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Utility of Ozone Treatment in the Maintenance of Water Quality in a Closed Marine System*

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Abstract

The use of ozone as an oxidative supplement to biological filtration and to control epizootic microbial outbreaks coincident with maintaining a biological filter was investigated in a 2,271-l (600 gallon) closed marine-water system. Under conditions of a relatively large biomass load (1.82 kg/380l), filter-bed effluent levels of total ammonia (0.135 ± 0.01 ppm), un-ionized ammonia (0.0074 ± 0.0006 ppm) and nitrite (0.17 ± 0.01 ppm) were maintained within acceptable limits. Reservoir ozonation (100 mg/h/380 l) further significantly reduced ($P < 0.005$) these levels. Nitrates were significantly elevated ($P < 0.005$) with ozonation. Cessation of ozonation elevated total ammonia, un-ionized ammonia and nitrite levels above acceptable limits within 24 h. Resuming ozonation rapidly reversed this trend. Ozone reduced the microbial content of the culture water. Ozonation is suggested as a means of maintaining oxidative flexibility when used as a supplement to biological filtration. Further, prevention of epizootic microbial outbreaks may be accomplished without danger to the biological filter provided a proper system design is utilized.

Introduction

Among the major limiting factors in the maintenance of marine animals in closed marine-water systems is the accumulation of toxic nitrogenous wastes (ammonium and nitrite) in the aquatic medium (Kawai *et al.*, 1964, 1965; King and Spotte, 1974). Bacterial populations in filter beds (Saeki, 1958; Kawai *et al.*, 1965) have been utilized in closed systems to effect the nitrification of ammonia to nitrate. Therefore, rapid conversion of ammonia to nitrate is of paramount importance. Unfortunately, reliance upon bacterial filtration may not insure the desired rate of nitrification as filter-bed bacterial populations are sensitive (Kawai *et al.*, 1965) to environmental perturbations (salinity, temperature, dissolved oxygen, dissolved organics, total system biomass, etc.), thereby limiting the flexibility necessary in marine-water systems used in research or mariculture. In addition, uncontrolled increase of microbial populations may occur to the point where the normal flora become infectious to the organisms of interest (Spotte, 1970).

Ultraviolet (UV) irradiation has been suggested (Shelbourne, 1964, 1971) as a means of controlling infectious agents. However, the applicability of UV in large-scale, high flow-rate systems does not appear practicable because of the limited UV penetration of water. Consequently, the use of ozone as an oxidation supplement to bacterial nitrification and as a bactericidal agent was evaluated in a closed marine-water system.

Materials and Methods

The closed marine-water system described previously (Honn and Chavin, 1975) was utilized in the present study (Fig. 1). Briefly, the system consisted of four 190-l (50 gallon) fiberglass tanks supplied by a 1,136-l (300 gallon) polyethylene reservoir. All plumbing was polyvinylchloride (PVC; type I, grade I; Celanese Piping Systems, Inc.). A magnetic-drive polypropylene centrifugal pump recirculated the artifical seawater (Instant Ocean Inc.) at 727 l/h (192 gallons/h), with a system turnover time

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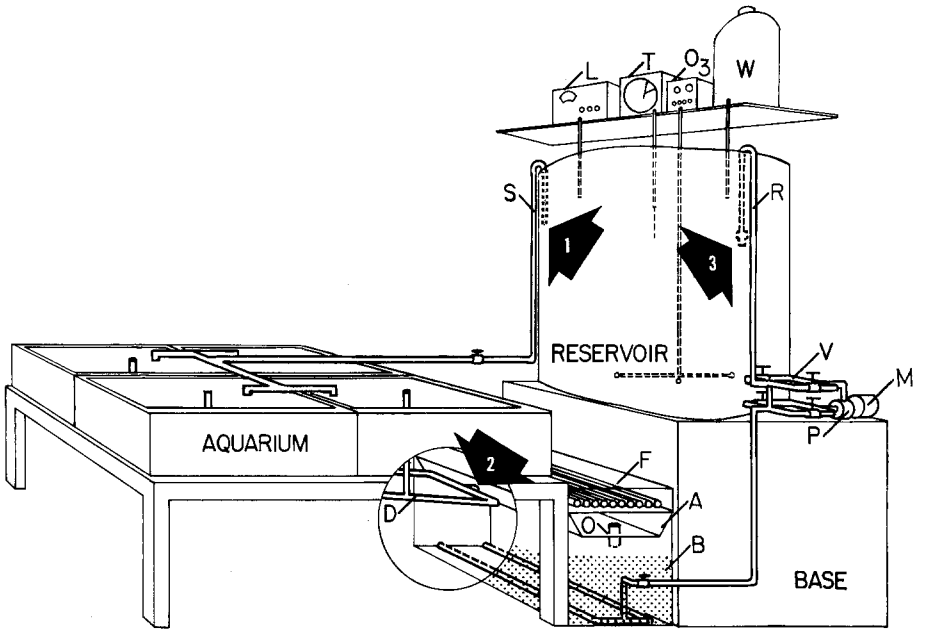

Fig. 1. Diagram of 2,271-1 (600 gallon) prototype marine-water system. System consists of four 190-1 (50 gallon) aquaria, one 1,136-1 (300 gallon) reservoir and one 380-1 (100 gallon) capacity algal bed-bacterial filter. The water to the aquaria is supplied by siphon action, and the aquarium overflow leads through a mechanical filter to an algal bed over which twelve 40-W cold-white fluorescent light tubes provide continuous (24 h/day) illumination. The water then flows through the dolomite and marble bacterial filter, 363 kg (800 lb) and is pumped into the reservoir. The pump is controlled by a liquid-level controller. Water lost by evaporation is replaced by metered inflow of freshwater into the reservoir. In addition, the reservoir temperature is monitored continuously. The reservoir is ozonated (300 mg/h). A: algal bed; B: bacterial filter; D: drain line; F: fluorescent light tubes; L: liquid level controller; M: motor; O: overflow; O_3, ozone generator; P: centrifugal pump; R: reservoir water return; S: reservoir siphon; T: temperature recorder; V: valve system for backflush of bacterial filter; W: fresh-water reservoir. Water samples were collected at three locations: (1) reservoir effluent; (2) aquarium effluent; (3) algal bed-bacterial filter effluent

of 2.6 h. The system also contained a mechanical filtration unit, algal bed and a bacterial filter. Ozonation of the reservoir (300 mg/h) was accomplished by a Purozone silent electrical discharge ozonator modified for continuous flow operation. Oil-free compressed air (Inoue and Sugino, 1959) dried by a single pass through a calcium chloride column (Cromwell and Manley, 1959) was supplied to the ozonator (500 l/h). Dissolved ozone concentrations were not determined.

Four nurse sharks (*Ginglymostoma cirratum* Bonnaterre) were maintained in the system throughout the study. The biomass load was 1.82 kg/380 l. Temperature (24°C ± 0.5C°), salinity (34.5‰) and pH (7.86 to 7.97) were constant throughout the entire period of study. Water samples were collected at 14 intervals in three locations (aquarium effluent, algal bed-bacterial filter effluent and reservoir effluent, Fig. 1) during a 3-week evaluation period. The samples were analyzed in triplicate for total ammonia, nitrite and nitrate content (Strickland and Parsons, 1968) to provide baseline conditions of the algal bed-bacterial filter and chemical (O_3) processing units. In addition, un-ionized ammonia levels (Trussell, 1972) were calculated from total ammonia values (Spotte, 1973).

Ozonation of the reservoir was discontinued at zero time, t_0, and water samples collected as described above at t_0 and at t_3, t_6, t_{12} and t_{24}-h post t_0. At 24 h (t_{24}), ozonation was resumed at the previous level (300 mg/h) and samples collected at r_3, r_6, r_{12}, r_{24} and r_{48}-h post t_{24}. Triplicate samples were analyzed throughout (Table 1).

Samples were plated in triplicate on plate-count agar (Difco) and the total number of bacteria/ml determined. As initial experiments did not demonstrate a significant difference ($P > 0.05$) between plate counts determined with plate-count agar (Difco) and marine agar (Difco), plate-count agar was used rou-

Table 1. Seawater nitrogen content, in ppm ($\bar{X} \pm$ SEM) at 14 sampling intervals during 3-week evaluation period designed to establish baseline conditions in a closed marine-water system

Nitrogen source (ppm)	Aquarium effluent	Algal bed-bacterial filter effluent	Reservoir effluent
Total ammonia	0.29 ±0.024	0.135 ±0.01	0.035 ±0.007
Un-ionized ammonia[a]	0.016±0.0013	0.0074±0.0006	0.0019±0.0003
Nitrite	0.51 ±0.046	0.17 ±0.01	0.05 ±0.01
Nitrate	15.96 ±0.84	6.84 ±0.06	24.8 ±1.0

[a]Calculated values (Spotte, 1973).

Table 2. Estimations of maximum acceptable limits (ppm) of ammonia, nitrite and nitrate in culture water

Total ammonia	Un-ionized ammonia	Nitrite	Nitrate	Culture water[a]	Source
100	-	-	-	DW	Grindley (1946)
50	-	-	-	FW	Grindley (1946)
0.3	-	-	-	FW	Brockway (1950)
-	-	-	7,000	FW	Trama (1954)
0.2	-	0.04	100	SW	Saeki (1958)
18	0.84	-	-	FW	Lloyd and Herbert (1960)
1.2	-	-	-	FW	Kawamoto (1961)
30.2	-	-	-	FW	Lloyd (1961)
0.3	0.006	-	-	FW	Burrows (1964)
-	-	-	102	SW	Hirayama (1966b)
-	-	-	50	SW	Kelley and Smith (1968)
3.4-420[b]	-	-	-	FW	Patrick *et al.* (1968)
67	-	-	120	SW	Hirayama (1970)
-	-	few	-	SW	Miklosz (1970)
0.1	-	0.1	20	SW	Spotte (1970)
0.1	0.01	0.1	20	SW	Spotte (1973)
0.1	-	0.1	20	SW	King and Spotte (1974)
0.1	-	-	20	SW	Spotte (1974)
0.1	-	-	-	FW	Water Research Centre (1974)

[a]DW: distilled water; FW: freshwater; SW: seawater.

[b]Species-dependent.

tinely. Data were analyzed by Student's *t* test for unpaired observations. Differences were accepted as significant when $P < 0.05$.

Results

The average ammonia, nitrite and nitrate levels at the three sampling locations during a 3-week evaluation were determined (Table 1) and found to be within "acceptable limits" (Table 2). The lowest experimentally documented levels of total ammonia (<0.1 ppm), un-ionized ammonia (<0.006 ppm), nitrite (<0.04 ppm) and nitrate (<100 ppm) were considered "acceptable limits" in the present report. Total ammonia levels in the aquaria effluents were reduced significantly ($P < 0.005$) by the algal bed-bacterial filter processing units. Ozonation of the reservoir significantly ($P < 0.005$) further reduced total ammonia levels below that present in the algal bed-bacterial filter effluent. The calculated un-ionized ammonia levels also were reduced. A significant ($P < 0.005$) reduction of nitrite levels occurred during algal bed-bacterial filter passage, but the nitrite

Table 3. Effect of ozone termination and resumption of ozonation on nitrogen sources in 3

Nitrogen source (ppm)[a]	Ozone terminated (h)				
	t_0	t_3	t_6	t_{12}	t_{24}
Aquarium effluent					
Total ammonia	0.24 ±0.012	0.29 ±0.009	0.30 ±0.02	0.52 ±0.03	0.59 ±0.02
Un-ionized ammonia[b]	0.013±0.0007	0.016±0.0006	0.017±0.0009	0.029±0.002	0.033±0.001
Nitrite	0.47 ±0.02	0.54 ±0.01	0.51 ±0.01	0.66 ±0.02	0.83 ±0.01
Nitrate	18.20 ±0.40	18.10 ±1.00	15.00 ±2.20	20.50 ±1.90	9.90 ±0.20
Algal bed-bacterial filter effluent					
Total ammonia	0.113±0.012	0.22 ±0.003	0.25 ±0.004	0.29 ±0.01	0.31 ±0.01
Un-ionized ammonia[b]	0.007±0.001	0.012±0.0003	0.014±0.0004	0.016±0.0001	0.02 ±0.001
Nitrite	0.18 ±0.01	0.29 ±0.01	0.33 ±0.012	0.42 ±0.01	0.45 ±0.01
Nitrate	14.60 ±1.20	16.80 ±1.70	15.30 ±1.40	18.30 ±1.50	8.10 ±2.10
Reservoir effluent					
Total ammonia	0.04 ±0.002	0.08 ±0.01	0.16 ±0.006	0.28 ±0.003	0.30 ±0.01
Un-ionized ammonia[b]	0.002±0.0001	0.004±0.001	0.009±0.001	0.015±0.0002	0.017±0.001
Nitrite	0.04 ±0.01	0.15 ±0.01	0.22 ±0.01	0.43 ±0.01	0.47 ±0.013
Nitrate	25.90 ±2.40	20.50 ±1.50	22.50 ±0.80	15.60 ±2.10	7.60 ±1.30

[a] Mean ± SEM.

[b] Calculated values (Spotte, 1973).

Table 4. Effect of ozone termination and resumption of ozonation on effluent bacterial con-

Effluent	Ozone terminated (h)				
	t_0	t_3	t_6	t_{12}	t_{24}
Aquarium	$1.2 \pm 0.09 \times 10^3$	$2.7 \pm 0.11 \times 10^3$	$1.47 \pm 0.22 \times 10^3$	$3.54 \pm 0.13 \times 10^3$	$4.9 \pm 0.18 \times 10^4$
Algal bed-bacterial filter	$1.35 \pm 0.08 \times 10^3$	$1.7 \pm 0.12 \times 10^3$	$3.7 \pm 0.9 \times 10^3$	$7.1 \pm 0.2 \times 10^4$	$1.87 \pm 0.4 \times 10^5$
Reservoir	$1.1 \pm 0.3 \times 10^2$	$2.3 \pm 0.28 \times 10^3$	$6.8 \pm 1.3 \times 10^3$	$1.4 \pm 0.12 \times 10^4$	$2.77 \pm 0.77 \times 10^5$

levels in the algal bed-bacterial filter effluent were not below the maximal (0.04 ppm) acceptable level. These levels, however, were reduced significantly ($P < 0.0005$) in the reservoir effluent post-ozonation and fell within the acceptable range ($P < 0.04$ ppm). Nitrate levels in the aquaria effluents were reduced significantly ($P < 0.005$) by the algal bed-bacterial filter units, however, they remained below the maximal (100 ppm) acceptable limit. Nitrate levels were elevated significantly ($P < 0.0005$) after passage through the ozonated reservoir, but remained below the acceptable limit.

Termination of ozone treatment resulted in increased nitrogenous waste levels (Table 3). At t_0, total and un-ionized ammonia were within acceptable limits, 0.1 and 0.006 ppm, respectively, in the reservoir effluent. However, t_3 levels were elevated significantly ($P < 0.01$) in all effluents sampled. Acceptable limits were present in the reservoir effluent only. The reservoir effluent total and un-ionized ammonia exceeded acceptable limits at t_{12}. At t_{24} these parameters were greatly elevated ($P < 0.005$) above t_0 levels. Resumption of reservoir ozonation at t_{24} resulted in a precipitous drop ($P < 0.0005$) in total and un-ionized ammonia levels within r_3 h in all effluents sampled. At r_6, ammonia levels in all effluents had returned to the t_0 range.

The changes in nitrite levels after termination of ozone treatment paralleled those described for ammonia. The nitrite levels were increased in all effluents at t_3, achieving a plateau level at t_{12}. These levels were significantly elevated in aquarium effluent ($P < 0.025$), algal bed-bacterial filter effluent ($P < 0.0005$) and reservoir effluent ($P < 0.0005$) at t_0. Resumption of reservoir

compartments of a closed marine system

Ozone resumed (h)				
r_3	r_6	r_{12}	r_{24}	r_{48}
0.31 ±0.010	0.11 ±0.01	0.20 ±0.01	0.17 ±0.01	0.22 ±0.01
0.016±0.001	0.006±0.0006	0.011 ±0.001	0.009 ±0.0003	0.02 ±0.01
0.78 ±0.001	0.48 ±0.009	0.39 ±0.01	0.34 ±0.05	0.40 ±0.02
12.20 ±1.60	30.00 ±1.60	25.20 ±1.20	21.80 ±1.70	15.30 ±1.30
0.13 ±0.0040	0.09 ±0.01	0.04 ±0.003	0.12 ±0.004	0.14 ±0.01
0.007±0.0002	0.005±0.0003	0.008 ±0.0002	0.007 ±0.0002	0.008 ±0.0004
0.35 ±0.003	0.20 ±0.003	0.17 ±0.01	0.17 ±0.02	0.19 ±0.01
13.00 ±1.00	15.80 ±1.10	23.40 ±1.60	14.30 ±1.20	9.40 ±1.80
0.07 ±0.003	0.03 ±0.01	0.026 ±0.002	0.035 ±0.003	0.03 ±0.02
0.004±0.0002	0.002±0.0003	0.0014±0.0002	0.0019±0.0004	0.0016±0.0002
0.22 ±0.01	0.11 ±0.02	0.08 ±0.01	0.04 ±0.002	0.04 ±0.005
20.40 ±1.80	38.90 ±1.60	28.30 ±1.80	23.20 ±1.40	18.60 ±2.30

tent (total bacteria/ml, means ± SEM) from 3 compartments of a closed marine system

Ozone resumed (h)				
r_3	r_6	r_{12}	r_{24}	r_{48}
2.4 ±0.17x10^3	1.9 ±0.54x10^3	1.2±0.15x10^3	1.7 ±0.2 x10^3	1.5 ±0.04x10^3
4.5 ±0.4 x10^4	2.96±0.13x10^3	5.2±0.15x10^3	1.98±0.12x10^3	1.93±0.10x10^3
0.42±0.06x10^2	1.3 ±0.17x10^2	1.2±0.05x10^2	0.93±0.1 x10^2	0.95±0.08x10^2

ozonation at t_{24} significantly ($P < 0.005$) decreased algal bed-bacterial filter and reservoir effluent nitrite levels at r_3. Aquarium effluent nitrite levels were reduced significantly ($P < 0.005$) at r_6. With ozonation, all effluent nitrite levels returned to t_0 levels at r_{12}.

After termination of ozone treatment, reservoir effluent nitrate levels demonstrated a significant ($P < 0.005$) downward trend below t_0 at t_{12}. Algal bed-bacterial filter effluent nitrates tended to increase during the first 12-h post t_0, while aquarium effluent nitrate levels remained relatively stable. All effluent nitrate levels fell significantly ($P < 0.0005$) lower than t_0 at t_{24}. The resumption of reservoir ozonation (t_{24}) resulted in a significant ($P < 0.005$) elevation of reservoir effluent nitrate levels at r_3, with a peak elevation at r_6. Significant peak elevation of aquarium effluent nitrate ($P < 0.0005$) and algal bed-bacterial filter nitrate ($P < 0.0005$) levels occurred at r_6 and r_{12}, respectively. At r_{48}, all effluent nitrate levels returned to pre-experimental range.

During normal operation of the closed system, bacterial counts were similar in the aquarium and the algal bed-bacterial filter effluents (Table 4), but were significantly ($P < 0.005$) reduced by ozonation (reservoir effluent, t_0). Termination of ozone treatment resulted in a significant ($P < 0.005$) increase in the reservoir effluent bacterial number at t_3 which continued to increase to t_{24} h ($P < 0.0005$). Similar significant increases in bacterial numbers were observed in the aquarium ($P < 0.005$) and algal bed-bacterial filter ($P < 0.0005$) effluents at t_{24} h. Ozonation produced a highly significant ($P < 0.0005$) drop in bacterial count of the reservoir effluent at r_3 to pre-experimental levels.

Similar decreases in bacterial number occurred in the aquarium and algal bed-bacterial filter effluents. The bacterial count of the algal bed-bacterial filter effluent increased at r_{12}; however, this increase was not reflected in the reservoir effluent.

Although response-time differences exist, with some parameters returning to pre-experimental levels within 3 h of resuming ozone treatment (r_3), in general, the total system was stabilized within 24 h (r_{24}).

Discussion and Conclusions

Perhaps the most important limiting factor in the maintenance of marine fishes and invertebrates in a closed system is the accumulation of nitrogenous wastes (Atz, 1964). Numerous investigators (Table 2) have attempted to determine "acceptable" limits of these compounds in culture water for both fresh-water and marine animals. However, considerable disparity exists for the values determined (Table 2). Modifying environmental factors such as pH, dissolved carbon dioxide (Lloyd and Herbert, 1960; Lloyd, 1961), dissolved oxygen (Downing and Merkens, 1955; Merkens and Downing, 1957; Lloyd, 1961) and temperature (Grindley, 1946; Lloyd, 1961; Wilson *et al.*, 1969), in addition to species differences (Kawamoto, 1961; Patrick *et al.*, 1968; Wilson *et al.*, 1969) contribute to this disparity. Further, the parameters used to determine toxicity vary considerably. Mortality, loss of equilibrium and decreased growth rate are commonly used parameters, but may obscure more subtle toxic effects (i.e., gill deformities; Burrows, 1964) occurring at lower concentrations of the substance studied. Ammonia toxicity is the most widely studied parameter (Table 2). Most reports deal with fresh-water teleosts, a few with marine animals, but only one with elasmobranchs (Sigel *et al.*, 1972). Nitrates are believed (Spotte, 1970) to be the least toxic nitrogenous waste, in fact, fresh-water (Trama, 1954) and marine (Oliver, 1957) animals have survived in waters containing high nitrate concentrations (>1000 ppm). Nevertheless, subtle toxicities cannot be excluded. Acceptable limits of nitrate vary considerably, however, the lowest level for which experimental support exists is 100 ppm (Saeki, 1958). Although lower (20 to 50 ppm) acceptable limits have been reported (Table 2), they appear to have been determined empirically and are open to reinvestigation. Considerably more effort must be expended to quantitatively establish the acceptable limits of nitrogenous compounds in culture water for various species under a variety of environmental conditions. However, in view of current knowledge, closed-systems design should minimize the nitrogen content of culture water.

Biological filtration is currently the major approach to sea-water processing in closed systems (Goldizen, 1970; Miklosz, 1970). Subsequent to an initial conditioning period (Goldizen, 1970), the filter bed bacterial populations stabilize (Kawai *et al.*, 1964) in dynamic equilibrium with the available energy sources (urea, ammonia, etc.). Thus conditioned, the system is only flexible within a given carrying capacity which is a function of the oxidizing capacity of the filter bed (Hirayama, 1966a). Increased carrying capacity may be achieved with the use of artificial ammonium sources as a conditioning agent (Siddall, 1974). Nevertheless, bacterial populations in the filter bed are unable to adapt to sudden transitions in salinity, dissolved oxygen, and temperature (Kawai *et al.*, 1964). Therefore, sole reliance upon biological filtration results in a limited carrying capacity. Such inflexibility is undesirable in a research installation. Further, increased use of closed systems for mariculture purposes (Winget *et al.*, 1973) places emphasis on the development of flexible closed-system management techniques.

Ozone is a strongly oxidizing molecule capable of effecting rapid oxidation of nitrite to nitrate in fresh-water effluents (Evans, 1972). Comparison of nitrite and nitrate levels (Table 1) in algal bed-bacterial filter effluents with reservoir effluents indicates that the oxidative capability of ozone may be utilized in sea-water systems. Further, ozone is capable of oxidizing ammonia to nitrate, although at a slower rate than the nitrite to nitrate conversion (Papko, 1957). Examination of algal bed-bacterial filter effluents and reservoir effluents (Table 1) also demonstrates this capability in seawater. An additional action of ozone upon ammonia in aqueous systems is the production of ionized ammonia (Papko, 1957), which would result in a further reduction of the biologically toxic (Warren, 1962) un-ionized form. As a result of the ozone-mediated conversion of un-ionized to ionized ammonia, the un-ionized ammonia levels in the reservoir effluent of the present system, although greatly below (Table 1) the maximum acceptable limit (Table 2), actually may be much less than calculated

as the calculation considers only temperature and pH as modifying factors (Spotte, 1973) but not the presence of ozone.

The use of ozone has been cautious as a result of its possible deleterious effects on the cultured organisms (MacLean *et al.*, 1973) and the biological filters (King and Spotte, 1974) upon which the culture systems depend. However, in the system used in the present study, spatial separation of cultured organisms and the filter bed from the site of ozonation, further aided by the relatively short half-life (15 min) of ozone in water (Layton, 1972), has not shown ozone-evoked effects. Thus, the existence of a functional filter bed in an ozone-treated closed system is evident (Table 1). Ozone has been reported to deplete trace elements, particularly manganese in sea-water systems necessitating partial (10%) water replacement (Spotte, 1970). Manganese, iron, aluminum and particularly rubidium levels were reduced in the culture water used in the present study, however, trace-element analysis of acid-digested algal samples accounted for the majority of the depleted elements (Honn and Chavin, unpublished observations). The return of these trace elements to the culture water has been discussed (Honn and Chavin, 1975). The need for ozonation to obtain flexibility in closed sea-water systems is demonstrated by suddenly increasing the biological load on the filter bed in an otherwise stable system (Table 1). In the present study this was accomplished by terminating reservoir ozonation (Table 3). It is evident within 3-h post t_0 (t_3) that the biological filter is unable to maintain total ammonia, un-ionized ammonia, and nitrite levels within acceptable limits (Table 2). Reservoir effluent levels of these parameters, due to the dilution effect of the large water reserve, do not exceed acceptable levels (Table 2) until t_{12}. Nevertheless, within 24 h of such increase in the biological load, nitrogenous wastes in the total system reach deleterious levels. The positive effect of ozone is clearly demonstrated within 3 h of resuming reservoir ozonation (Table 3, r_3) with stabilization of the entire system within 24 h. It is evident that sudden increases in biological load can be effectively managed if provisions are made for ozonation. This is especially important in research facilities wherein the increased biological load may result from experimental perturbations of the environment (i.e., salinity, temperature) which may have depressive effects on biological filters (Kawai *et al.*, 1964).

The effectiveness of ozone as a bactericidal agent in waters containing pathogenic and non-pathogenic organisms is well documented (Miller *et al.*, 1959; Kinman, 1972; Venosa, 1972) for freshwater and waste-water effluents. The need for control of infectious agents in open (Sanders *et al.*, 1972) and closed (Spotte, 1970) systems is clear. UV irradiation has been suggested (Shelbourne, 1964, 1971; Eagleton and Herald, 1968) as a bactericidal agent in closed sea-water systems, however, the suggested flow rate of 0.2 gallons/min/effective inch of UV lamp (Spotte, 1973) limits its applicability on a large scale. Chlorination has been suggested as a possible means of controlling epizootic outbreaks (Sanders *et al.*, 1972), however, its effectiveness on a mg/l basis is less than that of ozone (Venosa, 1972). Further, persistent residual chlorine may be deleterious to aquatic life whereas the only by-product of ozonation is molecular oxygen (Layton, 1972). Termination of ozone treatment (Table 4) in the present study resulted in increased bacterial populations in all effluents sampled within 24 h. Whether or not such represents an increase in infectious agents was not assessed, however, fish subjected to environmental stress (i.e., increased nitrogenous wastes) often succumb to infections resulting from outbreaks of normal flora. The resumption of ozonation (Table 4) produced a precipitous drop in reservoir bacterial content within 3 h, thereby obviating such potential dangers. Persistent low levels of bacteria in the reservoir effluent (Table 4) indicate the ability of ozone to check microbial outbreaks in closed systems.

The present findings are based upon the ozone dose rate of 0.132 mg/h/l sea-water in the entire system. The ozone dose rate in the reservoir (contact chamber) is 0.264 mg/h/l. However, absolute ozone consumption is dependent upon a number of factors including chemical oxygen demand, turbidity, salinity, microbial and/or planktonic densities, dissolved solids, etc. (Kirk *et al.*, 1972). Thus, other sea-water systems may vary in the ozone requirement necessary to produce comparable results.

The use of ozone in closed marine-water systems is recommended as an oxidative supplement to biological filtration both in the reduction of the levels of toxic nitrogenous wastes and in imparting flexibility to research and mariculture installations. Further, control of microbial contamination warrants the continuous use of ozone since the only residual byproduct is dissolved oxygen.

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