

2.4 Grasping of the generation of a hydroxyl radical[4)]

It is emphatically suggested that reduction in photo-reactivation phenomenon can be accomplished by photo-catalyst/UV radiation, a task imposed relative to UV radiation disinfecting before the preceding sub-section. Study was made of the method of measuring the amount generated of hydroxyl radicals to clarify whether this tendency stems from the amount of hydroxyl radicals generated as pointed out by the initial tentative theory.

2.4.1 Measuring principle

Because of the very strong reactivity and the short life of a hydroxyl radical, it is considered difficult to directly measure its reactivity. A method of trapping radicals has been devised, however, as a technique of measuring the reaction of radicals in a living body in the field of biochemistry, a method in which hydroxyl radicals are allowed to react with a given compound (a radical scavenger) to observe the resulting product[5)]. The authors et al., with their intent to attain the radical tapping method using dimethyl sulfoxide (DMSO) as a radical scavenger, verified the applicability of this method in water treatment.

The reaction between DMSO [$(CH_3)_2SO$] and a hydroxyl radical [· OH], expressed by the following formula, results in the formation of methane sulfinic acid (MSA: CH_3SO_2H). The amount of this MSA formed indicates the amount of hydroxyl radicals generated.

$$(CH_3)_2SO + \cdot OH \rightarrow CH_3SO_2H + \cdot CH_3$$

A methane radical [· CH_3] is formed as a reaction by-product, which leads to a chain reaction with other radical. It is considered, however, that this has no influence on the formation of MSA.

2.4.2 Experiment methods

As in the previous experiment of disinfecting, a Petri dish was used for the treatment by UV radiation or by photo-catalyst/UV radiation. However, an aqueous solution of 0.5M DMSO was used as a test solution instead of E. coli suspension. After UV-irradiating samples under various conditions, MSA formed was extracted with sulfuric-acidified n-butanol solvent, and further with acetic acid buffer solution. To the water phase of the recovered acetic acid buffer solution extract was added an aqueous solution of Fast Blue BB to allow it to react with MSA to form azo dye. After subjecting this dye to toluene/n-butanol solvent extraction and washing with n-butanol-saturated water, absorbance was measured. For quantification, a calibration curve was prepared by measuring the standard sample, in which sodium methane sulfinate as a standard substance is dissolved in DMSO, in the same manner. Then, the concentration of MSA was calculated from the absorbance of the sample measured based on the calibration curve.

2.4.3 Results

Fig. 3 shows the results. As the Figure clearly shows, the formation of MSA was most striking in the treatment by photo-catalyst/UV irradiation with a medium-pressure mercury lamp as a UV radiation source (marked ● in the Figure), going downward in the order of the treatment by UV irradiation with a medium-pressure mercury lamp as a UV radiation source (marked ■ in the Figure), and the treatment by photocatalyst/UV irradiation with a low-pressure mercury lamp as a UV radiation source (marked ○ in the Figure). In the treatment by UV irradiation with a low-pressure mercury lamp as a UV radiation source (marked □ in the Figure) the formation of MSA was almost invisible.

2.5 Considerations

The reduction in photo-reactivation phenomenon, one of the tasks to be considered in the future in UV radiation disinfecting, can be achieved by increasing a UV irradiation dose, that is, by prolonging treatment time. Moreover, the photo-catalyst/UV irradiation method could reduce the photo-reactivation phenomenon significantly (Fig. 2).

For the study of the mechanism of this photo-reactivation reduction effect, the amount of hydroxyl radicals generated was examined along with the establishment of a technique of measuring them to confirm that they can be measured by the radical trapping method using DMSO. Since a similar tendency was observed between the effect of disinfecting with photo-reactivation considered and the amount of hydroxyl radicals generated relative to the presence of a photo-catalyst and the difference in the kinds of UV radiation lamps, it is safe to consider that the presence of a hydroxyl radical greatly contributes to reducing photo-reactivation.

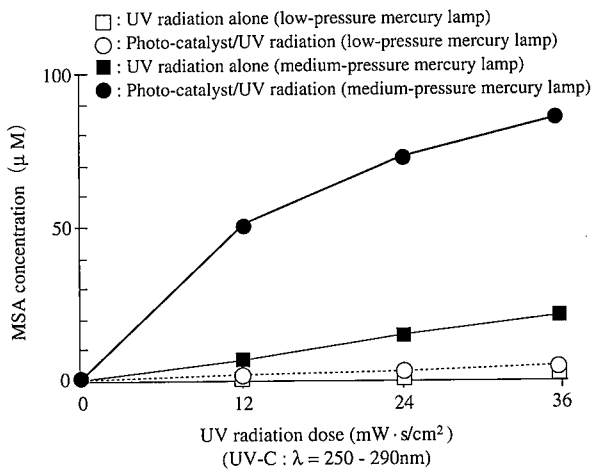


Fig. 3 Comparison of radical generated amount between UV radiation disinfecting and photo-catalyst/UV radiation disinfecting

3. Technique of inspecting Cryptosporidium protozoa and a proposal of technique for controlling them[6,7]

3.1 What is Cryptosporidium protozoan ?[8]

Water-related infections stemming from protozoa, including Cryptosporidium, have become a serious issue worldwide. For example, a very serious instance was reported that over 400 thousand people developed symptoms and over 100 people died out of over 4,000 people hospitalized in Milwaukee, U.S. in 1993. In Japan as well, more than 8,000 townspeople, accounting for 70% of the total townspeople, developed diarrhea in Ogose-machi, Saitama Prefecture in 1996. Although no deaths were reported, this triggered the need to take urgent steps against the recurrence of the disease.

Cryptosporidium is present with its infectious sporozoites enveloped in an oocyst in the environment. Because of its resistance to chlorine, the oocyst is considered difficult to control in the conventional water purification process. What is more, Cryptosporidium is a parasite on animals, and does not propagate in the environment. This makes the application of a culturing method to study it impossible, unlike that which has been performed with regard to microbes.

As a technique to counter Cryptosporidium, a method of physical removal by membrane separation is in use. The problems of cost and how to treat a separated oocyst are a part of the efforts in membrane separation. They have led to the study of the application of UV radiation disinfecting or photo-catalyst/UV irradiation disinfecting as a technique of inactivating Cryptosporidium. In case of inactivation by disinfection, a simple technique to ascertain oocyst's viability is indispensable, because the presence of an oocyst alone in treating water is not enough for evaluation of purification performance unlike physical removal by membrane separation.

There are two cases for ascertaining the viability of an oocyst: one is to ascertain its life or death as an organism, and the other, to ascertain whether it is sensitive enough as a parasite. As a method of distinguishing life from death of an oocyst, two proposals are made: one is for a vital dye staining method (microscopic observation after dyeing with fluorescent pigment), and the other, for an excitation test (microscopic observation of the production of a sporozoite from an oocyst). Again, as a method of distinguishing oocyst's infectivity, a test of infecting small animals is employed. It is necessary, however, to establish a new simple inspection method to replace the above methods because they are both extremely labor and time intensive.

3.2 Establishment of a technique to inspect Cryptosporidium protozoan[9]

A cultured cell experiment has come to attract our attention in recent years as an experimental system for inspection of Cryptosporidium without using animals[10]. It was reported that, by infecting a cultured cell with an oocyst or a sporozoite, excyctated beforehand[11], Cryptosporidium propagates in the cultured cell. By developing this expertise, a method is proposed of detecting and quantifying propagated oocysts by the enzyme-linked immuno-sorbent assay[12]. It is also reported that a sporozoite adhered to the cell surface can be detected by the enzyme-linked immuno-sorbent assay[13]. In either method, however, it is necessary to enhance detection sensitivity for evaluation of the extent of inactivation. Therefore, this point was examined.

3.2.1 Study of a technique to inspect viability

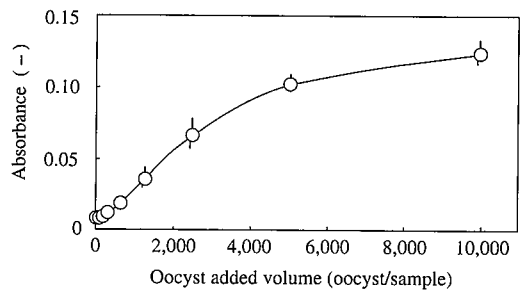
(1) Experiment methods: Human ileocecal epiterial cell HCT-8 were cultured, fixed with formalin, and allowed to contact oocyst suspension to react for two hours. This enables a living oocyst to excyctate to allow a sporozoite to adhere to the cell surface. After the reaction, we washed an unreacted oocyst, and detected the sporozoite adhered to the cell surface by the ELISA (Enzyme-linked immunosorbent assay) method using an anti-sporozoite antibody.

(2) Experimental results: Oocyst suspensions diluted to various concentrations were measured. **Fig. 4(a)** shows the results. After a detailed study of the host cell culture conditions and a detection reagent to be used for the ELISA method, changes in absorbance in proportion to the number of oocysts added were confirmed, and with the oocysts of about 10^2, a value lower than the one already reported by two digits was detected[9]. Sporozoites highly sensitive even when allowed to excystate before allowing to contact cultured cells were detected, and infected sporozoites as they are in oocysts without separating them to recover[9]. It is thought that this method enables to simply evaluate the viability of Cryptosporidium without microscopic observation when we turn our attention to the excystativity of an oocyst.

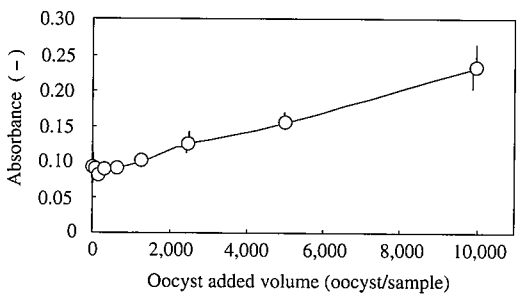
3.2.2 Study of a technique of inspecting infectivity

(1) Experiment methods: HCT-8 Cell was cultured, and allowed to contact oocyst suspension to react for two hours. After the reaction, theculture was continued for two days with the addition of a fresh culture medium after cleaning away non-reactive oocysts. This enables sporozoites that infected a host cell to propagate within the cell to produce oocysts. After fixing cultured substances with formalin, the oocysts that propagated within the cell were detected using the Elisa method through an anti-oocyst antibody.

(2) Experiment results: In the experiment, oocyst suspensions diluted to various concentrations were measured. **Fig. 4(b)** shows the results. After detailed study of the host cell culture conditions and a detection reagent to be used for the ELISA method, there was a successful increase in detection sensitivity from 10^4- to 10^5 per sample of the conventional animal tests to the extent of 10^2. It is thought that this method enables a simply evaluation of infection of



(a) Viability inspection



(b) Infectivity inspection

Fig. 4 Example of a calibration curve concerning inspection of the viability of Cryptosporidium protozoan

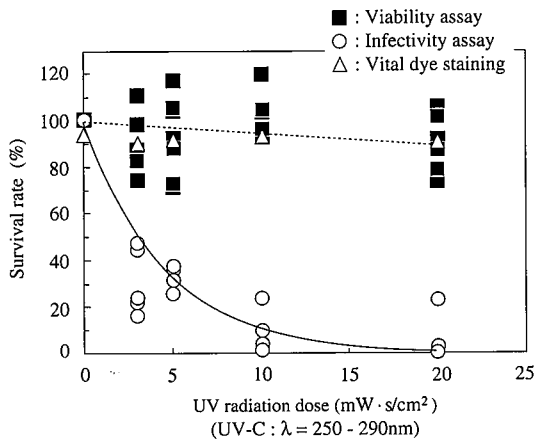


Fig. 5 Inactivation of Cryptosporidium protozoan by UV radiation

Cryptosporidium which otherwise would have been impossible to evaluate without an animal test.

3.3 Verification of the effect of UV radiation disinfecting

3.3.1 Experiment methods

After applying UV rays to the oocyst suspension, the viability of the oocycst (viability and infectivity) was measured using the above-described Elisa method for the cultured cell system. In the experiment, the effect of radiation dose was verified by varying radiation doses in several steps. Vital dye staining using fluorescent pigment for comparison with the conventional method was also evaluated.

3.3.2 Experiment results

The results of measurement of oocyst suspension after application of UV rays was divided by the value measured of a sample solution before application of UV rays to calculate the rate of survival. **Fig. 5** shows the results.

First, there was no observable change in the results of the experiment using the Elisa method for inspection of viability that corresponds to the conventional excitation test (marked ■ in the Figure) even with an increase in UV radiation doses. This tendency was similar to the results of evaluation using the conventional vital dye staining method (marked △ in the Figure). On the other hand, the results of Elisa for evaluation of infectivity (marked ○ in the Figure) showed a notable decrease in the survival rate in proportion to an increase in UV radiation doses.

3.4 Considerations

UV radiation disinfecting has been considered unfit for controlling Cryptosporidium because of its resistance to UV rays[14]. It is reported, however, that a plurality of research groups in the U.S. confirmed in the microscopic observation of cultured cell systems and animal tests that low-dose UV irradiation can reduce the infectivity of Cryptosporidium although powerful UV irradiation is required to exterminate Cryptosporidium. Results to support the aforementioned facts were also obtained[15].

The results suggest that the ability of Cryptosporidium to infect can be lessened although Cryptosporidium cannot be exterminated even through UV irradiation theretoto. This is based on the assumption that UV rays denature DNA, the object of genes, to impair DNA duplication resulting in the inability to propagate.

4. Conclusions

Because of its residual tendency, chlorination, hitherto employed widely, has the merit of long-lasting effect after its application. It is pointed out, however, that residual chlorine has an adverse influence on the ecosystem, regarding its discharge. It is also feared that disinfecting by-products, including trihalomethane, have negative impact on human health. In addition to the foregoing, pathogenic microbes, difficult to disinfect by chlorination, pose serious hygiene problems. The development and commercialization of disinfecting techniques to replace chlorination are therefore of the highest urgency.

UV radiation disinfecting is attracting public attention as one of the disinfecting techniques to replace chlorination, it is considered to generate no disinfecting by-products. It is necessary, however, to consider the phenomenon of photo-reactivation. In other words, the equipment design of UV radiation disinfecting is now evaluated in terms of the rate of survival immediately after the application of UV rays. In the supply of water after disinfecting through a closed conduit, the photo-reactivation phenomenon may not be a serious problem. It is necessary, however, to evaluate based on the rate of survival with a possibility of photo-reactivation included in cases where sewage water is discharged to a natural water area and where water is recycled for recreational or for other purposes.

Reduction of the photo-reactivation phenomenon, one of the problems in UV radiation disinfecting, can be achieved even by increasing UV radiation doses. As pointed out in this paper, it is also possible to reduce photo-reactivation without increasing UV radiation doses by combining it with a photocatalyst.

Since it is difficult to control pathogenic microbes, such as Cryptosporidium, with the conventional chlorination, the research into a technique to combat them is ardently being pursued on a worldwide scale. Disinfecting in water treatment has so far aimed at exterminating pathogenic microbes. It is now suggested, however, that reduction in infectivity means the achievement of a primary purpose of disinfecting[16]. In view of this, UV radiation disinfecting is likely to become a potential technique to combat Cryptosporidium. In fact, the US EPA is considering the approval of UV radiation disinfecting as a technique to counter Cryptsoporidium independent of other disinfecting processes.

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