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USING THE BIOLUMINESCENCE AND MICROBIOLOGICAL CONTACT METHODS IN SUSTAINING A PROPER HYGIENIC LEVEL IN FOOD PROCESSING PLANTS

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Abstract. The efficiency of bioluminescence applied to monitor the state of hygiene in a dairy processing plant was assessed in relation to the results of conventional microbiological methods. The used blotting tests were Envirocheck Contact DC with Agar CASO medium with added neutralizers. The analysed object was the surface of a beam stirrer in a fermentation tank. Swabs were collected following tank washing and disinfection after the completion of 15 production cycles. A high degree of correlation r = 0.91 was obtained at the reliability of comparison β = 0.906. The analysis of probability of distribution confirmed the feasibility of bioluminescence. Boundary values (112 and 171 RLU) were determined for bioluminescence for three object cleanliness ranges, based on microbial counts (cfu/cm^2). Over 13% surfaces were classified as conditionally clean (Alert).

Key words: bioluminescence, ATP, hygiene

INTRODUCTION

In compliance with the Ordinance of the European Committee no. 1441/2007 of 5 December 2007 enterprises of the agri-food sector, including the dairy industry, are required to provide for foodstuffs to meet the respective microbiological criteria and to ensure the maintenance of respective hygienic criteria for the processes of their production. The application of microbiological criteria should constitute an integral part of the implementation of procedures based on HACCP principles and other hygiene control procedures. In order to ensure the acceptability of the operating production process, agri-food sector enterprises have to meet respective process hygiene criteria. These criteria determine the contamination index value, which when exceeded shows that corrective actions are required in order to maintain process hygiene at a level imposed by the food legal regulations. In the assessment of process hygiene criteria it is admissi-

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ble to analyse the presence of alternative microorganisms in relation to respective microbiological limits, as well as investigate parameters other than microbiological ones. They are particularly applicable in the monitoring of hygienic condition of production lines on a daily basis. The determination of correlations between conventional microbiological methods and results recorded using modern ones will facilitate the application of rapid cleanliness tests for analysed objects as an effective tool in the monitoring of the hygiene condition [Bautista et al. 1992, Griffiths 1995, Squirrell et al. 2002, Cooper et al. 2007].

In food industry plants monitoring methods are most frequently used to determine hygienic quality. Conventional methods of microbiological analysis, such as the swabbing, wash-and-rinse, direct or blotting methods are time-consuming and laborious. Since the result of conventional analyses is obtained 48 or 72 h after swab collection, it is impossible to undertake corrective actions, as in practice the production cycle has been initiated much earlier. Safety assurance is facilitated when the cleanliness status of analysed objects is monitored by modern methods using physico-chemical properties of microorganisms, advances in genetics and findings concerning cell biochemistry [Griffiths 1996, Hawronskyj and Holah 1997, Cho and Yoon 2007]. An important advantage of these methods is the practically immediate result and easy performance [Deshpande 2001]. The shorter the time interval between the measurement and the result, the more effective the monitoring method in the food safety assurance system [Knaflewska and Pośpiech 2007, Rosmaninho et al. 2007, Sharma and Anand 2002]. However, the key factor in the appropriate use of these methods is to be able to properly interpret recorded results [Lappalainen et al. 2000, Larson et al. 2003].

The aim of the study was to assess the efficiency of washing and disinfection processes and to define the scope of application for bioluminescence in the assessment of hygienic status in a dairy processing plant prior to the commencement of production within the framework of hygiene and sanitary control at the plant.

MATERIAL AND METHODS

Collection of samples. The experiment was performed at a dairy plant in northwestern Poland, producing fermented dairy drinks. The analyses were conducted on the surface of a beam stirrer mounted within a fermentation tank. The object selected for analyses was manufactured from high-alloy austenitic steel type 316L. It is stainless chromium-nickel steel, cold rolled, annealed, etched, smooth and lustreless. Analyses were conducted after the completion of each of the 15 production cycles. The selected object was characterized by a high microbiological load.

Cleanliness of adjacent surfaces in the object was analysed after washing and disinfection processes by microbiological blotting tests and by bioluminescence. Swab samples were collected from clean and dry surfaces at least 2 h after and not later than 4 h after the completion of washing and disinfection procedures. The same surface was selected for each analysis.

Microbiological analysis. Blotting tests Envirocheck Contact DC by Merck KGaA (Darmstadt, Germany) were used to assess the efficiency of washing and disinfection of surfaces of technological line equipment at a dairy processing plant. The test is a rectangular plastic plate of approx. 9 cm^2, covered on both sides with a layer of agar me-

dium, and placed in a sterile vial. In the Envirocheck Contact DC test one side of the strip is covered by CASO trypticase soy agar (TSA). It is an agar medium with casein peptone and soy flour peptone for the determination of microbiological load before washing and disinfection. The other side of the strip is covered with CASO Agar with an addition of neutralizers such as Ploysorbate 80 (Tween 80), sodium thiosulfate, lecithin and histidine. Tween 80 neutralizes hexachlorophene and mercury compounds, sodium thiosulfate neutralizes halogen derivatives, lecithin neutralizes chlorhexidines, histidine in combination with Tween 80 and lecithin neutralizes aldehydes and substituted phenols. Quaternary ammonium salts are also neutralized by a combination of Tween 80 and lecithin. The application of neutralizers facilitates the growth of bacteria, which development was only inhibited as a result of washing and disinfection and which still remain capable of growing. The Agar CASO medium with neutralizers is used to investigate microbiological load on the surface following washing and disinfection.

Prior to use blotting tests were stored at 12 ±3°C. The smearing procedure was performed according to the manufacturer's recommendations. The cap on the tube was unscrewed and the Envirocheck slide was removed from the tube, taking care not to touch the agar surfaces. The slide was checked before use for any sign of dehydration or contamination. The terminal end of the paddle was held with two fingers against the surface to be tested. The spike was pressed down to bend the paddle, while still holding the slide by the cap. With a firm and even pressure one medium was pressed against the surface to be tested. Care was taken not to smear the agar over the test area. The procedure was repeated with the other side of the paddle on an area adjacent to the initial test site. The slide was replaced back into the tube and closed tightly.

Tests, after being pressed to the analysed surface and replaced back to their tubes, were transported to the incubation site within max. 6 h, in the dark and at a temperature up to 5°C. Tubes with tests were placed vertically in a WTB Binder microbiological incubator (Tuttlingen, Germany) at 37°C for 48 h. Microbial counts were given in cfu/cm^2 area.

Bioluminescence method. Cleanliness status by bioluminescence was assessed based on results of ATP measurements with a luminometer FireFly Charm Sciences Inc. (Malden, USA) and swabs PocketSwab Plus Charm Science Inc. (Lawrence, USA). The measurement procedure was performed following the instructions of the manufacturers of the meter 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. 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 ($\leq 5 - 0.44 \times Sd$), conditionally clean, i.e. Alert ($5 - 0.44 \times Sd <$ and $\leq 8 - 0.44 \times Sd$) or unacceptable, i.e. Fail ($> 8 - 0.44 \times Sd$) for the total number of object samples n = 15. 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. On this basis regression lines were plotted and the coefficient of determination was calculated, constituting the basis for the size of the correlation. Coefficients of determination express the size of common variance for the two analysed variables. Statistical calculations were performed using a data analysis software system STATISTICA (version 7.1) by StatSoft (2005).

RESULTS

Microbiological analyses conducted using blotting tests showed counts ranging from 0.8 to 5.3 cfu per 1 cm^2 area of the investigated object. In turn, the simultaneous application of swabs, next placed in a luminometer, showed the presence of 17 to 116 RLU on the surface of the object. The basis for the determination of a correlation between both methods was to calculate the coefficient of determination ($R^2 = 0.93$) and to analyse the probability density function (Table 1). High values of correlations were recorded for analysed variables, as it is evidenced by the reliability of correlation $\beta = 0.906$, i.e. close to 1. The reliable correlation of results obtained using blotting tests and by bioluminescence is shown by the low value $p < 0.001$. These data were recorded irrespective of the division of cleanliness scores of the same objects in successive production cycles.

Table 1. The analysis of correlation between bioluminescence results (RLU) and microbial counts (cfu/cm^2), $\alpha = 0.05$, *df* = 1.13, n = 15

	Mean log cfu ±Sd	Mean log RLU ±Sd	R^2	F	β	*p*
Pass	0.17 ±0.25	1.73 ±0.23				
Alert	0.68 ±0.06	2.06 ±0.01				
Mean	0.24 ±0.25	1.77 ±0.23	0.93	59.48	0.906	< 0.001

df – degrees of freedom.
R^2 – coefficient of determination.
F – density function.
β – density probability.
Sd – standard deviation.
p – value statistically significant.

The determination of the correlation between results of bioluminescence and conventional microbiological assays was facilitated by the conducted analysis of probability of normal distribution (Fig. 1). Almost 95% obtained results were found in the ellipse of covariance defined by its matrix. They were results of great importance for the determination of correlation (weight 10). Only 20% results were found outside the regression area defined at 95%. No deviations were found from the linear distribution of correlations of both variables. The calculated correlation coefficient of both methods was high ($r > 0.91$).

Experimental values recorded using blotting tests and boundary values calculated on their basis classified 87% experimental results to the class of adequate cleanliness (Pass) and 13% results as the admissible warning class (Alert; Table 2). The maximum result classified to the appropriate cleanliness level was the value of 3.3 cfu/cm^2. The difference between this value and the lowest result in the admissible range was 1.1 cfu/cm^2. In turn, this difference was equivalent to only 6 RLU recorded by bioluminescence. Thus, based on the linear distribution, the RLU range was predicted in relation to the independent variable characterized by microbial count (cfu/cm^2). Obtained ranges of prediction values for the admissible level of cleanliness were consistent with experimental data.

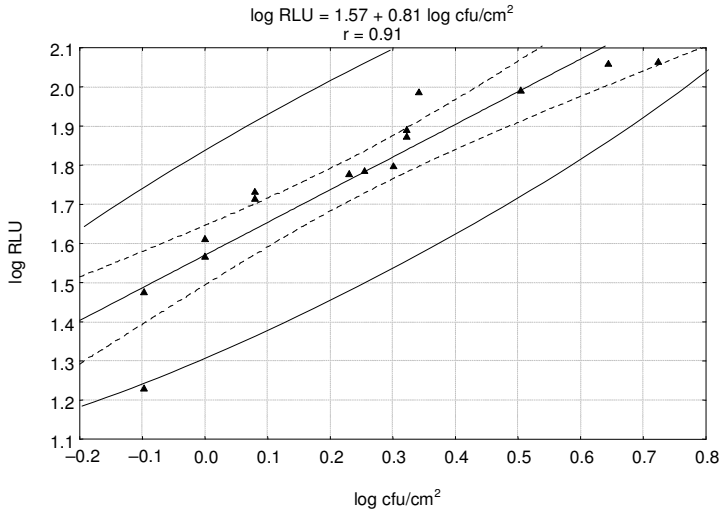

Fig. 1. Relative probability of normal distribution of results recorded using conventional microbiological testing and bioluminescence (weight = 10)

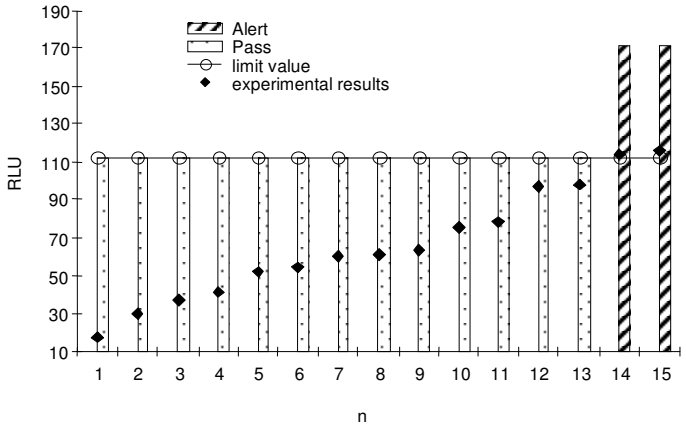

Fig. 2. Prediction and experimental ranges of object cleanliness based on bioluminescence results

A detailed analysis of measurement data recorded using bioluminescence and their prediction confirmed a lack of discrepancy between results obtained in the 15 successive cycles of the experiment (Fig. 2). Two of the 15 measurements taken with a luminometer were higher than the boundary value between the Pass and Alert ranges (112 RLU) and at the same lower than the boundary value between the Alert and Fail classes

(171 RLU). No result recorded in the experiment would indicate that the object surface was inadequately clean and thus requiring its repeated washing and disinfection.

DISCUSSION

Bautista et al. [1992], when assessing the hygienic status of surfaces using the conventional method and by bioluminescence, showed that in 74% analysed surfaces the results obtained by the traditional method and by bioluminescence were consistent. In 36% surfaces RLU results indicated that the surfaces were not sufficiently clean, although it was not confirmed using the conventional method. Prior to washing the authors on 20 surfaces measured 4 – 2191 RLU, while after washing they detected 2 – 285 RLU. In turn, Aycicek et al. [2006] found that 97.5% examined surfaces could be considered clean on the basis of results recorded by both the conventional method and bioluminescence. The other 2.5% 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 as clean by the authors on the basis of bacterial counts and which turned out to be 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 1435 to 90959 measured RLU. The suitability of bioluminescence in the assessment of cleanliness status was shown by Cooper et al. [2007] within the range of 83% to 100% prior to washing and 90% to 100% after surface washing. The authors decided that on average 84% surfaces they examined were clean based on RLU data, but only 66% based on the conventional microbial count method. Cho and Yoon [2007] used in their model studies the high dependence of microbial counts and results of RLU measurements to determine detection levels with a luminometer. A high correlation was also found for results of bioluminescence and the conventional method reported by Larson et al. [2003]. When examining 219 surfaces of 4 cm × 4 cm those authors 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 of the study was r = 0.82.

Table 2. Bioluminescence results corresponding to calculated and experimental values of microbiological testing determining cleanliness levels of the analysed object

Cleanliness levels for object		Results of microbiological method, cfu/cm^2	Bioluminescence results, RLU	
	cfu/cm^2	experimental values	experimental values	prediction
Pass	≤ 4.42	0.8-3.3	17-98	≤ 112
Alert	4.42 < x ≤ 7.42	4.4-5.3	114-116	112 < x ≤ 171
Fail	> 7.42	–	–	> 171

Pass – clean, Alert – conditionally clean, Fail – unacceptable.

CONCLUSION

A high correlation was determined in this experiment between results of blotting tests and those recorded by bioluminescence. However, the introduction of bioluminescence testing using a luminometer requires a pre-determination of cleanliness ranges based on results of conventional microbiological tests. This results from the necessity to calculate boundary values of dependent variables, RLU, on the basis of the independent variable, i.e. results of microbial count determinations on the analysed surface. Ranges of the hygienic status of the surface, defined as clean, conditionally clean and inadequately clean based on RLU, should be completely convergent with ranges determined as a result of prediction, as it was the case in the experiment. Analyses conducted using a luminometer showed that it is possible to monitor the presence of microorganisms as an indicator of surface cleanliness in a dairy processing plant.

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WYKORZYSTANIE BIOLUMINESCENCJI I MIKROBIOLOGICZNEJ METODY KONTAKTOWEJ W UTRZYMANIU ODPOWIEDNIEGO POZIOMU HIGIENY W ZAKŁADACH PRZEMYSŁU SPOŻYWCZEGO

Streszczenie. Ocenę skuteczności stosowania metody bioluminescencji w monitorowaniu higieny w zakładzie przetwórstwa mleka prowadzono w odniesieniu do wyników tradycyjnych metod mikrobiologicznych. Użyto testów kontaktowych Envirocheck Contact DC z podłożem Agar CASO z dodatkiem neutralizatorów. Obiektem była powierzchnia mieszadła belkowego w zbiorniku fermentacyjnym. Wymazy pobierano po procesie mycia i dezynfekcji zbiornika po zakończeniu 15 cykli produkcyjnych. Uzyskano wysoki stopień korelacji r = 0,91 przy wiarygodności porównania $\beta = 0{,}906$. Analiza prawdopodobieństwa rozkładu potwierdziła przydatność stosowania metody bioluminescencji. Ustalono wartości graniczne (112 i 171 RLU) metody bioluminescencji dla trzech zakresów czystości obiektu na podstawie liczby drobnoustrojów (cfu/cm^2). Powierzchni zaklasyfikowanych jako czystych warunkowo (Alert) było ponad 13%.

Słowa kluczowe: bioluminescencja, ATP, higiena

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