CONTROL OF BIOFILMS ON THE FOOD CONTACT SURFACES

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ABSTRACT

Three types of disinfectant solutions (chlorine, iodine and mixture of both) were applied on three different types of food contact surfaces (stainless steel, wood and plastic) to examine their ability to remove the already attached biofilms on these surfaces. Two different trials were carried out; the first trial is application of the disinfectant solutions at 25 °C and at specific pH of each solution and the second at 70 °C and at pH 5. A total of 60 samples (10 of each surface for each trial) were examined for the effect of these disinfectants. The results declared that iodine solution was more effective than chlorine especially in trial 2 in spite of isolation of persister spore-forming bacteria. There were no significant differences between reduction rates of iodine and mixture, while chlorine was significantly different and less effective in biofilm removal. This may be attributed to developed microbial resistance to chlorine as a result of chlorination of water supply. Moreover, the high efficacy of tested disinfectants in trial 2 may be aided by high temperature which has bactericidal effect and acidic pH which aids in break down of exopolysaccharide matrix.

INTRODUCTION:

Microbial bio films on surfaces cost the nation billions of dollars yearly in equipment damage, product contamination, energy losses and medical infections. Conventional methods of killing bacteria (such as antibiotics, and disinfection) are often ineffective with biofilm bacteria. The huge doses of antimicrobials required to rid systems of biofilm bacteria are environmentally undesirable and perhaps not allowed by environmental regulations. Efforts to control food borne pathogens and spoilage Microorganisms in the meat processsing industries have accelerated in recent years. Biofilm formation can compromise the sanitation of food surfaces and environmental surfaces by spreading detached organisms to other areas of processing plants. On occasion, parts of the biofilm slough off into the surrounding environment. After sloughing, the bacteria incorporated in the biofilm can contaminate other surfaces, as well as food products. For this reason, removal of biofilms in the food processing environment is critical (**Virginia and Jean, 2003**). Nowadays, the food hygienist faces a tremendous challenge in overcoming problems stemming from the formation and persistence of bacterial biofilms in food industry. So, the present study is aimed to determine the effect of three types of disinfectants which can be safely used in food industry to eliminate the biofilms. These objectives will be obtained through investigating the following points:

A. Trial 1: Studying the effect of three types of disinfectants that can commonly and safely used in food processing establishments (Chlorine, Iodine and mixture of both) at recommended concentrations at usual temperature and pH circumstances.

B. Trial 2: Studying the effect of these disinfectants on attached microorganisms on food contact surfaces at 70° C and pH 5.

MATERIALS & METHODS

Trial 1: Application of disinfectants at recommended concentrations at usual temperature and pH circumstances: A total of thirty samples (Ten samples of each surface) were collected from the washed, cleaned and stored stainless steel, wood and plastic food contact surfaces. The preparation of tested area was done as applied in food service establishments, Efficient washing and rinsing of tested surfaces by warm water and liquid soap to remove all debris and fat then rinsing with tape water to remove any traces of detergent then allowed to dry in closed and clean chamber. Then the area was measured (10 cm^2) by using a template (2X5 cm) and swabbed by 70 % alcohol to eliminate the surface contaminants.

Disinfection protocols: Three different disinfection solutions were applied (Chlorine, Iodine and mixture of both). The effect of these disinfection solutions were tested on the attached microorganisms on the experimental food surfaces as follows:

Preparation of disinfectant solutions: These disinfectants were diluted to the indicated concentrations as recommended by **CFR (2005)** with sterile distilled water. The pH value was determined before application of each disinfectant:

Chlorine: 200 ppm chlorine solutions were prepared from household bleach (Clorox®) by adding 4 ml Clorox® to one litre distilled water. The pH value was 8.

Iodine: 25 ppm iodine solutions were prepared by adding 0.25 ml Betadine® to one litre distilled. The pH value was 6.

Mixture of chlorine and iodine: From the previously prepared solutions, equal amounts of both disinfectant solutions were mixed. The pH value was 7.3.

Application of disinfectant soluteons: The tested area was divided into four parts (each part was measured by using a 2X5 cm template). The first part was covered with chlorine solution; the second was covered with iodine solution; the third was covered with the mixture of both solutions and the fourth part was considered as control. The contact time was 15 minutes and the temperature of solutions was 25 °C.

Detection of attached food contact surfaces biofilms: After the contact time, each experimental surface was rinsed with sterile distilled water to neutralize the disinfectant solution. Then the sampling procedures were carried out according to **Sinde and Carballo (2000)** and **Donald (2007)**.

Microbiological techniques: The microbiological methods for ACC, Staphylococcus aureus counts and Enterobacteriacae counts were carried out as recommended by **ISO.** And for Clostridium perfringens (MPN/cm^2) was determined according to **Beerens et al. (1980).** And total aerobic spore forming counts and coliforms counts were carried out as recommended by **APHA (1992).** The treatment efficacies and biofilms resistance to each disinfectant were measured by the reduction rate [Reduction rate = $(N_1-N_2) / N_1 x100$], where N_1 is the initial count of CFU/cm^2 on control surfaces and N_2 is the count of CFU/cm^2 after treatment on experimental surfaces.

Trial 2: Studying the effect of these disinfectants on attached microorganisms on food contact surfaces at 70° C and pH 5: A total of thirty samples (Ten samples of each surface) were collected from the washed, cleaned and stored stainless steel, wood and plastic food contact surfaces. The same procedures that carried in trial 1 were applied in trial 2 by using the same selective disinfectants with changes in pH and temperature of the disinfectant solutions. The temperature was elevated up to 70 °C and the pH value was adjusted at 5 by using lactic acid. And the reduction rates were recorded.

RESULTS AND DISCUSSION :

Reduction rates of chlorine in trial 1: The results showed that the reduction rate after application of chlorine in a concentration of 200 ppm at 25 °C and at pH 8 did not exceed 67 %. This higher percent of reduction rate was obtained in Clostridium perfringens counts on stainless steel surface. Meanwhile, the lower percent of reduction rate was obtained in aerobic colony counts on wood surface (8.17%). Nevertheless, no significant differrence between mean counts after application of chlorine on stainless steel and plastic surfaces, while, in general, wood surface represents high and significantly different counts.

Reduction rates of iodine in trial 1: The results were pointed out that the reduction rate after disinfection with iodine in a concentration of 25 ppm at 25 °C and at pH 6 did not exceed 85 %, and this occurs also in Clostridium perfringens counts on stainless steel surface. On the other hand, the lower percent of reduction rate was obtained in aerobic colony counts on wood surface (35.12%). No significant difference between the mean counts after application of iodine on stainless steel and plastic surfaces, while, wood surface represent high and significantly different counts.

Reduction rates of mixture in trial 1: The results were declared that the reduction rate after disinfection with the mixture of both disinfectants at 25 °C and at pH 7.3 did not exceed 85 %, and this occurs also in Clostridium perfringens counts on stainless steel surface. On the other hand, the lower percent of reduction rate was obtained in aerobic colony counts on wood surface (19.55%). No significant difference between mean counts after application of iodine on stainless steel and plastic surfaces, while, in general, wood surfaces represent high and significantly different counts with exception of total aerobic colony counts which are significantly different in the three surfaces. In addition, these data revealed that there was no significant difference between the reduction rates of the mixture and iodine. The chlorine resistant microorganisms isolated from tested food contact surfaces at 25 °C and at pH 8 and their incidence were Escherichia coli (16.67%), Bacillus cereus (16.67%), Bacillus stearothermophilus (13.88%), Clostridium perfringens (13.88%), Staphylococcus aureus (11.12%), Streptococcus faecalis (11.12%), Bacillus coagulance (11.12%), Bacillus subtilis (2.77%) and Pseudomonas putida (2.77%). And the iodine resistant microorganisms isolated from tested food contact surfaces at 25 °C and at pH 6 were Bacillus coagulance (16.39%), Bacillus subtilis (16.39%), Clostridium perfringens (13.12%), Bacillus cereus (13.12%), Bacillus stea-rothermophilus (11.48%), Escherichia coli (8.19 %), Strept-ococcus faecalis (8.19%), Pseudo-monas putida (6.56%) and Staphyl-ococcus aureus (6.56%). While the mixture resistant microor-ganisms isola-ted from tested food contact surf-aces at 25 °C and at pH 7.3 were Bacillus coagulance (17.38%), Bacillus subtilis (17.38%), Clostridium perfringens (17.38%), Bacillus cereus (15.23%), Bacillus stea-rothermophilus (15.23%), Escherichia coli (8.7%) and Staphylococcus aureus (8.7%).

Reduction rates of chlorine in trial 2: The results pointed out that the reduction rate after disinfection with chlorine in a concentration of 200 ppm at 70 °C and at pH 5 reached 100 % in many applications. This total removal of attached microbes was obtained in Staphylococcus counts, coliforms counts, and Enterobacteriacae counts after application of chlorine on stainless steel and plastic surfaces. In contrast, the application procedures on wood surface does not reached 100 % for the same bacterial groups. Meanwhile, the lower percent of reduction rate was obtained in Clostridium perfringens counts on stainless steel surface (29.71%). No significant difference between the mean counts after application of chlorine on stainless steel and plastic surfaces, while wood surface represents high and significantly different counts.

Reduction rates of iodine in trial 2: The results declared that the reduction rate after disinfection with iodine in a concentration of 25 ppm at 70 °C and at pH 5 reached 100 % in many applications. This high percent was obtained in Staphylococcus counts, coliforms counts, and Enterobacteriacae counts after application of iodine on stainless steel and plastic surfaces. But the application procedures on wood surface does not reached 100 % for the same bacterial groups. Meanwhile, the lower percent of reduction rate was obtained in aerobic spore forming counts on stainless steel surface (74.19%). No significant difference between mean counts after application of chlorine on stainless steel and plastic surfaces, while, in general, wood surface represent high and significantly different counts.

Reduction rates of mixture in trial 2: The results declared that the reduction rate of the mixture at 70 °C and at pH 5 reached 100 % in many applications. This was obtained in Staphylococcus counts, coliforms counts, and Enterobacteriacae counts after application of iodine on stainless steel and plastic surfaces. But the reduction rates on wood surface do not reach 100 % for the same bacterial groups. Meanwhile, the lower percent of reduction rate was obtained in aerobic spore forming counts on stainless steel surface (77.06%). No significant difference between mean counts after application of chlorine on stainless steel and plastic surfaces, while wood surface represent high and significantly different counts. The chlorine resistant microrganisms isolated from tested food contact surfaces after disinfection at 70 °C and at pH 5 and their incidence were Bacillus cereus (24.59%), Bacillus stearothermophilus (24.59%), Bacillus subtilis (19.68%), Clostridium perfringens (16.39%) and Bacillus coagulance (14.75%). While the iodine resistant microorganisms isolated from tested food contact surfaces at 70 °C and at pH 5 and their incidence were Bacillus stearothermophilus (26.92%), Bacillus cereus (19.23%), Bacillus subtilis (19.23%), Clostridium perfringens (19.23%) and Bacillus coagulance (15.39%). The mixture resistant microorganisms isolated from tested food contact surfaces at 70 °C and at pH 5 and their incidence were Bacillus stearothermophilus (25%), Clostridium perfringens (25%), Bacillus subtilis (20.83%), Bacillus cereus (16.67%), and Bacillus coagulance (12.5%). None of the reduction rates of tested disinfectants exceeded 85 % in trial 1, meanwhile in trial 2 the reduction rates were usually exceeded this percent and sometimes reached 100 %. And it is necessary to stress the fact that the improvement in reduction rates in trial 2 were obtained after increased application temperature up to 70 °C and adjustment of pH value 5 of the disinfectants. This can be explained by: 1) The high temperature gives an advantage over the bactericidal effect of the disinfectant when applied at lower temperatures. 2) The acidic pH of the disinfectant solutions (pH 5) aids in the breaking down and digestion of the exopolysaccharide matrix. This resulting in more effective penetration of the disinfectants and also prevent its exhaustion before reaching the protected microorganisms. The reduction rates of iodine and mixture are higher than chlorine at the same application circumstances. In addition, there are no significant difference between the reduction rates of iodine and mixture. The lower values of chlorine reduction rates in this study could be interpreted as the microrganisms can develop resistance to chlorine as a result of water chlorination and its repeated use in regular sanitation procedures in tested establishments. Moreover, it is clear that the effect of tested disinfectants on wood surfaces is lower than on other two surfaces. These this was attributed to the wood surfaces can protect these microorganisms due to its topographical properties which include pitted surface and hydrophilic nature. These properties provide physical protection for the biofilm from the cleaning solutions. This can occur to greatly lesser extend in stainless steel and plastic materials. Concerning the pH of disinfectant solution, **Norman (2006), Simone et al. (2007)** and **Panagiotis et al. (2009)** reported that the acidic pH has an advantage over alkaline pH. Meanwhile, opposite data reported by **Antoniou and Frank (2005)** and **Sharma et al. (2005)**. While **Gibson et al. (1999), Parkar et al. (2004)** and **Giaouris et al. (2005)** stated that the biofilm formation was found to be independent of the pH value. The reduced ability of chlorine to remove biofilms was in agreement with the data reported by **Keesha and Joseph (2006), Byun et al. (2007), Donald (2007), Mangalappalli-Illathu et al. (2008)** and **Yong et al. (2009).** In contrast, opposite results were recorded by **Jang et al. (2006), Norman and Robert (2006), Park et al. (2006)** and **Craig (2008).** On the other hand **Akiyama et al. (2004)** and **Thorn et al. (2009)** reported the improvement of iodine effect over other biofilm removing agents. Meanwhile, the iodine resistance was reported by **Barry and Gordon (1990)** and **Elisabeth et al. (2007).**

CONCLUSION:

The problem of biofilm control can be summarised in two points, the first one is prevention of bacterial attachment and the second is removal of already attached bacteria. So the biofilm control should be carried out by two parallel procedures, limiting of bacterial attachment as possible and removal of formed biofilms by suitable disinfectant.

Table (1): Mean values of reduction rates of disinfectants on the attached microorganisms on food contact surfaces

Disinfectant	Trials	Surface	Aerobic microorganisms	Spore forming microorganisms	Staphylococcus aureus	Coliforms	Enterobacteriacae	C. perfringens
Chlorine	No. 1	Stainless steel	17.91 %	19.04 %	19.37 %	25.57 %	30.15 %	67 %
		Wood	8.17 %	32.15 %	29.95 %	28.42 %	13.58 %	43.63 %
		Plastic	16 %	19.42 %	28.82 %	33.46 %	20.57 %	41.31 %
	No. 2	Stainless steel	65.75 %	32.97 %	100 %	100 %	100 %	29.71 %
		Wood	94.24 %	63.31 %	84.81 %	77.02 %	92.61 %	52.20 %
		Plastic	91.48 %	81.32 %	100 %	100 %	100 %	31.44 %
Iodine	No. 1	Stainless steel	51.14 %	42.85 %	43.05 %	47.81 %	42.06 %	85 %
		Wood	35.12 %	45.04 %	50.88 %	56.44 %	46.38 %	53.13 %
		Plastic	44.07 %	38.84 %	47.74 %	50.16 %	43.42 %	78.87 %
	No. 2	Stainless steel	92.05 %	74.19 %	100 %	100 %	100 %	77.63 %
		Wood	97.14 %	92.91 %	98.33 %	87.82 %	98.01 %	94.81 %
		Plastic	96.34 %	92.46 %	100 %	100 %	100 %	90.72 %
Mixture	No. 1	Stainless steel	55.04 %	45.02 %	44.22 %	46.02 %	41.26 %	85 %
		Wood	19.55 %	48.78 %	54.35 %	47.38 %	52.4 %	57.37 %
		Plastic	49.09 %	45.32 %	57.65 %	57.41 %	49.71 %	62.91 %
	No. 2	Stainless steel	93.15 %	77.06 %	100 %	100 %	100 %	79.55 %
		Wood	97.23 %	91.80 %	98.03 %	88.37 %	97.98 %	96.83 %
		Plastic	97.80 %	92.77 %	100 %	100 %	100 %	84.53 %

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الملخص العربي

السيطرة علي الأغشية الحيوية علي الأسطح الملامسة للأغذية

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تسبب الأغشية الحيوية على الأسطح الملامسة للأغذية ضررا كبيرا يقدر بنحو المليارات من الدولارات في السنة وذلك في فساد المعدات، والتلوث، والمشاكل الطبية. وقد ثبت أن الأساليب التقليدية في قتل البكتيريا (مثل المضادات الحيوية والتطهير) غالبا ما تكون غير فعالة مع الكائنات الحية الدقيقة في الأغشية الحيوية. وقد تسارعت الجهود المبذولة في السنوات الأخيرة لمكافحة مسببات الأمراض التي تنقلها الأغذية. ويمكن أن تتسبب الأغشية الحيوية في التأثير على نظم النظافة والتطهير في مؤسسات الخدمات الغذائية من خلال نشر الكائنات الحية الدقيقة إلى مناطق أخرى. ولذلك فإن إزالة الأغشية الحيوية في مؤسسات تجهيز الأغذية أمر بالغ الأهمية .وكان الهدف من هذه الدراسة هو محاولة الوصول إلى طرق السيطرة علي ظاهرة الأغشية الحيوية بإستخدام ثلاثة أنواع من المطهرات التي تستخدم بصورة آمنة في مجال صناعة الغذاء وهي الكلور واليود وخليط منهما لمعرفة قدرتها على القضاء على ظاهرة تكوين الأغشية الحيوية في مؤسسات الخدمات الغذائية وذلك تحت ظروف تطبيق مختلفة؛ الأول هو التطبيق عند درجة حرارة 25 درجة مئوية وعند درجة الحموضة الطبيعية لكل محلول، والثاني هو التطبيق عند درجة حرارة 70 درجة مئوية وعند درجة الحموضة 5. وقد تم فحص 60 عينة (10 من كل سطح لكل تطبيق) للوقوف على قدرة هذه المطهرات على إزالة الأغشية الحيوية. وقد أظهرت النتائج أنه في التطبيق الأول لم تتجاوز أيا من نسب التخفيض للمطهرات عن 85 ٪، بينما تجاوزت نسب التخفيض هذه النسبة في التطبيق الثاني بل ووصلت في بعض الأحيان إلى 100 ٪.