

# Halophile Bacteria: Experimental Control and Its Ecological Significance

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## ABSTRACT

*Dead Sea and other halophiles were used to determine susceptibility to two sterilizing agents (Peracetic Acid vapor and Ethylene Oxide gas). These spanned the whole spectrum of halophilism (20-30% to 2-5% NaCl tolerance): Halobacterium salinarium, Micrococcus morrhuae, Micrococcus halodenitrificans, Flavobacterium halmephilum, Pseudomonas beyerinckii. One hundred percent susceptibility was obtained by both methods and limiting conditions established. Acid vapor treatment was found superior; it achieved complete kill after very brief exposure; acid and end-products are readily soluble in water and the method is adaptable to mass production. Application to solar and other salts to eliminate contaminating potential is discussed.*

## INTRODUCTION

Solar salt operations occur on a world wide scale and are expanding in several areas (Lefond, 1969; Dickinson, 1970; and Fig. 1 this paper). In some places marine pans are the exclusive source of salt as in the Caribbean Islands. Elsewhere solar salt constitutes the bulk of the total salt production (California, Brazil, Australia, India, among others). Here attention is focused on that fraction of solar salt used as an important item in human and animal consumption, for example in the salting of fish and meat, in feed mixes and the treatment of hides. Applications discussed below can also be made to saline lake NaCl salt, as far as microbial content is involved.

## ORGANIC CONTENT OF SALT

During the formation of marine salt various organic components may be, and frequently are, incorporated in the crystals. Included among other items are algae, spores,

eggs, and so on (Tasch 1963, 1970) as well as halophilic bacteria (Reiser and Tasch 1960; Tasch and Todd 1973). Worldwide use of solar salts promotes food spoilage due to halophilic microbes in the salt. The question is whether there is a simple inexpensive method for treating contaminated solar salt so as to preclude such undesirable effects as red pigmentation of fish and hides due to *Halobacterium cutirubrum* and other red halophiles?

## PREVIOUS WORK

Kushner et al. (1965) suggested acidifying the salt brine with hydrochloric acid or acetic acid. Earlier, Gibbons (cit. Kushner et al., 1965) proposed mixing acid phosphate with salt used in preserving fish, or acidifying the brines. The concept here was that the cited red halophile was very responsive to changes in pH and would not grow under acid conditions. Gibbons also noted that salted acid on fish supported another type of contamination, known as "dunn." Other proposals in the past recommended heating the salt to kill contaminating bacteria or the use of mine salt (less likely to have microbial content), although Reiser and Tasch (1960) and others have shown that mine salt does indeed have a microbial content, methane bacteria as well as extreme halophiles. Costs eliminate the thermal treatment, and some less expensive method is needed.

As an extension of Gibbons' observations, Kushner, Masson and Gibbons (1965) performed the cited experiment at very low pH values, (for 0.01 M HCl, pH of 1.5; for 1.0% acetic acid, pH of 2.7) and found no viable bacteria. Decreased growth followed decrease in pH from 7.8 to 5.0 (for acetic acid).

Elsewhere, Kushner (1968) observed that very little was known about lethal agents for extreme halophilic bacteria. The portion of our research on the geomicrobiology

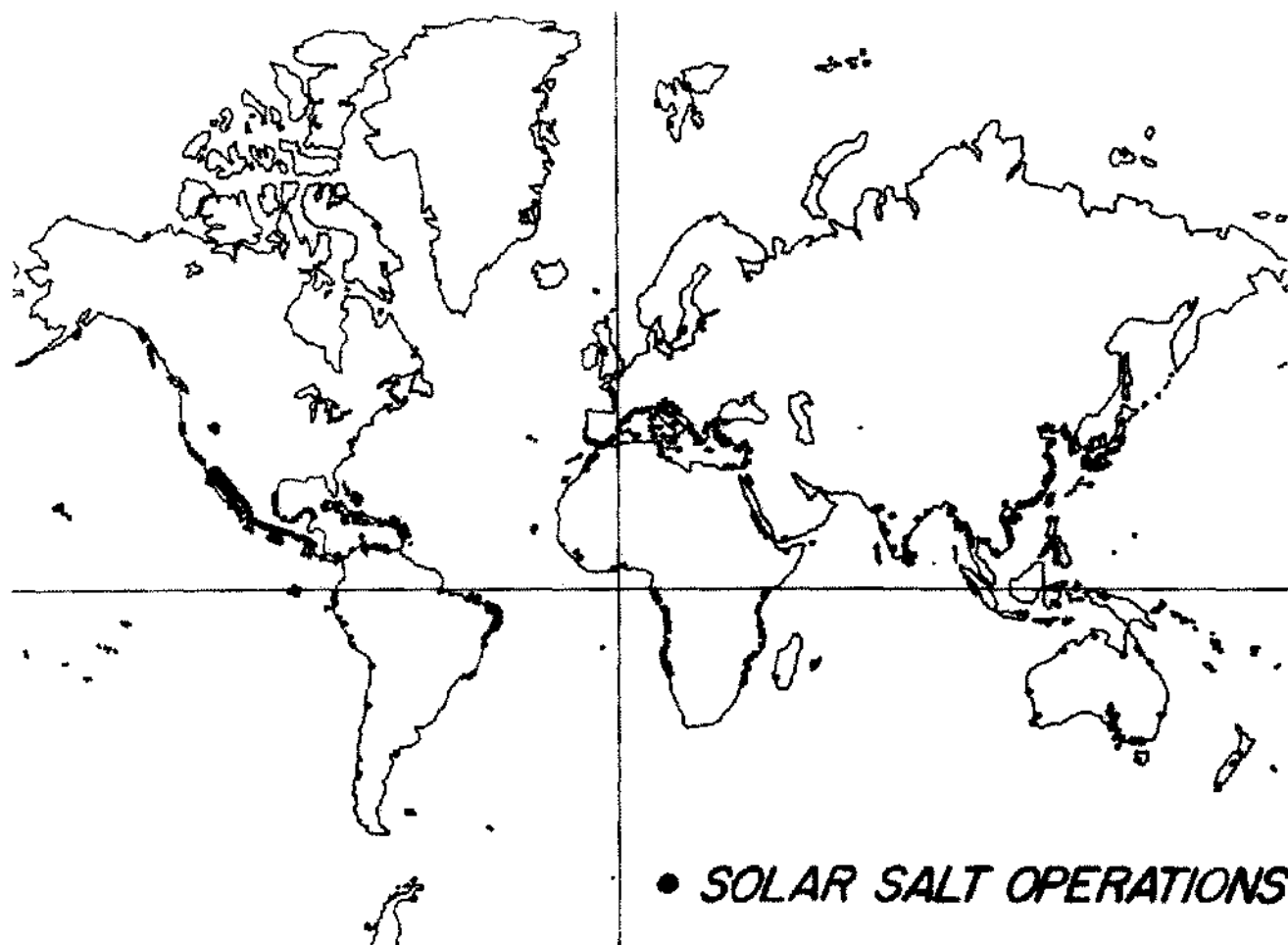


Figure 1.

of salt reported here was deliberately undertaken to determine lethal agents for the entire spectrum of halophiles, for those with a tolerance of 20–30% NaCl (the extreme halophiles) to those with a tolerance of 2–5% (slightly halophilic types).

## TWO STERILIZING AGENTS FOR SALT

The two sterilizing agents used were Peracetic Acid vapor and Ethylene Oxide gas. Pure cultures were obtained from the American Type Culture Collection (ATCC) and used in our experiments. (The forms used are listed in Table I in order of decreasing salinity tolerance; asterisked bacteria are from the Dead Sea).

Details of culture, media, controls and methodology are given in Tasch and Todd (1973). Peracetic Acid (PAA) treatment was performed in a petri dish (in a preparatory chamber). A dosage of 0.5 ml of acid was pipetted onto a filter pad in the petri dish cover and only vapor was in contact with the culture in the plate for one minute. These experiments disclosed a 100 percent susceptibility to PAA for each of the five test halophiles.

TABLE I

Spectrum of Halophilic bacteria used to test two sterilizing agents

Organism	ATCC Number	NaCl Tolerance (%)
<i>Halobacterium salinarum</i>	19700	28-32
<i>Micrococcus (=Paracoccus) morrhuae</i> *	17077	9-30
<i>Flavobacterium halmophilum</i> *	19717	3-24
<i>Pseudomonas beyerinckii</i>	19372	3-24
<i>Micrococcus (=Paracoccus) halodenitrificans</i>	13511	4-23

\*Dead Sea forms.

The second lethal agent used was Ethylene Oxide Gas (ETO). Experiments were performed in a Kewaunee Glove Box, adapted for gas sterilization. The chamber of the glove box was exposed to ETO—1.5 pounds/24 hours. Such exposure was followed by evacuation of the chamber by sterile (incinerated) air, for 24 hours. Experiments conducted in this sterile chamber yielded 100 percent susceptibility for all five halophiles exposed to the ETO.

Thus, two previously uncharacterized lethal agents for extreme halophiles, such as occur in solar salts, were con-

clusively demonstrated to be effective. Of these PAA is the quickest and most economical. Peracetic Acid possesses certain valuable properties (Greenspan et al., 1955) as a sterilizing agent for solar salt. These include: 1. The breakdown products acetic acid, water and oxygen are all non-toxic and the acid is water-soluble and readily diminished by evaporation; 2. PAA can be diluted with water from the 40% commercial product to as little as 1.0% for germicidal effectiveness (the strength of commercial liquid spray can preparations). (However, higher concentrations as our experiments showed require a fractional part of the time of exposure—a distinct advantage in any mass production arrangement); 3. PAA is a weaker acid than Acetic.

Greenspan et al. (1955) found that 1.0 ml of 1.0% PAA liquid spray, released through a stainless steel nozzle inactivated all bacterial spores (on strips) in a volume of 1.0 cubic foot in fifteen minutes. Our experimental results favor use of the vapor phase, since it will diffuse much more rapidly over an entire confined space.

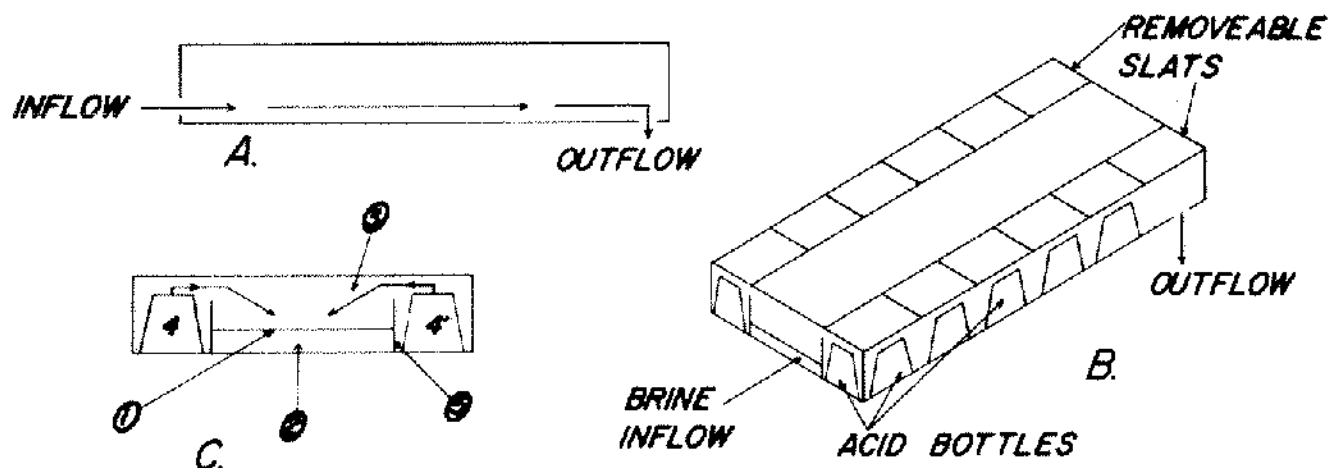
#### MASS PRODUCED STERILE SOLAR SALT

The setup required for sterilization of any large or small quantity of solar salt (halite) has very few specifica-

tions, as follows. Minor modifications might be required by different types of operation or daily production schedules (Fig. 2).

1. Restricted slot-like entrant to a trough to govern shallowness of entering brine layer.
2. A comparatively wide trough to allow a continuous shallow flow of brine. (Controlled rate of flow to permit 1 + minute exposure to PAA vapor for any given entering layer of brine.)
3. A suitable low chamber, non-corrosive under PAA vapor, that is completely sealed except for entrant and exit slots. Floor of chamber to contain trough margined by acid racks to hold unstoppered bottles of PAA.
4. To ensure most rapid, effective diffusion of PAA vapor, the ceiling of the chamber should be very low, close to the trough and the acid rack should bring the acid-bottle necks slightly above height of the brine layer.
5. Ceiling above the acid racks on either side of the trough should be removable to allow PAA bottles to be stoppered when not in use and/or replaced or refilled, when needed.
6. A relative humidity of greater than 40 + percent should be maintained in the chamber since it will facilitate PAA action (Portner and Hoffman, 1968). Our PAA ex-

### PAA SOLAR SALT TREATMENT CHAMBER



1. ENTRANT SLIT CONTROLS SHALLOWNESS OF BRINE FLOW.
2. SHALLOW CONTINUOUS INFLOW OF BRINE.
3. LOW CHAMBER, A FEW INCHES ABOVE "5".
- 4-4' PAA IN UNSTOPPERED BOTTLES.
5. WALLS OF SHALLOW TROUGH CARRYING BRINE.

Figure 2.

periments were performed under a relative humidity of  $54 \pm 6\%$ . In many solar operations the climate suffices to guarantee adequate RH. Elsewhere, this RH can be readily achieved by having a few unstoppered containers of distilled water on the acid rack on each side of the trough.

All materials needed are comparatively inexpensive. The acid alone will be the major cost. Even here, costs can be considerably reduced by either bulk purchasing or preparation of PAA solutions in one of two ways (Greenspan et al., 1955), i.e., reaction of hydrogen peroxide with either acetic anhydride or acetic acid. The commercial product of PAA is a 40% solution and the calculated weight loss percent is 0.011/day at 25°C. As already remarked, as little as 1.0% solution has been found effective as a sterilizing agent, hence the 40% solution has a satisfactory useful lifespan.

The PAA treatment does not add anything to the salt proper; the negligible quantity of acetic acid that might be incorporated in the brine (during its one minute exposure to PAA vapor) will be lost on evaporation (Kushner et al., 1965); and the treatment does not alter the pH. Further, the process requires no expenditures for any kind of mechanical or electrical input, except that customary in pumping brines.

A sterile solar salt (NaCl) should find an enlarged market.

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