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EVALUATION OF STEEL SURFACE CLEANLINESS LEVEL IN DAIRIES USING THE BIOLUMINESCENCE METHOD

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Abstract

The aim of the study was to evaluate the usefulness of bioluminescence in the determination of cleanliness of steel surfaces characterised by different roughness (0.6 and 0.8 μm). The applicability of the method was assessed based on correlations between the number of relative light units (RLU) measured with a luminometer and microbial counts determined by the conventional microbiological method (cfu/cm^2). The higher the roughness of surface was the higher was the microbiological contamination measured by these methods. Microbial counts classifying an examined surface as clean, acceptably clean and unacceptable clean were the basis for predictions of RLU ranges. High levels of proportional correlation were obtained for these methods.

Key words: dairies, hygiene, surface roughness, ATP bioluminescence method.

A significant factor influencing the high quality of milk products is sustaining of a proper hygienic level in dairy processing plants (12, 20). Cleanliness of surfaces in case of machines, technological line elements, and facilities involved in the production process is most frequently examined visually, using the sense of smell or touch. The application of these methods daily, each time after washing and disinfection does not show the actual cleanliness level. This pertains especially to situations when the examined surfaces are made from materials differing in roughness and thus also exhibiting different capacity to maintain the bacterial biofilm (11, 15, 16, 17). Reliable results of cleanliness examination are obtained when using microbiological methods. However, traditional microbiological testing methods such as the hygiene swabbing, wipe-rinse, direct or blotting methods are time-consuming and laborious. A result of traditional testing methods obtained 48 or 72 h after swabbing makes it impossible to undertake any corrective action, as in practice the production cycle has long been in progress. Safety may be guaranteed thanks to the application in the monitoring of cleanliness in examined objects of state-of-the-art testing methods using physico-chemical properties of microorganisms, advances in genetics and cell biochemistry (3, 7, 8). Remarkable advantages of these methods include prompt results and easy performance (1). However, the key issue for the appropriate application of these methods is the ability to interpret the recorded results (13, 14). The determination of a correlation between traditional microbiological methods and results of modern methods will facilitate an adequate utilisation of rapid tests assessing cleanliness of examined objects as effective tools monitoring their hygienic status (2, 4, 6).

The aim of the study was to evaluate the usefulness of the bioluminescence method in assessing the hygienic status in a dairy equipped with machinery characterised by different surface roughness, and in determining the range of cleanliness defined as good, acceptable, conditional warning level, and unacceptable.

Material and Methods

Objects examined and collection of samples. The experiment was conducted in a dairy, located in north-western Poland, producing rennet cheese. The analyses were performed for objects such as surfaces of devices being in contact with the dairy product. Two objects were selected for the analyses (n=20), differing in terms of the roughness of surfaces. The examined objects were manufactured from high-alloy austenitic steel type 316L-AISI (American Iron and Steel Institute), X2CrNiMo 17 13 2-DIN (Deutsche Industrie Normen). It is a stainless chromium-nickel steel, heat resistant and extremely corrosion-resistant. Types of plate surface: cold-rolled sheet, annealed, pickled sheet, smooth without lustre. Surface treatment: 2D-AISI, IIIb-DIN. Object A: paddle mixers, surface roughness Ra 0.6 (μm). Object B: flap valve, surface roughness Ra 0.8 (μm).

The cleanliness status of adjacent surfaces with an area of 100 cm^2 was examined using the traditional swabbing method and by bioluminescence. Swabs were collected from visually clean and dry surfaces at least 2

h and not later than 4 h after the completion of washing and disinfection procedures. Swabs were collected from an area limited by a 10 cm x 10 cm frame using a sterile swab by moving it five times parallel to one of the frame sides and next perpendicular, tilting it at an angle of 45°.

Microbiological analysis. Microbiological contamination of surfaces was determined by the traditional swabbing method. This consisted of the following stages: wiping the area limited by the frame with a swab moistened with a dilution fluid, rinsing the swab, preparation of dilutions, submerging the cultures of 1 cm^3 each onto two dishes, incubation in a microbiological thermostat WTB Binder (Tuttlingen, Germany), and recording microbial counts per 1 cm^2. The collected swabs were analysed within 2 h after collection. Standard diluents and microbial media (P-0054, BTL, Poland) were used in the experiment (5, 10). The composition of P-0054 is as follows: agar (15g/L), peptone (5.0 g/L), extract yeast (2.5 g/L), and glucose (1.0 g/L).

Bioluminescence method. The assessing of cleanliness status was based on results of ATP measurements with a luminometer (FireFly Charm Sciences Inc., USA) and swabs (PocketSwab Plus Charm Science Inc., USA). The measurement procedure was performed following the instructions of the manufacturers of the luminometer and swabs. The total testing time including the reading did not exceed 45 s. The result was given in relative light units (RLU).

Statistical analysis. The results of bioluminescence and those of the conventional microbiological method were compared following the division of object surfaces into those classified as clean, i.e. Pass (≤5–0.44xSd), conditionally clean, i.e. Alert (5–0.44xSd<and≤8–0.44xSd), or unacceptable, i.e. Fail (>8–0.44xSd) for the total number of object samples (n=20).

Pearson's linear correlation coefficients were calculated in order to determine the degree of proportional correlations between values of the conventional microbiological method and those obtained by bioluminescence. In order to eliminate the departures from linearity of Pearson's distribution, which might cause an increase in the sum squares of deviations from regression lines, scatter diagrams were analysed for results recorded for each examined object. Statistical calculations were performed using a data analysis software system STATISTICA (version 7.1) by StatSoft, Inc. (2005).

Results

The results of the studies indicate a proportional correlation between the total number of microorganisms and different surface roughness (Table 1). Significant statistical differences in the total number of microorganisms determined by the conventional microbiological method for objects A and B were found. At the same time, it was found that the higher the roughness of surface was, the higher were the RLU values determined by the bioluminescence method. High variation in the cleanliness levels for the examined objects resulted from different roughness of those surfaces and thus also varying effectiveness of washing and disinfection in order to remove the formed bacterial biofilm.

The probability of normal distribution was analysed to assess the suitability of results of microbiological tests and results obtained using a luminometer to determine their correlations (Fig. 1). Irrespective of the type of objects, no deviation was shown from linearity, which measures the dependence between the log of microbial count from 1 cm^2 of the analysed object and the log of number of relative light units RLU (Fig. 1). For each object, a regression line was plotted by the initial ordinate, within the range from 2.27 for object B to 2.49 for object A. In turn, the slope of the line fell within the range from 0.35 for object A to 0.45 for object B. Cho and Yoon (3) used in their model studies the high dependence of microbial counts and results of RLU measurements to determine detection levels with a luminometer. Such a high regularity of the results was reflected in the values of correlation coefficients r= 0.975 (object A) and r=0.987 (object B) between microbial counts assayed using the microbiological method and the number of relative light units measured with a luminometer.

The significant variation in the results for individual objects made it possible to determine three levels of surface cleanliness for each of the objects (Table 2). The proportion of samples for individual surfaces considered clean ranged from 45% to 80%, for those with the alert cleanliness level conditionally acceptable to initiate the production cycle ranged from 10% to 45%, while for those unacceptably dirty it was 10%. The number of RLU for each cleanliness level was defined on the basis of the boundary values of microbial counts on the analysed surface, determining these three ranges of cleanliness (Table 2).

Table 1

Analysis of dependence of bioluminescence results (RLU) on microbial counts (cfu/cm^2), α=0.05, df=1.18, n=20

Object	Mean log cfu	Mean log RLU	R^2	
A	0.40±0.28	2.63±0.10	0.951	P<0.001
B	0.69±0.17	3.02±0.07	0.974	P<0.001
	P<0.001			

df - degrees of freedom; R^2 - coefficient of determination; ± - SD;

Table 2
Results recorded using bioluminescence corresponding to calculated values obtained by the microbiological method defining cleanliness levels for individual objects

Object	Cleanliness levels for object	Results of microbiological method		Bioluminescence results (RLU)	
		(cfu/cm^2)	(%)	Experimental values	Prediction
A	Pass	≤3.97	80	≤450	≤475
	Alert	3.97<x≤6.97	10	520≤x≤550	475<x≤607
	Fail	>6.97	10	≥650	>623
B	Pass	≤4.03	45	≤910	≤951
	Alert	4.03<x≤7.03	45	1100≤x≤1220	951<x≤1222
	Fail	>7.03	10	≥1500	>1222

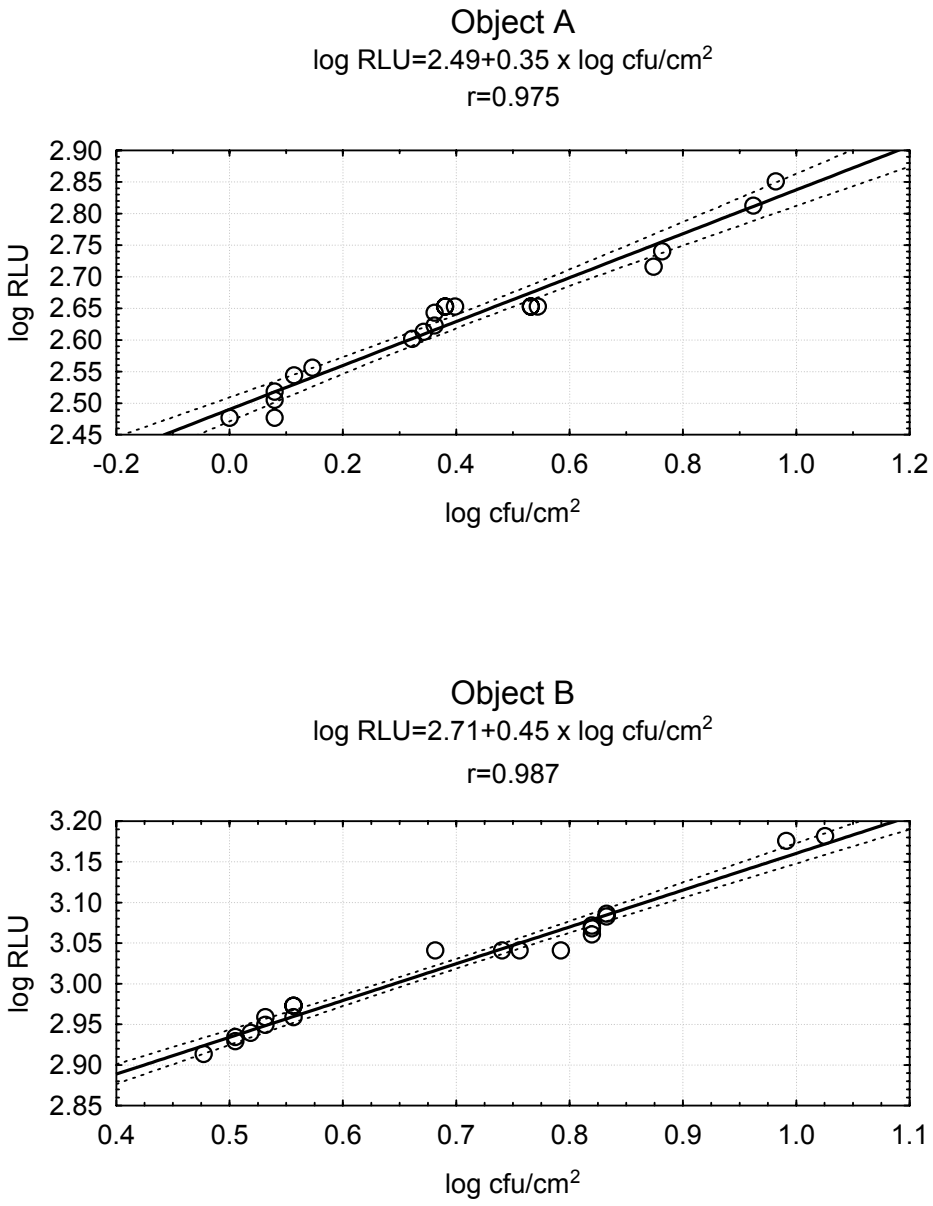

Fig. 1. Regression of variable RLU number of microbial counts (cfu/cm^2) for examined objects, α=0.05.

Discussion

The formation of biological film on an abiotic surface starts with the moment the first cell is deposited and the mechanism of the attachment reaction is a specific response of bacteria to environmental conditions. The viability of bacteria on abiotic surfaces indicates a potential hazard (18, 19). Hilbert *et al*. (9) when investigating adhesiveness of bacteria to different steel surfaces, detected from 6.19 to 7.17 cfu/cm^2. After the analysed surfaces were washed, bacterial counts decreased markedly to 0.3–4.69 cfu/cm^2. The authors selected for analyses steel surfaces with roughness ranging from 0.01 to 2.0 μm.

A high correlation was also found for results of bioluminescence and the conventional method reported by Larson *et al*. (14). When examining 219 surfaces of 4 cm x 4 cm, they detected 2.97 log cfu and at the same time recorded on average 2.61 log RLU at P=0.45. The value of measured RLU ranged from 0.8 to 4.6 log RLU, which corresponded to 15-44,000 RLU. The correlation coefficient calculated by the authors was r=0.82.

The established boundary values for cleanliness levels of examined surfaces included experimental data. Bautista *et al*. (2) when assessing the hygienic status of surfaces using the conventional method and by bioluminescence, showed that in 74% of the analysed surfaces, the results obtained by the traditional method and by bioluminescence were consistent. In 36% of surfaces, RLU results indicated that the surfaces were not sufficiently clean, although it was not confirmed using the conventional method. Prior to washing, out of 20 surfaces the authors detected 4-2,191 RLU, while after washing they detected 2-285 RLU. In turn, Aycicek *et al*. (1) found that 97.5% of examined surfaces could be considered clean on the basis of results recorded by both the conventional method and bioluminescence. The other 2.5% of investigated objects turned out to be clean based on ATP-bioluminescence results, although microbial count assessed by the conventional method did not show it. The percentage of objects assessed by the authors as clean on the basis of bacterial counts and as dirty based on RLU data was 74.6%. At the same time, the authors when examining 14 different objects, *e.g*. steel and plastic, showed a wide spectrum ranging from 1,435 to 90,959 measured RLU. The suitability of bioluminescence in the assessment of cleanliness status was shown by Cooper *et al*. (4) within the range of 83% to 100% prior to washing and 90% to 100% after surface washing. The authors decided that on average 84% of surfaces they examined were clean based on RLU data, but only 66% based on the conventional microbial count method. This proves the necessity to define detailed accurate RLU ranges for specific surfaces.

The investigations indicate a high correlation of results recorded using the conventional and bioluminescence assay methods. The application of ATP bioluminescence in a milk processing plant has to be preceded by microbiological tests in order to determine cleanliness levels for surfaces. The prediction of cleanliness ranges based on RLU data should be performed for each object separately. This results from the diversity of examined materials, their roughness, purpose and utilisation as well as attached bacterial biofilm. As a consequence, a wide range of measured RLU levels was recorded for the analysed surfaces. Ranges of the hygienic status of surfaces defined as clean, conditionally clean, and insufficiently clean based on RLU data should be – as in this experiment – completely consistent with the ranges defined as a result of prediction for the independent variable, i.e. microbial count assessed by the conventional microbiological method.

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