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ROLE AND COMPARISON OF ATP TEST WITH CLASICAL MICROBIOLOGICAL METHODS IN HYGIENE DESIGN

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Abstract

The important aspects of microbial control of the hygiene are monitoring process of areas that come into close contact with food and other surfaces in food plants. In the standard method of taking swabs from equipment, there is no immediate corrective action. The results are obtained after 24-48 hours and if the sanitation is not adequately performed, it could lead to contamination of products. Therefore, the application of bioluminescence to determine ATP, as central metabolites of microorganisms and also indicator of organic impurities, present unavoidable rapid method for the level of hygiene. This paper describes the comparison of these methods with standard microbiological method in one of the leading dairy in Serbia.

The effect of sanitation were performed on the pasteurization line, the number of fillers and on microfiltration line by using luminometer LIGHTNING MVP (Biocontrol, USA) which detects contamination by measuring the amount of ATP. To compare effectiveness we also used swabs taken from these places after the sanitation and determined the total microbial count and total coli form bacteria. The tests were performed at over 100 locations in order to get a more relevant picture of the effectiveness of sanitation measures.

Key words: *Food safety, microbial contamination, sanitation, ATP, bioluminescence, luminometer*.

1. Introduction

Sanitation is the applied science for obtaining hygienic conditions in production, handling, distribution and consumption of food. Sanitation involves the principles of distribution equipments and facilities, developing, implementing, maintaining, and improving hygiene practices and hygienic conditions with food processor. Proper implementation of sanitation reduces the incidence of diseases transmitted by food, resulting in improvement of product quality and extends shelf life and is a prerequisite for the application of HACCP. It is clear that the determination of the exact hygienic state of the process after application of sanitation measures contributes greatly to obtain the relevant images on the efficacy of sanitation (Notermans and Powell [1]).

Plant sanitation is an essential part of good manufacturing practices in order to get healthy and good products. Respect of good hygiene practices in food industry is required for taking all measures to ensure that the amount of nonconforming product has a minimal level and prevent damage to customer health. One of the important aspects of microbial control of the hygiene is monitoring process of areas that come into close contact with food and other surfaces in food plants. In the standard method of taking swabs from equipment, there is no immediate corrective action. The results are obtained after 24-48 hours and if the sanitation is not adequately performed, it could lead to contamination of products. Therefore, the application of bioluminescence to determine ATP, as central metabolites of microorganisms and also indicator of organic impurities, present unavoidable rapid method for the level of hygiene (Griffith [2], Bell *et al*., [3] and Leon and Albrecht [4]). The aims of this paper were to give comparison of microbiological and non-micro-biological methods for monitoring cleaning efficacy and suggest ways to manage an integrated programme of monitoring, in an attempt to ensure adequate and cost-effective cleaning or the level of surface hygiene in one of the leading dairy in Serbia.

The effect of sanitation were performed on the pasteurization line, the number of fillers and

on microfiltration line by using new generation luminometer LIGHTNING MVP (Biocontrol, USA) which detects contamination by measuring the amount of ATP. To compare effectiveness of cleaning we also used swabs taken from these places after the sanitation and determined the total microbial count and total *E.coli* bacteria.

2. Materials and methods

Monitoring of production lines

The tests were performed at over 27 locations in order to get a more relevant picture of the effectiveness of sanitation measures. The preliminary steps of the experimental program included the choice of the monitoring points in one of the biggest dairy plant in Serbia. Various swab points, along production line and covering almost the whole production process, have been selected. Surfaces or parts of equipment, total of 27 points, indicate a higher risk of contamination or problems in accurate sanitization were chosen. Frequency of sampling for comparison were chosen for 3 weeks from March to April in years 2011, 2010 and 2009 and number of samples were 122 each year. Various points were at the prefillers, different type of fillers, UHT fillers, Sidel Pet fillers, the pre-sterilized containers, line for microfiltration and others. The sampling has been performed after the sanitization procedures, and before the production starting. Also monitoring of rinse water was compared in 3 weeks period in March in years 2011, 2010, 2009.

Assessment of the microbial contamination on surfaces

The microbiological counts included determination of the total bacterial count developed on Merck plate count agar medium (CFU/mL or cm^2) and *Escherichia coli* bacteria at coliform Merck agar (CFU/mL or cm^2).The first kind of plates have been incubated at 30 °C for 48 h, and presence of fecal coliforms were confirmed in EC medium (*E. coli*, Merck) at 44.5 °C for 24-48 h.

Bioluminescence: each sample has been collected from an area of 10x10 cm, by using the swab devices from LIGHTNING MVP, The BioControl Systems Inc, USA. Then, the swab was introduced in a tube which cap contained all reagents for ATP determination, i.e. extracting solution and luciferinluciferase detection system. Once the cap was opened and the reagents had come into contact with the sample and introduced into the portable luminometer The luminescent assay measured the amount of ATP. ATP (adenosine triphosphate) is found in all cells, from food residue to microbial contamination and monitoring its levels on surfaces and in liquids is an ideal indicator of overall hygiene present in the tested area. The Lightning MVP utilises a photomultiplier tube which amplifies the signal and is claimed to provide more sensitive readings than those instruments which implement a photodiode. Also, since temperature will affect the strength of the ATP reaction, the Lightning MVP will compensate for this (BioControl [5]). MVP Surface Sampling Device for surface samples and MVP Liquid Sampling Device for rinse water samples were used.

The luminometer was set to default to Warn >2.5. Fail > 3.0 for all test points. Clean, caution and dirty results are based on the following scale (Figure 1):

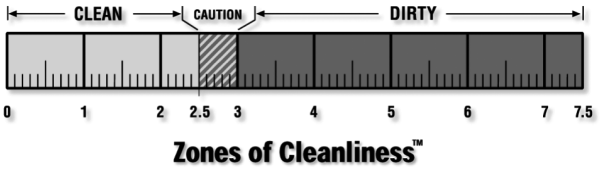

Figure 1. Zone of Cleanliness for LIGHTNING MVP

According to the default setting, any result zone 2.5 or below is considered clean, the range from zone 2.6 to zone 3.0 is the caution area, and any result above zone 3.0 is considered dirty not sanitized areas, with high risk of bacterial contamination and possible bacterial growth.

Statistical analysis

The data are presented as the means of four samples. Analysis of variance of the completely randomized design was calculated using the STATGRAPHICS PLUS 6.0 software. All means were compared to control values by the Turkey test, with the level of significance set at 5%.

3. Results and Discussion

The tests were performed at over 27 locations with 122 samples for each 3 years (total of 366 samples) in order to get a more relevant picture of the effectiveness of sanitation measures. Most part of data obtained during this study was under the threshold values below zone 2.5. It must be remembered that most of the points taken into consideration from production line were washed automatically. The main steps of the two procedures, starting from sampling till the determination of the results, are represented in Figure 2.

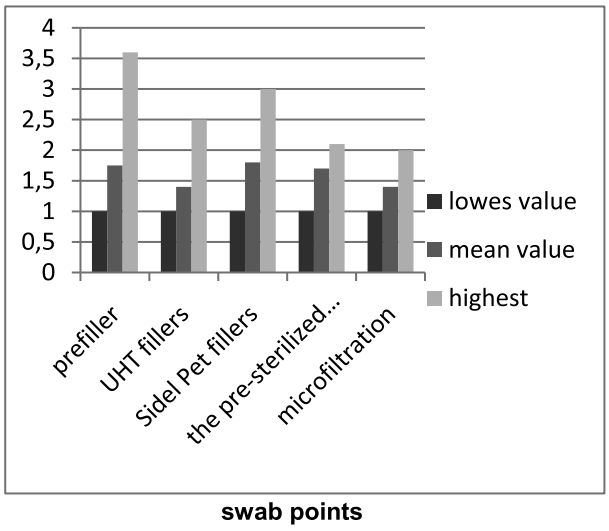

Figure 2. Value of zones of cleanliness at different swab spots for 2009-2011 using Surface Sampling Device

The highest values of zones of cleanliness were detected at prefiller with value of 3.6 and for Sidel Pet fillers with value 3.0. From the total count of swabs using by LIGHTNING MVP, 11.11% were at result above zone 2.5 (dirty) and 88.89% were below 2.5 (clean). So we compare these higher value zones of cleanliness with total number of bacterial colonies and *Escherichia coli* bacteria (Table 1).

Table 1. Correlation of higher value zones of cleanliness (CVZ) obtained for ATP measurements with mean number of total colonies of bacteria (CFU) and *E. coli* on agar plates

Place	CVZ	Total No of bacteria (CFU/cm²)	*E. coli* (CFU/cm²)
Prefiller	3.6	18	0
	2.5	5	0
	2.6	7	0
Sidel pet filler	3.0	15	0
	2.8	10	0
	2.6	8	0

It can be noticed that no E. *coli* were detected by classical microbiological methods and that for higher value zones of cleanliness (CVZ higher than 2.5) some value for total number of bacteria were detected. Both methods, ATP and classical microbiological, gave results above the threshold values, indicating not good hygienic conditions at that swab point and the sources of the contamination were identified. The high values of bioluminescent ATP test (CVZ), compared to only slightly high CFU counts indicated the presence of bacterial ATP. After measurements performed after the application of careful treatment (cleaning) gave CFU and CVZ values under the threshold values.

When CVZ values, using MVP Liquid Sampling Device for rinse water, were analyzed in samples for 4 containers for milk for 3 years, it was noticed that 3 containers occasionally had higher CVZ value (Figure 3).

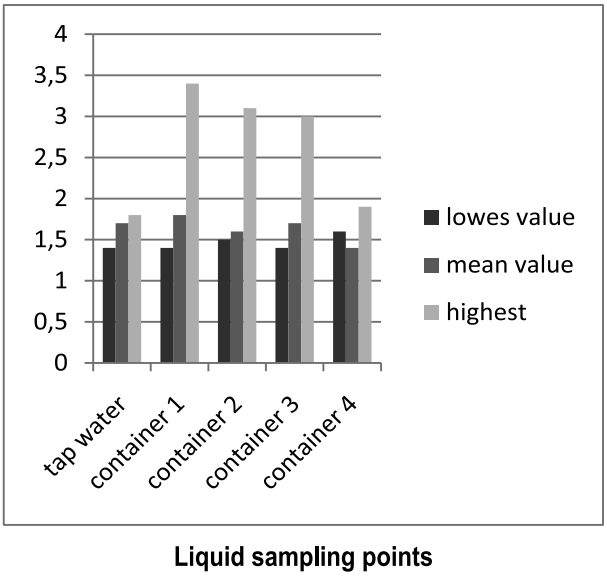

Figure 3. Value of zones of cleanliness for rinse water compared in 3 weeks period in years 2011, 2010, 2009 using MVP Liquid Sampling Device

The high values of bioluminescent ATP test (CVZ), compared to only slightly high CFU counts confirmed again the presence of bacterial ATP (Table 2)

Table 2. Correlation of higher value zones of cleanliness (CVZ) for rinse water obtained for ATP measurements with mean number of total colonies of bacteria (CFU) and *E. coli* on agar plates

Place	CVZ	Total No of bacteria (CFU/mL)	*E.coli* (CFU/mL)
Tap water	1.8	0	0
Container 1	3.4	60	0
Container 2	3.1	20	0
Container 3	3.0	8	0
Container 4	1.8	0	0

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4. Conclusions

- The data collected during this study confirm the very good reliability of the rapid luminescent ATP assay performed by LIGHTNING MVP in the estimation of the contamination with microorganisms, representing a short-time response and a valid alternative to the microbiological plate methods. This was also proved by Caputo et al. [6] but by using different bioluminescent instrument - UltraSnap swabs. However, it should be realized that in food environments there is often little value in trying to directly correlate surface counts to ATP readings. On the other hand Sala et al. [7] found the low correlation with general contamination level of the samples from the milk plant surfaces.
- Portable ATP bioluminescent-based tests are very common in many food processing plants, and the test is now a widely recognized method for rapid hygiene testing. However, not all ATP systems are the same, and some may provide false and variable results. However there was a difference in the repeatability, accuracy and sensitivity between systems. Potential sources of variability include the luminometer, temperature, sanitizer residues, storage condition of the swabs and operator technique. The BioControl Systems Lightning MVP is designed to overcome these problems through both the instrument and the swabs and was shown to give a good response to contaminants.
- The method and application are not intended to be a precise quantitative determination for ATP, and the test is not intended to be a direct replacement for the traditional culture microbiological test. It can be combined with microbiological methods to determine the effectiveness of surface cleaning and disinfection. It is intended as a post-cleaning verification test for the removal of product residues and is different to those of microbial enumeration methods and gives additional information that the microbial test cannot provide

5. References

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