(12) **CONTROL OF ALGAL GROWTH BY UV-RADIATION**

紫外線照射による藻類増殖抑制効果

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要旨

水道水源の湖や貯水池における藻類の異常増殖は、浄水場における砂ろ過の閉塞、異臭味、消毒副生成物前駆物質の生成など様々な問題を起こす。富栄養化防止あるいは藻類の異常増殖を抑制するためには様々な試みがなされているが、条件によって効果の差異が生ずる。特に硫酸銅などによる直接的な殺藻等の場合は、湖や貯水池の生態系への影響を考慮しなければならない。

本研究は紫外線照射による藻類増殖抑制効果について検討したものである。対象として用いた藻類は *Microcystis aeruginosa* と *Anabena vulgaris* である。これらの藻類に対して入射紫外線照射量として 450mWs/cm^2 を照射した後、培養したところ、藻類細胞数の減少が生じた。また 180mWs/cm^2 を照射した場合では、7日間増殖が抑制された。藻類由来有機物質が溶存している試料に紫外線を照射し、その後その試料に藻類を投入したところ、藻類の増殖が抑制されることが観察され、紫外線の残存効果が見られた。さらに紫外線照射後7日間冷暗所に保存した試料に藻類を投入した場合でも増殖抑制効果が残存することが確かめられた。

Abstract; Excessive algal growth in drinking water sources like lakes and reservoirs is responsible for filter-clogging, undesirable taste and odor, disinfection-by-product formation and toxin generation. Although various methods are currently being used to control algal bloom, their successes are limited. Many water utilities routinely use copper sulfate to control excessive algal growth. But there is a growing concern against its use mainly because it is non-specific to target algae and kills many non-target species. In this study, the scope of using UV-radiation to control algal growth was assessed using *Microcystis aeruginosa* and *Anabena vulgaris* as test species. For both of these species, an incident UV-dose of 450-mWs/cm^2 was found to be lethal. A smaller dose of 180-mWs/cm^2 prevented growth for about 7-days. It was also observed that UV-radiation on algal extracellular products has a significant residual effect and can contribute to algal growth control. The extent of residual effect depends on the UV-dose and can continue even for 7-days.

Key Words; Algae, *Microcystis aeruginosa*, *Anabena vulgaris*, Extracellular products, UV-radiation, Incident UV-dose.

1. INTRODUCTION

The presence of algae in drinking water source can have a significant impact on the treatment of

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that water. Algae impart undesirable taste and odor, cause filter-clogging and is also responsible for some disinfection-by-products and toxins. Hence control of algae in the source has received considerable attention. The most effective way to control algal growth is to reduce nutrient-load into the reservoir (Ryding *et al.*, 1989). But because of significant internal loading in most reservoirs and lakes, especially from bottom sediment, control of external nutrient-load alone is not sufficient to prevent seasonal algal blooms (Horne *et al.*, 1994). Hence, many kinds of in-lake management programs are practiced to control algal bloom. Hypolimnic aeration (Ljubisavljevic *et al.*, 1997), artificial destratification (Saito *et al.*, 1994a, 1994b, Simmons, 1997), biomanipulation (Hejzlar *et al.*, 1997), use of macrophyte and macrophyte extracted bioactive compounds (Nakai *et. al*, 1996) are some of the methods that has been tried. But despite all these efforts, excessive algal growth remains a major problem in many lakes and reservoirs. Till now, many water utilities routinely apply copper sulfate to control excessive algal bloom. But currently there is a growing concern against its use, mainly because it is non-specific to target algae (kill many non-target species). Other disadvantages associated to their use include— oxygen depletion caused by sudden death and decomposition of algal bloom, accelerated release of odor causing compounds and/or toxins, and accumulation of copper in sediments.

The objective of this study was to investigate the scope of using of UV-radiation to control algal growth. UV-radiation has the potential to inhibit algal growth without considerable adverse effect to the ecology of the lakes and reservoirs. *Microcystis aeruginosa* and *Anabena vulgaris* were selected for the experiments because of their frequent association with seasonal algal-bloom. In addition, indirect and residual effect of UV-radiation was also assessed by using *M. aeruginosa* as the test organism.

2. MATERIALS AND METHODS

Cultivation of Algae

Axenic cultures of blue green algae *Microcystis aeruginosa* and *Anabena vulgaris* were obtained from National Institute for Environmental Studies (NIES), Japan and was then grown in standard culture media (CT-media, JWWA, 1993). For *M. aeruginosa*, the pH was adjusted to 9.0 by buffering with bicine (N,N-2Bis(2-hydroxymethyl) glycine $C_6H_{13}NO_4$) instead of Tris (hydroxymethyl) aminomethane. Cultures were maintained at 25^{0}C in an incubation chamber with controlled humidity and lighting. Fluorescent lamps (FL20SW-B, GE/Hitachi) were used as the light source with an automated 16h/8h-light/dark cycle. The light intensity during the light phase was 1500-lux.

Effect of direct UV-radiation

Samples were prepared by inoculating exponentially growing cells into autoclaved media (P=5.23 mg/l) to give an initial concentration of about 10^6 cells/ml. To assess the effect of direct UV-

radiation (254 nm), 40-ml samples were irradiated in petridishes (depth of the sample = 0.67 cm) for 2, 5 and 10 minutes by a single low-pressure mercury lamp (10W, GL-10, National Co. Ltd). The intensity of UV-radiation at the surface of the sample was 1.5-mW/cm^2. The incident UV-dose is expressed as the product of the time of irradiation and the intensity of the irradiation at the surface. After irradiation the samples were incubated in an incubation chamber (1500-lux fluorescent light, 25^{0}C temperature, 16h/8h-light/dark cycle as mentioned before). The growth was measured by counting cells in haemocytometers by a phase contrast microscope. Only those cells showing no marked damage were counted, i.e., broken and faded cells were considered dead. All growth experiments were carried out in triplicate.

Indirect effect of UV-radiation

To assess the indirect effect of UV-radiation, the procedure outlined in Fig. 1 was followed. Algal extracellular product (ECP) was separated from the cells by filtering through 0.7-μm glass-fiber filter (GF/F, Whatman). Nutrient was added to the filtrate to give an initial phosphorous concentration of about 5-mg/l and pH was adjusted to 9.0 using NaOH. 30-ml samples were irradiated in petridishes (depth of the sample = 0.5 cm) under a low-pressure mercury lamp for 5, 10 and 20 minutes (intensity at the surface of the sample = 1.5-mW/cm^2). After irradiation, 20-ml samples were transferred to 60-ml glass tubes. Some of the irradiated samples were immediately inoculated with exponentially growing *M. aeruginosa* (test organism) to an initial concentration of about 10^5 cells/ml and were incubated at 25^{0}C under fluorescent light with a 16h/8h day/night cycle. To study the duration of the residual effect, some irradiated samples were stored in the dark for 1-7 days before inoculation with fresh *M. aeruginosa*. The indirect effect was assessed by monitoring the survival of freshly inoculated *M. aeruginosa* in the UV-irradiated ECP-solution. All the experiments were carried out in triplicate.

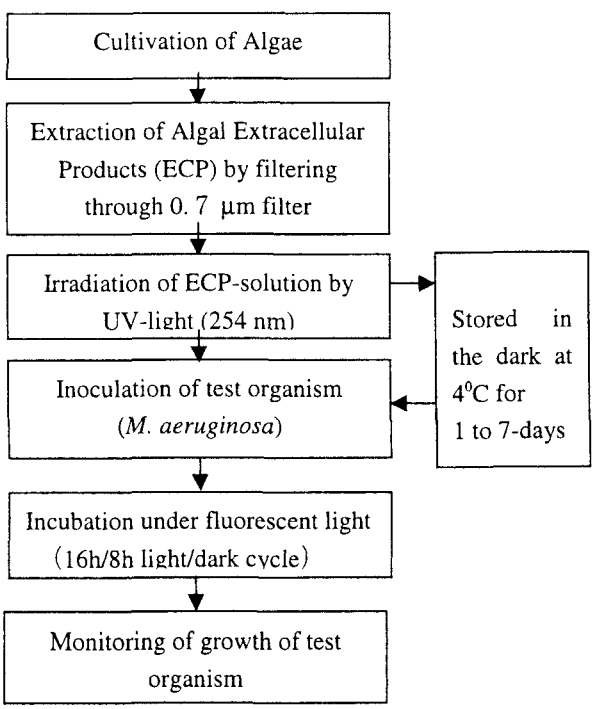

Fig. 1. Procedure followed for assessing the indirect effect of UV-radiation.

3. RESULTS AND DISCUSSION

Effect of direct UV-radiation

The effect of direct UV-radiation on *M. aeruginosa* and *A. vulgaris* is shown in Fig. 2(a) and 2(b). In both cases, there was no change in cell density immediately after irradiation. But under the microscope, the green color of the cells was found to be slightly faded. But during incubation the cells subjected to an incident UV-dose of 450-mWs/cm^2 and 900-mWs/cm^2 gradually started to die. And at the end of 7 days the cell densities in these samples were less than 2 percent of the respective controls. The fact that cells continue to die for a long time after irradiation, indicates that there may be considerable residual effect of UV-radiation. Interestingly, for both *M. aeruginosa* and *A. vulgaris*, there was little difference between the effect of 450-mWs/cm^2 dose and 900-mWs/cm^2 dose. Probably 450-mWs/cm^2 is sufficient to cause lethal cell damage and increasing the intensity further only increased the extent of cell damage. Another interesting observation was that although 180-mWs/cm^2 dose did not initiate any decay, it prevented the growth. And according to microscopic observation very few cell division took place during the seven days of incubation. This probably indicates damage to the reproduction system of cells. It was also observed that cells tend to settle to the bottom of the tube after irradiation; which could be particularly important in case of reservoirs and lakes because settling down from the epilimnion can mean effective death to the cell.

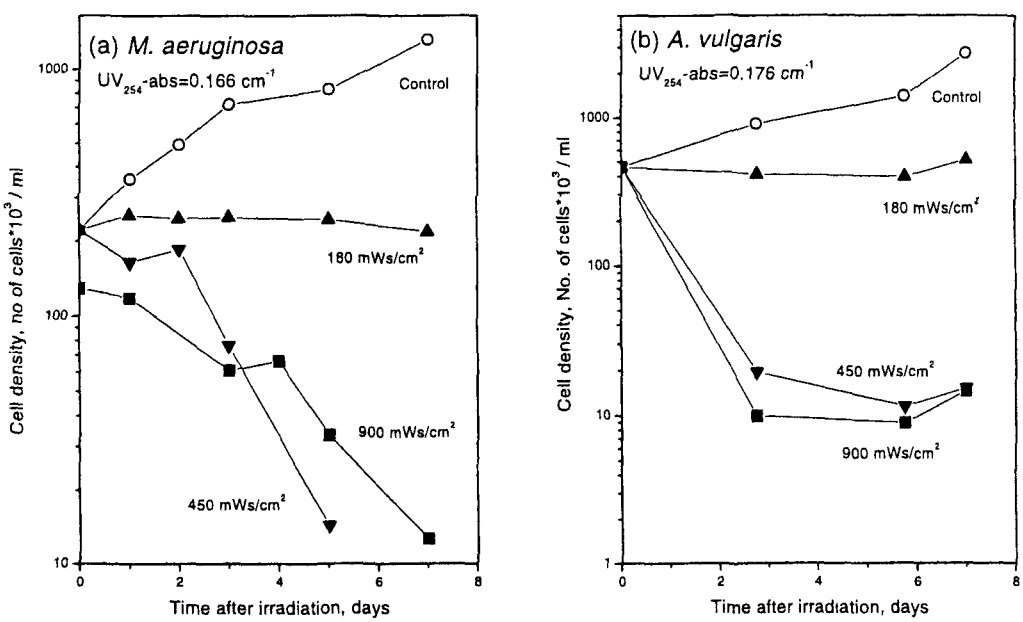

Fig. 2. Decay of algae after direct UV-radiation. (a) *Microcystis aeruginosa*, UV_{254}- absorbance of sample solution = 0.166 cm^{-1} (b) *Anabena vulgaris*, UV_{254}- absorbance of sample solution = 0.176 cm^{-1}. All the doses indicated in the figure are incident UV-dose, which is equal to the product of the intensity of UV-radiation at the surface of the sample and duration of irradiation.

Indirect (residual) effect of UV-radiation

A summary of the results of the indirect effects of UV-radiation on water containing algal-ECP is detailed in Fig. 3 and Table 1. Fig. 3 shows the growth profiles of the test organism (*M. aeruginosa*) with time and the residual algicidal effects are summarized in Table 1. As shown in Fig. 3(a), when ECP-solution from *M. aeruginosa* was irradiated by an incident UV-dose of 450-mWs/cm^2, it produced no residual effect on the growth of the test organism. But when the dose was

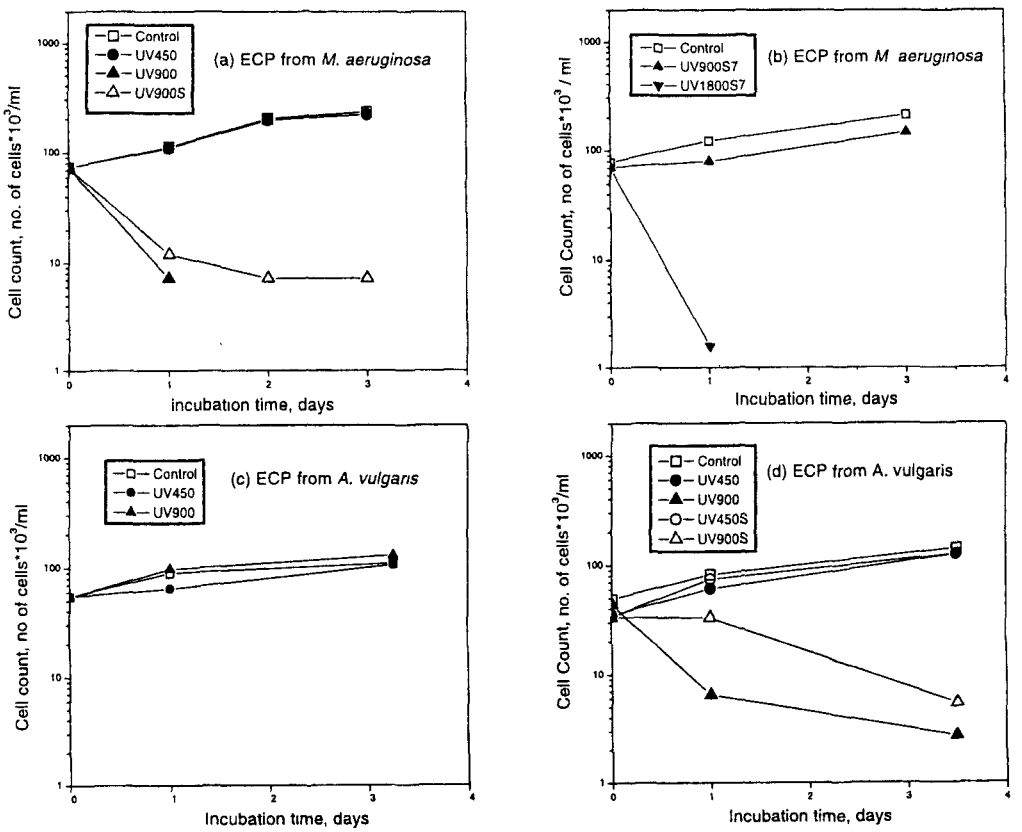

Fig. 3. Survival and growth of test organism (*M. aeruginosa*) in UV-irradiated algal-ECP-solution (a) ECP-from *M. aeruginosa* (b) ECP-from *M. aeruginosa* (c) ECP from *A. vulgaris* (d) diluted ECP from *A. vulgaris*. UV450 and UV900 indicate incident UV-dose of 450-mWs/cm^2 and 900-mWs/cm^2 respectively. UV450S and UV900S indicate samples that were stored in the dark for 24-hours before inoculation after incident UV-dose of 450-mWs/cm^2 and 900-mWs/cm^2 respectively. UV900S7 and UV1800S7 indicate samples that were stored in the dark for 7-days before inoculation after incident UV-dose of 900-mWs/cm^2 and 1800-mWs/cm^2. For the ECP from *M. aeruginosa* subjected to an incident UV-dose of 900-mWs/cm^2, no test organism survived after 2-days of incubation. Similarly, when a dose of 1800-mWs/cm^2 was applied, no test organism survived even after storage of 7-days before inoculation.

increased to 900-mWs/cm^2 the residual toxic effect caused complete mortality of all the test organism. The toxic effect persisted, although some exposed cells survived (after 3-days, cell concentration was only 3% of that in the control) when the irradiated samples were stored for 24-hours before it was inoculated with test organism. But when the storage period was increased to 7-days, no significant residual effect on the growth of test organism was observed for an incident UV-dose of 900-mWs/cm^2 (Fig. 3(b)). But for a higher dose (1800-mWs/cm^2) the effect persisted even after 7-days storage (Fig. 3(b)). So the duration of the residual effect probably depends on the dose of UV-radiation. When ECP-solution from *A. vulgaris* was irradiated, no algicidal effect was observed (Fig.3 (c)) even for an incident UV-dose of 900-mWs/cm^2. The probable reason was that the absorbance of this sample was so high (UV_{254} = 2.520 cm^{-1}) that the effective-dose (calculated by Beer-Lambert law) became too small to cause any significant effect. When this ECP was diluted (UV_{254} = 0.370 cm^{-1}), the results (Fig.3 (d)) were similar to those observed for *M. aeruginosa*.

Table1: Indirect (residual) effect of UV-radiation

	Incident UV-dose, mW-s/ cm^2	Identification	UV_{254}-abs cm^{-1}		Algicidal effect	Calculated Effective UV-dose mW-s/ cm^2
			Before irradiation	After irradiation		
Extra-cellular-products from *M. aeruginosa*	450		0.131	0.128	--	408
	900		0.131	0.129	+++	816
	900	Stored for 1-day in the dark at 4^{0}C	0.131	0.129	++	816
	900	Stored for 7-days in the dark at 4^{0}C	0.131	0.129	--	816
	1800	Stored for 7-days in the dark at 4^{0}C	0.131	0.129	+++	1631
Extra-cellular-products from *A. vulgaris*	450	Undiluted	2.520	2.500	--	114
	900	Undiluted	2.520	2.450	--	230
	450	Diluted	0.370	0.347	--	346
	450	Stored for 1-day in the dark at 4^{0}C	0.370	0.347	--	346
	900	Diluted	0.370	0.333	++	695
	900	Stored for 1-day in the dark at 4^{0}C	0.370	0.333	++	695

+++ Complete mortality of all the test organism

++ Cell concentration is less than 10% of that in the control

+ Cell concentration is less than 50% of that in the control

-- No significant difference in cell concentration as compared to the control

The residual effect of UV-radiation can be partly explained by the action of photosensitizers. These are compounds that are transformed from the low-energy ground state to a higher energy electronically excited state by absorption of photon and are capable of transferring their energy to

other molecules. The energy transfer may induce chemical reactions producing intermediate compounds like singlet oxygen, hydrogen peroxide and OH-radicals, which in turn may have toxic effect on algae.

Previously the humic substances have been shown to act as photosensitizers (Gjessing *et al.*, 1991, Hessen *et al.*, 1994). This study shows that extracellular organic matter from algae (which mainly consists of glycolic acid, polysaccharides and low molecular-weight sugars, and trace amounts of complex macromolecular carbohydrates, organic acids, amino acids and lipids) can also act as photosensitizers.

However, the action of photosensitizers alone is probably insufficient to produce the long-term toxic response. Because most toxic species (singlet oxygen, hydrogen peroxide, hydroxyl radical etc) produced by photosensitizers are very short lived but there was significant algicidal effect even when the irradiated sample was stored for 7-days, before inoculation of the test organism. Apparently UV-radiation starts a sequence of oxidation reactions that may last for longer duration.

4. CONCLUSIONS

This study shows that UV-radiation has the potential to be used as an effective means to control algae. As relatively smaller UV-dose (180 mWs/cm^2) can control the algal reproduction for a significant period of time, UV-radiation prior to the bloom period may act as an effective means to control seasonal algal bloom. This study also shows that UV-radiation of algal extracellular products produces significant residual effect on the growth of algae. The extent of residual effect depends on the concentration of extracellular products and also on the UV-dose. The fact that UV-radiation on algal extracellular products produces significant residual effect also adds to the merits of UV-radiation as a potential means of algae control. More over, as UV-radiation adversely affects the ability of planktonic algae to remain in suspension, it is possible that UV-radiation would be more effective in actual lakes and reservoirs.

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