pH and Low Level (10 ppm) Effects of HB2 Against *Campylobacter jejuni*

Background/Purpose

The contamination of food products by pathogenic organisms such as *Salmonella* or *Campylobacter* is an on-going problem that is addressed within the processing plant using antimicrobial products. The efficacy of Food Contact Substances (FCS) is important to assure a safe and reliable food supply. Poultry processing facilities are adopting new and improved chemical intervention steps of treating their poultry with FDA approved sanitizers as part of their HACCP programs. One of these new FCS approved for use by the FSIS is liquid hypobromous acid (HB2). This laboratory has shown the increased sensitivity (efficacy) of Salmonella at elevated pH regimes, particularly in the 7.5-8.5 range, and it important to establish if any pH response to hypobromous acid is evident for Campylobacter. Therefore, the purpose of this study was to determine whether pH adjustment of available bromine (Br_2) from activated HB2 has any impact on the relative efficacy against *Campylobacter jejuni.* Low challenge concentrations were chosen so the effects of the exposure could be tracked over reasonable time periods.

Methods

Test Systems:
Campylobacter jejuni bacteria (ATCC 33291)

Campylobacter jejuni bacteria (ATCC 33291) were cultured in Bolton Broth (Sigma Aldrich, lot number BCBB7257) containing 5% defibrinated sheep blood (Hardy Diagnostics, lot number 11243) by microaerophilic incubation (~20% oxygen) for two days at 42^{o} C. The bacteria were separated from the nutrient broth by centrifugation. One ml of the concentrated bacteria mixture was removed and plated on Campy Cefex Agar (Hardy Diagnostics, lot number 10315). This was repeated eight times to achieve a total of eight *Campylobacter* inoculated Campy Cefex Agar plates. The plates were kept under a microaerophilic atmosphere and incubated for 48 hours at 42^{o}C. After two days, the surfaces of the six

Campylobacter inoculated Campy Cefex Agar plates were aseptically scraped using a sterile L-shaped spreader to remove as many of the *Campylobacter* colonies as possible. The bacteria colonies were carefully resuspended in approximately 1550 ml of sterile phosphate buffer. The amount of *Campylobacter jejuni* bacteria was measured in the *Campylobacter*/phosphate buffer solution, to serve as the control, by serial dilution and plating on Campy Cefex Agar (Hardy Diagnostics, lot number 10315). Immediately thereafter, the remaining solution was split into three 500 ml samples. The three 500 ml samples were used to demonstrate the efficacy of 10 ppm Br_2 (from HB2) against *Campylobacter jejuni* at three different pHs.

Test Material Preparation:

HB-2 activation occurs by blending hydrogen bromide with a hypochlorite source. For this study, hypobromous acid was created on-site by combining hydrogen bromide (HB-2) and sodium hypochlorite. The actual Br_2 concentration measured before use in the experiment was approximately 3000 ppm as Br_2. This concentrated stock solution was then used to dose the three test solutions to 10 ppm available bromine (Br_2).

One at a time, a calculated amount of the concentrated stock solution was introduced to one of the 500 g test material samples. Immediately thereafter, the actual concentration of the test material was measured by using the modified DPD method (U.S. patent no. 7,651,724, *Howarth and Harvey*) and a HACH Chlorine Colorimeter. The value was multiplied by the mole weight ratio of chlorine to bromine (2.25), and the dilution factor of the test material. Each solution was adjusted to either pH 6, pH 7 of pH 8 ($\pm$0.1) using either hydrochloric acid or sodium hydroxide.

At the 1, 5, 10 and 20 minute time intervals the pH and actual concentrations of the test solutions were measured using the Modified DPD method. All plating was done in duplicate. One ml of the test solution was removed and plated without dilution on Campy Cefex Agar at 1, 5, 10 and 20 minute time intervals. At the same time another ml of each of the test solution was removed, serially diluted to 10^{-2} and 10^{-4} (using 99 ml Butterfield's Buffer) and subsequently plated on Campy Cefex Agar.

After plating, all Campy Cefex Agar Plates (including the control) were incubated under microaerophilic conditions for 48 hours at 42°C. After incubation, the plates were enumerated.

Results and Discussion

During the challenge testing, the residual Br_2, as well as the pH, was measured at the 1, 5, 10, and 20 minute time intervals. This data is reported in Table 1. It can be seen that over the course of the 20 minute contact time, the pH remained relatively stable throughout. However, the Br_2 concentration steadily declined throughout the test period at each pH, and was faster for the pH 8 sample compared to the pH 6 and pH 7 sample.

Table 1: *Campylobacter* pH and Residual Br_2 (ppm)

	pH 6		**pH 7**		**pH 8**	
Time (min)	**pH**	**ppm Br_2**	**pH**	**ppm Br_2**	**pH**	**ppm Br_2**
0	5.90	9.45	7.03	9.45	8.08	9.68
1 min	5.94	9.45	7.04	9.45	8.07	9.45
5 min	5.96	8.33	7.04	7.88	8.03	7.20
10 min	5.99	6.75	7.05	7.88	8.03	6.75
20 min	6.04	6.75	7.06	6.75	8.01	5.85

Table 2 shows the microbiological results of the *Campylobacter jejuni* suspension before treatment with Br_2, and at 1, 5, 10, and 20 minutes after the samples were dosed to a nominal 10 ppm Br_2. It can be seen that the Br_2 at 10 ppm remains effective from pH 6 to pH 8. The *Campylobacter* was eradicated between 5 and 10 minutes in all three solutions. As expected, the longer the contact time, the more bacteria was eradicated.

Table 2: *Campylobacter* Microbiological Results

pH (time)	Log_{10} CFU/ml Remaining	Log_{10} Reduction
Before treatment (pH 6)	5.53	N/A
(1 min)	0.78	4.75 (99.998%)
(5 min)	0.30	5.23 (99.999%)
(10 min)	0.00	>5.53 (>99.999%)
(20 min)	0.00	>5.53 (>99.999%)
Before treatment (pH 7)	5.53	N/A
(1 min)	0.85	4.68 (99.998%)
(5 min)	0.30	5.23 (99.999%)
(10 min)	0.00	>5.53 (>99.999%)
(20 min)	0.00	>5.53 (>99.999%)
Before treatment (pH 8)	5.53	N/A
(1 min)	0.70	4.83 (99.998%)
(5 min)	0.30	5.23 (99.999%)
(10 min)	0.00	>5.53 (>99.999%)
(20 min)	0.00	>5.53 (>99.999%)

Conclusions

- The purpose of this study was to determine whether pH adjustment of hypobromous acid solutions (from activated HB2) may affect the efficacy against *Campylobacter* bacteria in these artificial pH environments.

- In this experiment, cultures of *Campylobacter jejuni* were challenged at pH's 6, 7, and 8 at 10 ppm Br_2 (from HB2). The microbiological results shown in Table 2 clearly demonstrate that there is no difference in Br_2 efficacy against *Campylobacter* when comparing the three different pHs, i.e. Br_2 is equally effective over the pH 6 to pH 8 range. As expected, the log_{10} reduction in bacteria increased as the contact time increased.

- The R&D department of Enviro Tech Chemical Services has performed similar challenge studies using different antimicrobials (such as peracetic acid) against

Campylobacter at different pHs. The data obtained from those studies prove that the efficacy of the antimicrobial against *Campylobacter* was not dependent on pH. What was attempted here was to provide data that correlates with data previously obtained, but this time using the antimicrobial HB2. This study clearly confirms that using activated HB2 over a pH range of 6-8 has no significant effect on the relative efficacy of the HB2 on the *Campylobacter* under in the conditions used in this test. It also confirms that peracetic acid solutions similarly had no change in efficacy under the same conditions, therefore it is concluded that Campylobacter is not a pH sensitive organism.

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