BIOFILM RESEARCH:

AN UPDATE

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A RESEARCH UPDATE ON THE EFFECT OF CLEANERS AND SANITIZERS ON FOOD PROCESSING BIOFILMS

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Dublin, California

December, 1991

Biofilms consist of microscopic layers of microbial cells firmly attached to a surface and to one another. Imbedded in this film we may find bacteria, yeasts, molds, algae and particles of food debris and dirt. These films exist naturally throughout nature and have become increasingly important to food processors as they attempt to rid their facilities of food pathogens and spoilage organisms.

Biofilm development has been documented in varying degrees on teflon, glass, rubber and stainless steel surfaces, all of which are found in food processing environments. These biofilms have been implicated in disease processes, the plugging of petroleum pipe transport systems, the fouling of ship bottoms and in the corrosion of metals. We also believe them to be a source of recontamination in our food processing facilities when inadequate cleaning and sanitizing practices prevail.

The formation of these biofilms seems to be a universal microbial strategy for survival, in that these organisms attach themselves to surfaces in order to expend less energy and then proceed to flourish on the surrounding nutrients. Recent research has shown that these organisms are more resistant to attack by biocides than their counterparts suspended in solutions. Food processors' dilemma has been to find a satisfactory method of cleaning their equipment and surrounding environment to destroy, or at least control the numbers of these harmful organisms.

The second phase of our project involved growing biofilms consisting of pure cultures of pathogens on 1/2 " stainless steel chips and then subjecting these biofilms to various cleaners and sanitizers to determine their effects. In order to quantify our data a rapid method for enumerating the number of viable cells on our chips was developed using the Impedance Technology of the Vitek Systems Bactometer.

Comparative tests using the Bactometer, the Standard Plate Count methodology and Epifluorescent Microscopic direct counts were conducted until we were satisfied that the Bactometer's Impedance method could give us rapid, reproducible, quantifiable results. The Bactometer method uses Impedance Detection times which are a function of growth kinetics, lag time and the concentration of organisms in
a given sample. Since our one half inch Type #4, 304 finish stainless steel chips fit directly into the individual Bactometer wells we could enumerate the number of viable cells attached to the entire surface chips before and after treatment.

Growth curves were set up using known concentrations of each of our test organisms. Depending on the initial concentration, the Bactometer detection times varied, with the highest level of organisms giving the shortest detection times. Once the computer was given the concentrations that correlated to each detection time the Bactometer calculated (using regression curves) detection times for concentrations of zero through 10^9 cells/ml.

The biofilms were grown on the stainless steel chips in test tubes containing either diluted nutrient broth or diluted trypticase soy broth. Diluted broths were chosen because of early research showing that biofilms formed faster in low nutrient environments.

Pure cultures of Listeria monocytogenes (Jalisco strain), Escherichia coli, Salmonella enteriditis and Salmonella typhimurium were added to the broths containing the chips and the tubes were incubated at 30°C for 1, 5 and 10 day intervals. The broths were removed and replaced with fresh media every 48 hours, and the tubes were reinoculated with new organisms at the same time.

After the appropriate incubation time the chips were removed from the broths, rinsed four times with 5 mls of sterile Phosphate buffer and placed into a sterile test tube for treatment with either a Detergent solution and/or a Sanitizing solution of known concentration. Five chips of each organism were treated at a time. The chips were submerged in 5 mls of Detergent for five minutes, then the chips were rinsed four times with 5 mls Phosphate Buffer and either placed directly into the Bactometer wells or placed in a second solution of sanitizer. The chips remained in the sanitizer solutions for five

minutes before being removed, rinsed again with Phosphate Buffer (four times with 5 mls each) and then placed into the Bactometer for enumeration. Five controls chips were run in conjunction with each test to determine the average number of viable cells attached to the chips prior to treatment. The Bactometer held the treated chips in growth media at 30°C for 48 hours while the computer recorded the detection times for each well. Additional runs were conducted using only the sanitizer solutions.

The detergent solutions and sanitizing solutions used in this experiment were as follows:

MP	=	All purpose non-chlorinated alkaline detergent
M15	=	Chlorinated alkaline detergent, 80 ppm
CL	=	Chlorine solution, 200 ppm
I	=	Iodine solution, 25 ppm
Q	=	Quaternary Ammonium compound solution, 200 pm
OX	=	Oxonia Active sanitizer solution, 840 ppm hydrogen peroxide
MP/S	=	Data of combined treatments of alkaline detergent plus all sanitizer solutions
M15/S	=	Data of combined treatments of chlorinated alkaline detergent plus all sanitizer solutions
D/145	=	145°F alkaline detergent solution treatment

Tables 1-5 summarize the findings of our treatment methods. Initial concentrations of cells were 10^8 cfu/chip surface. The darkened portion of each bar shows the number of cells surviving the different treatment methods. Our research showed that 24 hour biofilms containing 10^5-10^6 cfu/chip surface were easily inactivated by all our treatments, therefore the data shown represents 5- and 10-day films where we achieved 10^8 attached cells per chip surface.

We found the Escherichia coli was very adept at setting up biofilms but it was not particularly resistant to treatments with either detergents and/or sanitizers. (TABLE 1.)

Listeria monocytogenes proved to be more difficult to treat with either detergents alone or with sanitizers. But when we combined the detergent with the sanitizer solutions, or used 145°F detergent

solutions (5 mins) as we do with CIP systems, the 5-day film of 10^8 organisms was all but inactivated. (TABLE 2.)

Salmonella enterilitis gave us a similar profile for the 5-day film (TABLE 3) but an interesting change was noted on the 10-day chips. (TABLE 4). For the 10-day film the Quaternary Ammonium Compound sanitizer was the most effective. This could be explained by the surfactants in the Quat solution which reduce the surface tension of the biofilm allowing greater penetration and more biocidal activity.

Salmonella tryphimurium showed results similar to Salmonella enteriditis with the Quats alone or the combination of detergents plus sanitizer or 145°F detergents giving the best results. (TABLE 5.)

We also investigated an interesting new technology using a cryogenic cleaning system to clean the chip surfaces. Chips covered with 10^7 cfu (colony forming units) of Listeria monocytogenes and 10^6 cfu Salmonella enteriditis biofilms were cleaned by the Coldjet Company using their liquid carbon dioxide cleaning system. The carbon dioxide was formed into dry ice pellets and propelled through a nozzle at high pressure. The thermal shock (-110°F) fractured the biofilm and allowed for easy removal of the attached cells.

After the cryogenic treatment the chips were placed into the Bactometer wells for detection of surviving cells. No survivors were detected on the treated chip surfaces. Epifluorescent microscopic examination showed only fragments of cell structures remaining on the stainless steel. We feel this new technique could have direct applications for food processors in the future. Both low pressure (80-90 psi) and high pressure (130-140 psi) air systems seemed adequate to remove these laboratory biofilms.

In reviewing our findings, the following conclusions can be drawn:

1. Resistance of biofilms to treatments with detergents and/or sanitizers increases over time. The longer manufacturers run their production lines without cleaning, the more difficult it becomes to kill the organisms attached to the surfaces of the equipment and those in the surrounding environment.

2. Resistance of these organisms to treatment seems to be genus, species, and even strain specific. Our research found differences between the Jalisco and Scott A strains of L. monocytogenes and between S. enteriditis and S. typhimurium.

3. While treatment with sanitizers alone gave only an average reduction of 50% in the number of surviving organisms, the right combination of hot detergents followed by appropriate sanitizers were shown to inactivate the biofilms we produced under laboratory conditions. This is good news for food manufacturers since many of the earlier research created serious doubt about our present cleaning methods.

4. Further research should be conducted on cryogenic cleaning systems to determine their effectiveness on food processing biofilms.

5. It is important to remember that the thoroughness of our cleaning procedures is equally or more important than the types of cleaners or sanitizers used. Equipment should be dismantled whenever possible and gaskets should be replaced and/or soaked in sanitizer solutions for a minimum of 1 hr. Areas which the cleaning and sanitizer solutions do not contact will undoubtedly still contain viable organisms which will recontaminate the line.

For further information, contact NFPA's Bonnie J. Humm or Lisa Nesbett at 510-828-2070.)

EFFECT OF DETERGENTS AND SANITIZERS ON BIOFILMS (5 DAYS @ 30° C)

Escherichia coli

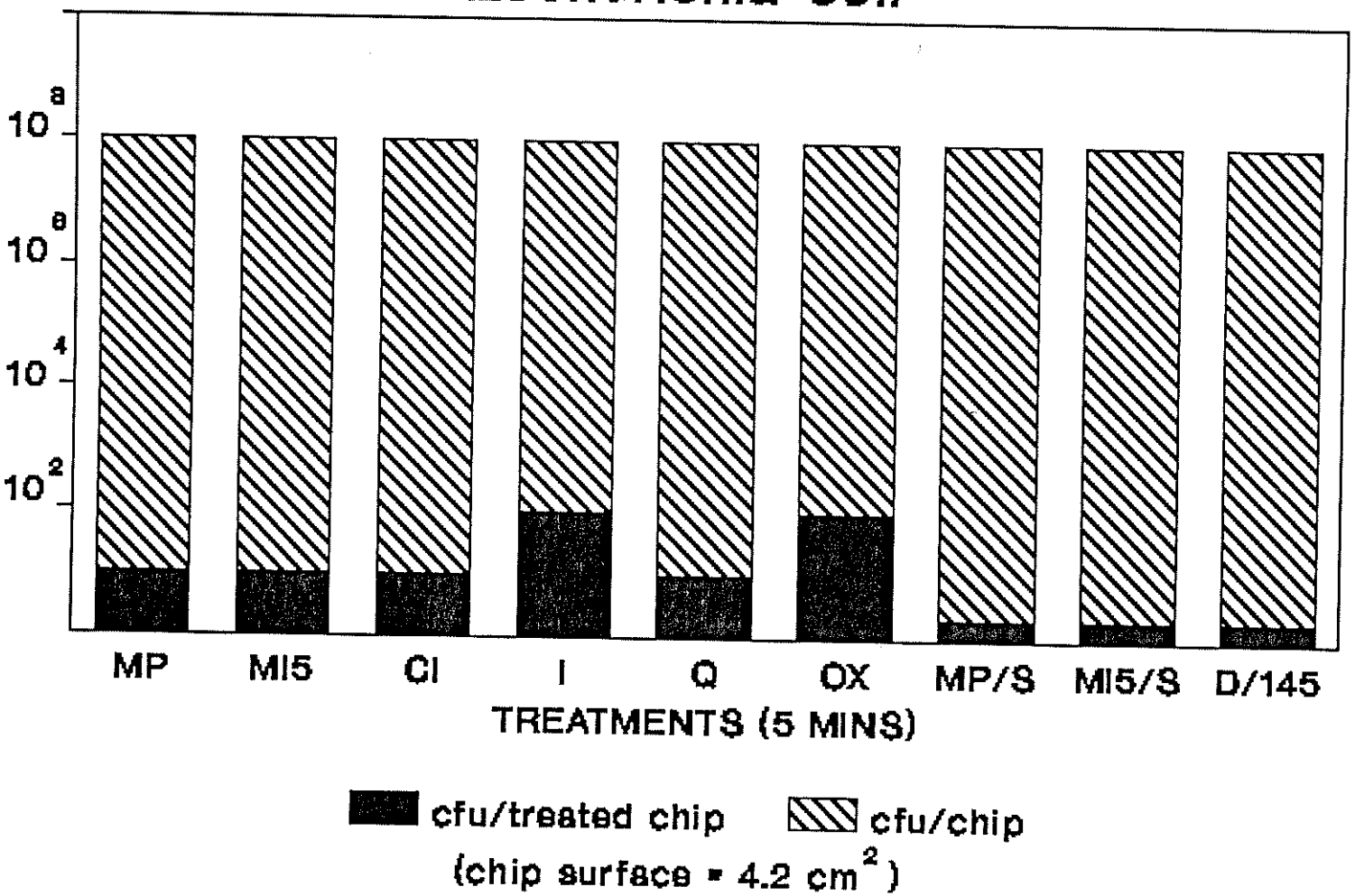

TABLE 1

EFFECT OF DETERGENTS AND SANITIZERS ON BIOFILMS (5 DAYS @ 30° C)

Listeria monocytogenes

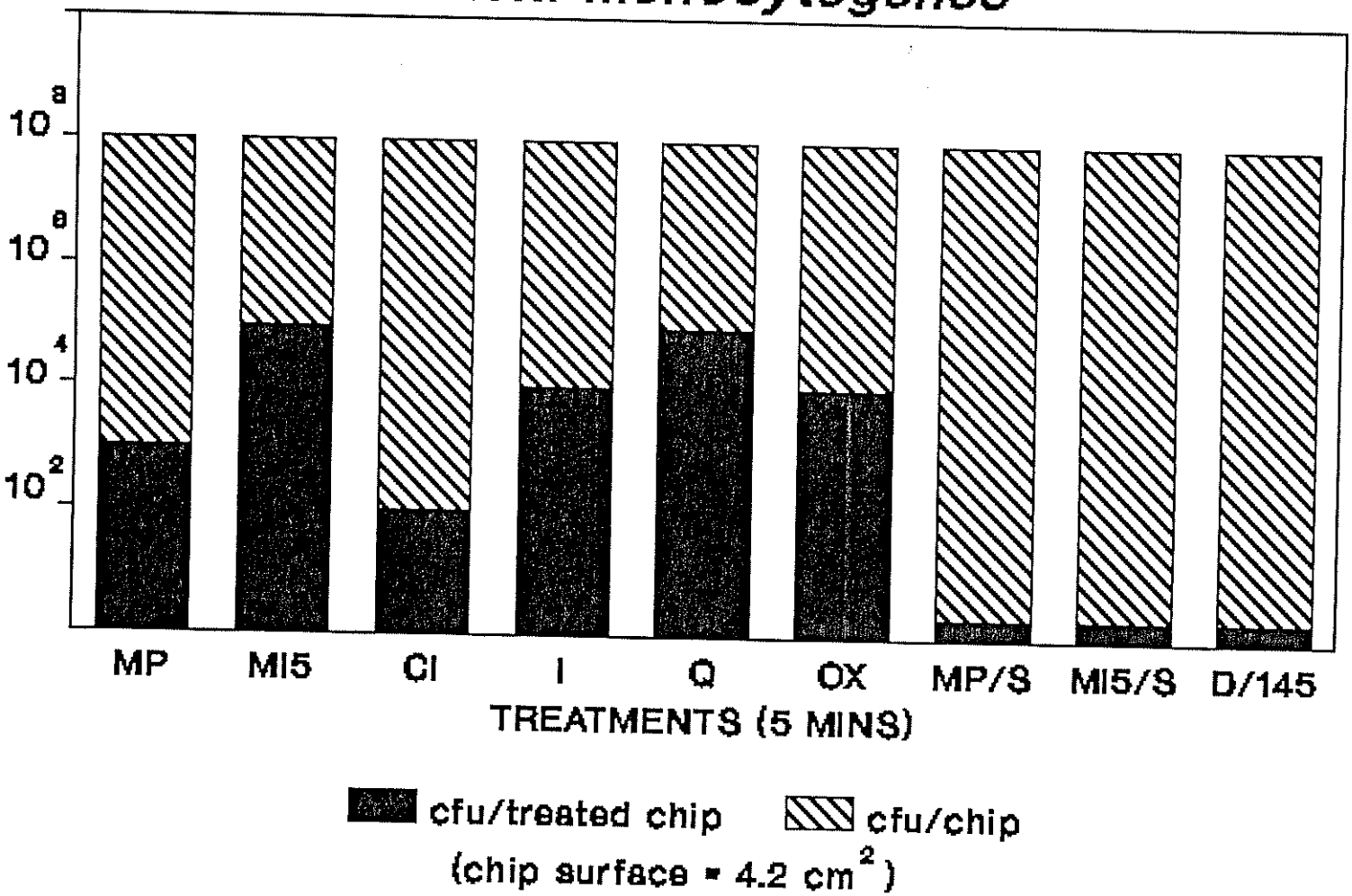

TABLE 2

EFFECT OF DETERGENTS AND SANITIZERS ON BIOFILMS (5 DAYS @ 30° C)

Salmonella enteritidis

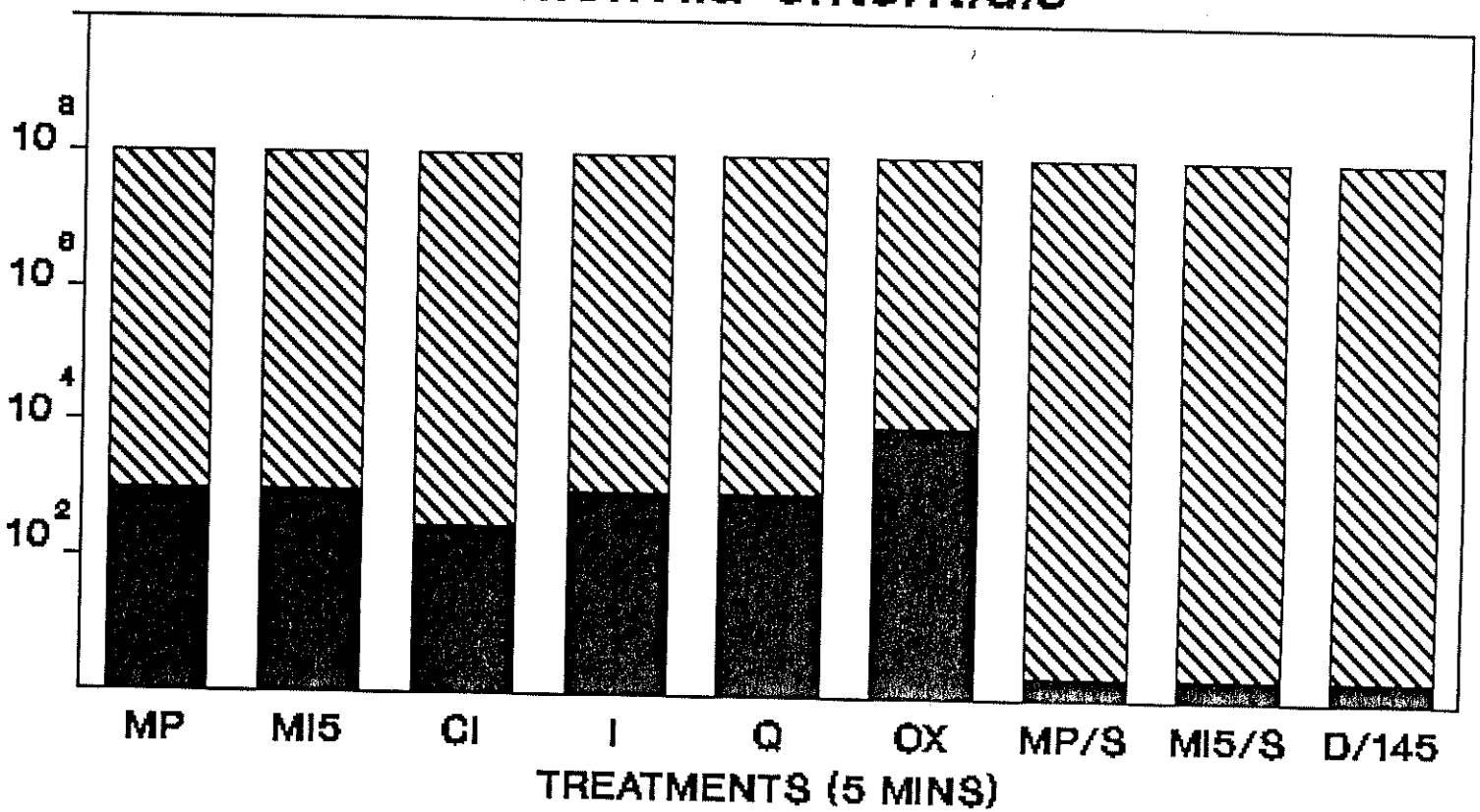

TABLE 3

EFFECT OF DETERGENTS AND SANITIZERS ON BIOFILMS (10 DAYS @ 30 °C)

Salmonella enteritidis

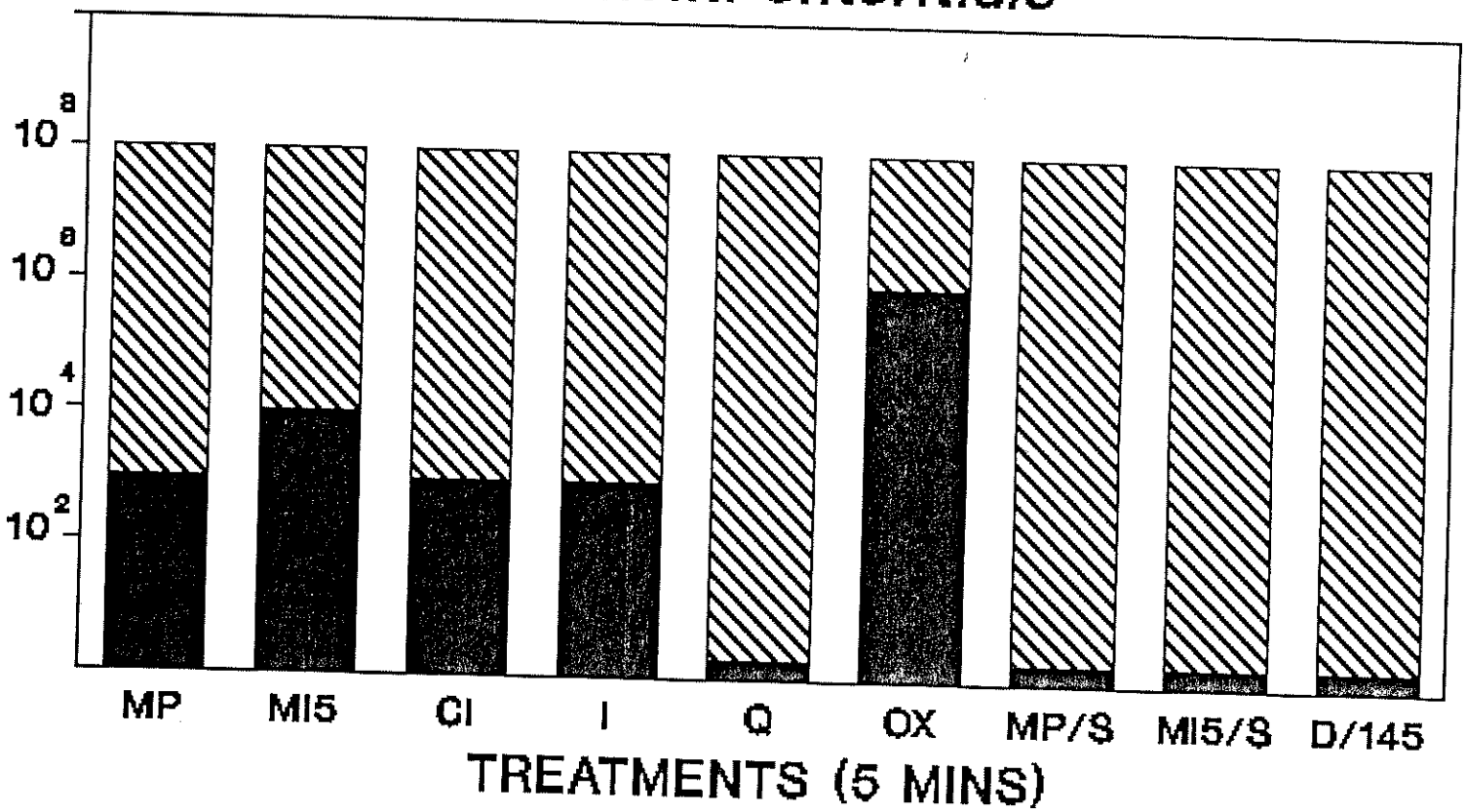

TABLE 4

EFFECT OF DETERGENTS AND SANITIZERS ON BIOFILMS (5 DAYS @ 30° C)

Salmonella typhimurium

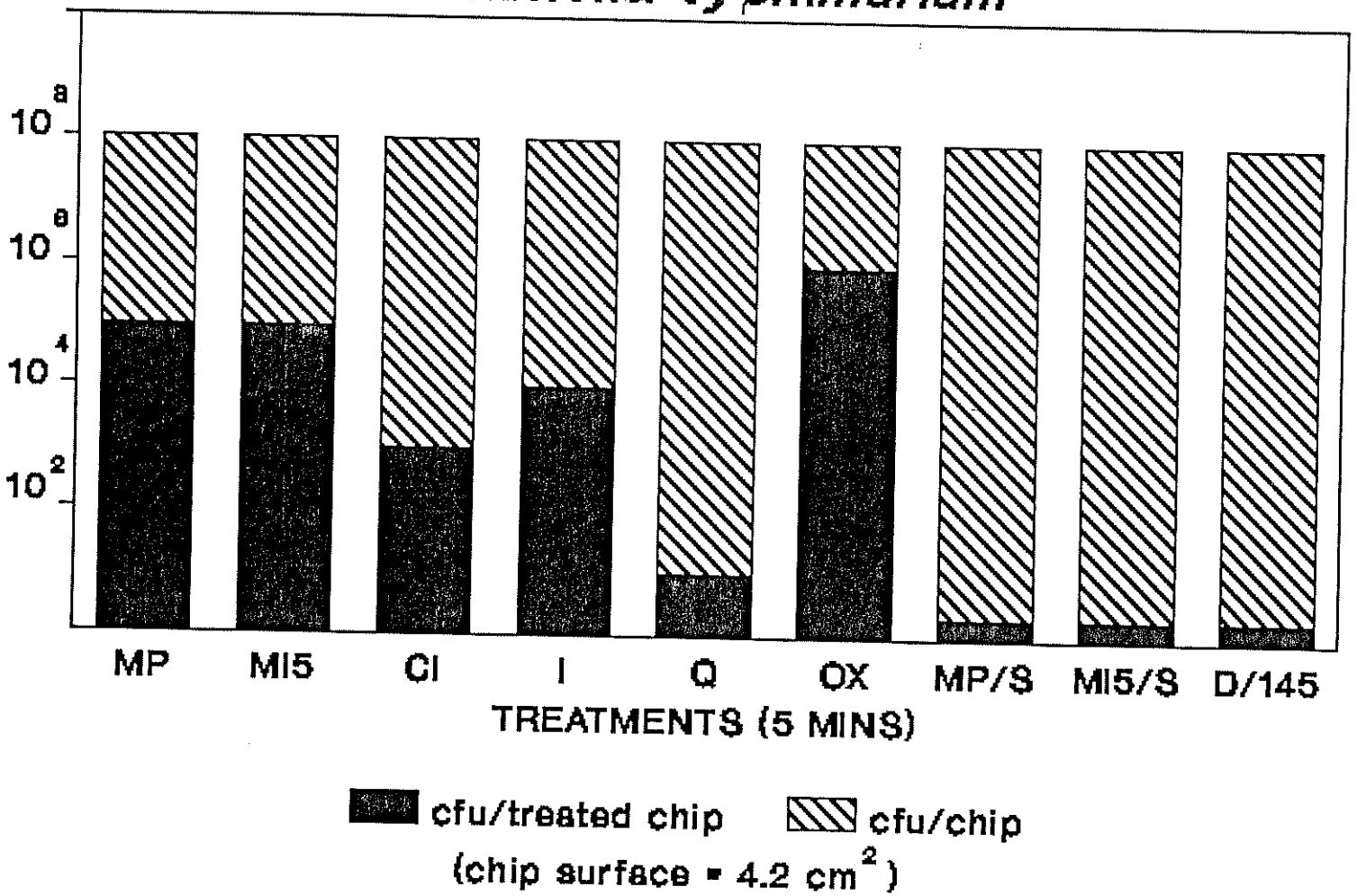

TABLE 5

CORRELATION AMONG ENNUMERATION METHODS

METHOD	TOTAL CFU/CHIP AVERAGE
3 mm BEADS (Vortexed 5 min/ Plated)	3.6×10^5
DIRECT MICRO COUNT	3.4×10^5
BACTOMETER	5.0×10^5

GROWTH MEDIA FOR BIOFILMS

TRYPTICASE SOY BROTH
WITH ADDED DEXTROSE

1:15 OF NORMAL STRENGTH

NUTRIENT BROTH

1:10 OF NORMAL STRENGTH

PROCEDURE

1. INCUBATED AT 30 °C
2. REMOVED AND RINSED FOUR TIMES WITH 5 mls STERILE PHOSPHATE BUFFER
3. TREATED WITH 5 mls OF DETERGENTS AND/ OR SANITIZERS IN STERILE TEST TUBE
4. RINSED AGAIN WITH 20 mls STERILE PHOSPHATE BUFFER
5. CHIPS ASEPTICALLY PLACED INTO BACTOMETER WELLS
6. STREAKED FOR IDENTIFICATION

CLEANERS

CLEANER	CATEGORY	pH	Cl_2 CONC.
MP	ALKALINE DETERGENT	12.0	0
MI-5	CHLORINATED ALKALINE DETERGENT	10.8	80 ppm

SANITIZERS

	CONC.	pH
CHLORINE	200 ppm	5.3
IODINE	25 ppm	3.2
QUAT	200 ppm	7.3
OXONIA ACTIVE	840 ppm	3.2

SURVIVAL OF BIOFILM ORGANISMS ON STAINLESS STEEL SURFACE USING COLD JET PROCESS

	LOW PRESSURE (80-90 psi)	HIGH PRESSURE (130-140 psi)
L. monocytogenes 10^7 cfu/surface	NEG	NEG
S. enteritidis 10^6 cfu/surface	NEG	NEG

8 Chips grown 5 days @ 30° C