EFFECTS OF POTASSIUM SORBATE SINGLY AND IN COMBINATION WITH BUTYL HYDROXYANISOLE, TERTIARY BUTYLHYDROQUINONE AND PROPYL GALLATE ON THE GROWTH OF STAPHYLOCOCCUS AUREUS S-6 AND SALMONELLA SENFTENBERG

by

ROGER EDWARD POERSCHKE

B.A., Emporia State University, 1973

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

in

Food Science

Department of Animal Sciences and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1981

Approved by:

J. E. Cunningham
Major Professor

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (continued)

INTRODUCTION

Since the early 1950's, sorbic acid and the potassium salt, potassium sorbate, have been widely used as effective antimycotic agents in various commercially prepared foods (Luck, 1976). More recently, sorbic acid and potassium sorbate have been studied for their antimicrobial activity against various food-borne illness and food spoilage bacteria. Antioxidants such as butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ), and propyl gallate (PG), commonly used to prevent rancidity in lipids and lipid-containing products, also possess antimicrobial properties against microorganisms. Recent studies using the combination of potassium sorbate and BHA or TBHQ have shown a synergistic antimicrobial effect against Staphylococcus aureus, Clostridium perfringens and Salmonella typhimurium in culture media. Due to the complex and varied systems that make up a food, the results of inhibition studies in a defined system of the agar or broth medium cannot be extrapolated directly to a food system. To date, no study has been reported that determines the effectiveness of potassium sorbate/antioxidant combinations in a food system. Also, the effects of a fairly substantial lipid content of a food on the antimicrobial effectiveness of the antioxidants have not been studied.

The objectives of the study reported here were to determine: (1) the effects of potassium sorbate singly and in combination with butyl hydroxyanisole, tertiary butylhydroquinone and propyl gallate on the growth of Staphylococcus aureus S-6 and Salmonella senftenberg in broth culture media at 37°C; (2) the effectiveness of potassium sorbate/antioxidant combinations, determined to be the most antimicrobial in broth

culture studies, on the growth of Staphylococcus aureus S-6 and Salmonella senftenberg in fresh ground beef held at 30°C; and (3) the effectiveness of potassium sorbate singly on the growth of test organisms.

LITERATURE REVIEW

Sorbates

Sorbic acid was first discovered in 1859 by A.W. Hofman, a German chemist, by reacting rowan berry oil with strong alkali. It was called sorbic acid after the scientific name of the mountain ash, Sorbus aucuparia Linne, which is the parent plant of rowan berry. In 1939, E. Muller (in Germany) and in 1940, C.M. Gooding (in the United States), discovered that sorbic acid had antimicrobial properties. Large scale production of sorbic acid commenced in the 1950's and its use as a food preservative increased gradually after being permitted in most countries (Luck, 1976).

Gooding (1945), noting the inhibitory properties of sorbic acid, obtained a patent for a process to inhibit growth of molds. Phillips and Mundt (1950) first suggested the use of sorbic acid in cucumber brines to control the surface yeasts. They noted that 0.1% sorbic acid in the brine would prevent surface scum formation without interfering with acid production. This was later confirmed by Jones and Harper (1952). However, Borg et al. (1955) reported that 0.1% sorbic acid inhibited growth of fermentative yeasts in cucumber fermentations, as well as acid fermentation. Costilow et al. (1955) reported that yeasts most prevalent in cucumber fermentations were completely inhibited by 0.01% sorbic acid in an 8% salt medium at pH 4.6. By increasing pH and/or

decreasing salt concentration, higher sorbic acid concentrations were needed for complete inhibition of the yeast tested. Costilow et al. (1956) next demonstrated that cultures of Pediococcus cerevisiae, Lactobacillus plantarum and Lactobacillus brevis isolated from cucumber fermentations were not greatly affected by sorbic acid concentrations up to 0.1%. Concentrations of sorbic acid lower than 0.1% were found to be effective against both surface and film-forming types of yeast in cucumber fermentations (Costilow, 1957).

Salt and sugar were found to have a marked synergistic effect on sorbic acid fungistasis by Gooding et al. (1955). Sorbic acid effectiveness was increased in high sugar systems even at pH values above 6.5. In the same article, sorbic acid was reported to be about four times as effective as propionates in protecting cheese, bread, and cake products. Smith and Rollin (1954) found sorbic acid superior to sodium benzoate as a fungistatic agent in protecting cheese and cheese products, which are generally very susceptible to mold spoilage. A level of 0.05% was shown to give full protection of products. Mold and yeast growth in smoked fish was delayed by 0.05-0.1% sorbic acid incorporated in the brining solution (Boyd and Tarr, 1955).

At approximately the same time, work on other uses of sorbic acid was being reported. York and Vaughn (1954) tested 20 species and types of Clostridium, including vegetative cells and spore suspensions, for their resistance to sorbic acid in beef liver infusion medium. Vegetative cells and spores of C. botulinum types C, D and E were inhibited by 0.5% sorbic acid, while 3.0% sorbic acid did not inhibit C. parabotulinum types A and B. Emard and Vaughn (1952), working with laboratory media, found Salmonella sp. and some Streptococcus faecalis strains

THIS BOOK
CONTAINS
NUMEROUS PAGES
WITH THE ORIGINAL
PRINTING BEING
SKEWED
DIFFERENTLY FROM
THE TOP OF THE
PAGE TO THE
BOTTOM.

THIS IS AS RECEIVED
FROM THE
CUSTOMER.

inhibited by 0.12% sorbic acid, while S. aureus was inhibited by 0.07% sorbic acid in glucose yeast extract media and 0.12% in liver infusion media. The same authors recommended sorbic acid as a selective agent in culture media for the isolation of catalase-negative lactobacilli and clostridia.

Following the works of the early and middle 1950's, litte progress was reported until the mid 1960's when Perry et al. (1964) reported extending the shelf life of poultry using a two-step process involving sorbic acid. The process consisted of an application of a 7.5% sorbic acid solution in a 70:20:10 propylene glycol, water and glycerine mixture at 140°F. Control samples spoiled after 5 days at refrigeration temperatures, while treated samples did not spoil until 18 days. Recently, the use of potassium sorbate dip in extending the shelf life of broiler parts and limiting salmonellae growth was examined by Robach and Ivey (1978). Use of a 10.0% sorbate dip significantly reduced the total plate count, compared to control parts after 5 days at 22°C. The 10% dip resulted in significantly lower salmonellae counts than untreated parts after 7 days at 10°C, and a 5% or greater dip markedly reduced the growth rate of the salmonellae at 10 and 22°C. Cunningham (1979) reported the organoleptic quality of broiler parts unaffected by 5 or 10% potassium sorbate dip.

Robach et al. (1980) reported reduced psychrotrophic plate counts in vacuum-packaged, oven-roasted whole turkey breasts and sliced turkey breast luncheon meat processed with 0.26% sorbate and stored 12 days at 10°C. The addition of 0.12% sorbic acid to the sliced product extended the time to reach 10^7 cells/g from 15 days in the controls to 42 days in the treated product when held at 4°C. Organoleptic evaluations indicated no significant differences between control and sorbate-treated products.

Food spoilage microorganisms of the genus Pseudomonas have also been found susceptible to sorbate. Bradley et al. (1962) found that 0.1% potassium sorbate inhibited the growth of Pseudomonas fragi at 10°C in creamed cottage cheese. Moustafa and Collins (1969) showed that 0.1% potassium sorbate delayed the growth of P. fragi at 13°C in skim milk adjusted to pH 5.2. Robach (1978) reported the addition of 0.05% sorbate inhibited growth of Pseudomonas fluorescens in trypticase soy broth at pH 5.5, whereas, 0.2% sorbate was required to delay growth in TSB at pH 6.0. Robach (1979) also found 0.2% potassium sorbate in TSB (pH 6.0) to inactivate or delay growth of two strains of Pseudomonas putrefaciens at 24°C.

Sorbates have been studied for their antimicrobial activity against various food poisoning bacteria. Park and Marth (1972) and Park et al. (1970) found Salmonella typhimurium was inactivated by 0.2-0.3% sorbic acid in media, milk and cheese. Tompkin et al. (1974) reported that 0.1% (w/w) potassium sorbate delayed the growth of S. aureus and salmonellae, as well as growth and toxin production of Clostridium botulinum in uncured pork sausage held at 27°C. The growth of Vibrio parahaemolyticus at 35°C in crab and flounder homogenates (pH 6.2) was shown to be delayed or prevented by 0.05-0.1% sorbic acid (Robach and Hickey, 1978).

Sorbic acid and its potassium salt have been studied in combinations with sodium nitrite to determine whether they could be used as a partial replacer of nitrite for botulism control in meat products. Ivey et al. (1978) tested low nitrite (1 and 40 ug/g) and potassium sorbate (0.13 and 0.26%) concentrations in bacon inoculated with C. botulinum spores and incubated at 27°C. Potassium sorbate was shown to significantly reduce the number of toxic-swollen packages and lengthen the time toxicity was

observed. The effectiveness of sorbic acid and low nitrite concentrations to inhibit botulinal growth in canned comminuted pork was studied by Ivey and Robach (1978). Sorbic acid at 0.2% level delayed growth and toxin production. Low nitrite levels (5 ug/g) and 0.1 or 0.2% sorbic acid greatly retarded growth and toxicity. Robach et al. (1978) found that addition of 0.1 and 0.2% sorbic acid extended the time for swelling in a comminuted chicken product. Combining the sorbic acid with 20 ug of nitrite/g delayed botulinal growth even more. Work by Sofos et al. (1979 a,b,c) showed that sorbic acid (0.2%) inhibited botulinal spore germination in mechanically deboned chicken meat, beef and pork frankfurter emulsions abused at 27°C. Sofos et al. (1980) reported that a combination of sorbate (0.26%), with reduced nitrite levels (40, 80 ppm), was effective in delaying botulinal toxin production in bacon held at 27°C.

Antioxidants

Antioxidants have long been used in lipids and lipid-containing products to prevent rancidity, but also have been found to possess antimicrobial activity. Ward and Ward (1967) tested the antimicrobial effectiveness of BHT against Salmonella senftenberg 775W. Only slight inhibition of the organism was found at a concentration of 1.0% BHT in brilliant green agar. Chang and Branen (1975) studied the effect of BHA on selected microorganisms which often pose a public health hazard in food products. 1,000 ppm of BHA was shown to totally prevent growth and aflatoxin production of Aspergillus parasiticus spores, while 150-200 ppm was necessary to inactivate S. typhimurium and E. coli in nutrient broth. Shih and Harris (1977), however, found BHA and 50/50 combination

with propyl gallate at 400 ppm levels to have little effect on another strain of E. coli in TSB at 35°C. BHA levels of 400 ppm were required to totally inactivate S. aureus. The difference in growth inhibition by BHA was reported to be the result of media or culture strain differences (Shih and Harris, 1977). Ayaz et al. (1978) reported 100% inhibition of S. aureus by 200 ppm BHA in brain heart infusion broth and 100 ppm BHA inhibited enterotoxin formation in the same organism. Fung et al. (1977) tested BHA and BHT against 6 toxigenic and 6 nontoxigenic strains of Aspergillus flavus. BHT was found not to be inhibitory, whereas, BHA inhibited A. flavus growth at 0.005-0.02 g per plate of solid media. The production of aflatoxins B_2, G_1 and G_2 was completely inhibited by 0.01g BHA.

The effectiveness of TBHQ against growth of various types of bacteria in agar medium was reported by Erickson and Tompkin (1977). A level of 30 ppm TBHQ (w/w) was found to totally inhibit S. aureus growth, while 0.01% TBHQ was effective against E. coli and soy flake bacteria. A level of 0.1% TBHQ was needed to be effective against P. aerugenosa and fresh meat bacteria.

Growth of Vibrio parahaemolyticus 04:K11 was inhibited in TSB + 2.5% NaCl by 50 ppm BHA, while 400 ppm BHA was required to inhibit growth in a crab meat homogenate (Robach et al., 1977). The higher level of BHA required for inhibition in the crab meat homogenate was thought to be due to partial inactivation of the antioxidant properties by the presence of oxidized crab meat lipids. Klindworth et al. (1979) found that 150 ppm BHA was inhibitory to three strains of Clostridium perfringens grown in fluid thioglycollate medium. The authors also found the antimicrobial activity of BHA against C. perfringens greatly

reduced in the presence of a lipid and surfactant in the media.

Just recently, Davidson and Branen (1980) studied the antimicrobial activity of BHA against two psychrotrophic food spoilage microorganisms, Pseudomonas fluorescens and Pseudomonas fragi. In TSB 100 ppm BHA delayed the growth of P. fluorescens at 22°C and totally inhibited growth at 7°C. In contrast, P. fragi was more resistant, even at 400 ppm BHA growth occurred at 7 and/or 22°C. The antimicrobial mechanisms of BHA against the same two organisms was also studied by Davidson and Branen (1980). They found that BHA created leaking of intracellular (cytoplasmic or periplasmic) proteins, and altered the percentages of major fatty acids in both P. fragi and P. fluorescens.

Sorbates in Combination with Other Compounds

Only until the past couple of years have any studies been performed on inhancing the antimicrobial effect of sorbates by addition of other compounds, mainly antioxidants, salt and EDTA. Klindworth et al. (1979) reported that combining varying amounts of sorbic acid and BHA produced a synergistic effect against C. perfringens in thioglycollate media. Robach and Stateler (1980) demonstrated that potassium sorbate (0.2%), in combination with sodium chloride (5-7%), TBHQ (25 ppm) or BHA (50-100 ppm), resulted in synergistic inhibition of growth of two strains of S. aureus (5-6 and 12600). Addition of EDTA (50 ppm) did not increase sorbate's activity against growth of strain S-6, but was synergistic with sorbate against growth of strain 12600. Davidson et al. (1981) reported a synergistic antimicrobial effect against S. typhimurium by potassium sorbate (0.05-0.1%) with BHA (50-100 ppm) in trypticase soy broth (pH 6.0) at 32°C. They also found potassium sorbate (0.05-0.1%), when combined

with BHA (50 ppm) or TBHQ (10 ppm), to be synergistic against S. aureus grown under the same conditions. Lahellec et al. (1981) reported S. aureus S-6 was more resistant to sorbate/antioxidant combinations than toxigenic strains 196, 137 and 326. The same authors found TBHQ was highly inhibitory to S. aureus strains with or without sorbate, and also reported that sorbate/BHA combination was more inhibitory than BHT or PG combined with sorbate.

Recent investigations have shown the combination of potassium sorbate with sodium chloride to be effective in the inhibition of Salmonella typhimurium and spore outgrowth of Clostridium sporogenes. LaRocco and Martin (1981) found that combinations of sorbate and NaCl were more effective in the inhibition of S. typhimurium than sorbate alone. The combination of 3% NaCl + 0.3% potassium sorbate was the most effective in inhibiting growth of S. typhimurium at 22°C or 35°C. Robach (1980) reported that at 37°C the addition of 3 or 5% NaCl increased the antimicrobial effectiveness of 0.1, 0.2 and 0.3% sorbate against C. sporogenes PA 3679. Also, the addition of 1, 3 or 5% NaCl enhanced the functionality of 0.1, 0.2 and 0.3% sorbate against the outgrowth of PA 3679 spores at 24°C.

Safety of Sorbates

Deuel et al. (1954) reported that sorbic acid was harmless to rats and dogs when incorporated in their diets to the extent of 5%. The same authors also reported sorbic acid toxicity to be lower than that of sodium benzoate. Sorbic acid is utilized in the body in a way similar to other fatty acids. Sorbic acid's half-life in the body is 40-110 min., depending on the dosage. Under normal conditions of digestion and

metabolism, sorbic acid is completely oxidized to CO_2 and H_2O, yielding potential energy as calories (Luck, 1976).

Sorbic acid and its potassium salt have been cleared for use and listed as products "Generally Recognized as Safe" (GRAS) by the FDA. On March 10, 1978, the FDA published a proposal to reaffirm the GRAS status of sorbic acid and potassium sorbate (USDA, 1978). The Select Committe concluded that the sorbates demonstrated very low acute or chronic toxicity for experimental animals; and they were metabolized in the animal by the normal fatty acid pathway. The World Health Organization has confirmed the harmlessness of sorbic acid by stipulating for it the highest acceptable daily intake (25 mg/kg of body weight) among food preservatives (Luck, 1976).

On May 16, 1978, a regulation was proposed by the United States Department of Agriculture (USDA, 1976) to reduce nitrite input in bacon to 40 ppm when used in conjunction with 0.26% potassium sorbate. Allergic reactions by some people testing bacon prepared in this manner were reported in news releases by the USDA, but in similar studies by Paquette et al. (1980), the addition of 0.26% potassium sorbate in commercially prepared bacon did not have any effect on the sensory qualities of the bacon and no adverse effects were noticed.

MATERIALS AND METHODS

Test Organisms and Media

Stock cultures of Staphylococcus aureus S-6 and Salmonella senftenberg were obtained from the Kansas State University Food Microbiology Stock Culture Collection. The microorganisms were grown on tryptic soy

agar (TSA, Difco) slants at 35°C for 24 hours. The slants were stored at 4°C and transferred every two weeks to maintain viability. All cultures used were prepared by inoculating a 250 ml shake flask containing 50 ml of sterile tryptic soy broth (TSB, Difco) with a loop of a slant culture of the appropriate organism. The flask was incubated in a shaker water bath (American Optical, Buffalo, N.Y.) for 12 hours at 37°C and 175 cpm.

Potassium Sorbate Solutions and Media

Potassium sorbate used in this study was obtained from the Monsanto Co., St. Louis, Missouri. Two percent solutions of potassium sorbate were prepared in distilled water and filter sterilized using a 0.45 Millipore filter (Millipore Corp., Bedford, Mass.). Stock solutions were stored at 4°C.

Antioxidant Solutions and Media

Antioxidants, butylated hydroxyanisole (BHA); tertiary butylhydroquinone (TBHQ); and propyl gallate (PG), were obtained from Eastman Chemical Products (Kingsport, Tenn.). Original tablets of BHA were ground into powder with a mortar and pestle. The BHA powder, TBHQ and PG were then dissolved in 95% ethanol to a concentration of 4% (w/v) to prepare the stock solutions of each and stored at 4°C when not in use.

Growth Inhibition Studies in Media

The growth media used in all growth studies was tryptic soy broth (TSB, Difco) adjusted to pH 5.5 with 5N HCl. Growth studies were done in 250 ml screw cap Erlenmeyer flasks. Appropriate amounts of TSB were autoclaved at 121°C for 15 min., cooled, and specific amounts of filter-sterilized 4% stock potassium sorbate aseptically added to yield 50 ml

TSB containing specific concentrations of potassium sorbate in full strength TSB.

In growth studies using antioxidants, appropriate aliquots of BHA, TBHQ or PG stock solution were added to specific amounts of TSB before autoclaving to yield the needed final concentrations in 50 ml TSB. Previous studies had indicated that autoclaving the antioxidants in the broth does not influence the antimicrobial activity (Klindworth et al., 1979). Studies that used sorbate/antioxidant combinations also used appropriate amounts of broth and antioxidant so that addition of sorbate gave the needed levels of both in 50 ml of full strength TSB.

The TSB was then inoculated with 1 ml of a 1:100 dilution in 0.1% peptone of the 12-hour shaker culture of the appropriate test organism. This yielded an initial inoculum level of approximately 10^5 cells/ml. Samples were withdrawn at intervals of 0, 4, 8, 12 and 24 hours and serial dilutions were made in sterile 0.1% peptone dilution fluid (pH 7.0). Test organisms were enumerated by duplicate pour plating using trypticase soy agar (TSA, Difco) as the enumerating medium. The plates were incubated at 37°C for 24 hours prior to counting.

Concentrations of potassium sorbate and antioxidants to be used in combination were determined by first adding each alone to the TSB. The concentration of each antimicrobial used were: potassium sorbate: 0.05, 0.1, 0.2 and 0.4%; BHA, TBHQ or PG: 50, 100, 200 and 400 ppm respectively. Concentrations which resulted in delayed or inhibited growth after 24 hour incubation were used in combination testing.

Growth Inhibition Studies in Fresh Ground Beef

Fresh ground beef was purchased at a local supermarket the same

day it was to be used. Ground beef was analyzed for moisture, fat and protein by standard proximate analysis methods (Table 1). Sterile plastic gloves were used in the handling of all meat samples. The ground beef was divided into 500 g sample sizes, placed in sterile plastic bags and refrigerated until mixing. A Kitchen Aid Mixer Model K4-B (Hobart Mgf., Troy, Ohio) was used to facilitate mixing of the potassium sorbate or sorbate/antioxidant into the ground beef samples. Mixing was done at the lowest mixing speed with the appropriate amount of preservative sprinkled into the meat and allowed to mix for one minute. Ground beef was then removed from the mixer, divided into 200 g amounts and placed in appropriately labeled sterile plastic bags. The samples were then inoculated with 5 ml of a 12-hour broth culture of the appropriate test organism serially diluted in 0.1% peptone to 10^3 organism/ml. This yielded approximately 100-200 test organisms per gram of ground beef sample. The inoculum was mixed as uniformly as possible into the meat sample by manipulating the polyethylene bag. The ground beef samples were then incubated at 30°C for 24 hours.

At 0, 4, 8, 12 and 24-hour time periods, 10 g ($\pm$ 0.1 g) samples were removed with an alcohol-flamed spatula, weighed in a sterile petri dish, aseptically placed in a 400 ml sterile, Stomacher Bag (Dynatek Lab, Inc., Alexandria, Vir.) and 90 ml sterile 0.1% peptone added. Samples were then mixed in a Stomacher Lab-Blender 400 (Model No. BA6021, A.J. Seward, London, England) for 1 min. and appropriate dilutions of the homogenates were made in sterile 0.1% peptone. Total viable cell counts were performed by pour plating TSA. The plates were incubated at 30°C for 36-48 hours prior to counting. Detection and enumeration of S. aureus S-6 involved direct plating of appropriate dilutions of the sample

Table 1

Composition of Fresh Ground Beef

	Ground Beef
Moisture %	44.6
Fat %	26.7
Protein %	17.7
pH %	5.7

homogenate onto the surface of Baird-Parker Agar (Difco) plates and spreading inoculum with a sterile bent glass rod. Duplicate plates were prepared for each dilution. Plates were incubated at 37°C for 30 hours, at which time colonies which were black and shiny and surrounded by clear zones extending into the opaque medium were counted. Plates were incubated for a further 18-hour period, at which time another count was taken of all colonies with the above appearance, as well as colonies which were shiny black, with or without clear zones. A number of colonies were submitted to a coagulase to confirm them to be S. aureus. Salmonella senftenberg was detected and enumerated by direct plating of appropriate dilutions of sample homogenate onto the surface of Brilliant Green Sulfa Agar, SS Agar and Bismuth Sulfite Agar (Difco). Inoculum was spread with a sterile bent glass rod and plates incubated at 37°C for 24 to 48 hours. Typical salmonellae growth was counted and a significant number of colonies submitted to biochemical confirmation by inoculating triple sugar iron (TSI) and lysine iron agar (LIA, Difco) slants. These slants were incubated at 37°C for 24 hours prior to reading.

Statistical analysis of the data obtained in the growth inhibition studies in ground beef was performed using Two-Way Analysis of Variance and Least Significant Differences (Snedecor and Cochran, 1976) with the aid of SASS program.

RESULTS AND DISCUSSION

Effect of Potassium Sorbate Alone

The effect of potassium sorbate on growth of Salmonella senftenberg is presented in Figure 1. As was expected, the higher the concentration, the greater the inhibitory effect of potassium sorbate on the test microorganism. Figure 1 demonstrates that with a 0.4% sorbate concentration, there was a 5-log cycle reduction in cell count at 24 hours, but all other levels showed less growth than the control. These results agree with studies that showed S. typhimurium inactivated by 0.2-0.3% sorbic acid in nutrient broth (pH 5.0-5.5) at 37°C and 0.3% sorbate efficacy the greatest in TSB at 35°C (Park and Marth, 1972; LaRocco and Martin, 1981). Figure 2 shows S. aureus S-6 growth inhibition by potassium sorbate to be less than that of S. senftenberg. At 0.4% sorbate, S. senftenberg showed a 5-log cycle decrease, while no increase or decrease of growth was observed in S. aureus S-6 at 24 hours.

Effect of Antioxidants

The concentration of BHA, TBHQ and PG found to delay growth of S. senftenberg are shown in Figure 3. The results in Figure 3 were determined from inhibition studies of each antioxidant, at various concentrations, on the test organisms (see Appendix). Concentrations of 200 ppm BHA resulted in delay and inhibition of S. senftenberg growth and 400 ppm BHA was found to totally inhibit S. senftenberg. These results agree with Chang and Branen (1975), who found 400 ppm BHA necessary for total inhibition of S. typhimurium. Another strain of S. typhimurium was totally inhibited by 150 ppm BHA in TSB (Davidson et al., 1981). Tertiary butylhydroquinone at 200 ppm delayed growth, while 400 ppm PG

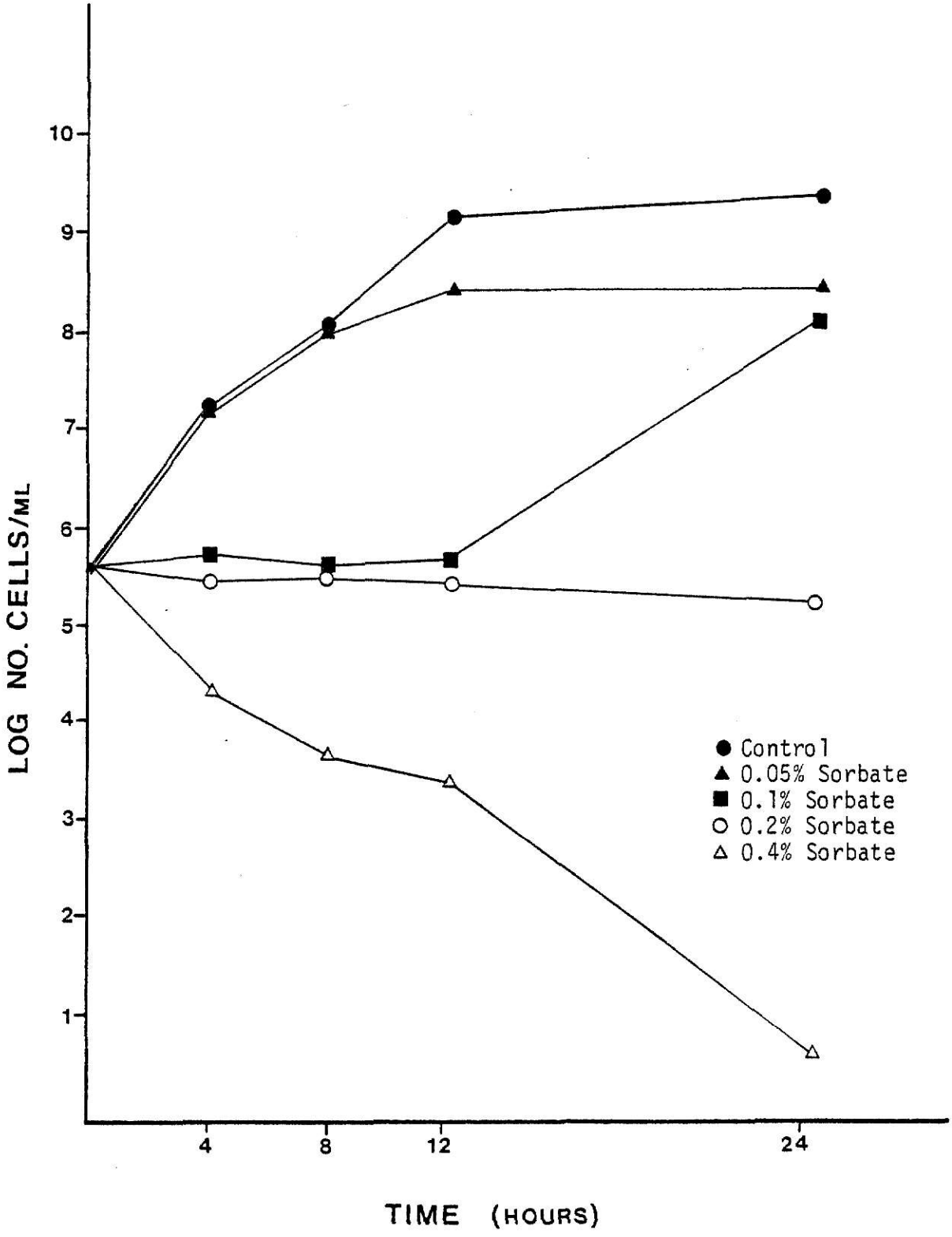


Figure 1. Effect of potassium sorbate on growth of Salmonella senftenberg in tryptic soy broth (pH 5.5) at 37°C.

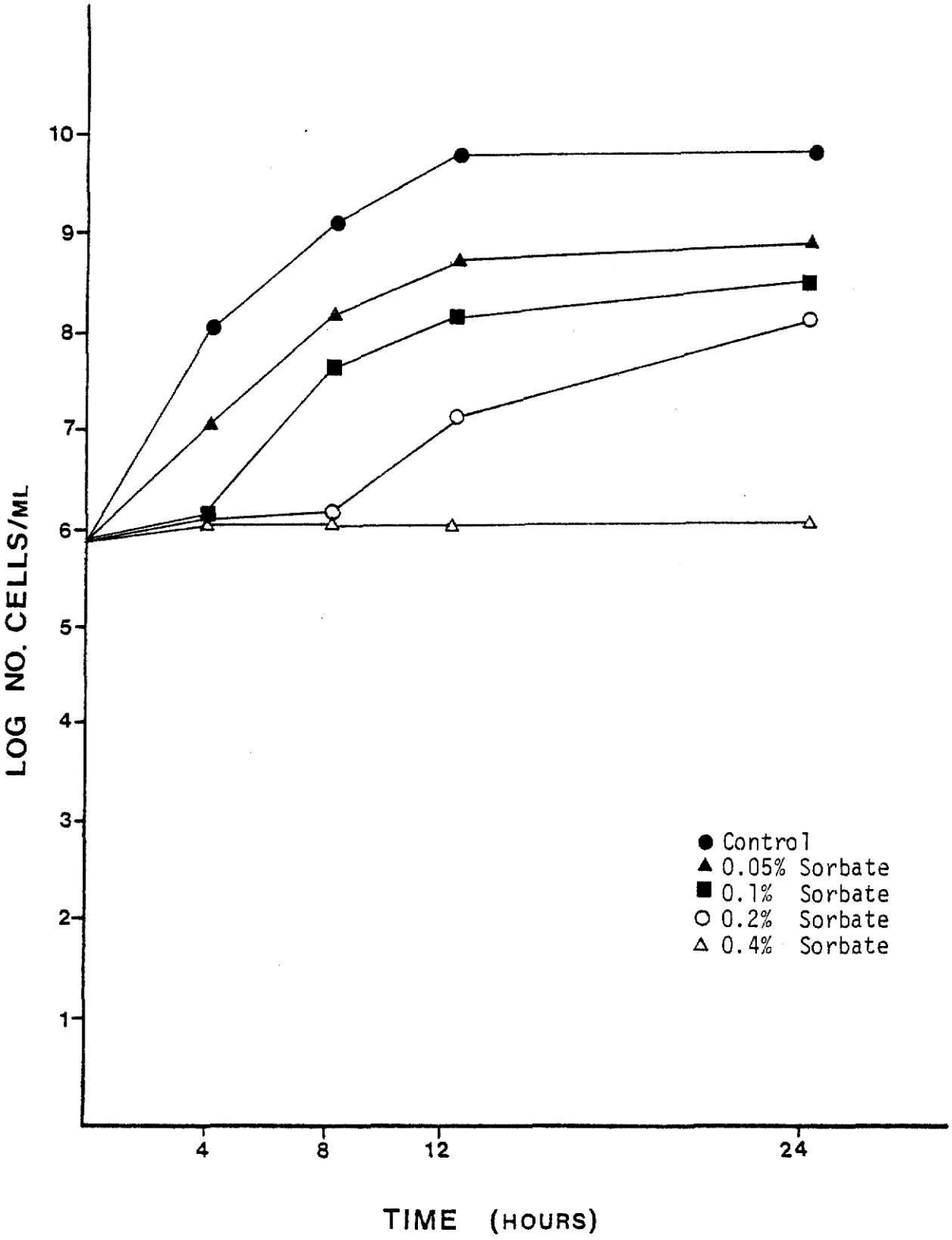


Figure 2. Effect of potassium sorbate on growth of Staphylococcus aureus S-6 in tryptic soy broth (pH 5.5) at 37°C.

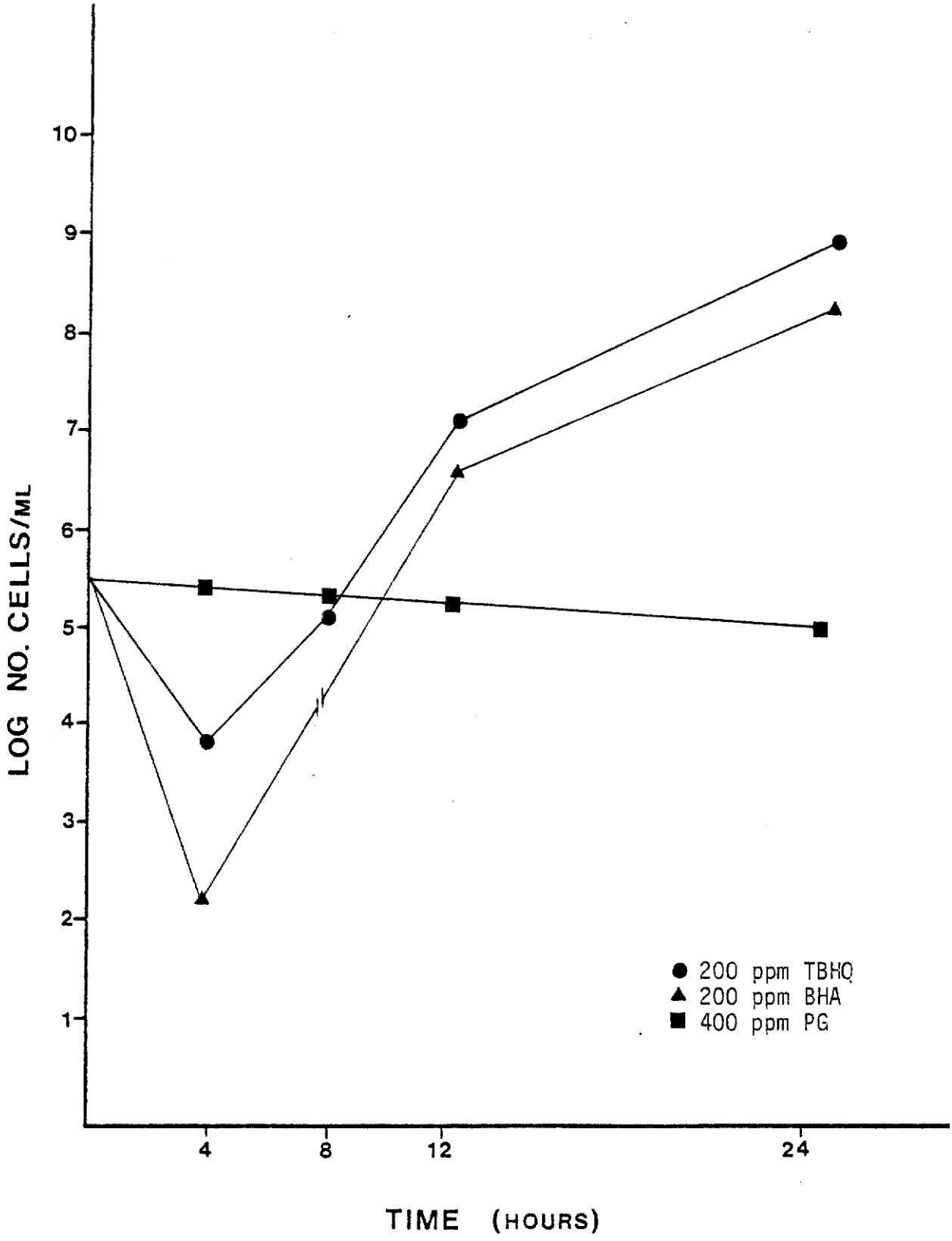


Figure 3. Levels of TBHQ, BHA and PG found to delay or inhibit growth of Salmonella senftenberg in TSB at 37°C.

was needed to effect growth of S. senftenberg. Previously, Davidson et al. (1981) reported S. typhimurium able to grow at 400 ppm TBHQ.

Delaying or varying inhibition of S. aureus S-6 growth was found at concentrations of 100 ppm BHA, 25 ppm TBHQ and 400 ppm PG (Figure 4). These findings agree with previous reports, which generally show S. aureus to be totally inhibited by concentrations of 200 ppm BHA and above (Chang and Branen, 1975) and 25 ppm TBHQ (Davidson et al., 1981; Robach and Stateler, 1980). Concentrations of 400 ppm PG were also required to show inhibition in S. aureus S-6. This result agrees with the work of Shih and Harris (1977), who reported PG at levels of 400 ppm to be only slightly inhibitory to S. aureus growth in TSB at 35°C. Tertiary butylhydroquinone had dramatically different effects on the two organisms. Staphylococcus aureus S-6 was almost totally inhibited by 25 ppm, whereas S. senftenberg was only slightly inhibited by 200 ppm and growth was observed at 400 ppm. This difference between the organisms may be due to the lipopolysaccharide layer in the cell wall of gram-negative bacteria screening out TBHQ, as theorized by Branen et al. (1980).

Effects of Combinations of Potassium Sorbate with BHA, TBHQ or PG in Media

The concentrations to be used in the combination tests determined from the above results were as follows: S. senftenberg -- 0.1% sorbate - 100 ppm antioxidants; 0.1% sorbate - 200 ppm antioxidants; 0.2% sorbate - 50 ppm antioxidants; 0.2% sorbate - 100 ppm antioxidants; 0.2% sorbate - 200 ppm antioxidants; S. aureus S-6 -- 0.1% sorbate - 25 ppm TBHQ; 0.2% sorbate - 25 ppm TBHQ; 0.1% sorbate - 100 ppm BHA or PG; 0.1% sorbate - 200 ppm BHA or PG; 0.2% sorbate - 50 ppm BHA or PG; 0.2% sorbate - 200 ppm BHA or PG.

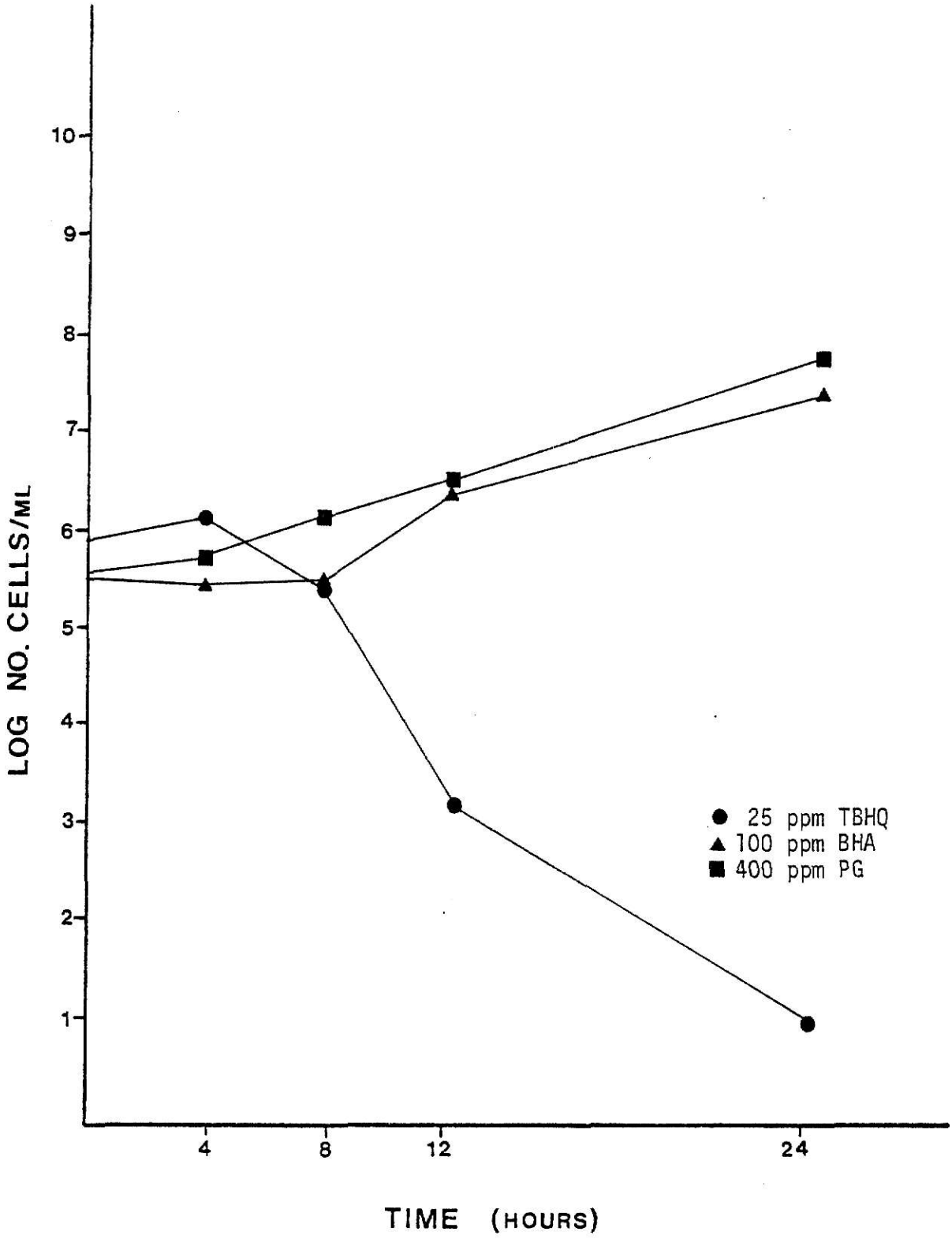


Figure 4. Levels of TBHQ, BHA and PG found to delay or inhibit growth of Staphylococcus aureus S-6 in TSB at 37°C.

Figure 5 shows the results of potassium sorbate/BHA combinations on the growth of S. senftenberg. All the combinations of sorbate and BHA delayed the initial growth by 12 hours and continued to show good delay and inhibition of growth through 24 hours, except the lowest concentration (0.1% sorbate - 100 ppm BHA). Combinations containing 200 ppm BHA were more effective against S. senftenberg than either 50 or 100 ppm BHA, with the 0.2% sorbate - 200 ppm BHA combination reducing counts to near zero at 24 hours.

The effects of potassium sorbate and TBHQ combinations on growth of S. senftenberg are shown in Figure 6. All the combinations of sorbate and TBHQ delayed initial growth by 8 hours, after which time combinations containing 0.2% potassium sorbate progressively reduced cell counts as TBHQ concentrations were increased. Once again, the highest concentration of TBHQ and sorbate was the most effective in reducing the S. senftenberg counts, which is to be expected.

Figure 7 depicts the effects of the potassium sorbate and PG combinations on the growth of S. senftenberg. All the combinations of sorbate and PG were found to be effective in delaying initial growth through 24 hours. None of the combinations tested allowed final growth at 24 hours above the initial inoculum level and, as to be expected, the highest concentration of sorbate and PG was the most inhibitory.

The concentration of 0.2% potassium sorbate and 200 ppm BHA was found to reduce the cell counts of S. senftenberg more than all the other sorbate/antioxidant combinations tested; only 3 cells/ml were found at 24 hours. The same concentration was found to be the most inhibitory in the sorbate/TBHQ and sorbate/PG combinations, with a 4-log cycle decrease at 24 hours from the initial inoculum level. Data reported in Figure 5

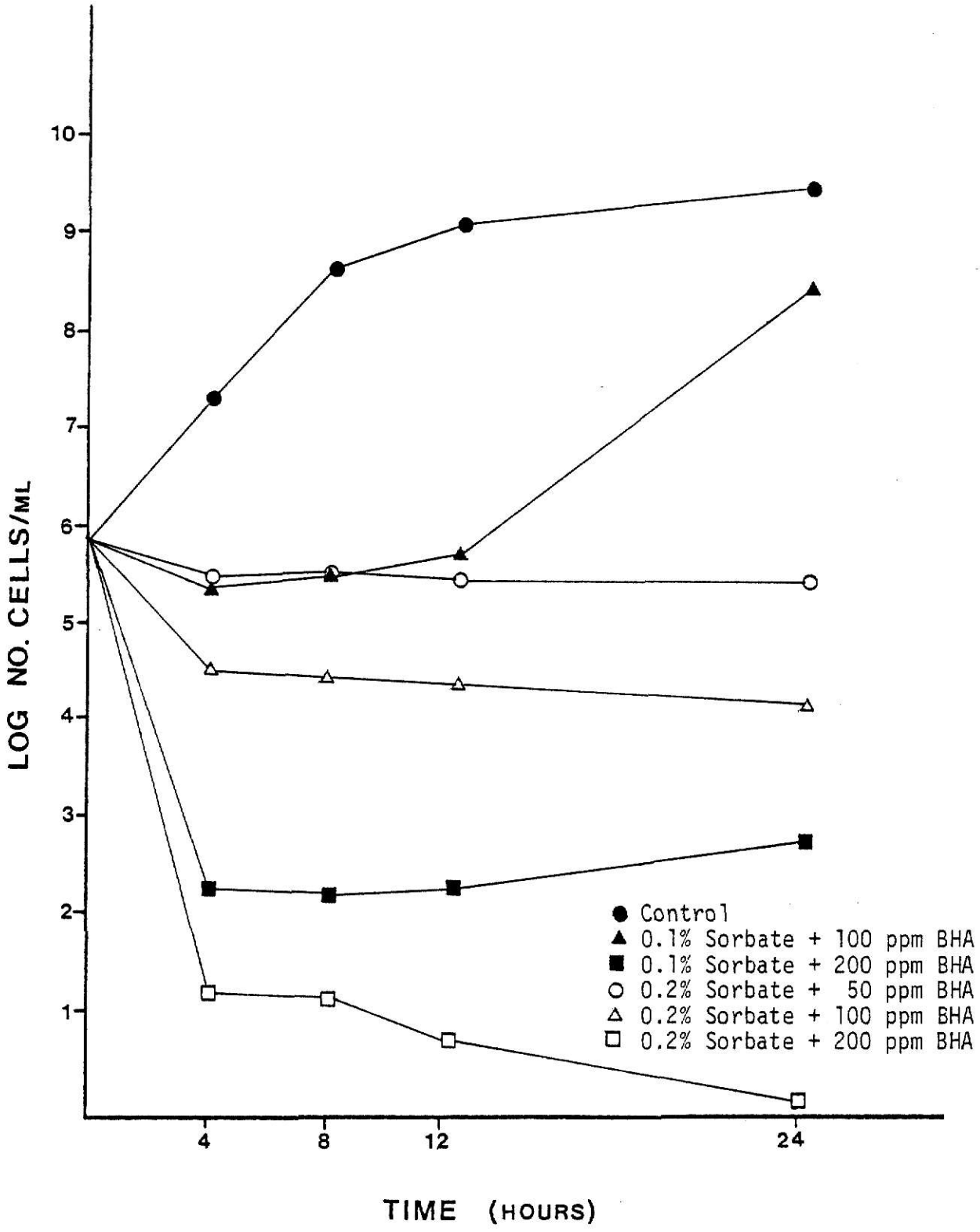


Figure 5. Effect of potassium sorbate and BHA on growth of Salmonella senftenberg in tryptic soy broth (pH 5.5) at 37°C.

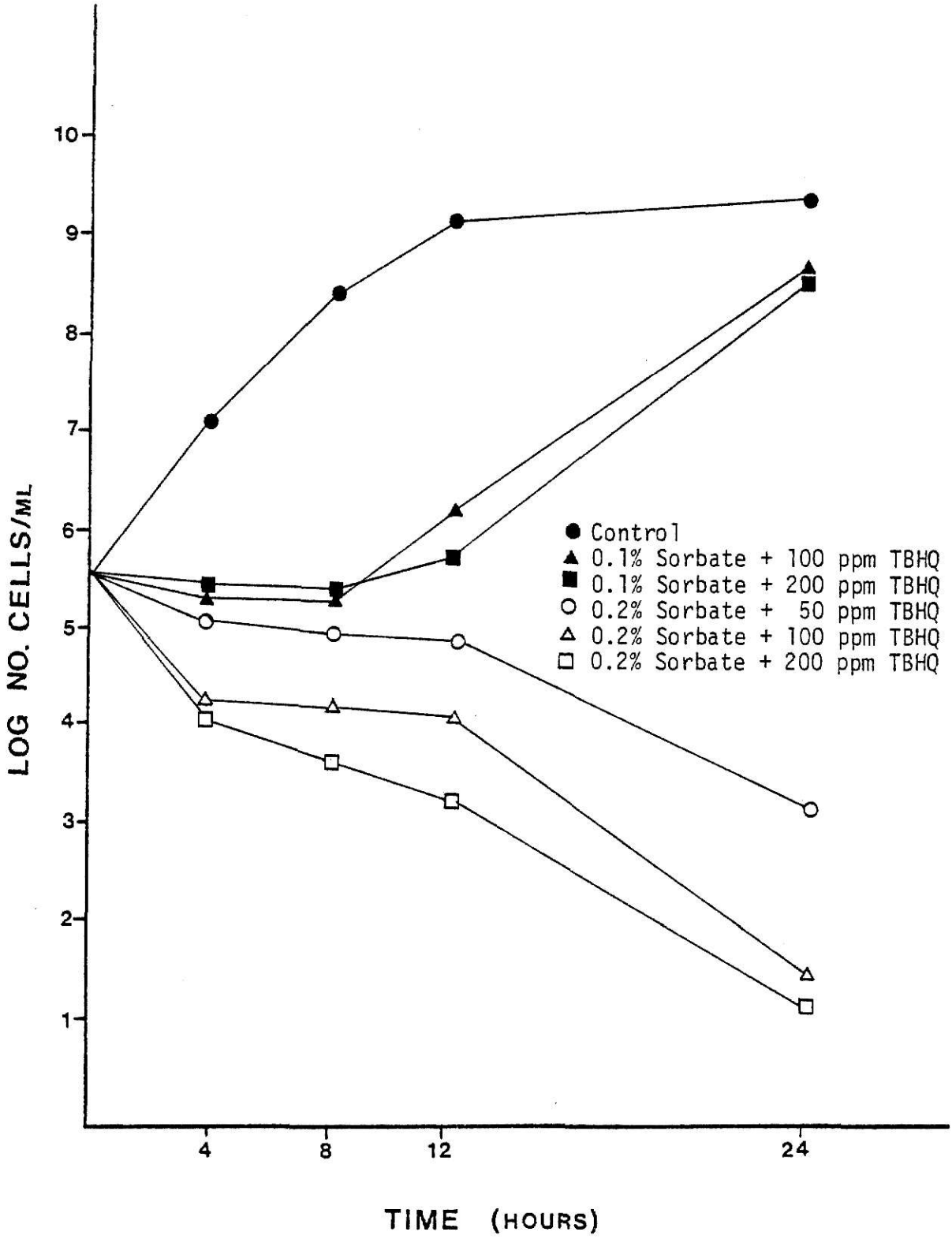


Figure 6. Effect of potassium sorbate and TBHQ on growth of Salmonella senftenberg in tryptic soy broth (pH 5.5) at 37°C.

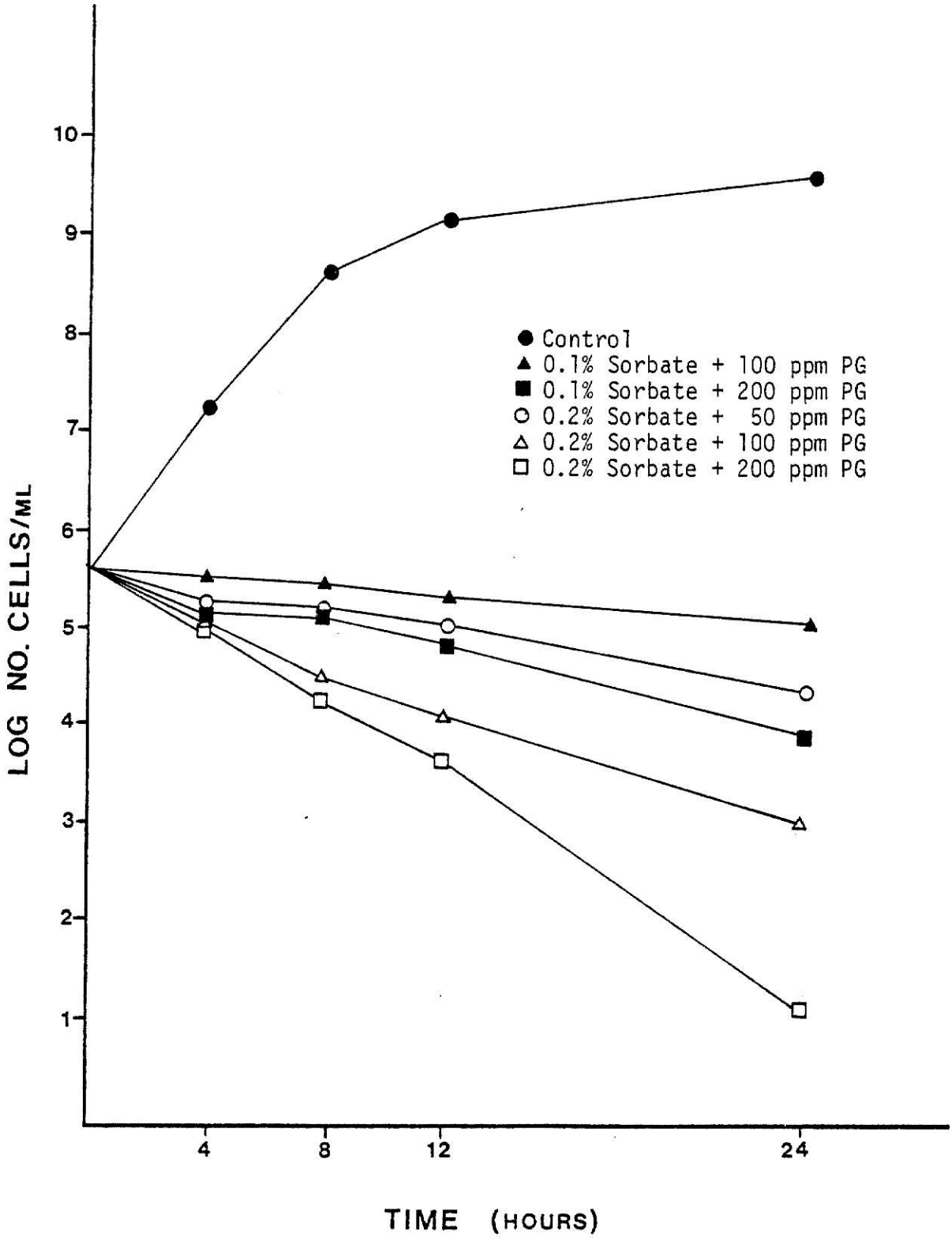


Figure 7. Effect of potassium sorbate and PG on growth of Salmonella senftenberg in tryptic soy broth (pH 5.5) at 37°C.

is not in agreement with Davidson et al. (1981), who found 0.1% sorbate - 50 ppm and 100 ppm BHA combinations 73% and 80%, respectively, inhibitory to S. typhimurium growth in TSB (pH 6.0) at 32°C. This difference could very well be attributed to the different specie tested, as well as dissimilarities in cultural conditions: 37°C vs. 32°C and shaking vs. nonshaking. Branen et al. (1980) have reported that other studies have shown microorganism strain used, media, or cultural conditions influence BHA activity. All the concentrations of the sorbate and PG combinations were found to show good synergism in reducing S. senftenberg counts through the 24-hour incubation period. The 0.1% sorbate - 100 ppm BHA combination was found to delay growth for 12 hours and the 0.1% - 100 ppm and 200 ppm TBHQ combinations to delay growth for only 8 hours.

The effects of potassium sorbate and antioxidant combinations on the growth of S. aureus S-6 are shown in Figures 8-10. All levels of sorbate and BHA combinations tested were shown to be bactericidial or bacteriostatic to the growth of S. aureus S-6. The concentrations of 200 ppm BHA with 0.1 and 0.2% potassium sorbate were totally inhibitory to growth at 8 hours of incubation. The 0.2% sorbate and 100 ppm BHA concentration showed a synergistic effect on S. aureus S-6 by limiting growth at 24 hours to approximately 1-log cycle below initial inoculum level. This is in general agreement with Robach and Stateler (1980), who found 0.2% sorbate and 100 ppm BHA, in combination, to synergistically inhibit growth of S. aureus S-6 in TSB (pH 6.0) at 37°C.

As seen in the preliminary studies (Figure 4), TBHQ at low concentrations is highly antimicrobial to S. aureus S-6. Potassium sorbate combined with TBHQ is shown in Figure 9 to have a more bactericidial effect on S. aureus S-6. Tertiary butylhydroquinone at the level of

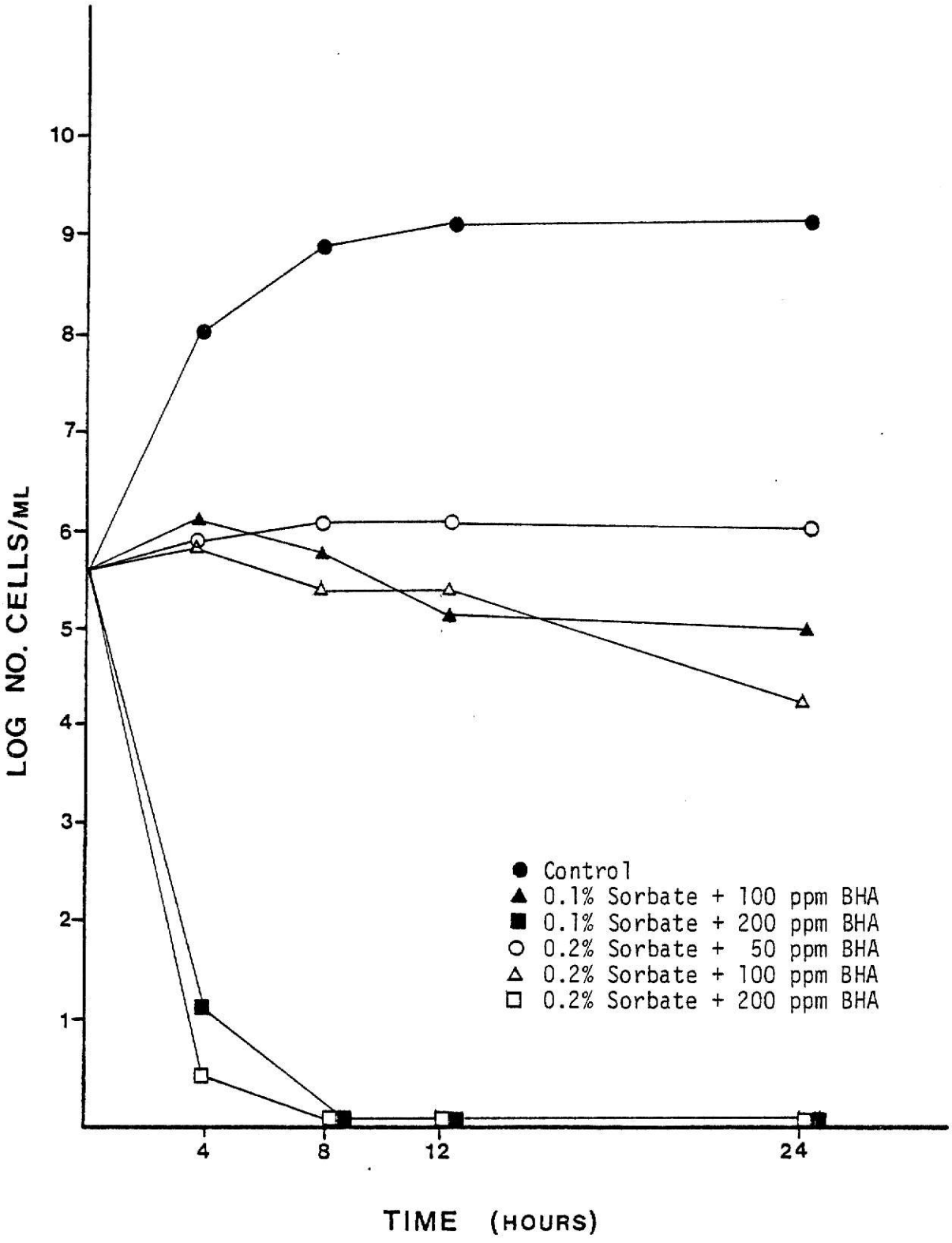


Figure 8. Effect of potassium sorbate and BHA on growth of Staphylococcus aureus S-6 in tryptic soy broth (pH 5.5) at 37°C.

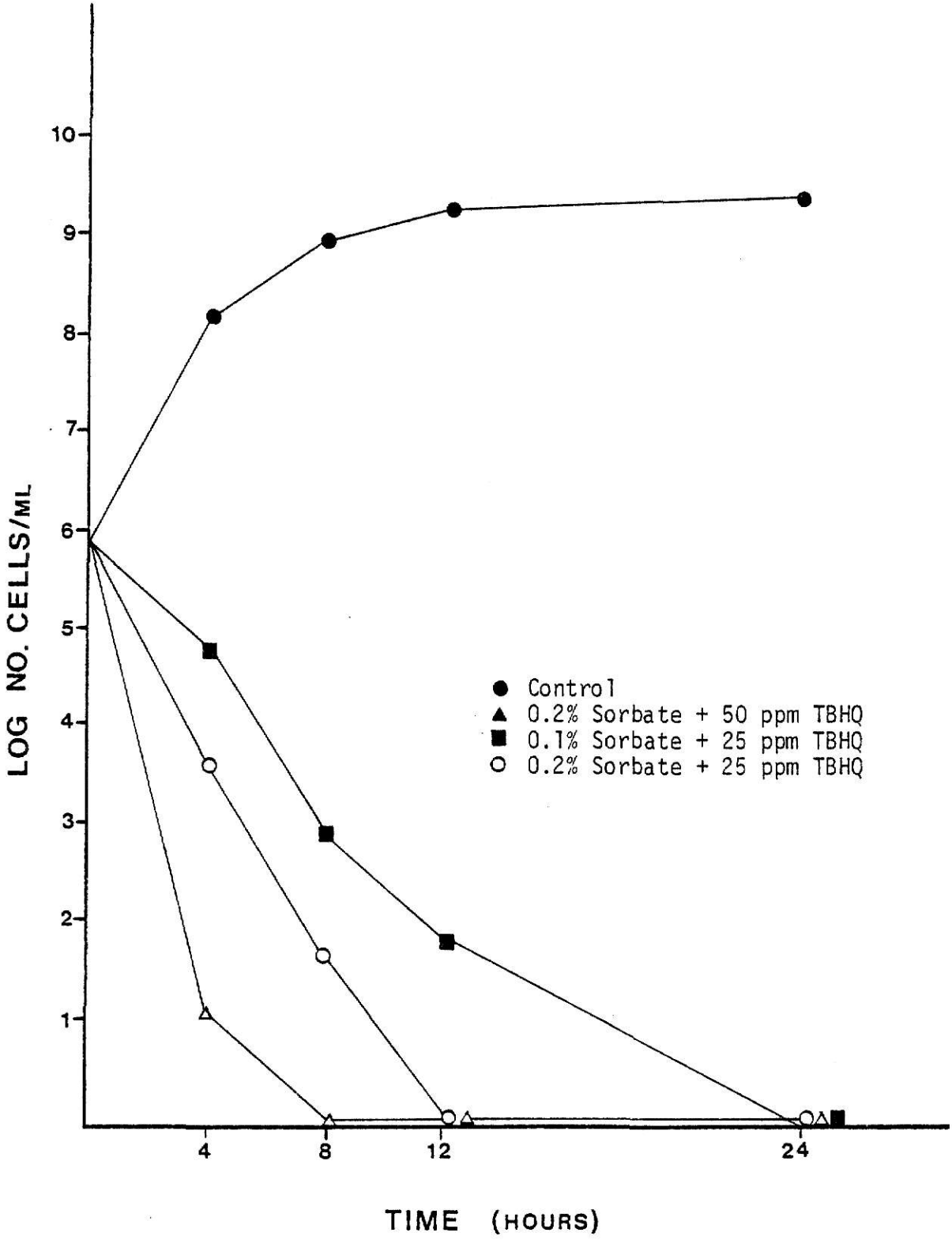


Figure 9. Effect of potassium sorbate and TBHQ on growth of Staphylococcus aureus S-6 in tryptic soy broth (pH 5.5) at 37°C.

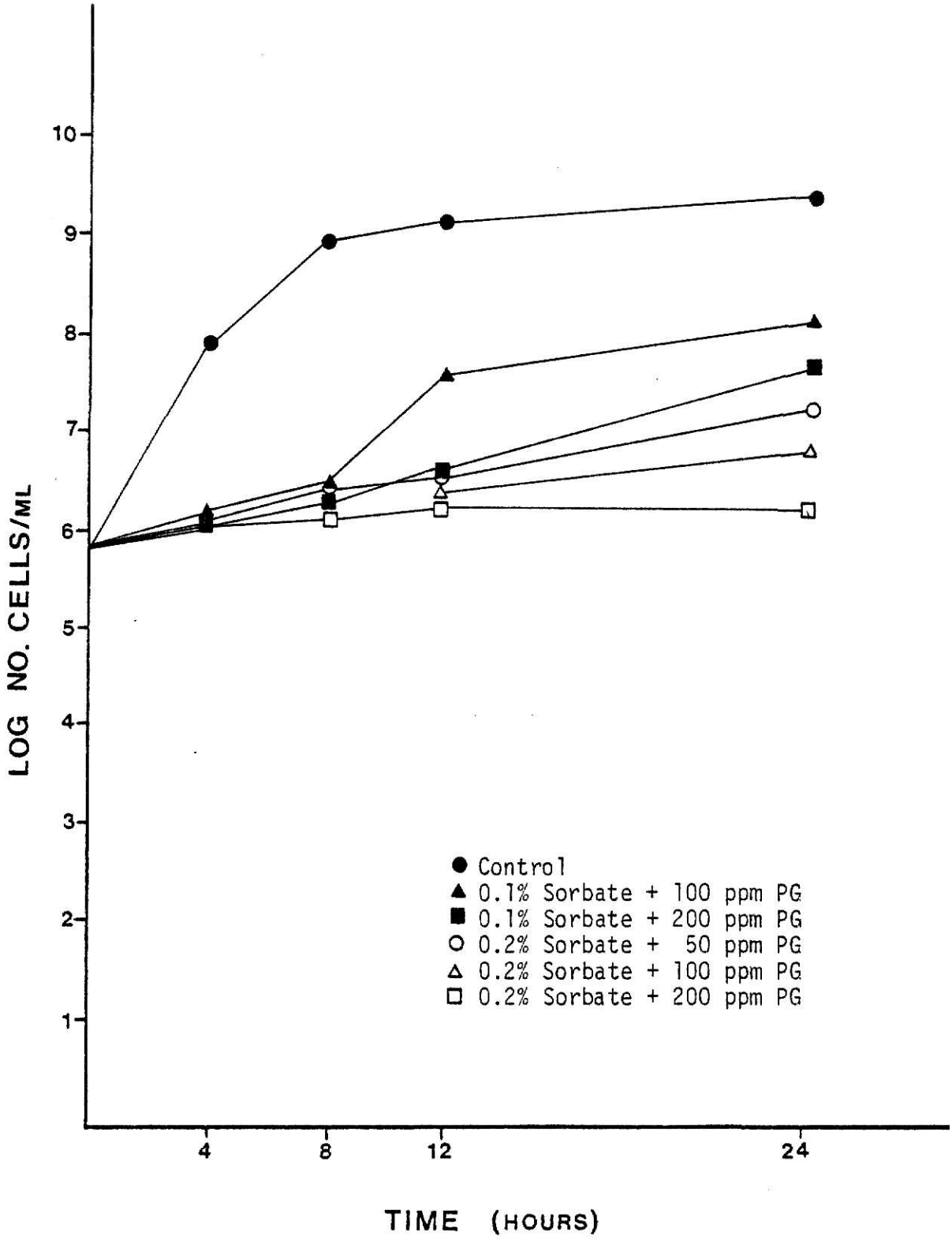


Figure 10. Effect of potassium sorbate and PG on growth of Staphylococcus aureus S-6 in tryptic soy broth (pH 5.5) at 37° C.

25 ppm when combined with 0.1 and 0.2% potassium sorbate, showed excellent antimicrobial effect against S. aureus S-6. At the 0.2% sorbate level, no growth was observed at 12 hours, while at 0.1% sorbate, no viable growth was recorded at 24 hours. These results are in agreement with the work of Robach and Stateler (1980), who reported 0.2% sorbate, combined with 25 ppm TBHQ, totally inhibited S. aureus S-6 growth at 24 hours in TSB (pH 6.0) at 37°C. The results are also in general agreement with Davidson et al. (1981), who found 0.1% sorbate and 10 ppm TBHQ caused a 12-hour delay in the growth initiation of S. aureus in TSB (pH 6.0) at 32°C.

Growth curve results of the effects of potassium sorbate and PG combinations on the growth of S. aureus S-6 are shown in Figure 10. All combinations tested inhibited growth, as compared to the control data. A slight synergistic effect was observed with the concentrations tested, but sorbate and propyl gallate combinations did not prove to be potent inhibitors of S. aureus S-6.

The combination of potassium sorbate and tertiary butylhydroquinone, at the level of 0.2% sorbate and 25 ppm TBHQ, was found to be the most effective in the inhibition of S. aureus S-6 growth in TSB (pH 5.5) at 37°C. The effectiveness of TBHQ against the S. aureus organism can be measured in the low concentration required to completely stop growth, whereas, the same effectiveness can be seen using BHA, but at levels of 200 ppm. The combination of 200 ppm BHA and 0.1 and 0.2% sorbate gave no viable cells at 8 hours. The potassium sorbate and propyl gallate concentrations tested were observed to be considerably less powerful in limiting the growth of S. aureus S-6 than the two other antioxidants. The concentration of 0.2% sorbate and 200 ppm PG permitted slight

increase in growth of the initial inoculum through the 24-hour incubation. This was expected, as the preliminary results showed 400 ppm PG alone to have little effect on S. aureus S-6.

The results of the growth inhibition studies conducted in TSB demonstrate that potassium sorbate, with BHA, TBHQ or PG, were in general, effective in synergistically inhibiting or delaying growth of S. senftenberg. Combinations of potassium sorbate, BHA and TBHQ were totally bactericidial to growth of S. aureus S-6 in TSB. Combining of potassium sorbate and propyl gallate was shown to suppress final growth of S. aureus S-6.

The importance of growth inhibition studies in media was to evaluate the antimicrobial effectiveness of combinations of potassium sorbate, BHA, TBHQ and PG and to find concentrations of each that were highly or totally antimicrobial to the two microorganism species in a defined system before applying the compounds to a complex food system.

Growth Inhibition Studies in Ground Beef

Due to the complex make-up of a food, the results of the inhibition studies in a defined system of the broth medium cannot be extrapolated directly to all food systems. Therefore, growth inhibition studies were conducted in fresh ground beef with concentrations and combinations of the potassium sorbate with BHA, TBHQ or PG that were shown to be highly effective in the inhibition of the test organisms in the media studies. Ground beef was chosen as the food system because it contains about 26% fat, which may limit the antimicrobial effect of the antioxidants, and as a food that may show promise for practical application. The high abuse temperature of 30°C was selected to allow for adequate growth of the test

organisms in the 24-hour test period.

The concentrations of the potassium sorbate, BHA, TBHQ and PG combinations tested were: 0.2% sorbate - 200 ppm BHA, TBHQ or PG against S. senftenberg; 0.2% sorbate - 50 ppm TBHQ, 200 ppm BHA and PG against S. aureus S-6.

Results of the sorbate/antioxidant combination against the growth of S. senftenberg are shown in Figure 11. All the combinations tested were found to suppress final growth of S. senftenberg compared to the control sample. The potassium sorbate - BHA combination was the most effective in limiting growth, with only 1/2-log cycle growth increase observed through 24 hours. Tertiary butylhydroquinone and sorbate combination was the least effective and allowed a 1-log cycle growth increase. The sorbate/propyl gallate treatment was found to be less effective than the sorbate/BHA treatment, but more effective than sorbate/TBHQ combination. None of the sorbate/antioxidant combinations tested were found to be highly effective against the growth of S. senftenberg in ground beef at the abuse temperature used. A growth increase was observed in all treatments at the end of the test period.

The effects of potassium sorbate, combined with BHA, TBHQ and PG on the growth of S. aureus S-6 are shown in Figure 12. Final growth of S. aureus S-6 was suppressed by the sorbate/antixodant combinations tested, as compared to the control sample growth. In all treatments, growth increase of the test organism was recorded at 24 hours. The sorbate and BHA or TBHQ treatments were shown to delay initial growth up to 8 hours, after which time a total of approximately 1-log cycle of growth was recorded for each at the end of the test period. The potassium sorbate and propyl gallate treatment was the least effective and a 2-log cycle

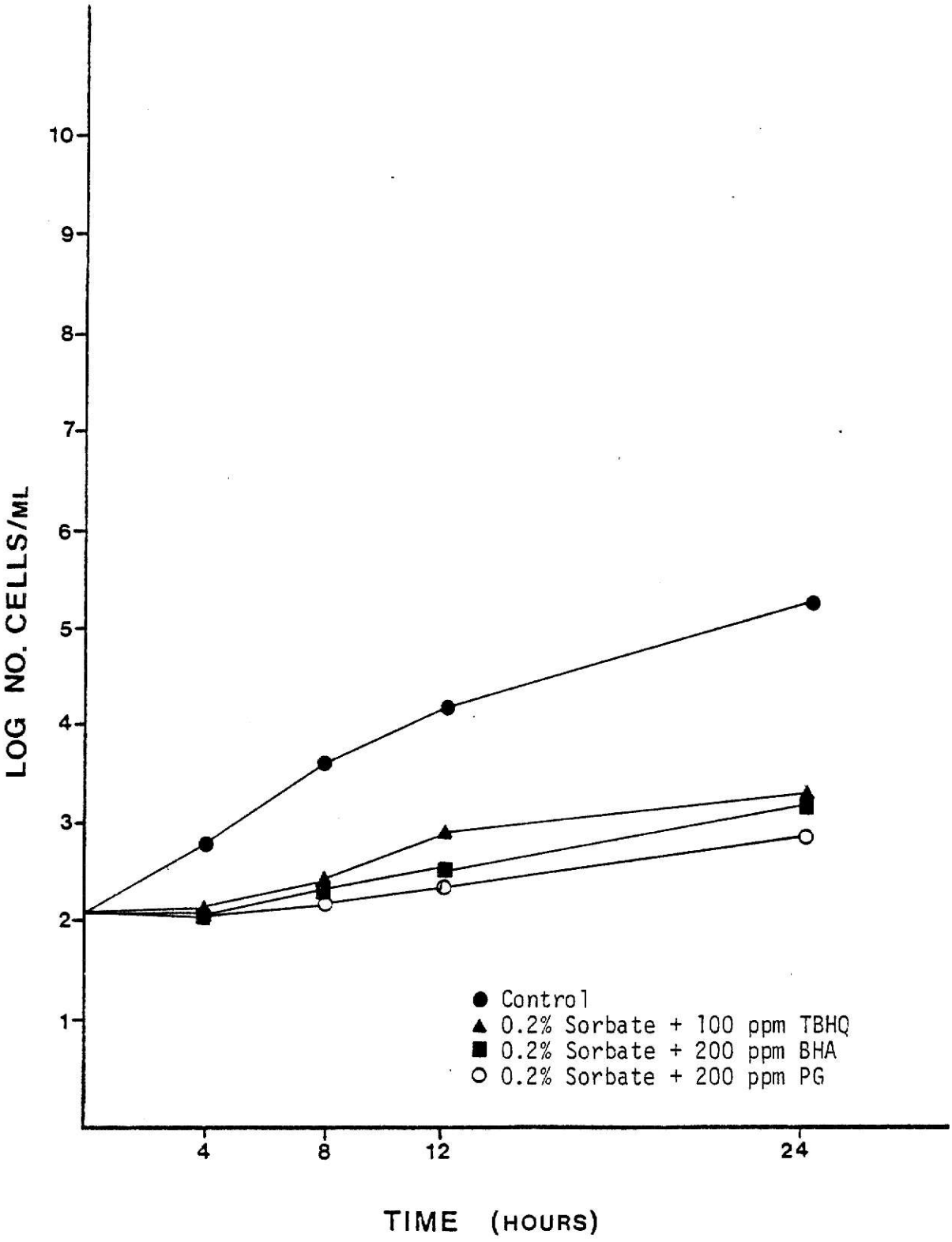


Figure 11. Effect of potassium sorbate, combined with TBHQ, BHA and PG, on growth of Salmonella senftenberg in fresh ground beef at 30°C.

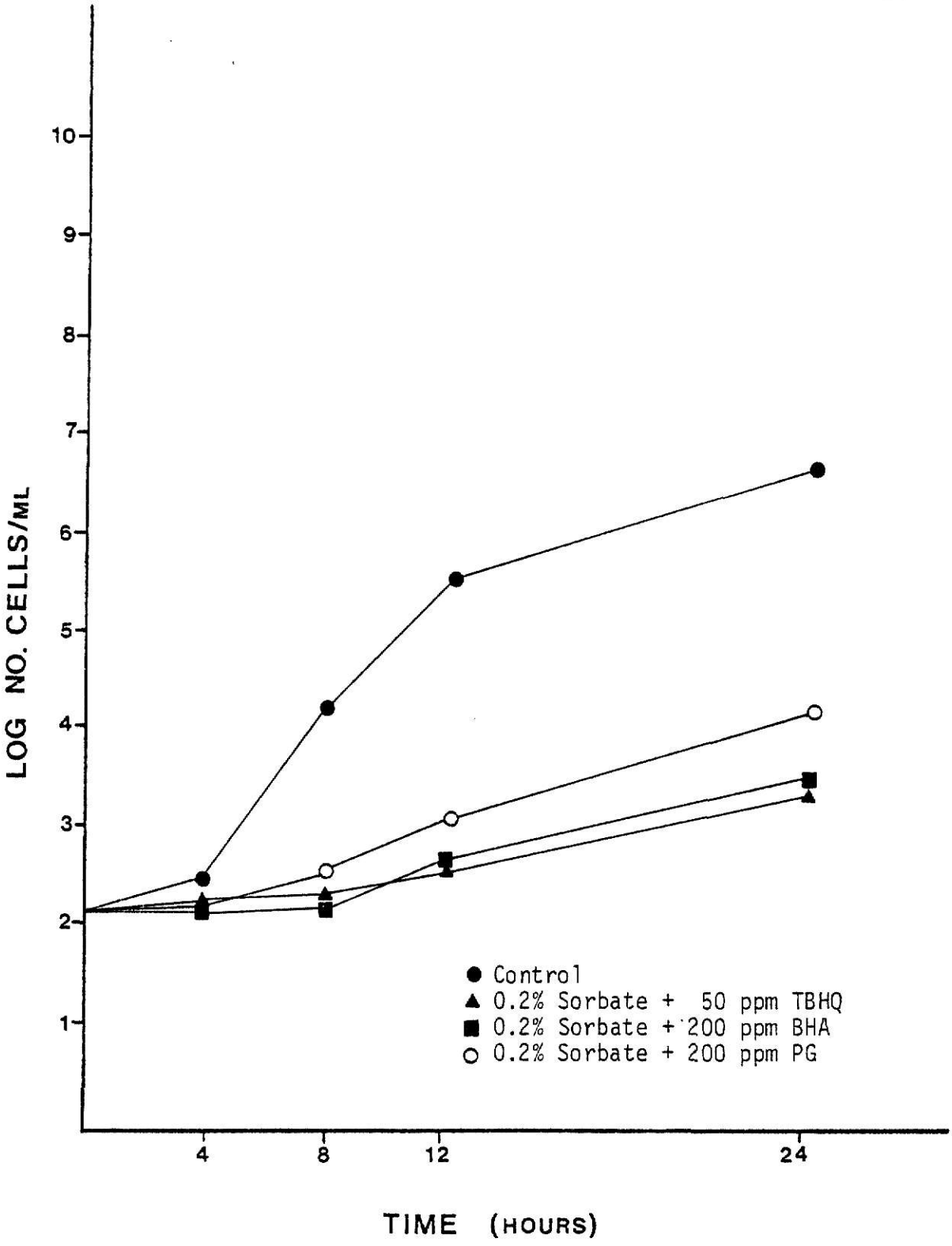


Figure 12. Effect of potassium sorbate, combined with TBHQ, BHA and PG, on growth of Staphylococcus aureus S-6 in fresh ground beef at 30°C.

increase of growth was observed at 24 hours.

The data in Figures 11 and 12 demonstrates that concentrations of potassium sorbate, BHA, TBHQ and PG, found to be totally or highly inhibitory to the growth of the test microorganisms in broth media, were only able to suppress final growth levels in fresh ground beef held at 30°C. The antimicrobial effectiveness of the antioxidants tested was found to be markedly reduced in a food system containing 26% lipid content. Unsaturated lipids are extremely reactive compounds which undergo autoxidation with the subsequent formation of free radicals and hydroperoxides (Dugan, 1976). Antioxidants, such as those tested, are active in preventing autoxidation by donating a hydrogen-free radical to the unstable lipid-free radical, terminating the chain reaction. The antimicrobial activity of the antioxidants may be destroyed due to the reaction between the antioxidant and a lipid molecule that involves the antioxidant molecule, making it no longer effective. Also, the hydrophobic nature of antioxidants and their solubility in lipid compounds might cause the antioxidants to be localized within the lipid portion of foods. If this happens, the antioxidant would be unavailable to act against microorganisms in other portions of the food.

Previous studies have shown the presence of lipids to decrease the antimicrobial activity of BHA. Robach et al. (1977) found Vibrio parahaemolyticus (04:K11) totally inhibited at 3 hours by 50 ppm BHA in trypticase soy broth containing 2.5% NaCl at 35°C. When a sterile crab meat homogenate was used for growth however, 400 ppm BHA was found to be necessary to totally inhibit growth. The authors stated that the marked decrease in the effectiveness of antioxidants to inhibit growth may have been due to a partial inactivation of the antioxidant properties by the

oxidized crab meat lipids. Klindworth et al. (1979) found the addition of corn oil, up to 5% (v/v), to trypticase sulfite neomycin agar, caused a reduction in the inhibition of C. perfringens by 200 ppm BHA, indicating some interaction between BHA and the lipid. Nevertheless, BHA's antimicrobial activity still remained, as greater inhibition was observed for lipid samples containing BHA than those without BHA.

Another factor that may influence the overall effectiveness of the potassium sorbate/antioxidant combinations against the test microorganisms is the presence and growth of the other microorganisms in substantial numbers in the ground beef. Aerobic plate counts of plain ground beef showed 10^6 organisms/g of sample, indicating a high initial load of microorganisms for the sorbate/antioxidant treatments to contend with. Potassium sorbate and the three antioxidants have been shown to be effective against types of spoilage bacteria present in meat (Robach, 1978 and 1979; Davidson, et al., 1980; Erickson and Tompkin, 1977). The effectiveness of the sorbate/antioxidant combinations against the test organisms could be diluted due to presence of the other microorganisms. Aerobic plate counts at the end of the 24-hour test period were approximately 1/2-log cycle lower for the sorbate/antioxidant treatment samples than the control.

Next, varying levels of potassium sorbate alone were investigated to determine the effect on the growth of the test organisms in ground beef. The results of 0.2, 0.4 and 0.6% potassium sorbate (w/w) levels on growth of S. senftenberg are shown in Figure 13. All levels of sorbate were found to be effective in limiting growth through 12 hours. The 0.2% level allowed a 1-log cycle growth in 24 hours, while 0.4 and 0.6% sorbate were more effective in limiting growth. Data for the efficacy of

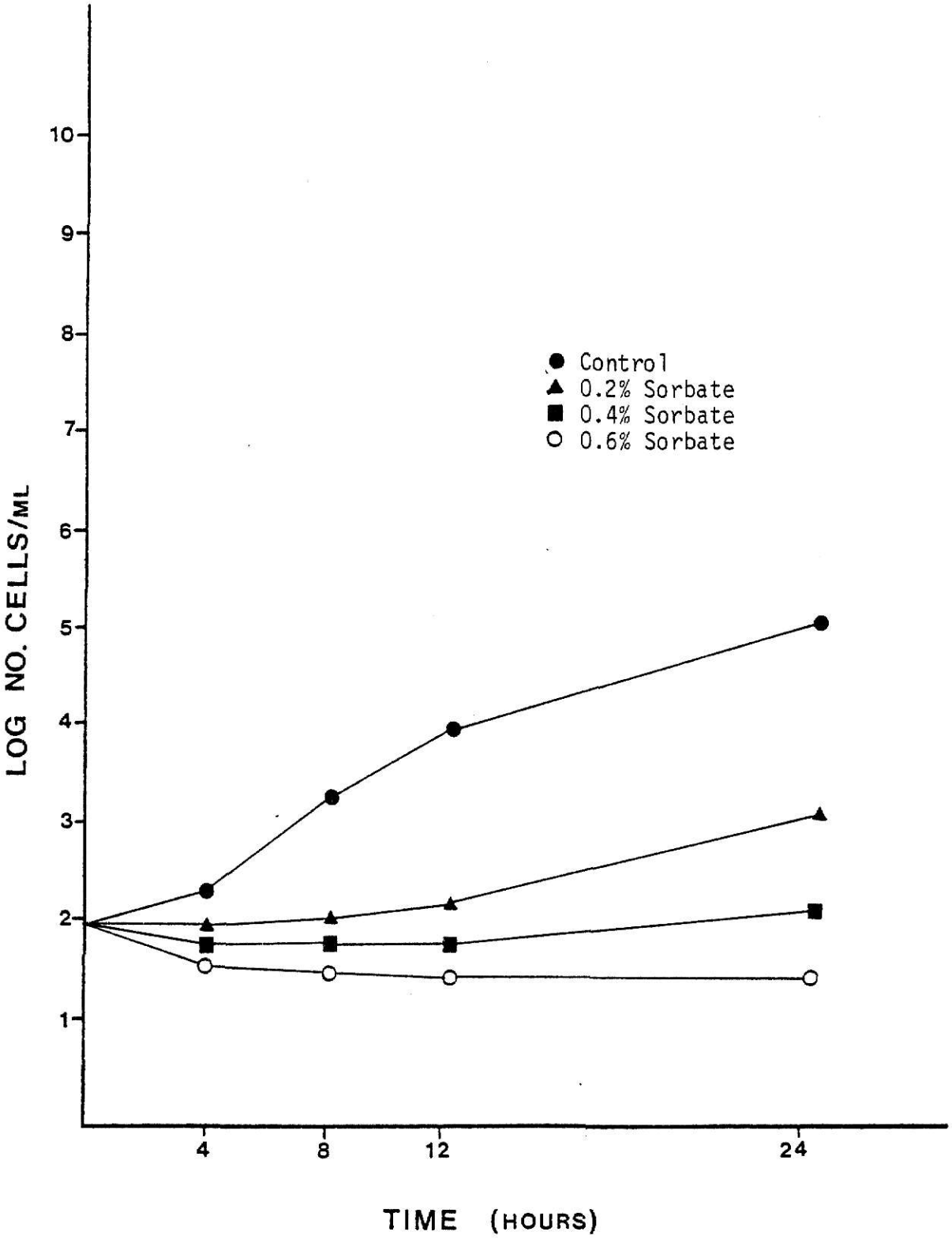


Figure 13. Effect of potassium sorbate on growth of Salmonella senftenberg in fresh ground beef at 30°C.

potassium sorbate levels against S. aureus S-6 is shown in Figure 14. Initial growth of the test organism was delayed 4 hours, after which time growth was observed to increase in all treatment levels.

Potassium sorbate alone was found to be more effective in delaying initial growth of S. senftenberg than S. aureus S-6. Counts of S. senftenberg were equal to or below the initial inoculum level through 12 hours, whereas, S. aureus S-6 growth was delayed only through 4 hours. Potassium sorbate alone in treatments, in general, was found to be as effective as the sorbate/antioxidant combinations against the growth of test bacteria. This finding lends support to the fact that due to autoxidation of the lipid portion of the ground beef, the antioxidants were being tied up or were solubilized in the fat portion and their antimicrobial properties diminished to a large extent.

The effect of sorbate/antioxidant and sorbate alone treatments at each time interval against the test microorganism, as compared to the control, was analyzed using Two-Way Analysis of Variance. All treatments tested at all sampling times were found to significantly ($P<0.05$) inhibit growth of both S. senftenberg and S. aureus S-6 in ground beef at 30°C. None of the potassium sorbate and antioxidant combinations were found to inhibit growth of the test microorganisms significantly better ($P<0.05$.) than another.

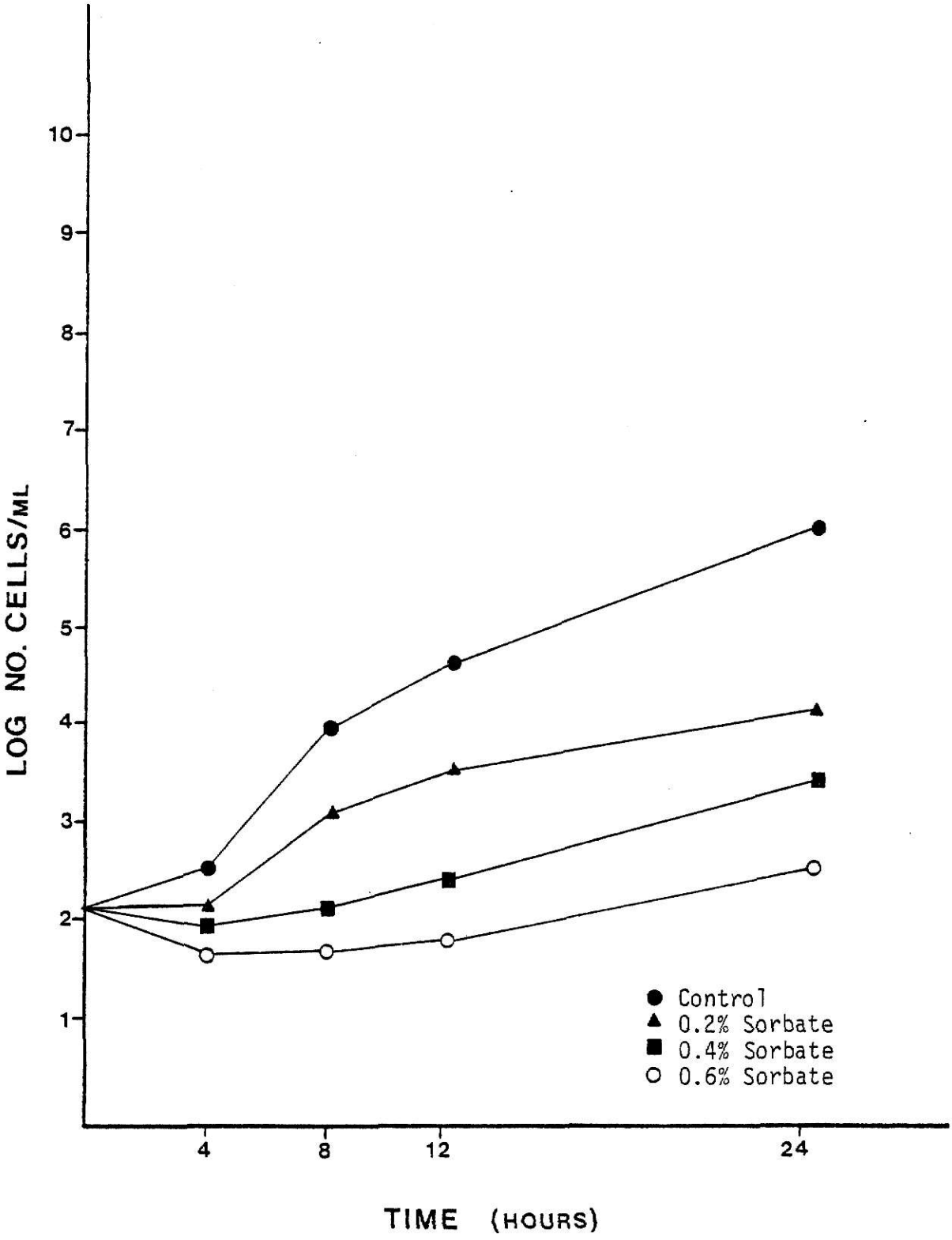


Figure 14. Effect of potassium sorbate on growth of Staphylococcus aureus S-6 in fresh ground beef at 30°C.

CONCLUSION

The results of growth inhibition studies in tryptic soy broth demonstrated that a concentration of 0.2% potassium sorbate, when combined with 200 ppm BHA, TBHQ or PG, yielded a synergistic antimicrobial effect against the growth of Salmonella senftenberg. Potassium sorbate at 0.1% concentration, combined with 200 ppm BHA, was totally bactericidial to Staphylococcus aureus S-6 at 8 hours in tryptic soy broth (pH 5.5) at 37°C. Tertiary butylhydoquinone at 25 ppm, when combined with 0.1-0.2% potassium sorbate, was shown to be totally antimicrobial to Staphylococcus aureus S-6 growth at 12 and 24 hours respectively under the same culture conditions. These same concentrations of potassium sorbate/antioxidant combinations, when added to fresh ground beef, inoculated with S. senftenberg or S. aureus S-6 and held at 30°C for 24 hours, were found to be less antimicrobial to the growth of the test microorganisms. Concentrations of 0.2-0.6% potassium sorbate alone in ground beef were found to be effective in limiting growth of S. senftenberg and S. aureus S-6, compared to control growth. All potassium sorbate/antioxidant and potassium sorbate alone treatments tested in ground beef were found to significantly inhibit ($P<0.5$) growth of the test microorganisms when compared to control treatments.

REFERENCES

Ayaz, M., Luedecke, L.O. and Branen, A.L. 1978. Antimicrobial effect of butylated hydroxyanisole and butylated hydroxytoluene on Staphylococcus aureus. J. Food Protect. 43:4.

Bradley, R.L., Harmon, L.G. and Stine, C.M. 1962. Effect of potassium sorbate on some organisms associated with cottage cheese spoilage. J. Milk Food Technol. 25:318.

Borg, A.F., Etchells, J.L. and Bell, T.A. 1955. The influence of sorbic acid on microbial activity in commercial cucumber fermentations. Bacteriol. Proc., Abstract 19.

Boyd, J.W. and Tarr, H.L.A 1955. Inhibition of mold and yeast development in fish products. Food Technol. 9:411.

Chang, H.C. and Branen, A.L. 1975. Antimicrobial effects of butylated hydroxyanisole (BHA). J. Food Sci. 40:349.

Costilow, R.N., Ferguson, W.E. and Ray, S. 1955. Sorbic acid as a selective agent in cucumber fermentations. I. Effect of sorbic acid on microorganisms associated with cucumber fermentations. Appl. Microbiol. 3:341.

Costilow, R.N., Coughlin, F.M., Robach, D.L. and Ragheb, H.S. 1956. A study of the acid-forming bacteria from cucumber fermentations in Michigan. Food Res. 21:27.

Costilow, R.N., Coughlin, F.M., Robbins, E.K. and Hsu, W.T. 1957. Sorbic acid as a selective agent in cucumber fermentations. II. Effect of sorbic acid on the yeast and lactic acid fermentation in brined cucumbers. Appl. Microbiol. 5:373.

Cunningham, F.E. 1979. Shelf life and quality characteristics of poultry parts dipped in potassium sorbate. J. Food Sci. 44:863.

Davidson, P.M. and Branen, A.L. 1980a. Inhibition of two psychrotrophic Pseudomonas species by butylated hydroxyanisole. J. Food Sci. 45:1603.

Davidson, P.M. and Branen, A.L. 1980b. Antimicrobial mechanisms of butylated hydroxyanisole against two Pseudomonas species. J. Food Sci. 45:1607.

Davidson, P.M., Brekke, C.J. and Branen, A.L. 1981. Antimicrobial activity of butylated hydroxyanisole, tertiary butylhydroquinone, and potassium sorbate in combination. J. Food Sci. 46:314.

Deuel, H.J., Alfin-Slater, R., Weil, C.S. and Smyth, H.F. 1954. Sorbic acid as a fungistatic agent for foods. I. Harmlessness of sorbic acid as a dietary component. Food Res. 19:1.

Emard, L.O. and Vaughn, R.H. 1952. Selectivity of sorbic acid media for the catalase negative lactic acid bacteria and clostridia. J. Bacteriol. 63:487.

Erickson, D.R. and Tompkin, R.B. 1977. Antimicrobial and antirancidity agent. U.S. Patent No. 4,044,160. August 23, 1977.

Fung, D.Y.C., Taylor, S. and Kahan, J. 1977. Effect of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) on growth and aflatoxin production of Aspergillus flavus. J. Food Safety. 1:39.

Gooding, C.M. 1945. Process of inhibiting growth of molds. U.S. Patent No. 2,379,294.

Gooding, C.M., Melnick, D., Lawrence, R.L. and Luckmann, F.H. 1955. Sorbic acid as a fungistatic agent for foods. IX. Physiochemical considerations in using sorbic acid to protect foods. Food Res. 20:639.

Ivey, F.J., Shaver, K.J., Christiansen, L.N. and Tompkin, R.B. 1978. Effect of potassium sorbate on toxiongenesis of Clostridium botulinum in bacon. J. Food Prot. 41:621.

Ivey, F.J. and Robach, M.C. 1978. Effect of sorbic acid and sodium nitrite on Clostridium botulinum outgrowth and toxin production in canned comminuted pork. J. Food Sci. 43:1782.

Jones, A.H. and Harper, G.S. 1952. A preliminary study of factors affecting the quality of pickles on the Canadian market. Food Technol. 6:304.

Klindworth, K.J., Davidson, P.M., Brekke, C.J. and Branen, A.L. 1979. Inhibition of Clostridium perfringens by butylated hydroxyanisole. J. Food Sci. 44:564.

Lahellec, C., Fung, D.Y.C. and Cunningham, F.E. 1981. Growth effect of sorbate and selected antioxidants on toxigenic strains of Staphylococcus aureus. J. Food Prot. 44:(in press).

LaRocco, K.A. and Martin, S.E. 1981. Effects of potassium sorbate alone and in combination with sodium chloride on the growth of Salmonella typhimurium 7136. J. Food Sci. 46:568.

Luck, E. 1976. Sorbic acid as a food preservative. Int. Flavors and Food Additiv. 7:122.

Moustafa, H.H. and Collins, E.B. 1969. Effects of selected food additives on growth of Pseudomonas fragi. J. Dairy Sci. 52:335.

Paquette, M.W., Robach, M.C., Sofos, J.N. and Busta, F.F. 1980. Effects of various concentrations of sodium nitrite and potassium sorbate on color and sensory qualities of commercially prepared bacon. J. Food Sci. 45:1293.

Park, H.S., Marth, E.H. and Olson, N.F. 1970. Survival of Salmonella typhimurium in cold-pack cheese food during refrigerated storage. J. Milk Food Technol. 33:383.

Park, H.S. and Marth, E.H. 1972. Inactivation of Salmonella typhimurium by sorbic acid. J. Milk Food Technol. 35:532.

Perry, G.A., Lawrence, R.L. and Melnick, D. 1964. Extension of shelf life of poultry by processing with sorbic acid. Food Technol. 18:101.

Phillips, G.F. and Muniot, J.O. 1950. Sorbic acid as an inhibitor of scum yeast in cucumber fermentations. Food Technol. 4:291.

Price, J.F. and Stevenson, K.E. 1979. Effects of sorbate and nitrite in bacon on color, flavor and Clostridium botulinum toxigenesis. Presented at the 39th Annual Meeting of the Institute of Food Technologists, St. Louis, Mo. Abstract No. 373.

Robach, M.C., Smoot, L.A. and Pierson, M.D. 1977. Inhibition of Vibrio parahaemolyticus 04:K11 by butylated hydroxyanisole. J. Food Prot. 40:549.

Robach, M.C. and Ivey, F.J. 1978. Antimicrobial efficacy of a potassium sorbate dip on fresh processed poultry. J. Food Prot. 41:284.

Robach, M.C. 1978. Effect of potassium sorbate on the growth of Pseudomonas fluorescens. J. Food Sci. 43:1886.

Robach, M.C. and Hickey, C.S. 1978. Inhibition of Vibrio parahaemolyticus by sorbic acid in crab meat and flounder homogenates. J. Food Prot. 41:699.

Robach, M.C. 1979. Influence of potassium sorbate on growth of Pseudomonas putrefaciens. J. Food Prot. 42:312.

Robach, M.C., To, E.C., Meydav, S. and Cook, C.F. 1980. Effect of sorbates on microbiological growth in cooked turkey products. J. Food Sci. 45:638.

Robach, M.C. 1980. Interaction of salt, potassium sorbate and temperature on the outgrowth of Clostridium sporogenes PA 3679 spores in a pre-reduced medium. J. Food Sci. 45:742.

Robach, M.C. and Stateler, C.L. 1980. Inhibition of Staphylococcus aureus by potassium sorbate in combination with sodium chloride, tertiary butylhyrdoquinone, butylated hydroxyanisole or ethylenediamine tetraacetic acid. J. Food Prot. 43:208.

Shih, A.L. and Harris, N.D. 1977. Antimicrobial activity of selected antioxidants. J. Food Prot. 40:520.

Smith, D.P. and Rollins, N. 1954. Sorbic acid as a fungistatic agent for foods. VII. Effectiveness of sorbic acid in protecting cheese. Food Res. 19:59.

Sofos, J.N., Busta, F.F. and Allen, C.E. 1979a. Sodium nitrite and sorbic effects of Clostridium botulinum spore germination and total microbial growth in chicken frankfurter emulsions during temperature abuse. Appl. Environ. Microbiol. 37:1103.

Sofos, J.N., Busta, F.F. and Allen, C.E. 1979b. Clostridium botulinum control by sodium nitrite and sorbic acid in various meat and soy protein formulations. J. Food Sci. 44:1662.

Sofos, J.N., Busta, F.F., Bhothipaksa, K., Allen, C.E., Robach, M.C. and Paquette, M.W. 1980. Effects of various concentrations of sodium nitrite and potassium sorbate on Clostridium botulinum toxin production in commercially prepared bacon. J. Food Sci. 45:1285.

Tompkin, R.B., Christiansen, L.N., Shaparis, A.B. and Bolin, H. 1974. Effect of potassium sorbate on salmonellae, Staphylococcus aureus, Clostridium perfringens and Clostridium botulinum in cooked, uncured sausage. Appl. Microbiol. 28:262.

United States Department of Agriculture. 1978. Proposal to affirm potassium sorbate and sorbic acid as GRAS. Fed. Regist. March 10, 43:9823.

United States Department of Agriculture. 1978. Substances used in preparation of bacon. Fed. Regist. May 16, 43:21007.

Ward, M.S. and Ward, B.Q. 1967. Initial evaluation of the effect of butylated hydroxytoluene upon Salmonella senftenberg 775W. Poultry Sci. 46:1601.

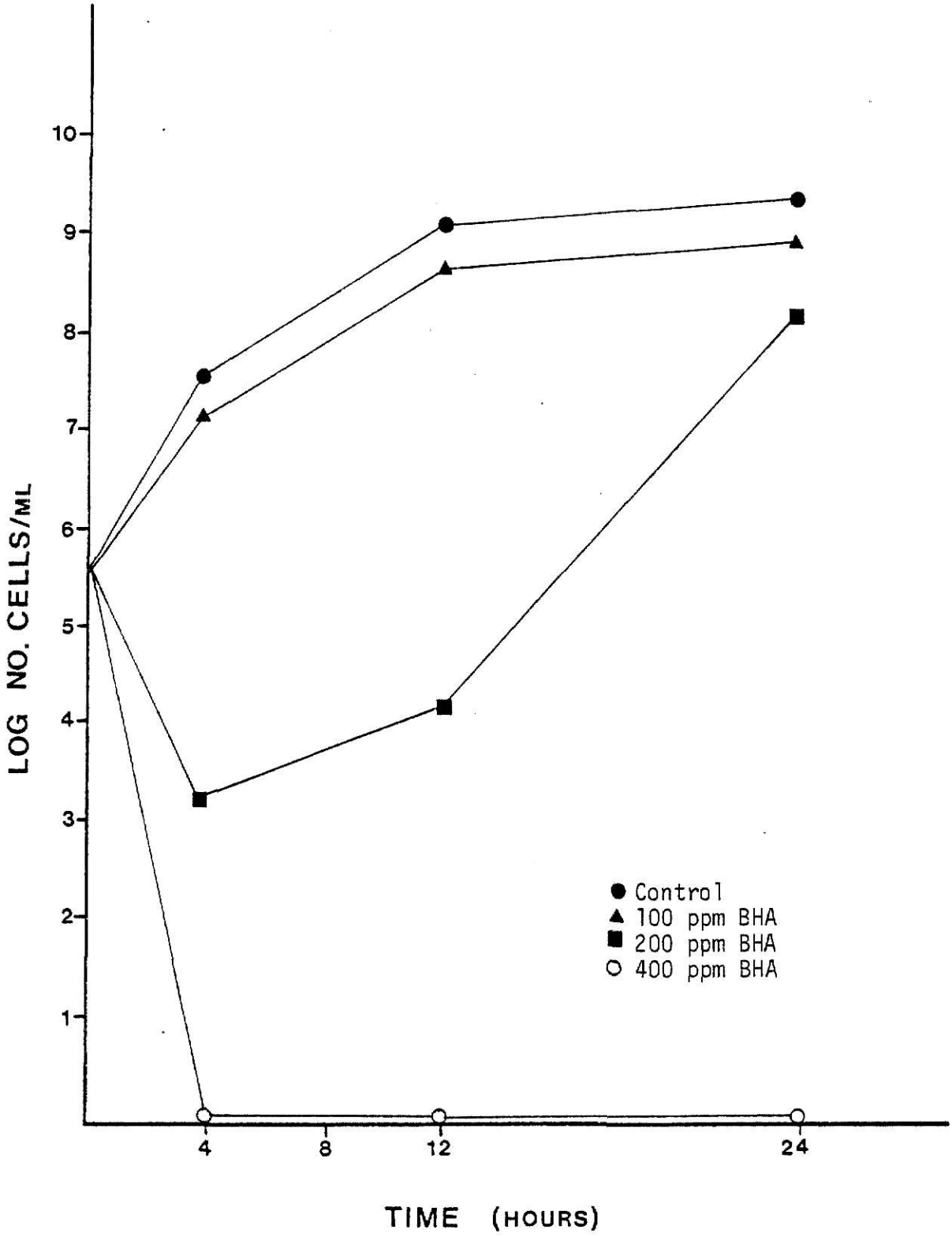
ACKNOWLEDGEMENTS

The author is grateful and indebted to his major professor, Dr. Franklin E. Cunningham, Department of Animal Sciences and Industry, for providing assistance in this study and for his helpful suggestions in the preparation of this thesis.

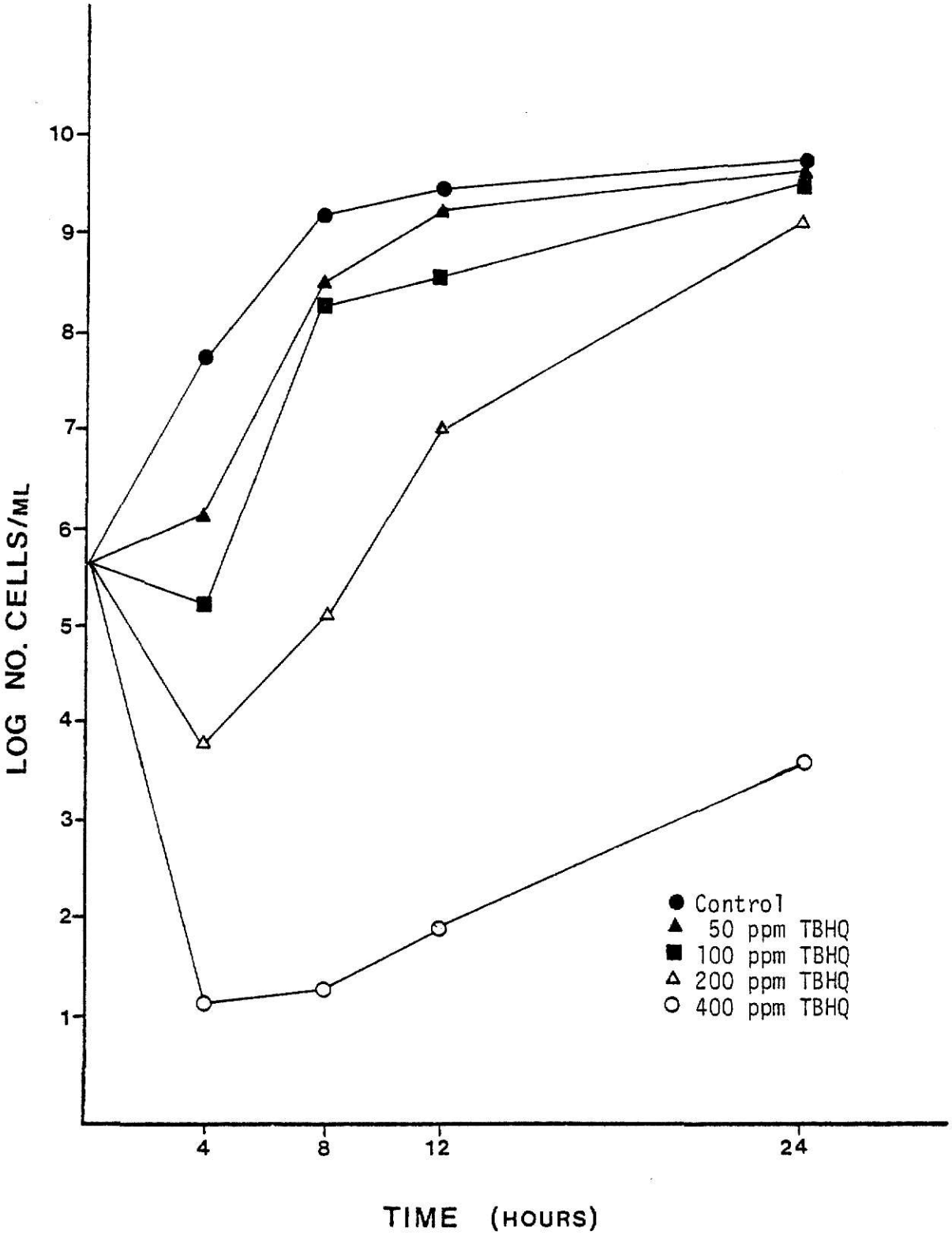
The author wishes to thank Dr. Daniel Y.C. Fung and Dr. Richard Bassette, Department of Animal Sciences and Industry, for serving on his committee and for their help and guidance as professors and friends during the author's studies at Kansas State University.

Most of all, the author wishes to thank his lovely wife, Marilyn, for her love, understanding and untiring assistance that aided in the completion of this manuscript and studies at this university.

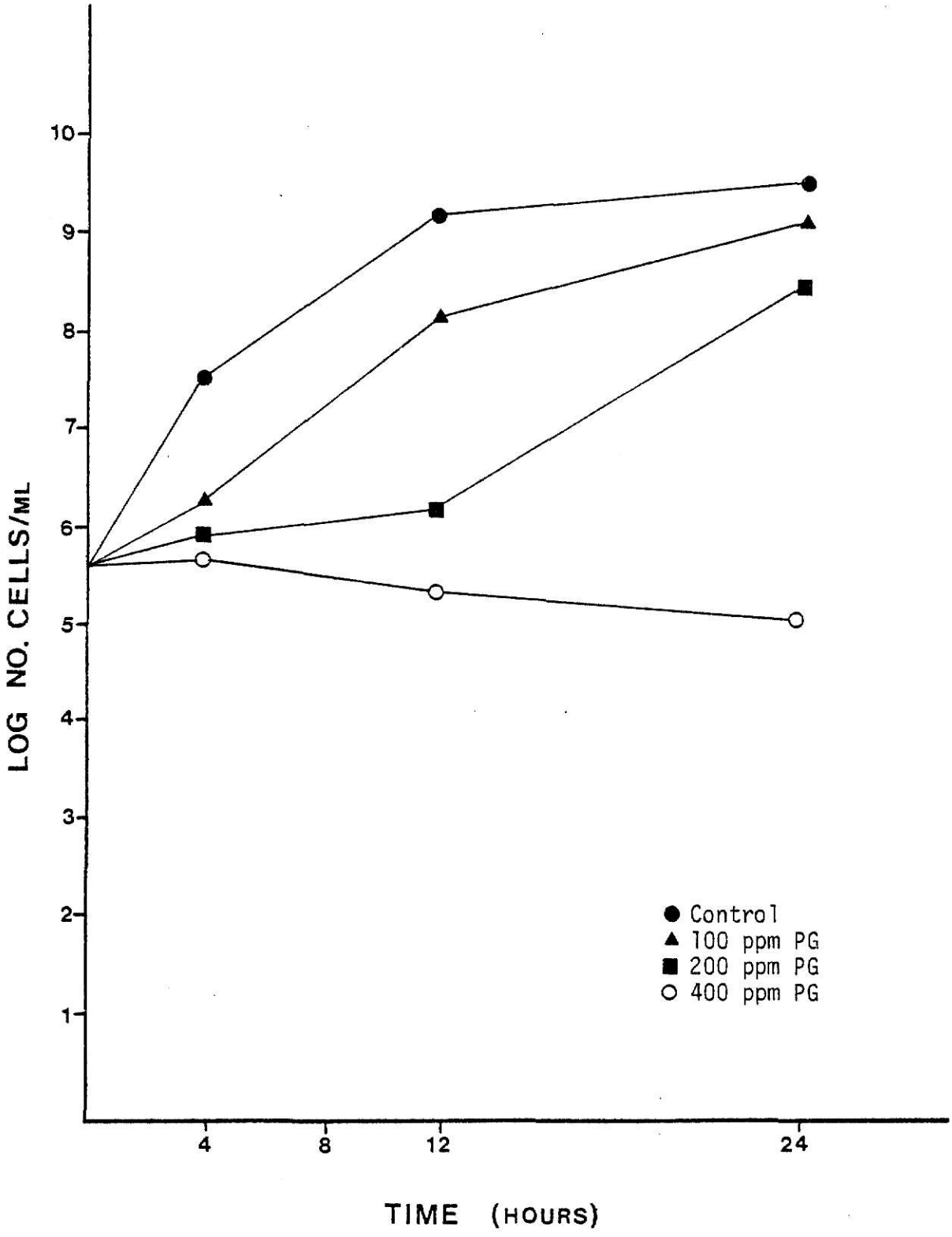
APPENDIX



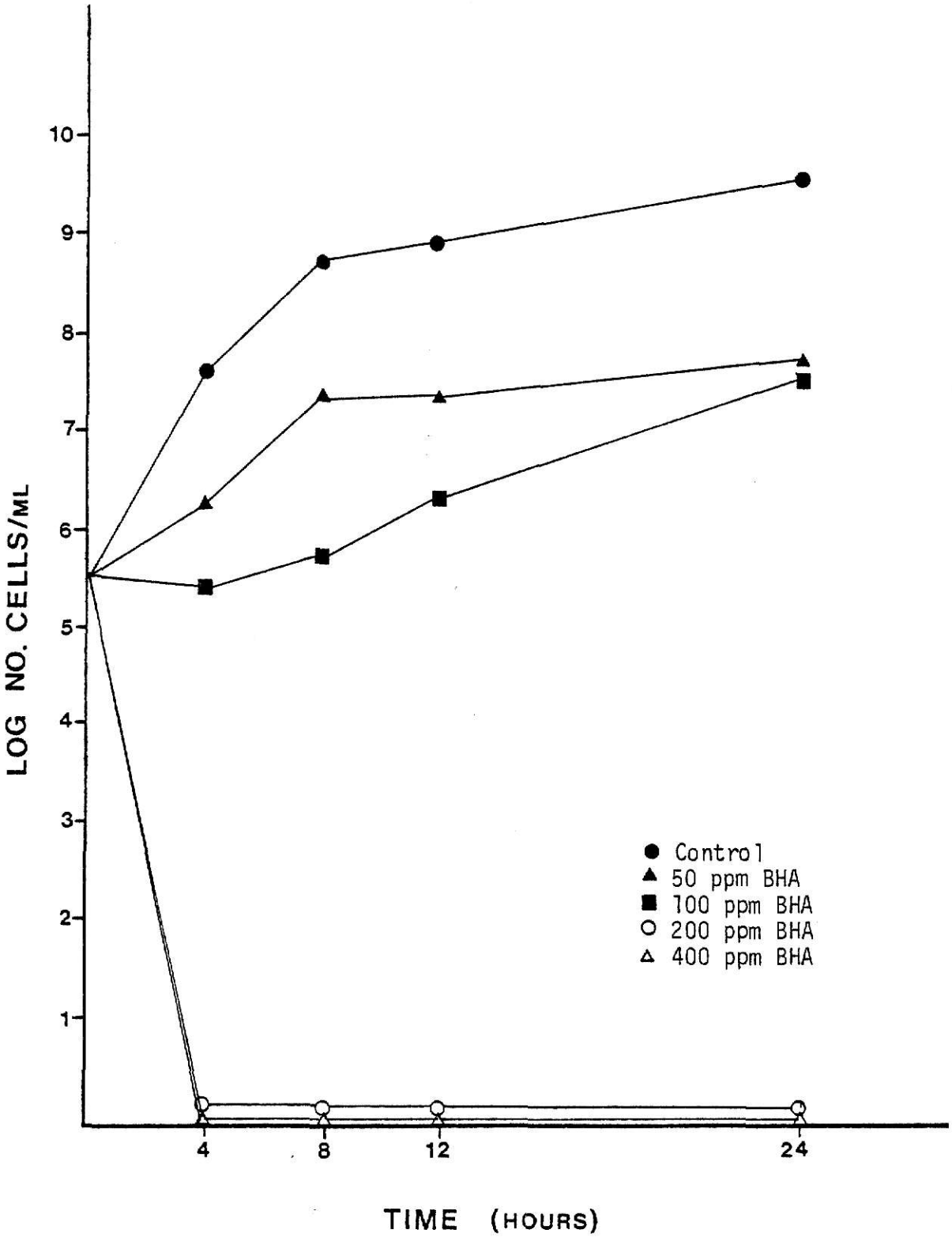
Appendix Figure 1. Effect of BHA on growth of Salmonella senftenberg in tryptic soy broth (pH 5.5) at 37°C.



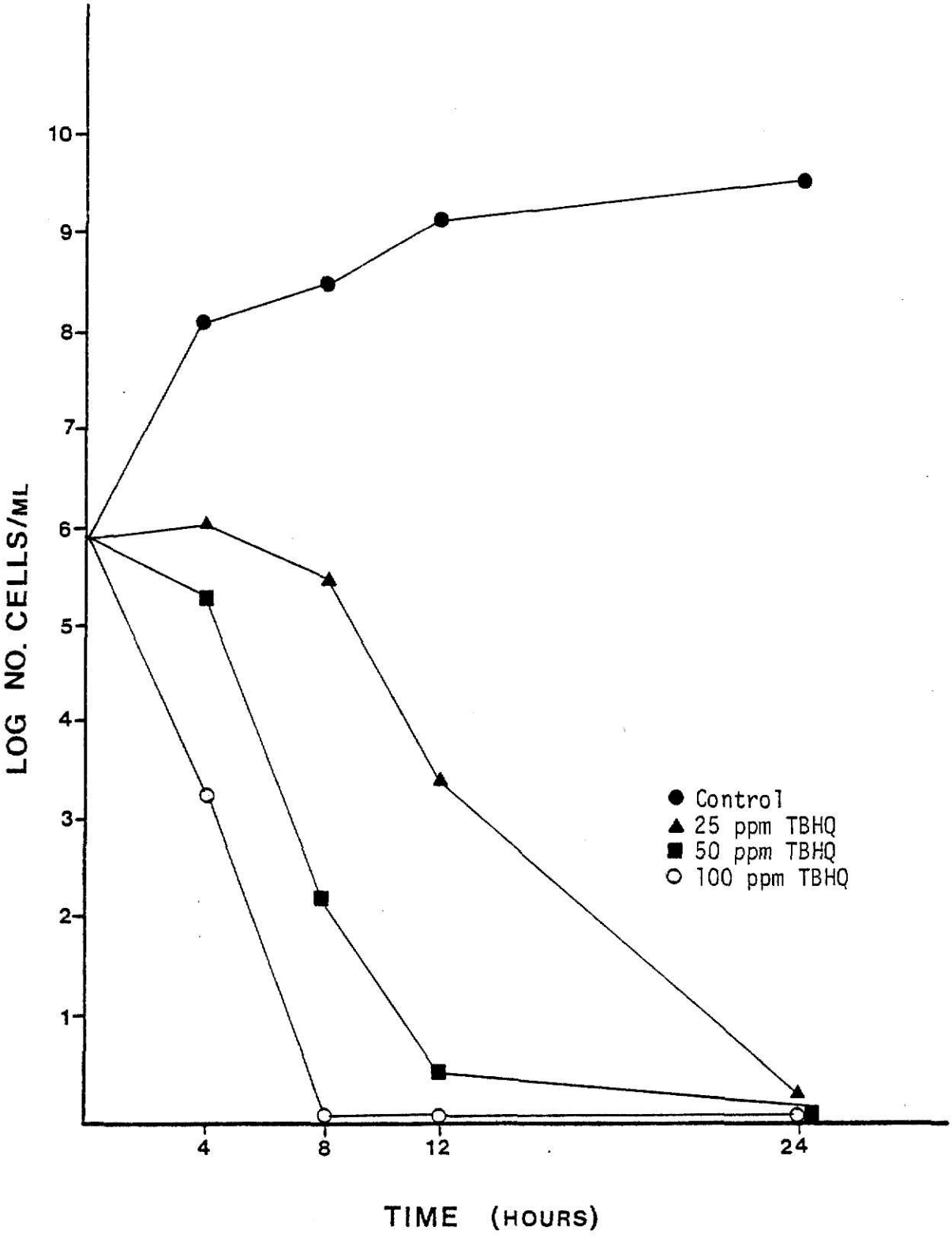
Appendix Figure 2. Effect of TBHQ on growth of Salmonella senftenberg in tryptic soy broth (pH 5.5) at 37°C.



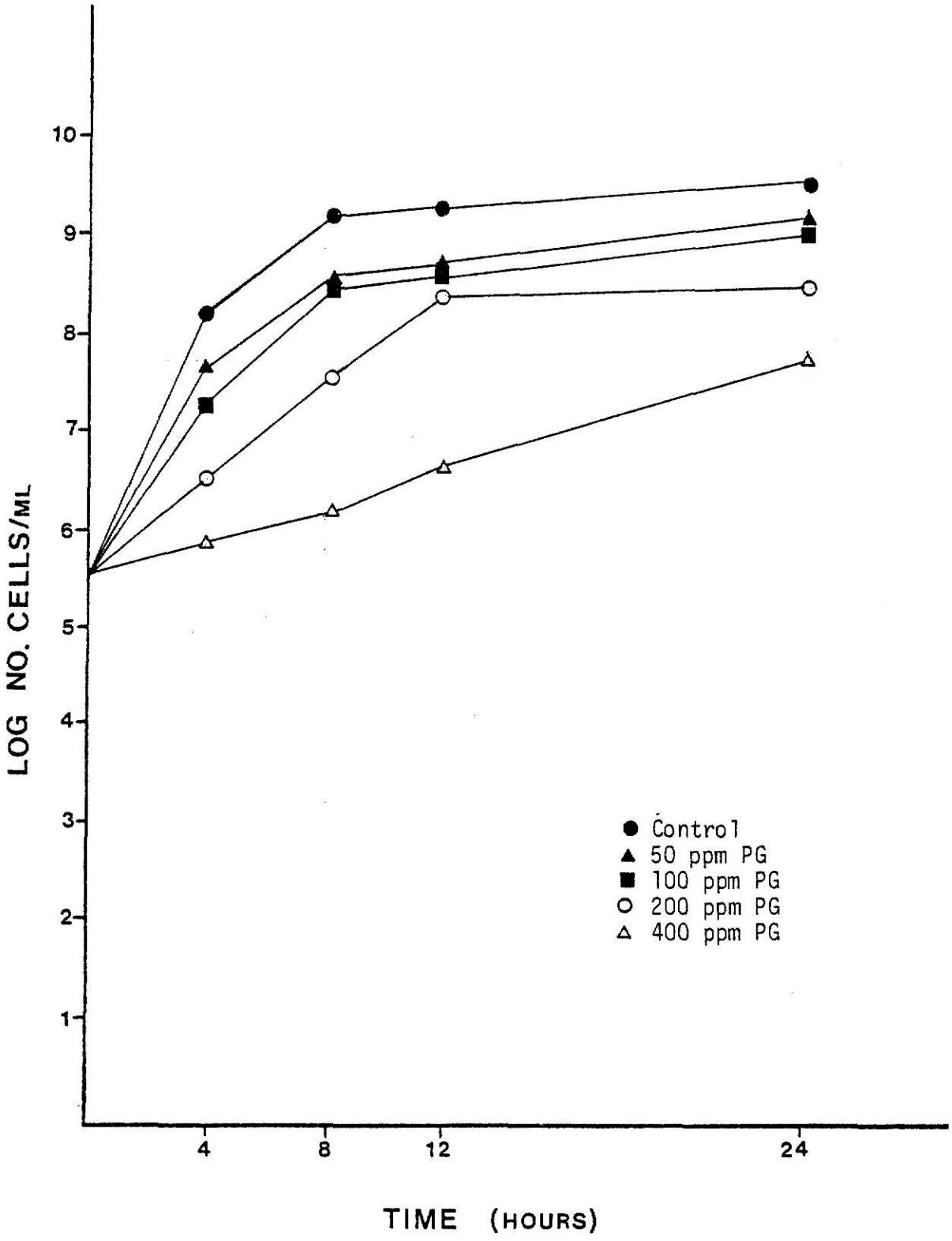
Appendix Figure 3. Effect of PG on growth of Salmonella senftenberg in tryptic soy broth (pH 5.5) at 37°C.



Appendix Figure 4. Effect of BHA on growth of Staphylococcus aureus S-6 in tryptic soy broth (pH 5.5) at 37°C.



Appendix Figure 5. Effect of TBHQ on growth of Staphylococcus aureus S-6 in tryptic soy broth (pH 5.5) at 37°C.



Appendix Figure 6. Effect of PG on growth of Staphylococcus aureus S-6 in tryptic soy broth (pH 5.5) at 37°C.

EFFECTS OF POTASSIUM SORBATE SINGLY AND IN COMBINATION WITH BUTYL HYDROXYANISOLE, TERTIARY BUTYLHYDROQUINONE AND PROPYL GALLATE ON THE GROWTH OF *STAPHYLOCOCCUS AUREUS* S-6 AND *SALMONELLA SENFTENBERG*

by

ROGER EDWARD POERSCHKE

B.A., Emporia State University, 1973

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

in

Food Science

Department of Animal Sciences and Industry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1981

ABSTRACT

The effect of potassium sorbate alone and in combination with butyl hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ) or propyl gallate (PG) on the growth of Salmonella senftenberg and Staphylococcus aureus S-6 was studied. The growth studies first were made using trypticase soy broth (pH 5.5) at 37°C. Certain combinations of sorbate with BHA, TBHQ and PG resulted in synergistic inhibition of growth of each test microorganism. The concentration of 0.2% potassium sorbate and 200 ppm BHA was found to be the most effective in synergistic inhibition of growth of S. senftenberg, whereas 0.2% potassium sorbate and 25 ppm TBHQ resulted in total inhibition of S. aureus S-6 at 12 hours.

The concentrations of potassium sorbate, combined with BHA, TBHQ or PG, that were the most effective in synergistic inhibition of growth of S. senftenberg and S. aureus S-6 in media, were used to study the effectiveness of sorbate/antioxidant combinations in fresh ground beef held at elevated abuse temperature (30°C). All the sorbate/antioxidant combinations tested resulted in suppressed final growth of both S. senftenberg and S. aureus S-6. The antimicrobial effectiveness of sorbate/antioxidant combinations in fresh ground beef was markedly diminished, compared to media studies. Potassium sorbate alone in ground beef was found to be effective in the limitation of S. senftenberg and S. aureus S-6 growth. All potassium sorbate/antioxidant and potassium sorbate alone treatments tested in ground beef were found to significantly inhibit ($P<0.05$) growth of the test organisms when compared to control samples.