

Use of photon emission to identify the degree of microbial contamination of liquid biological substances

Abstract. The incidence of foodborne infections has increased worldwide over the past few years, with Gram-negative and Gram-positive bacterial pathogens at the epicenter of most reported cases. The aim of this study was to determine the effect of microbial contamination, understood as the abundance of microorganisms per unit of liquid biological substance, on the photon emission rate of the aforementioned substances. Such identification will allow to parametrize the process of microbial multiplication in a given substance by the photon emission rate, which would create a fast and contact-free method of estimating the concerned abundance of microorganisms. It was found that with the increase in the abundance of microorganisms in suspension, the number of photons emitted from it increases and the trend of this increase can be described by a linear function.

Streszczenie. Częstość występowania zakażeń przenoszonych na żywność wzrosła na całym świecie w ciągu ostatnich kilku lat, a patogeny bakterii Gram-ujemnych i Gram-dodatnich znajdują się w epicentrum większości zgłaszanych przypadków. Celem badań było określenie wpływu zanieczyszczenia mikrobiologicznego rozumianego jako liczebność mikroorganizmów w jednostce płynnej substancji biologicznej na wielkość emisji fotonowej w/w substancji. Taka identyfikacja pozwoli sparametryzować proces namnażania mikroorganizmów w danej substancji stopniem emisji fotonowej, co stworzyłoby szybką i bezkontaktową metodę szacowania przedmiotowej liczebności mikroorganizmów. Stwierdzono, że wraz ze wzrostem liczebności mikroorganizmów w zawiesinie liczba emitowanych z niej fotonów rośnie a sam trend tego wzrostu można opisać funkcją liniową (**Wykorzystanie emisji fotonowej do identyfikacji stopnia zanieczyszczenia mikrobiologicznego płynnych substancji biologicznych**).

Keywords: photon emission, bacteria, production engineering, technology

Słowa kluczowe: emisja fotonowa, bakterie, inżynieria produkcji, technologia

Introduction

Electromagnetic ultra-weak radiation, invisible to the naked eye, accompanies living matter continuously and illustrates processes of cell transformation that involve free radical reactions. In the undisturbed state of a living object, the ultra-weak stationary photon emission reflects the oxidoreductive equilibrium state of the cell, whereas changes in the intensity of this emission and the rate of occurrence of these changes are correlated with the response of the cells to an appropriate stimulus from the environment. Photon emission is not visible to the naked eye. This is due to the number of photons emitted in the visible range - it lies below the absolute energy threshold of our eye's sensitivity. All these factors contributed to the fact that it was observed only in the 20th century and is not yet fully understood. The existence of ultra-weak photon emission was finally confirmed in the second half of the 20th century. The registration of this radiation required the use of appropriate measurement techniques. In the 1950s, a photomultiplier was invented, which contributed to the immediate development of research on the phenomenon of ultra-weak luminescence on biological and later animal systems. This development was initiated by an experiment of two Italian physicists Colli and Facchini, who, by using a photomultiplier, observed the existence of radiation in the visible light range of plant seedlings [1].

In recent years at the University of Agriculture University of Agriculture in Krakow a number of studies on photons have been carried out. The objects studied included: oil, bread, fruit, white, dark and milk chocolate [2-5]. The experiments conducted allowed us to observe logical relations between the number of photons emitted by the studied samples and the degree of their processing and concentration of active substances contained in them. These observations allowed us to conclude that a lower number of emitted photons is correlated with a higher content of substances negatively affecting the state of the food product, while a higher number of emitted photons is associated with health-promoting qualities of the food being the object of study. It is worth noting that despite the fact that the method of photon counting was discovered in the last century - it is still considered a novelty and is rarely

used [6]. It is important for the development of research to strive for the construction of better and more modern testing devices, which will be equipped with high quality photomultipliers giving the possibility of examining photon emission in different ranges of wave frequency [7].

Bacteria and viruses can survive on surfaces and then be transferred to the hands of a person and later infect food. Survival of microorganisms on inanimate surfaces depends on many factors, including species, humidity, temperature, materials, and surface properties. Bacteria tend to be found in higher numbers on porous surfaces and in moist conditions [8,9]. The incidence of foodborne infections has increased worldwide over the past few years, and a significant number of people are at risk [10]. Gram-negative and Gram-positive bacterial pathogens are at the epicenter of most reported cases [11]. In addition to causing infections, Gram-negative bacteria in food can also be toxic by producing endotoxins in the food. Endotoxins are ubiquitous heat-stable lipopolysaccharide (LPS) complexes found in the outer cell membranes of Gram-negative bacteria [12]. The potency of endotoxins varies among bacterial species [13]. Human exposure to endotoxins can lead to many health complications such as septic shock, development of microvascular abnormalities, multi-organ failure and disseminated intravascular coagulation [14]. There is a need for food surveillance to ensure consumer safety and to monitor factors associated with food production [15-17]. Such factors include poor handling practices, non-standardized processing methods, involvement of various microbes and unsanitary counter display practices that may facilitate their contamination with pathogenic microorganisms and their toxins [18].

Enterococci are Gram-positive, facultative anaerobic granules. They are inherently resistant and able to survive in a wide range of hostile conditions and can persist in the environment for long periods of time [19]. They are known as commensals in the gastrointestinal tract, and play an important role in the ripening of foods and the development of specific flavors of various cheeses and can also cause spoilage of some meats [20,21]. Enterococci have gained importance as a cause of nosocomial infections, but they also occur as food contaminants and have been linked to

dental disease. *E. faecalis* has great potential to spread virulence as well as antibiotic resistance genes through horizontal gene transfer [22,23].

Although *Pseudomonas aeruginosa* is well known as a leading human opportunistic pathogen, it is an underappreciated microorganism in food safety. Due to its high adaptability and low growth requirements (low nutrient and moisture content), *P. aeruginosa* is widely distributed in the environment and commonly transmitted by humans, which consequently can cause food safety problems. In food spoilage or food poisoning, *P. aeruginosa* is frequently reported in water, dairy products, meat and plant-based foods [24].

The aim of this study was to determine the effect of microbial contamination understood as the number of microorganisms in a unit of liquid biological substance on the photon emission of the above mentioned substance. Such identification allows to parameterize the process of microorganisms multiplication in the substance with the photon emission rate, which will create a quick and contact-free method of estimating the concerned abundance of microorganisms.

Material and methods

During the experiments, strains from the strain collection of the Laboratory of Experimental Research Techniques of Raw Materials and Biological Products: *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 278596, which are representatives of Gram positive and Gram negative bacteria, respectively. In order to restore vital functions, the test microorganisms were reductively inoculated on TSA solid media. The procedure was carried out using a laminar chamber creating sterile conditions to eliminate unwanted contamination. The culture was conducted for 24 hours at 37°C. Suspensions of the test microorganisms were then prepared with optical densities of 0.2; 0.4; 0.6; 0.8 and 1 on the McFarland scale, described e.g. in [25,26]. The optical density of the sample was measured using a DEN-18 densitometer. Photon emission of the suspensions in question was determined in the Laboratory of Experimental Research Techniques of Raw Materials and Biological Products, which is accredited by the Polish Centre for Accreditation No AB 1698 for this type of measurements. In order to perform the experiments, the author's measuring system was used (Fig. 1), which allows recording the number of photons emitted per unit time. The measuring stand consists of a photon emission detector HAMAMATSU photomultiplier R4220 type, a light-proof chamber, a central unit (computer) and a monitor (for data reading in BioLumi software).

Single photon counting method was used to determine the ultra-weak photon emission. The time interval of the single photon counting operation was experimentally determined each time depending on the tested material. The minimum length of the time interval of staying of the sample in the light-proof chamber was taken as the appropriate time when the difference in the number of photons counted between two directly adjacent one-minute time intervals is less than 10%. The ultra-weak photon emission measurement result is the absolute difference between the number of photons recorded by the photomultiplier in the light-tight chamber with material and the number of photons recorded by the photomultiplier in this chamber without material, according to the relation $L=A-B$ [photon], where: L - the number of photons emitted by the test sample, A - the number of photons emitted by the sample placed in the light-proof chamber, B - the number of indications (photons) generated by the empty light-proof chamber. Calibration of the sensor was

performed each time on the day of the measurements and consisted in determining the ratio of the system response to a standard radiation dose. The experimentally determined time interval for a single measurement was 30 minutes. Before photon analysis of the microorganism samples, the sample was each time weighed and thermally stabilized.

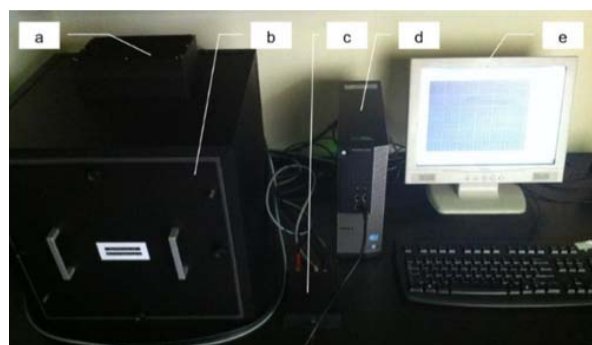


Fig. 1. Ultra-weak photon emission test rig: a - photosensitive element (photomultiplier R 1538-13), b - light-proof chamber, c - instrument interface, d - central processing unit (computer), e - BioLumi software interface

Results

In this study, the luminescence properties of two types of bacteria, viz: *Pseudomonas aeruginosa* ATCC 27859 and *Enterococcus faecalis* ATCC 29212. Figures 2 and 3 show the total number of photons emitted from the suspensions taking into account the different unit number of microorganisms in the suspension. The number of microorganisms was determined by photon emission measurement and the optical density method, which is recognized in this type of determinations.

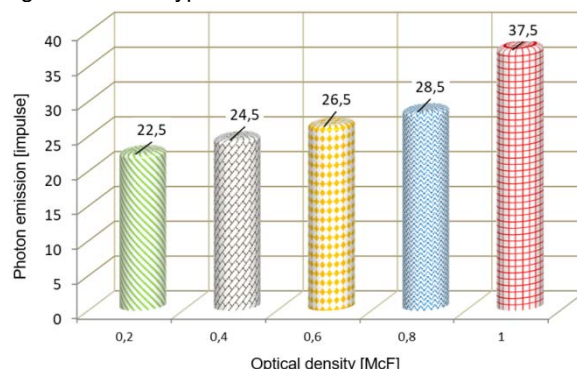


Fig. 2. Photon emission for suspension with microorganisms of the group *Enterococcus faecalis* ATCC 29212 in relation to the quantitative structure of microorganism

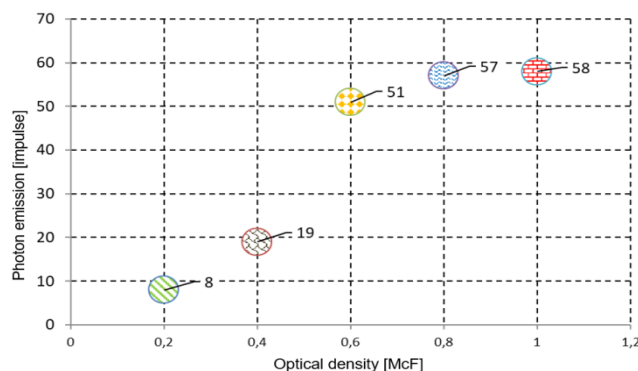


Fig. 3. Photon emission for the suspension with microorganisms of the group *Pseudomonas aeruginosa* ATCC 27859 in relation to the quantitative structure of microorganisms

It was found that for the analysis of *Enterococcus fecalias* ATCC 29212 microorganisms, the suspension with an optical density of 1 McF had the highest number of emitted photons, where the total number of photons was 38 pulses (Figure 2). In contrast, the summed number of emitted photons in the other suspensions with lower optical densities was less than 30 pulses in each case. A significant difference in the number of emitted photons of relatively about 30% was found between the suspension with an optical density of 0.8 McF and the suspension where the optical density was 1 McF. The differences in photon counts between the other suspensions were not statistically significant. It should be stated, however, that although there were no statistically significant differences in the results of the different compartments in the microbial counts, there was a great convergence in the results of determining the number of microorganisms in the suspension, by the single photon counting method and by the optical density method.

In the case of microorganisms *Pseudomonas aeruginosa* ATCC 27859, it was noted that the highest number of emitted photons was in the suspension with an optical density of 1 McF (Figure 3). In this case, the total number of photons was 58 pulses and this value was comparable to the number of photons recorded for the suspension of microorganisms *Pseudomonas aeruginosa* ATCC 27859, where the optical density was 0.8 McF and the total number of photons was 57 pulses. A significant difference in the number of emitted photons was found between the suspension with an optical density of 0.4 McF and the suspension where the optical density was 0.6 McF. In this case, there was almost a threefold difference in photon emission (32 photons). A slightly smaller, twofold difference in photon emission was observed between the first optical density interval of 0.2 McF (smallest unit number of microorganisms) and the optical density of 0.4 McF.

Analyzing the photon emission structure of the suspension with microorganisms of the group *Enterococcus fecalias* ATCC 29212 in individual measurement time intervals, it was noted that for an optical density of 0.2 McF, the highest value of photon emission was 21.5%, which was recorded in the interval 301-600 s (Fig.4). For an optical density of 0.4 McF, the highest value in the photon emission structure of more than 23% was recorded in the interval between 1501-1800s. On the other hand, for the optical density of 0.6 McF, the highest value of emission was observed in the interval between 301s and 600s, which was 19.6%. For an optical density of 0.8 McF, the highest photon emission value was observed in the measurement time interval between 1501s and 1800s, where 20.6% of the total number of emitted photons was found for this variant of microbial abundance.

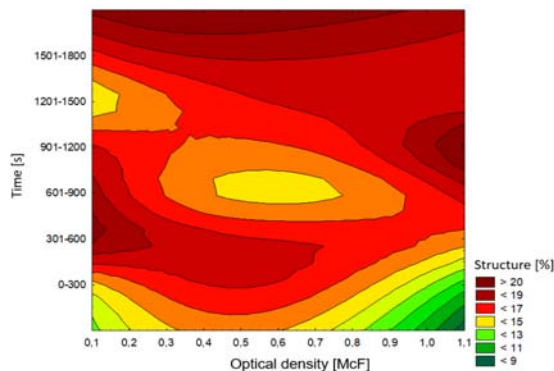


Fig. 4. Photon emission structure during suspension with microorganisms of the group *Enterococcus fecalias* ATCC 29212 in relation to the quantitative structure of microorganism

Analyzing the structure of photon emission for the abundance of the suspension of microorganisms with an optical density of 1 McF, it was found that the largest share of photon emission in relation to the total measurement interval was characterized by the time interval between 901-1200s, where the sum of emitted photons accounted for 19.6% (Fig. 4).

Considering the photon emission structure of the suspension with microorganisms of the *Pseudomonas aeruginosa* ATCC 27859 group in individual measurement intervals, it was noted that for the variant of optical density of 0.2 McF the highest value of the photon emission structure was 20% and occurred in the time interval from 1501s to 1800s (Fig. 5). On the other hand, for the optical density of 0.4 McF, the largest emission value was observed in the time interval from 301s to 600s, which was 26.3%. It should be noted that this was the highest value of photon emission contribution of all the density variants of the tested samples. On the other hand, for the optical density of 0.6 McF, the highest value of emission was observed in the time interval from 301s to 600s, which was 19.7%.

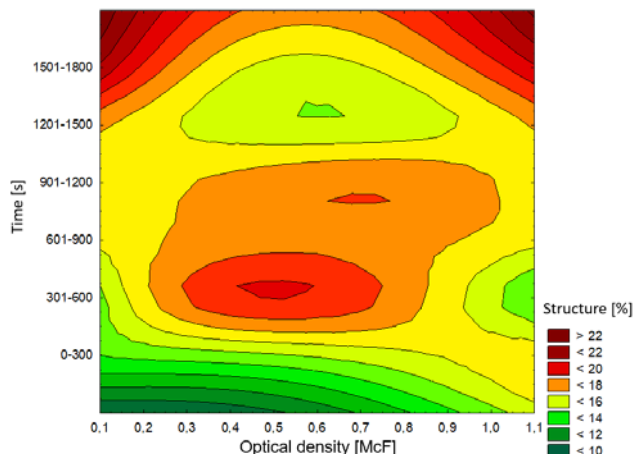


Fig. 5. Structure of photon emission during suspension with microorganisms of the *Pseudomonas aeruginosa* ATCC 27859 group in relation to the quantitative structure of the microorganisms

In the case of optical density of 0.8 McF, the highest value of photon emission was found in the measurement time interval from 901s to 1200s, where 20.6% of the total number of emitted photons was found for this variant of microorganism abundance. In the case of the structure of photon emission for the abundance of suspended microorganisms with an optical density of 1 McF, it was found that the largest share of photon emission in relation to the total measurement interval was characterized by the time intervals between 601-900s and 1501-1800s, where the sum of emitted photons accounted for 17.9% (Fig. 5).

It was observed that in the optical density range of the suspension with microorganism of the group *Enterococcus fecalias* ATCC 29212 from 0.2 McF to 1 McF, an increase in optical density of 0.1 McF will result in an increase in photon emission of 0.03847 pulses/gram of suspension. This relationship was described by a linear function of the form $y = 0.3847x + 1.3387$, where the coefficient of determination was 0.6 has the form, and the unit increase in photon emission is constant and proportional to the unit number of microorganisms. However, in the case of the relationship between the number of emitted photons of a suspension with microorganisms of the group *Pseudomonas aeruginosa* ATCC 27859 and the unit number of microorganisms identified in the subject suspension by optical density, it was found that in the range

of optical density from 0.2 McF to 1 McF, an increase in optical density of 0.1 McF will result in an increase in photon emission of 0.259 pulses/gram of suspension. This relationship was described by a linear function where the coefficient of determination was 0.7 and the equation has the form $y = 2.5936x + 0.1848$, where the unit increase in photon emission as in the case of the suspension with microorganism of the group *Enterococcus fecalias* ATCC 29212 is constant and proportional to the unit number of microorganisms in the suspension.

Conclusion

It was found that with the increase in the number of microorganisms in the suspension, the number of photons emitted increases and the trend of this increase can be described by a linear function or a second-degree polynomial with a coefficient of determination exceeding 0.9, except for microorganisms of the group *Enterococcus fecalias* ATCC 29212, where the matching coefficient was slightly over 60%. The method used to determine the abundance of *Enterococcus fecalias* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27859 by measuring the photon emission from the suspension showed a high convergence of results compared to the determination of the above mentioned parameter by the optical density method. Therefore, it can be concluded that the photon emission method is an effective way to measure the degree of contamination of liquid biological substances.

Authors: Anna Miernik MSc Eng, University of Agriculture in Krakow, Faculty of Production and Power Engineering, Balicka Av. 116B, 30-149 Krakow, E-mail: anna.miernik@urk.edu.pl; Paweł Kielbasa Assistant Professor, University of Agriculture in Krakow, Faculty of Production and Power Engineering, Balicka Av. 116B, 30-149 Krakow, E-mail: pawel.kielbasa@urk.edu.pl; Tomasz Drózdź Assistant Professor, University of Agriculture in Krakow, Faculty of Production and Power Engineering, Balicka Av. 116B, 30-149 Krakow, E-mail: tomasz.drozd@urk.edu.pl; Bartłomiej Wiśniowski MSc Eng, University of Agriculture in Krakow, Faculty of Production and Power Engineering, Balicka Av. 116B, 30-149 Krakow, E-mail: barteklive97@gmail.com

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