

**EXPLOITATION OF BIOLUMINESCENCE METHOD FOR EVALUATION
OF ENVIRONMENTAL HYGIENE IN SELECTED DAIRY PROCESSING PLANT**

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Abstract:

The time demanding conventional microbiological methods do not fulfil requirements of food hygiene practice that stress rapid obtaining of results when evaluating effectiveness of sanitation. The bioluminescence methods based on determination of ATP are new modern methods advantageous with regard to their rapidity, simplicity, low time, easy manipulation. The results obtained in a dairy plant showed that disinfection was effective in all sections with the exception of swabs taken from cottage cheese production. In this section we recorded on the Floor I and on a Table increased total counts of bacteria and of coliform bacteria. Contamination of this part of premises was supported by the ATP results. The mentioned counts changed after some time up to the beginning of production and corrective measures were taken on the basis of ATP swabs. Thus the practical use of bioluminescence method in food hygiene is justified as it allows to take corrective measures.

Key words: Sanitation, ATP method, hygiene, milk products

1. INTRODUCTION

Sanitation regimen is one of the most important components of the HACCP control system. Cleaning and disinfection as a part of everyday production practice are inevitable for correct food production and complying with legislative regulations. Inspection of hygiene and quality of cleaning and disinfection is a decisive factor of food safety and part of the basic requirements of the HACCP system in food operations (Vojtaššák 2003). Microbial adhesion and biofilm formation is a major risk factor for the food industry, because quite often it is recorded to occur on surfaces in contact with food (Jay, Loessner & Golden 2005). Microbial adhesion is due to the capture and attachment of microorganisms on the surface, then triggering growth process. Cell reproduction gives rise to colonies and establishment of biofilms which cell meat is thick enough to gather nutrients, residual levels and other microorganisms (Tsuneda et al. 2005). Use of sanitary means of enzyme-based as biocleaners, also known as "green chemicals" can serve as an appropriate solution to overcome the problem of biofilms in the food industry (Meyer, 2003).

The time demanding conventional microbiological methods do not fulfil requirements of food hygiene practice that stress rapid obtaining of results when evaluating effectiveness of sanitation, overall level of hygiene and quality of the final product (Vojtaššák 2003). Optimal food production is based on combination of permanent monitoring of hygiene level and obtaining results of laboratory examination early enough so in case of adverse results one can take necessary corrective measures that prevent impairment of the product and contamination of productive premises and, subsequently, also extensive

economical losses, danger to consumer's health and development of alimentary diseases (Lopašovský, Pavličová & Šiška 2006).

Currently we observe increased use of bioluminescence methods (Suková 2002). The bioluminescence methods based on determination of ATP are new modern methods advantageous with regard to their rapidity, simplicity, low time and personal demands, easy manipulation, detection of the presence of impurities and, most of all, possibility to take corrective measures.

Increased bacterial counts or presence of undesirable microflora are usually the final result of inadequate cleaning and disinfection. The primary problem involves insufficient elimination of organic residues from technological lines or working environment in food production premises. The residual organics are a suitable substrate for multiplication of micro-organisms resulting in undesirable contamination.

Finally, it is possible that ATP bioluminescence monitoring should not be used as a predictor of microbial contamination as some have claimed. Poulis et al. (1993) noted that there was a poor relationship between ATP readings and conventional microbiological culture methods in samples from food processing facilities. Colquhoun et al. (1998) compared three different ATP hygiene monitoring systems and found that they differed considerably in sensitivity and reproducibility.

Contamination of foodstuffs from the atmosphere is important to the health and economic. Microorganisms are usually found in the air trapped in dust particles or water droplets. Droplets and dust are also transmitted sporing microbes on walls, ceiling, floor, food and additives. Of these may then be out of the atmosphere. And it is for these reasons, evaluation of environmental hygiene in the production of dairy products is justified (Griger & Vařejka 1991).

2. MATERIALS AND METHODS

Inspection of sanitation effectiveness in a dairy plant was carried out by two methods, microbiological and bioluminescence.

Swabs were taken from various surfaces before and after disinfection from 3 sections of dairy processing plant producing quark cheese ("tvaroh"), yoghurt and cottage cheese. The sampling sites are shown in Tables 1–3.

Microbiological swabs were taken from the area of 10 cm² and evaluated for total bacterial counts, *E.coli* and moulds. Total bacterial counts were determined after cultivation on meat-peptone agar (MPA) at 37°C for 24 hours. *E.coli* were cultivated on Endo agar for 24 hours at 37°C and moulds at 22°C for 5 days on Sabouraud agar.

Bioluminescence was measured with the system HY – LITE NG, produced by Biotrace, using a portable luminometer to measure emitted light produced in a reagent chamber of a sampling pen supplied by the manufacturer. By means of the sampling pens we took swabs from the area of 100 cm². The system is based on reaction of ATP from the sample with enzyme luciferase in a reagent chamber of the pen and the intensity of emitted light is measured by the luminometer in relative luminescence units (RLU). The RLU value is directly proportional to ATP in the tested sample. Results presented in the Tables 1–3 are means of 5 swabs.

Using without instrumentation methods were determined number of microorganisms in the air in the dairy operation. At predetermined locations of operation were left running, open Petri dishes with the appropriate breeding ground for micro-organisms to sedimentation of aerosol air. Exposure time was determined according to the expected bacterial air pollution in 1-10 minutes. You still need to be converted to 5 minutes, it settles for five minutes at 100 cm² area of a number of microorganisms that present in 10 liters of air.

Total bacterial counts were determined after cultivation on meat- peptone agar (MPA) at 37°C for 24 hours. *E.coli* were cultivated on Endo agar for 24 hours at 37°C and moulds at 22°C for 5 days on Sabouraud agar. After the expiry of the exposure, the Petri dishes with broth incubated in an incubator for some time at a certain temperature, which is determined based on the type of microorganisms studied.

The number of embryos that sediment from 1 cm³ of air is expressed by following general relationship: $636 (a \times t / r^2)$

a: number of colonies

t: time converted to 5 minute sedimentation

r: radius of the test plate (cm)

Results presented in the Graphs 1-3 are means of 5 swabs.

3. RESULTS AND DISCUSSION

Were monitored following sections: Quark cheese production, yoghurt production, making room for COTAGGE cheese. In these parts of the plant were always microbiological swabs and ATP swabs taken from different places.

Place of sampling	Before disinfection				After disinfection			
	<i>E.coli</i>	TBC	Moulds	ATP	<i>E.coli</i>	TBC	Moulds	ATP
	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
	(CFU)	(CFU)	(CFU)	(RLU)	(CFU)	(CFU)	(CFU)	(RLU)
Floor I	0	80	18	70	0	40	11	12
Floor II	0	105	5	89	0	10	0	2
Floor III	1	134	7	101	0	2	1	-
Wall I	0	110	2	92	0	19	0	8
Wall II	1	104	6	89	0	20	2	8
Wall III	0	160	5	139	0	45	1	15
Table	1	34	1	18	0	1	0	-
Door	0	90	3	63	0	5	2	-

Table 1 The average value of microorganisms on surfaces in the test section of “Quark cheese production”

Legend: TBC - Total bacteria counts

Table 1 shows results obtained by cultivation of microbiological swabs and of ATP determination on tested surfaces in the section “Quark cheese production”. These results show that bacterial counts on the tested surfaces in this section of milk processing complied with hygiene requirements and the

disinfection in this section was effective. Microbial biofilms which form on all types of surfaces of technological systems in the dairy industry and on dairy farms adversely affect the quality and safety of final products, i.e. both foodstuffs and raw materials used for their production. The fact that a number of microorganisms are alimentary pathogens. Biofilms are usually formed by various species of microorganism, which protect each other against the effects of biocidal (antibacterial) agents and are resistant to these agents. The colonisation of surfaces of the open and closed piping systems, floors, waste, walls and ceilings of the production halls becomes a major problem in the selection of effective sanitation agents for their control (Vlkova et al. 2008). Monitoring of hygiene under operating conditions is ensured by traditional microbiological swabs and lately also by the ATP-detecting bioluminescence method capable of providing information about contamination of surfaces in less than 10 minutes (Green et al. 1999). Disinfectant or biocide is a common term for an agent used for killing, inhibiting, or restricting the growth of harmful organisms in all spheres of human activity (Keener et al. 2004). Sanitation processes are an inevitable part of food production as they influence hygiene and outcome of the entire production system (Bremner & Johnston 1996).

Place of sampling	Before disinfection				After disinfection			
	<i>E.coli</i>	TBC	Moulds	ATP	<i>E.coli</i>	TBC	Moulds	ATP
	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
	(CFU)	(CFU)	(CFU)	(RLU)	(CFU)	(CFU)	(CFU)	(RLU)
Floor I	0	220	3	138	0	13	3	-
Floor II	0	100	7	87	0	4	1	-
Floor III	18	74	14	39	0	10	0	6
Wall I	0	4	1	-	0	0	0	-
Wall II	0	14	3	-	0	0	2	-
Wall III	1	4	3	-	0	1	3	-
Table	0	3	2	-	0	1	0	-
Door	0	18	7	7	0	6	1	-

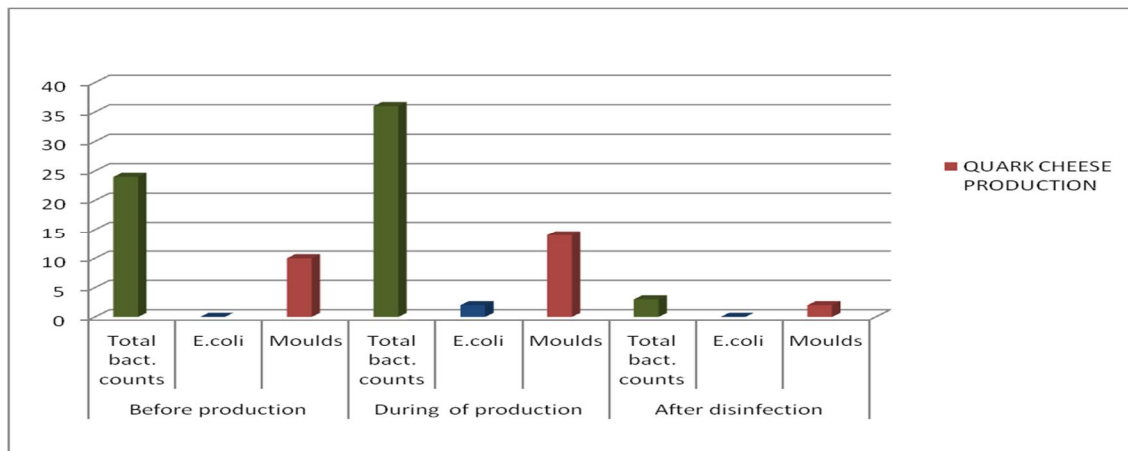
Table 2 summarizes results of microbiological swabs from surfaces in the section of yoghurt production.

Results in table 2 are obtained by the ATP-based method. It shows good hygiene before disinfection and indicates that the disinfection was adequate and effective. Disinfection in food processing plants should remove or devitalize all disease-producing germs and reduce the counts of other germs to a level, which has no negative influence on the products (Gracey & Collins 1992).

Place of sampling	Before disinfection				After disinfection			
	<i>E.coli</i>	TBC	Moulds	ATP	<i>E.coli</i>	TBC	Moulds	ATP
	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
	(CFU)	(CFU)	(CFU)	(RLU)	(CFU)	(CFU)	(CFU)	(RLU)
Floor I	23	180	5	110	1	104	4	89
Floor II	0	75	2	61	0	10	1	3
Floor III	3	102	3	104	0	5	0	55
Wall I	0	10	1	3	0	1	0	-
Wall II	0	3	1	-	0	0	1	-
Wall III	0	1	1	-	0	0	0	-
Table	0	13	5	4	1	11	4	55
Door	0	2	3	-	3	0	1	20

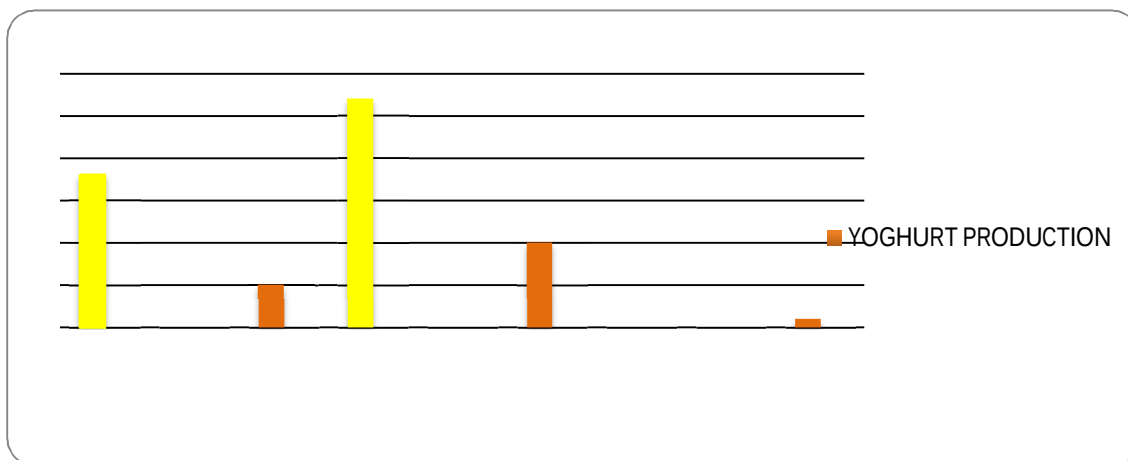
Table 3 The average values of micro-organisms on the test surface of the cheese factory in cottage cheese

Table 3 presents results obtained by both methods (microbiological and ATP) from surfaces in the section „production of cottage cheese“, aimed at testing of hygiene level of respective premises. Decreased counts of observed micro-organisms after disinfection indicate that the disinfection was carried out correctly and effectively. The only problematic sites were Floor I and worktable. The counts of CPM and *E.coli* were high even after disinfection. On the worktable we detected 2 *E.coli* colonies which suggests possibility of subsequent contamination either of raw material used in production or intermediate product or final product before packaging. Contamination during operation is indicated also by results obtained by the bioluminescence method. Moore and Griffith (2002) studied the dependence between the surface quality, cleaning and disinfection and the testing methods. The number of surfaces considered suitable for food production increased after cleaning but the agreement between the testing methods varied with regard to the type of processed food. Insufficient detection of proteins was most frequently observed on surfaces in bakeries while ATP-bioluminescence and the standard microbiological methods failed mostly in freezing facilities and cheese processing plants. The authors concluded that it is necessary to combine these methods when monitoring cleanliness in these facilities. Oulahal-Lagsir et al. (2000) accept the use of ATP-bioluminescence method for quantification of biofilm removal.

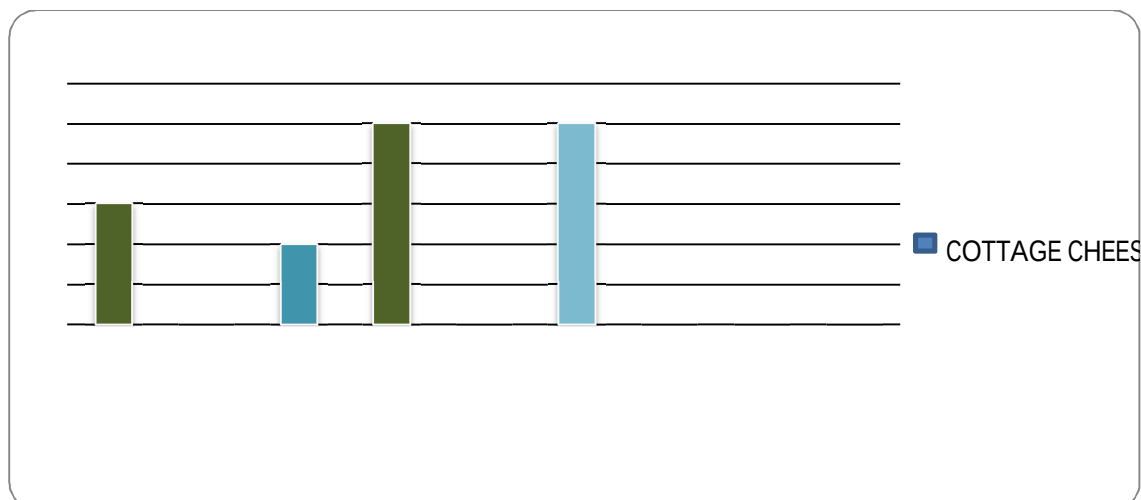


Graph 1 The average values of airborne microorganisms in the section „Quark cheese production”

In Graph1 are listed average values of airborne microorganisms in the section „Quark cheese production”. The resulting values exceed the standards set, so it can be concluded that the disinfection process is effective. So it can be concluded that effective disinfection process. CPM and mold in the process of production increased slightly, which is related to the process and the movement of persons.



Graph 2 The average values of airborne microorganisms in the section „Yoghurt production”



Graph 3 The average values of airborne microorganisms in the section „Cottage cheese ”

Graphs 2-3 describes the results of microbial control of air pollution in the dairy. The resulting values do not exceed the standard, so it can be concluded that an effective disinfection process. Values of total bacteria counts, *E. coli* and fungi in the production process increase slightly, reflecting the technological process and the movement of people in the building. Microorganisms in the atmosphere are not favorable environment for their growth, so it is only persist. The atmosphere is generally found spores and for their small size, resistance against desiccation and high number of spores from a single mold. Airborne spores can travel great distances. The bacteria in the air are more common than coca sticks, bacterial spores are rare in dust-free environment. Frequent air microflora staying sporogénne yeast (Griger & Vařejka 1991). Microorganisms are usually in the atmosphere are captured from dust particles or water drops. Dust and droplets are transferred and sporulated microbes on walls, ceiling, floor, food and additives. Of these may then be out of the atmosphere.

4. CONCLUSIONS

The aim of the study was to evaluate the effectiveness of sanitation in a dairy processing plant by means of two methods, microbiological and bioluminescence, and to compare the results.

To evaluate objectively the level of hygiene and sanitation in dairy processing plants it is necessary to carry out the examinations according to individual branches and production procedures considering their specificity. The most objective parameter is microbiological contamination that allows us to assess effectiveness of the disinfectant used or effectiveness of preceding mechanical cleaning that is essentially important for effectiveness of sanitation works and to take measures for improvement (Ondrašovičová et al. 2003).

The bioluminescence method is suitable for rapid inspection of sanitation effectiveness before starting the production or continuously during the specific work shift. In very short time it provides results about hygiene of surfaces, serves to detect shortcomings in sanitation and allows one to eliminate them. By using the system HY – LITE NG produced by Biotrace together with necessary accessories we obtained results within 1 minute.

Our results obtained in the selected dairy processing plant showed that the disinfection was effective in all three tested sections with the exception of some sampling sites in the section for production of cottage

cheese. In this section we detected increased total bacterial counts and coliform counts on Floor I and on Table. Contamination of these sites was confirmed also by the ATP-based bioluminescence method. These values changed after the time up to start of production and corrective measures were taken on the basis of ATP swabs. The presence of ATP in swabs indicates presence of any organic impurities that may serve as substrates for multiplication of micro-organisms and thus its higher values indicate either presence of micro-organisms or their potential nutrients. Thus the practical use of the bioluminescence method in food hygiene is justified as it allows one to take necessary corrective measures and comply with correct production practice.

In summary, ATP monitoring has been widely used, particularly in the food service industry, as a rapid screening method for measuring gross, general levels of cleanliness, i.e. the presence of organic matter, but it is not a reliable substitute for culturing in situations in which a measurement of microbial contamination is needed nor for correlating risk of infectious disease transmission. ATP bioluminescence should not be used as a substitute for quantitation of microbial load (Larson et al. 2003).

The results of microbial pollution control in dairy processing plant exceed established standards, so it can be concluded that an effective disinfection process. Values of total bacteria counts, *E. coli* and fungi in the production process increase slightly, reflecting the technological process and the movement of people in the building.

This study was supported by the Science Grant Agency (VEGA) project No. 1/0950/12.

REFERENCES

- Bremner, A, Johnston, M 1996, Poultry Meat Hygiene and Inspection. WB Saunders Company Ltd. TheUniversity Press, Cambridge, pp. 125-169.
- Colquhoun, KO, Timms, S & Fricker, CR 1998 'A simple method for the comparison of commercially available ATP hygienemonitoring systems' *Food Prot.*, vo. 61, no. 4, pp. 499–501.
- Gracey, JF, Collins, DS 1992, Meat Hygiene. 9th Edition, Bailliere Tindall, The Bath Press, London, pp. 122-131.
- Green, TA, Russell, SM & Fletcher, DL 1999' Effect of chemical cleaning agents and commercial sanitizeron ATP bioluminescence measurement' *J Food Protect*, vol. 62, pp.86-92.
- Griger, C & Vařejka, F 1991, 'Mikrobiológia požívateľín živočíšneho pôvodu': Príroda Bratislava, p. 288.
- Jay, JM, Loessner, MJ & Golden, DA 2005, Modern Food Microbiology: Springer-Science, Business Media, 7th ed, Inc., New York.
- Keener, KM., Bashor, MP, Curtis, PA, Sheldon, BW & Kathariou, S 2004 'Comprehensive review of *Campylobacter* and poultry processing' *Food Science and Food Safety*, vol. 3, pp. 105–116.
- Larson, EL, Aiello, AE, Duarte, CG, Linc, SX, Leed, L, Latta, PD & Lindhardt, CH 2003, 'Bioluminescence ATP monitoring as a surrogate marker for microbial load on hands and surfaces in the home' *Food Microbiology*, vol.20, pp. 735–739
- Lopašovský, L, Pavličová S & Šiška B 2006, 'Utilization method of bioluminescence at the monitoring at some food processing facilities', *Zborník z medzinárodnej konferencie, Rizikové faktory potravinového reťazca*, NITRA, pp. 210-21.

Meyer, B 2003 'Approaches to prevention, removal and killing of biofilms' *International Biodeterioration & Biodegradation*, vol. 51, pp. 249-253.

Moore, G & Griffith, C 2002 'A comparison of traditional and recently developed methods for monitoring surface hygiene within the food industry: an industry trial' *Int J Environ Health Res*, vol. 12, pp. 317-329.

Ondrašovičová, O, Vargová, M, Ondrašovič, M, Biswancel, H, Kašková, A & Nagy, J 2003, 'Sanitácia v mäso spracujúcich prevádzkach', *Sborník referátů ze semináře o údržbě masa*, Skalský Dvůr, pp. 51 – 55.

Oulahal-Lagsir, N, Martial-Gros, A, Bonneau, M, Blum, LJ 2000 'Ultrasonic methodology coupled to ATP bioluminescence for the non-invasive detection of fouling in food processing equipment - validation and application to a dairy factory' *J Appl Microbiol*, vol. 83, pp. 433-441.

Poulis, JA, DE Pijper, M, Mossel, DA & Dekkers, PP 1993 'Assessment of cleaning and disinfection in the food industry with the rapid ATP-bioluminescence technique combined with the tissue fluid contamination test and a conventional microbiological method' *Food Microbiol*, vol. 20, no. 2, pp. 109–116.

Suková, I 2002, 'Rychlá metoda hodnocení hygieny provozu', *Kvalita potravin*, č.1, p. 18.

Tsuneda, S, Aikawa, H, Hayashi, H, Yuasa, A & Hirata, A 2003 'Extracellular polymeric substances responsible for bacterial adhesion onto solid surface' *FEMS Microbiol. Lett.*, vol. 223, no. 2, pp. 287-292.

Vlčková, H, Babák, V, Seydlová, R, Pavlík, I & Schlegelová, J 2008 'Biofilms and Hygiene on Dairy Farms and in the Dairy Industry: Sanitation Chemical Products and their Effectiveness on Biofilms' *Czech J. Food Sci*, vol. 26, no. 5, pp. 309–323.

Vojtaššák, J 2003 'Kontrola hygieny pomocou detektoru Lightning MVP', *Maso*, roč. 14, č.5, pp. 33-35.