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Full Length Research Paper

Determination of the effectiveness of organosulfur compound in the leather industry by adenosine triphosphate (ATP) luminometer

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Organosulfur compound is the world's leading bactericide product for the leather industry. It was reported that it is highly effective for preserving salt and brine solutions, soaking liquors as well as processing materials. This research was conducted in a plant-processing cattle hide in Tuzla. Measurements were carried out at 24 h of wetting back- soaking baths into which was fed organosulfur containing commercial bactericide at the ratio of 0.02% at the beginning of soaking process every month for a year. The objective of this study was to examine the effectiveness of organosulfur compound commercially using adenosine triphosphate (**ATP) luminometer. According to ATP test, organosulfur compound had enough effective bactericidal activity during the soaking process.**

Keywords: Leather industry, soaking, organosulfur compound, ATP.

INTRODUCTION

Leather is an organic material. Untanned leather must be protected during stock, soaking and wetting back processes against the bacterial attack. Fungi and bacteria growth depends on the presence of organic substances in the medium, such as the proteins, fats, and blood that are dissolved with water addition; initiating their degradation by microbiological enzymatic processes, and their level depends on climatic conditions (temperature, humidity), storage periods, conditions of fabrication/stock, leather fat content and pH. Bacteria optimally grow between alkaline and slightly neutral pH. Standard conditions for bacteria are pH of 6.5 to 8 and temperature of 37°C. Bacteria are effective on leather proteins with their extracellular enzymes. As a result of bacterial activity that occurs, advanced dull and rough grain structure, grain looseness, grain peeling due to adverse effects, such as occurs in the skin become completely unavailable. Bacteria grow quickly and cause irreversible damage to both physical properties and aesthetic appeal of leather. Many studies have been conducted on this subject. Various types of bactericide are recommended for soaking float; microbial counts are made in order to determine their effectiveness. To determine the level of contamination, we can either estimate how many bacteria are present, or we can measure changes that occur as a result of bacterial activity.

Although various counting methods are used in order to count the bacteria that grow during the soaking wetting back process, naturally, those who are most practical and fastest are preferred in commercial practices. Among them, ATP (Bioluminescence Method) and dip slide method are most commonly applied (Bayramoğlu et al., 2009). Bayramoğlu et al. (2009) reported that ATP method is convenient to use in commerce for measuring the activities of biocides under the light of scientific data. Widespread use of ATP method leads to an increase in effectiveness in the leather sector, which leads to an

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Abbreviations: **ATP**, Adenosine triphosphate; **RLU**, relative light units.

increase in the insight of offering right products at right quality and time and direct customer satisfaction. On the other hand, other methods are available for the determination of contamination level of bacterial attack such as the pour plate method, the use of selective media, microscopic examination and 3 M Petrifilm method. In summary, there are a number of different techniques that may be used for monitoring bacteria, and they all result in approximations of population numbers or activity levels. In this research, we used organosulfur compound bactericide and ATP measurement method for checking the contamination level.

MATERIALS AND METHODS

Raw material

The raw material was Turkish domestic wet-salted hides.

Bactericides

Wide variety of bactericides and fungicides were used in the leather industry. The ones mostly eferred among by the leather manufacturers were chosen for the study. Organosulfur compound is the world's leading bactericide product for the leather industry. It was reported that it is highly effective for preserving salt and brine solutions, soaking liquors as well as processing materials (Buckman, 2002). This research was conducted in a plant processing cattle hide in Tuzla. Measurements were evaluated at 24 h of soaking wetting back baths into which was fed organosulfur containing commercial bactericide at the ratio of 0.02% at the beginning of the soaking process every month for a year. Soaking process was applied as a long soaking process in the propellers with 15 ton capacity.

Detection of microbial contamination using luceriferase/ATP luminometer

Description of methodology

ATP is a ubiquitous molecule that acts as a major energy storage medium for all living cells, including microorganisms. ATP consists of a ribose group with an adenine moiety on one side of the molecule and three phosphate groups on the other. Hydrolysis of these phosphate groups releases energy and drives a wide variety of biological reactions within the cell. The ATP molecule can thus be used as a marker of cellular contamination (Ceresa and Ball, 2004). The chemistry used to detect ATP comes from the mechanism that fireflies (fireflies enzymes utilises extracted from *Photinus pyralis*) use to produce light. Light is produced when the enzyme (luciferase) catalyzes a reaction between ATP and luciferin as follows (Josee, 2003):

$$\text{ATP} + \text{Luciferin} + O_2 \xrightarrow{\text{Luciferase}} \text{Luciferin} + H_2O + \text{Light}$$

A Luminometer, consisting of Photo-multiplier tubes, is used to record the number of photons released from the reaction mixture. The readings represent the average light released over the sampling time and is measured in Relative Light Units (RLU). The result is obtained within seconds. The result was digitally shown on the screen as RLU. RLU values are directly into the sample and therefore the total amount of ATP is related to degree of contamination in the sample. RLU values read graphics to facilitate the interpretation and expression can be done. Nonetheless, when the microbial-controlled sample emits too much light, the red light turns on and signals are overloaded. That means the registered value is higher than 500,000 RLU. The ATP test can be used to determine the amount of bacterial population by measuring the amount of ATP released when the organisms are killed by means of an extraction reagent. This results in the rupture (lysis) of the bacterial cell walls and the internal contents are released along with the ATP. The subsequent addition of a luciferin-luciferase reagent results in emission of light and is measured in the luminometer (Figures 1, 2 and 3). The biggest advantage of this method is the very short process time. Despite a certain incubation period in other methods, this method provided result in a very short time. It also caused less waste. However the high cost of kits could be considered as a drawback. There are some measurements for comparison (Rangarajan et al., 2003). This also reduces the delivery time to the customers.

Total ATP content in the sample will be determined by this test. The ATP content of sample comes from organic residues in the sample, sample microorganisms and food products wastes. The more organic residue, the more the ATP level. This cycle is proportional to the amount of light as a result of the test. Therefore, significant contamination of large amounts of sample results indicates that the results in terms of RLU compared with the sample are always a witness. This test is an important method that is used for the determination of bactericidal activity during soaking in leather production processes. This method is a reliable method for chemical suppliers to prove effective aspects of their products for their customers. The measurement values obtained from the samples taken per hour during soaking process have the possibility of graphical presentation and also it is an advantage for the suppliers of biocides.

RESULTS AND DISCUSSION

Tables 1 and 2 show the measurements at 24 h of the soaking wetting back floats into which was fed organosulfur containing commercial bactericide at the rate of 0.02% at the beginning of soaking-wetting back process every month for one year in a plant processing cattle hide in Tuzla. The changes in these measurements are shown in Figure 4. Raw hide is not sterilized and contains thousands of bacteria. The activities of these bacteria is restricted with the conversation process; however, when soaking wetting back process is initiated, which is the first stage of the leather processing, leathers are soaked in water; tried to be returned to its original conditions as they are slaughtered from the animal. In this case, bacteria activities which are restricted regain their activation. Typically, the ideal growth temperature of aerobic and mesophilic bacteria is 37°C. When this condition is taken into account, the bacterial growth is expected to be at maximum levels during the summer-time. Particularly, bactericide sellers emphasize to increase more the use of bactericide during summer-time. However, as a result of measurements made, it is clearly seen that some other factors become issue in addition to increase of bacteria during soaking float. In particular, the number of bacteria present at the beginning of soaking is the most important issue that

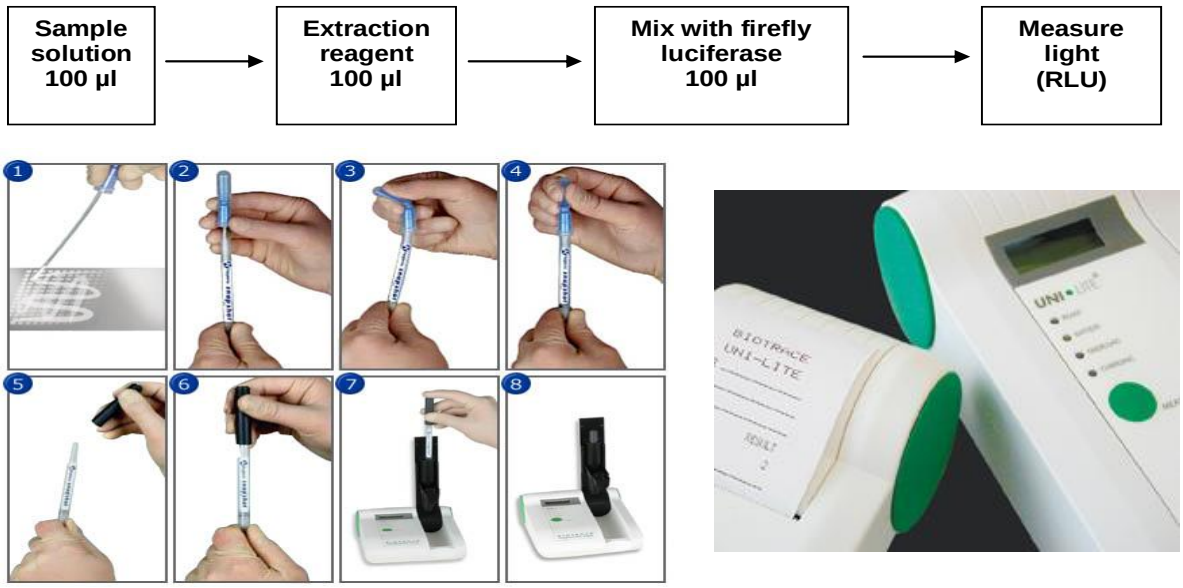

Figure 1. Biotrace uni- lite sampling procedure, hygiena.

Figure 2. ATP luminometer (Rangarajan et al., 2003).

Figure 3. The biotrace hygiene management guide, 2004

Table 1. ATP test results.

Tuzla	ATP result
January	2590 RLU
February	2015 RLU
March	1262 RLU
April	1856 RLU
May	1976 RLU
June	1750 RLU
July	1970 RLU
August	2280 RLU
September	2570 RLU
October	3125 RLU
November	3513 RLU
December	3035 RLU

Table 2. ATP reading for various aqueous solutions (Data from Dr. Attila G. Relenyi, "organic film and general organic fouling indices").

Sampling point	ATP reading
Drinking water	5-300 RLU
Natural aquifer source for refinery	300 RLU
Odorous well water	310,000 RLU
Sterile water (for babies)	1 RLU
Bottled water	212 RLU
Water cooling system	3,000 - 6,000 RLU

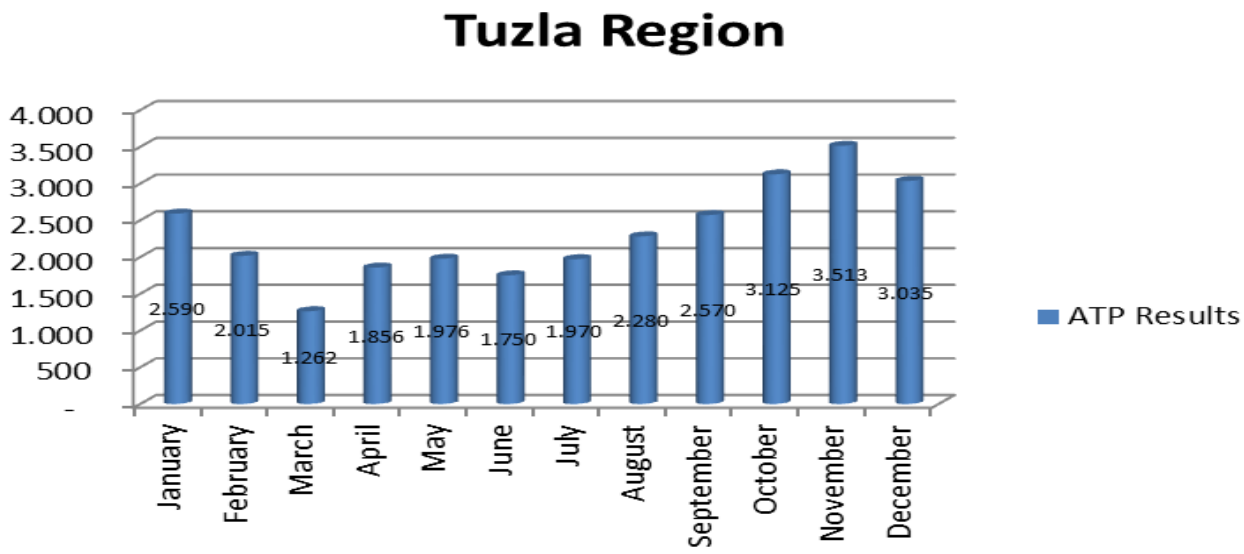

Figure 4. ATP test results evaluation.

must be taken into account. If the numbers of bacteria are much more at the beginning, since bacteria increase logarithmically, it is expected that the increase of bacteria number be much more during further soaking processes. During the animal cutting, blood must be removed from the leather; otherwise it prepares an enriched environment for bacteria to grow. Particularly, the moist present in the leather during summertime, increases the bacterial growth highly; thus, if soaking process will not immediately be carried out, it is advisable that leathers should

be applied with salt and stored in a dry place. Especially, since there are higher numbers of bacteria at the initial phase, higher risk will be in longer processes; it is advisable to carry out initial soaking process for several hours, even tough bactericidal agents are used. In this case, the number of bacteria will decrease further and thus the damaging possibilities of bacteria on leathers will have been reduced. Evaluating these kinds of factors is important in order to achieve money and time savings in leather production. Furthermore, taking these issues into account will sustain an improvement in product delivery time to customer.

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