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RADIOMETRIC DETERMINATION OF THE DEGREE OF MICROBIOLOGICAL DECONTAMINATION OF WORKING SURFACES IN FOOD INDUSTRIES

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Key words: microbiological decontamination, trace elements, cleaning of surfaces.

Bacteria ^{32}P were used to determine cleanability of various surfaces ised in food industries. Surfaces made of plastics, tephlon in particular, had the highest cleanability level as compared with those of steel or aluminium.

One of the essential factors determining the sanitary and hygienic levels within a technological process as a part of food industry operations is the cleanliness of working surfaces that have direct contact with raw materials. Classifications of different working surfaces, in terms of hygiene, rely on microbiological methods. Accurateness of these methods, however, is controversial, if one considers them as a possible reflection of reality. It follows from some studies [9] that preparation of surface smears by means of cotton-wool swabs renders it possible to collect 11% to 65% bacteria cells on surface. Only a little more than a half of those are countable in the further analysis by the dilution method. It is a rather well-known fact that determination of a variety of impurities is better accomplished by the technique of marked atoms.

There are many studies of the problem [7, 8, 9, 10, 11, 12, 13, 14]. Results of these analyses indicate that removability of bacteria from working surfaces depends on the following factors:

— kind of micro-organisms,
— kind of surface and surface treatment,
— kind of carrier, or the environment for microorganisms,
— degree of corrosion or mechanical surface damages,
— manner of washing and detergents used in the process,
— number of decontamination operations.

The aim of the study was to examine washability of bacteria from different types of working surfaces usually encountered in food industries.

MATERIAL AND METHODS

Bacillus subtilis, *Staphylococus aureus* and *Proteus vulgaris* were used in the study. They are not only typical representatives of microbiological pollution but also they show different morphological structures. The culture on the broth medium [3] was added with 10 ml neutral soultion of $KH_2{}^{32}PO_4$. After incubation the bacterial suspension was centrifuged. The resulting deposit was eluated with 0.85% NaCl solution or that of 0.01% LiCl in order to remove excessive quantities of the isotope. The eluation was performed by multiple centrifuging of the suspension until no presence of any radioactivity in the supernatant. After colorimetric standarization of the optical density of the suspension, as compared to the standard solution $BaSO_4$ [14], 0.5 ml suspension was placed on 5×5 cm working spaces purified and neutralized with ethanol and chloroform, and the carrier was evapoarated at 60°C. The carrier was composed of electrolyte solutions LiCl (0.01%) and NaCl (0.85%). Application of salt solutions of different ionic strength had an essential effect on mutual interaction of bacteria since the thickness of the electric layer surrounding any cell was ca 73 A in LiCl solution and ca 10 A in NaCl solution [14].

The reduction of ionic strength of the solution leads to an increase of thicknesses of monomial electric layers surrounding the cell, which involves an increment of repulsive force and energy [1]. For this reason the low-ionic strength LiCl solution is used in the microbiological determinations to prevent formation of the cell conglomerates [14]. It is confirmed by the results concerning determination of quantities of bacteria placed on the working surfaces. In the 0.85% NaCl solution there were:

3.27×10^6 cells of *Baccillus subtilis*
7.35×10^6 cells of *S. aureus*
13.75×10^6 cells of *P. vulgaris*

while in 0.01% LiCl there were:

10.0×10^6 cells of *B. subtilis*
40.0×10^6 cells of *S. aureus*
14.5×10^6 cells of *P. vulgaris*

with the same optical density of the suspension. Determinations were made with the dilution method and the given values are averages from 5 repeated series. Quantity of *P. vulgaris* were directly counted in a microscopic examination. In this case the ability of individual move-

ment of cells prevented any formation of conglomerates regardless of the ionic strength of the carrier.

The following typesof surface were investigated:

glazed wall tile (ceramics)	—	category of roughness:	14
polished stainless steel	(PSS)	„	10
scratched stainless steel	(SSS)	„	8-9
polished aluminium	(PAl)		10
scratched aluminium	(SAl)		6
smooth polystyrene	(SP)		7
corrugated polystyrene	(CP)		5-6
teflon	(T)		7-8

Roughness was determined on a surface analyser Zeiss Me-10 [16].

After evaporation of carrier radioactivity of the surface was measured with a USB-2 meter. Surfaces were washed in a specially set up apparatus warranting repeatedness of the experimental conditions: constant quantities, temperature, pressure, angle of incidence of water on the geometric centre of a contaminated area [6]. The washing medium was 250 ml distilled water at 80°C. After the washing radioactivity of the surfaces were measured again and then the surfaces were covered with nutritious agar [3] in order to determine the number of live cells surviving the operation. To confirm the presence of bacteria cells additional photographs were made of the polished aluminum plate. It was carried out with a Japanese JSM-50A electronic scanning microscope. Due to changes in the specific activity of the isotope, resulting from the period of half-life, results of determinations of the degree of decontamination of the surfaces were presented as a coefficient of washing facility:

$$K = \ln \frac{N_0}{N} \tag{1}$$

N_0 — net radioactivity of surfaces prior to washing (CPW),
N — net radioactivity of surfaces after washing (CAW).

There were ten repeated series for each bacteria species and for every carrier. The results were interpreted with the use of statistical analysis [5].

RESULTS AND DISCUSSION

As it follows from Table 1, the number of bacteria either inctivated or removed from the surfaces was very high: 99% to 100% for *B. subtilis* and 99.6% to 100% for *S. aureus*. It was assumed that a single colony that grew on the surface covered with nutritious agar corresponded to a single cell. It was virtually impossible to determined with this method the number of *P. vulgaris* because their ability to move individually caused the colonies to pour out all over.

Table 1. Number of bacteria on working surfaces after washing, % of quantity, microbiologically determined, of the initial level (extremal data)

Surface	*B. subtilis*		*S. aureus*	
	0.85% NaCl	0.01% LiCl	0.85% NaCl	0.01% LiCl
1	2	3	4	5
Plain steel	0.00-0.76	0.00-0.30	0.00-0.27	0.00-0.075
Polished steel	0.00-0.91	0.00-0.30	0.00-0.28	0.00-0.125
Scratched steel	0.00-1.07	0.00-0.30	0.00-0.001	0.00-0.075
Polished aluminum	0.00-0.67	0.00-0.30	0.00-0.200	0.00-0.145
Scratched aluminium	0.00-0.76	0.00-0.30	0.00-0.04	0.00-0.205
Ceramics	0.00-0.76	0.00-0.02	0.00-0.002	0.00
Corrugated polystyrene	0.00-0.09	0.00-0.15	0.00-0.136	0.00
Smooth polystyrene	0.00-0.04	0.00-0.06	0.00-0.41	0.00-0.10
Teflon	—	0.00-0.02	—	0.00

Note: the mark — stands for absence of results

Table 2. Results of analysis of the variation coefficient K

Source of variability	Free state	Squares summa	Average squares	F(calculated)
1	2	3	4	5
0	1	1674.0	1674.0	6977.19**
Microorganisms (1)	2	1420.0	710.0	2959.44**
Carriers (2)	1	12.27	12.27	51.15**
Surfaces (3)	8	44.0	5.5	22.92**
1×2	2	16.17	8.085	33.69**
1×3	16	29.12	1.82	7.58**
2×3	8	5.218	0.6523	2.72**
1×2×3	14	6.223	0.4445	1.85*
Error	468	112.30	0.2399	
Total	520	3320.00		

* stands for significance at 0.05. For the 0.05 level a term 'statistically significant difference' is used while for 0.01 — statistically highly significant.

** means that the value of Fcalc. is higher than the tabulated value at the level of significance equal 0.05 and 0.01 while

In the experiment the number of bacteria on surfaces after washing, determined microbiologically, depends on:

1) temperature effects,
2) mechanical removal of bacteria with a stream of water,
3) secondary contamination by the washing water,
4) improper selection of the culture conditions (the basic rather than selective medium was used here).

Practically it is not possible to determine the magnitude of effects of each of the listed factors on the data. Thus, it is also rather difficult to define the degree of bacteria removal from surfaces. The radiometric method used in the experiment, on the other hand, renders very precise results in this respect.

The radiometric measurements proved that there were bacteria still present on surfaces even if microbiological results spoke to the contrary. An analysis of variation (Table 2) showed that determination results

Table 3. Significance of contrasts of averaged K coefficients for surface microorganisms and carriers

No. Contrast	$F_{obl.}$	Significance $F_{cal.}$
1. *B. subtilis* × *S. aureus*	14.9864	++
2. *S. aureus* × *P. vulgaris*	3825.96	++
3. *B. subtilis* × *P. vulgaris*	3362.98	++
4. 0.85% NaCl × 0.01 LiCl	50.59	++
5. STZ × STR	0.36	−−
6. STR × STP	0.92	−−
7. STP × ALP	6.1050	+
8. ALP × ALR	0.5600	−−
9. ALR × PF	36.31	++
10. PF × PG	0.95	−−
11. PG × C	0.0004	−−
12. C × T	9.16	++
13. T × other surfaces	9.16-25.19	++
14. STZ, STR, STP, ALP, ALR, × PF, PG, C, T	170.669	++
15. *B. subtilis* (NaCl, LiCl) × *S. aureus* (NaCl, LiCl)	35.38	++
16. *S. aureus* (NaCl, LiCl) × *P. vulgaris* (NaCl, LiCl)	0.68	−−
17. *B. subtilis* (NaCl, LiCl) × *P. vulgaris* (NaCl, LiCl)	45.84	++

have a statistically very significant effect on all three parameters of variability: type of surface, microorganism species, and type of carrier. Their individual effects are not the same. Table 3 gives the results of the statistical assessment of the effects and it shows that *P. vulgaris* must be washed off under totally different conditions which are sufficient in regard to the two other species. Washability of surfaces made from the same material is similar. It is worth noting the results of Contrast 9: corrugated polystyrene washes in a different way from scratched aluminum. Contrast 14 confirms the assumption about better washing properties of plastics versus metals. As Contrast 13 indicates, teflon washes best of all the plastics. Effects of the carrier on washability of *S. aureus* and *P. vulgaris* are of random chacarter but it is highly significant as regards all the other cases.

Nevertheless, analysis of the comparisons does not provide a possibility for an absolute evaluation of the differences between particular

Table 4. Classification of sufraces according to the 14-point scale of the washing facility

No	Surface	Micro-organism	Carrier	Washing facility	K	N/00 N × 1 %
1	2	3	4	5	6	7
1.	Teflon	*P. vulgaris*	NaCl	1	5.1402	0.58
2.	Ceramics	*P. vulgaris*	NaCl	1, 2	4.7542	0.86
3.	Smooth polystyrene	*P. vulgaris*	NaCl	2	4.6679	0.94
4.	Teflon	*P. vulgaris*	LiCl	2	4.6152	0.99
5.	Corrugated pol.	*P. vulgaris*	NaCl	2, 3	4.3953	1.23
6.	Scratched steel	*P. vulgaris*	LiCl	2, 3, 4	4.3607	1.28
7.	Scratched steel	*P. vulgaris*	NaCl	2, 3, 4, 5	4.2903	1.37
8.	Ceramics	*P. vulgaris*	LiCl	2, 3, 4, 5	4.1933	1.51
9.	Smooth polystyrene	*P. vulgaris*	LiCl	3, 4, 5	4.1575	1.56
10.	Plain steel	*P. vulgaris*	NaCl	3, 4, 5	4.1571	1.57
11.	Plain steel	*P. vulgaris*	LiCl	3, 4, 5	4.0918	1.67
12.	Polished steel	*P. vulgaris*	NaCl	4, 5	4.0595	1.72
13.	Polished steel	*P. vulgaris*	LiCl	4, 5	4.0382	1.76
14.	Corrugated polystyrene	*P. vulgaris*	LiCl	5	3.9443	1.94
15.	Polished aluminium	*P. vulgaris*	LiCl	5, 6	3.6109	2.70
16.	Scratched aluminium	*P. vulgaris*	LiCl	6, 7	3.2307	3.95
17.	Polished aluminium	*P. vulgaris*	NaCl	7	2.7146	6.22
18.	Scratched aluminium	*P. vulgaris*	NaCl	7	2.7064	6.67
19.	Corrugated aluminium	*B. subtilis*	NaCl	8	1.3984	24.69
20.	Smooth polystyrene	*B. subtilis*	NaCl	8, 9	1.3886	24.94
21.	Ceramics	*B. subtilis*	NaCl	8, 9, 10	1.2910	27.49
22.	Scratched al.	*B. subtilis*	NaCl	8, 9, 10	1.1190	32.66
23.	Teflon	*S. aureus*	LiCl	8, 9, 10	1.1004	33.27
24.	Polished aluminium	*S. aureus*	NaCl	8, 9, 10	1.0049	36.60
25.	Plain steel	*S. aureus*	NaCl	8, 9, 10	0.9550	38.48
26.	Scratched steel	*S. aureus*	NaCl	8, 9, 10	0.9218	39.78
27.	Teflon	*B. subtilis*	LiCl	9, 10, 11	0.8568	42.45

c. d. tab. 4

1	2	3	4	5	6	7
28.	Polished ateel	*B. subtilis*	NaCl	10, 11, 12	0.8135	44.33
29.	Smooth polysterene	*S. aureus*	NaCl	11, 12, 13	0.7188	48·73
30.	Ceramics	*S. sureus*	NaCl	11, 12, 13, 14	0.681_0	50.65
31.	Corrugated polysterene	*S. aureus*	NaCl	11, 12, 13, 14	0.6755	50.89
32.	Smooth polystyrene	*S. aureus*	LiCl	11, 12, 13, 14	0.5867	56.61
33.	Polished aluminium	*S. aureus*	NaCl	11, 12, 13, 14	0.5569	57.29
34.	Corrugated polysterene	*S. aureus*	LiCl	11, 12, 13, 14	0.5225	59.30
35.	Ceramics	*S. aureus*	LiCl	11, 12, 13, 14	0.4830	61.69
36.	Scratched aluminium	*S. aureus*	NaCl	11, 12, 13, 14	0.4750	62.18
37.	Ceramics	*B. subtilis*	LiCl	11, 12, 13, 14	0.4359	64.66
38.	Scratched steel	*S. aureus*	NaCl	11, 12, 13, 14	0.4269	65.25
39.	Plain steel	*S. aureus*	NaCl	11, 12, 13, 14	0.3927	67.52
40.	Corr. polysterene	*B. subtilis*	LiCl	11, 12, 13, 14	0.3631	69.55
41.	Polished steel	*S. aureus*	NaCl	11, 12, 13, 14	0.3425	70.99
42.	Scratched aluminium	*B. subtilis*	LiCl	11, 12, 13, 14	0.3370	71.39
43.	Smooth polystyrene	*B. subtilis*	LiCl	12, 13, 14	0.3071	73.55
44.	Scratched aluminium	*S. aureus*	LiCl	12, 13, 14	0.2838	75.29
45.	Scratched steel	*S. aureus*	LiCl	12, 13, 14	0.2824	75.39
46.	Polished steel	*S. aureus*	LiCl	12, 13, 14	0.2739	75.63
47.	Plain steel	*S. aureus*	LiCl	13, 14	0.2589	77.19
48.	Scratched aluminium	*B. subtilis*	LiCl	13, 14	0.2505	77.84
49.	Polished aluminium	*S. aureus*	LiCl	13, 14	0.2450	78.27
50.	Polished steel	*B. subtilis*	LiCl	14	0.1548	85.65
51.	Scratched steel	*B. subtilis*	LiCl	14	0.1326	87.58
52.	Plain steel	*B. subtilis*	LiCl	14	0.1304	87.77

$N/N_0 \times 100$ — number of bacteria staying on surface after washing in % of the initial volume, as determined with the radiometric method
N — radioactivity of surface after washing (CAW)
N_0 — radioactivity of surface before washing (CBW).

factors. The information can be collected only from classification of surfaces. Its aim is to arrive at an optimum arrangement including all of the factors of variability. Such a classification was made with the Duncan multiplerange test in which K values are not essentially different from one another [5]. The results are shown in Table 4.

It follows from the presented data that 14 groups of arrangements were discernible in the experiment. They were characterized by differentiated and lowering facility of washing (Column 5, Table 4).

Regardless of the carrier, the most easily removed is *P. vulgaris*..The species is clearly different from others. Among the surfaces in every analyzed arrangment 'bacteria — carrier' teflon is always showing the best washability (Items 1, 4, 23, and 27, Col. 1, Table 4). In the experiment differences in the facility of washing result from:

1) differentiated force of the adhesion bond, and
2) different mechanical resistance to a washing medium.

These factors are determined by surface tension, or in other words, by free surface energy [1, 2, 4, 15]. The surface energy for plastics is rather minor: teflon — 18.5 dyn/sq·cm.; polystyrene — 33 dyn/sq·cm, while for aluminum it is ca 500 dyn/sq·cm. [15].

Effects on plastic surfaces are, therefore, much weaker than on metal surfaces, on which additional layers of oxides and a different type of surface treatment increase the energy state of it. This provides for low wetting of plastics by contaminants and reduces the effects of adhesion.

Application of carriers with different ionic force modifies the energy state of the surface of a cell. However, lack of descriptions of the cell surface energy does not permit a quantitative assessment of changes of forces between bacteria and the working surface.

The possibility that different resistance to the washing medium is involved can be confirmed with the analysis of the photographs. Clear, indentifiable cells of *B. subtilis* in 0.01% LiCl (Fig. 1) pass into a hazy picture for 0.85% NaCl carrier (Fig. 3). In this case the cells form a structural element of the NaCl layer on surface. In this configuration the probability of a direct contact between the cell and the surface is lower. It also follows from Fig. 5 that bacteria can settle on the carrier's crystals as well as panetrate micro-cracks of the surface (Fig. 6). The photographs show clearly that sedimentation of microbiological contaminants on surfaces may take several forms:

1) direct contact — surface: single cells
2) surface — crystal-cells
3) surface — a layer of cells-cells
4) surface — cells in a crystallized carrier.

As regards the experiment, Type 2) and Type 4) are characteristic

for conditions before washing and for a relatively high concentration of salt in the carrier. Types 1) and 3) are the dominating forms after the washing process is completed.

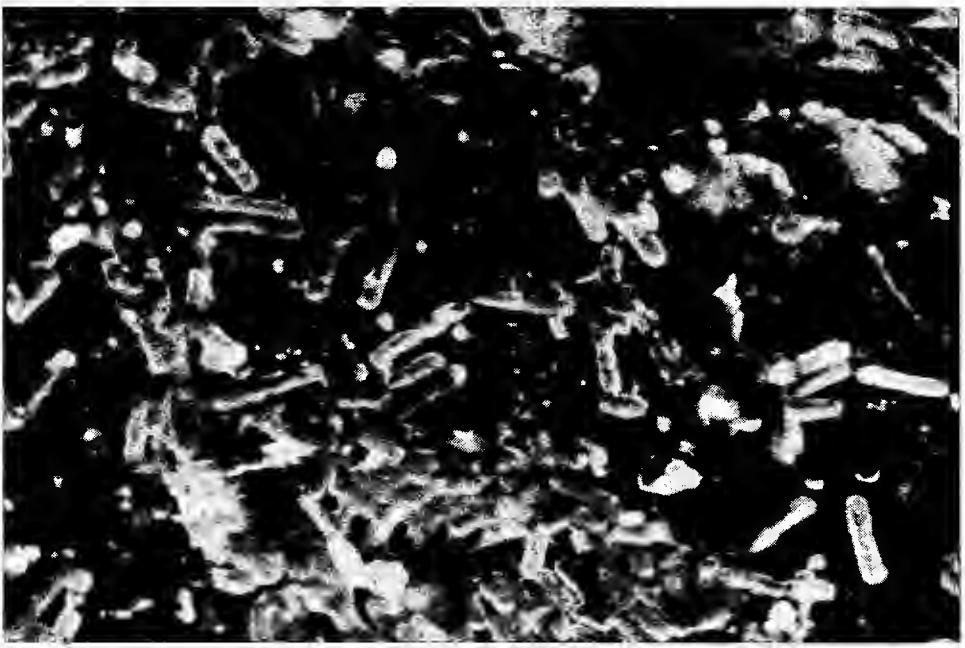


Fig. 1. *B. subtilis* on polished aluminium before cleaning. Carrier — 0.01% LiCl, magnification 3000×

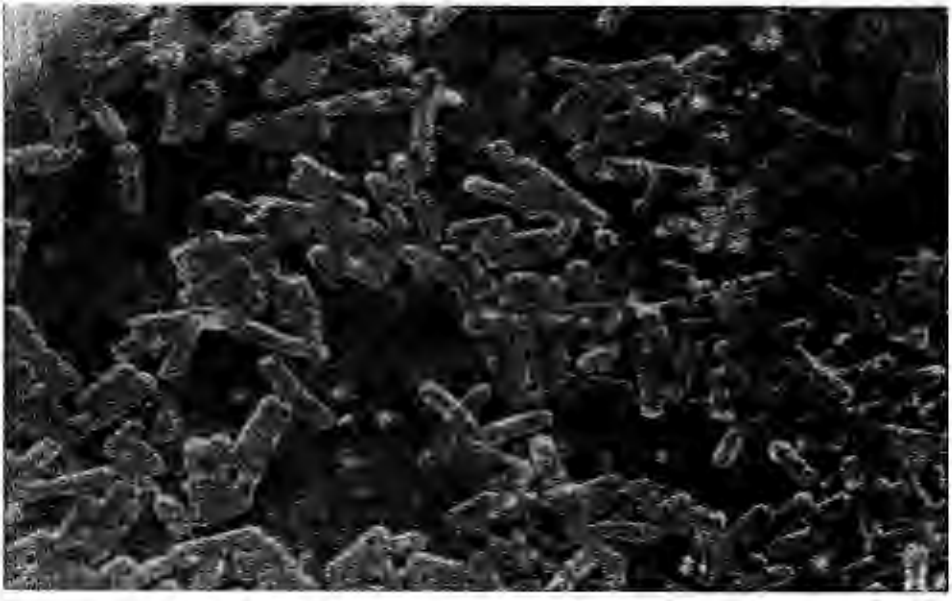


Fig. 2. *B. subtilis* on polished aluminium after cleaning. Carrier — 0.01% LiCl, magnification 3000×

Fig. 3. *B. subtilis* on polished aluminium before cleaning. Carrier — 0.85% NaCl, magnification 3000×

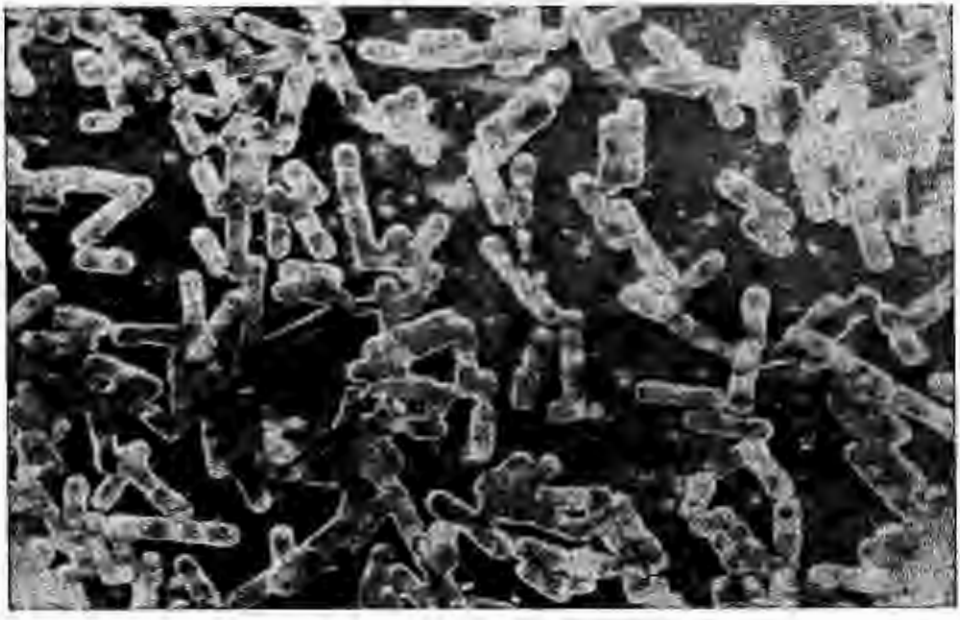
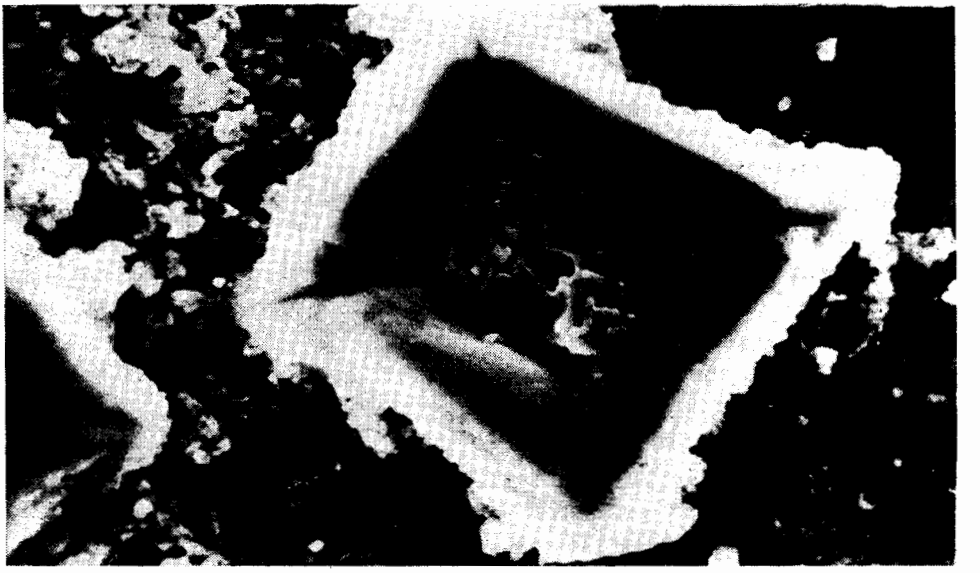


Fig. 4. *B. subtilis* on polished aluminium after cleaning. Carrier — 0.85% NaCl, magnification 3000×



Rys. 5. *S. aureus* on polished aluminium before cleaning. Carrier — 0.85% NaCl, magnification 1000×

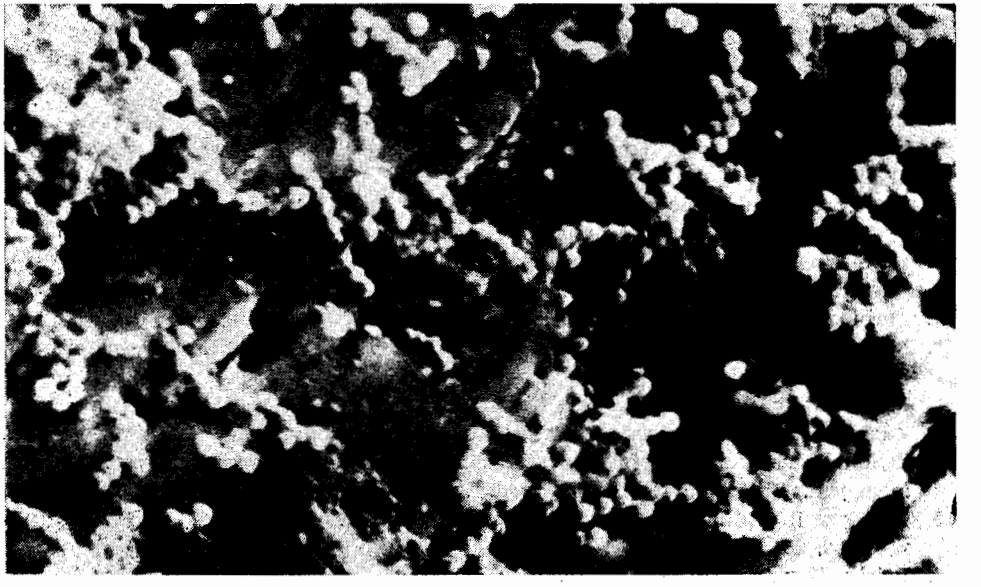


Fig. 6. *S. aureus* on polished aluminium before cleaning. Carrier — 0.01% LiCl, magnification 3000×

CONCLUSIONS

1. Different washability of the used species of bacteria was observed during the experiment. The easiest to wash off was *P. vulgaris*, regardless of carrier and type of surface.

2. Plastic surfaces are in general better washable in comparison with metallic ones. Among the tested surfaces teflon proved to be the most easily washable plastic surface.

3. As regard metal surfaces, the scratched as well as the polished surfaces turned up in the same groups of washability degree.

4. The radiometric method is superior to conventional methods of examining the mechanisms of decontamination processes. This is due to the possibility it provides for unequivocal determination of quantities of contaminats removed from surfaces.

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RADIOMETRYCZNE OKREŚLENIE STOPNIA DEKONTAMINACJI MIKROBIOLOGICZNEJ POWIERZCHNI ROBOCZYCH STOSOWANYCH W PRZEMYŚLE SPOŻYWCZYM

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Streszczenie

Przebadana została zmywalność mikroorganizmów z różnych rodzajów powierzchni roboczych przy użyciu bakterii zawierających ^{32}P. Znakowanie komórek przeprowadzono przez dodanie $KH_2{}^{32}PO_4$ do płynnego podłoża. Po inkubacji, hodowle *B. subtilis*, *S. aureus* i *P. vulgaris* odwirowywano wielokrotnie w 0,85% NaCl lub 0,01% LiCl w celu usunięcia nadmiaru izotopu, a następnie nanoszono na powierzchnie robocze. Pomiary radioaktywności powierzchni wykonywano przed i po zabiegu mycia w powtarzalnych warunkach.

Spośród wymienionych gatunków bakterii najłatwiejszy do zmywania okazał się *P. vulgaris*, niezależnie od zastosowanego nośnika i rodzaju powierzchni roboczej. Najłatwiejszą powierzchnią do zmywania okazał się teflon, najtrudniej zaś usuwano bakterie z powierzchni metalowych, niezależnie od stopnia ich chropowatości (tab. 4).

Różnice w łatwości zmywania pomiędzy *B. subtilis* i *S. aureus* oraz *P. vulgaris* i *B. subtilis* powodowane były także zastosowanym nośnikiem (tab. 3).

Wyniki oznaczeń radiometrycznych wykazały, że na powierzchniach roboczych znajdowały się bakterie nawet wtedy, gdy uzyskiwano negatywne rezultaty oznaczeń mikrobiologicznych (tab 1). Wskazuje to na większą przydatność opisywanej metody niż metod konwencjonalnych w badaniach nad mechanizmami procesów dekontaminacyjnych.

Fotografie wykonane techniką mikroskopii elektronowej wskazują na możliwość stawiania odmiennego oporu czynnikowi myjącemu przez bakterie i potwierdzają jednocześnie wyniki radiometryczne.