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An attempt at the specification of common cleanliness limits for abiotic surfaces in dairy processing plants based on ATP bioluminescence

Untersuchungen zur Festlegung allgemeiner Sauberkeitsgrenzwerte für abiotische Oberflächen in Molkereianlagen mittels ATP-Biolumineszenz

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Summary

The aim of this study was to specify a common cleanliness limit for abiotic surfaces in the processing plant environment based on ATP bioluminescence. Analyses were conducted on a total of 16 surfaces of 304L and 316L stainless steel and roughness indices Ra of 0.6 to 4.0 µm and IIa and IIIb finish. The machinery was located in five departments of three different milk processing plants. Counts of aerobic mesophilic bacteria were determined using the conventional microbiological method. The actual microbial loads of the surfaces ranged from 0.2 to 25.2 cfu/cm^2 and bioluminescence values ranged from 20 to 7800 RLU. Analyses were conducted only on clean surfaces (< 4 cfu/cm^2). Within one plant or even one department, common cleanliness ranges may not be determined for different devices on the basis of ATP bioluminescence. Admissible ATP bioluminescence per cm^2 was determined for the surfaces of a coagulation tank (2.5 RLU), a cheese curd press (3.2 RLU), a cheese vat (3.8 RLU), a moulding and pressing column (1.6 RLU) and a packing device (4.6 RLU) installed in different processing plants.

Keywords: bioluminescence, cleanliness, ATP, monitoring, dairy

Zusammenfassung

Das Ziel dieser Studie war die Bestimmung gemeinsamer Sauberkeits-Grenzwerte für abiotische Oberflächen mittels ATP-Biolumineszenz. Es wurden insgesamt 16 Oberflächen aus rostfreiem Stahl vom Typ 304L und 316L mit einer Rauheit von 0.6 bis 4.0 µm Ra und mit der Veredelung IIa und IIIb untersucht. Die beprobten Objekte befanden sich in fünf Abteilungen in drei Milchverarbeitungsbetrieben. Gleichzeitig wurden mit den Techniken der klassischen Mikrobiologie die aeroben, mesophilen Bakterien bestimmt. Die tatsächliche Oberflächenbelastung lag zwischen 0.2 und 25.2 KBE/cm^2, die Biolumineszenz betrug 20 bis 7800 RLU. Für die Auswertung wurden ausschließlich saubere Oberflächen (< 4 KBE/cm^2) genutzt. Innerhalb eines Betriebes oder einer Abteilung können keine allgemeingültigen Sauberkeits-Grenzwerte für verschiedene Geräte durch die ATP-Biolumineszenz festgelegt werden. Es wurden jedoch akzeptable ATP-Biolumineszenzen für Oberflächen von 1 cm^2 für Gerinnungsbehälter (2.5 RLU), Quarkbruchpressen (3.2 RLU), Käsekessel (3.8 RLU), Form- und Presssäulen (1.6 RLU) und Verpackungsmaschinen (4.6 RLU) bestimmt.

Schlüsselwörter: Biolumineszenz, Sauberkeit, ATP, Überwachung, Molkerei

Introduction

The quality of processed raw material and that of the resulting dairy products are closely dependent on microorganisms found in processing plants. Inadequate sanitary and hygienic conditions during the production process may cause potential contamination and spoilage of dairy products (Cais-Sokolińska and Pikul, 2008b; Cais-Sokolińska et al., 2010; Carrascosa et al., 2012). Any insufficient decontamination process on the equipment or negligent personal hygiene during food preparation may contribute to cross-contamination and transmission of foodborne pathogens and thus increase the risk of disease outbreaks (Aycicek et al., 2006; Nedeljković and Horvat, 2007). For this reason, it is essential to determine microbial loads on abiotic surfaces within the framework of hygienic and sanitary inspections of the plant both before and during the production process (Sharma and Anand, 2002a, 2002b; Tebbutt et al., 2007; Carrascosa et al., 2012).

Microorganisms in the production line environment are responsible for the formation of unique biological films, referred to as biofilms, on the surface of equipment and other technological facilities. The process of biofilm formation initiated during the deposition of single cells on abiotic surfaces progresses with varied kinetics (Julien et al., 2002). An interaction between adhesins of microbial cells and the surface of examined objects provides the biofilm with a stable structure. The roughness and the arrangement of acid resistant steel, the most frequently used material in machines and equipment in milk processing plants, determine the volume of retained liquid and thus the rate of microbial colonisation (Rosmaninho et al., 2007; Ok-Kyung et al., 2013). The greatest microbial colonisation rate was recorded for the surface made of 304L stainless steel in comparison to other working surfaces of 316L steel, Teflon or glass (Myszka et al., 2005). Such dependencies were also observed by Peng et al. (2001), when investigating the kinetics of biofilm formation on individual stainless steel surfaces. The rate and type of biofilm formation also depend on the availability of nutrients on abiotic surfaces (Cunliffe et al., 1999; Sanin et al., 2003; Myszka et al., 2005). Studies conducted by Jullien et al. (2002) showed that even minimal porosity of steel surfaces has a significant effect on phenomena occurring during cell adhesion. It was found that polishing of working surfaces does not reduce counts of pathogenic microorganisms in food products (Valcarce et al., 2002). For this reason, strict hygienic measures in processing plants are determinants of quality for the resulting products.

Monitoring the cleanliness of working surfaces in milk processing plants is a routine operation within the framework of internal and external audits. In view of the need to perform a large number of analyses and the time required to receive test results, new methods to control the hygienic status of production lines are being investigated. Such methods need to be rapid and easy to apply, but first of all they have to be repeatable and reliable (Jumaa, 2005; Knaflewska and Pośpiech, 2007). Among the tests based on the physico-chemical properties of microorganisms, measurement of bioluminescence is applied most commonly (Nedeljković and Horvat, 2007; Whitehead et al., 2008). The principle of bioluminescence measurement has been implemented systematically in food processing plants since the early 1990s. However, a significant limitation in the application of this method to the evaluation of the cleanliness of surfaces in food processing plants is connected with the requirement to perform simultaneous conventional microbiological smears for each examined object in a given processing plant. Only in such a case may individual cleanliness levels be determined for each of these objects. However, studies conducted to date have made it possible to observe comparable RLU values for objects made of austenitic chromium-nickel steel grades 304L and 316L, that are located in similar production departments, but in different milk processing plants (Cais-Sokolińska and Pikul 2008a, 2008b; Cais-Sokolińska et al., 2010). These observations have contributed to an extension of the scope of research for the determination of cleanliness limits for similar objects based on recorded RLU values. A good correlation has been reported between the results of traditional microbiological methods and ATP bioluminescence in dairies and dairy farms (Murphy et al., 1998; Vilar et al., 2008).

The aim of this study was to determine cleanliness limits for abiotic surfaces in the production line environment based on ATP bioluminescence measurements from actual microbial loads. Criteria were specified to be used as guidelines for the evaluation of cleanliness in similar objects located in different milk processing plants.

Materials and Methods

Analyses were conducted in three milk processing plants (denoted as A, B, C) that differed in their processing capacity. Surfaces of equipment in five different departments (denoted as I–V) were examined in each of the plants (Tab. 1). The basic machines, typically used in dairy technology, were selected. The tested equipment (object) surfaces were active working surfaces. These surfaces were in direct contact with the material. The equipment was distributed in five parts (I–V) of the dairy plant. This was a general type dairy plant with a broad range of products manufactured. It was not a unidirectional-specialized plant.

Surfaces were made of stainless steel grades 304L and 316L according to the American Iron and Steel Institute (AISI) with roughness indices Ra ranging from 0.6 to 4.0 µm. The steel had different finishes and was defined as hot rolled, cold rolled, annealed, pickled, mat, smooth and lustreless. The steel finish grades according to Deutsche Industrie Normen (DIN) were IIa and IIIb.

Microbiological contamination of machine surfaces was assessed before the start of production operations. Examined surfaces were visually clean, dry and with no residue of the processed product. Swabs were collected from

TABLE 1: *A list of tested surfaces of equipment in milk processing plants, n = 7.*

Object	Department	Plant	Sample denoted as
Milk tank	Milk processing room (I)	A, B, C	1, 2, 3
Coagulation tank	Curdling room (II)	A, C	4, 5
Cheese curd press	Curdling room (II)	B, C	6, 7
Cheese vat	Cheese room (III)	A, B	8, 9
Moulding and pressing column	Cheese room (III)	A, C	10, 11
Fermentation tank	Processed dairy products – fermented milk department (IV)	A, B, C	12, 13, 14
Packer	Packaging room (V)	B, C	15, 16

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visually clean and dry surfaces at least 2 h and not later than 4 h after the completion of washing and disinfection procedures. Tests were performed following guidelines presented by Carrascosa et al. (2012). Adjacent flat surfaces of an object were limited with templates in the form of a square frame with internal dimensions of 5 cm x 5 cm and smears were collected separately for each method. From the internal surface of tubes with Ø > 101.6 mm, smears were also collected using a template, reaching 10 cm deep. Swabs were moved five times parallel to one of the template sides and then perpendicularly, with swabs inclined at an angle of 45°. Results are given per cm^2.

Based on the counts of aerobic mesophilic microorganisms detected by the conventional microbiological method, the surfaces of analysed objects were divided in terms of their cleanliness into clean surfaces (< 4 cfu/cm^2) and inadequately clean surfaces (≥ 4cfu/cm^2). These ranges are consistent with the limits adopted in earlier studies by Cais-Sokolińska and Pikul (2008a, 2008b), Cais-Sokolińska et al. (2010), concerning the determination of microbiological contamination of surfaces in dairy facilities.

Analysis of microbiological contamination of surfaces using the smear method. Collected smears were transported to the incubation chamber within max. 4 h with no access to light at a temperature of max. 5 °C. Determination of the total count of aerobic mesophilic microorganisms was performed on submerged cultures of 2 cm^3 washings. Standard solvents and microbial growth media were used in the experiment (ISO 6610, 1992; ISO 6887, 2010). Each incubation was run in a WTB Binder heating chamber (Tuttlingen, Germany). Culture results are given per cm^2 surface. For this purpose, the number of microorganisms cultured from 2 cm^3 washings was multiplied by 10 and divided by 25 (or x 0.4).

The bioluminescence method. ATP bioluminescence was measured using a FireFly luminometer by Charm Sciences Inc. (Malden, USA). PocketSwab Plus swabs by Charm Science Inc. (Lawrence, USA) were used. The measurement procedure was consistent with the instructions of the device and swab manufacturers. Bioluminescence was recorded directly at the site of smear collection. The total test time including the reading did not exceed 45 s. The results are given in arbitrary relative luminescence units (RLU).

Statistical analysis. Based on the recorded results, Pearson's linear correlation coefficient was calculated, regression lines were plotted and the coefficient of determination as the basis for the assessment of the scale of their correlations was established. Scatter plots of all results were analysed in order to eliminate deviations that caused an increase in the sum square deviations from the regression line. Statistical calculations were performed using STATISTICA (data analysis software system), version 10 by StatSoft, Inc. (2011).

Results

The total microbial counts determined for 16 different surfaces located in production and warehouse lines in three different dairies ranged from 0.2 to 25.2 cfu/cm^2. On these surfaces, the numbers of relative luminescence units (RLU) recorded with a luminometer ranged from 20 to 7800 RLU.

In accordance with the assumptions for this experiment, only surfaces classified as clean, i.e. those on which the determined microbial count was below 4.0 cfu/cm^2, were selected for further analysis. On this basis, surfaces located in the milk processing room were rejected, because only 9.5 % of them met the above requirement. On clean surfaces, the highest bioluminescence value was 56 RLU/cm^2. When measured on 1 cm^2 dirty surfaces, bioluminescence ranged from 58 to 312 RLU.

A similar approach was adopted for the surface of fermentation tanks in the department of processed dairy products. In that department, only 19 % of surfaces were classified as clean. The mean microbial count on dirty surfaces of fermentation tanks was 4.6 cfu/cm^2, while recorded bioluminescence values ranged from 3.1 to 11.3 RLU/cm^2.

When analysing the surface of the coagulation tank in the cheese curdling room, it was found that on 92.8 % of examined surfaces, the recorded microbial count was on average 2.2 cfu/cm^2, which was lower than the required 4.0 cfu/cm^2. On the surface rejected from further analyses, on which the microbial count was 4.5 cfu/cm^2, the reading was 2.4 RLU per 1 cm^2. Thus, the next step was to eliminate measurements equal to or greater than 60 RLU/cm^2. The other results clearly indicated a lack of statistically significant differences between the mean numbers of microorganisms from the examined surfaces located in two different plants. In addition, no significant differences were found between the bioluminescence values measured on the surface of coagulation tanks (Tab. 2). As a result of the above analytical procedure, it was found that the maximum value of bioluminescence measured on the 25 cm^2 surface of coagulation tanks may not exceed 55 RLU for these surfaces to be considered clean at the microbial load of max. 3.0 cfu/cm^2. However, assuming a linear correlation and prediction for the threshold value of 4.0 cfu/cm^2, it was specified that a clean surface of coagulation tanks may show a maximum reading of 2.5 RLU per 1 cm^2 (Fig. 1). The accuracy of the established threshold limit is confirmed by the fact that it was lower than the lowest value of those rejected from the preliminary analysis.

Based on the measured values, we applied the above procedure and established the maximum value of bioluminescence per 25 cm^2 at the actual maximum microbial load that met the requirements of a clean surface in the cheese curd press. To be considered clean, the value of bioluminescence may not be equal to or greater than 81 RLU. The greatest difference between the maximum RLU per 25 cm^2 at the actual maximum microbial load (53 RLU) and the admissible predicted value (95 RLU) was shown when testing the same sized surface of the cheese vat. The admissible predicted RLU per 25 cm^2 surface of the moulding and pressing column was 48 % greater than the maximum RLU measured at the actual maximum microbial load (27 RLU/cm^2). Analysis of the correlations between bioluminescence and the recorded microbial count on the adjacent surfaces of packers indicated a high coefficient of determination ß = 0.62. The value of correlations for the tested variables r = 0.79 is high according to the scale proposed by Guilford (Gorsuch and Lehmann, 2010). The admissible predicted ATP value per 25 cm^2 surface of packers may not be equal to or greater than 114 RLU.

Discussion

In literature on the subject, we may find various criteria for the assessment of cleanliness of surfaces in food processing plants measured with a luminometer. Carrascosa et al. (2012) adopted the value ≤ 150 RLU for surfaces to be considered clean (suitable) and > 150 RLU as contaminated (not suitable). Other authors (Murphy et al., 1998) considered it suitable to establish three categories, reducing the cri-

TABLE 2: *Actual and predicted values of bioluminescence for surfaces considered clean in technological facilities of different dairies.*

Object (department, nr)*		Total microbial count (cfu/cm²)				Bioluminescence (RLU/cm²)			
		$\bar{x}$	SD	X_{min}	X_{max}	$\bar{x}$	SD	X_{min}	X_{max}
Coagulation tank	4	2.4	0.49	2.0	3.0	1.7	0.39	1.1	2.0
(II)	5	2.0	0.27	1.8	2.3	1.8	0.28	1.5	2.2
						maximum RLU value per 25 cm² at actual maximum microbial load			55
						Admissible forecasted RLU value per 25 cm²			63
Cheese curd press	6	1.9	0.32	1.4	2.0	2.6	0.56	1.8	3.1
(II)	7	1.9	0.97	1.3	3.1	2.5	0.59	1.7	2.9
						maximum RLU value per 25 cm² at actual maximum microbial load			78
						Admissible forecasted RLU value per 25 cm²			81
Cheese vat	8	0.7	0.34	0.3	1.2	1.0	0.23	0.8	1.5
(III)	9	1.3	0.17	1.1	1.5	1.6	0.38	1.2	2.1
						maximum RLU value per 25 cm² at actual maximum microbial load			53
						Admissible forecasted RLU value per 25 cm²			95
Moulding and pressing	10	0.4	0.17	0.2	0.7	0.7	0.15	0.4	0.8
column (III)	11	0.8	0.34	0.2	1.2	0.8	0.16	0.5	1.1
						maximum RLU value per 25 cm² at actual maximum microbial load			27
						Admissible forecasted RLU value per 25 cm²			40
Packer	15	2.8	0.53	2.4	3.4	4.4	0.17	4.1	4.5
(V)	16	2.7	0.46	2.2	3.0	4.4	0.15	4.1	4.4
						maximum RLU value per 25 cm² at actual maximum microbial load			113
						Admissible forecasted RLU value per 25 cm²			114

*denotation of tested surface as in table 1

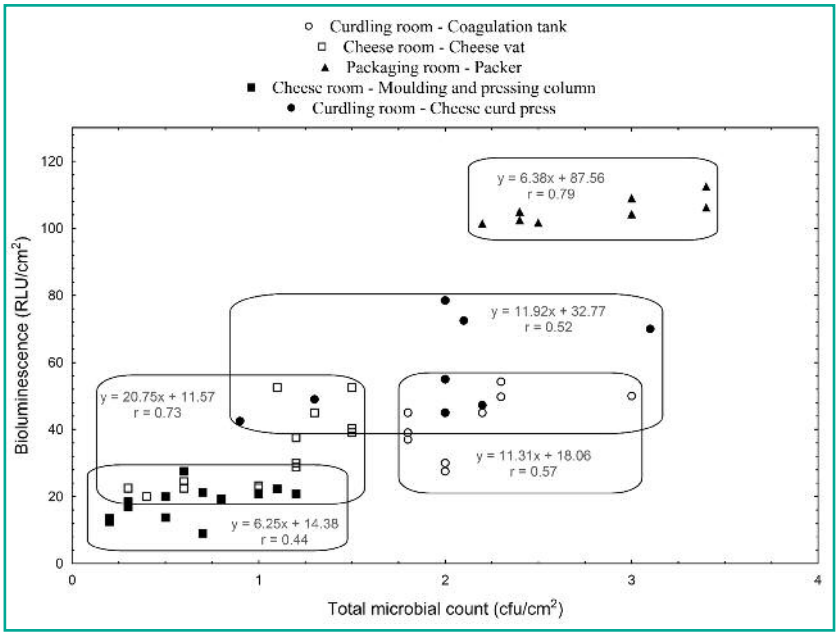

FIGURE 1:

teria to 100 RLU for clean surfaces, between 100 and 150 RLU for surfaces with deficient cleanliness, and a value above 150 RLU for dirty or very deficient surfaces. A study by Cais-Sokolińska et al. (2010) concerning the cleanliness of milk tanks located in different plants showed that such identical evaluation parameters may not be applied. That experiment was conducted in three plants processing goat milk. The surface of bulk tanks for milk after the pasteurisation process was examined. The tested surfaces varied in terms of their structure and finish. Two of the examined objects were manufactured from chromium-nickel austenitic steel grade 304L with low contents of 18-8 carbon. The other objects were made from high-alloy austenitic steel grade 316L. This is stainless chromium-nickel steel, heat resistant and extremely corrosion-resistant. Different results were obtained from bioluminescence measurements within each of the three ranges of hygienic cleanliness from 40 to 9650 RLU/100 cm². The higher the roughness of a tank surface, the significantly lower was the share of samples considered clean at ΔRLU = 35 %. For each of the three facilities, the maximum value of bioluminescence for the surface considered clean was established (≤ 5–0.44 x Sdin cfu/cm²). These values were 1.8, 2.1 and 6.3 RLU/cm². This shows that clean surfaces in one plant may be classified as dirty in another plant and vice versa. Cais-Sokolińska and Pikul (2008a) examined paddle mixers of surface roughness Ra = 0.6 µm and a flap valve of surface roughness Ra = 0.8 µm. These authors measured 45 RLU/100 cm² on the clean surface of paddle mixers, and up to 910 RLU/100 cm² on the flap valve. According to Aycicek et al. (2006), based on the results provided by the conventional method and bioluminescence, on average 97.5 % of tested surfaces may be considered clean. The other 2.5 % of tested objects turned out to be clean on the basis of ATP bioluminescence, although this was not indicated by the bacterial count assessed by the conventional method. These authors classified 74.6 % of objects as clean on the basis of bacterial count, and which also turned out to be dirty based on RLU results. In addition, 14 different surfaces, e. g. steel and plastic, showed a wide spectrum from 1435 to 90959 RLU. In turn, Cais and Pikul (2008b) established the relative probability of the normal distribution for the dependence of results provided by the conventional microbiological method and by bioluminescence (log RLU = 1.57 + 0.81 log cfu/cm²). A high degree of correlation r = 0.91 was obtained. This was found on the basis of analyses of the paddle mixer inside a fermentation tank in a dairy. The object selected for analysis was made

from high-alloy austenitic steel grade 316L. This is stainless chromium-nickel steel, cold rolled, annealed, pickled, smooth and lustreless. The experimental ranges of cleanliness for this object ranged from 0.8 to 3.3 cfu/cm^2 at prediction ≤ 4.42 cfu/cm^2 as well as values of bioluminescence from 17 to 98 RLU/cm^2 at prediction ≤ 112 RLU/cm^2.

Conclusion

Based on the measurements and assays, a similarity was observed between microbial load and ATP bioluminescence for selected surfaces in milk processing plants. This pertains to surfaces of technological line facilities made of stainless steel grades 304L and 316L and roughness Ra from 0.6 to 4.0 µm.

However, within one plant or even one department, common limits of cleanliness may not be established based on ATP bioluminescence. Each of the machines in the same milk processing plant in the milk processing room, the curdling room, the cheese room, the department of fermented drinks and the packaging room, was characterised by a different ATP bioluminescence. This was found even though the examined surfaces were clean and the actual microbial load was below 4 cfu/cm^2.

In contrast, the range for clean surfaces may be established for the same machines located in different plants. Examples include the coagulation tank, the cheese curd press, the cheese vat, the moulding and pressing column and the packer. For these machines, the admissible predicted RLU value per cm^2 was specified for a surface that may be classified as clean.

Conflict of interest

All the authors declare that there is no conflict of interests regarding the publication of this article.

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