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Evaluation of Methylene Blue and Rose Bengal for Dye Sensitized Solar Water Treatment

The experimental work described in this paper evaluated the efficacy of dyes as sensitizing agents for solar photochemical detoxification and disinfection of water. Methylene blue and rose bengal were evaluated as photosensitizing agents. While neither dye was effective for detoxification, methylene blue showed some efficacy for photodisinfection over natural sunlight. In all sunlight experiments, methylene blue effected a coliform reduction ranging from 96% at pH 7 and lower methylene blue concentrations, to 99.5% for all concentrations at pH 10 and the 10 mg/L concentration for pH 7. The 99.5% reduction at pH 7 is comparable to 74% reduction at the same pH using sunlight alone. Significant coliform reduction was also observed in dark experiments with 10 mg/L of methylene blue. [DOI: 10.1115/1.1498850]

Introduction

In some areas of the developing world, cross contamination of the water supply with both chemical and microbiological contaminants poses a problem. Innovative and cost-effective solutions must be sought to address this problem. The research reported here examined the feasibility of photosensitization as treatment for drinking water mildly contaminated with both chemical and microbiological contaminants. It was conducted as part of a broader research program that explored photochemical treatment of water for simultaneous disinfection and detoxification using TiO_2 alone and in combination with dyes [1,2]. Potential applications include point-of-use treatment, single and multiple family households, and small rural and suburban communities with specific emphasis on the developing world.

Cross-contamination of drinking water sources with microbiological contaminants has been observed in locations where maintenance of infrastructure is a difficulty or septic tank usage is common [3–5]. As lesser-developed countries seek to expand their economic base via industrial production, chemical contamination also increases. This is evident in some of the more recently industrialized areas of China [6]. While few studies are available that evaluate water quality as a function of both microbiological and organic contaminants, it is reasonable to expect that one of the consequences of rapid industrialization for economic development could lead to chemical contamination of drinking water supplies already tainted with microorganisms. The problem has already been seen in the Caribbean where a combination of leakage from sewerage lines and underground fuel storage tanks leads to contamination of wells and municipal drinking water sources by both microbiological and chemical contaminants [7].

Our goal for this study was to provide preliminary evidence of the efficacy of two dyes, methylene blue and rose bengal, for simultaneous photosensitized disinfection and detoxification. The aromatic hydrocarbons benzene and toluene, and the bacteria *Escherichia coli*, were used as model contaminants. The ultimate objective was the evaluation of conditions under which treatment by dye induced photodynamic action could effectively treat groundwater contaminated by leakage from sewer lines and underground fuel tanks.

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Background

Photosensitization has been studied for disinfection in the medical field [8] and in wastewater treatment for virus inactivation [9–12] and coliform destruction [13]. In a joint project, Israeli and U.S. researchers conducted laboratory, pilot, and field scale studies to evaluate photosensitization of wastewater and sewage effluent spiked with bromacil. In these studies, methylene blue and rose bengal were used as the photosensitizers [14–21].

As evidenced by the inclusion of bromacil in the wastewater studies, some viability for simultaneous treatment is indicated. However, the effects of photosensitization have not been reported over a broad range of organic chemical contaminants nor has work been reported that indicates treatment to levels acceptable for drinking water.

Experimental Approach

The experiments were designed to determine effects and interactions as a function of dye concentration, pH, and absence/presence of light on disinfection and detoxification. *Escherichia coli* and benzene and toluene were used as model microbiological and organic contaminants, respectively. The model contaminants were selected to simulate the dual contamination problem from sewage and fuel tanks described by Green [7]. All experiments were laboratory scale and conducted in sunlight.

In studies of methylene blue photodisinfection processes, pH values ranging from 8.6 to 10 were found to be optimum [9,17,18,22]. The same pH dependence was seen with methylene blue and rose bengal for the photodegradation of organic chemicals [23,24]. In photosensitization of bromacil using methylene blue and rose bengal, reaction rates increased as pH values increased, with the highest rates at pH 9–10 [19,21]. Based on this information, the experiments for this research were conducted in neutral (ph 7) and basic (pH 10) environments.

In pilot plant studies, Eisenberg et al. [21] found that concentrations of methylene blue ranging from 1 to 10 mg/l were sufficient for the photooxidation of bromacil. Acher and Juven [15] reported an increase in the photosensitized destruction of coliform in sewage effluent with a corresponding increase in methylene blue concentration, up to 5.0 mg/l. However, in pilot plant studies, smaller concentrations (<1.0 mg/l) were effective [18], and concentrations higher than 0.9 mg/l methylene blue hindered light penetration [14]. Therefore, photosensitization experiments for this research were conducted at several levels of concentration, ranging from 0.01 mg/l to 10 mg/l, with a control containing no dye.

Table 1 Descriptive statistics of measured conditions for all experiments

MB	Parameter	Mean	Std Dev	Min	Max
	Sunlight (all experiments), W/m^2	692	112	542	891
	Sunlight, pH 7, W/m^2	743	129	665	891
	Sunlight, pH 10, W/m^2	641	50	542	696
	Initial Coliform Density, cfu/L$\times 10^3$	784	365	27	1453
	Initial Benzene concentration, ppb	676	210	377	1218
	Initial Toluene concentration, ppb	314	139	136	770
RB	**Parameter**	**Mean**	**Std Dev**	**Min**	**Max**
	Sunlight (all experiments), W/m^2	781	56	715	856
	Sunlight, pH 7, W/m^2	815	60	746	856
	Sunlight, pH 10, W/m^2	746	30	715	775
	Initial Coliform Density, cfu/L$\times 10^3$	816	375	187	1680
	Initial Benzene concentration, ppb	560	262	296	1407
	Initial Toluene concentration, ppb	426	226	155	1026

A full factorial design was employed to incorporate the two pH levels and five dye concentrations (0, 0.01, 1.5, and 10 mg/L). for experiments conducted in sunlight and dark (for control). The matrix was completed three times for each of the two dyes, rose bengal (RB) and methylene blue (MB). The design resulted in a total of 20 reactor configurations per dye per experimental set. One reactor for each set of conditions represented a complete set of experiments, for a total of 40 reactors per set. Five reactors were run at a time. Each of four reactors contained a different concentration of a single dye (either methylene blue or rose bengal), and one reactor served as a control (no dye). All other parameters were the same for a given run.

Samples were taken from each reactor at 0, 5, 15, 30, 60, 120, and 240 min and refrigerated immediately. Three replicates were plated from each sample for microbiological analysis. The remainder of the 0, 60, and 240-min samples were refrigerated and saved for chemical analysis.

The reaction vessels were covered Pyrex® dishes with Pyrex® lids, allowing light transmission above wavelengths of $\lambda \geqslant 300$ nm. In order to eliminate problems of air stripping and ensure that the reactor was airtight, the reactor was equipped with a glass blown sampling port plugged with a butyl rubber septum (which did not come in contact with the liquid) and sealed at the contact point with parafilm. The reactors were filled to the rim of the vessel in order to minimize headspace, and placed on a flat horizontal table, allowing direct overhead light.

For the experiments conducted in sunlight, total solar radiation was measured and recorded over the duration of the experiment, and ranged from 542 W/m^2 to 892 W/m^2. Measurements were taken using an Eppley global radiometer placed on the horizontal directly beside the reactors. A metal reactor chamber painted in flat black was used for the dark experiments. External light was blocked via a hinged metal door that fit snugly over the chamber.

Cultures were prepared using trypticase soy broth nutrient and incubated for 24 hr at 35°C. Three serial dilutions of 1:100 were prepared using sterilized deionized water. Each reactor was inoculated with 45 μl of the *E. coli* using the first dilution. The third dilution was used to confirm that the cultures were viable. Initial bacterial densities in the reactors ranged from 10^3 to 10^4 colony forming units (cfu) per ml, with most values falling around 3.5 $\times 10^3$ cfu per ml. Bacteria were obtained from American Type Culture Collection (ATCC).

For evaluation of disinfection efficacy, duplicate petri dishes containing plate count agar nutrient were inoculated with 100 μl from each reactor. Two replicates were taken at 0, 30, 60, 120, and 240 min, yielding four counts for each set of conditions per experiment. The inoculated plates were spread and incubated for 24 h at 35°C. After 24 hr, the number of bacterial colonies on each plate were counted.

Benzene and toluene were analyzed by a modification of the EPA purge and trap method using an SR 8610 gas chromatograph with PID detector [25–27]. The method used was sensitive to a low concentration of about 1 ppb for the components in question. In order to ensure consistency of the data across experimental sets, the analyses were referenced to an internal chlorobenzene standard by the addition of a known concentration of chlorobenzene to each sample.

Results

Experimental sets were conducted on different days, and though efforts were made to minimize the differences between sets, both solar insolation and initial contaminant concentrations varied from one set to another. The mean, standard deviation, and range of these parameters for all experiments are shown in Table 1.

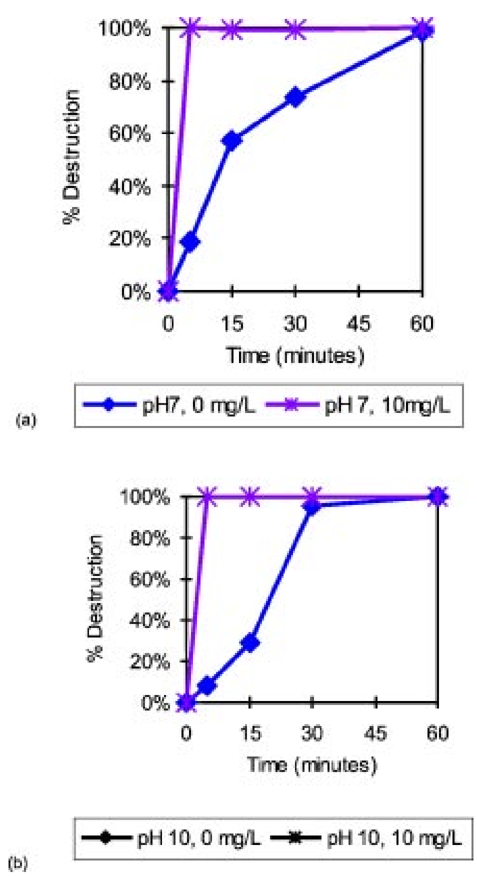

Fig. 1 Comparison of *E. coli* destruction in 10 mg/L MB versus sunlight alone

Disinfection. While disinfection in the presence of aromatic hydrocarbons was achieved with both rose bengal and methylene blue, simultaneous detoxification was not observed with either dye. Under the conditions tested, the presence of MB increased the disinfection rate of water contaminated with *E. coli* over sunlight alone.

At pH 10, all MB concentrations resulted in at least a 99.5% coliform reduction by 30 min of irradiation, compared to 96% reduction with sunlight alone. With 10 mg/L MB, complete coliform destruction was achieved by 5 min of irradiation. Figure 1 shows a comparison of coliform destruction using 10 mg/L MB versus sunlight alone at pH 7 and pH 10. In the experiments conducted at pH 7, the coliform reduction ranged from 99.5% with 10 mg/L MB to 96% with 0.1 mg/L MB by 30 min. Comparatively, only a 74% reduction was attained with sunlight alone for samples taken at the same time.

Regardless of pH, in the presence of at least 1 mg/L MB and sunlight (542–696 W/m^2), complete disinfection occurred within 5–30 min. However, in the absence of MB with the same intensity sunlight, complete disinfection required at least 60 min (Fig. 2).

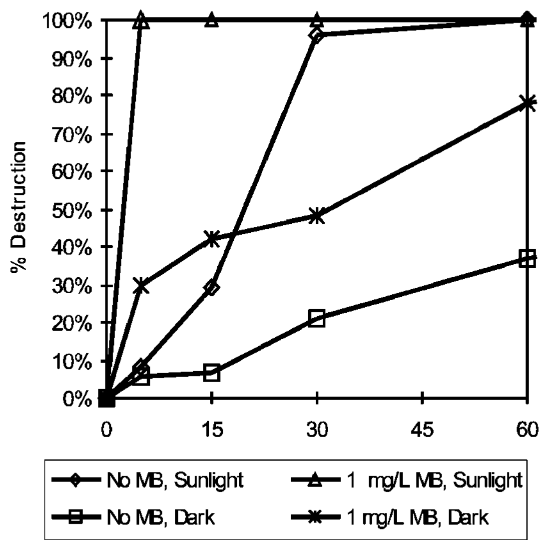

Fig. 2 Destruction of *E. coli* with 1 mg/L MB at pH=10, I_{avg} =641 W/m^2

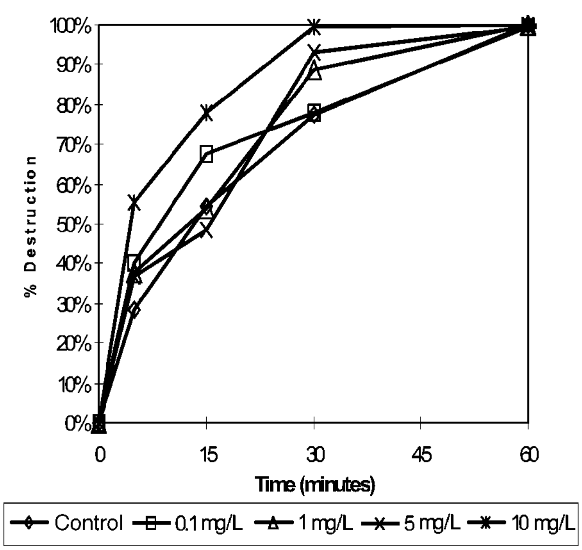

Fig. 3 RB destruction of *E. coli* in sunlight at pH=7, I_{avg} =746–856 W/m^2

Complete disinfection did not occur at all in the dark, although a 99% coliform reduction was observed with 10 mg/L MB in the dark.

Rose bengal was less effective for photochemical disinfection than was MB. The presence of RB had little, if any, positive effect on the disinfection rate over sunlight alone, although the experiments at pH 7 (Fig. 3) appeared to exhibit some photochemical disinfection, with an apparently faster rate of destruction with 10 mg/L RB. In the samples taken at 30 min, there was a steady increase in the percent destruction from 70% to >99% as RB concentration increased. However, by 60 min, all of the samples exhibited >99% reduction of *E. coli.* In the experiments conducted at pH 10, RB had no positive effect on disinfection over sunlight alone. Coliform reduction of greater than 99% was observed by 60 min in sunlight, both in the presence and absence of RB. Coliform reduction was about the same by 30 min regardless of the RB concentration, with a low of 81% for 10 mg/L RB and a high of 90% with 1 mg/L RB. The control, sunlight alone, had a coliform reduction of 88%. The differences are not significant, as all values fall within the average standard deviation of 21%. The average intensity of sunlight in these experiments ranged from 715 to 775 W/m^2.

Detoxification. While some reduction in both benzene and toluene concentration was observed with both dyes under every set of conditions, there was no identifiable relationship with either dye concentration or sunlight. The experimental values for both benzene and toluene showed fairly consistent reductions, both with and without dye. Figure 4 shows representative graphs for the concentration of aromatic hydrocarbon as a function of time in the presence of both methylene blue and rose bengal. The observed reduction of benzene and toluene is likely a function of the volatility and hydrophobicity of the components. A slight temperature increase, due to sunlight and friction from the magnetic stirrer, would lead to volatilization and migration of the hydrocar-

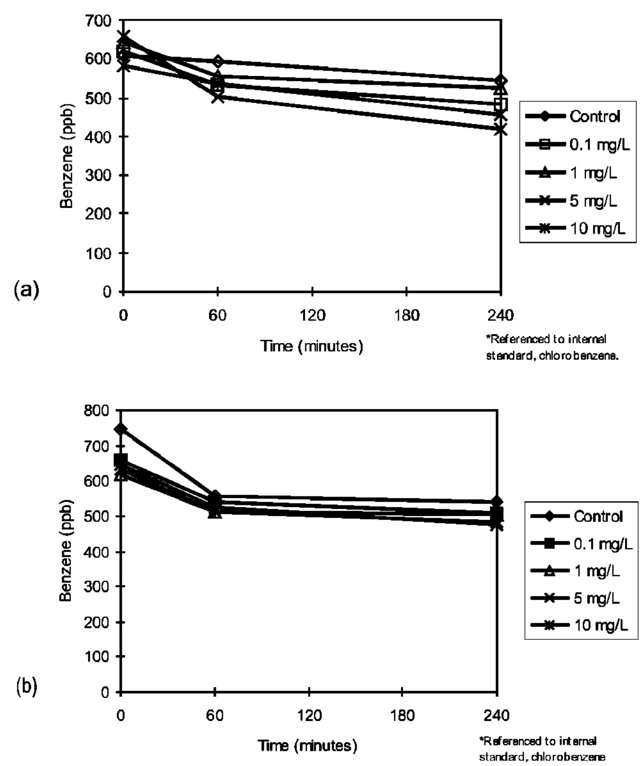

Fig. 4 Concentration as a function of time and dye concentration in sunlight; y-axis is organic concentration in ppb: (*a*) Benzene at pH=7 with MB, I_{avg}=665–891 W/m^2; (*b*) Benzene at pH=7 with RB, I_{avg}=746–856 W/m^2

Table 2 Calculated ANOM values for methylene blue photosensitized disinfection

Sample Set	Grand Average $\bar{\bar{X}}$	Avg Std Dev	Avg Range	Estimated SD(X)
t=5 min	0.39	0.14	0.31	0.20
t=15 min	0.28	0.13	0.31	0.18
t=30 min	0.23	0.13	0.30	0.18

bons to the butyl rubber septum and/or parafilm around the edge of the reactor top, providing the opportunity for the hydrophobic rubber components to absorb the volatile organics.

Discussion

Initial observation of the disinfection data for methylene blue photosensitization suggested that photochemical destruction might have taken place. Therefore, these data were evaluated for the effect of sunlight presence or absence, pH level, and dye concentration, using Analysis of Means (ANOM). The ANOM test compares the mean value for an entire data set under the selected parameter, providing an indication of the impact of that parameter. The fractional survival of colony forming units (cfu) after 5, 15, and 30 min were all analyzed in this manner. The analysis is presented graphically in the style of a signal chart, with calculated upper decision limits (UDL) and lower decision limits (LDL). Should the grand average values fall within the limits, the effect of a change in the parameter is negligible. However, values ranging outside of the limits indicate a significant effect. A relatively conservative α-level of 0.5 was chosen to minimize the probability of false alarms. A smaller α-level was not desirable as it might have resulted in missed signals and would not be appropriate for this type of exploratory research.

Grand averages were calculated separately for data at 5, 15, and 30 min using fractional survival of cfu. The grand averages were 0.39 ($\pm$0.14), 0.28 ($\pm$0.13), and 0.23 ($\pm$0.13) for 5, 15, and 30 min, respectively. The values calculated for use in ANOM are given in Table 2.

Effect of Sunlight. The absence or presence of sunlight was found to be statistically significant in all cases tested. With both lower and upper out of limits signals, as shown in Fig. 5, it is clear that the presence of sunlight is a critical factor for disinfection in these experiments.

Effect of pH. As shown in Fig. 6, no out of limits signals were obtained for the two pH levels tested for disinfection with methylene blue, indicating that there was no significant effect. This was inconsistent with the findings of Eisenberg et al. [20], who reported a very strong correlation with pH values and more efficient MB inactivation at a basic pH.

Effect of Dye Concentration. As shown in Fig. 7, signals were obtained for the control reactor on the upper side, and for 5 and 10 mg/L of MB on the lower side, indicating that the absence or presence of MB was significant at both 5 and 15 min. However, by 30 min (Fig. 7*c*), the presence of MB was no longer of significance, and disinfection in sunlight alone was just as effective.

Since MB concentration displayed some significance, each of the subgroup averages at 5 min was plotted against the control and the high concentration (10 mg/L) to determine the minimum significant concentration. These combinations allowed for the determination of optimum concentration range for the fastest disinfection.

When all of the MB concentrations were considered, the presence of at least 5 mg/L MB showed significance up to 30 min. By 30 min, the presence of MB no longer had an impact on the destruction of *E. coli*. Acher and Juven [15] reported that an increase in MB concentration from 0.5 to 5.0 mg/L caused an increase in the inactivation of coliform in both sewage water and tap water in sunlight. The results for destruction of *E. coli* found in this experiment were consistent with those that were reported by Acher and Juven [15].

Temperature. Though temperature was not a consistently measured parameter, an increase in temperature was observed, probably due to sunlight and friction from the magnetic stirrers. The combination of temperature and headspace increase would necessarily lead to volatilization of the chemical contaminants in the vapor space. Spot temperature checks with temperature strips on the outside reactor glass yielded values in excess of 35.5°C (96°F) in sunlight, which is not sufficient to cause disinfection by pasteurization [28,29].

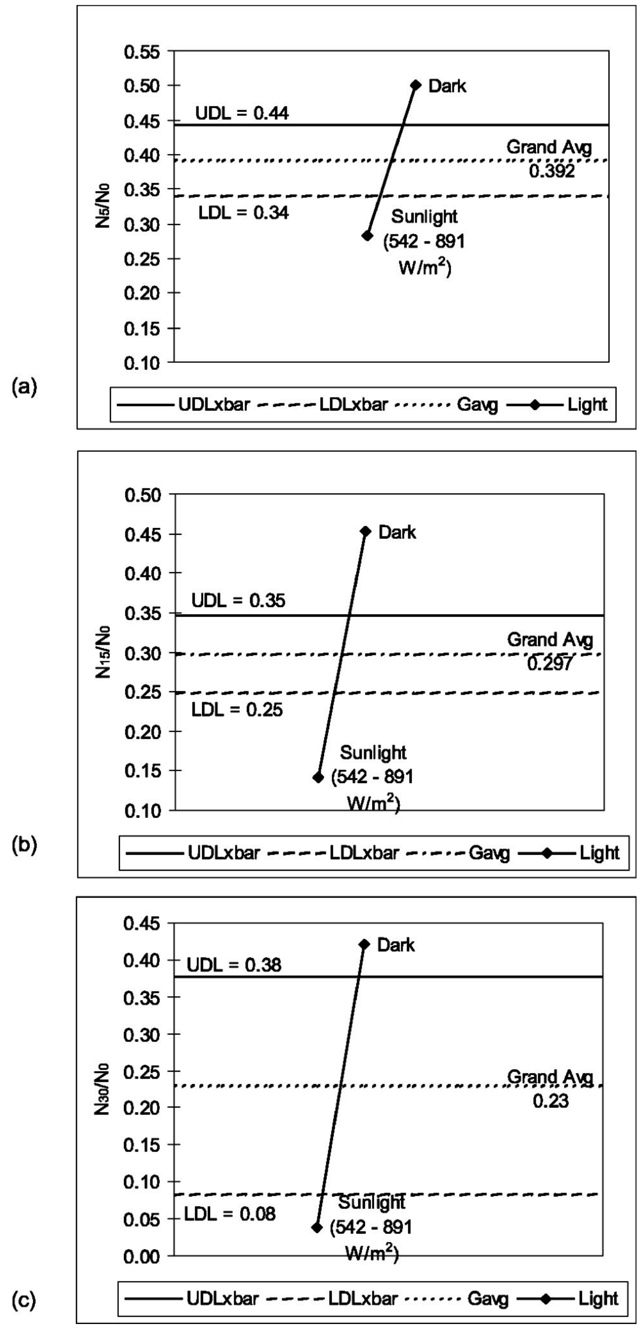

Fig. 5 Significance of sunlight, based on ANOM, in MB experiments: (*a*) 5 min, (*b*) 15 min, and (*c*) 30 min

Consistency with previous work. In order to provide confidence in the reactor system, a set of experiments was conducted to reproduce results found in the literature. Eisenberg et al. [20] were able to destroy bromacil in wastewater with MB in sunlight under a variety of pH conditions using several MB concentrations. While these experiments did not attempt to duplicate the work conducted by Eisenberg et al. [20], there was a desire to ensure that similar results could be obtained using the photosensitization reactor design. Photosensitization of water containing bromacil with 10 mg/L MB resulted in a 75% reduction, from 1448 ppb (±218) to 358 ppb (±48.5), after 4 hr of irradiation in sunlight versus a 13% reduction, to 1265 ppb, for the control reactor with no MB. This indicates clearly that bromacil destruction was effected under our conditions, consistent with the results of Eisenberg et al. [18].

Summary and Conclusions

Of the variables tested, sunlight, in terms of absence or presence, had the greatest impact on disinfection. Since sunlight is the driving force for the reaction and sunlight alone has disinfection capabilities [30], this is not an unusual finding. It does appear that the concentration of methylene blue has some impact on initial

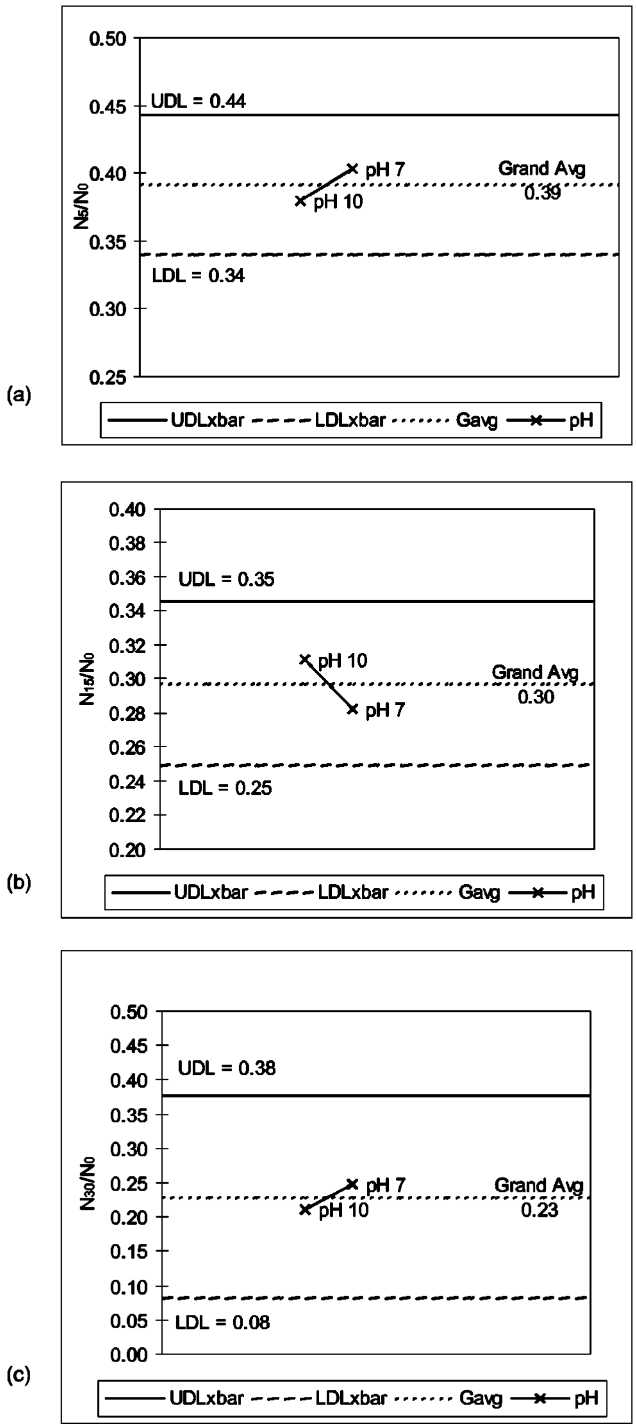

Fig. 6 Significance of pH, based on ANOM, in MB experiments: (*a*) 5 min, (*b*) 15 min, and (*c*) 30 min

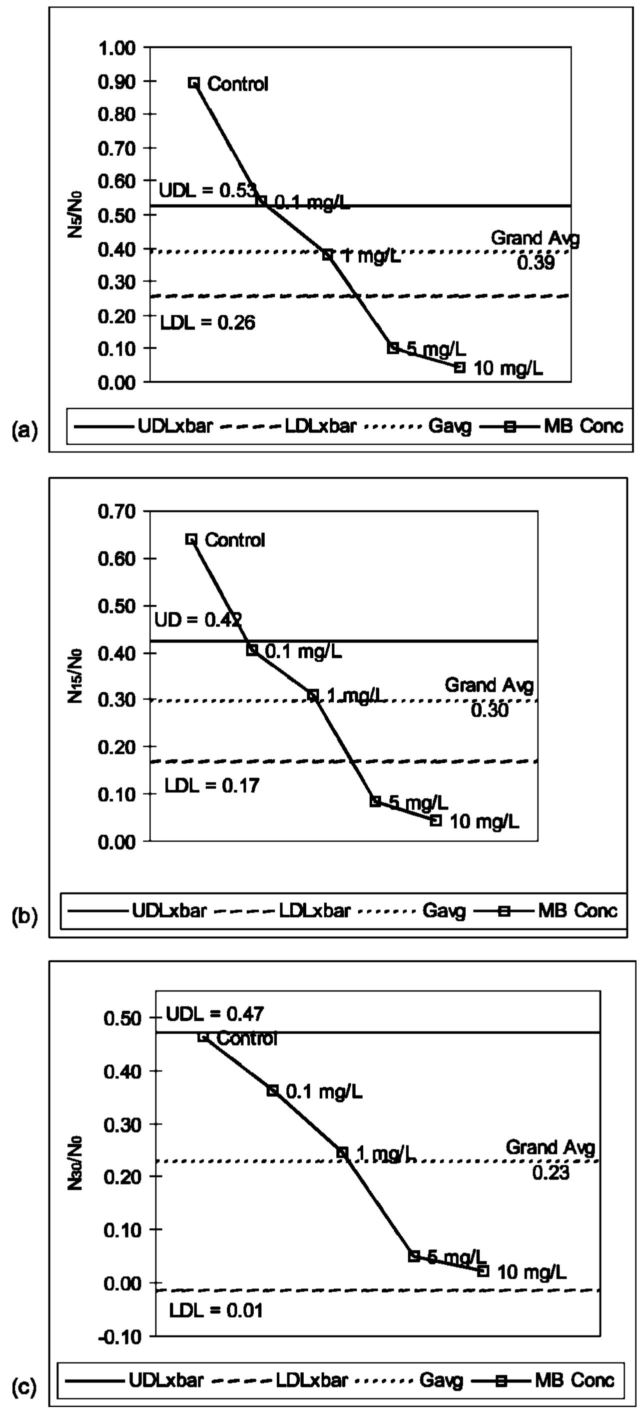

Fig. 7 Significance of MB concentration, based on ANOM, on disinfection in sunlight: (*a*) 5 min, (*b*) 15 min, and (*c*) 30 min

disinfection rates, indicating that the presence of methylene blue might reduce the required contact time for solar disinfection. There appeared to be minimal, if any, difference in performance with basic versus neutral pH, indicating, that, at least in this range, pH is not a significant factor.

Of the systems tested in these experiments, photosensitized disinfection with methylene blue was the only process that yielded any positive results. While disinfection occurred in the presence of rose bengal, it did not enhance the reaction to any degree. There were no indications at all that photosensitization occurred with respect to the chemical contaminants with either methylene blue or rose bengal. Consequently, with the exception of methylene blue photosensitized disinfection, these processes did not show promise for application.

Initial results indicate some promise for methylene blue disinfection; however, before the process is viable, additional, application specific, research is required. The positive aspects were pH and dosage of MB. Since pH was not a significant factor, no adjustments would be required for use of MB as a disinfectant. While dosage was of some importance, it would not require tight control.

One drawback to the use of MB as a disinfectant for drinking water was the presence of the dye. Since dyes have been known to be photosensitized and photolyzed [8], an initial expectation was that the dyes would completely mineralize themselves. While all of the MB disappeared, by visual inspection, from reactors at the lower dosages, by the end of a 4-hr experiment, some slight color was still visible at the higher dosages (5 and 10 mg/L). Therefore, separation of the dyes from the water may be required and is not a trivial task. The presence of dye in the water would likely be a deterrent for use as drinking water, particularly if the water were clear prior to treatment. It could also pose a hazard if harmful intermediates were formed. Additional work must be undertaken to determine if the dyes are capable of complete mineralization, or require additional processing for removal.

Most importantly, however, in order for this process to be determined feasible for application, it must be tested over a much broader range of microbiological contaminants. Site specific testing is a must, as species may differ from one locale to the other. Additionally, the process would need to be tested for inactivation of viruses and cysts.

Although the addition of MB increased the rate of disinfection over sunlight alone, the advantage is likely not enough to justify the addition of a foreign substance into a drinking water system, no matter how benign.

Acknowledgements

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