INACTIVATION OF PARALYTIC SHELLFISH POISON BY OZONE TREATMENT

by

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ABSTRACT

Paralytic shellfish poison obtained from New England shellfish during the 1972 *Gonyaulax tamarensis* red tide and shellfish poison from *Gonyaulax catenella* obtained in a purified form were treated with ozone gas in an attempt to detoxify the poisons. Detoxification, as measured by mouse injection, was achieved with both extracts in solution. A field study was conducted during the September, 1974 outbreak of paralytic shellfish poisoning in northern New England. Mussels held in raw seawater from the red tide area built up high levels of toxicity, while those held in ozonized red tide seawater remained non-toxic.

INTRODUCTION

Red tides, or blooms, of toxic dinoflagellates occur in marine waters throughout the world. Many of these dinoflagellates possess a paralyzing poison which may concentrate in the tissues of molluscs feeding on these toxic organisms. The poison accumulated by shellfish is commonly known as paralytic shellfish poison (PSP). Although not harmful to the molluscs themselves, it may become a serious problem when shellfish are eaten by animals higher in the food chain. The ingestion of poisonous shellfish has been responsible for many human and other animal mortalities for at least three hundred years (Halstead, 1965; Prakash *et al*, 1971). Red tides of *Gonyaulax catenella* in the Pacific northwest and *Gonyaulax tamarensis* on the northern Atlantic coast are the two principal causative agents of PSP in the United States and Canada (Schantz and Magnusson, 1964; Halstead, 1965; Sasner *et al*, 1973).

Schantz *et al* (1961) reported that *G. catenella* toxin could be oxidized with oxygen or reduced with hydrogen, producing a non-toxic derivative. Chin (1970) tested a number of chemical disinfectants against *G. catenella* toxin and noted that sodium hypochlorite was effective in

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neutralizing the toxin. The long period required for detoxification and the addition of chemical substances themselves toxic to marine life make these methods undesirable for treatment of seawater systems (Brungs, 1973). This report describes the alternative use of ozone gas, a powerful oxidizer, as a detoxifying agent.

Ozone has been used for a number of years in the disinfection of water and wastewater. It kills bacteria and viruses more rapidly than does chlorine (O'Donovan, 1965; Eisenhauer, 1968; Majumdar and Sproul, 1974). Spotte (1970) reported that dissolved organics could be removed from circulating aquarium water using ozone gas. As early as 1929, Voille reported the effectiveness of ozone in the sterilization of seawater and ozone is used routinely in France to disinfect seawater in shellfish depuration stations (Anonymous, 1972). Ozone has also been used to eliminate marine microorganisms which could be pathogenic to fish and shellfish (Blogoslawski *et al*, In Press, a).

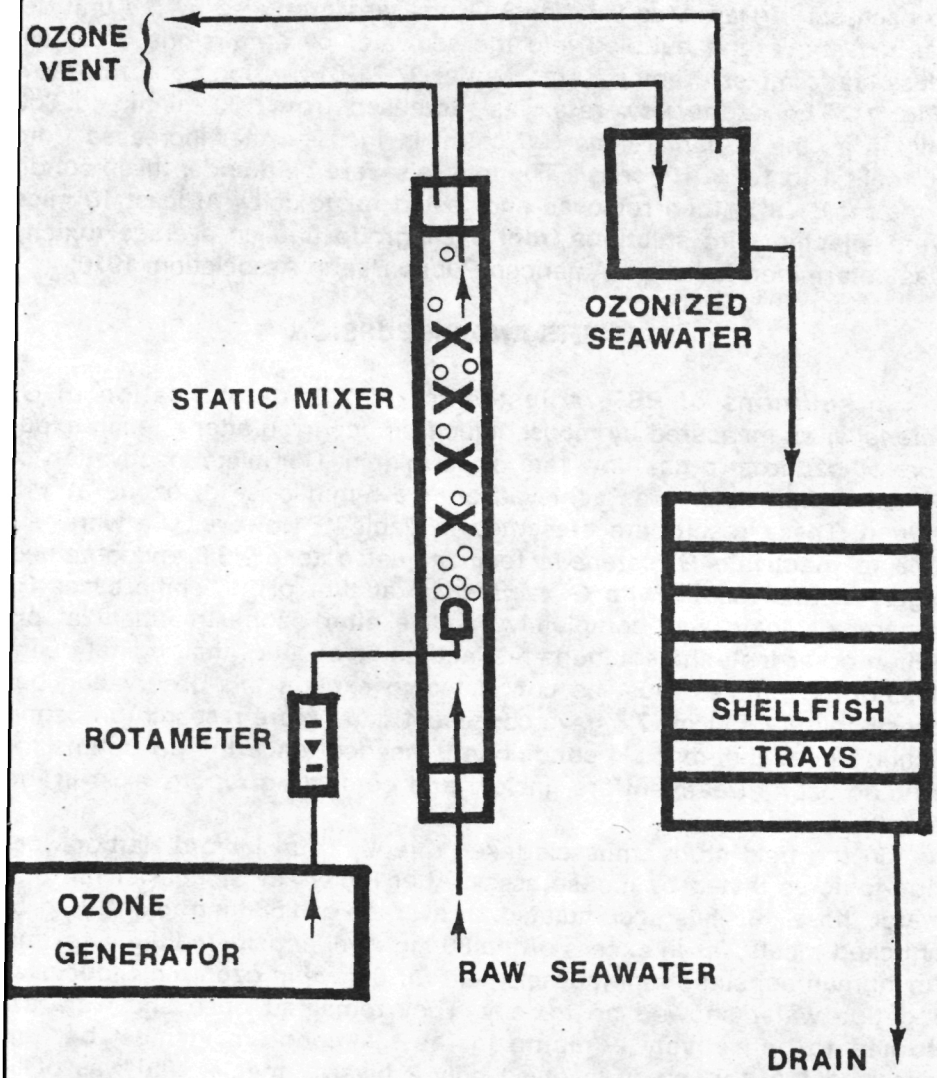
Toxic extracts from *Gymnodinium breve*, the Florida red tide dinoflagellate, were inactivated by ozone and the toxic properties of seawater from Florida red tides were nullified with this gas (Blogoslawski *et al*, 1973; In Press, b). The results of these studies have encouraged us to attempt detoxification of PSP using ozone gas.

MATERIAL AND METHODS

Gonyaulax tamarensis toxin was extracted from soft-shelled clams, *Mya arenaria*, by the procedure described in Prakash *et al* (1971). The clams were collected from Common Island, Hampton Harbor, New Hampshire, during a toxic red tide in the fall of 1972, and the resulting crude extracts possessed a toxicity of 3400 μg of toxin per 100 g of shucked meat. *Gonyaulax catanella* toxin, with a toxicity of 100 μg of toxin per ml of solution, was obtained from the U.S. Food and Drug Administration as a purified reference standard. Both toxins were diluted with distilled water and the pH of each solution was adjusted to either 3.8 or 7.8 with 0.1 N HCl or NaOH prior to ozonization. Twenty-five ml portions of toxin were treated with ozone in 100 ml test tubes at 20°C. Ozone gas (2% ozone in air mixture) was produced by a Pollution Control Industries generator (6 g/hr) and bubbled through the toxin solutions by means of extra-fine aquarium airstones (Halvin 66B) for 5 min. Gas flow rates were measured continuously with a Gilmont rotameter (size 1). Control solutions were treated with compressed air for 5 min. at 220 ml/min. Untreated samples were also used as controls. Toxicity was measured by intraperitoneal injection of 1 ml solution samples into 18-21 g Carworth Farms mice (CF-1 strain).

A field study was conducted in Gloucester, Massachusetts, during an outbreak of PSP in northern New England during September, 1974. This outbreak was caused by a *G. tamarensis* red tide. Blue mussels.

FIGURE 1



Field Study Ozone Treatment Apparatus

Ozone gas enters the static mixer and is mixed with raw seawater flowing up the mixer column. Ozonized seawater leaves the top of the column, is collected in a head tank, and gravity fed to a series of glassfiber trays containing shellfish. (Adapted from Thurberg, in press)

Mytilus edulis, and ribbed mussels, *Modiolus demissus*, were taken from Milford Harbor, Connecticut, to Gloucester and maintained in two flowing seawater systems in fiberglass trays. One system received untreated seawater from the red tide area, while the other received ozonized red tide seawater, both at pH of 8.4 and a temperature of 16°C. The flow rate to each set of trays was 0.6 l/min. Ozone was produced by the unit described above and bubbled into the seawater by an airstone in an all glass static mixer (Kenics Corp., Model 37-21-014) using cocurrent flow (Fig. 1). The ozone flow rate was increased from 100 ml/min to 600 ml/min as the *G. tamarensis* cell count in the seawater increased from 10^3 cells/1 to 1.2×10^7 cells/l. The mussels were held under these conditions for 8 days, then removed and tested for toxicity. At least 10 mice were injected with solutions from each group and an average toxicity was determined for each (American Public Health Association, 1970).

RESULTS AND DISCUSSION

In solutions of PSP, adjusted to pH 7.8, detoxification of *G. catenella*, as measured by mouse injection, occurred after a 5-min exposure to ozone at a gas flow rate of 55 ml/min. Complete inactivation of *G. tamarensis* toxin was achieved after a 5-min dose of ozone at 110 ml/min. These results are presented in Table 1. However, we were unable to inactivate *G. catenella* toxin adjusted to pH 3.8 and obtained highly erratic results with *G. tamarensis* at that pH. In some cases *G. tamarensis* toxin was completely inactive after ozone treatment at pH 3.8; in other tests the solutions retained some or all of the original toxicity. Further studies must be conducted to explain this observation because solutions at pH 7.8 gave consistent data. More research on ozone action under different pH conditions is needed since the pH optima for aquatic ozone treatment are unclear and conflicting reports exist in the literature.

In the field study, mussels taken directly from Milford Harbor were non-toxic as tested by mouse assay. After 8 days in Gloucester red tide water these mussels accumulated an average of 2586 mg toxin/100 g of shucked meats, far in excess of the 80 mg level considered the safe limit for human consumption. Mussels held for 8 days in ozonized Gloucester red tide water showed no toxicity. They remained open and were assumed to be actively pumping in the test medium. It must be emphasized that this study involved only 2 bivalve species and was of limited duration. Additional research is necessary before the results can be applied to practical situations. One such application might be the use of ozone in depuration stations where shellfish are purged of bacteria and viral contaminants. Ozone might prevent these shellfish from becoming toxic during red tides. During the recent bloom of *G. tamarensis* in Massachusetts, a depuration plant had to be closed because the ultraviolet-light treated intake water had become contaminated by this

TABLE 1

Inactivation of Paralytic Shellfish Poison at pH 7.8

Ozone dose ml O_3/min	Number of mice	Number of deaths	Death time (min)
Gonyaulax tamarensis			
220	5	0	—
110	10	0	—
55	10	1	12
27	10	8	12-14
0	10	10	5-6
Air control	20	19	6
Gonyaulax catenella			
220	15	0	—
110	15	0	—
55	15	0	—
27	10	10	4-7
0	10	10	5-7
Air control	10	10	5-7

toxic dinoflagellate. Based on our study, ozone treatment of the intake water might have allowed the plant to continue operations by eliminating the toxic properties of the water.

Many aquatic applications of ozone treatment are being examined at present, but very few studies have been conducted to determine the long-range effect of ozone residual and ozone byproducts on aquatic organisms. Recent studies suggest tissue damage and abnormal development of marine larvae in ozone-treated water (MacLean *et al*, 1973). It is clear, therefore, that additional research is needed before ozone can be used routinely to treat seawater. The use of ozone as an effective method of eliminating toxic red tide metabolites has been discussed, however, and future research may establish its safety and usefulness (Thurberg, In Press).

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Note: The use of trade names is merely to facilitate description and does not imply endorsement by the National Marine Fisheries Service.

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