Alternative Methods Testing for Assess the Microbial Contamination in Processing Plant

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Abstract. The two systems using ATP-bioluminescence were applied to evaluate the microbiological contamination level of surfaces and food in dairy plant, poultry slaughterhouse and retail unit, compared with standardized determination. The two systems utilized for ATP measurement were HyLyte and Lumitester PD 10 N. The low correlation with general contamination level of the samples from the milk plant surfaces and the absence of correlation for the others sampling objectives, let us to entitle to consider (though those studies are preliminary), that ATP bioluminescence should not be used as a substitute for quantitative methods establishing of microbial load. Still, the ATP monitoring system can be used as screening methods for general levels of cleanliness surface appreciation.

Keywords: ATP bioluminescence, hygiene monitoring, food industry

INTRODUCTION

Manufacturing of food today presents new variety of inherent challenges resulting from volume production. Any undesirable contamination leads to the loss of high value product, lost production time and most importantly release of contaminated products and product recall. These factors have as a result the use of monitoring and control systems to ensure the product safety. The most important control systems are Hazard Analysis at Critical Control Points (HACCP). The identification of hazards and critical control points, and routine monitoring at critical control points, allow corrective actions to be taken in order to obtain safe food. Hygiene monitoring, commonly consist in a visual inspection, is utilized to assess the hygienic status of critical control points. The implementation of European microbiological criteria for food in our country created the possibility of alternative methods utilization. Usually the commercial methods are simple adapted to industrial need, more rapid and economics than the traditional methods. The rapid methods were continuous investigated in the last years for the reason to found the most reliable and efficient methods (Larson EL et al. (2003), Murphy S. C.et al. (1998), Poulis J.A.et al. (1993), Siragusa G.R. et al. (1996).

An alternative procedure, ATP bioluminescence hygiene monitoring is now accepted in our legislation, too. The ATP-bioluminescence technique was used in this work to evaluate the level of surface and food contamination and trying to establish a correlation between those two techniques and standardized method (plating on the nutritive culture media). ATP bioluminescence is a sensitive technique, which detects bacteria by measuring light emitted when their ATP reacts with firefly Lucifer’s and Luciferin. There are a number of available commercial equipments utilizing ATP bioluminescence principles: the ATP reacts with the luciferin-luciferase enzymatic complex and the reaction releases light which intensity can be measured by a luminometer. The aim of this study was to evaluate the validity and reliability of using two rapid ATP monitoring systems as a marker for the contamination level on
different food plant processing surfaces and trying to establishing a correlation between microbial counts and ATP measurements on different surfaces.

MATERIALS AND METHODS

The evaluation of those two rapid ATP monitoring systems as a marker for the contamination level on different food plant processing surfaces was followed over one year period. The sampling was done using special swabs soaked in sterile physiological solution by wiping a surface of approximately 100 cm$^2$; later on, the tampons were placed in test tubes containing sterile saline solution, and then taken to the laboratory. For the standardized method the test tubes were shaken and 1 cm$^3$ of physiological solution was drawn and inoculated in two Petri dishes and than pour PCA culture medium and mix together. After 72 hours of incubation at 30°C the colony growing on this medium were counting (SR. ISO 4833/2003).

For the ATP level determination, the sampling was performed in a similar manner (from a surface around 25 cm$^2$), using specific swabs (according to the instruction manual from the manufacturer). The ATP detected by those techniques is from microbial origin or somatic, vegetal and animal cell origin. The higher ATP concentration on surface or food samples, the higher light intensity released as expressed in the Relative Light Unit (RLU).

The sanitation samples (100cm$^2$) were taken from: equipment surfaces, food surfaces and hand workers from a dairy plant, poultry slaughterhouse and a retail unit.

The surfaces from retail unit: beef carcasses, door surfaces, saw, table for pork cutting, table for beef cutting, worker apron, knife hatchet, mail gloves, filling machine, mincing machine wall, technological water, and hand workers.

The surfaces from poultry slaughterhouse - chickens feathers, knife scalding water, rubber fingers, carcasses after plucking, plucking table post evisceration carcasses surface, internal carcasses surface, carcasses after washing, carcasses after chilling, faience.

The surfaces from dairy plant - surface tampon vessel, milk from tank, surface plastic can, pasteurization milk tank, creamer 1, creamer 2, creamer cover, chilling tank, cream bucket.

The obtained results (cfu/cm$^2$ and RLU) were converted in log$_{10}$ for normalize the data experiment. The correlation coefficient was calculated to compare the obtaining results.

RESULTS AND DISCUSSIONS

The general level of surfaces and food contamination in the retail unit varied from the 0,85 log$_{10}$ (table for beef cutting) to 3,26 log$_{10}$ (worker apron). In generally the level of contamination were higher on the surfaces hard accessible were the microbes can populate and developed real biofilms, filling machine (3,01 log$_{10}$ or table for beef cutting (2,64 log$_{10}$). If we compared the obtaining results by ATP measurement with HyLite instrument and general contamination level we obtained the similarly results for carcasses surfaces (3,16 log$_{10}$ and respectively 3,99), mincing machine (1,62 log$_{10}$ and 1,78 log$_{10}$) and PVC wall (1,91 log$_{10}$ and 1,97 log$_{10}$).
In the case of ATP level measurement using the Lumitester instrument the similarly value were obtained just for the hatchet (0.82 $\log_{10}$ and respectively 0.95 $\log_{10}$).

The correlation coefficient obtained between the ATP measurement and the total number of germs determination were very low (0.05 for Hylite and a negative correlation 0.48 for Lumitester). In fact with those results we can affirm that that is not a correlation between the three tests.

The obtaining results in the poultry slaughterhouse are presented in the figure 2. The general level of contamination was higher than in the two other units tested. It is necessary to make a specification that in this plat the sanitation samples were taken during the processing operation and in the other two plants the probes were taken before starting the operation. The general level varied between 1.55 $\log_{10}$ on the faience surface and 4.74 $\log_{10}$ on plucking table and 4.84 $\log_{10}$ on chickens feathers. As we it can be observed the general contamination level was higher in the dirty zone compared to clean zone. The same differences of ATP measurement were observed between the dirty and clean zone, in a lot of sampled point the ATP measurement were less than 1 $\log_{10}$. For the samples taken from this plant as results from the figure 2, we didn’t obtain any similarly result between the ATP measurement and total number of germs determinations. Instead, the results obtain by standardized method were with 2 or 3 $\log_{10}$ higher.
Similarly results were obtained just for the two ATP measurement system LyLite and Lumitester, in case of carcasses surface ($0.7 \log_{10}$ and respectively $0.60$). The correlation coefficient was still in this case lower ($0.02$ for HyLite and $0.36$ for Lumitester). The results obtaining in the milk processing plant are presented in the figure 3.

For the milk processing plant samples the generally level of surface microbial contamination were high even though those were taken after sanitation program. The microbial loading varied from $0.35 \log_{10}$ for chilling tank and $3.81 \log_{10}$ for creamer 1. Just in case of surface plastic can the similarly results were shown for the two ATP measurement methods and standardized method ($2.15 \log_{10}$, $2.38$ and $2.64 \log_{10}$). The correlation coefficient obtained between the ATP measurement and the total number of germs determination were very low ($0.68$), in fact that was the highest coefficient for all those experiments.
The ATP level in some studies has been shown to have a good correlation with the level of hygiene especially with the general level of contamination. Murphy et al. (1998) were obtained a low to medium correlation for the samples taken from the surfaces in four milk plant. Poulis et al (1993) in other studies made in food processing facilities found a poor correlation between ATP measurement and standardized microbiological method. In our study as in the others we didn’t found a good correlation between ATP determination and general level of contamination (Larson et al. 2003). For the sampling from milk plant a lower correlation were established. The cause for the obtained results can be the limits of detection of microbial growth such has been reported in some monitoring system (about 104 cfu/100 cm²). Others causes for obtaining such a low correlation in our studies are, the limitation of this ATP bioluminescence measurement, because that detects ATP generically and cannot make any difference between the sources of this ATP.

CONCLUSIONS

✓ The low correlation with general contamination level of the samples from the milk plant surfaces and the absence of correlation for the others sampling objectives, let us to entitle to consider (though those studies are preliminary) that, ATP bioluminescence should not be used as a substitute for quantitative methods establishing of microbial load.

✓ Still, the ATP monitoring system can be used as screening methods for general levels of cleanliness surface appreciation.

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REFERENCES