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The photocatalytic production of organic-free water for molecular biological and pharmaceutical applications

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Abstract

The inability of conventional water-purification systems to meet the ultra-high purity needs of molecular biology and biopharmaceuticals reliably was attributed to their almost exclusive utilization of phase-transfer technologies. Water quality may unpredictably degrade when confronted by microorganism blooms or altered feed water characteristics.

Photocatalytic point-of-use water-purification systems fed by deionized water were demonstrated to meet the most stringent water-purity needs of the molecular biologist. The reliability of the photocatalytic water-purification technology was attributed to its ability to destroy organic contaminants rather than just effect their phase transfer.

Photocatalytically produced water was shown to be free of detectable microorganisms, DNA, endotoxins and RNAses. It is suitable for immunological studies involving tissue and other cell cultures because of its lack of detectable endotoxins. Because DNA was also undetectable, it is suitable for DNA and endotoxin zero-standards as well as pharmaceutical formulation. The photocatalytic water is a reliable substitute for diethyl pyrocarbonate-treated water used in RNA work, compatible with PCR and sufficiently free from other contaminants to be useful for most biochemical and enzymatic assays.

Key words: Photocatalysis; Pure-water; RNAse; DNA; Endotoxin

1. Introduction

In the molecular biology, biomedical and biopharmaceutical community, water-purity requirements are diverse and often stringent. Expensive bottled waters or costly and time-consuming water purification protocols are required to obtain water devoid of contaminants of biological origin. For many applications, conventional commercial water-purification systems are inadequate for reliably reducing the microbial and organic contamination to required levels. This is because conventional water-purification systems primarily utilize

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phase-transfer technologies such as distillation, ultrafiltration, reverse osmosis (RO), carbon adsorption and ion exchange (DI).

Phase-transfer technologies do provide a certain fractional reduction of influent contaminants to temporarily acceptable levels in the treated water. However, the phase-transfer media, such as carbon beds, ion-exchange beds (Sinha, 1990) and RO membranes (Durham, 1989), as well as other system surfaces (Patterson et al., 1991), can support the growth of microorganisms in the absence of proper maintenance. This, or changes in feedwater characteristics, can result in several orders of magnitude increase in organic contaminants impinging on the system. Since these contaminant concentrations are only fractionally reduced by traditional water purification systems, the treated water can become unacceptable even though all system components may be fully functional. The consequences of this for the researcher or analyst may be spurious data. Exemplary biological water contamination problems are discussed below.

Research involving RNA isolation, synthesis and manipulation as well as that involving high-sensitivity polymerase chain reactions (PCR) requires the use of carcinogenic compounds such as diethyl pyrocarbonate (DEPC) for deactivation of endogenous RNAses (Fedorcsak and Ehrenberg, 1966). While DEPC is traditionally employed to inactivate RNAses in water, it is contraindicated for PCR work (Coen, 1991) and its toxicity makes it undesirable to handle routinely in the laboratory.

The presence of endotoxins in water obfuscates much immunological research. This is because endotoxins are potent activators of immune cells including B lymphocytes, macrophages and neutrophils, even at vanishingly low concentrations.

In the pharmaceutical and biotechnology industries, release assay sensitivities for trace contaminants such as DNA and endotoxin are becoming increasingly important in that some analytical methods rely upon the use of aqueous standards with non-detectable levels of such contaminants. Highly sensitive assay technologies such as automated DNA analysis (Molecular Devices, Menlo Park, CA), various chromatographic and capillary electrophoretic methods and pyrogen testing demand extremely high-purity water when they are operated at their limits of sensitivity.

The microelectronics industry, which faces similar water purification problems, employs point-of-use or central DI water-purification systems, or both. Irradiation with UV light to maintain microbial control may also be employed. However, irradiation with UV light alone, although effective at killing microorganisms, does not efficiently destroy organic molecules in aqueous solution (Cooper et al., 1989).

A comprehensive and reliable solution to biologically contaminated water is a point-of-use, photocatalytic water-purification system. This novel and powerful water-purification technology destroys organic contaminants by oxidation under ambient conditions without the use of chemical oxidants. Photocatalysis compliments existing water treatment technologies by providing the final purification step. This paper will show that sterile water devoid of measurable DNA, RNAse, endotoxin and other biologically active molecules is rapidly and reliably produced by an AquaGen LM-400 photocatalytic water-purification system (Photo-Catalytics, Inc., Boulder, CO).

In previous studies, high concentrations of the microorganisms *Pseudomonas cepacia* (8×10^6 colony forming units (cfu) ml^{-1}), *Escherichia coli* (9×10^6 cfu ml^{-1} and the yeast *Rhodotorula rubra* (1×10^6 cfu ml^{-1} were shown to be reduced to less than 1 cfu ml^{-1} in 90 min, employing a photocatalytic water reclamation system developed for the National Aeronautics and Space Administration (NASA) by Photo-Catalytics, Inc. (Cooper and Ratcliff, 1990a). In other experiments, water contaminated by 36,000 PPB of total organic carbon (TOC), as a mixture of various organic compounds, and 1.7×10^6 cfu ml^{-1} *E. coli* was shown to contain less than 0.02 endotoxin units (EU) of endotoxin and 600 PPB TOC after 90 min of photocatalytic purification. Additionally, various aqueous organic contaminants were shown to be completely oxidized to carbon dioxide and inorganic anions under ambient conditions without the use of chemical additives

(Cooper et al., 1989; Cooper and Ratcliff, 1990a, b; Cooper and Nozik, 1987). The photocatalytic literature has abundantly demonstrated the ability of photocatalysis to oxidize multifarious aqueous organics (Hsiao et al., 1983; Pelizzetti et al., 1988; Tanaka et al., 1989).

In the photocatalytic water-purification system described here, a nontoxic, chemically stable, large band-gap semiconductor powder (the photocatalyst) is suspended in the water to be purified and exposed to ultraviolet (254 to 350 nm) light. Upon irradiating the photocatalyst with band-gap or higher energy photons, electron (e^-)/hole (h^+) pairs are created within the crystalline particles of the powder. These photogenerated holes and electrons can inject into solution species (oxygen, water and organic molecules, for example), resulting in the performance of redox chemical reactions. The detailed mechanism of organic oxidation is dependent on various factors. Fig. 1 depicts one of the generally accepted features of the process (Kormann et al., 1988; Turchi and Ollis, 1990). The electrons generated in the conduction band react with dissolved oxygen forming the superoxide (O_2^-) species. The superoxide subsequently undergoes further aqueous reactions, resulting in the production of powerfully oxidizing hydroxyl radicals. Additionally, the photogenerated holes are injected at the valence band edge at an energy of approx. +3.0 V vs. NHE. Thermodynamically, this is enough energy to oxidize almost all organic bonds (Ross et al., 1975). The presence of a catalytic surface and attack by the highly reactive oxidizing radicals

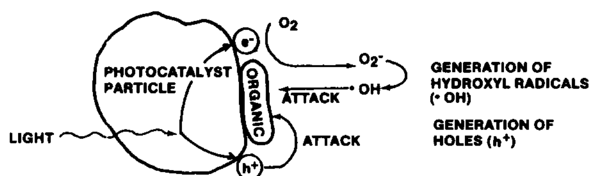
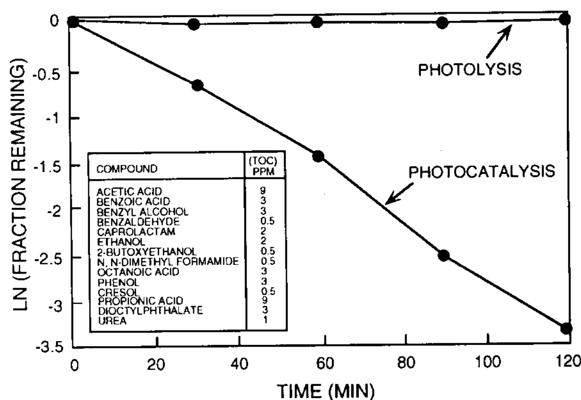


Fig. 1. Depiction of an organic molecule adsorbed onto an ultraviolet irradiated photocatalytic particle. Shown is the conduction band process of HO formation, the valence band generation of holes followed by attack on the organic. (Reprinted with permission from SAE Paper No. 891508, 1989 Society of Automotive Engineers, Inc.)



COMPOUND	(TOC) PPM
ACETIC ACID	9
BENZOIC ACID	3
BENZYL ALCOHOL	3
BENZALDEHYDE	0.5
CAPROLACTAM	2
ETHANOL	2
2-BUTOXYETHANOL	0.5
N, N-DIMETHYL FORMAMIDE	0.5
OCTANOIC ACID	3
PHENOL	3
CRESOL	0.5
PROPIONIC ACID	9
DIOCTYLPHTHALATE	3
UREA	1

Fig. 2. Comparison of photocatalytic and photolytic destruction of 40,000 PPB of TOC as mixed aqueous organics. TOC was measured on a Dohrman DC-80 TOC analyzer according to standard protocol. This procedure measures the complete photocatalytic oxidation of the organics to carbon dioxide. (Reprinted with permission from SAE Paper No. 891508, 1989 Society of Automotive Engineers, Inc.)

and holes makes photocatalysis a powerful method for oxidizing aqueous organics.

Fig. 2 compares the complete oxidation to carbon dioxide of a mixture of various aqueous organic molecules (tabulated in Fig. 2) by uncatalyzed photolysis with oxidation by photocatalysis, employing 254 nm light (Cooper et al., 1989). These compounds represented organics that were difficult to remove by other methods from wastewaters that NASA was attempting to recycle. The measurement of TOC showed the complete photocatalytic oxidation of the organics to carbon dioxide by photocatalysis. In addition to demonstrating the superior oxidizing ability of photocatalysis, it also suggests the ability of photocatalysis to oxidize completely organic molecules having the varied functional groups encountered in contaminants of biological origin.

In the design of the photocatalytic water-purification system employed in this work, the water-photocatalyst suspension was circulated in a loop exposed to ultraviolet light. This recirculating loop is impenetrable by the photocatalyst but allows free movement of water through the particle separator. For the production of water free of biologically active contaminants, we hypothesize that the first step in the purification process is the

adsorption of water-borne contaminants onto the photocatalyst particles. This is a reasonable assumption based on the observations of the behavior of various aqueous dyes and other organics which exhibited significant and rapid adsorption onto the high surface area presented by the photocatalyst (unpublished work, Photo-Catalytics, Inc. 1990). The adsorption of the organic molecules onto the photocatalyst causes their removal from the aqueous phase. Simultaneously, the ultraviolet illumination of the photocatalyst rapidly destroys the bound organics. The system is designed so that the rate of organic oxidation equals or exceeds the rate of organic ingress thereby avoiding the accumulation of organics on the particles. Once sterilized and put into operation, the photocatalytic water-purification system appears to retain sterility indefinitely. Apparently there is little opportunity for reverse contamination of the system under periodic operation.

In this communication, we present the utility of photocatalytically purified water in several commonly used procedures in molecular biology laboratories. The absence of contaminants in the photocatalytically produced water that interfere with in vitro RNA synthesis, cDNA synthesis or PCR amplification of DNA is demonstrated. The absence of endotoxins and microorganisms is also exhibited.

2. Materials and methods

2.1. Photocatalytic system preparation

Prior to installation, the inside of the photocatalytic water-purification systems was cleaned with 1 M KOH and rinsed with 18 M-ohm DI water. The systems were charged with photocatalyst and allowed to pass approx. 20 l of photocatalytically purified water.

A brief history of the machines employed in these tests, installed at different facilities, is appropriate. Each facility had performed verification tests before the system saw general usage. Those tests are not reported here. In all cases the systems had been in operation for several months prior to the performance of the tests reported here. Central house DI water systems at three facilities were used as the feedwater sources. Once installed, the units required no maintenance.

2.2. DNA tests

The presence of DNA in various aqueous samples was quantified using two independent methods. These were the Molecular Devices Threshold total DNA assay (Menlo Park, CA) and a modification of the Chemiprobe DNA detection test (FMC BioSupport Materials, Pine Brook, NJ). The Threshold assay was operated according to manufacturers instructions. The Chemiprobe assay was performed as follows. Briefly, contaminating DNA found in the samples was labeled according to the kit instructions. Labeled DNA was immobilized on Nytran nylon membrane (FMC: 0.45 μm) and immunochemically recognized and quantified using a dual antibody system. DNA levels were determined by comparison to a calibrated standard curve of salmon sperm DNA (Sigma, St. Louis, MO) treated in an identical fashion to the samples. In both cases, labeled DNA found in each sample was quantitatively detected using potentiometric or densitometric analysis for the Threshold and FMC systems, respectively. All samples were taken in 1.5 ml microfuge tubes which had previously been shown to be DNA-free.

2.3. Endotoxin assays

Samples intended for endotoxin testing were collected in borosilicate glass tubes that had been baked at 250°C for at least 4 h. Pyrogen levels were quantified using a limulus amoebocyte lysate (LAL)-based gel clot assay obtained from Haemachem (St. Louis, MO).

2.4. Northern hybridization

Northern hybridization was performed to confirm the absence of significant RNAse activity in the various water preparations (Williams et al., 1992). The presence of mRNA transcripts encoding the B cell activation marker FcϵRII (CD23)

in EBV-transformed B lymphocytes was investigated. Total RNA was extracted from the lymphocytes in RNAzol (Cinna/Biotecx, Friendswood, TX) according to the manufacturer's instructions. Total RNA was exposed to oligo $(dT)_{25}$ linked to magnetic polystyrene beads (Dynabeads; Dynal, Inc., Great Neck, NY). RNA bound to the magnetic beads was extensively washed, and enriched mRNA eluted per the manufacturer's instructions. The purified RNA was re-suspended in either DI water, DEPC-treated water, or photocatalytically treated water in the absence of RNAse inhibitors. After 2 h at 37°C, the mRNA (2.0 μg) was applied to a formaldehyde-containing agarose gel, electrophoresed, and electrophoretically transferred to nylon paper. Full-length FcϵRII (CD23) cDNA was prepared from DNA obtained from the PCR expansion of cDNA derived from FcϵRII (CD23)-containing plasmids (obtained courtesy of Dr. T. Kishimoto). ^{32}P-labeled FcϵRII (CD23) cDNA was generated using a commercially available random hexamer primers DNA labeling kit (BRL, Bethesda, MD) with [^{32}P]ATP (New England Nuclear, Boston, MA). The ^{32}P-FcϵRII-cDNA was allowed to hybridize at 45°C for 24 h. After extensive washing and drying, filters were exposed to X-ray film (Kodak X-omat; Rochester, NY) and developed.

2.5. Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR was performed to demonstrate that photocatalytically treated water would not interfere with the enzymatic activities of either reverse transcriptase or Taq polymerase. The presence of FcϵRII (CD23)-specific transcripts in the EBV-transformed B lymphocytes was detected through the application of reverse transcription followed by the polymerase chain reaction (RT-PCR; Borish et al., 1992; Brenner et al., 1989). Primers specific for FcϵRII cDNA were synthesized on an Applied Biosystems 381 DNA synthesizer (Foster City, CA) (5′: ATGGAGGAAG GT-CAATATTC; 3′: TGGCTTTCCAAGTTCTTG-GA). These primers can be used to amplify a DNA fragment of 220 bp. The mRNA (1 μg) prepared as described above was washed, denatured (65°C for 5 min), and exposed to MMLV reverse transcriptase (600 U; Bethesda Research Labs, Gaithersburg, MD) in the additional presence of RNAsin (40 U; Promega, Madison, WI), oligo d(T) 12-18mer (Promega), dNTP mix (10 mM each; Promega) and the supplied RT buffer. The reaction was allowed to continue for 1 h at 42°C, followed by heating at 95°C for 5 min to denature the reverse transcriptase. Aliquots of cDNA (1:50 dilution) were then subjected to PCR in the presence of primers (100 ng each), Taq Polymerase (1 U; Promega), dNTP mix (1.25 mM each), and supplied reaction buffer. Negative controls were performed with samples lacking cDNA, Taq polymerase, or with cDNA synthesized from resting monocyte RNA. The DNA thermal cycler was programmed to perform 40 cycles with the following conditions: 94°C – 1 min, 55°C – 2 min, 72°C – 3 min. At the completion of the PCR, 9 μl of PCR product was mixed with 1 μl of gel loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol) and electrophoresed in 1% agarose in the presence of 0.5 μg ml^{-1} ethidium bromide. The amplified DNA bands were visualized with an ultraviolet transilluminator.

2.6. Ribonuclease assay

Two sets of experiments were performed. In one set the photocatalytic system was spiked with 10 ml of a ribonuclease cocktail (10 mM Tris-HCl pH 7.5, 5 mM EDTA, 100 mM NaCl, 20 μg ml^{-1} ribonuclease A (RNAse A) and 1 μg ml^{-1} RNAse T1) and injected into a closed 750 ml photocatalytic system at the input port. After irradiation with ultraviolet light for 5 min, water samples were collected following rejection of the first 200 ml. In the second set of experiments, the house DI water was connected to the photocatalytic system, operated normally and samples were taken before and after treatment. The photocatalytically purified spiked output water, the input RNAse cocktail (diluted 80-fold to equal the dilution factor of the 750 ml photocatalytic system) and the untreated house DI water, which normally feeds the photocatalytic system, were tested for single-stranded RNA (ssRNA) substrate degradation. The tests were further differentiated

by the exclusion and addition of magnesium ions to the samples. Tris-HCl (pH 8.0) was added to the samples to a final concentration of 10 mM. In some samples, $MgCl_2$ was added to a final concentration of 1 mM. A 588 μl volume of each sample was incubated with 12 μl of ssRNA (12 pmol) in DEPC-treated water at 30°C for the indicated times. After 0, 2, 4, 8 and 24 h of incubation, 100 μl was removed from each sample and the RNA collected by ethanol precipitation. The RNA was redissolved in 5 μl formamide loading buffer, heated to 95°C for 3 min and analyzed on a 8% polyacrylamide, 8 M urea sequencing gel (Maxam and Gilbert, 1980).

The ssRNA substrate was approx. 222 nucleotides long, synthesized in vitro by SP6 polymerase transcription of plasmid pG158 (Morrissey and Kirkegaard, 1991) digested with EcoR1 restriction endonuclease. The RNA transcript was internally labeled using [^{32}P]UTP (DuPont New England Nuclear) to a final specific activity of 25,000 cpm pmol^{-1}. The labeled RNA molecules were purified by phenol extraction and ethanol precipitation and resuspended in a small volume of DEPC-treated water.

2.7. Sterility tests

DI water and photocatalytically treated DI water were tested for sterility. One ml aliquots of water were added to complete medium consisting of RPMI 1640 supplemented with HEPES buffer (pH 7.20; 0.15 M) and L-glutamine (2 mM; Gibco) in the absence of antibiotics. CM readily supports the growth of most fungal and bacterial species (personal observation). The culture media containing the water samples were incubated overnight in Corning culture flasks at 37°C in a CO_2 environment. Contamination was determined via microscopic inspection of the culture flask at 400 ×.

3. Results

3.1. DNA

Results from both DNA assays clearly demonstrated the ability of photocatalytic technology to remove contaminating DNA from water. Samples analyzed using the Threshold and modified Chemiprobe assays were taken from the outlet of the photocatalytic system at 15 min intervals over a period of 60 min. A sample of the feedwater was also taken from a point immediately upstream from the unit's inlet and tested. As shown in Table 1, between 100 to 200 pg ml^{-1} of DNA could be detected in the feed water. All water produced by the photocatalytic unit was shown to be either at or below detectable limits of both assays.

Table 1
DNA removal by photocatalysis as detected using the threshold and modified chemiprobe assays

Sample	DNA concentration (pg ml^{-1})	
	chemiprobe	threshold
Pre-unit inlet	100–150	100–200
0 min	N.D. [a]	N.D. [a]
15 min	N.D.	N.D.
30 min	N.D.	N.D.
45 min	N.D.	N.D.
60 min	N.D.	N.D.
50 pg control	25–50	N.T. [b]
25 pg control	N.T. [b]	25

[a] N.D. = none detected. The threshold of sensitivity for both assays was approx. 5 pg ml^{-1} of DNA.
[b] N.T. = not tested.

3.2. Endotoxins

Water samples were taken at 15 min intervals over a period of 60 min from the photocatalytic system. As shown in Table 2, endotoxins were reduced to below the detection level (0.036 EU

Table 2
Endotoxin removal through the use of photocatalysis

Sample	EU ml^{-1}
Feedwater	0.894
Effluent	
0 min	< 0.036 [a]
15 min	< 0.036
30 min	< 0.036
45 min	< 0.036
60 min	< 0.036

[a] The sensitivity of this assay was 0.036 EU ml^{-1}.

ml^{-1}) of the LAL assay through the time course of this test.

3.3. Northern hybridization

Northern hybridization was performed for the B lymphocyte activation marker FcϵRII (CD23) after exposure of the enriched mRNA for 2 h to DI water, DEPC water, and photocatalytically treated water. Results are shown in Fig. 3. The presence of FcϵRII (CD23)-specific mRNA was readily demonstrated with both the DEPC and photocatalytically treated water. However, the effects of RNAses were obvious after 2 h exposure of the mRNA to DI water. Thus, the photocatalytic water is free of detectable RNAses.

3.4. RT-PCR

To confirm that photocatalytic treatment would not interfere with either reverse transcriptase or Taq polymerase reactions, RT-PCR was performed. Results are displayed in Fig. 4 and demonstrate amplification of DNA corresponding to the FcϵRII (CD23) RNA.

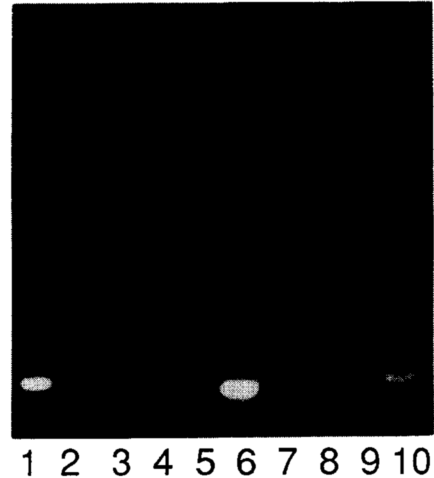


Fig. 4. RT-PCR for FcϵRII of cytokine-stimulated monocytes. Monocytes were stimulated with cytokines for 16 h and total RNA extracted. Complimentary DNA was obtained with MMLV reverse transcriptase. The cDNA was subjected to PCR with FcϵRII-specific primers with lanes stimulated as follows: 1, IL-4 (+ve control); 2, –mRNA; 3, –Taq; 4, resting monocytes; 5, M-CSF; 6, GM-CSF; 7, IL-2; 8, IL-6; 9, IFN-α; 10, IFN-Γ. (Reproduced with permission from J. Immunol. 149, 2823 (17)).

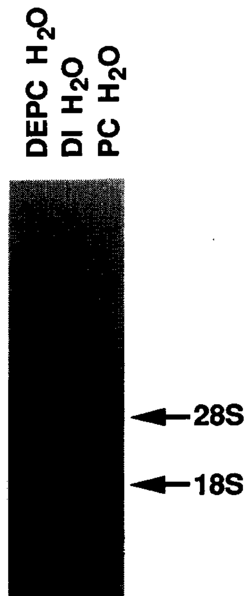


Fig. 3. Northern hybridization performed for B lymphocyte activation marker FcϵRII (CD23) after 2-h exposure in DEPC and photocatalytically purified water (destructive effect of RNAases shown in untreated DI water).

3.5. Ribonucleases

We tested the ability of photocatalysis to inactivate ribonucleases, even following direct injection of RNAses A and T1 into a closed photocatalytic system. The integrity of ^{32}P-labeled single-stranded RNA molecules (Fig. 5, lane I) in 10 mM Tris-HCl (pH 8.0) in the absence of magnesium ions was monitored by gel electrophoresis and autoradiography. After incubation at 30°C with the input RNAse cocktail for 0, 2, 4 and 8 hours, no intact RNA was visible (Fig. 5, set A). The disappearance of the sample without incubation at 30°C indicated that an amount of RNAse in great excess of that normally encountered in DI water systems was used in this experiment. After 5 min of exposure to the photocatalytic process, however, the water was sufficiently RNAse-free that, even after 8 h of incubation at

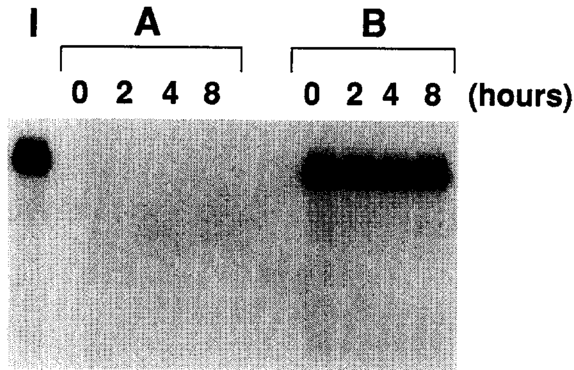


Fig. 5. Autoradiograph of ^{32}P-labeled ssRNA 222 nts. long, incubated at 30°C in: DEPC water (lane I); RNase A and T1 cocktail (set A); photocatalytically treated water (set B).

30°C, the single-stranded RNA remained intact (Fig. 5, Set B).

Fig. 6 displays similar experiments, with incubations performed in the presence of magnesium ions. Sets A and B show the time course of incubation of ssRNA with input DI water and DI water subjected to photocatalysis for 5 min. Although the Mg^{2+}-dependent cleavage of RNA (Butzow and Eichhorn, 1965) was observed in both sets A and B, the rate of RNA degradation was clearly slower in the photocatalytically treated water. Set C, in which the ssRNA was incubated with the diluted input RNAse cocktail as described for Fig. 5, shows the complete degradation of the RNA by the RNAses injected into the photocatalytic system. Set D shows, again, that these RNAses were destroyed by 5 min of photocatalytic treatment.

3.6. Sterility

Untreated DI water demonstrated overnight growth of contaminants, shown to be Gram-negative bacilli (approx. 250 cfu ml^{-1}). However, for the photocatalytically produced water, no microorganism growth occurred even after one month of culture.

4. Discussion

The test data reported here represent a 'snapshot' of the performance of three photocatalytic water-purification systems after months of operation. At the time of this writing, these systems have seen from 17 to 24 months of use with no apparent degradation in performance.

4.1. DNA

Quantitative detection of generic DNA provides an example of how organic-based interference of specific assays can impact analysis. Traditional hybridization assays generate a signal based upon the complementary binding between labeled probe and target DNA. As a result, only

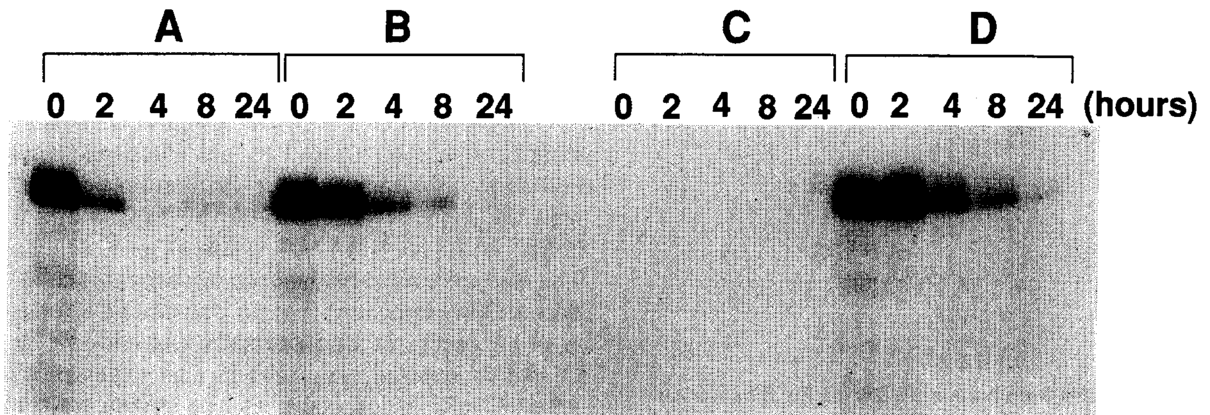


Fig. 6. Autoradiograph of ^{32}P-labeled ssRNA 222 nts. long, incubated at 30°C with Mg^{2+} in: DI water (set A); photocat. water (set B); RNase A and T1 cocktail (set C); photocatalytically treated cocktail (set D).

those pieces of DNA complementary to the probe are detected. Other sequences do not hybridize to the probe and are, therefore, functionally invisible to the assay.

In contrast, the threshold assay, which measures total DNA, provides a similar signal for approx. the same levels of DNA from bacterial, phage, fish and mammalian sources (Kung et al., 1990). Therefore, extreme precaution must be taken when executing this type of assay to avoid contaminating the sample through the use of 'dirty' water and reagents.

Choosing a water source for use in total DNA assays is not a trivial task. Studies involving a variety of water sources typically show 10 to 20 pg ml^{-1} of background levels of contaminating DNA in samples derived from distilled and deionized water, HPLC water, water for irrigation and water for injection. In industrial quality control labs, the problem of finding suitable aqueous DNA zero standards is further aggravated by the need for low or sub-picogram assay sensitivity. The harder such assays are pushed toward their limits of detection, the more significant background levels of DNA become in deciphering test results. In these types of assays, high background in blanks and standards can make the reporting of accurate DNA levels in product difficult, at best.

In industrial quality control and process development such confusion can be very costly. The primary concern in testing these types of products for parenteral use is, of course, the presence of oncogenic and other DNA of mammalian origin. Release criteria, relative to nucleic acids, for such products have been set in the Food and Drug Administration's Points to Consider at less than 10 pg of DNA per dose. Small amounts of contaminating DNA from laboratory water used to prepare reagents can result in artifactually elevated DNA levels above the specified allowances for release of product. The financial loss, then, is realized as a function of the number of lots of final product which are rejected because they do not meet DNA specifications.

Water supplied by the photocatalytic purification system has been shown to be free of detectable DNA using two independent assay systems. That this result can be achieved is significant due to the inherent problems associated with other DNA detection systems as described elsewhere (Briggs and Panfili, 1991; Kung et al., 1990).

4.2. Endotoxin

Both DNA and endotoxin testing are examples of specific assays which can result in failed lots of product due to artifactually elevated assay values. This is usually a result of contaminated water, sample tubes and, of course, poor technique. We have demonstrated that one variable which can be eliminated from this list is the water through the use of a photocatalytic water-purification system. Consistent with the discussion of ultra-low DNA-free water production, the photocatalytic system produces ample quantities of endotoxin-free water in minutes.

4.3. Northern hybridization

The ability to perform Northern hybridization is absolutely dependent on the absence of RNAses. Active RNAses contaminating any of the sequential steps of the Northern hybridization, including RNA extraction, will preclude obtaining meaningful data. Traditional approaches toward the removal of RNAses entail the use of suspected carcinogenic compounds such as DEPC. This is a time-consuming procedure and the prolonged absence of RNAse activity after DEPC treatment is not guaranteed. The ability to have a safe on-demand RNAse-free water source is obviously advantageous. Fig. 3 demonstrates the inability to perform Northern hybridization with untreated DI water owing to the presence of RNAse activity. It also demonstrates the successful performance of Northern hybridization in the photocatalytically purified water owing to the destruction of RNAses.

4.4. RT-PCR

The use of DEPC to eliminate RNAse activity in water may introduce contaminants that inhibit the activity of reverse transcriptase and Taq polymerase. Therefore, DEPC-treated water may in-

terfere with successful application of reverse transcriptase and the PCR reactions (Coen, 1991). Photocatalytically treated water contains no such inhibitors and its compatibility with RT-PCR is demonstrated in Fig. 4.

4.5. Ribonuclease

Photocatalysis clearly destroyed active ribonucleases that were directly injected into the system (Figs. 5 and 6). This was most evident in samples incubated in the absence of magnesium ions (Fig. 5), in which no detectable RNAses were observed even after 8 h of incubation at 30°C. Neither RNAse A nor T1 requires magnesium for its nuclease activity (Hirs et al., 1953; Uchida and Egami, 1966). Therefore, it appears that photocatalysis destroyed all detectable ribonuclease in the input RNAse cocktail. In the presence of magnesium ions, however, metal-catalyzed RNA hydrolysis (Butzow and Eichhorn, 1965) made long incubations of ssRNA less informative. However, much more full-length RNA remained after incubation in photocatalytically purified water, even when RNAses had been deliberately introduced into the DI feed water.

In this test the EDTA was responsible for the addition of 7700 PPB of TOC to the system. Normally maintained DI systems provide water having on the order of 100 PPB of TOC. A salient point is that the photocatalytic system managed to destroy an inordinately high level of RNAses in a TOC background that was approx. eighty times higher than normal.

4.6. Sterility

This study reports the reduction of 250 cfu ml^{-1} of Gram-negative bacteria to zero. In tests at four other installations, undetectable levels of microorganisms in the photocatalytically treated water were also observed.

Data from the NASA studies discussed in section 1 of this paper, imply that an AquaGen LM-400 system would have the ability to destroy a mixture of microorganisms at a rate of approx. 5×10^8 cfu ml^{-1} min^{-1}. The high purity water industry considers 10^5 cfu ml^{-1} to be an extremely high bacteria count for a DI water system (Marquardt, 1988). The average reactor residence time in the photocatalytic water purification system is 2 min, therefore, it should have sufficient capacity to sterilize water from almost all DI systems.

5. Conclusion

The unreliability of conventional water-purification systems, in molecular biological and biopharmaceutical applications, is attributed to their almost exclusive utilization of phase-transfer technologies. These phase-transfer technologies provide only fractional reduction of influent contaminants. They may not be able to produce the needed water quality when confronted by microorganism blooms or altered feed water characteristics. This can result in spurious experimental data which is costly in terms of wasted time, labor and materials.

The most stringent water-purity needs of molecular biology have been demonstrated to be met by the photocatalytic point-of-use water-purification system fed by DI water. The system has sufficient oxidative capacity to accommodate wide variations in input DI water quality. The reliability of the photocatalytic water-purification technology is attributed to its ability to destroy organic contaminants rather than just effect their phase transfer. The unit reported on here produces water at a rate of 0.5 to 1 liter per minute.

Photocatalytically produced water is suitable for immunological studies involving tissue and other cell cultures because of its lack of detectable endotoxins. Since DNA is also undetectable, it is suitable for DNA and endotoxin zero-standards as well as pharmaceutical formulation. The photocatalytic water is a reliable substitute for DEPC-treated water used in RNA work, compatible with PCR and sufficiently free from other contaminants to be useful for most biochemical and enzymatic assays.

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