THE GERMICIDAL PROPERTIES OF TRICHLOROCYANURIC ACID

by

KENNETH FREDERICK FORTNEY

A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1958

APPROVED:

Redacted for Privacy

Professor and Chairman of Department of Bacteriology

In Charge of Major

Redacted for Privacy

Chairman of School Graduate Committee

Redacted for Privacy

Dean of Graduate School

Date thesis is presented August 6, 1957

Typed by Dorice McLachlan

ACKNOWLEDGMENT

The writer takes this opportunity to express his sincere appreciation to Dr. Paul R. Elliker, Chairman of the Department of Bacteriology, and major advisor, whose assistance, encouragement and confidence made this study possible; and to Dr. Walter B. Bollen, Professor of Bacteriology, for his helpful criticisms and suggestions.

The writer also wishes to express his thanks to Mrs. Helen Hays for technical advice and helpful criticisms.

Finally, to my wife, Virginia, without whose sacrifice the opportunity to carry on advanced study and to engage in this project would never have been possible, goes my most affectionate gratitude and appreciation.

TABLE OF CONTENTS

TABLE OF CONTENTS
continued

TABLE OF CONTENTS
continued

TABLE OF CONTENTS
continued

LIST OF FIGURES AND TABLES

LIST OF FIGURES AND TABLES
continued

LIST OF FIGURES AND TABLES
continued

LIST OF FIGURES AND TABLES
continued

THE GERMICIDAL PROPERTIES OF TRICHLOROCYANURIC ACID

INTRODUCTION

At the present time germicides available for sanitizing operations in the food and dairy industry are sodium and calcium hypochlorites, quaternary ammonium compounds, iodophors, dichlorodimethylhydantoin and chloromelamines. Each compound has shown certain disadvantages which limits its use as a sanitizing agent.

A new organic chlorine compound, trichlorocyanuric acid (TCCA), was developed and preliminary studies by the manufacturer indicated that it had germicidal activity comparable to the hypochlorites. TCCA has one advantage of being a powdered product that could prove more stable in the dry form than the liquid hypochlorites. Additional information was needed on the effectiveness of TCCA against various forms of food spoilage organisms under various environmental conditions.

The objective of this study was to compare TCCA as a germicide with a hypochlorite, quaternary ammonium compound, an iodophor and chloramine-T plus potassium iodide against nonsporeforming bacteria, bacterial spores, lactic bacteriophage, yeasts and molds.

If a study of TCCA proved it to be more effective than other sanitizers now in use, such information could be beneficial to the food and dairy industry.

LITERATURE REVIEW

Early Development and Use of Hypochlorite Solutions

The chlorine compounds, especially the hypochlorites have long been recognized as effective germicidal agents. For instance, Robert Koch (75, p. 465) in 1881 investigated the bactericidal properties of hypochlorites. This was the first time that hypochlorites were shown to have a role in disinfection. A report to the American Public Health Association in 1886 (12, p. 8) by its committee on disinfectants was very favorable on the use of hypochlorite solutions as disinfectants. The present application of chlorine compounds as disinfectants or sanitizing agents resulted from their use during World War I when they were known as Dakin's solution.

The Effect of Concentration on Germicidal Activity

One would expect that germicidal efficiency is directly proportional to concentration of germicide. The work of some investigators has shown that this is not always true. Ram Ayyar (80, p. 9) found that chlorine at 20 ppm. was more effective than 200 ppm. against spores of Bacillus subtilis. Charlton and Levine (12, pp. 36-40) reported that several commercial calcium hypochlorite solutions; Perchloron, H.T.H., and H.T.H. 15, exhibited almost the

same germicidal power at 2 levels of concentration, 100 ppm. and 1000 ppm. available chlorine. Johns (40, p. 603) found that the germicidal efficiency of H.T.H. 15 was increased by dilution. In explanation of their findings Charlton and Levine (12, pp. 37, 40) and Johns (40, p. 604) have suggested that the lower alkalinity of the less concentrated hypochlorite solutions accounted for their level of germicidal activity.

The Effect of pH on Germicidal Action

The introduction of the glass electrode marked the beginning of a new era. With its development, pH of a hypochlorite could be accurately measured.

Johns (43, pp. 556, 558) noted the ineffectiveness of a hypochlorite containing trisodium phosphate against mixtures of bacteria from milk cans and Streptococcus lactis as compared to other hypochlorites not containing this salt. Investigations have indicated that trisodium phosphate was the factor involved in the retardation of bactericidal activity. The influence of trisodium phosphate upon the germicidal activity was associated with increased hydroxyl ion concentration. Mallmann and Schalm (55, p. 5) and Johns (42, p. 206) reported the decrease in germicidal efficiency of chlorine when alkali was added. Myers and Johnson's (64, pp. 41, 42) findings substantiate those of

previous workers. The hypochlorite which exhibited low germicidal effectiveness contained large amounts of alkali, while those of high germicidal effectiveness contained small amounts of alkali. Costigan (14, pp. 1-7) showed that a hypochlorite of low alkalinity was more efficient against nonsporeforming Micrococcus pyogenes var. aureus and Eberthella typhosa than one of a higher alkalinity. Rudolph and Levine (80, pp. 22-27) showed the effect resulting from a change in hydrogen ion concentration on germicidal efficiency of hypochlorite solutions. A concentration of 25 ppm. of available chlorine killed 99% of Bacillus metiens spores in 2½ minutes at pH 6, in 5 minutes at pH 8, and in 120 minutes at pH 10. Rudolph and Levine (80, p. 38) and Marks, Wyss, and Strandskov (56, p. 303) suggest that changes in killing time appear to be closely associated with changes in concentration of undissociated hypochlorous acid. Holwerda (12, p. 48) has calculated the quantity of hypchlorite present as undissociated HOCl for various levels of pH. He has shown that with a decrease in pH there is an increase in undissociated HOCl. Butterfield (9, pp. 1853-1855) found that hydrogen ion concentration influenced the germicidal efficiency of a sodium hypochlorite solution. Experiments using Escherichia coli and E. typhosa at temperatures of 2° to 5° C. and 20° to 25° C. showed that the pH effect on

germicidal activity was not so marked at the lower temperature range. In order to obtain 100% kill at either temperature level with progressive increases in pH, an increase in chlorine concentration was required.

The Effect of Organic Matter on Germicidal Action

The efficiency of chlorine and chlorine compounds is markedly reduced by the presence of organic matter (60, p. 336). Johns (41, p. 218) has concluded that when a large quantity of organic matter is added to a test solution as in the phenol coefficient method of testing germicides, the germicidal activity of Chloramine-T was much less than with hypochlorites. Studies at O.S.C. (18, p. 7) have suggested that hypochlorite solutions in most instances show higher germicidal activities in the absence of organic matter. When the germicidal activities of the quaternary ammonium compounds are compared to the hypochlorites, in low concentrations and in the presence of 1% skim milk, the quaternaries were more active than the hypochlorites. When the concentration of the compounds is increased to 100 ppm. and 200 ppm. the hypochlorites have shown greater activity than the quaternaries. Johns (44, pp. 93, 95) found that hypochlorite and quaternary ammonium compounds retain some activity against Staphylococcus aureus in organic matter of concentrations up to 2%. Preliminary

tests indicated that there was a slight stimulation of activity of the quaternary ammonium compound against E. coli in the presence of small quantities of skim milk.

Effect of Temperature on Germicidal Action

Alkaline solutions of sodium hypochlorites containing 50, 100, and 200 ppm. available chlorine have shown no chlorine losses when kept at 55° C. for 180 minutes (75, p. 473). Investigations have shown that there is a definite increase in germicidal activity with increase in temperature. Quisno and Foter (74, p. 113) found that at 37° C. germicidal activity of cetyl pyridinium chloride, a cationic detergent, was twice as great as at 20° C. against Staphylococcus aureus or E. typhosa. Friberg and Hammarstrom (23, p. 131) have demonstrated that with sodium hypochlorite the bactericidal as well as virucidal effects increase with increased temperatures. Rudolph and Levine (80, p. 30) found that in general, the killing time of B. metien spores exposed to a calcium hypochlorite was reduced from 60 to 65% with a rise of 10° C. Costigan (15, p. 59) showed that hypochlorite solutions of low alkalinity containing 200 ppm. available chlorine when heated to 50° C. destroyed Mycobacterium tuberculosis in 2½ minutes; when heated to 55° C., it destroyed the organism in 1 minute; and when heated to 60° C. the organism

was destroyed in 30 seconds.

Effect of Hard Water Salts on Germicidal Action

There can be present in a water supply certain ions that retard the activity of a quaternary ammonium compound. Ridenour and Armbruster (76, p. 507) showed that calcium and magnesium exert an adverse effect by decreasing the bactericidal action of a quaternary. When magnesium or calcium was increased from 0 to 40 ppm., the decrease in activity against E. typhosa was from 99.9% to 50%. Mueller and Seeley (47, p. 723, 724) found that cations such as calcium, magnesium, or ferric iron decreased the germicidal activity of a quaternary. A quaternary of 200 ppm. in the presence of 1000 ppm. of calcium or magnesium gave 100% destruction of E. coli within 8 minutes of contact. In the presence of 10 ppm. of ferric iron the quaternary was completely inactivated. The bactericidal properties of hypochlorite products, liquid or powder, in concentrations recommended for sanitizing were not affected by water hardness (33, p. 147). Buffering action of hard water salts may lower the pH value of a hypochlorite and increase its germicidal activity (45, pp. 56, 57).

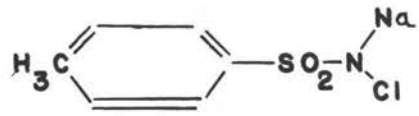
Organic Chlorine Compounds Used as Germicides

A number of organic chlorine compounds are being used as disinfectants. Of these, only the most frequently encountered will be discussed.

Chloramine. In 1931, Berliner (7, p. 1320) included all amino (- NH_2) or imino (= NH) groups where the hydrogen was in part or completely replaced by chlorine as chloramines. Today the word chloramine applies to monochloramine, NH_2Cl. Chloramine has also been used as a synonym for chloramine-T. The history of this compound, its discovery and germicidal properties, began in the early 1900's. In 1910, Rideal (25, p. 142) found that when ammonia was present in sewage the efficiency of chlorine increased as a disinfectant. Race (25, p. 142) found that when .1 ppm. ammonia was added to .2 ppm. chlorine the activity was equivalent to .6 ppm. chlorine. Gainey and Lords (25, pp. 142, 143) explanation of the greater activity of chlorine in the presence of ammonia was that the two compounds combine to form chloramine. Chloramine being more stable than chlorine extends its germicidal activity over longer periods of time. Weber and Levine (91, pp. 725, 727) have studied a number of factors which affect the activity of chlorine and chloramine. The test organism employed was spores of B. metiens. They found

that at reactions which were more acid than pH 9.4, chlorine was more effective than chloramine whereas at more alkaline conditions chloramine was more efficient. A rise of temperature of 10° C. using chlorine resulted in a 50 to 60% reduction in killing time. With chloramine, a rise of 10° C. affected a reduction of 68 to 74% in killing time.

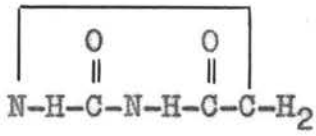
Chloramine-T. Chloramine-T occurs as a white to yellow crystalline powder which is soluble in water. This compound, which has the following chemical composition, slowly decomposes on exposure to air with liberation of chlorine.



In the presence of acidic substances decomposition is accelerated. Considerable study has been given to the germicidal properties of chloramine-T. Weber (89, pp. 505-509) concluded that two factors should be considered before using chloramine-T as a bactericide: (1) pH and (2) concentration. Chloramine-T was found to be less active in absence of organic matter, than a hypochlorite against M. aureus. Generally longer exposure times were required for chloramine-T than were necessary for the hypochlorite.

Chloramine-T at pH 8.3 and a concentration of 50 ppm. required 31.5 minutes for a 99.999% kill as compared to less than 5 seconds for the hypochlorite at pH 8.5 and concentration of 50 ppm. for the same degree of kill. As previously cited, Johns (41, p. 218) found that chloramine-T was less active than hypochlorites in the presence of organic matter. Investigations of Charlton and Levine (12, pp. 28, 38) using B. subtilis spores confirmed the work of Johns. They found that a calcium hypochlorite solution containing 20 ppm. available chlorine at pH 8.0 to 8.2 gave 99% kill within 4.8 minutes. A chloramine-T solution containing 4000 ppm. at pH 8.4 required 24 hours for a 99% kill.

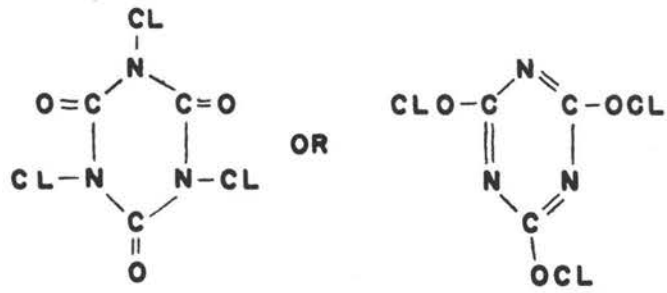
Chlorinated Hydantoins. In 1861, Baeyer (86, p. 403) discovered hydantoin. This compound as formulated below is a white, odorless solid and is only slightly soluble in water.



Hydantoins and their substitutions products have found a number of uses other than as medicinal products. Chlorinated hydantoins have been recommended for use as bleaching agents, antiseptics and germicides (77, 78, 79). A germicide rinse mixture (72), stable for long periods

when stored as a solid even at high temperatures and humidities in the presence of alkaline detergents, has been prepared from chlorinated hydantoins. "During World War II, 1,3 - dichloro-5, 5 - dimethylhydantoin received much study, and was furnished to a limited extent to the Armed Services, in the form of a compounded germicidal formulation for the rinsing of mess kits and other utensils, and related services in the field. The material proved to be successful, but had serious limitations, the chief of which was its slow rate of solution" (83, p. 285). Since World War II, 1,3 - dichloro-5,5 - dimethylhydantoin through special processing has been improved on from the standpoint of solubility. A new compound, Antibac, which contains as its active agent 1,3 - dichloro-5,5 - dimethylhydantoin has been tested and compared with the commercial hypochlorites. Sotier (83, pp. 287-291) found that with increase in temperature or decrease in pH the germicidal activity of Antibac increased; also, its activity was greater than a hypochlorite in the presence of organic matter. Johns (46, pp. 134-136) investigated the effectiveness of Antibac against M. pyogenes var. aureus, Pseudomonas aeruginosa and E. coli. The results reported indicated that the compound was no better and was sometimes less active than other hypochlorites tested.

Trichlorocyanuric Acid. The various chlorine liberating compounds with the exception of certain chloramines, have not been accepted for sanitizing operations. Each compound has shown one or more disadvantages; high cost, limited solubility, and lack of stability. Recently a new compound, trichlorocyanuric acid (TCCA), has been developed. Its structural formula is listed as follows:



To obtain maximum stability and solubility various formulations have been prepared. Each formulated mixture, a powder type product, contains the same active ingredient (TCCA) but not the same salt; some formulations have sodium bicarbonate while others contain one of the phosphate salts. Each TCCA compound contains approximately 17% available chlorine. One hundred percent TCCA is very insoluble even in water at temperatures up to 82° C. However, the various formulations of TCCA go into solution quite easily, even at room temperature. TCCA is very stable when dry; no loss of chlorine occurs when heated for 45 hours at 52 to 55° C.

Application of Sanitizers in Dairy and Water Sanitation

Dairy Sanitation. Since so many of our present day foods are by-products of the dairy industry, it seemed advisable to focus some attention on a number of problems related to this field.

Widespread losses of pasteurized products such as cottage cheese, milk, butter and cheddar cheese have resulted through the activities of psychrophilic bacteria (68, pp. 200, 201). Several defects contributed by the activities of these organisms have been observed. Elliker and Horrall (19, p. 926) found that butter became flat through loss of diacetyl and later developed a surface taint caused by contaminating psychrophilic bacteria. Parker, Smith and Elliker (71, p. 887) reported loss of flavor of cottage cheese which was proceeded by certain physical changes including white to yellowish slime accompanied by putrid and fruity odors. Roject and Burgwald (4, p. 383) found that psychrophilic bacteria which develop at refrigeration temperatures were responsible for deterioration of pasteurized milk. Olson, Parker, and Mueller (68, p. 203) suggest that contamination of pasteurized dairy products results from inefficient sanitized equipment or water supplies. Parker, Coldwell and Elliker have studied the effects of a quaternary

ammonium compound and hypochlorite solutions against various psychrophilic bacteria isolated from defective milk products. High and low concentrations of organisms were used to simulate contaminations which could be found on equipment or in water. Concentrations of available chlorine at 10 ppm. and 100 ppm. gave 100% destruction within 15 seconds against high and low numbers of psychrophiles. The activity of a quaternary ammonium compound was variable, in some cases 100% kill was not obtained within 5 minutes (69, p. 138).

Bacteriophage specific for lactic acid bacteria used in cheesemaking have caused damage in the cheese industry for years. Bacteriophage has been known to destroy lactic acid starter cultures in cheese milk with resulting starter failure and the necessary lack of acid development during the cheesemaking process. Anderson and Meanwell (5, pp. 62, 63) found evidence that phaging of a mixed starter culture was an important factor in the occurrence of slowness of cheesemaking. Smith, Parker and Elliker (82, p. 218) found in Oregon dairy plants that 21 out of 23 cases of starter failure were caused by bacteriophage. Possible sources of phage for the lactic streptoccci might be the same as those of the host cell e.g. milk, dairy products, and by-products, dairy utensils and environment of dairies. Johns (47, p. 120) has observed phage in a

milk supply but individual cow samples yielded no phage. Nichols and Wolf (66, p. 304) found cheese to be a good source of bacteriophage. Whitehead and Hunter (92, p. 65) isolated phage in the atmospheres of commercial cheese plants. Finely divided particles of whey emitted from whey separators was found to be the primary source of the phage. Wolf, Nichols and Ineson (94, p. 291) claim that starter failure is frequently due to the contamination of bulk or mother starters by air-borne phages. The control of bacteriophage is a difficult problem. Proper sanitation is one method of control. Whitehead and Hunter (93, p. 66) state that chlorine is the most effective agent for destruction of bacteriophage. Wolf, Nichols and Ineson (94, p. 314) have found that mists containing .003 ppm. to .02 ppm. available chlorine were satisfactory for destruction of air-borne bacteriophage. Parker and Elliker (70, p. 53) obtained complete destruction of S. cremoris strain W phage with 50 ppm. sodium hypochlorite solution and an exposure time of 15 seconds. Prouty (73, pp. 214, 218) determined the rate of phage destruction by six quaternary ammonium compounds. Exposures of cheese plant equipment to 200 ppm. of quaternary was considered adequate for destruction of phage. Parker and Elliker (70, p. 53) found that quaternaries at concentrations of 200 ppm. destroyed phage within 15 seconds.

Yeast and molds have caused serious losses especially in the butter industry. Neill (65, p. 129) attributes the source of mold infected butter to butterboxes, parchment paper and aerial contamination of butter during its manufacture. Macy (52, p. 39) has implicated parchment paper as a source of many molds in butter. He found a large number of samples of parchment to be heavily seeded with mold spores. He concluded that parchment paper was of good quality until it reached the creamery. At the creamery packages were broken and the paper was left exposed to dust contaminations. Macy, Combs and Morrison (53, p. 401) have shown that churns may be a source of mold in butter. Neil (65, p. 131) found with rotary cut Saranac boxes that the exterior and interior of the boxes and the parchment paper wrappers containing butter were contaminated with molds. The molds present were Cladosporium, Penicillium and Mucor. Martin and Julien (57, p. 46) have associated butter spoilages by molds with loss of flavor and development of discolorations. Molds are ubiquitous organisms and their spores are easily detached. These float about in the air and are always available for contaminating careless handled products. There are measures to reduce mold contamination. Vernon (85, p. 256) claims that pasteurization of cream used for buttermaking should eliminate nearly all the molds present

in the raw material. Elliker (17, p. 8) asserts that molds in all dairy products are quickly destroyed by approved pasteurization practices. Macy and Olson (54, p. 533) have observed the prevention of surface mold growth of laboratory seeded butter wrapped in parchment treated with sodium or calcium propionate. Belani (6) found that a germicide Amicrol TH in concentrations of .005 to .50% was capable of destroying molds. Gershenfeld and Ruthenberg (29, pp. 263, 264) found heavy contaminations of a cultured sour cream. The source of contamination was the filler and lid, hood over bottles, capping machine, walls of culture room and ceiling over incubator tank. To eliminate contamination, the walls of the culture room, the ceiling over the incubating tank, the hood over the bottles and the capping machine were rinsed in a chlorine solution. The filler and lid were rinsed with 100 ppm. to 200 ppm. solution of chlorine. Morrison, Macy and Combs (62, p. 412) found chloramine-T and alkaline solutions of hypochlorite at concentrations of 350 and 283 ppm. respectively, ineffective in treatment of churns containing a microflora of various molds. "In general, the control of mold growth in or on butter requires strict attention to proper sanitation of plant equipment and to the control of air-borne infection. Specifically the important points in this regard are: (a) adequate pasteurization;

(b) proper sanitization of pipelines and pumps that carry the pasteurized cream; (c) proper sanitation of churn and printing equipment; (d) prevention of air-borne contamination by avoiding excessive air currents within the plant; (e) adequate ventilation; (f) the use of hot water or hot salt brine for treatment of tubs and parchment wrappers and liners used in packaging salted butter, or use of "Mycoban" in the case of unsalted butter; and (g) cleanliness in the habits of personnel handling the butter" (22, p. 450).

Yeasts are widespread in nature and could be expected in butter under certain conditions. The organisms in fresh butter come from various sources: sweet cream; vats, pumps, coolers, churns or piping, and air. Macy, Combs and Morrison (53, p. 401) found yeasts consistently present in churns used for buttermaking. Olson and Hammer (67, p. 617) show that air is a source of yeast in dairy plants. Agar plates exposed to 3 dairy laboratories contained yeast. The presence of yeast in butter can result in specific flavor defects and discolorations. Hammer (34, p. 457) and Elliker (16, p. 79) described yeasty flavors that result through the growth of lactose-fermenting yeast. Foster, et al. (22, p. 449) reported red and pink discoloration on butter surfaces resulting from contamination of certain species of Torula or Monilia. Yeast in butter

have the same significance as molds. Their presence has been contributed in part to insanitary utensils and equipment. The result of faulty pasteurization could permit survival of yeast with development of flavor defects in butter. Elliker (17, p. 8) reported that yeast are destroyed by approved pasteurization procedures. The churn has been a leading source of butter contamination. A churn cleaning and sanitizing procedure has been recommended by the Minnesota Agricultural Experimental Station (22, p. 431). This procedure recommends that prior to churning cream 200 ppm. of available chlorine be used for rinsing the churn.

Water Sanitation. Water sanitation today follows a well established practice known as break-point chlorination. This method, the chemistry involved, and advantages of its use will be discussed.

Griffin and Chamberlin (32, p. 378) found that when chlorine was added to water containing ammonia, there was a rise in chlorine residual proportional to the amount of chlorine added. With further increases of chlorine a rapid drop occurred in chlorine residual and ammonia-nitrogen. A point was reached where the nitrogen completely disappeared. With still further increases in chlorine the residual increased proportional to the amount added and

slight amounts of nitrogen reappeared. When the residual chlorine concentrations were plotted, the curve was shown to have a hump followed by a dip. The bottom of the dip was referred to as the break-point. The work of Fair *et al.* (20, p. 1054) suggested that the reaction of ammonia and chlorine occurred as follows:

$$NH_3 + HOCl = NH_2Cl + H_2O$$
$$NH_2Cl + HOCl = NHCl_2 + H_2O$$
$$NHCl_2 + HOCl = NCl_3 + H_2O$$

Gainey and Lord (26, p. 272) explained the reaction of chlorine when added to water containing ammonia. The first reaction involved the formation of chloramine, the chlorine residual increased proportionally to the chlorine added. High concentrations of chlorine caused increased decomposition of chloramine and corresponding decrease in chlorine residual according to the equation:

$$NH_2Cl + NHCl_2 = N_2 + 3\ HCl$$
$$2NH_2Cl + Cl_2 = N_2 + 4\ HCl$$

With further additions of chlorine a point called the break-point was reached. At this point water contained neither chloramines nor chlorine. Additions of chlorine beyond the break-point resulted in increased concentrations of

available chlorine. Allen and Brooks (1, pp. 158, 159) have described the significance of chlorination beyond the break-point. Chlorination beyond this point resulted in free chlorine and nitrogen trichloride, NCl_3. Residual chlorine beyond the break-point destroyed bacteria more quickly than an equal concentration on either the upward or downward portion of a curve before the break-point. The explanation was that free chlorine was more active than chloramine. Nitrogen trichloride has certain unpleasant properties which makes it undesirable in water. The effects of this compound were said to be negligible providing chlorination did not proceed far beyond the break-point. Geiger and Maloney (28, pp. 361, 363) have suggested that hypochlorite alone was more effective than hypochlorite plus ammonium chloride in water low in organic matter. In waters high in organic matter, hypochlorite plus ammonium chloride was more effective than hypochlorite alone. Chang and Morris (11, pp. 1010-1012) have evaluated the germicidal properties of elemental iodine as an emergency disinfectant for water supplies. Iodine concentrations of 5 to 10 ppm. were found effective against many water-borne pathogenic organisms, enteric bacteria, cysts of Entamoeba histolytica and leptospira. The initial concentration of organisms was 10^6 per ml. Iodine dosages of 7 to 8 ppm. reduced various enteric bacteria to 5 or less organisms

within 10 minutes. Iodine gave complete destruction of cysts of E. histolytica (30 per ml.) in 10 minutes. Studies showed that Leptospira icterohaemorrhagiae was destroyed within 10 minutes by 10 ppm. iodine.

Something should be said regarding the efficiency of chlorine as a disinfectant for the purification of water. Gainey and Lord (26, p. 266) "If the organisms to be destroyed are bacteria, it has been found in practice that after satisfying the demand, 0.1 to 0.2 ppm. of chlorine is adequate to accomplish purification." Elliker (16, p. 81) has disclosed the success in eliminating spoilage bacteria that may enter a dairy product, as cottage cheese, through its rinse water. It has been suggested that a water supply should be chlorinated with 5 to 10 ppm. chlorine.

MATERIALS AND METHODS

Buffers. In experiments where buffers were employed, the following systems were used at a concentration of M/50: pH 4 and 5, sodium acetate and acetic acid; pH 6 and 7, monosodium phosphate and disodium phosphate; pH 8 and 9, sodium borate and boric acid.

Diluents. Bacterial cells and yeast suspensions, for exposure to germicide, were prepared with sterile .85% saline; and suspensions of bacterial spores and bacteriophage in whey filtrates were diluted with sterile distilled water. Sterile 99 ml. tap water blanks were used for diluting yeast, bacterial cells and spore germicide mixtures following exposure to germicide. Watkins (88, p. 28) when testing various diluents of a phage whey filtrate found that a buffered saline solution gave higher plaque counts. Serial dilutions of Streptococcus cremoris phage therefore were made with 9 ml. dilution blanks of a buffered saline solution of the following composition: 8.5 g. NaCl per liter of M/100 phosphate buffer at pH 7.2.

Germicide Solutions. Stock solutions were prepared to obtain a concentration of approximately 1000 ppm. These solutions were titrated a day prior to actual testing. Appropriate dilutions were made of each stock solution on the day of germicidal testing. The germicides were

diluted with either a buffer, or distilled or hard water. Since there is a variation in natural hard waters, a standard reference water (84, p. 7020) has been used and shall be designated as U.S.D.A. hard water.

The quaternary ammonium compound (QAC) used was alkyl dimethyl ethyl benzyl ammonium chloride. The concentration was determined by a method developed by Furlong and Elliker (24, p. 226).

The hypochlorite used was a typical commercial sodium hypochlorite. New chlorine compounds which are trichloro derivatives of cyanuric acid have been evaluated and shall be designated as TCCA 1, 2, 3, 4 and 5. The main differences between the various TCCA compounds is the presence or absence of various phosphate salts. TCCA used was in powder form and hydrolyzes to form hypochlorous acid which is the active ingredient. The available chlorine of TCCA and sodium hypochlorite was determined by iodometric titrations (4, pp. 98-100).

Several different types of iodine compounds were evaluated in this study. Two different iodophors were studied. These were designated as I-1 and as I-2. Both compounds were in liquid form and the iodine was complexed with a non-ionic carrier of the poly ethylene gylcol nonyl phenol type. There was one important difference between the iodophors. I-2 contained phosphoric acid and I-1 was

a near neutral product with no added acid. The available iodine of the iodophors was determined by titrating the iodine to a colorless endpoint with a standard sodium thiosulphate solution. No starch indicator was used with these titrations. A new iodine compound just recently developed was designated as CT-KI. This compound was a mixture of chloramine-T and potassium iodide. The available iodine was determined by iodometric titrations.

When buffered, the pH level of the germicides was considered near optimum for combined activity and stability.

Inactivators. The inactivator for the QAC consisted of 2.2 g. of Asolectin, 15.6 ml. of Tween 80 and 40 ml. of M/4 phosphate buffer which was diluted to 1 liter with distilled water.

The chlorine and iodine compounds were inactivated with sodium thiosulfate. The quantity of inactivator used was dependant upon the chlorine or iodine demand. Predetermined calculations showed that 80 mg. of sodium thiosulfate per liter would inactivate 50 ppm. chlorine and 100 ppm. iodine. To prepare the inactivator the sodium thiosulfate was diluted volumetrically with distilled water containing 40 ml. of M/4 phosphate buffer per liter.

The pH of all inactivator solutions was adjusted to pH 7.2 with a Beckman Model G pH meter. Inactivator solutions were dispensed into test tubes in amounts that

would give a final volume of 9 ml. after autoclaving for 15 minutes at 15 pounds pressure.

Preparation of Bacterial and Yeast Suspensions

Twenty-four old bacterial cultures were streaked onto bottle slants of tryptone glucose yeast agar (TGY agar) and incubated as follows for 24 hours: *P. viscosa*, *P. fragi*, *P. fluorescens*, and *Alcaligenes metalcaligenes* at 25° C. and other bacteria at 30° C. for 24 hours. Sterile .85% saline was used to remove the bacterial growth and the suspensions were filtered through Whatman No. 2 filter paper to remove large particles of agar and bacterial clumps. Bacterial suspensions were standardized in a Beckman Model B spectrophotometer at a wave length of 440 mu to give the desired concentration of cells.

Forty-eight hour old yeast cultures were streaked onto bottle slants of Difco's potato dextrose agar and incubated at 30° C. To obtain a satisfactory quantity of cells, *Saccharomyces cerevisiae* var. *ellipsoideus* was incubated for 48 hours, *Candida mycoderma* and *Candida pseudotropicalis* for 72 hours. Yeast suspensions were made with sterile .85% saline and filtered through a double thickness of cheesecloth. The suspensions were standardized in the same manner as the bacterial suspensions.

Preparation of Bacterial Spore Suspensions

The spore suspensions were prepared as outlined by Miller (61, p. 53). Two-hundred and fifty ml. Erylenmeyer flasks containing 100 ml. of a nutrient broth were inoculated with 1 ml. of a 48 hour old pure culture of Bacillus globigii. The nutrient broth consisted of 5 g. of tryptone, 5 g. of yeast extract and 3 g. of beef extract which was made up to 1 liter with tap water. The pH was adjusted to pH 6.8-7.0 with a colorimeter. The organism was grown on a mechanical shaker for 7 days at a temperature of 28° C. to 30° C. The suspensions were then centrifuged, washed 3 times with sterile distilled water, resuspended and filtered through several thickness of cheesecloth to remove bacterial clumps. The suspension was heat shocked at 80° C. for 10 minutes and the stock suspension of spores was stored in a refrigerator.

Preparation of Bacteriophage Filtrate

The preparation of the phage filtrate was essentially the same as outlined by Watkins (88, p. 18). There was a slight modification of the final preparation of the phage filtrate from the precipitated milk. Watkins separated the precipitated milk protein from the whey by centrifugation. To prevent possible phage contamination,

the separation was carried out by filtration.

Phage stock filtrates of Streptococcus cremoris W strain were prepared by inoculating 200 ml. of skim milk containing 10% reconstituted dry milk solids with 1 ml. of phage filtrate and a culture of the sensitive host. For a control 200 ml. of skim milk was inoculated with the host cell. The cultures were incubated at 30° C. until the control demonstrated coagulation. When the phage-host system failed to coagulate under similar conditions of incubation it was concluded that the phage was present in significant numbers for harvest. A solution of 10% lactic acid was added to the milk containing the phage to the point of precipitation of the milk protein. The precipitated protein was removed by filtering through sterile Buchner funnels containing a filter pad of cotton between a double thickness of cheesecloth. The clear phage filtrate was sterilized subsequently by filtering through a sterile Seitz filter. The filtrate was transferred to a sterile bottle containing 1 g. of calcium carbonate to neutralize the acidity of the whey. The prepared phage filtrate was stored in the refrigerator and high titers were maintained over long periods of time.

Technique of Obtaining Standardized Mold Spores

The technique of obtaining the mold spores followed

the method of McCallan and Wilcoxin (59, pp. 13, 16) with slight modifications. This method was employed because it reduced spore germination variation due to stimulants from the substrate. The principle modifications were: (1) molds were grown on petri dishes containing Difco's potato dextrose agar instead of test tubes and (2) deionized water was used in place of distilled water.

Approximately 5 ml. of deionized water was added to 7 day old cultures and the spores were lightly rubbed off with a sterile rubber policeman. The spores were filtered through a sterile Buchner funnel containing cheesecloth to remove pieces of mycelium and dislodged agar. The spore suspension was centrifuged to remove soluble nutrients from the growing medium. The supernatant was decanted and the spores resuspended in sterile deionized water. The spore suspension was standardized with a hemocytometer to a concentration of 1 X 10^6 to 3 X 10^6 spores per ml. as recommended by McCallan.[1]

Methods of Testing Germicidal Activity

Bactericides and Sporicides. The test method of Weber and Black (90, pp. 1406-1415) with slight modifications was used to determine the efficiency of the various germicides. The germicides and test agent, bacterial cells, yeast or

---

[1]Personal communication.

bacterial spores, were made up to twice their desired final concentration. A 5 ml. aliquot of the test organism was pipetted into a 30 ml. widemouth medication bottle. At zero time, as determined by an electric timer, 5 ml. of a well mixed germicide was added to the test organism. The time exposure period or contact time was variable and dependant upon the test organism. The maximum exposure period for bacteria and yeast was 5 minutes. Since bacterial spores are more resistant, their periods of exposure were increased; the maximum contact time was for 20 minutes. After a designated period of contact, 1 ml. of the germicide-test organism mixture was withdrawn and placed into an appropriate inactivator to stop the germicidal activity. Dilutions were made from the inactivator and .1 ml. or 1 ml. aliquots were transferred to sterile petri dishes. The plates were poured with an agar medium and allowed to incubate. The time of incubation varied as follows: Bacteria and bacterial spores were incubated for 48 hours; yeast, S. ellipsoideus for 48 hours, and C. mycoderma and C. pseudotropicalis for 72 hours. The temperature of incubation was varied: M. caseolyticus, E. coli, bacterial spores and yeast were incubated at 30° C. and P. viscosa, P. fragi, P. fluorescens, and Alcaligenes metalcaligenes at 25° C.

The pH of all germicide-test organism mixtures was determined at the end of each run. All studies, with one exception, were conducted at temperatures of 25° C. P. viscosa, P. fragi, P. fluorescens, and Alcaligenes metalcaligenes were studied at temperatures approaching 0° C.

Initial and final plate counts of each test organism were determined to establish the number of organisms exposed to each germicide and to determine the percentage number of cells or spores killed.

The plating medium when testing chlorine or iodine compounds was Difco's potato dextrose agar for yeast and TGY agar for bacteria and bacterial spores. TGY agar was prepared from individual constituents and contained 5 g. of tryptone, 2.5 g. yeast extract, 1 g. glucose and 15 g. of agar per liter of tap water. The pH was adjusted with a colorimeter to pH 7.0 to 7.2. When testing QAC against yeast, bacteria or bacterial spores 1 g. of Asolectin and 7 ml. of Tween 80 was added to a liter of Difco's potato dextrose agar or TGY agar.

Sporicidal studies involving organic matter in the form of skim milk were conducted in a normal fashion but with one modification: 2.5 ml. of 4% skim milk was added to 2.5 ml. of bacterial spores 1 minute prior to the addition of 5 ml. of germicide.

Virucides. The two methods for assaying lactic phage destruction, plaque count and resazurin reduction, were those outlined by Watkins (88, pp. 23-24).

In the plaque method, after dilutions were prepared from the inactivator, 1 ml. aliquots of the phage dilution was added to .25 ml. of a 5 hour old T-19 broth culture of Streptococcus cremoris. One minute was allowed for the adsorption of the phage onto the host cells. At the end of the adsorption time 3 ml. of semi-solid T-19 agar was poured into a petri dish containing previously hardened T-19 agar. All petri dishes were incubated right side up for 12 hours at 30° C. Following incubation plaques were counted and the percent destruction of phage was determined.

The medium designated T-19 was developed by Hanneson (35, p. 72). This medium contains the following ingredients: tryptone, 20 g.; yeast extract, 5 g.; gelatin, 2.5 g.; dextrose, lactose and sucrose, 5 g. (each); NaCl, 4 g.; sodium acetate, 1.5 g. and 15 g. of agar per liter of distilled water. The pH was adjusted with a colorimeter to pH 6.8. The semi-solid agar medium contained 8 g. of agar instead of 15 g.

In the resazurin reduction method, serial dilutions were carried out to the titer where surviving phage was diluted out. One ml. of each dilution was added to duplicate tubes containing 9 ml. of skim milk, .1 ml.

resazurin solution (.05%) and 1 drop of a 5-hour old host culture. Control tubes were prepared containing no phage. A color change of bluish-purple through pink to white was the result of the reduction of resazurin by the bacterial cells. The inhibition of reduction indicated the presence of phage. Test tubes were incubated at 30° C. and observations for reduction were made at hourly intervals for 8 hours. The last dilution showing inhibition of reduction gave the titer of the surviving phage.

In both the plaque and resazurin assays the virucides and virucide-phage mixtures were maintained at 25° C.

Fungicides. A study was made to observe the activity of various compounds against mold spores and mold mycelia.

McCallan and Wellman's method (58, p. 452), with modifications, was employed for studying the fungicidal properties of compounds against mold spores. The principle modifications were: (1) contact time between spores and fungicide was 2, 5, 10, and 20 minutes, (2) fungicides were removed from spores by centrifuging at approximately 930 X G. for 20 seconds. Nine ml. of each fungicide, at one concentration level, was pipetted into a set of 4 test tubes. Each test tube represented 1 contact time, such as 2, 5, 10 or 20 minutes. At zero time 1 ml. of a known concentration of spores was added to the fungicide. One

minute prior to the termination of each contact time, the fungicide was removed from the spores by centrifuging and decanting. Approximately 10 ml. of deionized water was then added to the spores. The fungicidal action was considered to terminate upon the addition of the first wash water. The spores were centrifuged again and wash water was decanted. The spore washing process took place a total of 3 times. Finally, the spores were resuspended in 10 ml. of .3% ultrafiltered orange juice. The orange juice was used to stimulate spore germination. Two pairs of drops of each spore suspension were placed on special cleaned glass slides and the fungicidal effects were determined by the slide germination method (3). Glass slides containing spore suspensions were supported with a bent glass rod within a large moist chamber (Figure 1). At the end of 20-24 hours incubation the spores were examined microscopically for germination using either the high or low dry objective. Two-hundred spores were counted for each time period and for every compound. The count of 200 spores consisted of approximately 50 spores from each of the 4 spore suspension drops. Glass slides containing the control or untreated spore suspensions were counted. Two counts were tallied simultaneously: (1) total spores which included germinated plus non-germinated spores, and (2) germinated spores. A certain percentage of non-viable

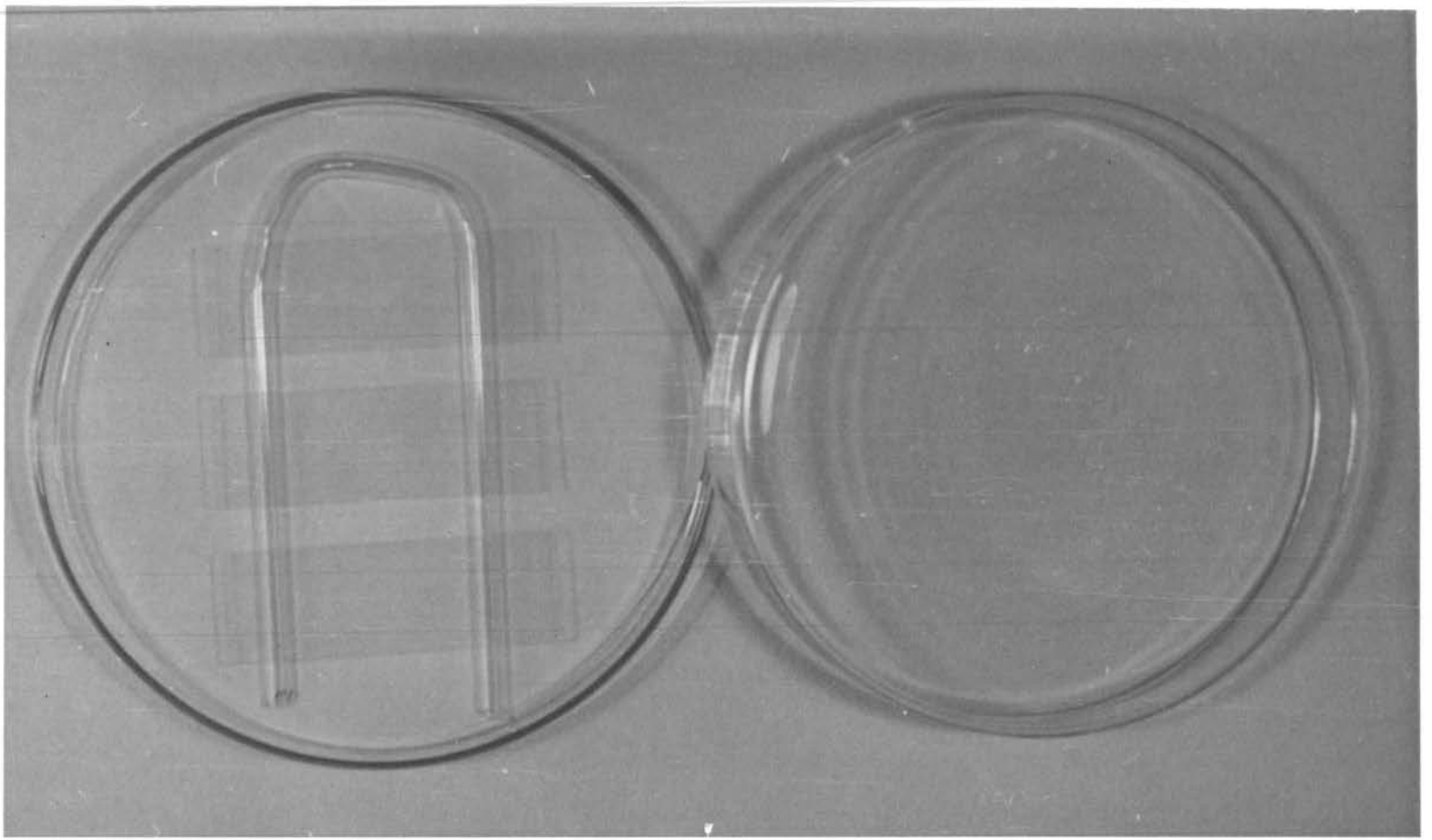


Figure 1. Moist chamber assembly for spore slide germination tests.

spores was normally encountered. The percentage of non-viable spores was considered to be the same in all spore suspensions. The total count that would give 100 viable or germinated spores was determined for each control. The same total count was applied to all treated spore suspensions. The difference between the viable cell count and 100 was considered the percent kill.

Petri dishes containing 1% plain sterile solidified agar were used to study a Penicillium species to eliminate aggregation of floating spores which would give doubtful cases of germination. The testing procedure was the same method listed above except that 4 separate drops of treated washed spore suspensions were placed on a solid agar surface instead of a glass slide. Gattani (27, p. 115) and Loegering (50, p. 932) found a high degree of reproducibility of germination using plain agar without added nutrients. They have suggested that this was due to the elimination of water drops as a germinating medium.

Ultrafiltered orange juice has been mentioned as a spore germinating stimulant. A standard procedure (2, p. 355) was followed for its preparation. The following modification was made: a Seitz filter was used instead of a Berkfeld cylinder, type W. The juice of 3 good quality oranges was filtered through cheesecloth, followed by Whatman No. 42 filter paper and finally through a Seitz

filter. Twenty-five ml. of the filtrate was diluted volumetrically to 250 ml. and 5 ml. volumes were dispensed into small sterile test tubes. This constituted 10% ultrafiltered orange juice. When needed, 15 ml. were diluted volumetrically to 500 ml. with deionized water. This constituted .3% orange juice, the concentration used for stimulating spore germination. The 10% ultrafiltered orange juice was stored at freezing temperatures.

Deionized water has been used in all mold studies because of the toxic effects exhibited by copper and other heavy metals. Horsfall (37, pp. 108-117) has given a complete review of copper and its fungicidal activities. Golworthy and Green (31, p. 495) report the effects of low concentrations of ionized copper on the germination of conidia of Sclerotina fructicola and Glomerella cingulata. The spores of both species were injured by solutions of copper at concentrations as low as .25 ppm. At this level, Sclerotina fructicola gave 16% germination and Glomerella cingulata 2%, while the controls showed 94% and 100% germination, respectively. In preparing deionized water, distilled water was passed through an Alloe deaminizer containing anion and cation exchange resins.

A new method was developed for assaying the effects of fungicides against mold mycelium. The technique reduced the neutralizing effect that organic matter

imparts on hypochlorite solutions.

Previous methods employed in botany and related fields have served as a background for the development of this technique. Fleming and Smith (21, pp. 13-15) found that cellophane disc cultures prepared by seeding mold spores on cellophane were convenient for preparing permanent culture slides. They used cellophane cultures for examination of mold spore germination. Yuill (96, p. 377) prepared cultures of Aspergillus on squares of cellophane to study conidia nuclei characteristics. Giolitti and Bertani (30, p. 281) studied the development of various members of the Actinomycetes using the cellophane culture technique. Humphrey and Fleming (39, p. 17) described a mold mycelium inhibition test. A petri dish containing solidified agar-preservative mixtures was inoculated with mycelium (5 to 6 mm. square) cut from a petri dish culture.

The method in this study consists of culturing mold on discs of sterile cellophane which are laid on the surface of an agar medium in petri plates. When cultures were developed 20 to 24 hours old and with no signs of sporulation, a heat sterilized wire (9 mm. in diameter) was used for cutting and or burning out small discs supporting mold mycelium. Disc cultures were removed with sterile forceps and placed in petri dishes containing

sterile deionized water to remove any soluble nutrients of the culture medium. The discs were placed growth side down in petri dishes containing the fungicide. Contact time between fungicide and the culture was for 20 minutes. At the end of the exposure period the cellophane discs were removed from the fungicide, rinsed in sterile deionized water to remove the chemical and placed growth side down on the surface of an agar medium. Petri plates were incubated at 25° C. for 7 days. Observations were made for growth at the periphery of each cellophane disc. The principal details of this method follow:

Test cultures in this study were representative of 3 genera: Penicillium, Aspergillus and Rhizopus. Aspergillus niger was supplied from the stock culture collection of the Bacteriology Department at O.S.C. A Penicillium sp. was isolated from a cheese storage room of the O.S.C. dairy plant and a black sporulating Rhizopus sp. was isolated from contaminated bread.

Spores used for seeding the cellophane discs were prepared from petri plate cultures incubated at 25° C. for 7 days. Plating medium used was Difco's dehydrated potato dextrose agar.

PART I

BACTERICIDAL STUDIES ON NONSPOREFORMING BACTERIA

The primary objective of this study was to establish the bacterial properties of TCCA against representatives of the more resistant species of nonsporeforming bacteria.

Various types of bactericides were used for comparison in order to evaluate the efficiency of TCCA. The reference compounds employed represented sanitizers now in use in the dairy and food industry.

NaOCl has been the most widely used sanitizer because of high activity against various microorganisms and bacterial spores. Nevertheless, the following disadvantages are associated with its use: (1) it shows low stability at low pH levels, and (2) chlorine is dissipated rapidly from solution in the presence of organic matter.

Preliminary studies showed that TCCA-4 and NaOCl were comparable in stability (Figures 2 and 3). However, all TCCA compounds have demonstrated more acidic properties than NaOCl (Table 1). These findings suggested that TCCA possessed properties that might provide some advantages as a sanitizing agent. Further study seemed justified because of its possible future importance as a germicide.

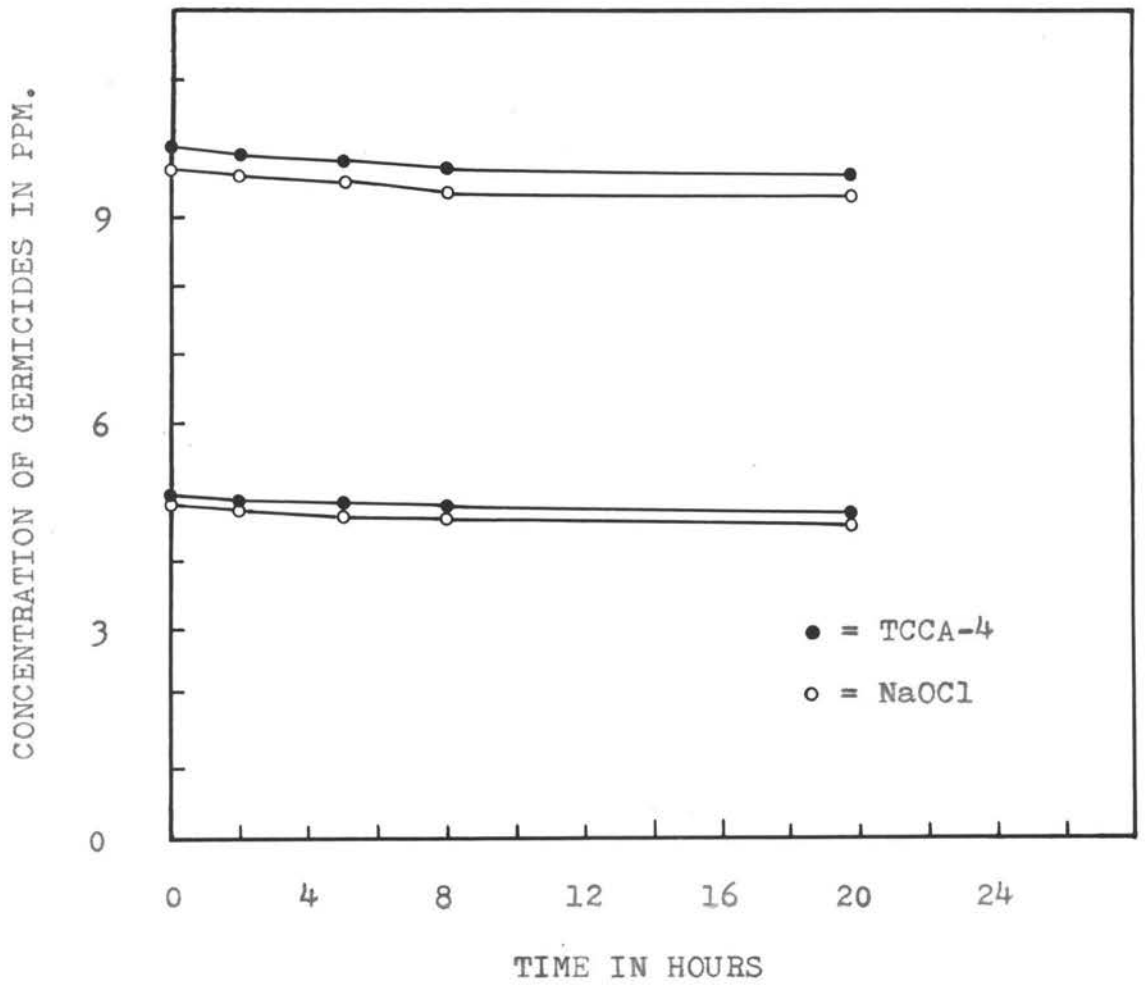


Figure 2. Comparison of the stability of 5 and 10 ppm. concentration of TCCA-4 and sodium hypochlorite at pH 4.6 at 25°C.

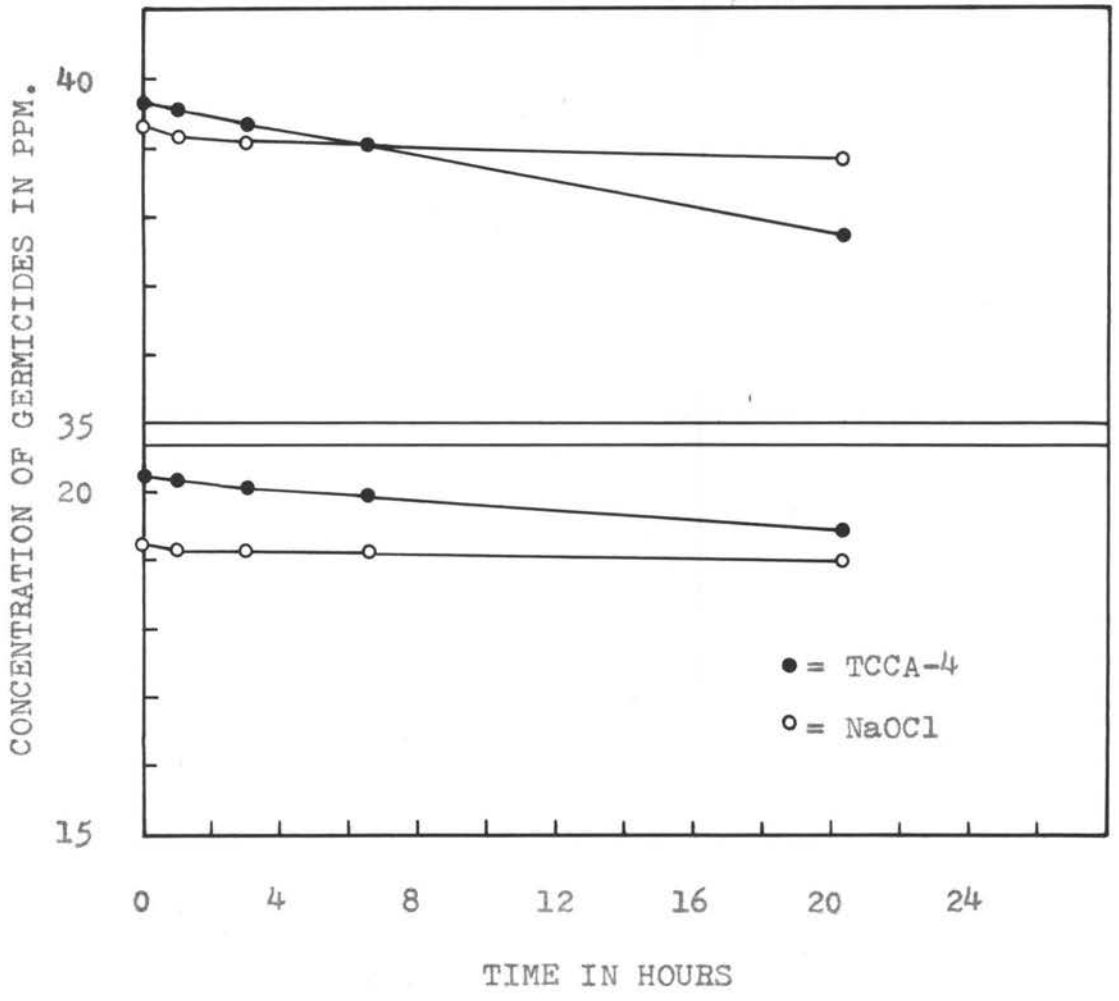


Figure 3. Comparison of the stability of 20 and 40 ppm. concentration of TCCA-4 and sodium hypochlorite at pH 4.6 at 25°C.

TABLE 1

Effect of concentration and diluent on the pH of various trichlorocyanuric acid formulations and sodium hypochlorite.

Water	Conc. germ.	TCCA-1	TCCA-2	TCCA-3	TCCA-4	TCCA-5	NaOCl
	ppm.						
Tap	5	7.3	7.4	7.0	7.15	7.4	8.0
	10	7.2	7.6	7.1	7.0	7.5	8.3
	25	7.1	7.7	6.9	6.8	7.65	8.7
	50	7.05	7.8	6.85	6.8	7.7	9.05
	100	6.95	7.8	6.9	6.8	7.75	9.4
Distilled	5	6.5	7.2	6.7	6.35	7.3	7.4
	10	6.6	7.4	6.95	6.55	7.45	8.1
	25	6.8	7.5	7.1	6.8	7.6	9.15
	50	6.8	7.6	7.2	6.8	7.7	9.55
	100	6.8	7.6	7.15	6.9	7.7	9.8

Results

Comparison of Activities of Trichlorocyanuric Acid, Sodium Hypochlorite, Iodophor and a Quaternary Ammonium Compound against M. caseolyticus and E. coli.

M. caseolyticus and E. coli were employed as representative organisms for tests on nonsporeforming types. M. caseolyticus was considered a typical thermoduric type which represents a sanitizing problem on food equipment. E. coli is one of the common test organisms used for this purpose. Both organisms were subjected to exposure to various concentrations of the bactericides for different time intervals. Results of the trials are shown in Tables 2 and 3.

Data showing the rate of destruction of TCCA-1, TCCA-2, TCCA-3, TCCA-4 and TCCA-5 as compared to NaOCl against M. caseolyticus have not been tabulated because complete destruction was obtained in all but one trial. Bactericides were studied at concentrations of 12.5 and 25 ppm. In all cases except with TCCA-2 at 12.5 ppm., 100% was obtained within 15 seconds. TCCA-2 required 30 seconds for 100% destruction.

Comparisons also were made with four bactericides: TCCA-5, NaOCl, QAC, and I-1, in concentrations of 12.5 and 25 ppm. at pH values considered to provide high activity

TABLE 2

Rate of destruction of Micrococcus caseolyticus by buffered trichlorocyanuric acid, sodium hypochlorite, a quaternary ammonium compound and an iodophor. Average of duplicate trials.

Germicide	Conc.	Final pH	Percent cells[1] killed at following exposure periods in seconds:				
			15 sec.	30 sec.	60 sec.	120 sec.	300 sec.
	ppm.		(%)	(%)	(%)	(%)	(%)
TCCA-5	12.5	8.0	100.000	100.000	100.000	100.000	100.000
	25.0	8.0	100.000	100.000	100.000	100.000	100.000
NaOCl	12.5	8.0	100.000	100.000	100.000	100.000	100.000
	25.0	8.05	100.000	100.000	100.000	100.000	100.000
QAC	12.5	8.0	96.405	99.785	99.997	100.000	100.000
	25.0	8.0	99.989	100.000	100.000	100.000	100.000
I-1	12.5	5.0	100.000	100.000	100.000	100.000	100.000
	25.0	5.0	100.000	100.000	100.000	100.000	100.000

[1]Average concentration of cells exposed --- 115 X 10^6 per ml.

TABLE 3

Rate of destruction of Escherichia coli by buffered trichlorocyanuric acid, sodium hypochlorite, a quaternary ammonium compound and an iodophor. Average of duplicate trials.

Germicide	Conc.	Final pH	Percent cells[1] killed at following exposure periods in seconds:				
			15 sec.	30 sec.	60 sec.	120 sec.	300 sec.
	ppm.		(%)	(%)	(%)	(%)	(%)
TCCA-5	12.5	8.0	100.000	100.000	100.000	100.000	100.000
	25.0	8.0	100.000	100.000	100.000	100.000	100.000
NaOCl	12.5	8.0	100.000	100.000	100.000	100.000	100.000
	25.0	8.0	100.000	100.000	100.000	100.000	100.000
QAC	12.5	7.95	18.250	20.380	35.792	52.924	70.521
	25.0	8.0	49.718	53.374	82.711	94.734	99.979
I-1	12.5	5.0	100.000	100.000	100.000	100.000	100.000
	25.0	5.0	100.000	100.000	100.000	100.000	100.000

[1]Average concentration of cells exposed --- 159 X 10^6 per ml.

of the compounds. M. caseolyticus was employed as the test agent. Table 2 shows the results of duplicate trials. TCCA-5, NaOCl, and I-1 at 12.5 ppm. gave 100% destruction within 15 seconds. The QAC was the least active bactericide. At 12.5 ppm., the QAC required 120 seconds for 100% kill.

Results not tabulated show that concentrations of 12.5 and 25 ppm. of all TCCA compounds and NaOCl gave 100% kill with E. coli exposed for the minimum period of 15 seconds. Table 3 shows a comparison of activity of TCCA-5, NaOCl, a QAC and I-1 at 12.5 and 25 ppm. TCCA-5, NaOCl and I-1 gave 100% destruction of E. coli in 15 seconds at a concentration of 12.5 ppm. The QAC showed the least activity; 100% kill was not obtained within 5 minutes at concentrations of 25 ppm.

Effect of Number of Bacteria in Test Suspensions on Viability during Extended Holding Periods at Low Temperatures.

During a preliminary bactericidal study, untreated saline suspensions of P. fluorescens and P. fragi at 2° C. showed decreases in titer within the 2-hour test period. These observations were made on suspensions containing low numbers of cells prepared at 25° C. from suspensions of concentrations of approximately 200 X 10^6 cells per ml. The number of viable cells was determined by plate count

at the end of 48 hours of incubation at 25° C. Such decreases in titer were considered undesirable because they raised the possibility of loss of cells through other factors than the bactericides during test periods.

Experiments were conducted to explain the reduction that had occurred in cell numbers. Results indicated the following measures were necessary to remedy reductions in viability in cell suspensions prior to bactericidal tests: (1) cell suspensions containing approximately 200 X 10^6 cells per ml. should be placed in an ice bath until their temperatures reach 0° C. and (2) subsequent cell dilutions should be made in .85% saline at 0° C. from suspensions containing at least 200 X 10^6 cells per ml.

Bactericidal Property of Trichlorocyanuric Acid, Sodium Hypochlorite and an Iodophor on Water Bacteria at 2° C.

Reports have suggested that water supplies used for the washing of butter and cottage cheese could harbor Pseudomonas and Alcaligenes species that affect the keeping quality of these products. Water supplies are employed at temperatures approximating 2° to 5° C. to reduce temperature of butter or curd. The pH of the water supply frequently is reduced to accelerate activity of bactericides and provide more desirable curd characteristics in cottage cheese manufacture. Chlorination of water at

5 to 10 ppm. usually is considered sufficient to destroy spoilage bacteria in most water supplies. This study compares the activity of TCCA-4 with compounds that have been recommended for water sanitation. In order to similate plant conditions, the germicides were studied at low concentrations and at high and low pH values. Numbers of organisms used in the test suspensions approximate those frequently encountered under practical conditions.

Results not tabulated show that concentrations of 5 ppm. of NaOCl and TCCA-4 at pH 4.6, 6.0 and 8.0 produced 100% kill in 30 seconds with P. fragi, P. fluorescens, P. viscosa, and A. metalcaligenes at concentrations of approximately 10,000 cells per ml. Determinations were not made with I-2 at pH 4.6. However under the test conditions it also accomplished 100% kill in 30 seconds at pH 6.0 and 8.0.

Table 4 shows the effect of TCCA-4, NaOCl and I-2 on the same organisms at a higher pH level such as might be encountered in highly alkaline water supplies. The bactericides were employed in concentrations of 5 ppm. and at pH values of approximately 10. There were no significant differences in the activities of TCCA-4 and NaOCl. Both compounds gave 100% kill in 30 seconds. Higher resistance to I-2 was shown. The rate of the destruction by I-2 for the 300-second exposure was 97.6% for P. fragi,

TABLE 4

Rate of destruction of Pseudomonas fragi, Pseudomonas fluorescens, Pseudomonas viscosa and Alcaligenes metalcaligenes by 5 ppm. of a buffered trichlorocyanuric acid compound, sodium hypochlorite and an iodophor.

Organism	Germicide	Final pH	Percent cells[1] killed at following exposure periods in seconds: 30 sec. (%)	60 sec. (%)	120 sec. (%)	300 sec. (%)
P. fragi	TCCA-4	9.9	100	100	100	100
	NaOCl	9.8	100	100	100	100
	I-2	9.8	11.5	39.1	74.4	97.6
P. fluorescens	TCCA-4	9.85	100	100	100	100
	NaOCl	9.8	100	100	100	100
	I-2	9.85	6.1	32.4	69.8	99.5
P. viscosa	TCCA-4	9.95	100	100	100	100
	NaOCl	9.9	100	100	100	100
	I-2	9.9	0	1.5	39.7	90.2
A. metalcaligenes	TCCA-4	9.85	100	100	100	100
	NaOCl	9.8	100	100	100	100
	I-2	9.8	2.8	1.6	0	30

[1]Concentrations of cells exposed:

P. fragi --- 76 X 10^2 per ml.
P. fluorescens --- 137 X 10^2 per ml.
P. viscosa --- 95 X 10^2 per ml.
A. metalcaligenes --- 126 X 10^2 per ml.

99.5% for P. fluorescens, 90.2% for P. viscosa and 30% for A. metalcaligenes. At the end of 30 seconds of exposure the percent kill of these organisms was less than 12%.

Discussion

The data presented indicates that the bactericidal efficiency of TCCA generally was as great as that of NaOCl. TCCA and NaOCl were found to be more effective than I-2 at pH 10. Cantor and Shelanski (10, p. 135) found with certain vegetative cells that 50 ppm. of an iodophor was equivalent to a hypochlorite of 200 ppm. available chlorine. These results were based on a capacity test. The superiority of I-2 could indicate that it was less adversely affected by the additions of successive increments of organic matter. The decrease in activity of I-2 in the present studies could be attributed to hydrolysis of molecular iodine to the less active hypoiodous acid. Wyss and Strandskov (95, pp. 261, 264) have shown that an equilibrium exists between hypoiodous acid and molecular iodine in aqueous solutions as follows:

$$I_2 + H_2O \rightleftharpoons HOI + I^- + H^+$$

The sporicidal action of iodine was considered primarily the result of molecular iodine. At pH values above 7.5 hypoiodous acid concentrations and killing time increased.

The results of the slow rate of destruction of *E. coli* and *M. caseolyticus* by the QAC when compared to the action of a NaOCl confirms the work of Hays (36, p. 67) and Miller (61, p. 45).

In preliminary experiments to study methods of holding organisms to be subjected to bactericidal tests, the rate of mortality due to temperature changes seemed to be related to cell concentration. Cells in high concentrations, as compared to those in low numbers, were less affected by sudden temperature changes. The effect of cell concentration upon the rate of mortality of bacteria when exposed to unfavorable conditions has been implied by several workers. Watkins and Winslow (87, p. 250) studied the germicidal effect of sodium hydroxide on different concentrations of bacteria. They noted that the slope of the survivor curves were more abrupt with lower concentrations of cells. It has been suggested that the lower rate of mortality associated with high cell concentrations was due to the production by the cells of substances which formed a protective zone about each cell.

With low numbers of organisms, approximately 10,000 cells per ml. and pH of approximately 10, *A. metalcaligenes*, demonstrated greater resistance to I-2 than *P. viscosa*, *P. fluorescens* or *P. fragi*. These results suggest that

A. metalcaligenes was less susceptible to the action of hypoiodous acid which is present in iodine solutions at higher pH levels.

PART II

SPORICIDAL STUDIES ON BACILLUS GLOBIGII

Numerous investigations have been cited in the literature showing the use of bacterial spores as test agents for the evaluation of compounds as sporicides. Rudolph and Levine (80, p. 39) have described the following advantages for their use: (1) spores tend to show less variation in resistance when compared to vegetative cells and (2) the highly resistant properties of spores permits the study of temperature, pH, concentration, and other factors that can affect the efficiency of sporicides. The advantages of using bacterial spores as test agents and their association with food spoilage suggested that the following studies be made.

The spores of B. globigii have been chosen as the test agent because sporicidal studies of Hays (36, pp. 37-40) has shown that these organisms were very resistant and produced easily counted colonies.

Results

Effect of Variations in pH on Activity of Trichlorocyanuric Acid.

Data presented by various investigators amply illustrate the tremendous effect that pH exerts upon the

germicidal efficiency of most chlorine solutions. The sporicidal activity of TCCA-5 was studied at various pH levels to establish the range at which it was most effective.

Figures 4 and 5 show the rate of destruction of B. globigii spores by TCCA-5 in concentrations of 100 and 200 ppm. in a pH range of 5 to 9. At both concentrations the sporicidal activity of TCCA-5 was shown to increase with decrease in pH.

At a concentration of 100 ppm. the sporicidal activity of TCCA-5 was optimum at pH 7.4 or less; 100% kill occurred within 10 minutes. At pH 8.2 there was slightly less activity while at pH 8.9 less than 75% destruction occurred in 20 minutes.

TCCA-5 at 200 ppm. was most active at pH 7.8 or less. At this pH range 100% kill occurred in 5 minutes. At pH 8.2 there was slightly less destruction and at pH 8.9 only 86% kill occurred in 20 minutes.

Comparison of Various Buffered Trichlorocyanuric Acid Compounds and other Sporicides.

Various TCCA compounds were included in this study to show any differences in sporicidal activity of compounds containing the same active ingredient but slightly different formulations. Several different types of sporicides

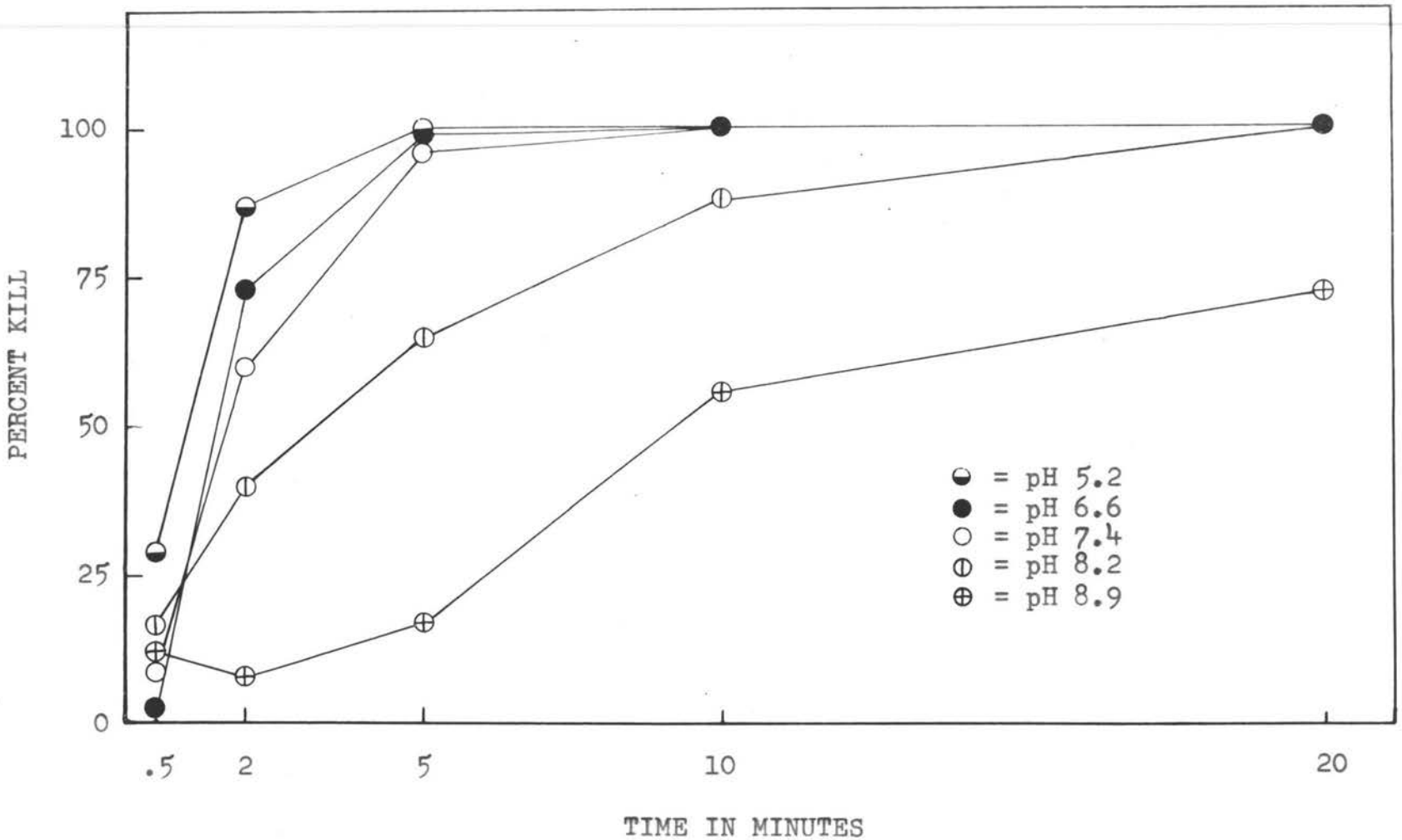


Figure 4. Effect of pH on destruction of spores of B. globigii by 100 ppm. TCCA-5. Concentration of spores exposed -- 108 x 10^3 per ml.

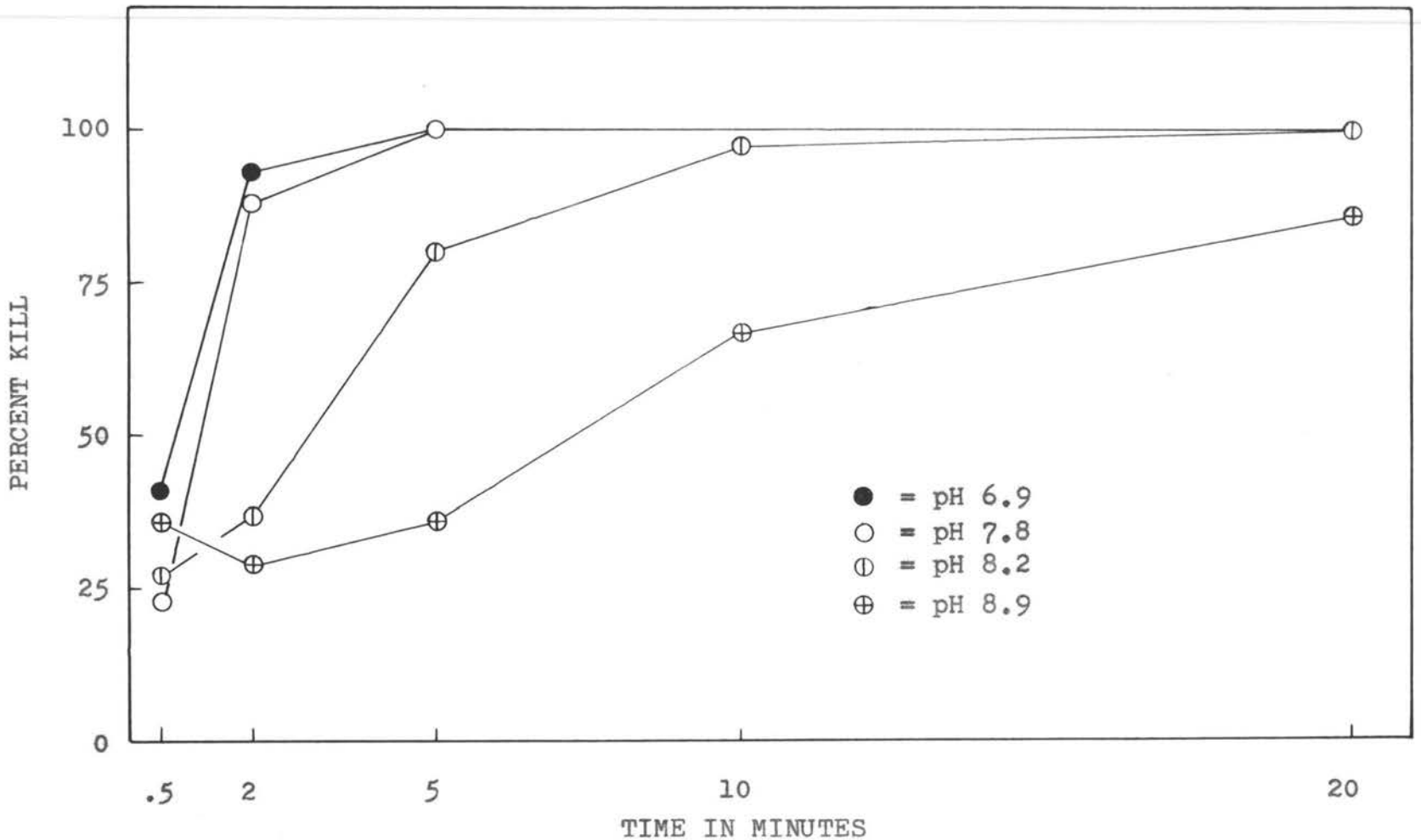


Figure 5. Effect of pH on destruction of spores of B. globigii by 200 ppm. TCCA-5. Concentration of spores exposed -- 123 x 10^3 per ml.

that are used for dairy and food plant sanitation were employed to evaluate the efficiency of TCCA. To properly evaluate the sporicidal activities of the germicides required that they would be tested under identical conditions. The chlorine germicides were buffered at pH 8.0 and I-1 at pH 5.0 to provide high activity of the compounds. Results of the trials are shown in Tables 5 and 6.

Table 5 shows the rate of destruction of various TCCA compounds as compared to NaOCl in concentrations of 100 and 200 ppm. and buffered at pH 8.0. The results show the average of triplicate trials. NaOCl showed slightly greater sporicidal activity than the TCCA compounds. NaOCl failed to give 100% destruction of the spores in 5 minutes but killed 100% in 10 minutes in concentrations of 200 ppm. The TCCA compounds demonstrated small differences in activity. TCCA-1, TCCA-3 and TCCA-4 showed slightly greater activity than TCCA-2 and TCCA-5.

Results in Table 6 show the average of triplicate trials. The data indicate that the order of activity of 4 different germicides at 100 and 200 ppm. was as follows: NaOCl > TCCA-5 > I-1 > QAC. The NaOCl gave 100% kill in 20 minutes at 100 ppm. TCCA-5, I-1 and QAC gave less than 100% destruction in 20 minutes at concentrations of 200 ppm.

TABLE 5

Rate of destruction of spores of Bacillus globigii by various buffered trichlorocyanuric acid compounds and sodium hypochlorite. Average of triplicate trials.

Germicide	Conc.	Final pH	Percent spores[1] killed at following exposure periods in minutes: .5 min.	2 min.	5 min.	10 min.	20 min.
	ppm.		(%)	(%)	(%)	(%)	(%)
TCCA-1	100	8.05	20.939	36.663	79.594	93.383	99.254
	200	8.0	31.634	62.502	94.563	99.808	99.997
TCCA-2	100	8.2	27.042	32.445	59.544	80.209	96.874
	200	8.2	29.000	44.819	77.174	96.808	99.602
TCCA-3	100	8.15	26.977	40.121	71.233	94.643	99.250
	200	8.1	26.881	64.466	95.242	99.677	99.981
TCCA-4	100	8.1	21.629	38.273	74.329	92.430	99.709
	200	8.2	26.745	59.286	91.656	99.769	99.990
TCCA-5	100	8.3	31.579	42.677	51.094	76.337	90.073
	200	8.25	31.224	24.428	53.872	93.352	99.786
NaOCl	100	8.1	21.864	75.966	98.303	99.976	99.969
	200	8.05	53.794	89.130	99.976	100.000	100.000

[1]Average concentration of spores exposed --- 116 X 10^3 per ml.

TABLE 6

Rate of destruction of spores of Bacillus globigii by buffered trichlorocyanuric acid, sodium hypochlorite, a quaternary ammonium compound and an iodophor. Average of triplicate trials.

Germicide	Conc.	Final pH	Percent spores[1] killed at following exposure periods in minutes.				
			.5 min.	2 min.	5 min.	10 min.	20 min.
	ppm.		(%)	(%)	(%)	(%)	(%)
TCCA-5	100	8.1	16.000	43.893	78.497	97.501	99.989
	200	8.2	25.347	64.552	95.448	99.839	99.996
NaOCl	100	8.1	39.686	78.737	94.188	99.965	100.000
	200	8.1	67.991	96.313	99.995	99.996	100.000
QAC	100	8.0	16.549	21.648	27.701	33.064	44.257
	200	8.0	21.929	13.412	25.013	29.956	32.403
I-1	100	5.2	37.655	52.852	68.952	66.363	82.648
	200	5.1	40.832	52.713	61.569	72.962	80.493

[1]Average concentration of spores exposed --- 109 X 10^3 per ml.

The Effect of Phosphate Addition on the Sporicidal Activity of Trichlorocyanuric Acid at pH 8.0.

Differences were observed in the sporicidal activity of TCCA-1, TCCA-2, TCCA-3, TCCA-4 and TCCA-5 when under similar test conditions (Table 5). It was apparent that some factor in the more active TCCA compounds (TCCA-1, TCCA-3 and TCCA-4) was causing the increased germicidal activity. Sodium tripolyphosphate was incorporated in all the TCCA formulations which gave the greater kill. The association of tripolyphosphate with the more active TCCA compounds suggested that it was the factor responsible for the higher rates of kill.

A new TCCA formulation was prepared which contained TCCA-5 plus 50% by weight of sodium tripolyphosphate. The amount of salt added to TCCA-5 was equivalent to that present in the more active TCCA compounds. When the germicide was adjusted to give a final concentration of 200 ppm. available chlorine, the final concentration of tripolyphosphate was 710 ppm. All test solutions were buffered at pH 8.0. Results in Figure 6 suggest that the activity of tripolyphosphate may have been additive to the effect of TCCA-5. The activity of TCCA-5 plus tripolyphosphate shows greater kill than TCCA-5 alone. The diluent, a borate buffer, gave no kill in 20 minutes.

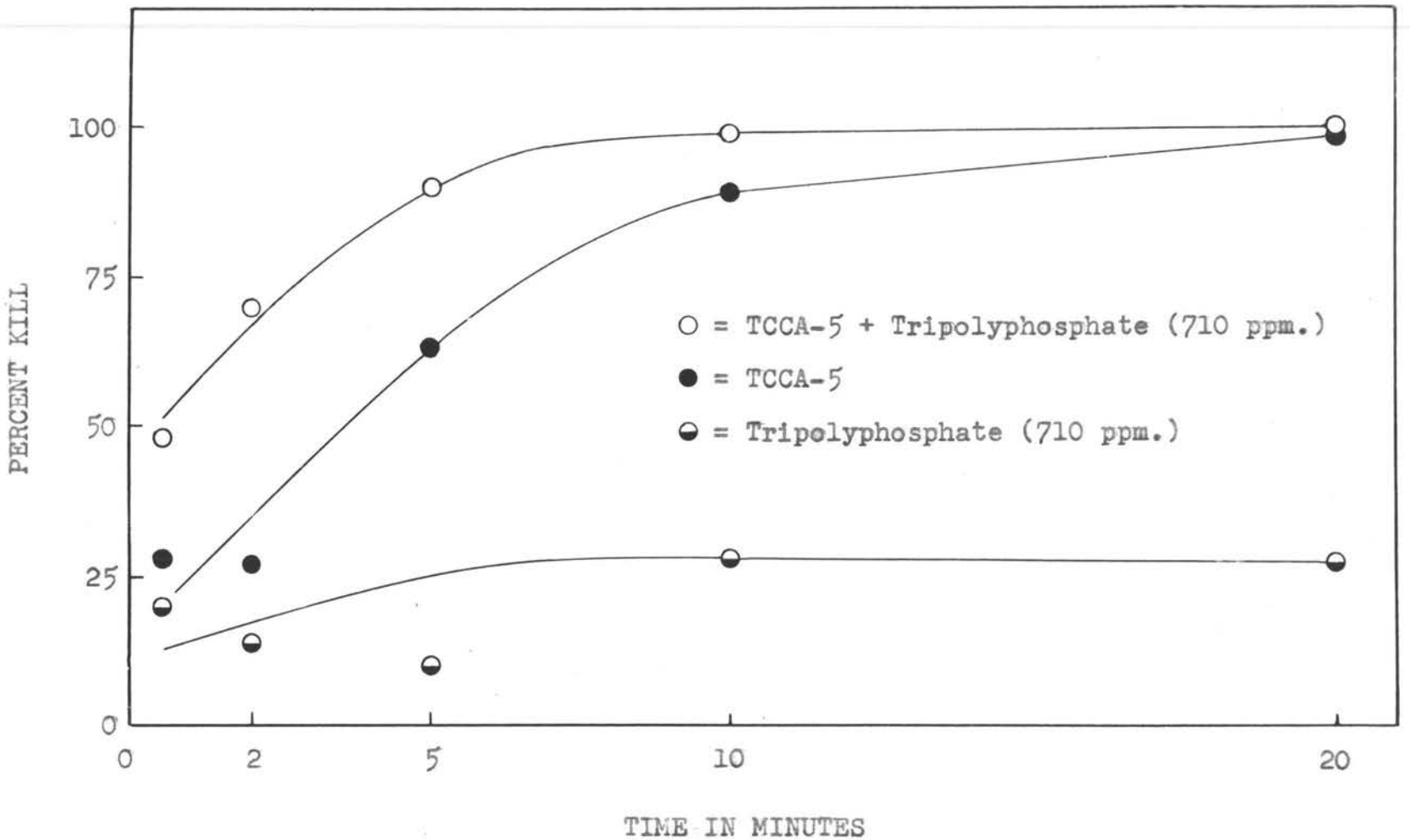


Figure 6. Effect of added phosphate on the sporicidal activity of 200 ppm. buffered TCCA-5 against spores of B. globigii. Average concentration of spores exposed -- 100 x 10^3 per ml.

Comparison of Various Unbuffered Trichlorocyanuric Acid Compounds and Sodium Hypochlorite.

Germicides in practical application are prepared by dilution in tap water. In view of these conditions, an experiment was performed where germicides of the desired concentration were prepared in tap water.

Table 7 compares the effect on bacterial spores of TCCA-1, TCCA-2, TCCA-3, TCCA-4 and NaOCl. These results are the averages of duplicate trials. In a concentration of 100 and 200 ppm. NaOCl showed less sporicidal activity than TCCA. NaOCl at 200 ppm. gave less than 100% destruction in 20 minutes. All TCCA compounds at 200 ppm., with the exception of TCCA-2, gave 100% kill within 20 minutes.

The Effect of Organic Matter on Various Buffered Trichlorocyanuric Acid Compounds and other Sporicides.

Under recommended sanitizing procedures, equipment and utensils are cleaned and rinsed prior to chemical treatment. In some instances a germicide must act in presence of small amounts of organic matter left after insufficient cleaning. Tests have been conducted with organic matter to determine the efficiency of sporicides under adverse conditions. Results of these studies are shown in Tables 8 and 9.

TABLE 7

Rate of destruction of spores of Bacillus globigii by various unbuffered trichloro-cyanuric acid compounds and sodium hypochlorite. Average of duplicate trials.

Germicide	Conc.	Final pH	Percent spores[1] killed at following exposure periods in minutes.				
			.5 min.	2 min.	5 min.	10 min.	20 min.
	ppm.		(%)	(%)	(%)	(%)	(%)
TCCA-1	100	7.15	45.087	84.234	99.726	99.987	99.995
	200	7.1	62.835	98.707	99.996	100.000	100.000
TCCA-2	100	8.1	37.721	40.506	77.258	93.236	99.920
	200	8.1	27.860	58.342	92.209	99.787	99.996
TCCA-3	100	7.6	31.344	80.617	99.869	99.987	100.000
	200	7.4	61.973	99.180	100.000	99.996	100.000
TCCA-4	100	7.5	31.237	84.862	99.796	99.995	100.000
	200	7.4	67.935	98.995	99.991	100.000	100.000
NaOCl	100	8.6	25.670	30.016	33.157	49.305	66.285
	200	9.0	36.214	23.657	27.141	38.440	74.095

[1]Average concentration of spores exposed --- 118 X 10^3 per ml.

TABLE 8

Rate of destruction of spores of Bacillus globigii in 1 percent skim milk by various buffered trichlorocyanuric acid compounds and sodium hypochlorite. Average of triplicate trials.

Germicide	Conc.	Final pH	Percent spores[1] killed at following exposure periods in minutes:				
			.5 min.	2 min.	5 min.	10 min.	20 min.
	ppm.		(%)	(%)	(%)	(%)	(%)
TCCA-1	200	7.4	25.925	38.665	37.600	51.441	72.973
TCCA-2	200	7.9	32.031	32.647	39.078	42.377	58.806
TCCA-3	200	7.5	25.810	40.380	44.183	65.740	81.529
TCCA-4	200	7.25	23.941	47.486	47.399	62.910	76.465
TCCA-5	200	7.8	28.332	33.008	46.689	49.494	58.210
NaOCl	200	7.7	47.591	82.953	96.859	99.730	99.949

[1]Average concentrations of spores exposed --- 133 X 10^3 per ml.

TABLE 9

Rate of destruction of spores of Bacillus globigii, in 1 percent skim milk, by trichlorocyanuric acid, sodium hypochlorite, a quaternary ammonium compound and an iodophor. Average of triplicate trials.

Germicide	Conc.	Final pH	Percent spores[1] killed at following exposure periods in minutes:				
			.5 min.	2 min.	5 min.	10 min.	20 min.
	ppm.		(%)	(%)	(%)	(%)	(%)
TCCA-5	200	7.9	36.474	37.443	53.024	63.940	80.381
NaOCl	200	7.7	70.941	97.484	99.980	99.993	99.984
QAC	200	7.9	87.423	96.111	96.862	98.979	99.304
I-1	200	5.1	37.701	55.871	58.957	69.292	79.779

[1]Average concentration of spores exposed --- 133 X 10^3 per ml.

The data in Table 8 show the effect of organic matter (1% skim milk) on the action of 200 ppm. of TCCA-1, TCCA-2, TCCA-3, TCCA-4, TCCA-5 and NaOCl. The results are the average of triplicate trials. The results show that the activity of TCCA is below that of NaOCl. However, none of the compounds gave 100% kill within a time period of 20 minutes. No striking differences were noted in the activity of the various TCCA compounds.

Table 9 shows the rate of kill by TCCA-5, NaOCl, I-1 and QAC during various time intervals and in the presence of 1% skim milk. The data indicate that NaOCl and the QAC were similar in activity and more efficient than TCCA-5 or I-1. I-1 and TCCA-5 gave similar results on the spores. However, none of the compounds effected 100% kill within a period of 20 minutes.

Discussion

The data reported emphasize the tremendous importance of pH on the germicidal efficiency of TCCA-5. The effect of pH on the action of TCCA-5 parallels the work of Rudolph and Levine (62, p. 25). These investigators have shown with spores of B. metiens that the killing time of calcium hypochlorite decreases with decrease in pH.

All TCCA compounds have shown less activity for spores of B. globigii than NaOCl under similar conditions of pH

and concentration. TCCA hydrolyzes in aqueous solution with formation of hypochlorous acid. If the germicidal effect of TCCA is due to release of hypochlorous acid, then the lower rate of destruction could be due to incomplete hydrolysis.

TCCA formulations have shown differences in action on spores of B. globigii under similar conditions of pH and concentration. The more efficient TCCA compounds, TCCA-1, TCCA-3 and TCCA-4, contain sodium tripolyphosphate. This salt has shown a slight degree of toxicity for the bacterial spores.

MacGregor (51, pp. 63-64) has shown evidence to support the idea that chelating agents, including the polyphosphates, may affect germicidal action of QAC's by influencing cell permeability. However, there is insufficient information available on the bacterial spore coat to indicate that its permeability might be affected by tripolyphosphate.

Tests have consistently shown the QAC to be more active for B. globigii spores in the presence of organic matter than in its absence. The most likely reason for this unusual occurrence is that the organic matter may have lowered the resistance of the spores by initiating first stages of germination. Studies on effect of organic matter on QAC action on nonsporeforming types indicate that organic matter interferes with the QAC action on the cell.

PART III

VIRUCIDAL STUDIES OF *STREPTOCOCCUS CREMORIS* W PHAGE

Investigations by Watkins (88, p. 61) has shown that TCCA was very effective against 144 F strains of *S. cremoris*.

Parker and Elliker (70, p. 52) report a variation in resistance to virucides by different lactic phage strains. Under the conditions of their test, the most resistant phage was W strain of *S. cremoris*.

Since it has been demonstrated in numerous studies that phage infection may be controlled in part through plant sanitation, it seemed advisable to study the effect of TCCA as a virucide for the destruction of one of the more resistant lactic phages.

Results

Plaque Count Studies Comparing Various Buffered and Unbuffered Trichlorocyanuric Acid Compounds with other Virucides.

Collins (13, p. 901) has shown the effects of small numbers of phage particles for their host cells. The addition of 3 particles to .5% host culture in skim milk resulted in mass lysis of the host bacteria. The plaque

count method was selected as an assay method because the technique yields quantitative results. This should be a consideration since under plant conditions the presence of a few resistant phage particles could have serious consequences.

Results of this study, not tabulated, indicated that 10 ppm. of TCCA-1, TCCA-2, TCCA-3, TCCA-4 and NaOCl effected 100% inactivation of S. cremoris W phage in 15 seconds. The virucides were prepared in buffer at pH 8.0 and in distilled water.

Comparisons also were made with four virucides: TCCA-5, NaOCl, QAC, and I-1 in concentration of 25 ppm. The chlorine compounds and the QAC were buffered at pH 8.0 and I-1 at pH 5.0. The same compounds were compared in activity when unbuffered. Table 10 shows that TCCA-5 and NaOCl gave 100% destruction of the phage within 15 seconds. When the virucides were buffered, the QAC was the next active compound and I-1 the least active. I-1 was more active than the QAC when prepared in distilled water. I-1 gave 100% kill in 30 seconds and the QAC required 5 minutes for 100% destruction.

TABLE 10

Rate of destruction of Streptococcus cremoris W phage by 25 ppm. trichlorocyanuric acid, sodium hypochlorite, quaternary ammonium compound and iodophor. Results based on plaque counting method. Average of duplicate trials.

Germicide	Final pH		Percent phage[1] killed at following exposure periods in minutes:				
	Unbuffered	Buffered	15 sec. (%)	30 sec. (%)	60 sec. (%)	120 sec. (%)	300 sec. (%)
TCCA-5	7.5		100.000	100.000	100.000	100.000	100.000
		8.2	100.000	100.000	100.000	100.000	100.000
NaOCl	7.1		100.000	100.000	100.000	100.000	100.000
		8.2	100.000	100.000	100.000	100.000	100.000
QAC	7.05		88.857	98.257	99.943	99.988	100.000
		8.0	95.945	98.564	99.727	99.975	99.992
I-1	7.0		99.992	100.000	100.000	100.000	100.000
		5.1	91.636	95.909	98.200	98.764	99.909

[1]Concentration of phage exposed --- 55 X 10^5 particles per ml.

Resazurin Reduction Studies Comparing Various Buffered and Unbuffered Trichlorocyanuric Acid Compounds with other Virucides.

This work was carried out to determine if the virucides used with the plaque method would give the same order of activity when compared by a different assay method. Table 11 compares the effect of TCCA-1, TCCA-2, TCCA-3, TCCA-4 and TCCA-5 with NaOCl, a QAC and I-1 on bacteriophage. The virucides were unbuffered and diluted in distilled water to concentrations of 25 ppm. The results show that all TCCA compounds were equivalent to NaOCl; these compounds gave 100% kill in 15 seconds. I-1 was the next most active compound providing 100% destruction within 30 seconds. The QAC was the least active virucide; 60 seconds were required for 100% inactivation.

Discussion

The results of this study show that the virucidal activity of I-1 was greater at pH 7 than at pH 5. Raising the pH of an iodine solution will increase the conversion of free iodine to iodide and hypoiodous acid. Results suggest the phage to be more sensitive to the hydrolysis products of molecular iodine than to free iodine.

TABLE 11

Rate of destruction of Streptococcus cremoris W phage by 25 ppm. unbuffered trichlorocyanuric acid, sodium hypochlorite, quaternary ammonium compound and iodophor. Results based on resazurin reduction method. Average of duplicate trials.

Germicide	Final pH	Percent phage[1] killed at following exposure periods in minutes:				
		15 sec.	30 sec.	60 sec.	120 sec.	300 sec.
		(%)	(%)	(%)	(%)	(%)
TCCA-1	6.65	100.000	100.000	100.000	100.000	100.000
TCCA-2	7.5	100.000	100.000	100.000	100.000	100.000
TCCA-3	6.2	100.000	100.000	100.000	100.000	100.000
TCCA-4	6.45	100.000	100.000	100.000	100.000	100.000
TCCA-5	7.9	100.000	100.000	100.000	100.000	100.000
NaOCl	7.3	100.000	100.000	100.000	100.000	100.000
QAC	6.5	99.583	99.996	100.000	100.000	100.000
I-1	6.25	99.995	100.000	100.000	100.000	100.000

[1]Concentration of phage exposed --- 24 X 10^5 particles per ml.

The plaque count and resazurin reduction in tubes of milk are two common methods for the enumeration of lactic bacteriophage. Results suggest that both methods are comparable in showing the order of activity of different virucides.

PART IV

COMPARATIVE EFFICIENCY OF VARIOUS GERMICIDES IN DESTRUCTION OF YEASTS

Little information was found in the literature indicating the germicidal efficiency of sanitizers for yeasts. This study was conducted to provide information on the germicidal activity of various compounds against yeasts that are involved in spoilage of food products.

Reddish (75, pp. 492, 493) shows that hard water salts can affect the activity of certain germicides. Water hardness can be quite variable over a given area, ranging from soft water (50 ppm. or less) to hard water (150 ppm. or more). It appeared desirable therefore to compare germicides both at low and high levels of hardness. Distilled water was included to represent soft water and a U.S.D.A. synthetic water preparation to represent hard water.

Results

Destruction of Low Numbers of Yeasts

The effects of various germicides was studied on low concentrations of yeasts, approximately 100 X 10^3 cells per ml. Lower concentrations of yeasts than bacteria were

employed because of their probable greater resistance to sanitizers and also because yeasts are found on food equipment in lower numbers. The following yeasts were selected for this study: C. mycoderma, C. pseudotropicalis and S. ellipsoideus. The activity of 4 different germicides: TCCA-4, NaOCl, a QAC and I-2, in concentrations of 12.5 and 25 ppm., is shown in Tables 12, 13, and 14. The results of each table represent triplicate trial runs.

Table 12 shows the activity of 4 germicides against C. mycoderma. The results indicate that the order of effectiveness was as follows: I-2 > NaOCl > TCCA-4 > QAC. With the exception of the QAC all compounds at 25 ppm. accomplished 100% destruction in 15 seconds. The QAC at 25 ppm. required 60 seconds for 100% destruction.

A comparison of the action of 4 germicides on C. pseudotropicalis is shown in Table 13. The trend in activity of the germicides was similar to the results obtained with C. mycoderma. However, C. pseudotropicalis was more resistant to the action of TCCA-4, NaOCl and the QAC. The iodophor, I-2, in concentration of 12.5 ppm., accomplished 100% destruction in 15 seconds. The NaOCl at 12.5 ppm. was slightly more active than TCCA-4; 100% kill occurred in 60 seconds. TCCA-4 required 120 seconds for 100% destruction of the cells. The QAC was the least

TABLE 12

Rate of destruction of Candida mycoderma by buffered trichlorocyanuric acid, sodium hypochlorite, quaternary ammonium compound and an iodophor. Average of triplicate trials.

Germicide	Conc.	Final pH	Percent cells[1] killed at following exposure periods in seconds:				
			15 sec.	30 sec.	60 sec.	120 sec.	300 sec.
	ppm.		(%)	(%)	(%)	(%)	(%)
TCCA-4	12.5	8.0	90.947	99.969	100.000	100.000	100.000
	25.0	7.9	100.000	100.000	100.000	100.000	100.000
NaOCl	12.5	8.0	96.775	99.994	100.000	100.000	100.000
	25.0	7.9	100.000	100.000	100.000	100.000	100.000
QAC	12.5	8.0	77.695	100.000	100.000	100.000	100.000
	25.0	7.9	99.988	99.997	100.000	100.000	100.000
I-2	12.5	5.05	100.000	100.000	100.000	100.000	100.000
	25.0	5.0	100.000	100.000	100.000	100.000	100.000

[1]Average concentration of cells exposed --- 249 X 10^3 per ml.

TABLE 13

Rate of destruction of Candida pseudotropicales by buffered trichlorocyanuric acid, sodium hypochlorite, quaternary ammonium compound and an iodophor. Average of triplicate trials.

Germicide	Conc.	Final pH	Percent cells[1] killed at following exposure periods in seconds:				
			15 sec.	30 sec.	60 sec.	120 sec.	300 sec.
	ppm.		(%)	(%)	(%)	(%)	(%)
TCCA-4	12.5	7.9	64.102	95.061	99.944	100.000	100.000
	25.0	7.9	97.126	99.953	100.000	100.000	100.000
NaOCl	12.5	8.0	95.373	99.962	100.000	100.000	100.000
	25.0	7.95	99.966	100.000	100.000	100.000	100.000
QAC	12.5	8.0	45.391	84.677	99.294	99.995	100.000
	25.0	7.9	98.571	99.991	100.000	100.000	100.000
I-2	12.5	5.0	100.000	100.000	100.000	100.000	100.000
	25.0	5.0	100.000	100.000	100.000	100.000	100.000

[1]Average concentration of cells exposed --- 95 X 10^3 per ml.

TABLE 14

Rate of destruction of Saccharomyces ellipsoideus by buffered trichlorocyanuric acid, sodium hypochlorite, quaternary ammonium compound and an iodophor. Average of triplicate trials.

Germicide	Conc.	Final pH	Percent cells[1] killed at following exposure period in seconds.				
			15 sec.	30 sec.	60 sec.	120 sec.	300 sec.
	ppm.		(%)	(%)	(%)	(%)	(%)
TCCA-4	12.5	8.0	21.724	58.600	97.609	99.996	100.000
	25.0	7.9	89.992	99.983	100.000	100.000	100.000
NaOCl	12.5	8.0	51.036	95.991	100.000	100.000	100.000
	25.0	7.9	99.437	100.000	100.000	100.000	100.000
QAC	12.5	8.0	8.759	25.098	75.179	99.334	99.993
	25.0	7.9	59.857	98.587	99.990	100.000	100.000
I-2	12.5	5.0	100.000	100.000	100.000	100.000	100.000
	25.0	5.0	100.000	100.000	100.000	100.000	100.000

[1]Average concentration of cells exposed --- 123 X 10^3 per ml.

active germicide; at 12.5 ppm., 300 seconds was required for 100% kill.

S. ellipsoideus (Table 14) showed more resistance to the action of TCCA-4, NaOCl, and the QAC than C. pseudotropicalis. The order of germicidal action was the same as in studies with C. mycoderma and C. pseudotropicalis. I-2 was the most active germicide; at 12.5 ppm., 100% kill occurred in 15 seconds. NaOCl was more active than TCCA-4 and the QAC was the least active germicide. Comparing the activity of NaOCl and TCCA-4 at 12.5 ppm. shows the following relationship: NaOCl required 60 seconds for 100% destruction and TCCA-4 required 300 seconds for 100% kill. The QAC at 12.5 ppm. did not accomplish 100% kill in 300 seconds.

Destruction of High Numbers of Yeasts

This study was made to determine the germicidal activity of sanitizing agents under adverse conditions. Investigators have reported that the greater the number of organisms the more difficult it was to sterilize areas of contamination. The following germicides were used in this investigation: TCCA-4, NaOCl, I-2, a QAC and CT-KI. In the study with low numbers of yeasts, C. mycoderma was found to be the most susceptible and S. ellipsoideus the most resistant to the action of the germicides. These

yeasts were used in the present study. The effectiveness of the germicides was determined at 2 levels of concentration in the presence of 500 ppm. U.S.D.A. synthetic hard water and in distilled water. The results of this study are shown in Tables 15, 16, 17, and 18. Each table shows the results of triplicate trial runs.

Tables 15 and 16 show the activity of 5 germicides against C. mycoderma. In distilled water (Table 15) the results suggest the following order of activity: CT-KI > NaOCl > I-2 > TCCA-4 > QAC. In concentrations of 12.5 and 25 ppm. TCCA-4, NaOCl, CT-KI, and I-2 were equivalent in germicidal activity after the 30 second period. These germicides gave 100% destruction of the yeast in 60 seconds. The QAC at 50 ppm. failed to accomplish complete destruction in 300 seconds. In U.S.D.A. hard water (Table 16) CT-KI and I-2 at 12.5 ppm. accomplished 100% destruction in 30 seconds. NaOCl was more active than TCCA-4. At 12.5 ppm. NaOCl effected 100% kill in 60 seconds. TCCA-4 failed to accomplish 100% destruction at 12.5 ppm. for an exposure period of 300 seconds. The QAC was the least active germicide; 50 ppm. required 300 seconds for 100% kill.

Tables 17 and 18 show the activity of various germicides on S. ellipsoideus. In distilled water (Table 17)

TABLE 15

Rage of destruction of Candida mycoderma by trichlorocyanuric acid, sodium hypochlorite, quaternary ammonium compound, iodophor and chloramine-T plus potassium iodide. Germicides were prepared in distilled water. Average of triplicate trials.

Germicide	Conc.	Final pH	Percent cells[1] killed at following exposure periods in seconds:				
			15 sec.	30 sec.	60 sec.	120 sec.	300 sec.
	ppm.		(%)	(%)	(%)	(%)	(%)
TCCA-4	12.5	5.6	99.676	99.942	100.000	100.000	100.000
	25.0	6.0	100.000	100.000	100.000	100.000	100.000
NaOCl	12.5	6.4	99.934	99.999	100.000	100.000	100.000
	25.0	6.8	100.000	100.000	100.000	100.000	100.000
QAC	25.0	5.3	18.534	24.138	65.517	93.491	99.258
	50.0	5.6	89.405	98.349	99.612	99.941	99.986
I-2	12.5	3.0	99.933	99.988	100.000	100.000	100.000
	25.0	2.7	99.991	100.000	100.000	100.000	100.000
CT-KI	12.5	5.9	99.994	99.999	100.000	100.000	100.000
	25.0	5.9	99.998	100.000	100.000	100.000	100.000

[1]Average concentration of cells exposed --- 58 X 10^5 per ml.

TABLE 16

Rate of destruction of Candida mycoderma by trichlorocyanuric acid, sodium hypochlorite, quaternary ammonium compound, iodophor and chloramine-T plus potassium iodide. Germicides prepared in U.S.D.A. hard water (500 ppm.). Average of triplicate trials.

Germicide	Conc.	Final pH	Percent cells[1] killed at following exposure periods in seconds:				
			15 sec.	30 sec.	60 sec.	120 sec.	300 sec.
	ppm.		(%)	(%)	(%)	(%)	(%)
TCCA-4	12.5	7.8	67.843	99.430	99.996	99.999	99.997
	25.0	7.6	99.529	99.998	100.000	100.000	100.000
NaOCl	12.5	7.8	99.823	99.999	100.000	100.000	100.000
	25.0	7.7	100.000	100.000	100.000	100.000	100.000
QAC	25.0	7.7	0.538	23.235	57.157	84.167	99.441
	50.0	7.8	72.902	97.490	99.929	99.997	100.000
I-2	12.5	6.9	99.997	100.000	100.000	100.000	100.000
	25.0	5.8	99.999	100.000	100.000	100.000	100.000
CT-KI	12.5	7.7	99.999	100.000	100.000	100.000	100.000
	25.0	7.5	100.000	100.000	100.000	100.000	100.000

[1]Average concentration of cells exposed --- 51 X 10^5 per ml.

TABLE 17

Rate of destruction of Saccharomyces ellipsoideus by trichlorocyanuric acid, sodium hypochlorite, quaternary ammonium compound, iodophor and chloramine-T plus potassium iodide. Germicides prepared in distilled water. Average of triplicate trials.

Germicide	Conc.	Final pH	Percent cells[1] killed at following exposure periods in seconds:				
			15 sec.	30 sec.	60 sec.	120 sec.	300 sec.
	ppm.		(%)	(%)	(%)	(%)	(%)
TCCA-4	12.5	5.4	62.703	99.027	99.999	100.000	100.000
	25.0	5.7	95.233	99.941	100.000	100.000	100.000
NaOCl	12.5	6.0	99.882	100.000	100.000	100.000	100.000
	25.0	6.6	100.000	100.000	100.000	100.000	100.000
QAC	25.0	4.8	24.594	20.946	14.189	31.351	61.756
	50.0	5.2	64.324	81.756	99.067	99.995	100.000
I-2	12.5	3.1	100.000	100.000	100.000	100.000	100.000
	25.0	2.8	100.000	100.000	100.000	100.000	100.000
CT-KI	12.5	5.2	100.000	100.000	100.000	100.000	100.000
	25.0	5.5	100.000	100.000	100.000	100.000	100.000

[1]Average concentration of cells exposed --- 37 X 10^5 per ml.

TABLE 18

Rate of destruction of Saccharomyces ellipsoideus by trichlorocyanuric acid, sodium hypochlorite, quaternary ammonium compound, iodophor and chloramine-T plus potassium iodide. Germicides prepared in U.S.D.A. hard water (500 ppm.). Average of triplicate trials.

Germicide	Conc.	Final pH	Percent cells[1] killed at following exposure periods in seconds:				
			15 sec.	30 sec.	60 sec.	120 sec.	300 sec.
	ppm.		(%)	(%)	(%)	(%)	(%)
TCCA-4	12.5	7.6	49.687	99.651	100.000	100.000	100.000
	25.0	7.4	93.859	99.999	100.000	100.000	100.000
NaOCl	12.5	7.8	98.495	100.000	100.000	100.000	100.000
	25.0	7.7	100.000	100.000	100.000	100.000	100.000
QAC	25.0	7.5	6.093	34.687	61.562	99.281	99.940
	50.0	7.6	78.906	99.203	99.973	100.000	100.000
I-2	12.5	6.9	99.253	99.963	100.000	100.000	100.000
	25.0	5.6	99.914	100.000	100.000	100.000	100.000
CT-KI	12.5	7.7	99.898	99.998	100.000	100.000	100.000
	25.0	7.5	99.991	100.000	100.000	100.000	100.000

[1]Average concentration of cells exposed --- 32 X 10^5 per ml.

CT-KI and I-2 were equivalent in germicidal activity. At 12.5 and 25 ppm. both compounds effected 100% destruction in 15 seconds. NaOCl was more active than TCCA-4. At 12.5 ppm. NaOCl gave 100% kill in 30 seconds. TCCA-4 in concentrations of 12.5 ppm. required 120 seconds for 100% destruction. The QAC was the least active germicide; at 50 ppm. the germicide required 300 seconds to destroy 100% of the cells. In hard water (Table 18) the order of germicidal activity was as follows: NaOCl > CT-KI > I-2 > TCCA-4 > QAC. The differences in activity of the germicides, excluding the QAC, were at the 15 and 30 second exposure periods. At 12.5 ppm. NaOCl accomplished 100% destruction in 30 seconds. CT-KI, I-2 and TCCA-4, in concentration of 12.5 ppm., required 60 seconds to effect 100% kill. The QAC at 50 ppm. required 120 seconds to accomplish 100% destruction.

Discussion

S. ellipsoideus, in low concentrations, was more resistant to the action of TCCA-4, NaOCl, and the QAC than C. mycoderma or C. pseudotropicalis. The germicidal resistance of S. ellipsoideus could not be related to sporulation since stains failed to reveal ascospores. It therefore appeared to represent an inherent difference between species of yeasts.

A study involving high numbers of yeasts has brought out a relationship between pH, species of yeast and germicidal activity of I-2 and CT-KI. S. ellipsoideus was more susceptible to a low pH level of I-2 and CT-KI and C. mycoderma to a high pH level. CT-KI as a powder contains chloramine-T and potassium iodide which react together when dissolved in water to provide free iodine. When hydrolyzed, iodine is in equilibrium with hypoiodous acid. Raising the pH of an iodophor or CT-KI increases the concentration of hypoiodous acid. The possibility exists that S. ellipsoideus is more susceptible to molecular iodine and C. mycoderma to hypoiodous acid.

The germicidal action of the QAC was not reduced in the presence of hard water ions which suggests that the cell sites were not inactivated with the ions and were free to adsorb the QAC. This observation is of considerable interest in view of the marked difference in effectiveness of QAC against bacteria in presence and absence of hard water ions.

C. mycoderma, C. pseudotropicalis and S. ellipsoideus appear less susceptible to the action of TCCA-4, NaOCl and QAC than M. caseolyticus, a representative bacterial species. The difference in resistance might be related to the structure of the cells. Yeast cells are much larger than bacterial cells and possess relatively thick

cell walls. The reduced activity of the QAC could be related to the proportionally smaller surface area of the yeast cells. MacGregor (51, pp. 62, 63) suggested that the killing effect of a QAC depended upon the amount coming in contact with a cell. He found that an adsorption of 9.05×10^7 molecules was required to kill a single cell of P. aeruginosa.

PART V

FUNGICIDAL STUDIES OF MOLD MYCELIA AND MOLD SPORES

At the present time molds represent one of the most serious causes of food spoilage. Therefore, destruction of mold spores and mycelia on equipment and building surfaces represents an important problem in preventing entrance of molds to food products during production and processing. Relatively little information is available on the effectiveness of various food equipment sanitizing agents against mold spores and mycelia.

Horsfall and Rick (38, pp. 316-318) reported that a number of heterocyclic nitrogen compounds had fungitoxic properties. Schuldt and Wolf (81, pp. 383-391) have demonstrated that certain derivatives of TCCA have high fungicidal activity. No information was found in the literature relating the fungicidal activity of TCCA against molds. Therefore, a study was conducted with TCCA against mold mycelia and mold spores. To aid this investigation a method was developed to test the action of fungicidal compounds on mold mycelia.

Results

Comparison of Activities of 4 Fungicides Against Spores of Asperigillus niger, Penicillium sp., and Rhizopus sp.

A study was conducted to determine the fungicidal activity of various sanitizing compounds against mold spores. Table 19 shows the effect of TCCA-4, NaOCl, I-2 and CT-KI on 7 day spores of 3 different genera of molds. The fungicides were prepared in deionized water in concentrations of 25 ppm.

Results with spores of Aspergillus niger show that the order of fungicidal activity was as follows: I-2 > CT-KI > NaOCl > TCCA-4. I-2 was the most active compound providing 100% spore destruction in 5 minutes. At the 5 minute exposure period TCCA-4 was less active than NaOCl. However, the 2 fungicides showed similar activities at other exposure periods. Both compounds required 20 minutes for 100% kill. CT-KI was slightly less active than I-2 during the 2 minute and 5 minute exposure periods; however, 100% kill occurred in 10 minutes.

I-2 and CT-KI showed equivalent fungicidal activity against spores of Penicillium sp. Both compounds accomplished 100% kill within a 2 minute exposure period. TCCA-4 was slightly less active effecting 100% destruction

TABLE 19

Rate of destruction of spores of Aspergillus niger, Penicillium sp. and Rhizopus sp. by 25 ppm. trichlorocyanuric acid, sodium hypochlorite, an iodophor, and chloramine-T plus potassium iodide. Average of duplicate trials.

Organisms	Fungicides	Final pH	Percent spores[1] killed at following exposure periods in minutes: 2 min.	5 min.	10 min.	20 min.
			(%)	(%)	(%)	(%)
A. niger	TCCA-4	7.55	0	50	98	100
	NaOCl	6.4	0	15	98	100
	I-2	2.8	94	100	100	100
	CT-KI	6.1	74	98	100	100
Penicillium sp.	TCCA-4	7.5	0	0	69	100
	NaOCl	6.6	0	89	100	100
	I-2	2.8	100	100	100	100
	CT-KI	6.2	100	100	100	100
Rhizopus sp.	TCCA-4	7.9	10	66	99	100
	NaOCl	6.45	9	92	93	100
	I-2	2.7	99	100	100	100
	CT-KI	6.3	12	29	74	97

[1]Following concentration of spores were exposed: A. niger --- 219 X 10^4 per ml. Penicillium sp. --- 247 X 10^4 per ml. Rhizopus sp. --- 216 X 10^4 per ml.

in 10 minutes and NaOCl showed the slowest activity requiring 20 minutes for 100% kill.

The order of fungicidal activity against spores of Rhizopus sp. was as follows: I-2 > TCCA-4 > NaOCl > CT-KI. I-2 Gave 100% destruction in 5 minutes. TCCA-4 and NaOCl required 20 minutes for 100% kill. CT-KI failed to show 100% destruction; however, 97% kill occurred in 20 minutes.

Comparison of Activities of 4 Fungicides Against Mycelia of Aspergillus niger, Penicillium sp., and Rhizopus sp.

Information on comparative effect of fungicides is important both on spores and mycelia. The previous section indicated that 4 fungicides initiated 100% destruction of mold spores, with one exception, in 20 minutes. This study was carried out to learn if mold mycelia were more or less resistant than spores of the same species. Table 20 and Figures 7, 8 and 9 show the rate of destruction by TCCA-4, NaOCl, I-2 and CT-KI on 20 to 24 hour old mycelia of 3 genera of molds during an exposure period of 20 minutes. The fungicides were prepared in deionized water at various concentrations ranging from 25 ppm. to 600 ppm. Presence or absence of growth following exposure was used as an index of fungicidal activity.

Results with Aspergillus niger show that TCCA-4, NaOCl, I-2 and CT-KI were toxic for the mycelia in concentrations

TABLE 20

Rate of destruction of mycelia of Aspergillus niger, Penicillium sp. and Rhizopus sp. by various concentrations of trichlorocyanuric acid, sodium hypochlorite, an iodophor, and chloramine-T plus potassium iodide during a 20 minute exposure period.

Fungicide	Conc.	Final pH	Organism		
			A. niger	Penicillium sp.	Rhizopus sp.
	ppm.				
TCCA-4	25	7.05	-	+	+
	50	7.0	-	+	+
	100	7.0	-	+	-
	200	6.9	-	-	-
	600	6.75	-	-	-
NaOCl	25	9.25	-	+	+
	50	9.7	-	+	+
	100	9.9	-	-	+
	200	10.2	-	-	-
	600	10.4	-	-	-
I-2	25	2.75	+	+	-
	50	2.5	-	-	-
	100	2.2	-	-	-
	200	2.0	-	-	-
	600	1.6	-	-	-
CT-KI	25	6.6	-	+	+
	50	6.6	-	-	+
	100	6.6	-	-	-

\+ = Growth

\- = No Growth

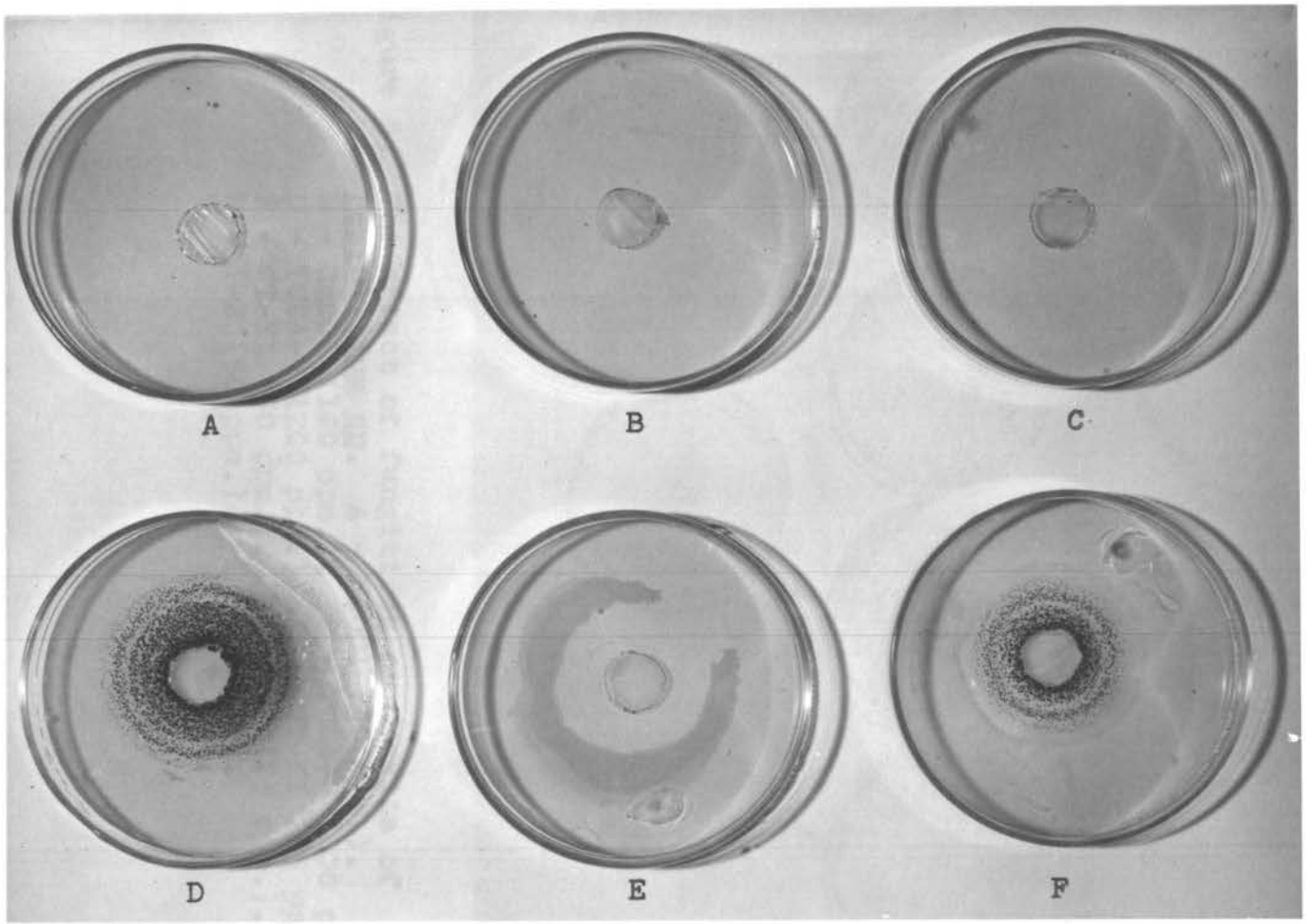


Figure 7. The effect of fungicides on mycelia of Aspergillus niger.
A - NaOCl (25 ppm.). B - TCCA-4 (25 ppm.). C - CT-KI (25 ppm.).
D - I-2 (25 ppm.). E - I-2 (50 ppm.). F - control.

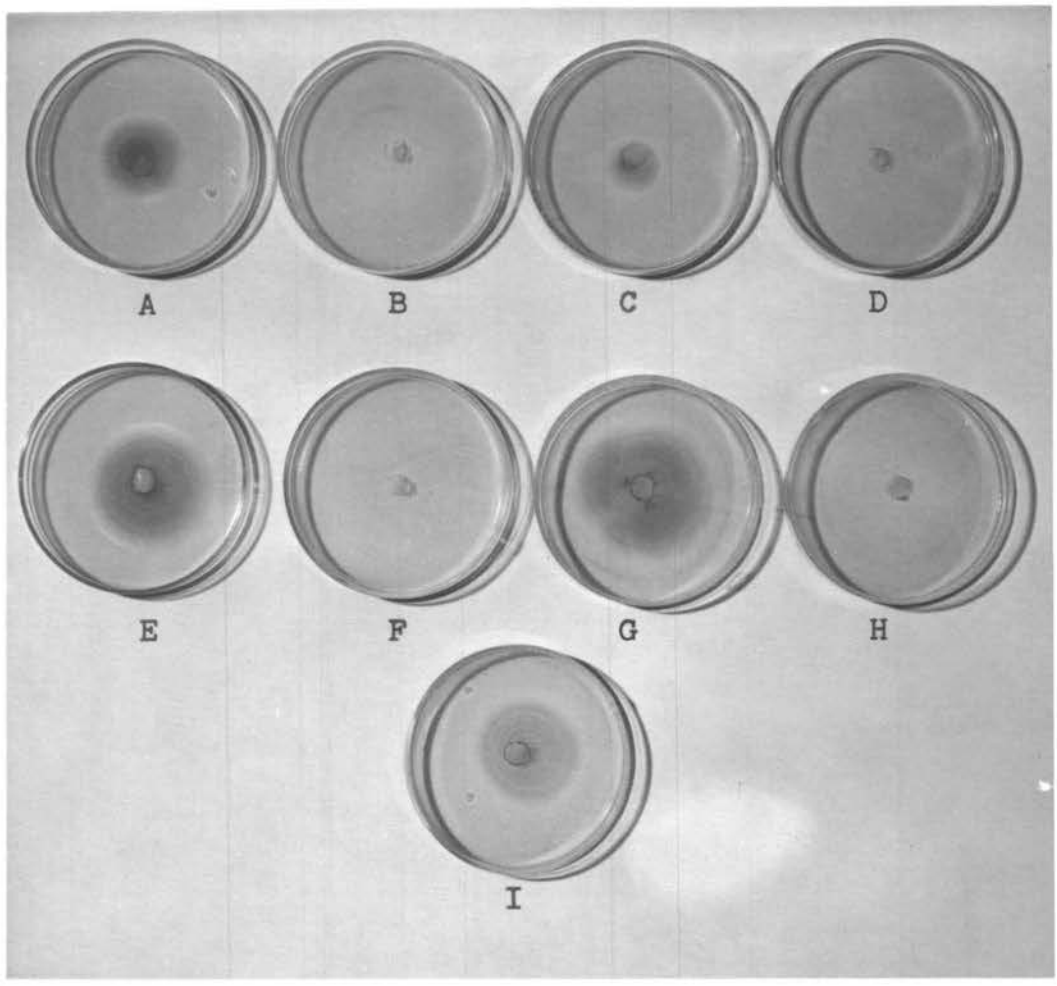


Figure 8. The effect of fungicides on mycelia of Penicillium sp. A - NaOCl (50 ppm.). B - NaOCl (100 ppm.). C - TCCA-4 (100 ppm.). D - TCCA-4 (200 ppm.). E - CT-KI (25 ppm.). F - CT-KI (50 ppm.). G - I-2 (25 ppm.). H - I-2 (50 ppm.). I - control.

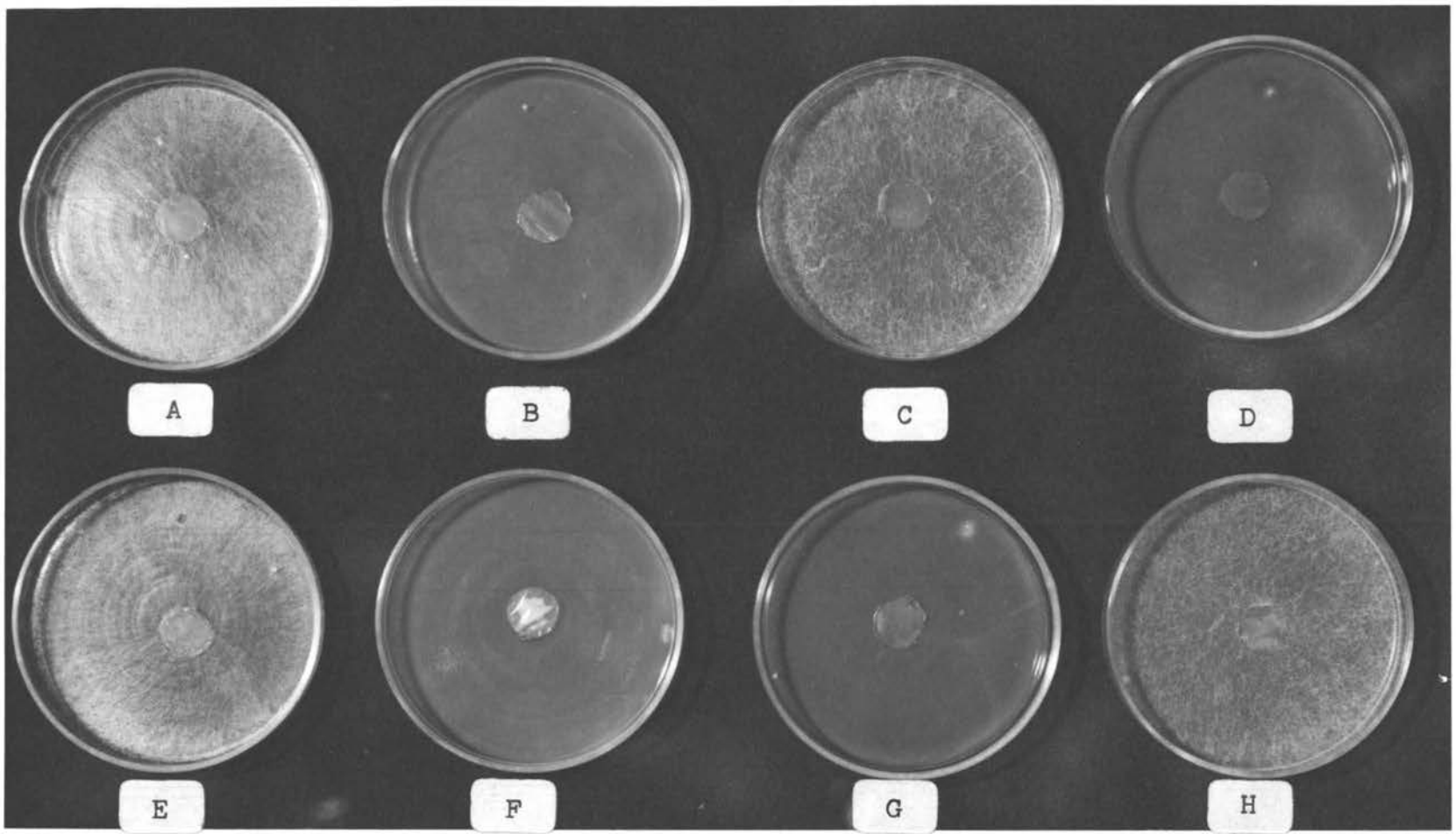


Figure 9. The effect of fungicides on mycelia of Rhizopus sp. A - NaOCl (100 ppm.). B - NaOCl (200 ppm.). C - TCCA-4 (50 ppm.). D - TCCA-4 (100 ppm.). E - CT-KI (50 ppm.). F - CT-KI (100 ppm.). G - I-2 (25 ppm.). H - control.

of 25 ppm. I-2 destroyed mold mycelia at 50 ppm. but not at 25 ppm.

I-2 and CT-KI showed equivalent fungicidal activity against mycelia of Penicillium sp. Both compounds in concentrations of 50 ppm. destroyed mycelia. NaOCl showed slightly less activity; concentrations of 100 ppm. available chlorine were required to destroy mycelia. TCCA-4 showed the least activity as growth occurred after exposure to 100 ppm. but not at 200 ppm.

I-2 showed more fungicidal activity for mycelia of Rhizopus sp. than TCCA-4, NaOCl and CT-KI. CT-KI and TCCA-4 were more active than NaOCl. I-2 showed fungicidal activity in a concentration of 25 ppm. available iodine. TCCA and CT-KI demonstrated equivalent fungicidal action. Both compounds destroyed the mycelia at concentrations of 50 ppm. but not at 25 ppm. NaOCl was not active at 100 ppm. but destroyed mycelia at 200 ppm.

Discussion

McCallan and Wellman (42, pp. 454, 455) report that iodine had a lower LD_{50} value in ppm. than NaOCl for spores of Penicillium expansum and Rhizopus nigricans during short contact times. The results of this study support the finding of McCallan and Wellman. The iodine compounds, with the exception of CT-KI against Rhizopus sp.,

showed greater activity for mold spores during early contact periods than the chlorine compounds.

Few investigators have described the comparative effects of the same fungicides on both mycelia and spores of the same organism. Results of this investigation suggested that mycelia of the test organisms were in some instances more resistant than the spores of the same species in the presence of fungicides. This was apparent when the mycelia of the molds showed growth after being exposed to 25 ppm. fungicide during a period of 20 minutes. Explanation for these differences may be due to: (1) cell structure, since species of Penicillium and Aspergillus are multicellular whereas mold spores are single cells and (2) mold mycelium as a mass of intertwined hypae could be less readily penetrated by the fungicide than free floating mold spores.

SUMMARY AND CONCLUSIONS

Bactericidal studies indicated that TCCA-1, TCCA-3, TCCA-4 and TCCA-5 were equivalent in activity to NaOCl against M. caseolyticus. The germicides effected 100% destruction of this organism in 15 seconds. TCCA-2 at 12.5 ppm. showed less activity than TCCA-1, TCCA-3, TCCA-4, TCCA-5 and NaOCl. All TCCA compounds were equivalent to NaOCl in germicidal action against E. coli. The germicides effected 100% destruction in 15 seconds. TCCA-5 was equivalent to NaOCl and I-1 against M. caseolyticus and E. coli. The 3 different germicides accomplished 100% destruction in 15 seconds. The QAC was the slowest acting bactericide against M. caseolyticus and E. coli.

Results indicated that the following measures were necessary to remedy reductions of viable bacteria during extended test periods at low temperatures: (1) suspensions containing approximately 200 X 10^6 cells per ml. should be placed in an ice bath until temperatures reach approximately 0° to 2° C. and (2) subsequent cell dilutions should be made in .85% saline at 0° to 2° C. from suspensions containing at least 200 X 10^6 cells per ml.

A bactericidal study was made with TCCA-4, NaOCl and I-2 on P. fragi, P. fluorescens, P. viscosa and A. metalcaligenes. The germicides were studied at 2° C. in

concentrations of 5 ppm. and at low and high pH values. The number of organisms used in the test suspensions was approximately 10,000 cells per ml. Results show that TCCA-4 and NaOCl, at pH 4.6, 6.0, 8.0 and 10, accomplished 100% destruction in 30 seconds. I-2 effected 100% destruction at pH 6.0 and 8.0 but failed to accomplish 100% kill at pH 10. The decrease in activity of I-2 might be attributed to hydrolysis of molecular iodine to the less active hypoiodous acid.

The data reported emphasize the importance of pH on the sporicidal activity of TCCA-5 against spores of B. globigii. At 100 and 200 ppm. an increase in kill followed a decrease in pH. Results demonstrated that a pH of 7.8 or less was more sporicidal than higher pH levels.

Buffered NaOCl showed slightly greater sporicidal activity than TCCA-1, TCCA-2, TCCA-3, TCCA-4, and TCCA-5, a QAC and I-1. TCCA-5 was more active than QAC and I-1. The QAC was the least active sporicide. All TCCA compounds demonstrated relatively minor differences in activity. TCCA-1, TCCA-3 and TCCA-4 showed slightly greater activity than TCCA-2 and TCCA-5. The more efficient TCCA compounds contained sodium tripolyphosphate. This salt has shown a slight degree of toxicity for the bacterial spores. There is insufficient information on the bacterial spore coat to indicate that its permeability might be affected by

polyphosphate. Unbuffered TCCA-1, TCCA-2, TCCA-3 and TCCA-4 showed greater sporicidal activity than NaOCl. Tests also were conducted with organic matter (1% skim milk) to determine the efficiency of buffered sporicides under adverse conditions. At 200 ppm. all TCCA compounds showed less activity for spores of B. globigii than NaOCl. No striking differences were noted in the activity of the different TCCA compounds. NaOCl and the QAC were similar in activity and more efficient than TCCA-5 or I-1. TCCA-5 and I-1 demonstrated equivalent activities against the spores. None of the compounds accomplished 100% kill in 20 minutes. The QAC has shown greater activity for B. globigii spores in the presence of organic matter than in its absence. The most likely reason for this occurrence is that organic matter may have lowered the resistance of the spores by initiating first stages of germination.

Virucidal studies were made on one of the more resistant lactic phages, W strain of S. cremoris. The data by plaque count method indicated that buffered TCCA-1, TCCA-2, TCCA-3 and TCCA-4 were equivalent to NaOCl in virus destruction. The virucides accomplished 100% destruction in 15 seconds. Results by plaque and resazurin reduction tests indicate that TCCA-5 and NaOCl were more active than QAC and I-1. I-1 was more active against bacteriophage than the QAC when prepared in distilled water (pH 7.0)

than when buffered at pH 5.0. These results suggest that the phage was more sensitive to hypoiodous acid than to free iodine. Results also suggested that the plaque count and resazurin reduction methods are comparable in showing the order of activity of different virucides.

Studies were made with 4 buffered germicides on low concentrations of C. mycoderma, C. pseudotropicalis and S. ellipsoideus at approximately 100 X 10^3 cells per ml. The trend in activity of the germicides was the same for the 3 yeasts. The order of effectiveness was as follows: I-2 > NaOCl > TCCA-4 > QAC. C. mycoderma was found to be the most susceptible and S. ellipsoideus the most resistant to the action of the germicides. The germicidal resistance of S. ellipsoideus could not be related to sporulation, but appeared to represent an inherent difference between species of yeasts.

A study was made to determine the germicidal activity of TCCA-4, NaOCl, I-2, a QAC and CT-KI against high concentrations of C. mycoderma and S. ellipsoideus. The effectiveness of the germicides was determined in the presence of 500 ppm. U.S.D.A. synthetic hard water and in distilled water. The iodine compounds, with the exception of I-2 in distilled water, were slightly more active on C. mycoderma than the chlorine compounds. The QAC was the least active germicide in both distilled and hard water.

When S. ellipsoideus was the test agent, the iodine compounds showed slightly greater germicidal activity than the chlorine compounds in distilled water. In hard water NaOCl was slightly more active than CT-KI, I-2 and TCCA-4. CT-KI and I-2 were more active than TCCA-4. The QAC was the least active germicide in both distilled and hard water. In these studies, the germicidal action of the QAC was not reduced in the presence of hard water ions which suggests that the cell sites were not inactivated with the ions and were free to absorb the QAC. This observation is of considerable interest in view of marked decreases in QAC activity that occur with bacteria in the presence of hard water ions.

A study was conducted to determine the fungicidal activity of TCCA-4, NaOCl, I-2 and CT-KI on mold spores and mycelia of A. niger, Penicillium sp., and Rhizopus sp. The iodine compounds, with the exception of CT-KI on Rhizopus sp., showed greater fungicidal activity for mold spores than the chlorine compounds. Results indicated that I-2 was more destructive for the mycelia of Penicillium sp. and Rhizopus sp. than TCCA-4 and NaOCl. CT-KI showed less fungicidal activity for mycelia of Rhizopus sp. than I-2. I-2 was slightly less active for mycelia of A. niger than TCCA-4, NaOCl and CT-KI. Results of this investigation

suggested that mycelia of the test organisms were in some instances more resistant than the spores of the same species in the presence of fungicides. This difference may be explained on the basis of cell structure and penetration.

BIBLIOGRAPHY

1. Allen, L. A., and Eileen Brooks. Some factors affecting the bactericidal action of chlorine. Journal of Applied Bacteriology 15:155-165. 1952.

2. American Phytopathological society. Committee on Standardization of fungicidal tests. Test tube dilution technique for use with the slide-germination method of evaluating protectant fungicides. Phytopathology 37:354-356. 1947.

3. American Phytopathological society. Committee on Standardization of fungicidal tests. The slide-germination method of evaluating protectant fungicides. Phytopathology 33:627-632. 1943.

4. American Public Health Association. Standard methods for the examination of water, sewage, and industrial wastes. 10th ed. New York, American Public Health Association, Inc., 1950. 522 p.

5. Anderson, E. B., and L. J. Meanwell. The problem of bacteriophage in cheese making. I. Observations and investigations on slow acid production. Journal of Dairy Research 13:58-72. 1942.

6. Belani, E. Fighting bacteria and mold in dairies and cheese factories. Zeitschrift fur Fleisch-und Milchhygiene 52:112-113. 1942. (Abstracted in Chemical Abstracts 38:1739. 1944.)

7. Berliner, J. F. T. The chemistry of chloramines. Journal of the American Water Works Association 23:1320-1333. 1931.

8. Burgwald, L. H., and D. V. Josephson. The effect of refrigerator storage on the keeping qualities of pasteurized milk. Journal of Dairy Science 30: 371-383. 1947.

9. Butterfield, C. T., et al. Influence of pH and temperature on the survival of coliforms and enteric pathogens when exposed to free chlorine. Public Health Reports 58:1837-1866. 1943.

10. Cantor, A., and H. A. Shelanski. A capacity test for germicidal action. Soap and Sanitary Chemicals 27 (2):133-137. 1951.

11. Chang, Shih Lu, J. Carrell Morris. Elemental iodine as a disinfectant of drinking water. Industrial and Engineering Chemistry 45:1009-1012. 1953.

12. Charlton, David, and Max Levine. Germicidal properties of chlorine compounds. Ames, Iowa, 1937. 60 p. (Iowa Engineering Experiment Station. Bulletin 132.)

13. Collins, E. B. Relation of different numbers of bacteriophage and bacteria to population changes and acid production. Journal of Dairy Science 34:894-904. 1951.

14. Costigan, S. M. Comparison of the germicidal efficiency of hypochlorites of high and low alkalinity. Journal of Bacteriology 34:1-7. 1937.

15. _____. Effectiveness of hot hypochlorites of low alkalinity in destroying Mycobacterium. Journal of Bacteriology 32:57-63. 1936.

16. Elliker, P. R. Fine points of sanitation that up cottage cheese quality. Food Engineering 26(11): 79-82. 1954.

17. _____. Pasteurization effect on bacteria, yeasts, moulds and enzymes. Corvallis, 1948. 12 p. (Oregon. Agricultural Experiment Station. Technical paper 582.)

18. _____. The application of quaternary compounds in dairy sanitation. Journal of Milk and Food Technology 13:1-15. 1950.

19. _____, and B. E. Horrall. Effect of growth of Pseudomonas putrefaciens on diacetyl and flavor of butter. Journal of Dairy Science 26: 943-949. 1943.

20. Fair, G. M. et al. The behavior of chlorine as a water disinfectant. Journal of the American Water Works Association 40:1051-1061. 1948.

21. Fleming, Alexander, and George Smith. Some methods for the study of moulds. Transactions of British Mycological Society 27:13-19. 1944.

22. Foster, Edwin M. et al. Dairy microbiology. Englewood Cliffs, N. J., Prentice-Hall, Inc., 1957. 492 p.

23. Friberg, L., and E. Hammarstrom. The action of free available chlorine on bacteria and bacterial viruses. Acta Pathologica et Microbiologica Scandinavica 38:127-134. 1956.

24. Furlong, T. E., and P. R. Elliker. An improved method of determining concentration of quaternary ammonium compounds in water solutions and in milk. Journal of Dairy Science 36:225-234. 1953.

25. Gainey, P. L., and Thomas H. Lord. Microbiology of water and sewage for engineering students. New York, Burgess Publishing Co., 1950. 319 p.

26. _____. Microbiology of water and sewage. New York, Prentice-Hall, Inc., 1957. 492 p.

27. Gattani, M. L. The agar plate spore germination method for testing fungicides. Phytopathology 44:113-115. 1954.

28. Geiger, K. H., and P. J. Maloney. Enhanced effectiveness of chlorination. Canadian Journal of Public Health 43:359-367. 1952.

29. Gershenfeld, Louis, and W. W. Ruthenberg. Mold and yeasts in dairy products. American journal of Pharmacy 116:256-267. 1944.

30. Giolitti, Giovanni, and Mariangela Bertani. A method for the microscopical study of Actinomycetes. Journal of Bacteriology 65:281-282. 1953.

31. Goldsworthy, M. C., and E. L. Green. Effect of low concentrations of copper on germination and growth of conidia of Scleratina fructicola and Glomerella cingulata. Journal of Agriculture Research 56:489-505. 1938.

32. Griffin, A. E., and N. S. Chamberlin. Some chemical aspects of break-point chlorination. Journal of the New England Water Works Association 55:371-383. 1941.

33. Hadfield, W. A., and G. R. Goetchius. Quaternaries vs. hypochlorites as disinfectants. Soap and Sanitary Chemicals 27:124-149. 1951.

34. Hammer, Bernard W. Dairy Bacteriology. 3d ed. New York, John Wiley and Sons, Inc., 1948. 492 p.

35. Hannesson, Gudlaugur. Factors related to growth stimulation and inhibition of lactic streptococci. Master's thesis. Corvallis, Oregon State College. 1955. 170 numb. leaves.

36. Hays, Helen A. Sporicidal properties of sodium hypochlorite, iodophors and quaternary ammonium compounds. Master's thesis. Corvallis, Oregon State College. 1956. 87 numb. leaves.

37. Horsfall, James G. Fungicides and their action. Massachusetts, Chronica Botanica Company, 1945. 239 p.

38. _____, and Saul Rich. Fungitoxicity of heterocyclic nitrogen compounds. Contributions from Boyce Thompson Institute 16:313-347. 1951.

39. Humphrey, C. J., and Ruth M. Fleming. The toxicity to fungi of various oils and salts particularly those in wood preservation. 1915. 38 p. (U. S. Department of Agriculture. Bulletin 227)

40. Johns, C. K. The evaluation of the germicidal potency of chlorine compounds. I. Hypochlorites. Scientific Agriculture 14:585-607. 1934.

41. _____. The evaluation of the germicidal potency of chlorine compounds. II. Chloramine-T products. Scientific Agriculture 15:218-227. 1934.

42. _____. The influence of alkalinity upon the efficiency of hypochlorite. International Association of Milk Sanitarians. Annual Report 20:197-209. 1931.

43. _____. Speed of germicidal action of chlorine compounds upon bacteria commonly occurring in milk. Scientific Agriculture 10:553-563. 1930.

44. _____. Influence of organic matter on the germicidal efficiency of quaternary ammonium and hypochlorite compounds. Canadian Journal of Research 26:91-104. 1948.

45. _____. Factors affecting activity of chemical germicides. Milk Plant Monthly 39: 54-58. 1950.

46. _____. The germicidal effectiveness of a new chlorine compound. Journal of Milk and Food Technology 14:134-136. 1951.

47. _____. Further studies on bacteriophage in relationship to cheddar cheese-making. Journal of Dairy Research 13:119-122. 1943.

48. _____, and C. E. Chaplin. Influence of alkalinity on germicidal action of hypochlorites. Journal of Dairy Research 16:322-326. 1949.

49. Lesser, M. A. Hypochlorites as sanitizers. Soap and Sanitary Chemicals 25:119-125. 1949.

50. Loegering, W. Q. A satisfactory medium for germination of urediospores of Puccinia graminis tritici. Phytopathology 31:952-953. 1941.

51. MacGregor, Dugal Roy. The bactericidal mechanism of quaternary ammonium compounds. Ph.D. thesis. Corvallis, Oregon State College. 1952. 110 numb. leaves.

52. Macy, H. Treatment of parchment paper used for wrapping butter. The Milk Plant Monthly 16: 38-40. 1927.

53. _____, Combs, W. B., and H. B. Morrison, Jr. The churn as a source of molds in butter. Journal of Dairy Science 14:398-403. 1931.

54. _____, and J. C. Olson. Preliminary observations on the treatment of parchment paper with sodium or calcium propionate. Journal of Dairy Science 22:527-534. 1939.

55. Mallman, W. L., and O. Schalm. The influence of the (OH) ion on the germicidal action of chlorine in dilute solutions. East Lansing, Michigan, 1932. 20 p. (Michigan State College. Engineering Experiment Station. Bulletin 44)

56. Marks, H. C., Wyss, O., and F. Strandskov. Studies on the mode of action of compounds containing available chlorine. Journal of Bacteriology 49:299-305. 1945.

57. Martin, G., and J. P. Julien. Off flavors in Quebec butter. Canadian Dairy and Ice Cream Journal 30:44-46, 54. 1951.

58. McCallan, S. E. A., and R. H. Wellman. Fungicidal verses fungistatic. Contributions from Boyce Thompson Institute 12:451-464. 1942.

59. _____, and Frank Wilcoxin. An analysis of factors causing variation in spore germination tests of fungicides. I. Methods of obtaining spores. Contributions from Boyce Thompson Institute 11:5-20. 1939.

60. McCulloch, Ernest C. Disinfectants and sterilization. 2d. ed. Philadelphia, Lea and Febiger, 1945. 472 p.

61. Miller, Donald D. Fundamental factors affecting germicidal activities of certain hypochlorite and quaternary ammonium compounds. Ph.D. thesis. Corvallis, Oregon State College, 1952. 167 numb. leaves.

62. Morrison, H. B., Macy, H., and W. B. Combs. Preliminary studies of churn sanitation. Journal Dairy Science 14:404-415. 1931.

63. Mueller, W. S., and D. B. Seeley. Effect of some water constituents on a quaternary salt. Journal of Dairy Science 31:723-724. 1948.

64. Myers, R. P., and A. H. Johnson. A critical study of the methods for determining bactericidal properties of chemical sterilizers. Proceedings of the International Association of Milk Dealers (Laboratory Section) 25:21-55. 1932.

65. Neill, J. C. Butter boxes and mould growth. New Zealand Journal of Agriculture 53:129-139. 1936.

66. Nichols, A. A., and J. Z. Wolf. The persistence and recovery of bacteriophage in cheese. Journal of Dairy Research 13:302-307. 1944.

67. Olson, H. C., and B. W. Hammer. Numbers of microorganisms falling from the air in dairy plants. Journal of Dairy Science 17:613-623. 1934.

68. Olson, J. C., Parker, R. B., and W. S. Mueller. The nature, significance and control of psychrophilic bacteria in dairy products. Journal of Milk and Food Technology 18:200-203. 1955.

69. Parker, R. B., Coldwell, A. L., and R. R. Elliker. Psychrophilic bacteria a sanitation problem. Journal of Milk and Food Technology 16:136-139. 1953.

70. _____, and P. R. Elliker. Destruction of lactic acid streptococcus bacteriophage by hypochlorite and quaternary ammonium compounds. Journal of Milk and Food Technology 14:52-54. 1951.

71. _____, Smith, V. N., and P. R. Elliker. Bacteria associated with a gelatinous or slimy curd defect of cottage cheese. Journal of Dairy Science 34:887-893. 1951.

72. Peters, F. T. Rinse compositions. U. S. patent 2,422,255. June 17, 1947. (Abstracted in Chemical Abstracts 41:5266. 1947)

73. Prouty, C. C. Inactivation of bacteriophage of the lactic acid streptococci of starters by quaternary ammonium compounds. Journal of Milk and Food Technology 12:214-218. 1949.

74. Quisno, R. A., and M. J. Foter. Cetyl pyridenium chloride. I. Germicidal properites. Journal of Bacteriology 52:111-117. 1946.

75. Reddish, G. F. (ed.) Antiseptics, disinfectants, fungicides, and chemical and physical sterilization. Philadelphia, Lea and Febiger, 1954. 841 p.

76. Ridenour, G. M., and E. H. Armbruster. Some factors affecting the properties of quaternary ammonium compounds as sanitizers. American Journal of Public Health 38:503-511. 1948.

77. Rogers, A. O. 1, 1- methylenebis (3 - chloro - 5, 5 - dimethyl hydantoin.) U. S. patent 2,404,096. July 16, 1946. (Abstracted in Chemical Abstracts 40:6096. 1946.)

78. _____. 1, 3 - Dichloro - 5 methyl - 5 isobutyl hydantoin. U. S. patent 2,398,599. April 16, 1946. (Abstracted in Chemical Abstracts 40:3773. 1946.)

79. _____. Chlorinated hydantoins. U. S. patent 2,398,598. April 16, 1946. (Abstracted in Chemical Abstracts 41:3773. 1946.)

80. Rudolph, A. S., and M. Levine. Factors affecting the germicidal efficiency of hypochlorite solutions. Ames, Iowa, 1941. 48 p. (Iowa Engineering Experiment Station. Bulletin 150)

81. Schuldt, Paul H., and Calvin N. Wolf. Fungitoxicity of substituted s- Triazines. Contributions from Boyce Thompson Institute 18:377-393. 1956.

82. Smith, V. N., Parker, R. B., and P. R. Elliker. Importance of bacteriophage as a cause of slow starters. Journal of Milk and Food Technology 15:218-219. 1952.

83. Sotier, Alfred L. Antibac - a new type of chlorine sanitizer. Journal of Milk and Food Technology 15:285-293. 1952.

84. U. S. Department of Agriculture. Agricultural Research Service. Regulations for the enforcement of the federal insecticide, fungicide, and rodenticide act. Interpretation concerning labeling claims for germicides, disinfectants, and sanitizers recommended for use in hard water areas. Federal Register 21:7020. 1956.

85. Vernon, T. R. Influence of moulds and yeasts on keeping quality of butter. IV. Of the mycology of dairy products. Dairy Industries 2:255-256. 1937.

86. Ware, Elinor. The chemistry of the hydantoins. Chemical Reviews 46:403-470. 1950.

87. Watkins, J. H., and C. E. A. Winslow. Factors determining the rate of mortality of bacteria exposed to alkalinity and heat. Journal of Bacteriology 24:243-265. 1932.

88. Watkins, Sprague H. Destruction of lactic acid streptococcus bacteriophage by various bactericidal agents. Master's thesis. Corvallis, Oregon State College, 1955. 93 numb. leaves.

89. Weber, George R. Effect of concentration and reaction (pH) on the germicidal activity of chloramine-T. Public Health Reports 65:503-512. 1950.

90. _____, and L. A. Black. Laboratory procedure for evaluating practical performance of quaternary ammonium and other compounds proposed for sanitizing food utensils. American Journal of Public Health 38:1405-1417. 1948.

91. _____, and Max Levine. Factors affecting germicidal efficiency of chlorine and chloramine. American Journal of Public Health 34: 719-728. 1944.

92. Whitehead, H. R., and G. J. E. Hunter. Starter cultures for cheese manufacture. Further attempts to eliminate failures due to bacteriophage. Journal of Dairy Research 12:63-70. 1941.

93. _____, and _____. The action of chemical disinfectants on bacteriophages for the lactic streptococci. Journal of Dairy Research 11:62-66. 1940.

94. Wolf, J. Z., Nichols, A. A., and J. P. Ineson. Mists containing hypochlorite as germicides in destruction of air-borne bacteriophages attacking lactic streptococci. Journal of Dairy Research 14:291-315. 1946.

95. Wyss, Orville, and Frede B. Strandskov. The germicidal action of iodine. Archives of Biochemistry 6:261-268. 1945.

96. Yuill, Edward. The number of nuclei in the conidia of Aspergilli. Transactions of the British Mycological Society 33:324-331. 1950.