

ATP MONITORING AS AN EXPRESS METHOD TO DETERMINE CONTAMINATION OF OBJECTS

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Introduction. Formulation of the problem

The raw materials, foods and water are favourable environments for various microbiota. So they can become sources not only of spoilage agents, but also of pathogens causing food poisoning and enteric infection. For this reason, the main task of each food enterprise is providing people with high-quality and absolutely safe products. This can be achieved by systemically controlling the sanitary and microbiological characteristics of water, the

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Abstract. The article presents the results of determining the contamination of water and different surfaces. The research was aimed at estimating water safety and the sanitary-hygienic condition of surfaces. To examine the water samples and the surfaces of household objects for contamination, we used the traditional method and the bioluminescence-based express method. The bioluminescence method is based on determining the total amount of ATP (bacterial, somatic, and extracellular) on contact surfaces and in water. The level of ATP (adenosine triphosphate) was determined with a luminometer *Lumitester* PD-30 (*Kikkoman*, Japan) according to the manufacturer's instructions, using special test systems. The ATP bioluminescence method is commercially more available for simple and quick sanitary-hygienic control according to the HACCP principles or international standards. The traditional method of determining the contamination of water and other materials consisted in inoculating water or wipe samples from the controlled surfaces on a general nutrient medium, with their further cultivation under appropriate conditions. The strongest luminescence reaction was observed in sea water, which can be explained by the presence of organic substances in it, while the bioluminescence values for the potable (bottled) and filtered water tested were the closest to those in the control test. The results of testing the sanitary-hygienic condition of surfaces show that the amount of adenosine triphosphate exceeded the limits in almost all tested objects. However, only slight adenosine triphosphate overconcentration was observed on the internal surface of new (plastic) food containers. The studies performed have shown that the bioluminescence-based express method can be used as primary control that gives immediate information about the contamination of both surfaces and liquids. Using the bioluminescence method can shorten the time of the study and thus reduce the cost of the test. However, to determine the qualitative and quantitative composition of the tested object's microbiota, the classical microbiological control is needed.

Keywords: ATP (adenosine triphosphate), AMP (adenosine monophosphate), bioluminescent ATP-metry, express method, luminometer, luciferase, luciferin

primary and secondary raw materials an enterprise receives, the technological process and final products. Besides, the sanitary-hygienic condition of the equipment, instruments, packaging materials, workers' hands and clothes, etc. must be constantly controlled. The sanitary microbiological control of water quality determines the level of its epidemiological safety according to the central water supply standards listed in StSanRR (State Sanitary Rules and Regulations) No 383/1940 "Potable water. Hygienic requirements to the quality of water in the

central potable water supply system” of 3 February 2005. The water quality is estimated by a complex of organoleptic, chemical, and bacteriological parameters. When the quality of water does not meet the requirements of organoleptic and other integral indices, it is recommended to determine the total microbial count. Microbiological control of water and sanitary condition at enterprises is done by traditional microbiological techniques. But the traditional methods have some serious disadvantages: they are time-taking, laborious, and do not allow detecting organic contaminations with animal or plant materials that are favourable environments for bacterial growth. Besides, the traditional methods can only be implemented if an enterprise has good laboratories and qualified staff. That is why, recently, a lot of attention has been paid to the express method of controlling the microbiological quality of water and foods, and the sanitary condition of surfaces and materials. This method, compared to the traditional ones, allows not only speeding up the test, but also extending the shelf life of foods.

Analysis of recent research and publications

Bioluminescent ATP-metry is a promising method that provides quick control of water and surfaces. Recently, it has been successfully used in microbiology and food industry. Bioluminescence is a result of a chemiluminescent reaction in which chemical energy is converted into light energy [1]. The method consists in determining the amount of intracellular ATP (adenosine triphosphate) of living cells present on various surfaces and in various fluids. ATP is known to be the main carrier of chemical energy in all living cells (in animals, microorganisms, plants, etc.). In a cell, ATP passes the energy to other molecules by being split into more low-energy compounds (ADP and AMP). In 2014, using a genetically encoded fluorescent ATP indicator *QUEEN* helped determine the absolute ATP content in single *E. coli* cells. The results showed that, even within one cell population, the ATP level had a positively-skewed distribution, and the average concentration of ATP in one cell was 1.54 ± 1.22 nM. The results of fluorescent and bioluminescent analyses were almost identical [2].

In one work, the bioluminescent analysis was used to study how cellular adhesion on glass surfaces effected on their metabolic activity and, consequently, on the ATP level. It was shown that the adhesion of gram-positive bacilli and *E. coli* on the surfaces resulted in an increase in the intracellular ATP level by 2–5 times compared to the level typical of the cell suspension [3]. The bioluminescence method was shown to be effective in determining the viable count in brucellosis vaccines [4]. The researchers established the optimum parameters of preparing live brucellosis vaccines and the conditions of penetration of *Brucella* cells into the host cells. Using firefly luciferase allowed improving the bioluminescent method of determining the content of somatic cells in milk by the ATP concentration [5,6]. In this work, the

researchers developed criteria of mastitis diagnostics by the level of bacterial ATP in milk. The control of bacterial contamination by luminescence is widely used in all developed countries as a quick quantitative laboratory test for the chemical toxicity and safety of water samples and water extracts from various environmental objects. For example, in [7], the bioluminescence method was used to determine the quality and safety of Sakmara River water. In this work, the heavy metal concentration was studied, with the use of a genetically engineered luminescent strain *Escherichia coli* K12 TG1 and constitutive genes of the natural marine microorganism *Photobacterium leiognathi* 54D10 in the lyophilised state.

A lot of works consider the express analysis of wipes from technological surfaces, dishes, equipment. The authors [8] used bioluminescent ATP-metry to determine the contamination level of the surfaces of equipment at meat factories. The investigations revealed a correlation between the quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM), coliforms, enterobacteria, and the total ATP content on technological surfaces.

Thus, knowing the concentration of intracellular ATP in any object or fluid allows estimating the content of viable cells and evaluating the sanitary-hygienic condition of various surfaces and materials.

The high rate of luminescent reaction allows quickly detecting hazardous production areas and foresee possible contamination of specific surfaces or other objects. This gives time to take all necessary measures well before the start of a technological line, and to avoid bacterial defects. In addition, it guarantees complying with the HACCP (Hazard Analysis and Critical Control Points) requirements.

Taking the above into account, it is reasonable to use the discussed expressed method to test various liquids (including water) and surfaces for biological contamination.

The purpose of this work was to prove the practical importance of the express method for determining biological contamination levels of water and various surfaces at food enterprises. For this purpose, it was necessary to achieve the following **objectives**:

1. To familiarise ourselves with the modern high-sensitivity device that uses bioluminescence to determine intracellular ATP;
2. To assess the levels of contamination of different water samples and surfaces using the classical method and the express one.

Research materials and methods

The research was carried out at the Biochemistry, Microbiology, and Nutrition Physiology Department of Odessa National Academy of Food Technologies.

The test samples to assess the level of microbiological contamination were taken from different water sources and surfaces of food production equipment. The water samples were taken in

compliance with the State Standard (DSTU) 7525:2014 “Drinking water. Quality control requirements and methods,” with sterile distilled water used as the control sample.

Intracellular ATP was determined with a portable luminometer *Lumitester PD-30* (Kikkoman, Japan). The luminometer operates by recording light radiation, the intensity of which is directly proportional to the concentration of adenosine triphosphate. That is, ATP is used as an indicator to determine the presence of living bacterial, animal, and plant cells both in liquids and on various surfaces [9].

This device and its testing reagents use a new biotechnological development – bioluminescence in the cyclic reaction (ATP cycling method) involving the enzyme pyruvate orthophosphate dikinase (PPDK) (Fig. 1). The high sensitivity of this development is due to the presence of both ATP and AMP [10].

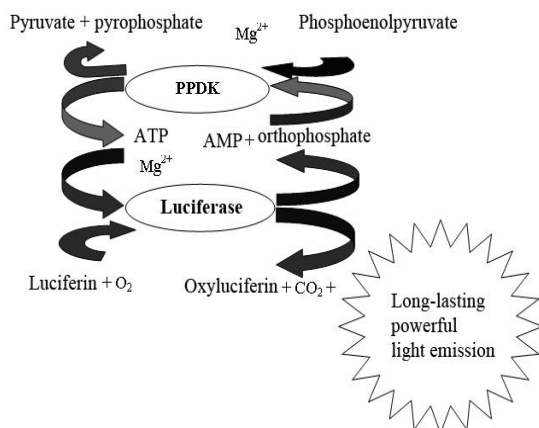


Fig. 1. Cyclic bioluminescent reaction involving pyruvate orthophosphate dikinase

This diagram shows that firefly luciferase produces light in the presence of ATP and luciferin. AMP produced in this reaction is converted again into ATP by PPDK, which allows obtaining a high-level and stable luminescence [11,12].

The kit of the device includes single-use test systems for analyses of water (*LuciPac Pen-Aqua*) and of surfaces, equipment, and food industry materials (*LuciPac Pen*).

The test system *LuciPac Pen* is a plastic test-tube (185 mm long and 10 mm in diameter) that contains a sterile cotton swab, a reagent to isolate ATP, and a reagent to induce glowing.

The *LuciPac Pen-Aqua* kit contains no harmful substances and consists of the following elements: stick handle, sample collector, luminescent reagent, and ATP-isolating reagent. The chemical agents to determine bioluminescent ATP constitute a lyophilised mixture containing all the necessary components for the reaction to take place: luciferin, luciferase, magnesium acetate, buffer solution components, and stabilisers. The test kits for analysis were stored in the refrigerator at a temperature of 2–8°C.

The test kit was kept at room temperature for 30 minutes before being used. In the course of measuring the intracellular ATP, the luminometer *Lumitester PD-30* was not tilted by more than 45° so as to avoid deviations in the results.

ATP bioluminescence testing of water was performed in the following sequence: the sample collector was removed from the tube and immersed in the liquid to be tested, making sure no air bubbles remained in the sample collector's comb, and then was returned into the tube. Then, the tube was shaken so that the entire sample liquid entered the reaction chamber for complete dissolution of the chemical agent. The prepared test system was placed in the measuring chamber of the luminometer, and the device was turned on. The luminometer was calibrated automatically. After 10 seconds, the result measured in relative light units (RLU) was shown on the device's display indicating the ATP level [13]. 1 RLU corresponds to 1 femtomole (10⁻¹⁵ mol) of ATP.

The QMAFAnM (CFU/cm³) was determined by the traditional classical method of inoculating 1 cm³ of a water sample on meat peptone agar (MPA), with subsequent cultivation at 30 ± 2°C for 48 hours [14].

LuciPac Pen swabs were used to analyse dry and moist surfaces. The surfaces were tested for hygienic and biological contamination in the following way: according to the instruction, the swab from the tube was soaked in tap water and used to wipe the surface to be tested. Then it was put back into the tube, the latter shaken intensively and put into the measuring chamber to determine the RLU level. At the same time, the traditional method was used to control the hygienic state of the surfaces: by wiping the microbes from 100 cm² of the surface followed by inoculating them on MPA [15].

A 25 cm² frame was used to wipe microbes off the flat surfaces, as described in [16]. The wiping was done with sterile synthetic swabs in order to avoid external biological contamination. The wiping fluid was then placed into a Petri dish with MPA, then cultivated for 48 hours at 30±2°C, and the QMAFAnM per 1 ml of the wiping fluid was determined. The surfaces of glassware (plates, wineglasses) were tested the same way by wiping their internal and external (2 cm away from the edges) surfaces with sterile swabs.

Results of the research and their discussion

In this work, we studied the level of hygienic and microbiological contamination of various surfaces and water. The first stage of the work was the analysis of some samples of water: distilled water, tap water, bottled still water, filtered water, pump water, and seawater. The results of the research are shown in Table 1.

Table 1 – Level of water contamination

Water sample	Classical method (QMAFAnM, CFU/cm ³)	Bioluminescent method, RLU
Distilled water (control)	0	0
Tap water	5	35
Bottled still water	1	3
Filtered water	5	8
Pump water	2	23
Seawater	26	167

It is clear from the table that the bioluminescence analysis has shown the most intensive luminescence in the tap and pump water samples compared to the control. These levels of ATP in such samples can be explained by their contamination with organic material due to violations in the purification technologies and rules of operating the water supply sources. However, the QMAFAnM in these samples does not exceed 5 CFU/cm³, which meets the accepted standards. The

other samples of potable water can be considered conditionally pure because their ATP levels are close to the control sample. The results of testing sea water have shown that both the total bacterial count and the bioluminescence value are high compared to those of the control. Such bioluminescence in this sample can be due to the presence of not only living bacteria, but other organic objects as well.

The correlation coefficient between the data obtained by the classical method and by the bioluminescence method, as determined by means of the *Statistica 10* application when measuring water pollution, was (CFU/RLU)_r=0.983.

The next stage of the research was determining the hygienic state of surfaces used in restaurants, cafés, and other food outlets. The following surfaces were tested: a cutting board, a kitchen knife, a plastic food container, glassware (a plate and a wineglass). The results obtained are shown in Table 2.

Table 2 – Levels of microbial and organic contamination of surfaces

Tested objects	Classical method, QMAFAnM	Bioluminescent method, RLU	Wiping technique
Wooden cutting board	219 CFU/cm ² of surface	17.218	100 cm ² in any location
Kitchen knife	35.800 CFU/cm ³ of wiping fluid	102.856	Both sides of the blade and the handle
Food container	150 CFU/cm ³ of wiping fluid	392	Entire internal surface
Glassware (plate)	10.600 CFU/cm ³ of wiping fluid	1.832	Entire internal surface
Glassware (wineglass)	9.500 CFU/cm ³ of wiping fluid	1.641	Entire internal surface and partly external surface

Table 4 shows that all tested objects had a high level of ATP. The most contaminated object was the kitchen knife. The ATP level on it was 205 times as high as the normal level provided for by the manufacturer. High levels of the total microbial count also indicate high-level contamination of the knife surface. The ATP level on the cutting board was also high compared to the normal levels recommended by the manufacturer. These ATP and QMAFAnM values indicate high-level contamination of the tested surface due to the material it is made of. A wooden board has a rough surface where food residues can remain in the pores and are a good source for microbial development. Moderate contamination was found by the express method on the wineglass and the plate. The QMAFAnM on the glassware surfaces are 11–19 times as large as that provided for in the sanitary requirements described in [17]. The least amount of extracellular ATP and total bacterial count was found on the plastic food container, indicating its insignificant contamination. This is explained by the technological aspects of plastic foodbox manufacturing. The high quality of polyethylene which

the container is made of provides its durability and usability. These properties prevent quick emergence of cracks where both food residues and various microbiota can enter. The correlation coefficient between the data obtained by the classical and the bioluminescence method, when measuring surface pollution, was also determined by means of the *Statistica 10* application and was (CFU/RLU)_r = 0.902. The above-limit values of the QMAFAnM and ATP levels in all the objects tested indicates violation of their sanitary treatment, making it necessary to rewash and disinfect them before use.

Conclusions

1. The use of *Lumitester PD-30* and its test systems has been proved to be effective for assessing the contamination levels of liquids (water) and monitoring the cleanness of various objects (equipment, tools, etc.) in food processing, production, and retail businesses.

2. Seawater and tap water has proved to be the most polluted water samples compared to the control

sample. Other samples of the water collected can be defined as conditionally pure due to a slight increase in the ATP levels compared to the control sample.

3. The total ATP level on various surfaces has been determined. High contamination was detected on the surfaces of the cutting board and the kitchen knife. Tests for these items have shown the ATP level to be 86–200 times as high as the recommended threshold.

4. The study has found high correlations between the data obtained by the classical method and by the bioluminescence one.

5. Therefore, the bioluminescence method for determining the residual amount of ATP can be used as one of the express microbiology methods to assess the quality of water and the cleanness of surfaces. It allows obtaining the results in 1 or 2 minutes, while the traditional test method requires almost 48 hours. If a high ATP level is detected at a tested object, immediate action must be taken to prevent faulty production. Moreover, this control system based on determining ATP can also be used in environmental practices to measure water contamination with bacteria or other biological tissue residues.

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АТФ-МОНІТОРИНГ ЯК ЕКСПРЕС-МЕТОД ВИЗНАЧЕННЯ КОНТАМІНАЦІЇ ОБ'ЄКТІВ

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Анотація. У статті представлено результати індикації забруднення води та промислових поверхонь. Дослідження проводили з метою оцінки безпеки води та санітарно-гігієнічного стану поверхонь. Для дослідження контамінації зразків води та побутових поверхонь використовували традиційний метод та експрес-метод на основі біолюмінесценції. Метод біолюмінесценції ґрунтується на визначенні сумарної кількості АТФ (бактеріальної, соматичної та позаклітинної) на контактних поверхнях та у складі води. Рівень АТФ (аденозинтрифосфат) визначали за допомогою приладу *Lumitester PD-30* (Kikkoman, Японія) відповідно до інструкції виробника із застосуванням спеціальних тест-систем. Метод біолюмінесценції АТФ комерційно більш доступний для простого та оперативного проведення санітарно-гігієнічних заходів відповідно принципам НАССР або міжнародним вимогам. Традиційний метод діагностики забрудненості води та інших матеріалів проводили посівом води або змивів мікроорганізмів з поверхонь на поживне середовище загального призначення з подальшим культивуванням у відповідних умовах.

Встановлено, що найбільша реакція світіння спостерігалась у воді морській, що може пояснюватись як прояв присутності в ній інших органічних речовин, тоді як значення біоломінесценції при дослідженні питної (бутильованої) та фільтрованої води найбільш наближені до контролю. Результати аналізу санітарно-гігієнічного стану поверхонь показали, що кількість аденозинтрифосфату перевищена майже в усіх тест-об'єктах. Однак, незначне перевищення концентрації аденозинтрифосфату спостерігалось при змиві з внутрішньої поверхні нової тари (пластикової) харчової. Проведені дослідження демонструють, що експрес-метод на основі біоломінесценції можна використовувати як первинний контроль, який надає оперативну інформацію щодо забрудненості не лише поверхонь, а також рідин. Використання методу біоломінесценції дозволяє скоротити час проведення дослідження і тим самим зменшити вартість дослідіду. Проте, у разі необхідності визначення якісного і кількісного складу мікробіоти в тест-об'єктах необхідно проводити класичний контроль.

Ключові слова: АТФ (аденозинтрифосфат), АМФ (аденозинмонофосфат), біоломінесцентна АТФ-метрія, експрес-метод, люмінометр, люцифераза, люциферин.

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