Pilot Testing for Inactivation of Non-Indigenous Seawater Organisms by Ozonation and Ultraviolet Sterilization

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Abstract

Research was conducted to develop and implement controls to result in a negligible risk of the release of non-indigenous species (NIS) including foreign pathogens (parasites, protozoa, bacteria, and viruses) into the Ocean. To remove viable NIS and prevent discharge of possible invasive species to the Ocean, a pilot study – using a pilot-scale treatment plant – was performed to evaluate the effectiveness of various treatments for inactivating at least 99% of NIS from seawater effluent discharge without chemical additions. For evaluation of NIS, the following five indicator species were selected: brine shrimp nauplii (*Artemia salina*), marine rotifers (*Brachionus plicatilis*), heterotrophic bacteria, marine green algae (*Nannochloropsis* spp.), and mussel larvae (*Mytilus* spp.).

The pilot test skid included mechanical filtration, UV sterilization, and a foam fractionator unit for ozone contacting. The objective of the pilot testing was to determine the combination of treatments, along with their corresponding doses and exposure periods, required for the desired level of inactivation. These data will be used for sizing of ozonation and UV sterilizer equipment required in the full-scale treatment system to deliver similar kill rates. The pilot testing concluded that filtration (or micro screening) will be required, in addition to both ozonation and UV sterilization, to meet the treatment goals.

Key words: Ozone; area of special biological significance (ASBS); brine shrimp nauplii (*artemia salina*); exotic species; giant kelp (*macrocystis pyrifera*); heterotrophic bacteria; indicator organisms; indigenous species; marine green algae (*nannochloropsis* spp.); marine rotifers (brachionus plicatilis); mechanical filtration; microorganism; mussel larvae (*mytilus* spp.); national pollutant discharge elimination system (NPDES) non-indigenous species (NIS); ocean; pacific topsmelt (*atherinops affinins*); pilot test skid; purple sea urchins (*strongylocentrotus purpuratus*); seawater; sterilization; toxicity testing; treatment optimization; ultraviolet.

Introduction

Determination of engineering controls to mitigate the release of exotic organisms from the Birch Aquarium at Scripps (BAS), together with minimizing any increase in flows to the municipal sewer system, is the main focus of this paper. BAS operates under a permit, issued by the San Diego Regional Water Quality Control Board, requiring implementation of administrative and/or engineering controls for a negligible risk of nuisance pollutants discharged into the Ocean. At the same time, the San Diego Municipal Sewer District has requested zero increases in discharges to the sewer system. Meetings with representatives from the California Department of Fish and Game (DFG) were also crucial in establishing goals and limitations for the design of the treatment processes. Attempting to meet the requirements of the regulating agencies, which at times have conflicting issues, is the crux of the challenges presented within.

The BAS is part of the Scripps Institution of Oceanography (SIO) located on the campus of the University of California, San Diego (UCSD). The BAS is a public aquarium dedicated to inspiring respect for ocean life and education of conservation issues while the SIO is known world-wide for it leadership in oceanography and environmental stewardship. The facility includes a seawater intake where ocean water is pumped from the Scripps pier, through sand filtration to research laboratories, display tanks at the BAS, educational exhibits, and behind the scenes holding and quarantine tanks. A portion of the overall volume of water runs on an open flow-through basis, while the majority of the water is re-circulated with sand media filtration. In the end, nearly all water is returned to the Ocean while a small amount of medicated water is discharged to the sanitary sewer.

Many of the tanks at the BAS require this open system seawater flow for maintaining invertebrate and algae species in the displays.

After use, the seawater is discharged from two beach outfalls and enters Area of Special Biological Significance (ASBS) number 31, also known as the San Diego-Scripps ASBS. Nuisance pollutants can include foreign specimens collected for research and display, both intentionally and unintentionally, known as non-indigenous species (NIS).

To set criteria for achieving the required goals, a pilot study (using a pilot-scale treatment plant) was performed to evaluate the effectiveness of various treatments at killing at least 99% of NIS from seawater effluent discharged from BAS without chemical additions. The pilot test skid, designed by TJP, Inc. (TJP) and built by Ozone Water Systems (OWS), included mechanical filtration, an ultraviolet light (UV) sterilizer, and a foam fractionator with ozone injection. Mechanical filtration, of various pore size selections, was evaluated to prevent the passage of multicellular organisms that have been found to be resistant to large UV and ozone exposures. In the case of UV sterilization, the concern is “shadowing” of the UV light. In the case of ozonation, particulate material may capture microorganisms and prevent oxidation and/or add to the oxidation reactions with the available ozone.

UV sterilization has the distinct advantage of inactivating a wide range of pathogens while producing negligible disinfection byproduct. It is generally accepted that UV disinfects by fusing adenine and thiamine molecules within microorganism DNA. UV

light is present as a broad spectrum below the wavelength spectrum of visible light and within the UV spectrum is a bell shaped curve where the UV light has a germicidal effect. This bell-shaped curve exists between 200 to 320 nm, with a peak germicidal effect at 265 nm. Please note, however, that the degree to which any organism is inactivated by a UV system is also species-specific – i.e. a given dose will not have a blanket effect across species groups.

Ozone is an oxygen molecule comprised of three oxygen atoms bonded together by a single bond and a double bond, where the double bond is delocalized over all three oxygen atoms. The delocalization of the double bond makes ozone an extremely powerful oxidizing agent (McMurry and Fay 1995). With seawater, the major residual oxidant is typically hypobromous acid (HOBr) due to the relatively fast reaction of ozone with the bromide ion found in natural seawater (Hoigné 1985). It is noteworthy that HOBr is also a disinfecting compound (similar to hypochlorous acid – HOCl).

Disinfection occurs when ozone destroys the integrity of the cell wall of a microorganism. In addition to disinfection, ozonation oxidizes certain organic materials including grease and oils, and inorganic compounds. During the disinfection/oxidation process, biological oxygen demand (BOD) and chemical oxygen demand (COD), as well as color and odor are reduced, while dissolved oxygen concentration is enhanced (i.e. generally improves water quality). These benefits, along with the decomposition of the ozone molecule during oxidation make the use of ozone desirable at BAS since the discharge of chemical additives to the ASBS is prohibited. In addition, water treated by

the NIS plant will mix with other water prior to discharge into the ocean outfall; the residual oxidants are expected to react with compounds in the untreated water, resulting in a non-detectable residual at the end of the long run of pipe prior to the Ocean discharge.

For compliance with the Ocean water discharge permit, both engineering controls and administrative controls are required; however, only procedures relative to the engineering controls are presented in this paper – while the administrative controls such as standard operating procedures, organism quarantining, and staff training are not discussed. The objective of the engineering controls is to design a treatment system based on the findings of the treatment pilot study that, in conjunction with administrative controls, will result in a negligible risk of the release of exotic species, including foreign pathogens. The treatment system design objectives include:

1. Mechanical redundancy to increase elimination of NIS
2. Durable and corrosion-resistant materials to reduce equipment failure
3. Prevention of short-circuiting of microorganisms past treatment barriers
4. Effective treatment during start/stop procedures
5. Monitoring devices and sample ports to measure treatment effectiveness
6. Efficiency and sustainability of the treatment system
7. Limited or insignificant increased discharges to the municipal sewer

This evaluation of NIS treatment plant effectiveness using a broad range of organism types/sizes is the first of its kind and data generated during this study will scientifically justify the engineering of the full-scale plant. Furthermore, while the Regional Water Quality Control Board stipulates that a nuisance shall not be created, a list of standard indicator organisms did not exist. As part of our work on the pilot treatment system we worked in collaboration with the BAS, SIO and DFG in developing the following indicator organisms.

- Brine shrimp nauplii (*Artemia salina*)
- Marine rotifers (*Brachionus plicatilis*)
- Heterotrophic bacteria
- Marine green algae (*Nannochloropsis* spp.)
- Mussel larvae (*Mytilus* spp.)

Additionally, toxicity testing was performed to demonstrate that the selected process treatment does not result in a "nuisance" discharge into the Ocean.

Due to the unique nature of this project, an appropriate test regime was employed in a step-wise manner to ensure that sources of variability were controlled and accounted for. This methodology has included determination of adequate sampling regimes and treatment optimizations which are still in progress for testing of all five indicator organisms. Upon startup of the final treatment process a reevaluation will occur to replicate test results for each species to ensure reproducibility and low variability.

Testing discussed in this study was conducted between August 2006 and May 2008. All treatments took place at BAS and were performed by Nautilus Environmental (Nautilus) with assistance from BAS staff. Evaluation of all pre- and post-treated samples was performed at Nautilus for all species, with the exception of heterotrophic bacteria which was evaluated by the City of San Diego Metropolitan Wastewater Department, Environmental Monitoring & Technical Services Division, Marine Microbiology Laboratory (City Lab).

Finally, it should be noted that the target kill rates from the pilot study may not be achievable in the full scale treatment systems due to factors unforeseen at the time of this writing. In collaboration with DFG, BAS/UCSD/SIO is making the best effort possible to achieve the most desirable results (negligible risk) within the constraints of economic, engineering, and construction limitations.

Materials & Methods

Part 1 - Test Skid

The pilot test skid set-up, shown in *Figure 1*, allowed for the following testing procedures:

- UV and/or ozonation, without bag filtration
- Bag filtration alone
- Bag filtration followed by UV

- Bag filtration followed by ozonation
- Bag filtration followed by UV then ozonation

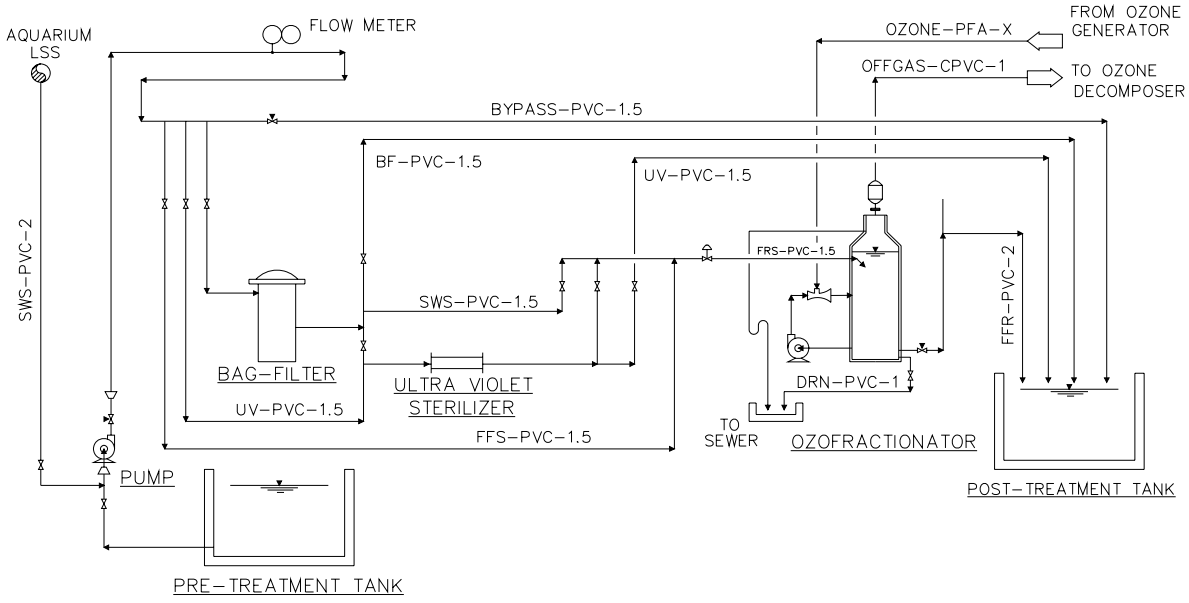

Figure 1. NIS Pilot Treatment System – Piping and Instrumentation Diagram

Part 2 - Sample Materials & Transport

The seawater used during the pilot treatment study was obtained from the re-circulating system at BAS. Specifically, seawater travels through a set of vertical column pumps installed at the end of the Scripps pier, is pumped through the pressure sand filters at the base of the pier, travels up to the holding reservoir on the hill above BAS, and is then flows by gravity down to the aquarium saltwater supply lines.

Nautilus personnel provided all materials required for the collection and analysis of pre- and post-treatment samples. All samples were collected using pre-cleaned equipment and

techniques following standard methods published in EPA for the collection and analysis of environmental samples (EPA/600/R-95/136).

Part 3 - Test Organism Sources & Handling

Brine shrimp nauplii (*Artemia salina*) were supplied by BAS. Nauplii were used for testing at approximately 24 to 48 hours post-hatch. Marine rotifers (*Brachionus plicatilis*) were purchased from Reed Mariculture (Campbell, CA). Rotifers were supplied with aeration during holding and were used for testing anywhere from 24 to 72 hours post-hatch. Back-flush effluent from the BAS seawater filters was used as the source of heterotrophic bacteria. To ensure an adequate concentration of bacteria for testing purposes, the testing schedule was discussed with BAS maintenance staff and the system was not flushed for approximately 12-24 hours prior to testing. Marine green algae (*Nannochloropsis* spp.) were provided for testing by BAS. Cultures of *Nannochloropsis* were used anywhere from 4 to 7 days old. Adult mussels (*Mytilus* spp.) were collected from Mission Bay (San Diego, CA) by Nautilus personnel. Heat treatment was used to spawn mussels at Nautilus and eggs were fertilized and allowed to develop into free-swimming veliger larvae prior to use in testing (approximately 24 hours post-fertilization).

Part 4 - Testing Procedures

Initial Skid Set-Up

Prior to all experimental trials, the pilot treatment skid was carefully examined to ensure all equipment was operating correctly and treatment variables such as flow-rate were within acceptable targeted ranges. System components for UV treatment consist of a

Wedeco CHI-10 unit, with one 80 W low-pressure high output lamp and a UCA sensor (part #I35168) to monitor actual UV intensity. The ozone generation system includes an oxygen separator (AirSep AS-12), an Ozotech model OZ2BTUSL cold plasma corona discharge ozone generator producing a maximum of 6.6 g O_3 /hour, an RK2 (RK20PE) foam fractionator was used for ozone contacting, and oxidation reduction potential (ORP) measured using a Signet ORP sensor.

Prior to sample addition, the skid was rinsed/flushed with domestic freshwater and then with clean seawater to ensure no organisms were retained that could subsequently be released during treatment trials and bias test results. Between test runs where the foam fractionator was used, the vessel was drained, rinsed with freshwater and then re-circulated with freshwater as part of the entire system flush. No additional cleaning agents were used during testing, and there was no physical scrubbing of equipment between runs. When filtration was used, pre-cleaned bag filters (FSI X-100 filter housing, felt bags) were used for each run (i.e. a single bag filter was not cleaned and re-used during a single day of testing). Bag filters were cleaned using tap water (sprayed inside and out) and were allowed to dry completely before re-use.

Treatment Optimization

The goal of this phase of the study was to narrow the list of variables in the most cost and time efficient manner possible. The order of the treatments proceeded from most efficient to least efficient with regard to operating costs and effort. Brine shrimp (*Artemia salina*) were used initially to evaluate and refine treatment variables due to the immediate accessibility of this species. Once an acceptable treatment/removal process

was identified, the same treatment was performed using an additional indicator species. Treatment variables were adjusted as necessary to achieve the target kill rate for that species, and so on. Target system efficiency values were 99% for brine shrimp, marine rotifers, mussel larvae, and marine green algae, and 99.9% for heterotrophic bacteria. UV and ozone doses were both dependent upon flow rate. Three flow regimes, three ozone generator output settings, as well as system re-circulation were all employed during this phase of testing to deliver different doses of UV and ozone alone and in combination.

During each test run, a uniform organism density was targeted in the pre-treatment tank; actual density varies according to species. The target density for brine shrimp, marine rotifers, and mussel larvae was 5 organisms per ml. The target density for marine green algae was 50,000 cells per ml. Initial density of heterotrophic bacteria was dependent on seawater filter condition at the time of discharge.

Prior to addition of organisms, 100-200 gallons of clean seawater were added to the pre-treatment tank and circulated through the system and back into the pre-treatment tank for a period of ten minutes. Please note that a volume of 200 gallons was always preferred for optimum system operation, but sometimes had to be lowered to achieve the organism target density. Following circulation, a 1-L sub-sample was collected (baseline). Organisms then were added to the pre-treatment tank, agitated to achieve a uniform density, and a 1-L sub-sample to verify initial organism density was collected by opening a clean 1-L plastic bottle at mid-depth in the center of the tank (pre-circulation).

Organisms then were circulated through the system and returned to the pre-treatment tank continuously for a period of ten minutes utilizing no active treatment to promote uniform distribution throughout the system. After circulation, treatment was initiated and the flow of water was directed from the pre-treatment tank, through the chosen filter combination, and into the post-treatment tank. The first 15 gallons of post-treated water were collected and discarded to account for the volume of water in the system when the filtration was activated and to ensure all organisms collected had been exposed to the treatment. The post-treatment tank was then rinsed with clean seawater to discard any organisms that may have spilled into the tank during bypass.

Once the treatment run had commenced and non-treated water had been bypassed, six 125-ml samples were collected from both the pre- and post-treatment tanks at appropriate time intervals (depending on flow rate). Collections were performed using a pre-cleaned 125-ml plastic bottle secured to the end of a pre-cleaned PVC sampling pole. Sample collection locations within each test tank were at mid-depth near the four corners and midline of each tank and were collected at 1-minute, 2-minute, or 4-minute intervals for 20, 10, or 5 gpm flow rates, respectively. Each sub-sample was transferred to a clean 1-L plastic bottle to yield a 750-ml composite sample representative of the entire treatment period.

Methods for processing samples to collect treatment effectiveness data were species-specific. Each of these five methods is outlined briefly below.

Immediately upon completion of the test run using brine shrimp, five 25-ml sub-samples were collected from each of the 1-L sample container (baseline, pre-circulation, pre-treatment, and post-treatment) and placed in 30-ml glass shell vials for density counts. Samples were preserved using 4 ml of 10% formalin buffered in seawater. Survival of brine shrimp was evaluated microscopically on-site between trial runs by pouring a portion of the remaining sample volume through a 35-µm screen and counting the number of alive and dead organisms retained on the screen under a dissecting scope at 25X magnification. Please note that the number and volume of sub-samples examined depended on how dense the organisms were in the samples. For example, three 30-ml sub-samples were counted for pre-circulation and pre-treatment samples because organisms were dense. However, in order to find a sufficient number of organisms in some of the post-treatment samples, it may have been necessary to examine the entire remaining sample volume. Sample densities were determined off-site at Nautilus, generally within 48 hours of sample collection and preservation, by scoring the total number of organisms in each vial under a compound microscope at 100X magnification.

For marine rotifers, sample and data collection procedures were the same as those previously described for brine shrimp with one exception. Survival of rotifers in the baseline, pre-circulation, pre-treatment, and post-treatment samples was determined by counting the number of live rotifers in ten 1-ml sub-samples placed in a Sedgewick-Rafter slide under a dissecting microscope at 50X magnification; the mean for all ten counts was calculated.

For data analysis and interpretation, the following parameters were defined and applied to data collected for these first two species:

Initial Density – Density and survival were determined in sub-samples from the pre-circulation sample as described for each species. Density for each replicate was then multiplied by the mean proportion of surviving or normal organisms. Therefore, the final definition of initial density is the number of live organisms confirmed in the pre-treatment tank prior to any circulation through the skid.

Final Density – the number of organisms counted in each 25-ml sub-sample divided by the sample volume.

Survival – The number of live organisms counted divided by the total number of organisms counted.

Final Survival – Because survival data were found to be slightly skewed due to the loss of dead organisms that had broken apart during treatment, survival rates were applied to final density counts, and this product was then divided by the initial density to yield "final survival." This number was used to determine treatment effectiveness and took into account any organisms that were caught up in the system, were not retained on the screen for survival determination, or were not alive at the time of organism addition to the system.

System Loss – This term applies to the organisms that were inherently lost in the system that could not be recovered or otherwise accounted for and is represented by the pre-treatment sample data. Calculating this parameter allows for greater understanding of the post-treatment data by defining what proportion of the kill rate was due to actual exposure to the treatment and what proportion to lack of recovery from the system.

System Efficiency – For pre-treatment sample data, system efficiency is equivalent to system loss. For post-treatment sample data, it is defined as kill rate plus system loss.

For mussel larvae and marine green algae, testing and sampling protocols were the same as those previously described for brine shrimp and marine rotifers. However, unlike brine shrimp and rotifers, system efficiency values for mussels and algae were based on post-treatment viability since survival cannot be evaluated visually with these species.

For mussel larvae, viability tests evaluating embryo-larval shell development for 48 hours following treatment were performed at Nautilus and consisted of baseline, pre-circulation, pre-treatment, and post-treatment samples tested concurrently with appropriate laboratory controls. Testing was performed in accordance with US EPA (1995).

For marine green algae, baseline, pre-circulation, pre-treatment, and post-treatment samples were also tested. Green algae were cultured for a period of 96 hours following the method outlined in ASTM (1997).

While the same treatment principles for the other test species applied to heterotrophic bacteria as well, special consideration was needed for this test organism due to the high probability that cross-contamination would occur. In other words, it would be very difficult to clear the system of heterotrophic bacteria. Therefore, additional changes were made to the experimental design for this species. The procedure employed is outlined below.

The pre-treatment tank was filled with water back-flushed from the seawater filtration system located adjacent to the pilot skid testing area in the life support system (LSS) yard at the BAS. Measurable concentrations of heterotrophic bacteria were verified in filter back-flush water on three occasions prior to commencement of testing. A 250-ml pre-circulation sample was collected as soon as the pre-treatment tank was filled. Water was then circulated through the system with no treatment for a period of ten minutes, the system shut down to prepare for treatment, and a 250-ml pre-treatment sample collected. Treatment commenced, the first 15 gallons of water were bypassed, the post-treatment tank was cleaned, and treated water collected in the post-treatment tank. Upon completion of the test run, the post-treatment sample was collected. Please note that all samples were collected as discreet samples, rather than time-series composites as with the other test species. Time series sampling was not necessary for this test species because organisms were not added to clean water, and so it was assumed that the distribution of bacteria in the test water would not be affected by the changing water volume in the treatment tanks over the course of the treatment. It was also assumed that collection of a

single sample would reduce the likelihood of sample contamination. Therefore, all three samples were collected at mid-depth in the center of the test tank by submerging and opening pre-cleaned and autoclaved 250-ml bottles.

Samples were placed in a cooler with ice immediately following collection and transported by Nautilus staff to the City of San Diego Metropolitan Wastewater Department, Environmental Monitoring & Technical Services Division, Marine Microbiology Laboratory (City Lab) for density determination. Appropriate chain-of-custody (COC) procedures were followed. Heterotrophic bacteria concentrations were measured in each sample by the City Lab using a pour plate method (method no. SM9215B), as provided in "Standard Methods for the Examination of Water and Wastewater, 18th Edition (Franson et al. eds. 1992)." Results were provided to Nautilus via email within three business days of sample collection.

For data analysis and interpretation, the following parameters were defined for data collected using mussel larvae, marine green algae, and heterotrophic bacteria:

Pre-treatment Density – The concentration of live/normally developed target test organisms measured/counted in the baseline sample plus that in the pre-circulation sample.

Post-treatment Density – The concentration of live/normally developed target test organisms measured/counted in the post-treatment sample.

Post-treatment Viability – The proportion of organisms exposed to treatment that exhibit measurable post-treatment normal development (mussel larvae) or population growth (marine green algae and heterotrophic bacteria; equal to the post-treatment density divided by the pre-treatment density times 100.

System Efficiency – Demonstrates how effective the treatment was at removing/killing target organisms; equal to 100 - post-treatment viability.

Results

Part 1 – Treatment Optimization

Results for the treatment optimization phase of this study are summarized in *Tables 1, 2* and *3*. Please note that while system loss data were collected, only overall kill rate data are presented in order to simplify interpretation.

Table 1. Summary of System Efficiency Values for Each Species and Treatment Combination Tested – Without Filtration

Treatment[a]	System Efficiency Values				
	***Artemia* spp.**	***Brachionus plicatilis***	***Nannochloropsis* spp.**	***Mytilus* spp.**	**Heterotrophic bacteria**
UV dose of 58,000 µW-s/cm^2	68.3	--	--	--	--
UV dose of 113,000 µW-s/cm^2	82.2	--	--	--	--
UV dose of 227,000 µW-s/cm^2	89.6	--	--	--	--
UV dose of 58,000 µW-s/cm^2 followed by 1.5 mg/L ozone	62.3	--	--	--	--
UV dose of 227,000 µW-s/cm^2 followed by 5.8 mg/L ozone	82.8	--	--	--	--
UV dose of 454,000 µW-s/cm^2 followed by 11.6 mg/L ozone	98.2	--	--	--	--
UV dose of 681,000 µW-s/cm^2 followed by 17.4 mg/L ozone	98.6	--	--	--	--
UV dose of 908,000 µW-s/cm^2 followed by 23.2 mg/L ozone	100	--	--	--	--

[a] UV lamp intensity of 4500 µW was measured using a UCA sensor (part #I35168). Dose was calculated using the following formula: lamp intensity (µW)*exposure chamber volume (gal)/flow rate (gal per s). The Ozotech ozone generator (#OZ2BTUSL) has a maximum ozone output of approximately 6.6 g/h. Dose was calculated using the following formula: (Maximum unit output (mg/min)/flow rate (L per min))*generator output setting (%).

Table 2. Summary of System Efficiency Values for Each Species and Treatment Combination Tested – With Bag Filtration Alone

Treatment	**System Efficiency Values**				
	***Artemia* spp.**	***Brachionus plicatilis***	***Nannochloropsis* spp.**	***Mytilus* spp.**	**Heterotrophic bacteria**
50-µm bag filtration at 5 gpm	99.6	--	--	--	--
50-µm bag filtration at 10 gpm	--	80.6	--	--	--
25-µm bag filtration at 10 gpm	--	98.6	--	--	--

Table 3. Summary of System Efficiency Values for Each Species and Treatment Combination Tested – With Filtration and Disinfection

Treatment[a]	System Efficiency Values				
	***Artemia* spp.**	***Brachionus plicatilis***	***Nannochloropsis* spp.**	***Mytilus* spp.**	**Heterotrophic bacteria**
50-µm bag filtration followed by UV dose of 58,000 µW-s/cm^2	--	83.5	--	--	--
50-µm bag filtration followed by UV dose of 113,000 µW-s/cm^2	--	87.7	--	--	98.3
50-µm bag filtration followed by UV dose of 227,000 µW-s/cm^2	99.7	80.5	--	--	--
50-µm bag filtration followed by UV dose of 113,000 µW-s/cm^2 and 2.9 mg/L ozone	--	100	100	100	>99.9
25-µm bag filtration followed by UV dose of 113,000 µW-s/cm^2	--	--	97.3	--	--
25-µm bag filtration followed by UV dose of 227,000 µW-s/cm^2	--	97.7	97.4	--	--
25-µm bag filtration followed by UV dose of 454,000 µW-s/cm^2 (double pass at 5 gpm, not lower flow rate)	--	--	100	--	--
25-µm bag filtration followed by UV dose of 454,000 µW-s/cm^2 (double pass at 5 gpm, not lower flow rate) and 1.9 mg/L ozone	--	100	--	--	--
25-µm bag filtration followed by UV dose of 454,000 µW-s/cm^2 (double pass at 5 gpm, not lower flow rate) and 1.2 mg/L ozone	--[b]	100	--[b]	--[b]	--[b]

[a] UV lamp intensity of 4500 µW was measured using a UCA sensor (part #I35168). Dose was calculated using the following formula: lamp intensity (µW)*exposure chamber volume (gal)/flow rate (gal per s). The Ozotech ozone generator (#OZ2BTUSL) has a maximum ozone output of approximately 6.6 g/h. Dose was calculated using the following formula: (Maximum unit output (mg/min)/flow rate (L per min))*generator output setting (%).

[b] Please note that the testing phase of the project is not yet complete. Performance of this treatment is planned for this species to complete the study.

Based on the results obtained with brine shrimp shown in Table 1 above, it was determined that delivery of the doses of UV and ozone required to achieve the proper kill rate without preliminary filtration were not feasible. Thus, 50-µm and 25-µm filtration were added as possible variables to the treatment scheme. Smaller filter pore sizes were not considered at this time because addition of a waste product that would occur with finer micron size bag filtration was not in line with the goals of the study. Target kill/removal rates were achieved with 50-µm filtration for brine shrimp (Table 2).

Testing then began with marine rotifers using 50-µm filtration as the base minimum treatment followed by the lowest UV dose and no ozone. UV dose was increased, with no increase in rotifer mortality indicating that this species was not sensitive to UV exposure. Ozonation was added to the treatment scheme at a dose of 2.9 mg/L and the 99% kill rate achieved (Table 3).

Testing proceeded using this treatment (50-µm bag filtration followed by UV dose of 113,000 µW-s/cm^2 and 2.9 mg/L ozone) and was successful at achieving the target kill rates for all indicator organisms.

Part 2 – Toxicity Testing

At this point during the study, the post-treatment sample water was evaluated for toxicity using three marine species: giant kelp (*Macrocystis pyrifera*), purple sea urchins (*Strongylocentrotus purpuratus*), and Pacific topsmelt (*Atherinops affinins*). The post-

treatment sample was determined to be toxic to purple sea urchins in excess of the toxic unit limit in UCSD's National Pollutant Discharge Elimination System (NPDES) permit – while detailed results are not presented or discussed in this paper. However, the results from the toxicity study showed that the treatment scheme had to be modified to yield a treatment that was effective at removing NIS and produced effluent that was non-toxic upon discharge to the Pacific Ocean.

To allow for a lower ozone dose, a smaller filtration pore size (25-µm) was confirmed to remove 99% of marine rotifers (Table 2), and then testing commenced with marine green algae, the species most likely to be UV-resistant, to determine the UV dose needed to kill 99% of this species. A dose of 454,000 µW-s/cm^2 was required to achieve the target kill rate (Table 3).

Following determination of the UV dose that would be used to complete the testing process, additional data, beyond the type of testing that had been performed to date, were needed to help determine the appropriate ozone dose that would achieve the treatment goals and result in a non-toxic discharge.

First, a standardization curve of ozone generator output versus total oxidant level was established using N,N-diethyl-p-phenylenediamine (DPD) free chlorine tests at flow rates of 5 and 10 gpm. Since Hypobromous acid (HOBr) is the most prevalent residual oxidant in ozonated seawater, and DPD tests have been shown to be very accurate in measuring levels of HOBr in seawater (Phillips, et al. 2004), DPD was considered a reliable test in

this scenario. This curve was generated to try to understand, as well as quantify the relationship between ozone dose and the residual toxicity of the effluent water for the purpose of efficiently choosing ozone doses for testing. Results are shown in Figure 2 below.

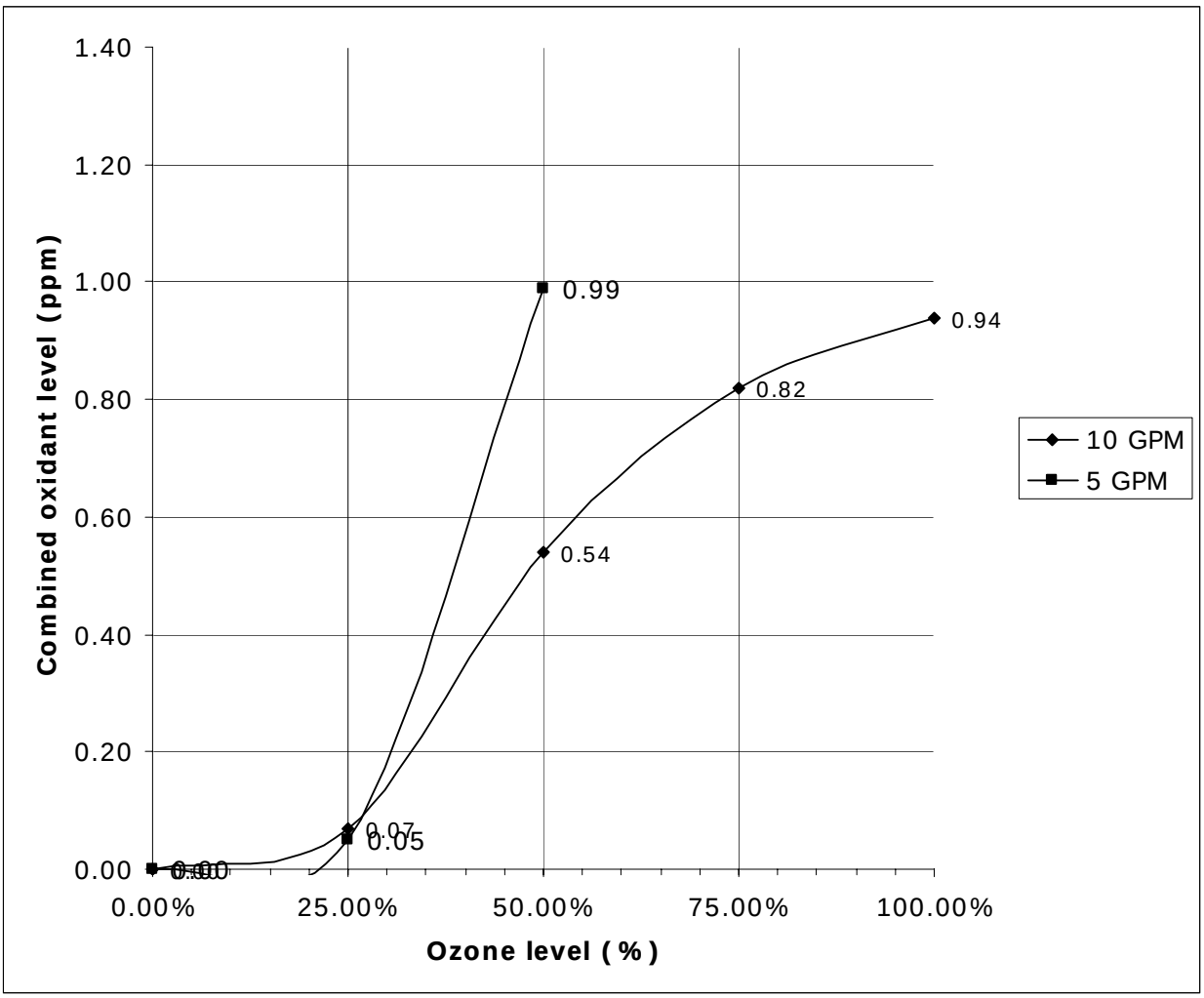

Figure 2. Ozonation Standard Curve – Generator Output versus Total Residual Oxidants

During the same period, both a toxicity reduction study and a toxicity threshold study were performed with purple sea urchins. The purpose of the first study was to determine if the 2.9 mg/L ozone dosed water could be rid of residual oxidants through a combination of carbon filtration and aeration. The purpose of the second study was to

determine the highest exposure concentration of ozone (and residual oxidants) that could be tolerated by this species without additional manipulation or treatment. In other research tests and in the author's experience, the use of carbon filtration and aeration have proven beneficial in reducing the toxicity of ozonized water, destruction of residual ozone, reducing ozonation byproducts, and improving the survival of fish larvae in saltwater systems (Ozawa 1991). However, our toxicity reduction study showed that neither carbon filtration nor aeration alone or in combination could adequately strip the treated water of residual oxidants for survival of purple urchins. The results from the toxicity threshold study were more promising – indicating that 1.2 mg/L was the highest exposure concentration that would yield a non-toxic effluent at the point of discharge.

At other facilities where treated water may be discharged, it may be possible to chemically treat ozonated water with sodium thiosulfate injection to remove toxic residual oxidants; however, because chemical additions to the ASBS are strictly prohibited by the State of California, this is not an option at the BAS location. While activated carbon filtration and aeration were unsuccessful in lowering the residual oxidant concentration to a non-toxic for purple urchins, these treatment processes are know to be beneficial following ozonation with other test fish species and could result in a more energy efficient treatment system where the sea urchins are not a concern.

With consideration of the toxicity testing, the final treatment scheme that is currently being performed with all five species is 25-µm filtration followed by 454,000 µW-s/cm^2

UV and 1.2 mg/L ozone. At the time of this report, testing had been completed with marine rotifers and a 100% kill rate was achieved (see Table 3).

Part 3 – Preliminary Design of Future NIS Treatment Systems

Based on the overall engineering goals for this project, and the results to date for the pilot treatment study, preliminary design treatment sequences for the final NIS treatment plant are:

1. 25-µm Filtration
1. Ozonation at a dose of 1.2 mg/L with a contact time of at least 2 minutes
2. UV light sterilization at a dose of 454,000 µW-s/cm^2
3. Discharge to the Ocean Outfall

Please note that the exposures to UV and ozone will occur in the opposite order compared to exposures during the pilot study. Application of UV downstream of ozonation may assist with decomposition of dissolved organics in a process called "advanced oxidation"; a process which has seen great success recently at freshwater drinking water treatment plants. The extent to which this arrangement may be effective with saltwater is not well known, however anecdotal evidence suggests that the effect of UV on ozonated freshwater is much different than it is on ozonated seawater. Regardless of advanced oxidation chemistry, location of UV after ozone treatment is expected to reduce the concentration of dissolved ozone and dissolved oxygen in the outgoing water by allowing a greater residence time to off-gas.

Conclusion

With the pilot plant testing study, we concluded that it will not be possible to achieve the NIS treatment system goals without the use of mechanical filtration. However, results also indicate that treatment goals can possibly be obtained using 25-µm filtration as a base treatment to remove larger organisms that are more resistant to exposures to UV and ozone. Limiting the use of filtration to the relatively larger pore size of 25-µm allows for the possibility of installing micro-screen drum filters with the following benefits: 1) very low discharge of backwash water to the sewer compared to other types of filtration, and 2) less material waste and minimal manual cleaning needs compared to the bag filters used in the pilot test.

Test results also show that inactivation of the indicator organisms is not possible with either UV or ozone alone, while dosing of both UV and ozone will allow for meeting treatment objectives for organisms that will pass through the filter. While the UV dosage determined to be effective in this study is high compared to conventional standards, a lower dose of UV paired with a higher dose of ozone are not possible due to the unique circumstances of discharge of the treated effluent to an ASBS.

Verification of the effectiveness of the 25-µm filtration followed by 454,000 µW-s/cm^2 UV and 1.2 mg/L ozone treatment is still needed for heterotrophic bacteria, marine green algae, and marine rotifers. However, based on results to date, it is likely that the target kill rates will be achieved with these species.

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