Effects of Copper Alloy Surfaces on the Viability of Bacterium, *E. coli* O157:H7

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ABSTRACT

E. coli O157:H7 has been associated with several large-scale food recalls by processors in the United States. This bacterium was found to be nonviable in a few hours when placed on copper surfaces, but survived for many days on stainless steel. The copper alloys tested included coppers, brasses, bronzes, copper-nickels and nickel silvers. The results confirm that the antibacterial effect is present in all the tested copper alloys, and increases with the copper content of the alloy. Furthermore, as expected, this action was faster at the higher temperature. The bacteria were found to be nonviable on almost all of the copper alloy surfaces in the range of one to six hours at 20°C. It took longer, with a minimum time of three hours, at 4°C. This anti-microbial attributes of copper alloys should be useful beyond food processing applications.

INTRODUCTION

Escherichia coli (E. coli) comprise a group of strains of bacterium commonly found in the intestines of cattle, sheep and humans. Although most strains are harmless to humans, several strains are known to be toxic. Verocytotoxigenic *E. coli* (VTEC), particularly *E. coli* O157: H7 is a food-borne pathogen, which has caused several outbreaks of hemolytic colitis. In the elderly and children, VTEC infections can lead to hemolytic uremia syndrome, a life-threatening condition usually treated in intensive care and often requiring blood transfusion and kidney dialysis. It is believed that the number of organisms required to produce infection is quite low and ingestion of as low as 10 to 50 individual bacteria may be sufficient. Thus, very small numbers of pathogens can contaminate work surfaces, with the potential to transfer to uncontaminated raw, processed or precooked foods. Consequently VTEC infections represent a significant and serous public heath problem.

Results from a preliminary study [1] indicated that copper (UNS C10200), and to a lesser extent a 60%Cu-40%Zn-containing brass, Muntz metal (UNS C28000), inhibited the growth of *E. coli* O157, while no inhibition was observed on stainless steel (UNS S30400) surfaces. The focus of the present study is on the inhibition effects of the surfaces of 25 commercial wrought copper base alloys, as well as stainless steel, on *E. coli* O157:H7. Stainless steel (UNS S30400) is widely used in food preparation and processing applications. The objective is to identify those copper alloys that can provide the best combination of microbial inhibition capability, durability, maintainability and corrosion resistance for food processing applications.

MATERIALS AND METHODS

The chemical compositions of the alloys utilized are listed in **Table I** . As can be verified, they range from coppers, to brasses and bronzes, copper-nickels and copper-nickel-zinc alloys (nickel silvers). The other materials tested include polyethylene, from a household chopping board, and stainless steel (UNS S30400), as well as a commercial silver ion containing coating on stainless steel, which is being promoted for its antimicrobial properties.

Small, 1cm x 1cm coupons of each material were sheared for testing samples. *E. coli* was grown aerobically at 37°C in Tryptone Soya Broth (TSB) and stored on microbeads at minus 20°C. Microbeads were removed and thawed at room temperature in TSB and incubated at 37°C until used. For each experiment, cultures were between 15 hours and 20 hours old. A 20-µl culture was placed on each coupon and allowed to air dry. After incubation, at either 4°C or 20°C, the coupons were removed at predetermined times and transferred into 10-ml autoclaved phosphate buffered saline (PBS), and the number of viable organisms remaining after various incubation times was determined, using plate counts on nutrient agar (NA). The 20°C incubation time corresponds to room temperature, while the 4°C incubation time corresponds to refrigeration temperature. The tests were repeated on an average of five times at 20°C and three times at 4°C. Furthermore, each data point at each temperature represents the mean bacterial count measured on several coupons. The number of coupons varies from alloy to alloy and also with temperature and ranged from two to six coupons, with four being the average.

Table I – Nominal Alloy Composition (Weight %)

Alloy UNS No.	Cu	Zn	Sn	Ni	Al	Mn	Fe	Cr	P	Si	Ti	Mg
Copper												
C10200	100											
C11000	100											
C18080	99						0.1	0.5			0.1	
C19700	99						0.7		0.3			0.1
Brass												
C21000	95	5										
C22000	90	10										
C23000	85	15										
C24000	80	20										
C26000	70	30										
Y90*	78	12		3		7						
Bronze												
C51000	95		5						0.2			
C61500	90			2	8							
C63800	95				3					2		
C65500	97					1				3		
C66300	86	10	2				2					
C68800	74	23			3							
Cu-Ni												
C70250	96			3						0.7		0.2
C70600	89			10			1					
C71000	79			21								
C71300	75			25								
C71500	70			30								
C72900	77		8	15								
Cu-Ni-Zn												
C73500	72	10		18								
C75200	65	17		18								
C77000	55	27		18								

Stainless Steel												
S30400	0			8			74	18				
Plastic												
Polyethylene*	0											
Ag ion coating* on UNS 30400	0											

*no UNS alloy number

RESULTS

A semi-log plot of time in minutes versus bacteria count for alloy UNS C10200 is shown in **Figure 1**. At 20°C, the bacteria count decreases by about ½ of 1 order of magnitude (one-half log) within 45 minutes. The count begins to falloff at 45 minutes, shows four log drop at 60 minutes and reaches zero at 75 minutes. The zero point indicates that the bacteria are no longer viable. A similar pattern is seen at 4°C, but the times are longer, in that activity decreases as temperature decreases. A falloff occurs between 75 minutes and 180 minutes at 4°C. In alloy UNS C10200, the tests were repeated five times at 20°C and four times 4°C. At 20°C, each data point represents five to nine different coupons, while exactly five coupons were used for each data point at 4°C. Figure 1 represents data from a total of 58 coupons.

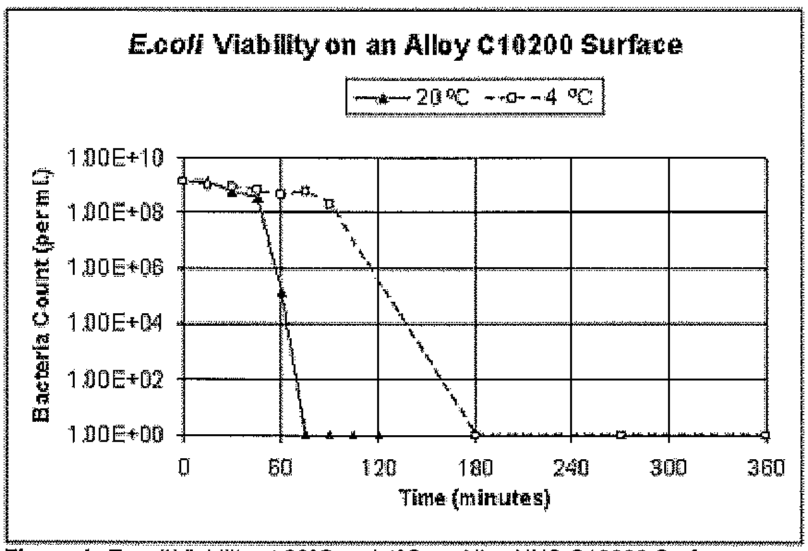

Figure 1. *E. coli* Viability at 20°C and 4°C on Alloy UNS C10200 Surfaces

In order to compare alloys within a family, as well as to make comparisons between families of alloys, certain features of the graphical data, such as Figure 1 for UNS C10200, were tabulated for all the alloys tested. This tabular format is a concise summary of the data on all 25 copper alloys, plus stainless steel, polyethylene and a silver ion-containing coating on stainless steel. The tabulated values include the time at which the initial drop-off in bacterial count is observed and the time at which a zero (viable) bacteria count was measured. These values are tabulated for all alloys at 20°C and 4°C, as show in **Table II.** The coppers, UNS C10200, C11000, C18080 and C19700 exhibited very similar behaviors, as shown in Table II. Figure 1 for alloy UNS C10200 is representative of the latter alloy family.

Table II – Elapsed Time of Initial Bacteria Count Drop-off and Zero Count

Alloy UNS No.	%Cu	Elapsed Time (minutes) at 20 °C			Elapsed Time (minutes) at 4 °C		
Coppers		**Rep**	**Initial Drop-off**	**Zero Count**	**Rep**	**Initial Drop-off**	**Zero Count**
C10200	100	5	45	75	4	90	180
C11000	100	6	75	90	4	180	270

C18080	99	5	45	75	3	180	270
C19700	99	5	45	75	4	90	180
Brass							
C21000	95	5	60	90	3	90	180
C22000	90	3	45	60	4	75	180
*C22000**	90	2	90	105			
C23000	85	5	30	60		not tested	not tested
C24000	80	4	60	75	4	270	360
*C24000**	80	2	90	105			
C26000	70	3	90	120	3	not seen	not reached
*C26000**	70	2	180	270			
Y90**	78	5	90	120	3	180	270
Bronze							
C51000	95	5	60	105	3	180	270
C61500	90	4	105	180	3	not seen	not reached
C63800	95	5	60	90	3	90	180
C65500	97	5	45	65	3	90	270
C66300	86	2	45	50	3	90	270
*C66300**	86	1	60	70			
*C66300**	86	2	90	180			
C68800	74	4	120	270	3	not seen	not reached
Cu-Ni							
C70250	96	5	90	105	4	90	270
C70600	89	5	90	105	4	180	360
C71000	79	5	90	120	3	not seen	not reached
C71300	75	4	75	120	3	270	360
C71500	70	4	105	not reached	3	not seen	not reached
C72900	77	5	120	360	3	not seen	not reached
Cu-Ni-Zn							
C73500	72	5	60	90	3	not seen	not reached
C75200	65	6	90	105	4	not seen	not reached
C77000	55	4	90	not reached	3	not seen	not reached
Stainless Steel							
S30400	0	6***	not seen	not reached	2	not seen	not reached
Plastic							
Polyethylene**	0	3	not seen	not reached		not tested	not tested
Ag ion coating** on S30400	0	9****	not seen	not reached	4	not seen	not reached

*replicate tests taken at different time intervals-see text. ** no UNS number.
***2 reps@270 min, 2 reps@2days & 2 reps @ 28 days. ****5 reps@360 min, 4 reps@2 days

The inhibition effects of the brasses on *E. coli* is similar but generally less than that of the coppers, as can also be seen in Table II. In addition, several of the brass alloys exhibit a secondary peak, as shown in **Figure 2** for UNS C22000. Furthermore, as can be determined from Tables II and III, the actual bacteria counts plotted in Figure 2 represent the mean of a total of five replicate tests at 20°C. The data for the five replicate tests for alloy UNS C22000 at 20°C is shown in Table III. In Replicate 1, the bacteria count was determined to have fallen to 2.06E+06. At 180, 270 and 360 minutes, bacteria counts were taken on the remaining three coupons and the viable bacteria count was found to be zero. One can go through the same thought process for Replicates 2 through 5. From **Table III**, it can be seen that five coupons were uses for Replicates 1 and 2, nine for Replicate 3, six for Replicate 4 and seven for Replicate 5, for a total of 32 coupons. The mean of the five replicates was plotted, as shown in Figure 2. Thus, the secondary peaks are anomalies related to the number of replicates and times at which bacteria counts were measured. It is obvious that these secondary peaks do not represent a return to life and subsequent bacteria growth.

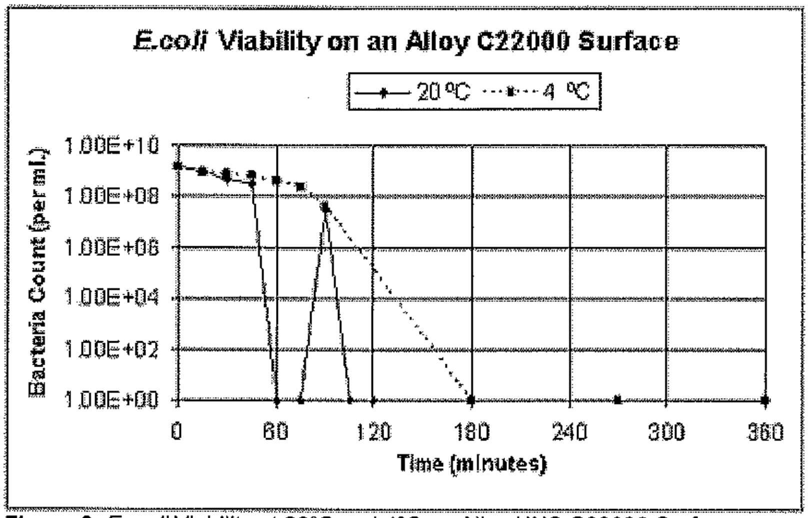

Figure 2. *E. coli* Viability at 20°C and 4°C on Alloy UNS C22000 Surfaces

Table III – Bacteria Counts at 20°C from Five Replicate Tests of UNS C22000						
Time (minutes)	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Rep 5**	**Mean**
0	1.35E+09	1.46E+09	1.52E+09	1.46E+09	1.82E+09	1.52E+09
15			1.16E+09	7.90E+08	1.25E+09	1.07E+09
30			8.90E+08	3.60E+08	1.37E+08	4.62E+08
45			1.01E+09	1.24E+07	3.70E+05	3.41E+08
60			0		0	0
75			0	0	0	0
90	2.06E+06	1.73E+08	0	0	0	3.50E+07
105			0			0
120			0			0
180	0	0				0
270	0	0				0
360	0	0				0

Bronze has historically been considered to be a copper-tin alloy. In modern times the term bronze is applied to a broad range of alloys which vary widely in competition, as can be seen in Table I. Not unexpectedly, they also vary considerably in inhibition effect on *E. coli*, as shown in Table II. In **Figure 3**, the inhibition effect a bronze, UNS C68800 on *E. coli* at 20°C is shown. This inhibition effect varies with the copper content. No inhibition effect is apparent at 4°C in this 74% Cu-containing bronze.

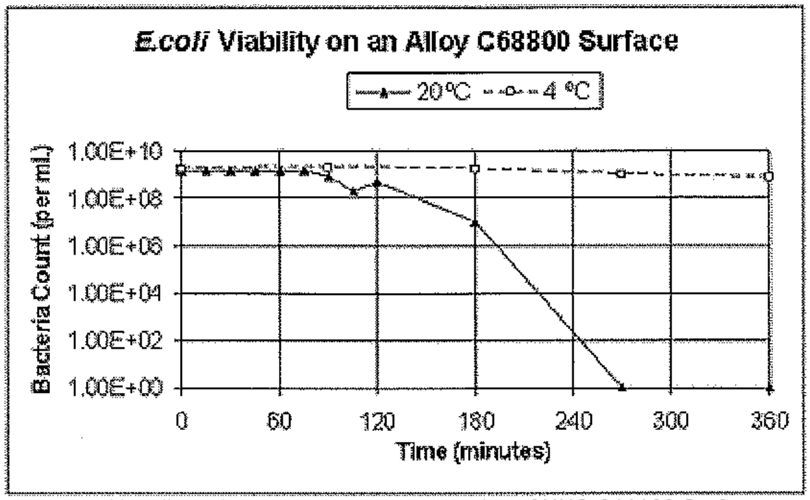

Figure 3. *E. coli* Viability at 20°C and 4°C on Alloy of UNS C68800 Surfaces

This double peak phenomenon was observed in three of the seven brasses tested. The only other alloy of the 25 coppers tested in which a double peak was observed is UNS C66300. Although UNS is C66300 classified as a bronze, it is similar to 10% zinc-containing brass but also contains 2% of both Sn and Fe. It is believed that this double peak behavior is related to the relatively poor corrosion resistance of the brass alloys relative to the other copper alloys tested. The brass alloys showed a higher degree of tarnishing than all of the other alloys during these tests.

In contrast to the brasses and bronzes, the copper-nickel alloys seem to follow a more predictable pattern, as shown in Table II. In alloy UNS C70600, the viability of *E. coli* begins to drop at 90 minutes and falls to zero at 105 minutes at 20°C and 360 minutes at 4°C, as shown in **Figure 4**. The copper-nickel alloys follow the same pattern of the degree of inhibition varying with of copper content as can be seen in Table II.

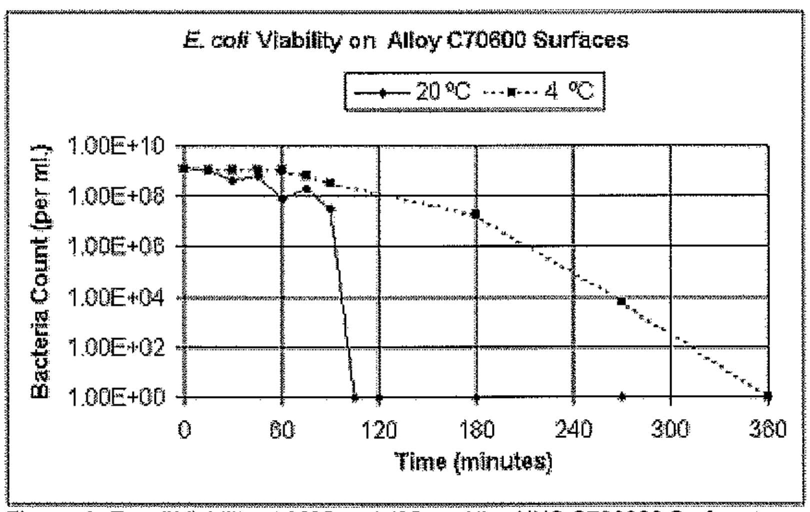

Figure 4. *E. coli* Viability at 20°C and 4°C on Alloy UNS C706000 Surfaces

The results for the copper-nickel-zinc alloys tested, which are commonly called nickel silvers because of their color, are summarized in Table II. UNS C73500 and C75200 follow the typical pattern of falloff to zero bacteria count in 90 to105 minutes. In contrast, UNS C77000 seems to show an initial decrease, which is not sustained, as shown in **Figure 5**. This may be a result of the low copper content of the latter alloy, which, at 55% Cu, is the lowest of all the copper alloys tested. At 4°C, the inhibition effect of the three alloys tested, UNS C73500, C75200 and C77000, is almost nil, as shown in Table II.

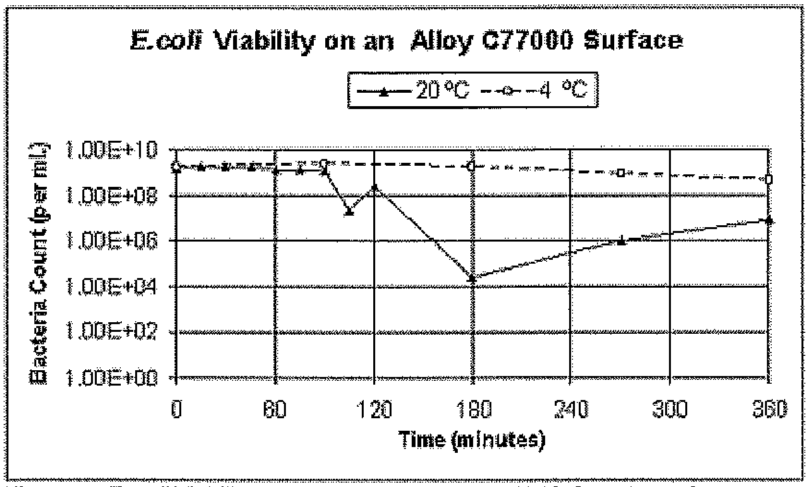

Figure 5. *E. coli* Viability at 20°C and 4°C on Alloy UNS C77000 surfaces

A polyethylene kitchen chopping board was cut into coupons and also tested. It shows very little inhibition effects on *E. coli* at 20°C, as shown in **Figure 6.** In stainless steel alloy UNS S30400 a very slight drop in bacteria count at 20°C is seen, as shown in Figure 6. Long-term, 28-day tests on UNS S30400 at both 20°C and 4°C show an approximate five log drop in bacteria count, as can be seen in **Figure 7**.

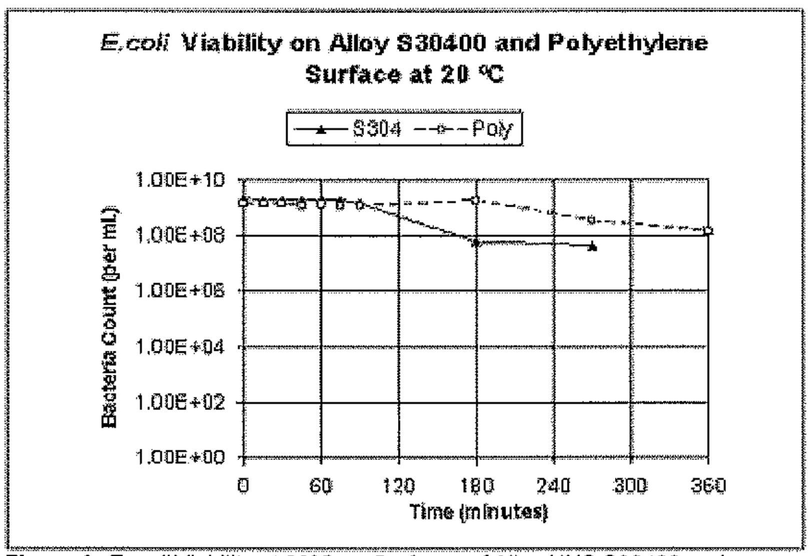

Figure 6. *E. coli* Viability at 20°C on Surfaces of Alloy UNS S30400 and Polyethylene

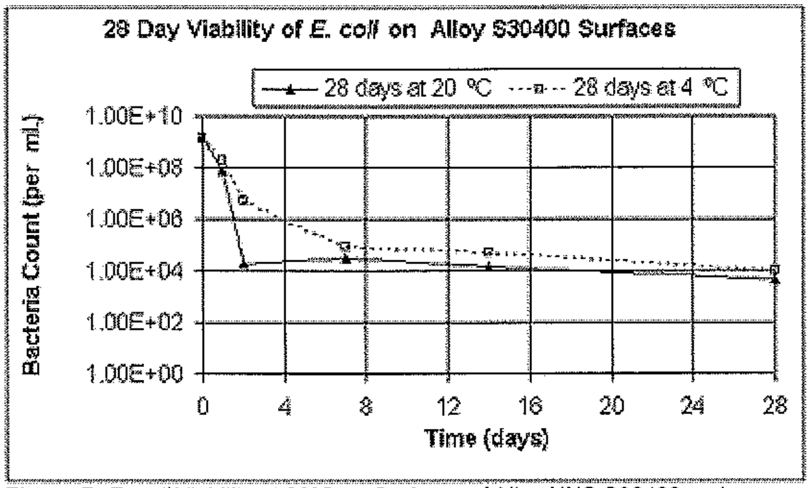

Figure 7. *E. coli* Viability at 20°C on Surfaces of Alloy UNS S30400 and Polyethylene

A silver ion-containing coating commercially applied to stainless steel alloy, UNS S30400, was also evaluated. As shown in **Figure 8**, a four log drop in bacteria count was observed after two days, which is less than the five log drop seen in on uncoated UNS S30400 also after two days, as shown in Figure 7.

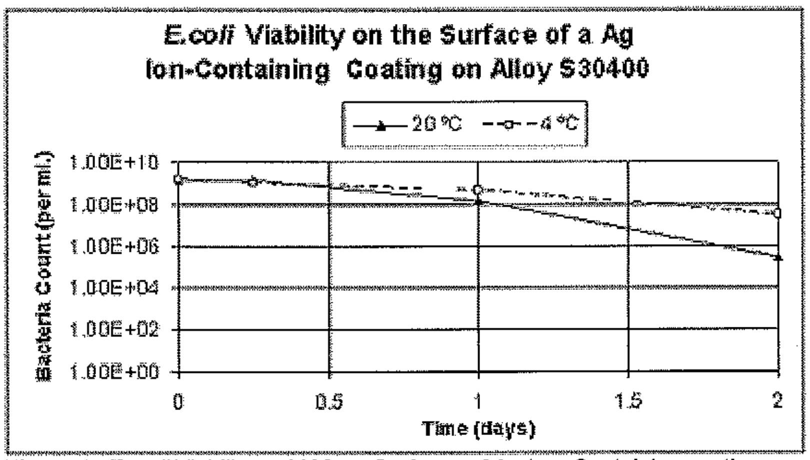

Figure 8. *E. coli* Viability at 20°C on Surfaces of Ag Ion -Containing coating on Alloy UNS S30400

DISCUSSION

Several trends are apparent from this study. The inhibition effects of a given alloy on *E. coli* decreases, as temperatures decrease from 20°C to 4°C. Furthermore, in general, the inhibition effects decrease as copper content of the alloys decreases, as can be seen in **Figure 9**. This is representative of the data from all 25 copper alloys tested at two temperatures. A computer graphics program was utilized to calculate and establish the trend line for each temperature, as shown in Figure 9. The scatter in data points is most likely related to the variation in the corrosion resistance of this broad range of copper alloys. One would expect variation in corrosion resistance of various copper alloys at a given copper content. For example, at 70% Cu, UNS C26000, a brass with 30% zinc, would typically have lower corrosion resistance than UNS C71500, a 30% nickel-containing copper-nickel alloy.

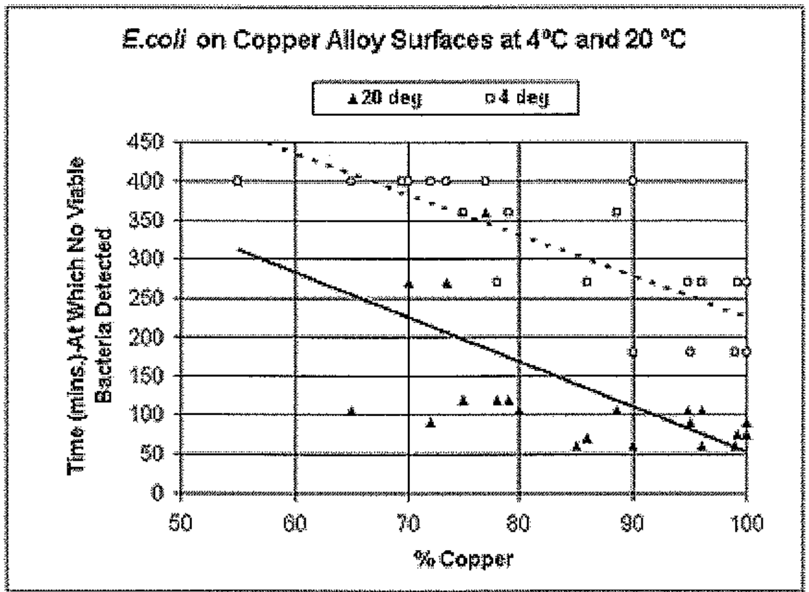

Figure 9. Time at Which No Viable Bacteria Detected at 20°C and 4°C on Surfaces of 25 Copper Alloys

In contrast to the copper alloys, the stainless steel, UNS S30400, a popular material for food processing equipment, has little or no inhibition effect. Its bacteria count is sustained at about 1E+08 through 270 minutes, which is not much lower than that found on polyethylene. During the first two days of 28-day exposure, alloy UNS S30400 shows a five log drop in bacteria count, to a little above 1E+04 and remained at that level for the 28 days. Similarly, the antimicrobial silver ion coating on stainless steel showed a four log drop a little above 1E+05 in two days, which is one log higher than seen on uncoated stainless steel. This is still a high bacteria count for both materials, especially when ingestion of only 10 to 50 individual bacteria may be sufficient to cause infection. Thus stainless steel surfaces and silver ion-containing coating still have a potential to adversely affect human health after two days. This persisted for 28 days in the case of uncoated UNS S30400.

Now that the effects of copper alloys on the viability of *E. coli* has been demonstrated on multiple samples of 25 copper alloys at two temperatures, efforts will focus on the development of additional information needed for the adoption of copper alloys by food processing equipment manufacturers. This includes durability, cleanability, the effects of common disinfectants and corrosion resistance in environments that directly relate to the intended application, food processing equipment and food preparation surfaces.

Results[2] from another phase of the present study indicate that *Listeria monocytogenes*, another important food-borne bacterium which threatens human health, is also inhibited by copper alloys. A few preliminary data points also indicate that a similar inhibition effect is seen on MRSA (Methicillin Resistant *Staphylococcus aureus*), a bacterium that is creating health problems in hospitals and nursing homes. Infection can occur as a result of humans touching contaminated surfaces. The specification of copper alloys, for door handles, door push plates, faucets, bedrails, stair and corridor rails and other hardware, holds the promise of being an effective passive approach to controlling MRSA in healthcare facilities. Other target markets include mass transit systems, public buildings, schools, as well as gyms.

Future work will evaluate the inhibition effects of copper alloys on other types of bacteria, other pathogens and molds that contribute to respiratory distress, a type of infection that is associated with "Sick Building Syndrome".

ACKNOWLEDGMENT

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