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Rapid checks on sanitized surfaces ensure microbial-free food processing in industry

Pasquale CAPUTO[a], Elida FERRI[a], Gianluca GUARNIERI[b], Graziella LASI[c], Simone GOZZI[d], Fabio Milesi[e] and Stefano GIROTTI[a]

[a]*Department of Metallurgic Science, Electrochemistry and Chemical Techniques, University of Bologna, Via S. Donato 15, 40127 Bologna, Italy*
[b]*Labo-Scientifica S.r.l., Via L. Corsari 23/A, 43100 Parma, Italy*
[c]*Granarolo S.p.A., Via Cariano 27/2, 40127 Bologna, Italy*
[d]*Camst S.c.r.l., Via Tosarelli 318, 40050 Villanova di Castenaso (BO), Italy*
[e]*JohnsonDiversey S.p.A., Via Milano 150, 20093 Cologno Monzese (MI), Italy*

Abstract. An effective monitoring of microbial contamination on surfaces and plant components is of fundamental importance in food industry, refectories and work canteens to ensure products quality and safety. The classic microbiological tests inform about the sanitization procedures effectiveness at least after 1-2 days, the rapid luminescent ATP assay after about 5 minutes, on site. In this study the presence of microorganisms at High Quality production line and worker's canteen of a dairy industry has been investigated. Plates count and the ATP assay have been performed in parallel. The data obtained from the two methods resulted in good agreement, indicating the rapid ATP test as a simple but reliable tool to verify immediately the effectiveness of the sanitization treatment, directly by the personnel involved in the cleaning activities. The final results showed that a good sanitation treatment allows the maintenance of satisfying hygiene conditions all weekend long.

Keywords: Microclimatic parameters, microbial contamination, ATP luminescent assay, food industry.

1. Introduction

The monitoring of microbial contamination of surfaces and/or of the air is of fundamental importance in all situation in which not optimal sanitary conditions can lead to the spread of infectious diseases or to the contamination of food, drug or medical products. These controls and the necessary cleaning procedures are usually performed at refectories and worker's canteens, food and drug industries, analysis and quality control laboratories, research laboratories, lecture and reading halls, etc. [1].

The hygienic safety of the products from industries and workshops operating in the food sector, according to recent regulatory rules [2-4], must be ensured through the identification and control of the critical phases of production processes. The contamination of the foodstuffs by micro organisms proliferated on not correctly sanitized plants and surfaces is one of the most important of such critical phases.

The contemporary presence of a residual bacterial population and of food debris, which can represent a growth medium, is a situation to be avoided at any point of a processing plant. For this reason, it is essential to have analytical methods allowing a timely identification of all unwanted biological contaminations by monitoring the effectiveness of the cleaning procedures usually applied, and then the application of all appropriate actions to continue a safe production process.

The correct application of HACCP directions [5-6] requires the preparation of a written document (the Standard Operating Procedure, SOP) reporting all the procedures for disinfection/sanitation of the environment, to avoid misunderstandings and to

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ensure uniform interpretation by all actors involved in this activity.

Starting from all the above considerations, the dairy industry Granarolo SpA (Bologna, Italy) and the ready-meals supplier Camst (Bologna, Italy) planned a hygiene monitoring program to evaluate the contamination of working surfaces and equipment by using a quick based on bioluminescence. This analysis is based on the determination of ATP extracted from cells and revealed by using luciferin-luciferase [1, 7-8]. It requires few minutes to give a reliable assessment of biological contamination, comparable to the microbiological plate count test. Moreover, the luminescent assays offer a significant costs reduction. In this study the high quality production lines for soft cheeses at Granarolo plant and its worker's canteen, managed by Camst, have been tested before and after the sanitization procedures, by the classic microbiological methods and the rapid bioluminescent assay, in order to compare the results.

2. Experimental

2.1. Monitoring of high quality production lines and worker's canteen.

The preliminary steps of the experimental program included the choice of the: (i) monitoring points: surfaces or parts of equipment which shape, location or particular uses indicate a higher risk of contamination or problems in accurate sanitization, (ii) frequency of sampling and (iii) number of samples, determined on the basis of the size and characteristics of the selected area.

Various points, along the High Quality production line, and covering the whole production process, have been selected and monitored (figure 2): the trolleys transporting the curdled milk to the production line, the Archimedes' screw (A. screw) and the rollers for the cutting and transport of the mozzarella cheeses, the chute to the packaging area, the pre-sterilized containers for chesses packaging. The sampling has been performed on Friday at 17:00, after the sanitization procedures, and on Monday, at 4:00, before the production starting, from March to May.

At the worker's canteen three benches for food preparation, the trays and the self-service counter have been tested (Figure 3). The sampling has been performed on Friday (at 17:00, after the sanitization) and on Monday (at 7:00, before the start of cooking activities). In both cases, the sites have been sanitized by cleaning products supplied by JohnsonDiversey Company, following standard procedures. When no anomalous situations were detected, all the data collected during 5 weeks were reported as the average value.

2.2. Assessment of the microbial contamination on surfaces

Microbiology: RODAC plates (Liofilchem S.r.l., Via Scozia, Zona Industriale 64026 Roseto degli Abruzzi (Te), 6 cm diameter, containing non selective agar medium or a chloramphenicol-added medium have been put into contact, for 1 min, with the surfaces. The first kind of plates have been incubated at 37°C for 24-48 h, to allow the growth of bacteria; the second kind, with chloramphenicol, for 48-72 h, to evaluate the presence of mould and yeast colonies. The microbiological counts included the bacterial colonies developed on the non-selective medium, as well as the mould and yeast colonies growth on chloramphenicol-containing medium.

Bioluminescence: each sample has been collected from an area of 10x10 cm, by using the UltraSnap swabs (Hygiena International, Watford, UK). Then, the swab was introduced in a tube which cap contained all reagents for microbial ATP determination, i.e. extracting solution and luciferin-luciferase detection system. Once the cap was opened and the reagents had come into contact with the sample, the tube was shaken vigorously and introduced into the portable luminometer "Sistem SURE II" (Hygiena International, Watford, UK), to measure the emitted light, directly proportional to the ATP content in the sample. The luminescent assay measured the amount of ATP extracted from all cells, bacterial or not, still vital or not, present in the tested area.

The threshold values for the bacterial counts (CFU) and relative light output (RLU) to be respected in the production and packaging of food, should not exceed the values of 30 CFU and 50

RLU, respectively. Results below these values indicate surfaces with no risk of bacterial contamination; values in excess of 15 units (in CFU or RLU) can define a risk of contamination because bacterial proliferation after a certain time, while values greater than 60 CFU and 100 RLU identify not sanitized areas, with high risk of bacterial contamination and bacterial growth.

The possible interferences produced by the cleaning products on the bioluminescent emission have been determined at different concentrations, the commonly employed 1:100 dilution and the more concentrated 1:1 dilution.

The main steps of the two procedures, starting from sampling till the determination of the results, are represented in Fig. 1; above the traditional microbiological method and the bioluminescent fast assay, under.

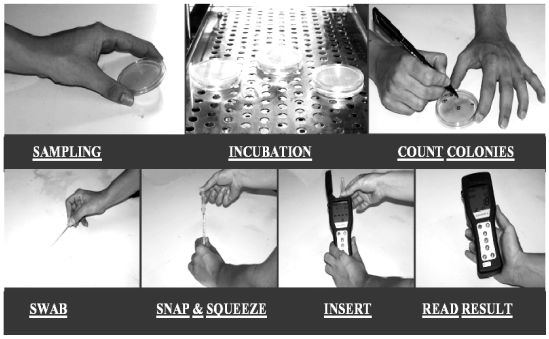

Fig. 1: Main steps of the two procedures.

3. Results and discussions

The preliminary tests investigating the influence of the cleaning products showed that no effect on the bioluminescent reactions has produced even at the higher concentrations.

Most part of data obtained during this study was under the threshold values reported earlier. It must be remembered that most of the points taken into consideration at the High Quality production line were washed automatically; the only exceptions were the rollers and the chute, washed manually.

On the contrary, all cleaning and sanitizing procedures at the worker's canteen were made manually. As it is possible to observe in Table 1, no one sampling area showed CFU or RLU values above the respective thresholds. Concerning the packaging containers, reaching the dairy industry already sterilized, the data from the Friday and Monday sampling were obtained, obviously, from different containers.

The only one situation displaying not satisfying hygienic conditions was found at the worker's canteen, the bench C, on which was presents a large Teflon chopping-board and the bench was in close contact with the only one sink of the kitchen. Both methods gave results above the threshold values (see Table 2), indicating not good hygienic conditions, and the sources of the contamination were identified. First, the high values of bioluminescent ATP test, compared to only slightly high CFU counts indicated the presence, after cleaning, of food residues (non bacterial ATP), probably retained by the not uniform surface of the chopping-board and representing the optimal substrate for bacterial proliferation.

Moreover, the short distance from the sink produced continuously a re-contamination of the bench. A more careful treatment of the chopping-board surface and the separation of the bench from the sink by a partition wall solved the problem. Measurements performed after the application of these changes gave CFU and RLU values under the threshold values.

From Tables 1 and 2 is possible also to note that during the week-end the increase in both values was minimal, and any case they remained under the thresholds indicating a permanent effect of the sanitization treatments.

The microbiological and luminescent average values of all sampling activities are showed in Figure 4 and 5.

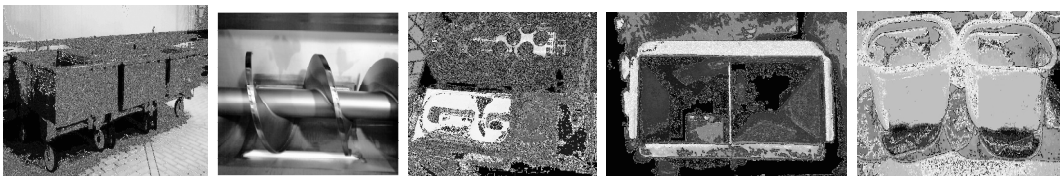

Fig. 2: sampling points of the High Quality production line of mozzarella, at the Granarolo dairy industry.

Fig. 3: Sampling points at the worker's canteen of Granarolo dairy industry.

Table 1: Mean number of colonies (CFU) on agar plates, and mean light emission (RLU) obtained for ATP measurements..

Place	CFU		RLU	
	Friday	Monday	Friday	Monday
Trolley	13	13	15	29
A. screw	0	0	0	3
Roller	4	7	8	9
Chute	1	7	3	10
Packaging	11	3	18	6

Table 2: Mean number of colonies (CFU) on agar plates, and mean light emission (RLU) obtained for ATP measurements.

Place	CFU		RLU	
	Friday	Monday	Friday	Monday
Bench A	25	28	40	48
Bench B	5	13	34	37
Bench C	33	46	77	88
Tray	6	11	8	13
Self-service	2	7	4	7

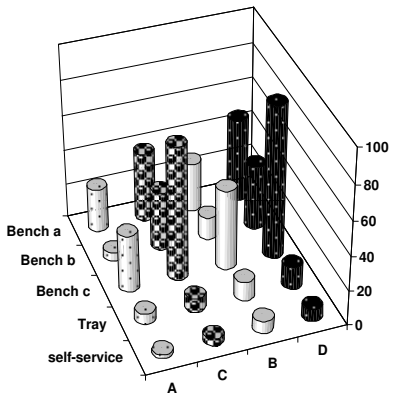

Fig. 4: CFU and RLU average values in the various sampling point at the worker's canteen of Granarolo industry. (A: CFU, Friday. B: CFU, Monday; C: RLU, Friday. D: RLU, Monday)

The values of the Coefficient of Variation were all below the 20%, with two exceptions: the trolley at the beginning of the production line (the sampling was performed each time on different trolleys); the trays, chosen randomly by the operator at each sampling session.

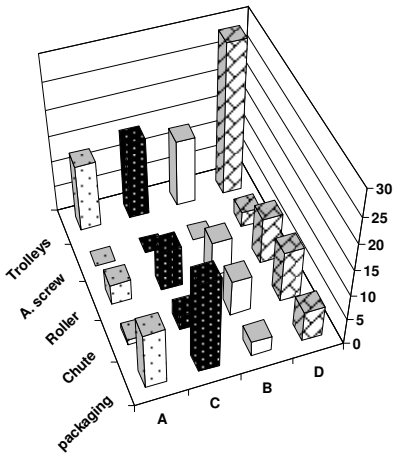

Fig. 5: CFU and RLU average values at the High Quality production line at Granarolo dairy industry. (A- CFU Friday; B- CFU Monday; C- RLU Friday; D- RLU Monday)

4. Conclusions

The data collected during this study confirm the very good reliability of the rapid luminescent ATP assay in the estimation of the contamination by organic residues (somatic cells) and/or micro organisms, both definitely to be absent in food processing areas, representing a short-time response and a valid alternative to the microbiological plate methods.

This easy, quick and cheap evaluation of the overall hygiene conditions of the surfaces allows checking immediately the effectiveness of the sanitization treatments, offering the possibility of a timely correction of any non optimal conditions revealed by the assay, and then avoiding delays in the production process or the not necessary introduction of contamination risks. Moreover, to recognize immediately the uncorrected cleaning procedures helps to improve, by appropriate courses, the training of the entrusted personnel.

5. Acknowledgement

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6. References

*E-mail address: stefano.girotti@unibo.it

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