KEY WORDS

ultraviolet radiation

disinfectant

photoreactivation

coliform bacteria

wastewater

ABSTRACT

Constance M. Dumais: Photoreactivation of UV-Irradiated coliforms in Wastewater Effluent. (under the direction of Dr. J. Donald Johnson).

Ultraviolet (uv) radiation of wastewater is currently being considered as an alternative to chlorination. However, the problem of the extent of photoreactivation (PR), and under what conditions it would occur most severely has not yet been defined. The parameters of optimal exposure time, sunlight intensity, nutrient content, temperature, and uv dose were examined. These same parameters were also studied under laboratory conditions, using an artificial PR light source. During strong summer sunlight, 60 minutes of exposure gave the maximum amount of repair at temperatures near 25°C. At higher (36°C) or lower (17°C) temperatures, the level of PR declined. Nutrient attenuation, achieved by diluting the sample 1:10 or 1:40 in receiving water did not decrease PR, however a very large decrease in nutrients did negatively affect the level of survival after PR. Under optimal conditions, the effective level of disinfection at a given uv dose was decreased by approximately 67-75%.

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LITERATURE REVIEW

Recently, in public health literature, the safety of chlorine as a disinfectant in water and wastewater treatment has come into question, (31, 49). The formation of potential toxic or carcinogenic byproducts, such as trihalomethanes, or THM's, has caused researchers to examine possible alternatives to chlorination. One such possibility currently under consideration is ultraviolet (uv) light.

UV light has long been known to be lethal to microoganisms. The first published evidence in 1878 by Downs and Blunt, demonstrated the bactericidal properties of short wave radiation (47). In 1903, Bernard and Morgan determined the region of lethal action to be between 226.7 - 228.7nm (5). Two years later, Bang published findings which placed the most effective germidical wavelengths at approximately 250nm. In 1910, this property of uv light was first applied to disinfecting water by Cernovodeanu and Henri, and Henri, Helbronner, and Recklinghausen (47). They effectively disinfected water, to which a variety of bacteria had been added.

There are several advantages to using uv as a disinfectant over chlorine. These include the high efficiency of uv disinfection at a cost which approaches that of conventional methods (35); that the chemical composition of the water is not altered; that no harmful byproducts are formed; that the taste of the water is not changed; and that the maintenance of the machinery involved is not difficult, needing only routine cleaning (5, 29, 47). However, because there is no disinfectant residual in the water, process control can be difficult, and there is no protection from post-treatment

contamination. Also the water quality prior to uv-irradiation must be relatively good, in order to achieve adequate disinfection, thus increasing the cost of operation. Finally, there is the problem of regrowth of damaged, but not killed microbes. There are several repair processes within the cell, which can reverse uv caused lesions in the cells DNA. The most effective of these is a light induced enzymatic repair system, known as photoreactivation.

Initially, the mechanism which led to cell death from irradiation was unknown. It was speculated that the uv light might act directly on cellular components, such as proteins, enzymes, or nucleic acids, or indicrectly, by forming toxins within the cell (3). Later research showed the action spectrum of uv light on the cell corresponded very well to the absorption spectrum of nucleic acids, suggesting that they might be the target of the uv photons. As the importance of nucleic acids and DNA became understood, initiated by Watson and Crick's work in the 1950's, it became apparent that damage to this cellular component could be lethal, cause mutations, and account for many of the other effects attributed to exposure to uv light. By the early 1970's, the uv photoproducts were well characterized.

When photons of uv light are absorbed by the DNA, there is usually some local denaturization of the double helix (19, 44). (See Fig. 1) This is often caused by dimerization of adjacent pyrimidine bases. These dimers can occur between any of the cyclobutanes, and all types have been isolated, including Thimidine-Thimidine (T-T), Cytosine-Cytosine (C-C-), and Thimidine-Cytosine (T-C). Thimidine dimers predominate, and are also the most easily reversed by the process of photoreactivation. Hydration products of bases are also

fairly common events, and also causes denaturization of the DNA. The frequency of chain breakages increase, leaving the DNA susceptible to forming cross linkages with other pieces of genetic material or proteins. At least some of these cross linkages may be attributed to the error prone excision repair system, which is stimulated by irradiating with germicidal wavelength light. These lesions can lead to the types of uv damage observed in microbes, including death, mutations, and delay of DNA synthesis and cell replication cycles, making uv light an effective germicidal agent.

Organismal survival is a function of both intensity and exposure time, as per the equation:

$$D\ (mWatt\text{-}sec/cm^2) = I\ (MW/cm^2) \times T\ (sec) \quad (equ.1)$$

where D is the does, I is the light intensity, and T is the time.

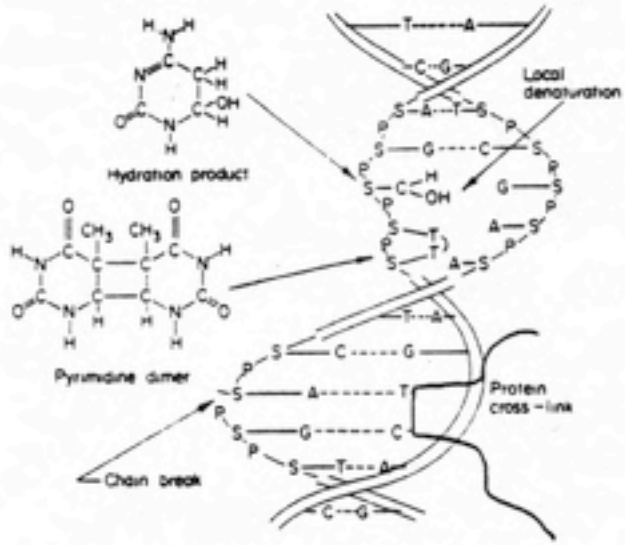
As early as 1929, Gates reported that the Bunson-Roscoe Law of Reciprocity did not apply to uv irradiation, over a large range of intensities (11). That is to say "that when the product of intensity and exposure time is constant, a constant photochemical reaction results". This was at least partially repudiated in subsequent literature (24, 34). Survival can be represented by the following equation:

$$\log(N_s/N_o) = f(Dose) \quad (eqn.2)$$

where N_o is the initial population, and N_s is the number of survivors after irradiation. Equation 2 is derived from Luckiesh and Holiday's (29, 47). Survival data is usually plotted as log survival vs. uv dose. The resulting dose-survival curves for uv disinfection are often sigmoidal in shape (2). The initial shoulder indicates a

deviation from one-hit or one-target kinetics, which may be due to threshold dose levels or redundancy in the genome (16). There has also been speculation that the shoulder may also be caused from the repair of the photoproducts. This is somewhat substantiated by the observation that Micrococcus radiodurens exhibits an exceptionally large shoulder, and has one of the most efficient repair systems of any organism tested (24). Dose-survival curves include a straight line portion which follows Chick's Law, where increases in uv dose results in proportionally lower survival and the Law of Reciprocity appiles. Often at high uv doses, there is a leveling off, after which further increases in dose do not give the expected decrease in survival. This has been largely attributed to particulate protection in wastewater disinfection. Unsettled clumps of organic matter or aggregates of cells can shield bacteria imbedded within them by absorbing the uv light (33).

The efficient disinfection of water by uv light depends on a variety of factors. Physical-chemical characteristics of the water to be treated are important. Highly turbid or colored water may absorb uv light, making it unavailable for disinfection. The removal of particles and aggregates will improve the efficiency of the irradiating light (36). Some mixing of the water in the uv reactor facilitates the exposure of microbes to the uv, effectively increasing the dose seen by a given organism by increasing the exposure time. The intensity can be enhanced by lining the reactor interior with reflective material. However, too much foreward dispersion will allow a significant portion of the microbes to short circuit through the reactor , receiving a smaller uv dose, and decreasing disinfection efficiency. Most of the survivors from a



Schematic illustration of the various alterations found in DNA extracted from cells that have been irradiated with ultraviolet light. [Adapted from R. A. Deering, *Sci. Am.* **207**, 135 (1962).]

FIGURE 1: from P.C. Hanawalt. Radiation damage and repair in vitro. In "An Introduction to Photobiology", C.P. Swanson, editor. Prentice Hall, Inc. NJ. 1968.

given volume of water passing through a reactor are those which short circuit through the unit (35). There is a tradeoff between allowing some degree of mixing and not causing too much foreward dispersion. A recent study demonstrated that ideal plug flow is more effective than completely mixed type flow patterns in uv disinfection (6, 35).

Although uv light absorbed by the DNA will produce some type of lesion, not all of these are fatal. There exists several possibilities where uv induced damage could be biologically undetected (15). For example, the lesion could be located in a portion of the DNA molecule that does not contain vital information or is duplicated elsewhere in the genome or that is not being transcribed or translated at the time of irradiation. Polyploid organisms are known to require roughly twice the uv dose in order to achieve a level of disinfection comparable to those containing only a single copy of the genetic information. Kinetic studies show that the inactivation curves, similar to those generated when there are two-targets per cell.

When measurable endpoints, such as mutation or death are observed, the level of survival can be greatly affected by post-irradiation conditions. There are several processes by which the damaged cell can resume normal function. Some involve spontaneous electrochemical reactions, such as the disintegration of dimers, or dehydration of the bases. Some repair schemes involve exchange of complementary genetic material to replace the damaged portion of the chromosome. Some are enzymatic systems. Not all the repair is specific for uv induced lesions. The most effective of these is photoreactivation, which is specific for reversing cyclobutane dimers formed as a result of uv irradiation.

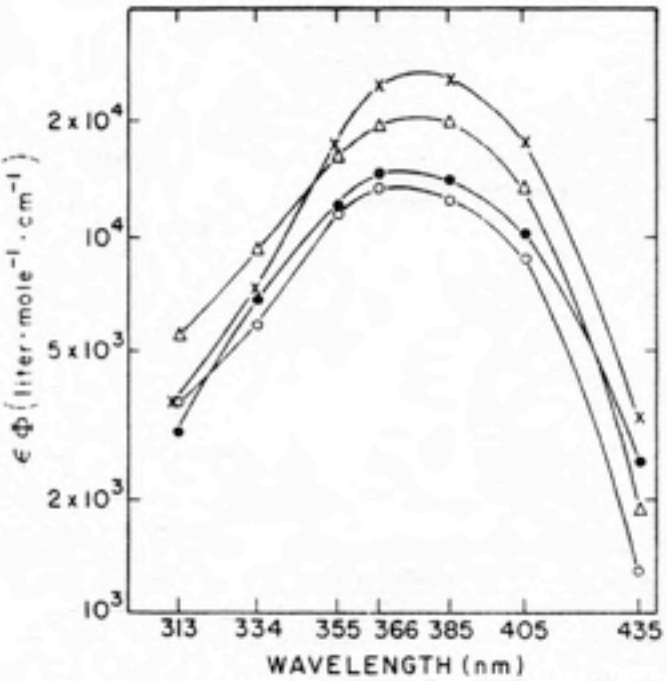
Photoreactivation (PR) refers to the highly specialized enzymatic removal of pyrimidine dimers, initiated by post-irradiation illumination with light of wavelengths in the range of 300-500nm (23). The reversal of uv damage was first observed by Whitaker, in 1942. However, it was Kelner, in 1949, who perceived the importance of exposure to near-uv light after disinfection, and correlated it to the ability of bacteria to reverse the otherwise fatal effects of the germicial light (25). Later that same year, Dulbecco published similar findings in bacteriophages (8). Since that time, the ability of organisms to use 300-500nm light to stimulate the enzymatic repair of pyrimidine dimers has been studied extensively (See Fig. 2). It has been found in almost all bacteria, viruses, and yeast (23), as well as in protozoans and higher animal cells (3, 12, 14, 27). PR is able to reverse a variety of cellular effects caused by uv light, including death, mutations, and delay of cell division, protein synthesis, and DNA replication.

Early experimentation concentrated primarily on determining the extent of photoreactivability in cultured organisms, as well as the influences of irradiation and illumination conditions on organismal survival. Experiments often involved irradiation with commercially available germicidal lamps, having a peak output at 253.7nm. Estimation of uv dose was made by plotting log survival vs. time, assuming the output of the lamps was the same, regardless of age, manufacturer, etc. Disinfection was followed by illumination using either fluorescent lights, black lights, or Quartz-iodide lamps as the pR source (1, 17). Both treatments were commonly performed on cell suspensions of non-nutritive buffer solutions, although some

researchers plated the uv-irradiated samples prior to exposure to PR light. Phage studies were performed on irradiated viruses which were allowed to absorb onto host cells before being photoreactivated.

Definitions in early literature were inconsistent and confusing, until Jagger, in one of the first comprehensive reviews, suggested a standardized nomenclature (23). Jagger defined PR as "the restoration of uv radiation lesions in biological systems with light of wavelengths longer than that of the damaging radiation". He proposed that PR be used in lieu of the other terms sometimes used, such as photorestoration or photoreversal. Since then, the term photoreactivation has been further narrowed to include only that enzymatic restoration of uv damage involving the photoreactivating enzyme (PRE). Inactivation has been defined as when an organism is unable to give rise to a colony under normal (i.e. standard laboratory) growth condition. Varying growth conditions, in some cases, may stimulate the formation of a colony which would not have occurred had a standard nutrient media been used (30, 45). Irradiation refers to the germicidal light, and illumination to PR light.

PRE was first isolated by Rupert and Harm in 1960, extracted from yeast (40). Prior to this, other evidence indicated the enzymatic nature of PR. Factors known to influence the extent of recovery due to PR included temperature, pH, exposure time, and to some degree, light intensity and nutrient conditions of the post-irradiation environment. Factors which inhibit cell growth and division, i.e. those which delay transcription and translation of the damaged DNA



Absolute action spectra for photoenzymatic repair of various biological systems. Stationary phase *E. coli* B_{s-1}-160 cells (×); stationary phase cells of *Saccharomyces cerevisiae* uvr1-2, which were mutated to increase the PRE content (•); *Haemophilus* tfansforming DNA in vitro with yeast PRE either at low purification (○) or at high purification (△). (From W. Harm, in: *Molecular Mechanisms for Repair of DNA*, Part A, P. C. Hanawalt and R. B. Setlow, eds., Plenum Publ. Co., New York, 1975, pp. 89–101.)

FIGURE 2: from Harm, Walter. Biological Effects of Ultraviolet Radiation. Cambridge Press. Cambridge, Ma. 1980. p. 88.

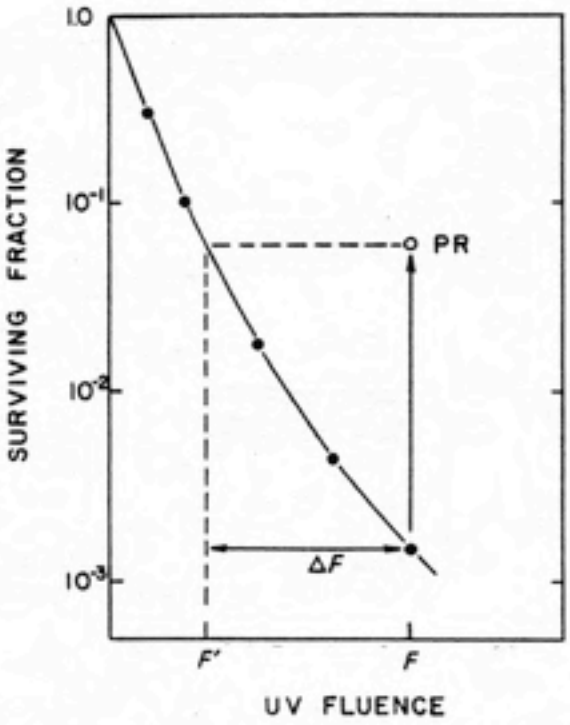
will, in general, promote survival (16). However, those which interfere with enzyme mobility, or otherwise inhibit its ability to function, will decrease the amount of repair possible. PR is the most effective of all the enzymatic repair schemes, because it is able to split the dimers directly. It has been estimated that approximately 80% of the uv lesions are T-T dimers (38). PR is also the least error prone of the repair schemes.

Kelner initially considered the "photoreactivable sector" of a given microbe to be constant. That is to say, at a given uv dose, the proportion of cells able to recover is constant, and is calculated as the number of survivors after PR (N_1) divided by the number of survivors after irradiation (N_d). This led to the proposal of the dose-reduction ratio, hypothesizing that the uv dose could be reduced by a constant factor. PR curves, plotted as log-survival vs. uv dose, yield parallel curves with a higher survival than that of the unphotoreactivated sample. This holds only for pure cultures. The resulting recovery was expressed as the dose or fluence decrement, written as:

$$\Delta D = D - D^1 \qquad \text{(eqn. 3)}$$

(19, 20), where ΔD is the dose decrement, D is the uv dose, and D^1 is the equivalent uv dose, after PR (see Fig. 3). Jagger reported PR as a percentage of the initial population, represented by the following equation:

$$\%PR = X\ (N_o - N_d)/(N_1 - N_d)\ 100\% \qquad \text{(eqn. 4)}$$



Representation of the extent of photoreactivation by the fluence decrement ΔF (see text for details). The open circle, marked PR, represents the survival obtained after exposure to fluence F with subsequent photoreactivation. (From W. Harm, in: *Molecular Mechanisms for Repair of DNA*, Part A, P. C. Hanawalt and R. B. Setlow, eds., Plenum Publ. Co., New York, 1975, pp. 89–101.)

FIGURE 3: from Harm, Walter, Biological Effects of Ultraviolet Radiation. Cambridge Press, Cambridge, Ma. 1980. p. 84.

where N_o is the initial population, N_d the number of survivors after irradiation, and N_1 the number of survivors after PR. Dulbecco also thought the number of lesions which can be photorepaired was constant (9), and termed this fraction the "photoreactivable sector" which he defined as follows:

$$1 - a = \frac{\text{slope of straight line portion of survival curve after PR}}{\text{slope of straight portion of curve without PR}}$$

According to Kelner, low PR light intensity produced lower ratios or recovery, given the same total dose of PR light. When the optimum PR light intensity was reduced by 10%, recovery was reduced by 30% (26). Dulbecco, however, found no such decrease in efficiency with decreasing intensity in bacteriophages. He did report a decline in infective units at high PR doses, when illumination was continued after the maximum recovery level had been reached. This was due to damage sustained by the host-phage complex by the near-uv light (9).

The kinetics of PR have been studied extensively in the 1960's and 1970's. The early reports show a nearly linear response with time under stricly controlled laboratory conditions (8). Kelner also mentions a linear response with intensity and exposure time, but not across as large a range as with phages (25). Temperature dependence was also recognized early in the literature as an important factor in the degree of photoreactivation. In W. Harm's 1975 review of the kinetics of PR, he included the spectrum and intensity of the PR light, amount of PRE in the cell, temperature, pH, ionic strength, and presence of inhibitors as mediating parameters in recovery (20). Rupert, in 1966 proposed the following Michaelis-Menton reaction for

PR:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow[h\nu]{k_3} P + E \qquad \text{(eqn. 5)}$$

where E is the PRE; S, the substrate, is the pyrimidine dimer; ES is the enzyme-substrate complex; and P, the product, is the repaired DNA (40).

The Michaelis-Menton equation differentiates between chemical and enzyme kinetics, allowing for the expression of enzyme saturation (28). At low substrate concentrations, the increase in velocity is proportional to the increase in substrate. At higher concentrations, velocity is no longer proportional to the substrate, and becomes independent with respect to S, approaching zero order kinetics. The velocity is then dependent on the enzyme concentration. A typical Michaelis-Menton reaction scheme is written as follows

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES$$

$$ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + S \qquad \text{(eqn. 6)}$$

(notations are as previously described). PR follows Michaelis-Menton kinetics, with two exceptions: the final, photolytic step is not reversible, therefore there is no k_4, and this step is absolutely dependent on light of wavelengths between 300-500nm.

Each component of the reaction can be studied separately, by exposing samples incubated in the dark for a known amount of time, to flashes of intense PR light. The dark incubation allows the ES to form, and the flash of PR must be bright enough to convert all the ES to P + S. The formation constant, k_1, can be determined in the following way: the number of T-T dimers can be quantified for a

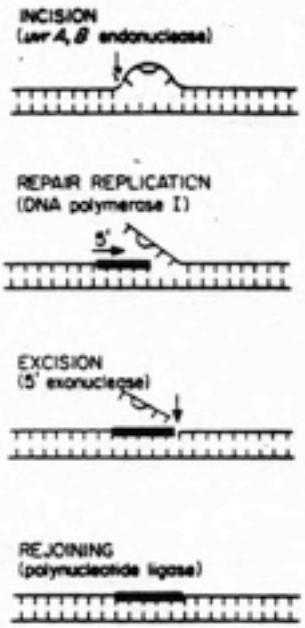
given uv dose and organism, by converting E to the number of repaired dimers per individual, which is converted to the number of ES complexes present at the time of the light flash. By experimentation, they were able to determine the optimum incubation time and flash intensity to convert all the ES to product. The amount of PRE is controlled by adding it as a cell free extract, which allowed the calculation of the number of repaired dimers. From the measured time, light intensity, and number of repaired dimers, k_1 can be calculated. Similar manipulations of dark and light phases permitted the determination of k_2 and k_3, as well as the effect of the various parameters mentioned earlier. Both k_1 and k_2 show a strong temperature dependence, however the photolytic step is only affected by high or low temperature.

The final, dimer splitting step in the reaction is absolutely dependent on light between the wavelengths 300-55nm. When discrete flashes of light were used, Rupert found that the disappearance of the first half of the ES complex was temperature independent, and proportional to the intensity of the light flash. The disappearance of the second half of the complex was slower, temperature dependent, and not proportional to the intensity. He attributed this to the heterogeneous composition of the substrate, i.e. the dimers are no longer primarily T-T, the preferred substrate for PRE. As the repair process continues, the proportion of C-C and T-C dimers increases, and they are not as easily split by PRE, and so the process slows (37).

Photoreactivation is not the only enzymatic repair system operating in the damaged cells. Excision, or dark repair, as it is often called in the literature, also reverses uv lesions. It differs

from PR in several ways. Excision repair is not specific for uv induced damage, but rather functions more as a general repair system, operating continuously . It is not light dependent, and it is much more error prone than is PR. Several enzymes systematically remove and resynthesize the area around the lesion. The first step in the process is the recognition of the damage as a distortion in the double helix. An incision is made in several base units before the damaged portion of the DNA, and the lesion is then excised. The DNA is resynthesized, and the new piece is reconnected to the strand by a polynucleotide ligase. (See Fig. 4) Unlike PR, all organisms tested have this excision repair system, except for some specially developed mutants (20, 45).

Dark repair was first reported to reverse uv lesions by Roberts and Aldous, in 1949 (36). They stipulated several factors for controlling the considerable variability in organismal response to uv irradiation, which include the phase of the growth cycle of the culture ot be irradiated, growth media and temperatures used during culturing as well as post-disinfection. Irradiated organisms, which are allowed to remain for a time in a nutrient poor liquid suspension before plating will have higher survival rates than those plated immediately after treatment. This was later termed liquid holding recovery (LHR). They also noticed that synthetic media was more favorable for survival than other types of media commonly used in enumeration techniques, eg. Difco Broth. For low to medium uv doses, maxium survival rates were seen after 2-4 hours of pre-incubation in non-nutrient buffers, while high uv doses require a longer period of time to achieve maximum recovery. Although these trends correlate well with more recent literature no mention was made as to the



A general model for the major pathway of excision repair. An enzyme recognizes the lesion, shown here as a cyclobutane-type pyrimidine dimer, and makes an incision cut in the DNA strand. Repair replication (heavy line) commences using the opposite strand of DNA as the template. Finally, the damaged section of the DNA is excised, and the break in the DNA strand is sealed. The vertical arrows indicate the locations of nuclease cuts in the damaged DNA strand and the horizontal arrow indicates the direction of repair replication, beginning at the 3'OH end of the DNA strand. [Modified from P. C. Hanawalt, *Endeavour* **31**, 83–87 (1972).]

FIGURE 4: from Smith, K. C. The Science of Photobiology. K. C. Smith, ed. Plenum Press, NY. p. 133.

exclusion of light during the pre-incubation by the authors, since the effect of light was not recognized. However, in 1964, Castellani et al. reported that the degree of recovery after uv irradiation between photoreactivated organisms and those held in LHR was comparable, under conditions of strictly controlled light (4). Maximum PR could be obtained within 5 - 10 minutes of exposure to PR light, while 4 - 6 hours was required to reach the same degree of recovery using only the excision repair system. Over that 4 - 6 hour period, approximately 10% of the organisms lost the ability to photoreactivate. Since about the same level of recovery was obtained by both systems, they concluded that excision repair functioned on essentially all the photorepairable lesions. However, of all the types of photoproducts formed, PR only splits cyclobutane dimers, while excision repair can reverse all types of damage. One would therfore expect the dark repair process to be more efficient, except that it is more prone to error than is PR, and so the recovery levels are similar.

There are several other schemes by which organisms can reverse the effects of uv. Viruses, in particular, have evolved a variety of ways to repair their damaged genome, using the host cells enzymatic repair systems. THese schemes have been reviewed by Hanewalt (16) and Harm (20), and are briefly summarized below:

1) Multiplicity Reactivation: When a host cell is infected by both uv-irradiated and unirradiated phages of the same species, there is a stimulation of recombinational events. Through a random exchange of genetic material between the damaged and undamaged genomes, achieved by subverting the host cells' repair enzymes, a viable copy of DNA can be

generated for the irradiated phages, enhancing survival.

2) Cross Reactivation: As with multiciplicity reactivation, the random exchange of genetic material can yield viable copies of genomes of irradiated phages, once inside the host cell. However, in this case, the phages are of different types, only one of which is irradiated. It is a "donor-recipient" relationship, rather than a two-way exchange of DNA. Both mechanisms rely on the stimulation of recombination of uv light, and the formation of viable viruses is a chance event. The increased frequency of recombination simply improves the likelihood that a viable genome can be formed. It is thought that the excision repair system helps by increasing the frequency of strand breakage, which in turn promotes recombination.

3) Suppression of Prophage Induction: This tends to enhance the survival of the host cell by the presence of uv inducible prophages, i.e. conditions which inhibit induction of the prophage, such as a lesion in its DNA increases the uv resistance in the host cell. This is also a chance happening, and depends on whether or not the lesion is formed in the prophage genome.

4) Host Cell Reactivation (HCR): Irradiated viruses may actually subvert the host cells own repair enzymes in order to repair their DNA. Although the enzymes do not function as effectively on the foreign DNA, phages able to to use the host's excision repair system do have higher survivals than those lacking this ability. When the host is also

irradiated, the phage DNA usually does not compete well for the enzymes.

5) UV Reactivation (UVR): Seemingly in contradiction to HCR, phage survival is enhanced when the host is given a small dose of uv after infection. It is frequently found in those instances where HCR is also possible. UVR can be reduced by photoreactivation, and is found in excision repair proficient bacteria, which may indicate that the stimulation of the dark repair is at least partially responsible for the reversal of the uv effects.

6) Spontaneous Decay of Photoproducts: Within about 2 - 3 hours of formation, the lesions may spontaneously degrade, i.e. hydration products can dehydrate or dimers split due to chemical instability. After that time they may become irreversible (4). Environmental conditions, including temperature and pH influence the reversal to the pre-irradiation state. When cells are incubated at 45°C for up to 3 hours, beginning immediately after exposure to uv light, followed by normal growth conditions (37°), the rate of decay increases. Acid catalysis of T-T dimers may occur spontaneously in the cell, under certain conditions. Catalase synthesis reportedly also enhances the decay of photoproducts.

7) Direct Reversal of Photoproducts: This occurs at very high uv doses. Germicidal light energy is more efficient at forming dimers, howver it may monomerize dimers, via 230-240nm wavelengths. Dimerization far outweighs the other,

being approximately 300-1000X faster. At very high levels of uv irradiation, an equilibrium is established, where, as the substrate of undimerized adjacent pyrimidines becomes exhausted, the rate between the two opposing processes becomes roughly equal.

8) Photoprotection: Organisms illuminated with light of wavelengths longer than those of the primary germicidal uv light before irradiation are more resistant to the effects of uv radiation. The increase in survival may be largely due to the inhibition of DNA and protein synthesis by the near uv light. This delay allows other repair systems to located and reverse the damage caused by the germicidal light.

9) Indirect Photoreactivation: The mechanism which promotes survival survival is similar to photoprotection, in that normal cell function is suspended temporarily, permitting the detection amd repair of the lesions. It is different from PR in that it is not temperature dependent, nor does it follow the typical saturation kinetics, although it is the longer wavelengths responsible for the increase in survival. Pyrimidine dimers are not split directly, but are thought to be removed via the excision repair mechanism.

10) Post-Replication Repair or Recombinational Repair: This complements excision repair in the cell, using the parental DNA strand as a template to fill the gaps caused by excision of photoproducts. Partial DNA replication must have already occurred before this mechanism will function. Dark repair, without PR, may account for 95% recovery after uv irradiation. However, there may be as many as 100+ potentially lethal lesions in the genome, possibly due to insufficient time or inability to complete repair at all sites. Post-replication repair is able to further reverse the remaining damage. It is not possible to completely eliminate all the effects of uv radiation, because the genetic material is not the only target of the uv photons. Other cellular components, such as proteins and so on, may be severely damaged, and these contribute to the mortality of the microbes. However, any strategy which enhances repair of the DNA enhances survival (See Fig. 5).

A model for postreplication repair of UV radiation-damaged DNA. (a) Dots indicate photochemical lesions produced in the two strands of DNA. (b) DNA synthesis proceeds past the lesions in the parental strands leaving gaps in the daughter strands. (c) Filling of the gaps in the daughter strands with material from the parental strands by a recombinational process (depends upon functional *recA*$^+$ genes). (d) Repair of the gaps in the parental strands by repair replication. The reader is cautioned that steps (c) and (d) are highly schematized, and will probably be modified as additional data become available. (Modified from reference 19.)

FIGURE 5: from Smith, K. C. The Science of Photobiology. K.C. Smith, ed. Plenum Press, NY. p. 135.

PROJECT OBJECTIVES

Experiments were designed to reach the following objectives. They were divided into two phases: laboratory and field. The laboratory phase was designed to determine the effects of the following parameters on PR:

1) intensity of PR time
2) optimal exposure time
3) temperature
4) nutrients
5) uv dose

After the optimal conditions, as relevant to wastewater disinfection were established in the laboratory, a similar set of parameters was tested in the field, including:

1) exposure time
2) nutrients
3) uv dose
4) effect of incubation in Pyrex bottles

Field experiments were conducted between May 22, 1984 and July 3, 1984, when sunlight intensity was close to that of full summer sun. Water temperature in the stream was also near the optimum, as determined in the laboratory phase, being between 24 - 26°C.

MATERIALS AND METHODS

In all PR experiments, initial uv disinfection of samples of the secondary effluent from the Mason Farm Wastewater Treatment facility in Chapel Hill, North Carolina, was accomplished by irradiating them under the collumineting beam apparatus (35). (See Fig. 6). This allows the accurate replication of uv doses. Intensity of the uv light at the surface of the effluent was made with an IL-500 radiometer, equipped with an SEE-240 detector, calibrated to a National Bureau of Standards lamp (See Fig. 7). Effluent samples of 1.0cm depth were continuously stirred during irradiation. The average intensity was calculated as in Qualls, et. al. (35). Absorbance was measured at 253.7nm for each sample, and the uv dose corrected so as to be consistent over time. This was done by adjusting the exposure time to compensate for the actual uv intensity the organisms were exposed to, eg. by increasing the exposure time if the uv absorbance of the sample was high or the lamp output low.

For the first phase of this study, an artificial PR light source was chose, to permit the control of intensity. The light source was required to emit light in the 300-500nm range, with as little as possible below 300nm. Wavelengths above 500nm do not significantly affect PR (25). To accomplish this, three 15 Watt GE black light fluorescent lamps (F15T8-BL) were used. These lamps had the advantage of emitting most of their energy at approximately 360nm, with a significant portion of their spectrum between the optimum range for PR. Although there was a small amount of light below 300nm, the glass tubing around the lamps did not transmit a measurable amount of germicidal light, as measured with the IL-500

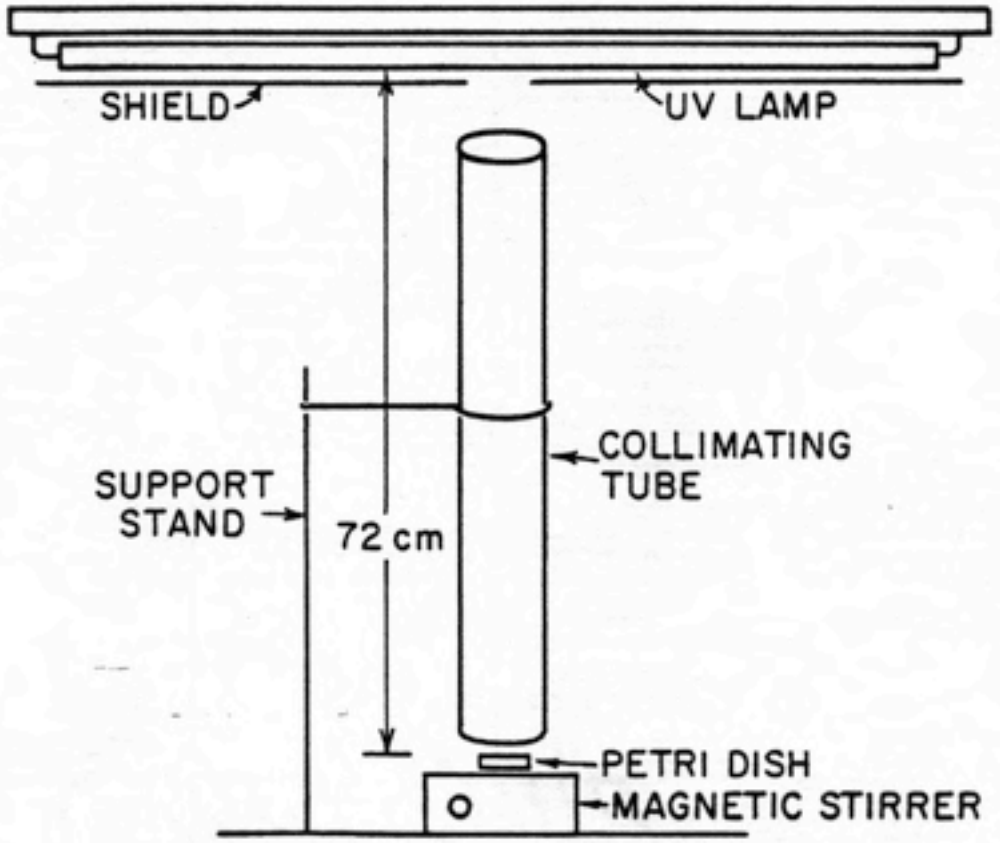


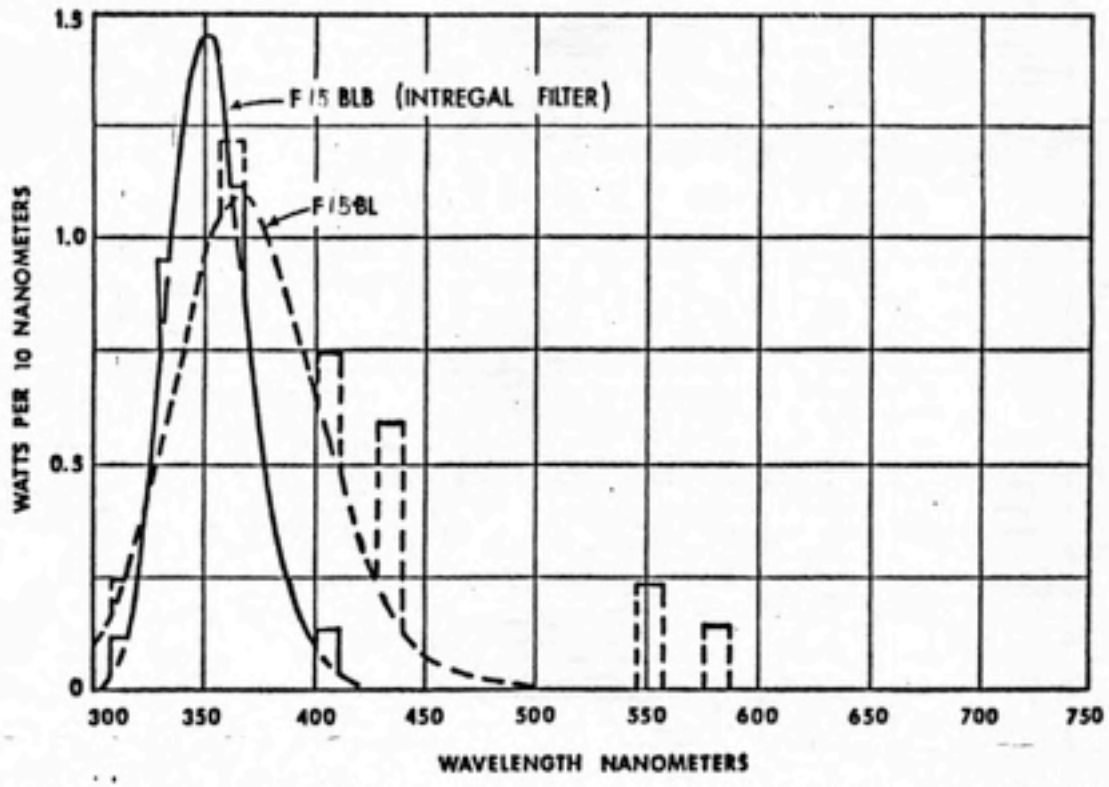
FIGURE 6: Columinating Beam Apparatus, from Qualls, et. al. THe role of suspended particles in ultraviolet disinfection. J. WPCF, 1983.

radiometer at PR light intensities below 0.5 mWatt/cm^2. (See Fig. 7).

PR light intensities were also measured wiht an IL-500 radiometer and SEE-400 detector. The detector was fitted with two filters, SCS-320 and CF-470, for wavelength measurement between 300-420nm, to match the PR action spectrum of E. coli, adapted from Jagger (23). (See Fig. 8). Intensity inside the PR chamber could be increased by lining it with reflective material, such as aluminum foil, and decreased by removing one or two lamps.

Following uv irradiation in the laboratory phase, samples were filtered through 0.45 micron Gelman filters and placed on pads soaked in m-Endo broth, as per Standard Methods procedure (46). Illumination was performed in an enclosed chamber which effectively excluded extraneous light. The temperature in the chamber was approximately 25^{o}C. Plates were kept moist by adding sterile, non-nutrient phosphate buffer as necessary. Possible toxic effects of the black light were determined on samples with either nutrient or buffer soaked pads. No significant toxicity was seen in the first 60 minutes of illumination, however after 2 hours, a decrease in survival was seen in some samples placed on m-Endo broth soaked pads. All handling of uv irradiated samples was performed excluding light below 500nm, by using a GE Gold fluorescent lamp (25). Dark controls were filtered and kept in the dark at room temperature for the duration of the illumination.

For experiments examining temperature effects, the PR chamber was placed in either a 37^{o}C or a 4^{o}C incubator, or kept at room temperature. The cold temperature decreased the lamp output, and so the PR exposure time was adjusted to maintain a consistent dose level



APPROXIMATE INITIAL SPECTRAL ENERGY DISTRIBUTION

SPECTRAL DATA: F15 BL & F15BLB

FIGURE 7: Energy distribution of F15BLB blacklight lamps, courtesy of General Electric, Nela Park, Ohio.

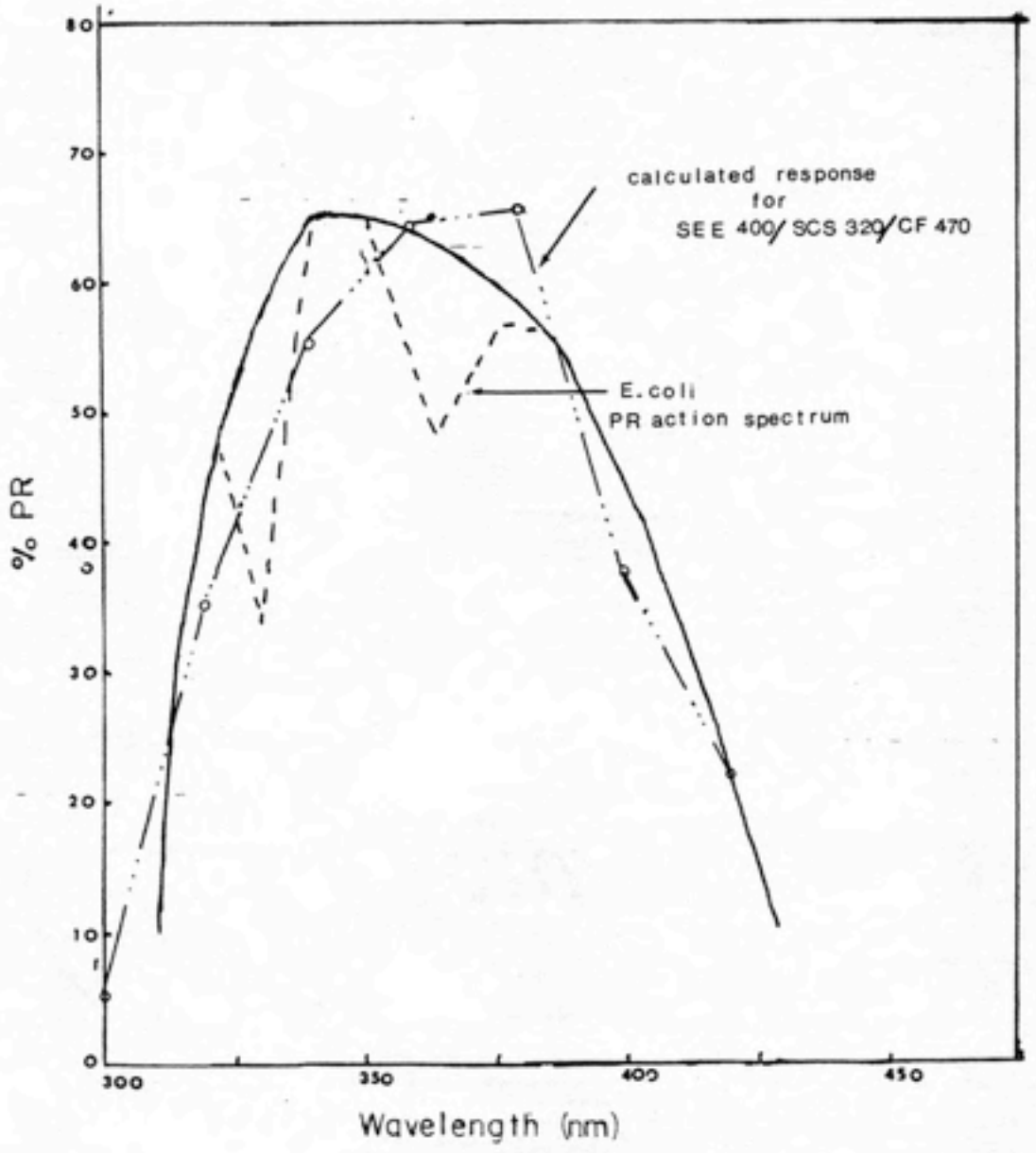


FIGURE 8: Light measurement by the IL 500 Radiometer, equipped with SEE-400 detector and SCS-320/CF-470 filters, courtesy of International Light, Inc., Newburyport, MA.

of 600mWatt-sec/cm^2, which was found to yield maximum recovery, at intensitites of 1.25mWatt/cm^2 or lower. Optimum PR in the laboratory was reached under the following conditions: exposure at 25°C for 60 minutes at 0.11mWatt/cm^2, on samples filtered and placed on pads soaked in nutrient broth.

Due to significant (i.e. greater than 10%) differences in intensity within a relatively small area, petri dishes the size of the detector were used, so that the intensity could be measured at exact intervals, and the dose controlled for each replication. The large volumes of irradiated effluent required for accurate enumeration made it impractical to illuminate unfiltered samples.

In the field phase, disinfection was accomplished in the same manner as previously described. However, irradiated samples were incubated in Morgan Creek, exposed to sunlight in Pyrex bottles before filtering. Dark controls were also placed in Pyrex bottle, and wrapped in aluminum foil to exclude light, and incubated in the creek with the photoreactivated samples. Pyrex bottle do not absorb light above approximately 290nm. Absorbance increases rapidly below that wavelength, while excluded some of the disinfecting effects of sunlight. Therefore, we were able to examine the effects of PR, without additional killing from shorter wave light emitted by the sun.

Sunlight intensity was measured in the field, approximately 20' from the creek. The detector was placed in an unshaded area, elevated above the ground so that it was out of the shadows caused by plant growth on the banks of the creek. Experiments were performed between 11:00-1:00 o'clock, when the sun is directly overhead, to

allow for both maximum intensity for exposure and accurate measurement by the SEE-400 detector.

Since Chapel Hill wastewater effluent is generally of higher nutrient concentration than the receiving stream, the treated effluent was diluted to simulate as closely as possible actual stream conditions. In one series, sterile, non-nutrient phosphate buffer was used as the diluent, in 1:10 and 1:40 dilution ratios with irradiated effluent. In a second series, Morgan Creek water was filter sterilized and used to dilute the effluent in the same ratios. During the time of experimentation, Morgan Creek has the following characteristics, as measured by the Orange Water and Sewer Authority (OWASA):

TABLE 1:

PARAMETER	RANGE	MEAN	POP. STD. DEV.
BOD(mg/L)	0.0 - 2.8	0.94	± 0.81
COD(mg/L)	6.1 - 23.7	12.5	± 4.55
AMONIA	0.01- 0.06	0.03	± 0.016
DO	7.3 - 8.3	7.91	± 0.33
TURBIDITY	0.2 - 16.0	2.76	± 4.99
pH	7.1 - 7.5	7.34	± 0.13

For the secondary effluent, during the same time period, the characteristics are as follows (also obtained from OWASA):

TABLE 2:

PARAMETER	RANGE	MEAN	POP.STD.DEV.
BOD(MG/L)	0.5 - 2.7	1.37	± 0.56
COD(MG/L)	28.9 - 42.4	33.41	± 3.95
AMONIA	0.01- 0.85	0.16	± 0.25
DO	7.2 - 8.7	7.8	± 0.41
TURBIDITY	0.2 - 0.3	0.21	± 0.02
pH	6.8 - 7.2	6.95	± 0.15

The creek temperature ranged between 24 -26°C, nearly the optimum for PR, as determined in the laboratory phase of this study. This facilitated good temperature control during the incubation of the sample in the stream. Absorbance of light in the wavelength range of 300-55-nm was 0.012 - 0.112/cm, increasing below 300nm. At 254nm, the absorbance of Morgan Creek water was approximately 0.175/cm.

In determining optimum temperature, intensity, exposure time, and nutrient conditions in both field and lab phases, uv doses of 4 and 9 or 16mWatt-sec/cm^2 were used, yielding log survivals of -2.0 and -3.0, respectively. For assessing the effect of uv dose, samples were irradiated at levels of 4, 9, 16, and 26mWatt-sec/cm^2, giving -2.0, -3.0, -3.5, and -4.0 logs survival, respectively. Actual log survivals for each separate experiment varied somewhat with the sample taken on that particular day and the uv sensitivity of the coliform population.

Results are presented as either log survivals, before and after PR, or as %PR. Statistical evaluation was performed using a SAS model for analysis, and p-values reported refer to the 99% confidence interval, unless otherwise specified.

RESULTS AND DISCUSSION

This study proposed to determine the optimum level of recovery of uv-irradiated coliforms, due to photoreactivation. Since PR is an enzymatic process, known to be dependent on growth conditions, the following parameters were examined: nutrient levels in wastewater secondary effluent and receiving water, temperature, PR light intensity and exposure time, and uv dose. One portion of the study was performed in a laboratory setting, using an artificial PR light source, in order to control the parameters of temperature and intensity. UV irradiations were done in such a way as to accurately reproduce the disinfecting dose levels given on each sampling day, for each effluent sample. Variations in log survivals after each treatment are therefore presumably due to changes in the uv sensitivity of the coliform population over time. This is not an unreasonable assumption, since the types, relative proportions, and physical state of organisms in the wastewater change with the organic and inorganic composition of the water, time of day, etc. In order to minimize this variation, samples were taken at approximately the same time of day for each experiment. It was assumed that, although the coliform group is a heterogenous population, their response would be, at least qualitatively, similar to *E. coli*, which comprise the largest portion of this group. Since the nineteenth century, *E. coli* has been routinely used as a model for disinfection processes. However, there may be important physiological differences between laboratory cultured and "naturally" occurring organisms, such as the

relative resistance to disinfectants of some of the Gram negative bacteria (7). We also assumed that the Law of Reciprocity applied within the narrow ranges of intensity used in applying both uv and PR doses.

EFFECT OF EXPOSURE TIME TO PR LIGHT:

In both the laboratory and the field, samples of uv irradiated secondary effluent were exposed to PR light for 20, 60, and 120 minutes. The level of photoreactivation increased with time at the fixed intervals, until maximum recovery was obtained at 20 and 60 minutes, for the laboratory and field samples, respectively. Further exposure had a negative effect on recovery, and survival declined. (See Figs. 9, 10 and Tables 3, 4). There is precedence for this in the literature. This may, in part, be due to the loss of the ability to photoreactivate over time (4). Several authors report a time limit of 2 - 4 hours at room temperature, beyond which damage becomes permanent (8, 13, 20). Furthermore, light below 315nm still causes dimer formation, although not as efficiently as with the 254nm wavelength. As the organism loses its photorepair capabilities, these new lesions become increasingly biologically important. The other repair mechanisms, mentioned in the literature review section, may not be able to keep pace with the lesion formation. In addition, longer wavelengths of PR light, (365nm) may also form lesions in the DNA, again at a slower rate, and of a different type than the 254nm light (22, 34). Thus, while PR is operating, the rate of lesion formation due to damaging components in the near-uv light is much less than the rate of repair. As time elapses, all the repair

systems begin to slow, yet lesions are continually being formed, and recovery declines.

There may be another factor contributing to the declining survival after prolonged exposure to PR light, which is saturation of the enzyme system. There are a limited number of PRE molecules in the cell, and no indication in the literature that the number increases after irradiation (19, 38). Again, as the amount of damage caused by elements of the PR light, the system becomes overloaded, and unable to match the rate of lesion formation. Also, since PR acts only on pyrimidine dimers, there is a certain amount of overlap with other repair mechanisms, especially the excision repair. That is to say, excision repair can mend all types of photoproducts (dimers, hydration products, strand breaks, and protein cross-linkages) whereas PR is only capable of splitting T-T dimers efficiently. The two mechanisms essentially compete for the same substrate (dimers), which are gradually repaired, so that they are no longer the major cause of lethality. The excision repair alone must reverse the remaining lesions, which can comprise about 20% of the total damage from the original irradiation, and more from subsequent illumination. This is not the most efficient use of the various repair mechanisms by the cell, which can be seen as fighting against time to repair as much of the damage as possible. As the organism gets ready to replicate its DNA and divide, time for repair has essentially run out, but for two rather inefficient repair systems (excision repair and post-replication repair).

In a study done in Northwest Bergen County, New Jersey, on uv treated secondary effluent, by O.K. Scheible, the optimum exposure time was also found to be 90 minutes (41, 42). In that report, samples of irradiated effluent were placed in borosilicate bottles, either transparent to light or shielded. They were exposed to sunlight for 10, 30, 90, or 180 minutes. Ultraviolet doses were larger than those used in our study, reported as 57 and 380 $mW\text{-}s/cm^2$ in NJ. The general trends were in good agreement, increasing up to a point, beyond which continued illumination had a detrimental effect. The need for a longer exposure time may have been due to the higher uv doses, which may require more time to repair the higher level of damage.

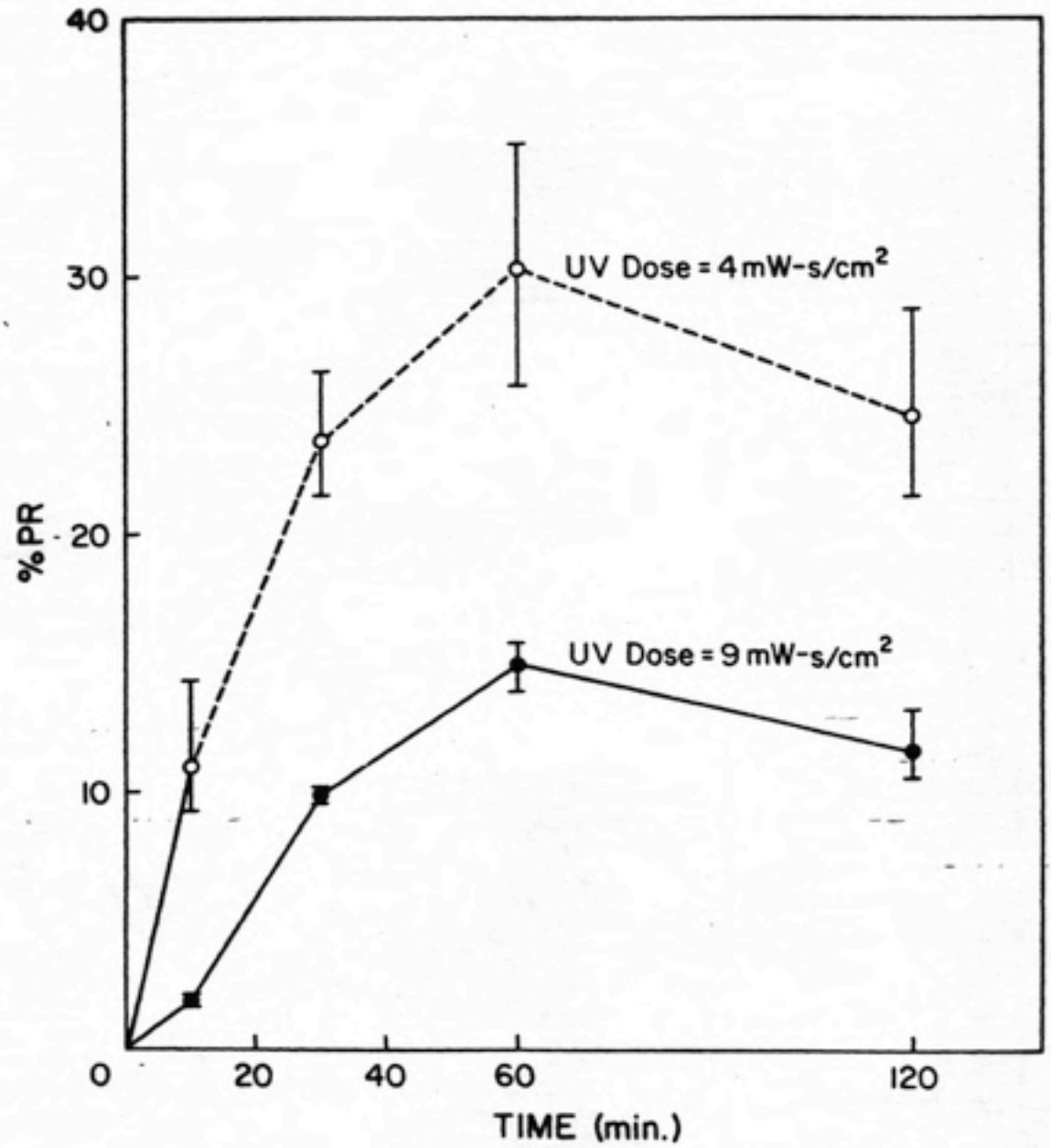


FIGURE 9: Percent PR vs. Time of exposure to sunlight for uv doses 4 and 9mWatt-sec/cm2. Bars indicate range.

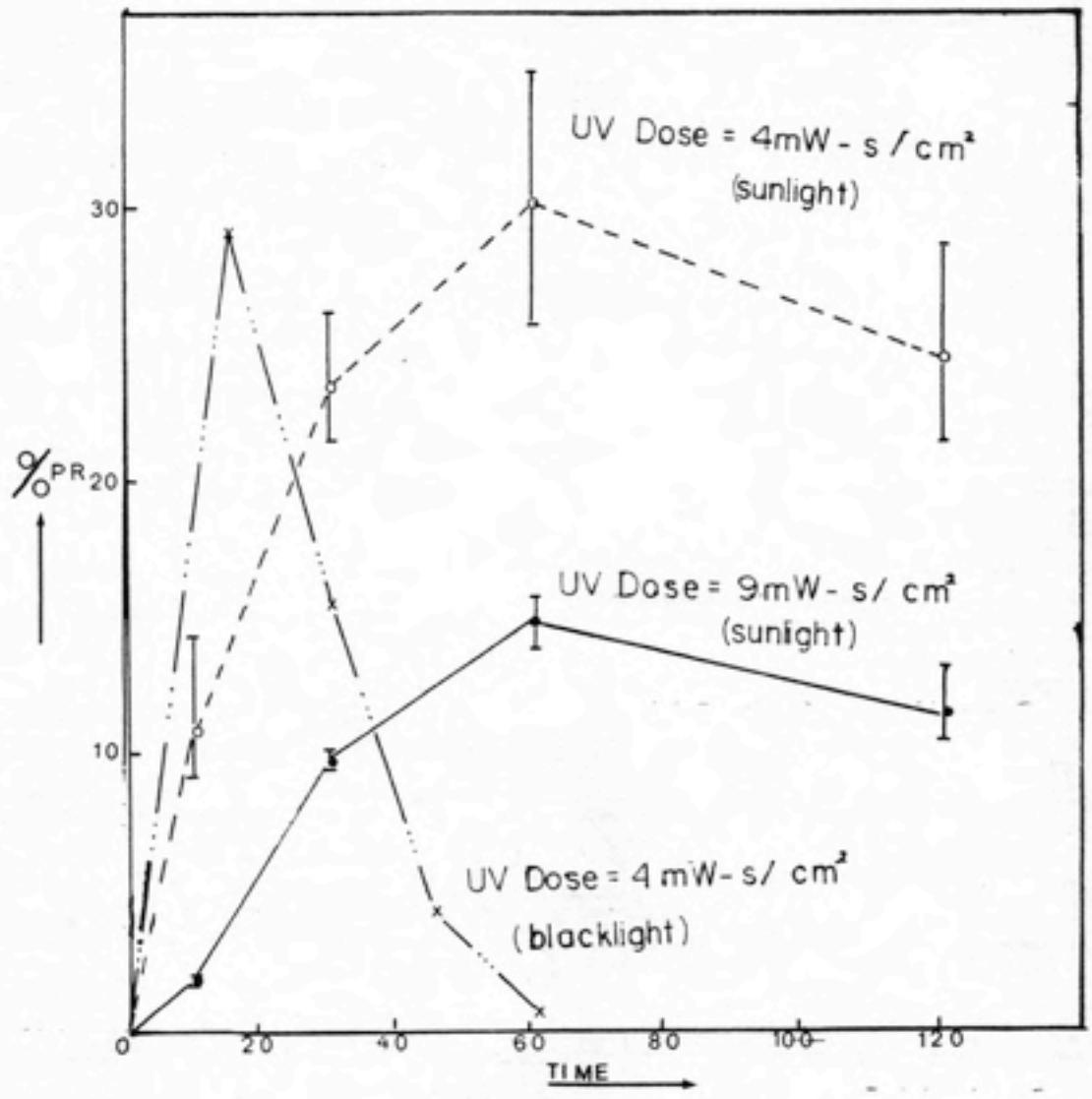


FIGURE 10: Percent PR vs. Time of exposure to sunlight for uv doses 4 and 9mWatt-sec/cm2 and blacklight for uv dose 4. Bars indicate range.

Table 3: Effect of Exposure Time, Laboratory Experiments. PR Light Intensity = 0.22 mw/cm^2

2/24/84					2/29/84				
UV Dose	PR exposure time (min)	Ave. cfu/ml	Logs	%PR	UV Dose	PR exposure time (min)	Ave. cfu/ml	Logs	%PR
0	-	2683	-	-	0	-	1717	-	-
4	0	43	-1.88	-	4	0	80	-1.33	-
	20	290	-0.97	9.3		20	103	-1.22	1.4
	60	133	-1.32	3.3		60	10.0	-2.23	-4.3
	120	45	-1.86	0.08		120	7.0	-2/39	-4.5
16	0	1.3	-3.46	-	16	0	1.4	-3.09	-
	20	6.0	-2.67	0.2		20	446	-1.58	2.6
	60	14.0	-2.27	0.5		60	27.8	-1.79	1.5
	120	17.0	-2.21	0.6		120	24.4	-1.85	1.3

Table 4: Effect of Exposure Time. Incubated in Pyrex bottles in Morgan Creek

6/12/84 Sunlight I = 3.65 - 3.90					6/14/84 Sunlight I = 2.95 - 3.9 (mW/cm²)				
UV Dose	PR exposure time (min)	Ave. cfu/ml	Logs	%PR	UV Dose	PR exposure time (min)	Ave. cfu/ml	Logs	%PR
0	-	1920	-	-	-	-	2550	-	-
4	0	5.7	-1.53	-	4	0	19	-2.3	-
	10	313	-0.79	16		10	317	-0.91	11.0
	30	787	-0.39	41		30	747	-0.53	23.6
	60	810	-0.37	42		60	907	-0.45	30.4
	120	557	-0.54	29		120	770	-0.52	24.6
9	0	1.63	-3.07	-	9	0	13.3	-2.28	-
	10	117	-1.22	6.2		10	51	-1.70	1.9
	30	219	-0.94	11.4		30	251	-1.01	9.8
	60	89.0	-1.33	4.6		60	383	-0.82	15.0
	120	31.6	-1.78	16.4		120	299	93	11.7

EFFECT OF PR LIGHT INTENSITY

During the first series of experiments for determining the optimum exposure time, the PR light intensity was 0.225 mWatt-sec/cm^2, giving an optimum PR dose of 600mW-s/cm^2. If the Law of Reciprocity holds for photoreactivation of coliforms, then this dose could be reached at higher or lower intensities by altering the exposure time. If it dose not hold, then light intensity must be considered as an independent variable.

The effect of PR light intensity was tested at uv dose levels of 4 and 9 mW-s/cm^2. PR light intensity was varied as follows: 1.27-1.12, 0.86 - 0.80, 0.50 - 0.43, 0.22, and 0.11 mW-s/cm^2, as measured by the IL-500 radiometer with the SEE-400 detector. (See Fig. 11 and Tables 5a, 5b). Increasing intensity approximately 10-fold resulted in a statistically significant lower level of recovery at both uv doses ($p = 0.02$ at uv dose 4, and 0.0001 at uv dose 9).

This was perhaps not unexpected, as germicidal wavelength energy becomes increasingly efficient with intensity. According to Gates (11), there is a 75% loss in killing efficiency by germicidal lamps with a 50% decrease in intensity of wavelengths between 239-302nm. With blacklight, here is a near-uv component (i.e. 300 - 315nm), which may become more important at high intensities. Effects from other damaging portions of the PR spectrum may also become more biologically important. Peak (34) report increasing 365nm damage with increasing intensity. In a review of near-uv light effects of bacteria, Jagger (22) reports a synergystic effect between near and far-uv radiation at high fluence rates (intensities), which can be induced by blacklight illumination of E. coli. The 334nm component of

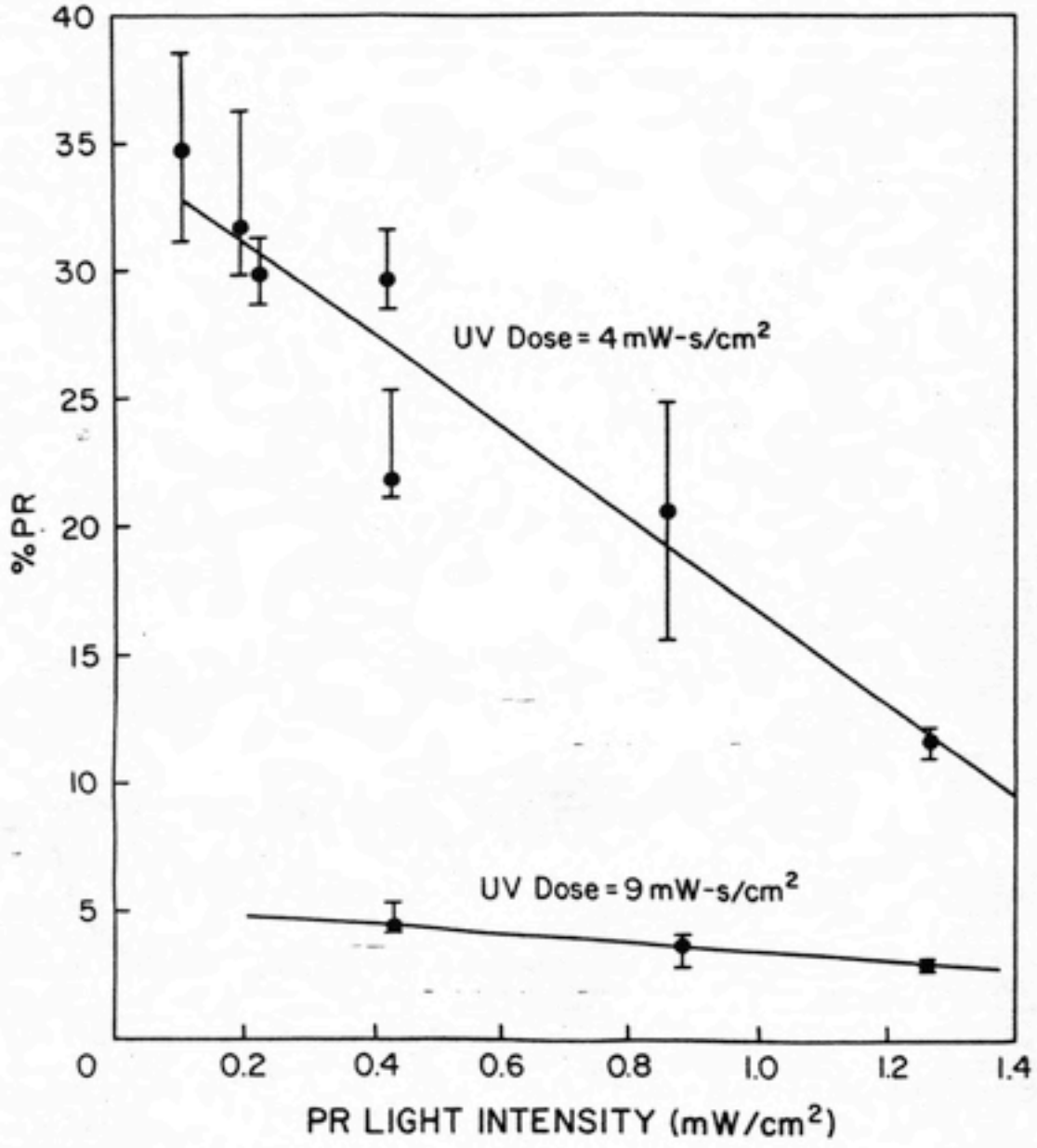


FIGURE 11: Percent PR vs. Intensity for uv doses 4 and 9mW-s/cm^2 using blacklight as the PR light source. Bars indicate range.

Table 5a: EFFECT OF PR INTENSITY

UV Dose ($mW\text{-}s/cm^2$)	Dark Control	Cells/ml PR Intensity* 1.12 (mW/cm^2)	.80 (mW/cm^2)	.50 (mW/cm^2)
	960			
0	800			
	880			
	32	182	266	250
4	32	162	194	244
	30	180	246	274
	Log S -1.45	-0.70	-0.57	-0.54
	% PR	17.8	27.7	25.8
		15.4	19.2	25.1
		17.5	25.3	28.6
	1.8	19.8	31.4	34.0
9	2.4	23.2	24.4	34.8
	2.0	23.4	26.6	37.2
	Log S -2.63	-1.60	-1.51	-1.40
	% PR	2.0	3.3	3.6
		2.4	2.5	3.7
		2.4	2.8	4.0

* PR Dose = 600 $mW\text{-}s/cm^2$.

Table 5B: EFFECT OF PR INTENSITY

UV Dose mW-s/cm^2		Dark Control	Cells/ml PR Intensity 1.27	.86	.43
0	700				
4	19.0		160	152	136
			124	178	142
			132	152	176
	Log S -1.57		-0.70	-0.64	-0.67
9	2.0		25	23	39
			19	28	32
			21	31	32
	Log S -2.54		-1.51	-1.41	-1.31

*The times of exposure were varied so that the PR Doses were the same (600 mW-s/cm^2).

the spectrum is thought to cause this increase in mortality. Jagger proposes the overstimulation of the excision repair system as a possible mechanism to account for this. When the sections of excised DNA become too numerous or too large, the strand breaks may overlap, and so the integrity of the molecule is destroyed.

This does not appear to be the case for sunlight exposure in Pyrex bottles. (See Table 10), where the sunlight intensity ranged from 1.9-4.5mW/cm^2, with no signficant change in the amount of photoreactivation. Some of the light intensity may be lost due to reflection and refraction of light waves as it passes through the various interfaces of air, water, glass, and effluent.

EFFECT OF TEMPERATURE ON PR

Photoreactivation is temperature dependent, largely due to the formation of the enzyme-substrate complex (eg. 18, 19, 39). The second step, which is the photolytic reaction, is reported to be stable over a wide range of temperatures, from 2 - 40°C (38).

There was a significant temperature effect also found in this study of uv-irradiated coliforms. (See Fig. 12 and Table 6). Recovery increased almost logrythmically from 17-25°C at both uv doses tested. Raising the temperature further, to 36°C, did not promote survival at either uv dose. The PR dose given in each case was 600mWsec/cm^2. The difference in temperature had a statistically significant effect at uv dose 9 ($p=0.0025$), but not at uv dose 4 ($p=0.0748$). This may be due to the high variability in log survivals at the lowest uv dose, some of which showed lower levels of killing than would be expected, rather than a lack of temperature effect at low temperatures.

TABLE 6: EFFECT OF PR TEMPERATURE

UV Dose (mW-s/cm²)		Dark control	Cells/ml PR Temperature* 16C	25C	36C
		1740			
0		1300			
		1500			
	16C	40,40	266	394	332
	25C	32,28	282	368	362
4	36C	34,34	202	378	306
Log S		-1.64	-0.78	-0.60	-0.66
% PR			15.6	24.3	20.1
			16.7	22.5	22.1
			11.3	23.2	18.4
	16C	1.4,3.0	18.0	53.4	57.2
	25C	1.0,2.6	28.8	49.8	52.4
9	36C	3.6,2.4	26.6	47.6	51.4
Log S		-2.81	-1.79	-1.49	-1.45
%PR			1.0	3.4	3.6
			1.8	3.1	3.3
			1.6	3.0	3.2

* PR Dose = 600 mW-s/cm².

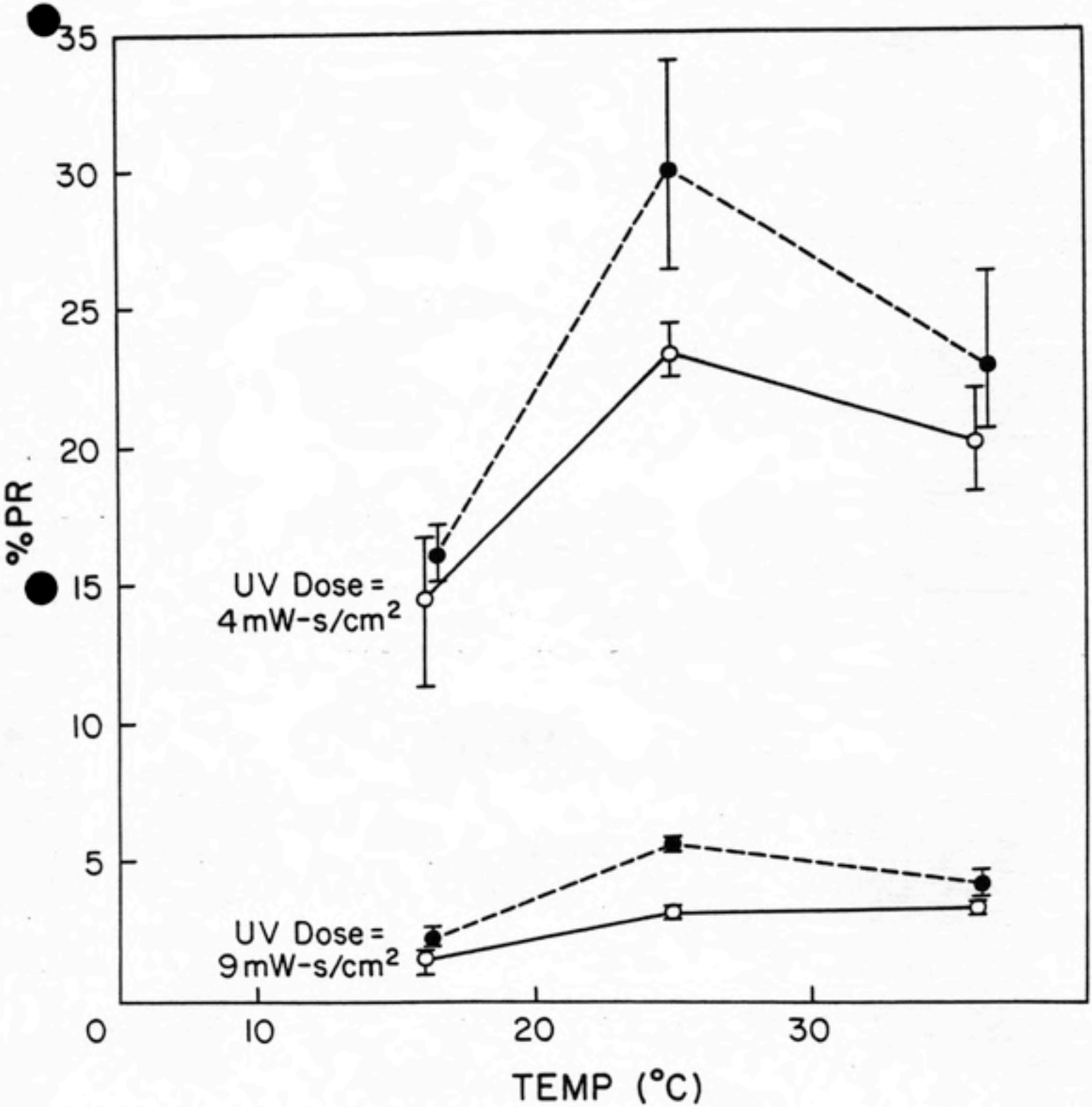


FIGURE 12: Percent PR vs. Temperature, for uv doses 4 and 9mW-s/cm^2 using blacklight lamps as the PR light source. Bars indicate range.

There is ample precedent in the literature, indicating that a variety of microbes reach maximum recovery in the 24-26°C temperature range (eg 1, 9, 17). In a recent study comparing E. coli and S. aureus, Adkins and Allen found 23 - 25°C to yield the highest levels of photoreactivation in both organisms (1). Kelner, in the early literature showed PR to increase with temperature between 20 - 40°C. for several bacteria tested (26). In many early experiments, however, temperature was not controlled, nor was heat from the lamps, which can be considerable. Increasing the temperature from 25° to 36° decreased the intensity output of the lamps, as did decreasing the temperature from 25° to 17°C. This was controlled for in our study by adjusting exposure time to compensate for the change in PR light intensity, giving a dose of 600mW-s/cm^2 for all treatments. Although several studies have used fluorescent lamps, of both blacklight and day-glow types, the effect of changing temperatures on the lamps themselves were not reported. This has little bearing on the field results per se, however should be considered when laboratory setups are used to simulate "real world" conditions.

Harm (17) explained the decrease in PR at low temperatures by increasing viscosity within the cell. The enzyme's movement from one lesion to the next may be hampered, and so decrease the repair rate. Only at very low temperatures, i.e. below 0°C, did the site conformational changes in the enzyme-substrate complex and decrease in the metabolic rate as significantly affecting PR.

The Bergen County study included temperature as an independent variable, and conducted a series of exposures between Feb. and Aug. There was a statistically significant difference in survival

reported, with a correlation coefficient of 0.75. However, there was a high degree of variability in the photoreactivated samples, and the increasing intensity of the sun was not considered as a variable. Intensity does affect PR, and this increase in intensity is concomitant with the rising water temperature in their experiments. This report suggests that perhaps they should be considered separately. These were all conducted in either the chlorine contact chamber, or in the spillway leading to it. Bottles were placed in the channel in some cases, or samples were collected directly from the contact chamber or downstream in others. The actual PR dose received by any sample would vary. Also, no attempt was made to measure the sunlight intensity, nor was there any mention of the meteriological conditions (overcast, sunny, or variable), and so generalizations are difficult to make from this data, even though the authors attribute 50% of the correlation coefficient to temperature effects.

EFFECT OF NUTRIENT LEVELS ON PR

UV treated secondary effluent was diluted with either a sterile, non-nutrient buffer, or filter sterilized Morgan Creek water collected 1/4 mile upstream from the effluent outfall. The filtered creek water contained less than one coliform per 100ml. Samples were again irradiated at two uv dose levels, as previously described. No significant difference was found between undiluted samples and those diluted with sterile creek water at either uv dose tested. The p values were 0.61 and 0.13 and 9mW-sec/cm^2, respectively. However, when diluted with phosphate buffer, sample (p=0.06 and 0.0002 for uv doses 4 and 9, respectively). (See Tables 7, 8).

TABLE 7 . Effect of nutrient on photoreactivation.

UV Dose	6/8 Treatment	Dilution*	cfu/ml	%PR	6/11 Treatment	Dil*	cfu/ml	%PR
0			3980				1387	
	Dark		78		Dark		120	
	Light	Undiluted	1250	30.0	Light	Und.	530	32.4
			1190	28.5			530	32.4
			1120	26.7			440	25.3
4								
		1:10	1570	38.2		1:10	680	44.2
			1330	32.1			570	35.5
			1550	37.7			560	34.7
		1:40	1456	35.3		1:40	568	35.4
			1392	33.7			488	29.0
			1168	27.9			560	34.7
	Dark		0.4		Dark		1.5	
	Light	Undiluted	117	2.9	Light	Und.	69	4.9
			164	4.1			82	5.8
9			185	4.6			71	5.0
		1:10	207	5.2		1:10	108	7.7
			218	5.5			87	6.2
			189	4.7			89	6.3
		1:40	147	3.7		1:40	150	10.7
			206	5.2			99	7.1
			210	5.3			80	5.7
Sunlight Intensity (mW/cm^2) =			3.9 - 4.5				3.25 - 3.30	

* Filter-sterilized Morgan Creek water was used for dilution.

Table 8 Nutrient Effects Diluted on Sterile Phosphate Buffer

Sunlight I =
3.2 - 3.35

UV Dose	Dilution Ratio	Ave. cfu/ml	Ave. Log Survival	Ave. %PR
0	-	3653	-	-
4	dark control	42.7	-1.93	-
	undiluted	1110	-0.52	29.6
	1:10	1207	-0.48	32.2
	1:40	626.7	-0.77	16.2
9	dark control	2.0	-3.27	-
	undiluted	14.6	-1.40	3.9
	1:10	113	-1.51	3.0
	1:40	85.3	-1.63	2.3

In the literature, the lack of much of a nutrient effect has been documented (3.13). Hanawalt describes conditions which inhibit cell division as being favorable for repair. Indeed, conditions which slow metabolism, such as low nutrient availability may provide extra time for all the enzyme systems operating in the cell to optimize reversal of lesions before normal cell function resumes. If cells were to attempt to transcribe a portion of DNA essential for survival or to replicate DNA immediately after irradiation, the chances of survival are small. This also accounts for the difference in uv sensitivity during the lifecycle of bacteria (30). Organisms are known to be more resistant to radiation effect during the stationary phase when DNA replication and protein synthesis are slow, than during other, more active phases of growth. Coliforms in wastewater effluent have nearly exhausted the food supply, and so have a relatively slow metabolic rate. Morgan Creek is a more dilute nutrient environment, compared to the wastewater, and therefore would not stimulate growth. This postpones DNA replication further, giving the cell more time to reverse damage sustained during disinfection. There is relatively little absorption of near-uv light by Morgan Creek water. However, absorption of light below 300nm increases, which includes the damaging portion of sunlight making it unavailable for further disinfection. Therefore, photoreactivating wavelengths would be the main component of light reaching the organisms. It has already been stated that near-uv light highly favors PR over possible damaging effects. One would therefore expect the number of coliforms to increase downstream, after uv treatment, especially under conditions which promote PR, the most efficient of the repair systems.

EFFECT OF UV DOSE ON PR

In both phases of this investigation, a uv dose-survival curve was generated including uv doses of 4, 9, 16, and 26mWs/cm^2 (uv dose 42 was added to the field experiments), giving from 1.5 - 4.0 logs of kills. Samples of the irradiated secondary effluent were allowed to photoreactivate under optimum conditions, as recorded in previous experiments (i.e. at 600mWs/cm^2 in the lab, or for 60 min in Morgan Creek). (See Figs. 13, 14 and Table 9, 10). PR consistently yielded a log or better of recovery, even at low uv doses. As the disinfection dose increased, the proportion of survivors due to PR increased, so that at high doses, there was 1.5 - 2.0 logs of recovery, in the lab. Field conditions were more variable. Sunlight intensity ranged from 1.9 - 4.5 mW/cm^2, over the 4 days of experimentation. There was no significant difference in PR due to the changing intensity, a difference of 0.5 log units being statistically significant (6).

This has serious implications in uv disinfection, since there can be more recovery at higher uv doses. Also, perhaps at high doses, 60 minutes of exposure to sunlight is not adequate for optimal recovery. There are more lesions, the majority of which must be reversed in order for the cell to survive, as was the case in the Bergen County study.

The data was plotted as follows: the number of survivors at a given uv dose without PR (i.e. the dark control) on the right ordinate vs. the number of survivors at the equivalent uv dose, after PR on the abcsissa, as adapted from Jagger (23). According to him, since the resulting line is straight, there is a direct, constant reduction of the uv dose as a result of PR. The slope of the line

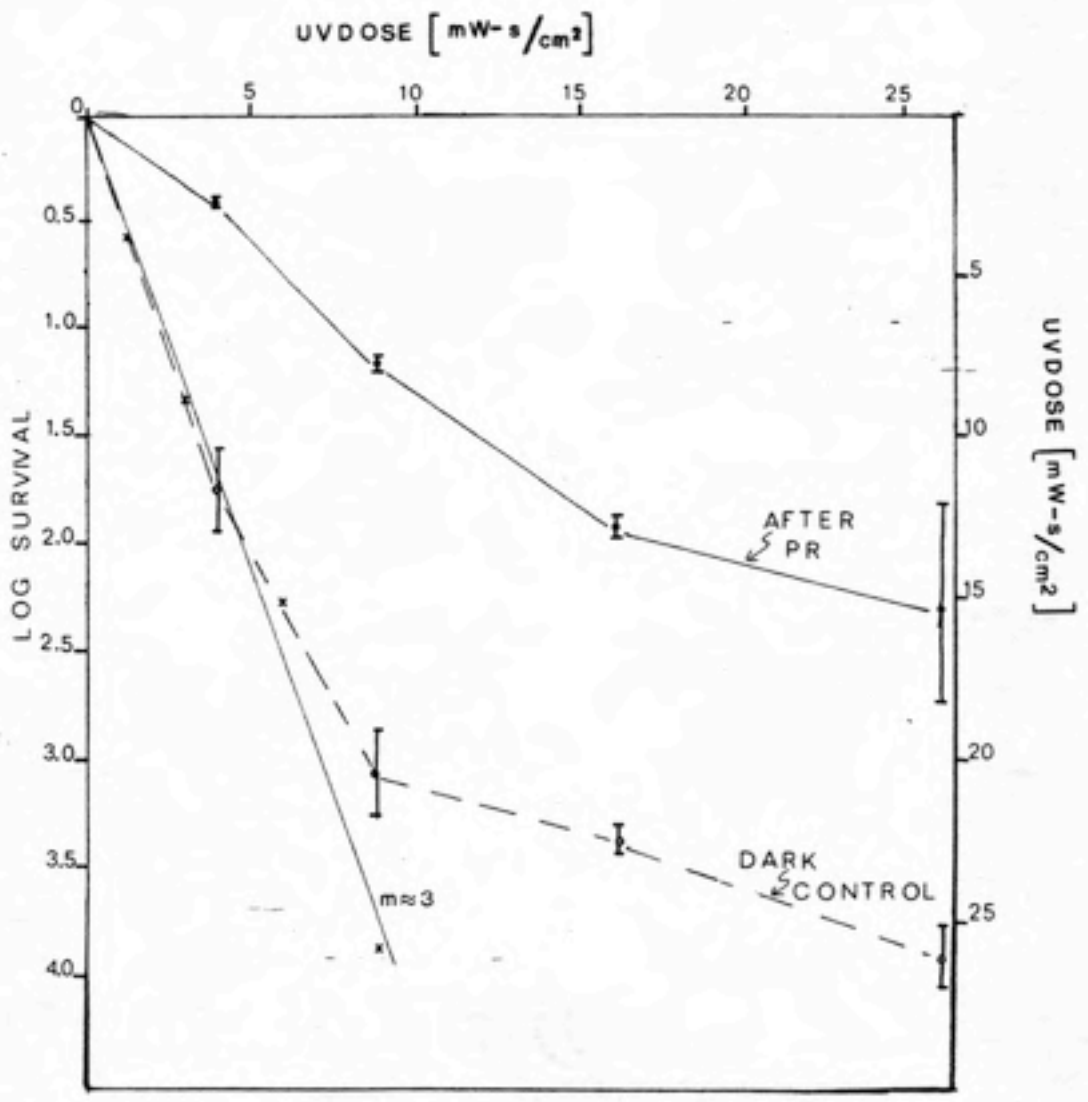


FIGURE 13: a)Log Survival vs. UV Dose. Parallel survival curves before and after exposure to PR light in the laboratory. PR dose = 600 mW-s/cm^2. Bars indicate range; b) UV Dose Reduction Factor - shows the ratio of the uv dose at a given dark survival (right ordinate) to the uv dose yielding the same survival after exposure to PR light (abscissa). Slope = 3.

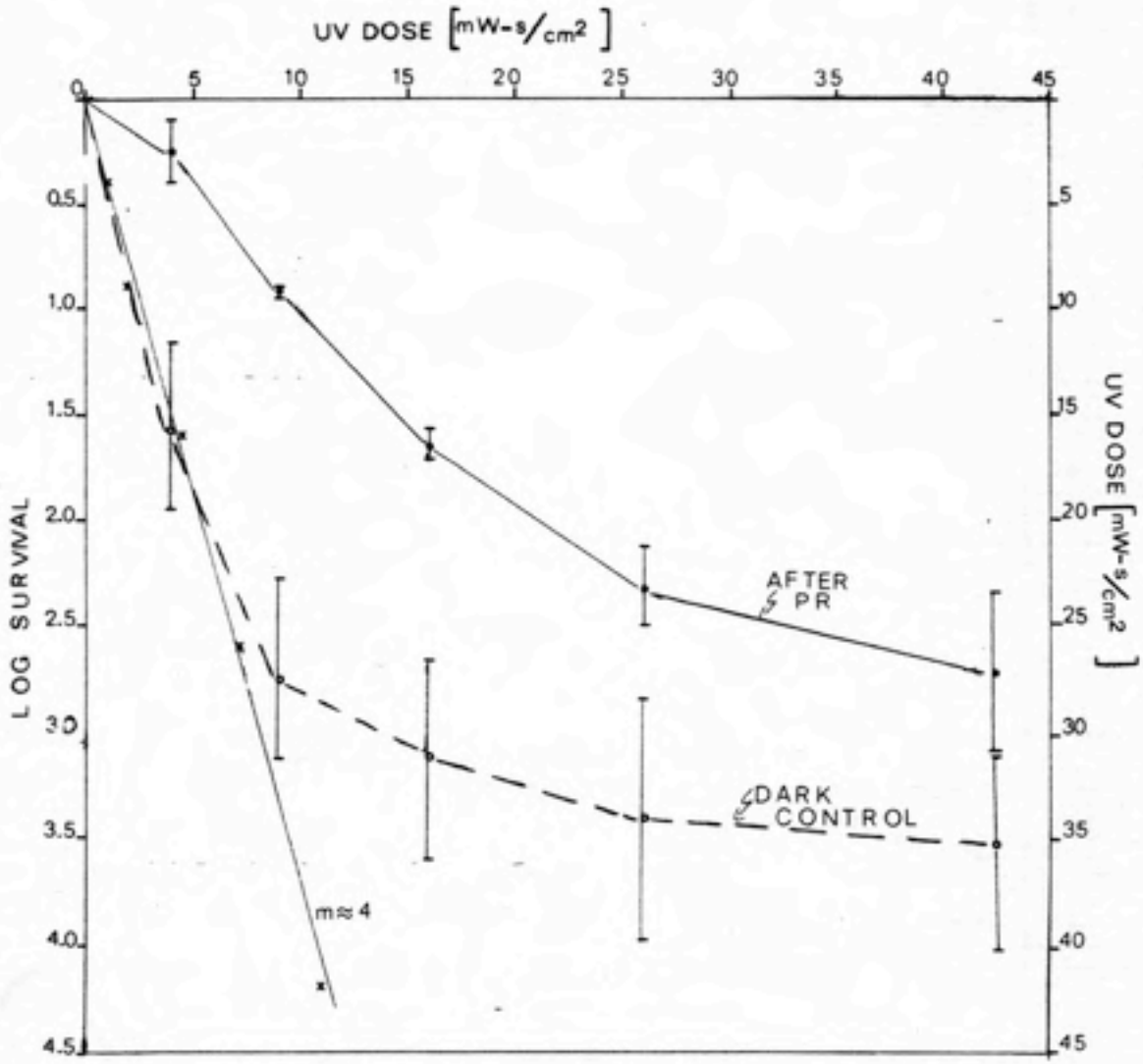


FIGURE 14: a) Log Survival vs. UV Dose. Parallel survival curves before and after exposure to sunlight for 60 minutes. Bars indicate range. b) UV Dose Reduction Factor - shows the ratio of the uv dose at a given dark survival (right ordinate) to the uv dose yielding the same survival after exposure to PR light (abscissa). Slope = 4.3.

Table 9 PHOTOREACTIVATION OF UV-IRRADIATED COLIFORMS

UV Dose ($mW\text{-}s/cm^2$)		1 cells/ml	1 logS	1 %PR	2 cells/ml	2 logS	2 %PR	3 cells/ml	3 logS	3 %PR
0		8433			3350			3480		
4	Dark	91	-1.97		88	-1.58		98	-1.55	
	Light	3400	-0.37	39.7	1313	-0.14	37.4	1343	-0.41	36.8
9	Dark	4.9	-3.24		2.8	-3.08		4.8	-2.86	
	Light	528	-1.20	6.2	231	-1.16	6.8	243	-1.16	6.9
16	Dark	3.2	-3.42		1.4	-3.38		1.8	-3.29	
	Light	120	-1.86	1.4	40.5	-1.92	1.2	38.5	-1.96	1.1
26	Dark	0.9	-3.96		0.3	-4.05		0.65	-3.73	
	Light	17.0	-2.70	0.2	55.9	-1.78	1.7	7.0	-2.70	0.2

UV Absorbance	.242	0.248	.216
PR Dose ($mW\text{-}s/cm^2$)	600	600	600
PR Intensity ($mW\text{-}s/cm^2$)	.212 - .238	.212 - .238	.212 - .238

Sample source: Secondary effluent from the Mason Farm plant.

TABLE 10 UV Dose-survival Curves of Coliforms Before and After Photoreactivation.

UV Dose		6/22 cfu/ml	6/22 logS	6/25 cuf/ml	6/25 logS	6/27 cfu/ml	6/27 logS	6/28 cuf/ml	6/28 logS
0		4,333		1,200		3,213		3,420	
4	D	201	-1.34	33	-1.56	221	-1.16	41	-1.92
	L	3920	-0.1	483	-0.4	1,537	-0.32	1,270	-0.43
9	D	3.2	-3.14	1.2	-3.01	10.5	-2.49	18.0	-2.28
	L	583	-0.9	151	-0.9	416	-0.89	387	-0.95
16	D	1.2	-3.59	.45	-3.43	6.8	-2.67	1.1	-3.5
	L	111	-1.6	32.5	-1.57	86	-1.57	67	-1.71
26	D	6.2	-2.86	.13	-3.95	2.6	-3.09	0.37	-3.97
	L	19.1	-2.37	8.5	-2.15	13.0	-2.39	10.2	-2.53
42	D			.12	-4.01	2.5	-3.10	-	-
	L			2.56	-2.67	15.5	-2.32	2.77	-3.09

Sunlight intensity (mW/cm^2) = 2.95 - 3.6 4.5 - 4.3 3.9 - 4.1 1.9 - 3.6

PR Time = 60 minutes

yields a "dose reduction factor" which will predict the amount by which the uv dose will have to increased to compensate for PR. In the laboratory where the PR dose was held constant at 600mW-s/cm^2, the uv dose is effectively decreased by two-thirds. In field conditions, with the sunlight intensity varying from 1.0 - 4.5mW/cm^2 over the four days of experimentation, the uv dose was decreased by approximately 75%.

The following example will help illustrate this in more practical terms. If we assume that a uv dose of 26mW-s/cm^2 will yield 3.5 logs of disinfection without PR, then we can predict survival after PR. Assuming optimum conditions of bright sunlight intensity, relatively warm water temperatures (24-26^o), and adequate exposure time (60 min.), then we can calculate from the data that the effective uv dose will be reduced by a factor of 3-4. Therefore, in order to compensate for the effects of PR, the initial uv dose must be increased by the same factor. Therefore, a uv dose of between 78-104mW-s/cm^2 is necessary, to ensure 3.5 logs of kill, even after PR.

PYREX BOTTLE VS. OPEN BEAKER

One final set of experiments was performed, to determine whether the pyrex bottle significantly influenced coliform survival, by decreasing the intensity of the incident sunlight on the samples while incubating in the stream, or by absorbing germicidal components in the sunlight. It has been reported that sunlight can be an effective disinfectant for aquatic microbes (21, 43). Effluent with

uv doses of 4 and 9, and 26mWsec/cm^2 were placed in either pyrex bottles of open beakers, and incubated for 60 minutes in the creek. There was no significant difference in survival between the two treatments, except at the uv dose 26. (See Fig. 15 and Table 11). In unirradiated samples, which were either wrapped to exclude sunlight or incubated exposed to the sun in a pyrex bottle or beaker, there was a slight but significant lethal effect. This was also seen in an earlier study of PR with Chapel Hill effluent (10), where the number of coliforms in effluent samples incubated in sunlight declined slightly.

Data from all experiments were also analysed for statistically significant interaction between treatment and sampling day using the SAS model as before, and analyzing at both the 95 and 99% confidence intervals. It was found that the differences between samples taken on different days had a definite effect on the results. That is to say, in most cases, the coliform population varied from day to day, and this influenced their response to uv irradiation as well as to PR. There was no difference between the days during the bottle vs. beaker experiments. There was a significant difference in the response of irradiated samples, however there was no interaction between the two. The difference attributable to sampling day had no consistent effect on the treatments, neither synergystic nor antagonistic, in the two days of experimenting for the "beaker vs. bottle" series. It would appear that the sample from the first day was more resistant to uv disinfection than the second day, when the uv absorbance was also higher. This illustrates one of the difficulties in this study, namely that the wastewater

characteristics changed over time, and this can have a significant effect on its response to both disinfection and PR. Thus, generalizations are difficult to make with high degrees of confidence, since some of the data appear to be contradictory. Increasing the number of replications of each experiment would perhaps allow a better understanding of which factors might be most influential, which controllable, and which discountable.

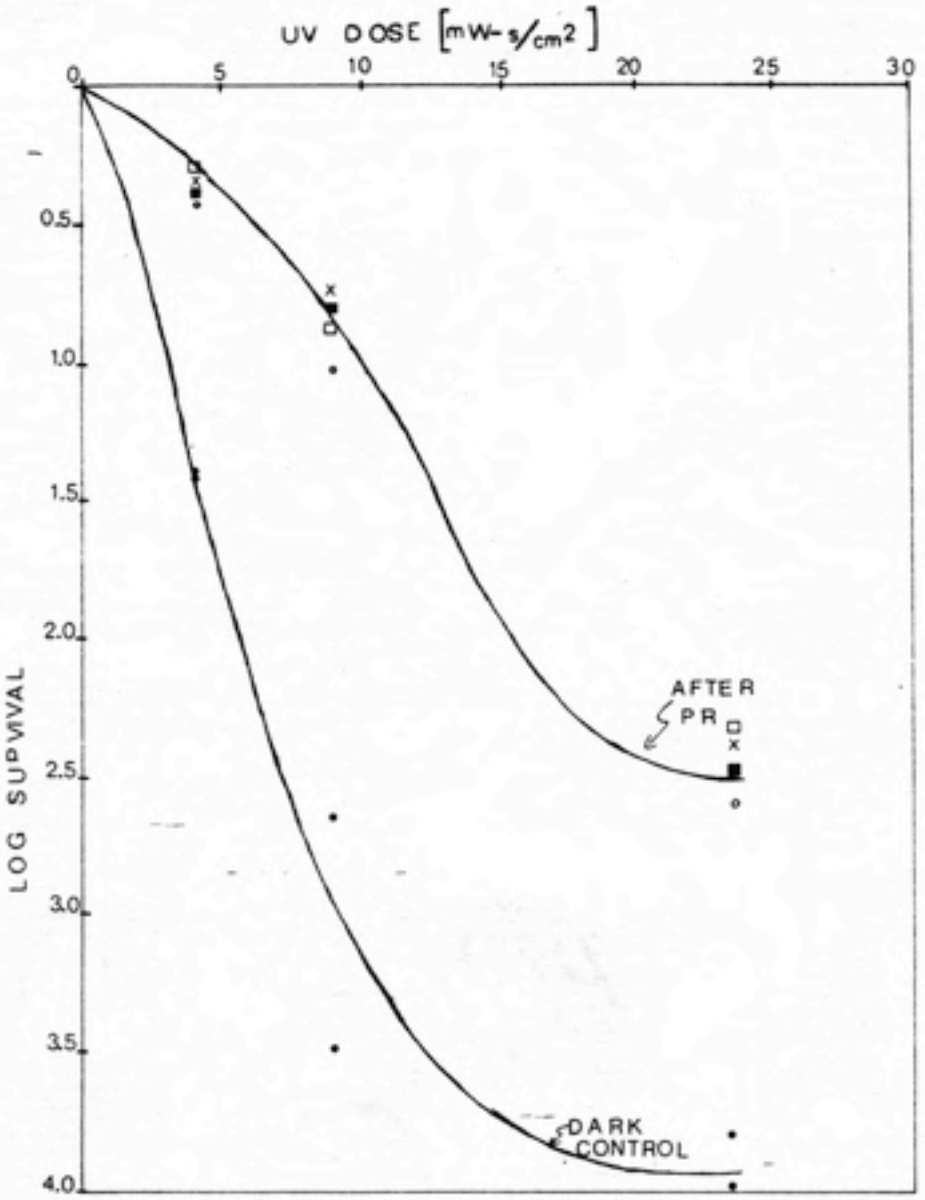


FIGURE 15: Log Survival vs. UV Dose, for doses 4, 9, and 22mW-s/cm^2, exposed to sunlight for 60 minutes, in Pyrex bottles or open beakers.

TABLE 11

UV and PR Survival Curves - Open Beaker vs. Pyrex Bottle

UV dose		7/2 cfu/ml	7/2 log S	7/3 cfu/ml	7/3 log S
0		5,760		5,886-7	
4	dark	232	-1.39	243	1.38
	beaker	3080	-0.27	243.3	-0.38
	bottle	2350	-0.39	2672.7	-0.34
9	dark	13.0	-2.65	2.0	-3.47
	beaker	780.7	-0.87	1093.3	-0.73
	bottle	558.3	-1.01	991.7	-0.77
26	dark	1.0	-3.76	0.48	-2.43
	beaker	24.7	-2.37	21.8	-2.43
	bottle	14.8	-2.59	28.2	-2.32

exposure time (sunlight) - 60 min

Sunlight I -

July 2nd = 1.34 - 1.8 mW/cm^2

July 3rd = 3.35 - 3.70 mW/cm^2

CONCLUSIONS

Although the results are somewhat variable, there are nonetheless several conclusions which can be drawn:

1) UV light is and effective disinfectant, however it has limitations.
2) PR can have a large impact on the level of disinfection, greatly reducing the effect of the initial uv dose, by as much as 75%.
3) Factors, such as sunlight intensity, temperature, water quality of the receiving stream, and distance downstream, will influence PR. Conditions which favor PR include fairly bright sunlight (above 2.0 mW/cm^2), relatively low nutrient content of the receiving stream, warm water (24-26^{o}C), and adequate exposure time (60 min.).
4) These must be taken into account, if uv is to be used as a disinfectant, and that when conditions are favorable for PR, the uv dose must be increased to compensate for the post-irradiation regrowth. These limitations lie on the flat part of the survival curve, where increasing the uv dose dose not yield proportionately lower survival. Wastewater quality will in part, determine the level of disinfection possible. The uv dose may have to be increased by 3 - 4 times the equivalent dose without PR, in order to maintain adequate levels of disinfection.

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