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Fluorescence Analysis of E. coli Bacteria in Water

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Abstract

The fluorescence analysis of Escherichia coli (E. coli) bacteria was done. It has been established that a luminescent signal from the one of metabolites (reduction form of nicotinamide adenine dinucleotide, NADH) can be adopted as a vitality indicator of the bacteria. This signal was chosen as an analytical signal. It was determined that the nature of this signal is fluorescence. In order to eliminate influence of the light scattering on this fluorescence signal optimal conditions were chosen.

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Keywords: Escherichia coli (E. coli), metabolism, NADH, fluorescence, analytical signal, light scattering, analysis conditions.

1. Introduction

Bacteria - these tiny single-celled organisms are present in the bodies of all living creatures, including humans. Bacteria play a vital role in processes such as decomposition and digestion. One of them is well-studied bacteria Escherichia coli.

Escherichia coli (E. coli) are rod-shaped, Gram-negative bacteria which are present in warm-blooded organisms in their intestines (Fig.1). They are facultative anaerobes forming metabolic products such as lactate, ethanol, succinate, carbon dioxide and acetate[1]. This bacterium is a main model organism in microbiology. They are also used in biological engineering. Most of E. coli strains are harmless, but some serotypes can cause serious food poisoning in their hosts.

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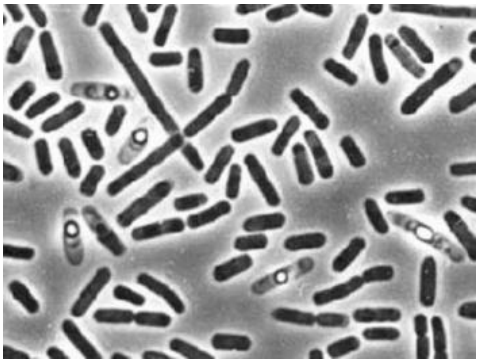

Fig.1. Escherichia coli

E. coli lives not only in living organisms, but also in water where it hits household and agricultural effluents. According to the United States Environmental Protection Agency (USEPA) *E. coli* is the best indicator of health risk from water contact in recreational waters[2]. The presence of *E. coli* in water is an indication of recent contamination from sewage or animal wastes, which may have many disease-causing organisms[2]. The presence of E-coli in water negatively affects its quality, using of this water can cause severe poisoning, which sometimes can be lethal[3]. Therefore, simple and rapid determination of E-coli presence in water is a topical problem. E-coli must be indicator bacteria. There are some criteria for indicator organisms:

1. The organism should be present whenever enteric pathogens are present
2. The organism should have a longer survival time than the hardiest enteric pathogen.
3. The organism should not grow in water.
4. The organism should be found in warm-blooded animals intestines.
5. The organism should be useful for all types of water.

None of the types of indicator organisms that are currently in use fit all of these criteria perfectly, however, when cost is considered, the use of indicators becomes necessary.

Currently existing methods for water quality determination are time consuming and lengthy because of sowing of samples on special nutritional medium and subsequent counting of the formed bacterial colonies. It is needed to create rapid and inexpensive methods for quantification of bacteria without losing its accuracy, that is why, firstly, culture of E. coli bacteria must be explored.

In this paper, as a research method luminescence analysis has been chosen. In the process of metabolism E. coli produces a substance nicotinamide adenine dinucleotide (NAD) (reduced form NADH), has a clear and stable luminescent signal[4]. According to the presence of this signal it is possible to judge the presence of bacteria in the investigated object. According to the value of this signal it is possible to determine the quantity of bacteria.

2. Materials and Methods

All investigations were done with a spectrofluorimeter “Fluorat 02 Panorama”. As an investigation object a medical drug “Colibacterin” containing E. coli bacteria was chosen. The content of one vial was dissolved in 15 ml of 0.9% sodium chloride. 3 ml of the suspension were put into a cuvette, luminescence spectrum was obtained on the excitation wavelength of 360 nm. This wavelength corresponds to the excitation of the NADH substance[5] (Fig.2). Then, an initial suspension was diluted to 5, 25 and 75 times, in order to reduce the turbidity of the sample and reduce the influence of light scattering of the cell on the luminescence signal of NADH.

To determine the nature of the luminescence signal (fluorescence or phosphorescence) the study of strobe parameters influents on signal was done. By varying the time delay and strobe duration the nature of this luminescent signal was determined. To reduce the influence of the device setting on the signal a full correction for the signal in a luminescence channel was chosen.

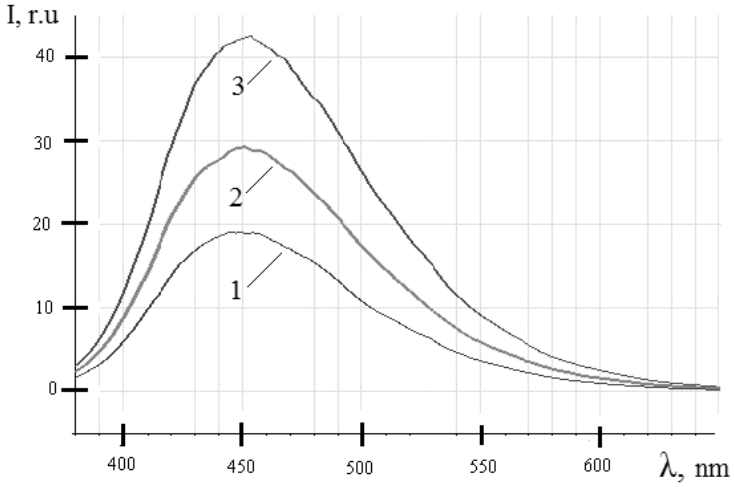

Fig.2. Luminescence spectrum of NADH: excitation wavelength – 360 nm,
1 – Concentration of NADH $C_м = 10^{-5}$, 2 - concentration of NADH $C_м = 10^{-4}$, 3 - concentration of NADH $C_м = 10^{-3}$

3. Results and Discussion

The first stage was dilution of the initial suspension in 75 times in order to reduce the effect of light scattering by the bacterial cells on the luminescence signal. The content of bacteria in the initial suspension was $1*10^9$ cells. The bacterial content in the analyzed suspension was $8 * 10^7$ bacteria.

To determine the presence of bacteria in the analyzed sample, a luminescence spectrum of the suspension at the excitation wavelength of 360 nm was obtained (Figure 3). The intensity of the signal is 11 units; it proves the presence of bacteria in the sample.

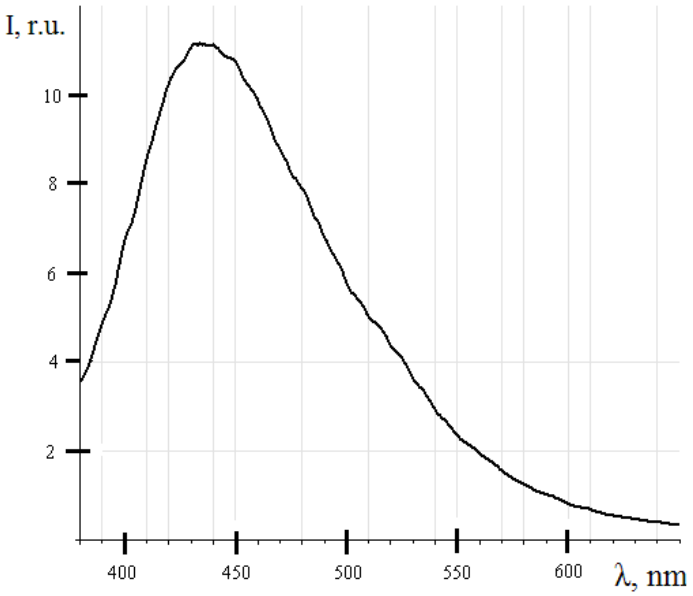

Fig. 3. Luminescence signal of NADH in E-coli

The position of the maximum of NADH luminescence peak in E-coli is different from the position of the peak maximum of the NADH standard solution. This difference is 20 nm. It is explained by the fact that binding of NAD with its dehydrogenase leads to the shift of the luminescence maximum to shorter wavelengths up to 440 nm.

It is necessary to select optimum conditions of the signal correction and strobe settings for improving the luminescence signal.

The second stage was determination of optimal parameters for the analysis. The effect of the strobe settings changing on a luminescence signal was investigated. Fig. 3 shows luminescence spectra of the suspension, which has been obtained for various parameter values of the strobe. Changing the delay time four spectra were obtained at a maximum duration parameter.

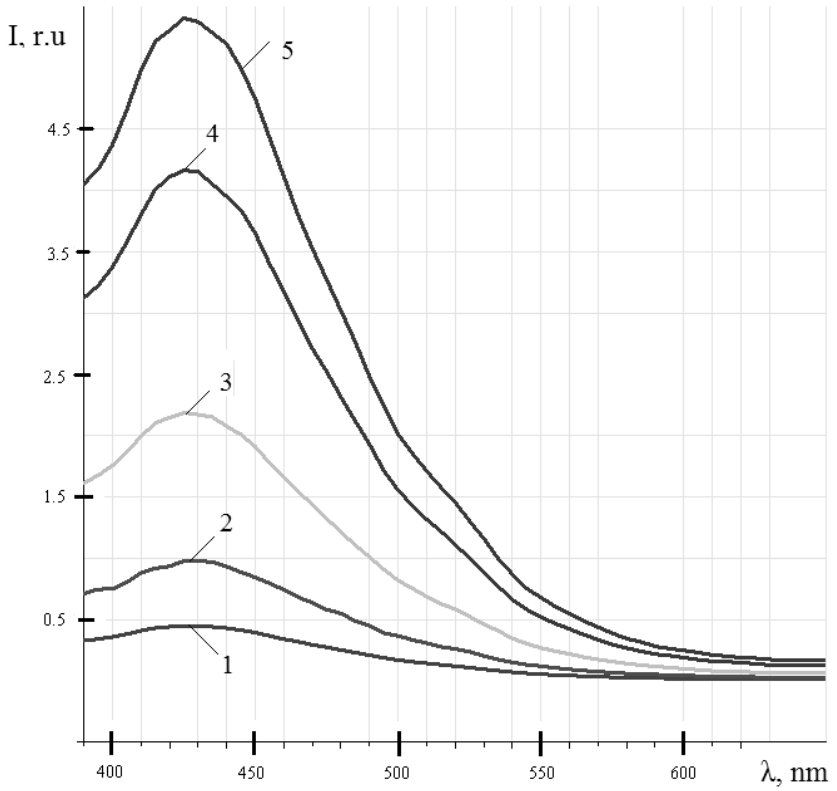

Fig. 4. Dependence of the NADH luminescence signal intensity on changing of strobe parameters. Excitation wavelength of 360 nm:
1 - Delay time-5 microseconds, duration-100 microseconds;
2 - Delay time-4 microseconds, duration-100 microseconds;
3 - Delay time-3 microseconds, duration-100 microseconds;
4 - Delay time-2 microseconds, duration-100 microseconds;
5 - Delay time-1 microsecond, duration-100 microseconds.

The figure shows that the highest signal intensity is achieved when the value of delay time is 1 microsecond.

As the delay time reduction leads to the signal increase, therefore, the nature of the signal is luminescence. It has been found that the duration of strobe does not have a significant effect on signal intensity (Fig.4). That is why in this study the average value of duration of 40 microseconds was selected.

Those spectra were obtained with a full correction of the signal in order to remove the influence of the device's noise on the NADH luminescence signal.

4. Conclusion

In this paper, optimal conditions of fluorescence analysis of Escherichia coli were chosen.

For establishing an analytical signal model the system based on the medication "Colibacterin" containing coliform bacteria was prepared. It was determined that a luminescent signal from one of the bacterial metabolites (NADH) can be an analytical signal. It was proved that excitation wavelength of 360 nm and emission wavelength of 460 nm correspond to NADH. It was determined, that in the objects such as E.coli bacteria, maximum of NADH emission peak is 440 nm. It is explained by the fact of binding of NAD with its dehydrogenase.

During the experiment it was established that a NADH luminescence signal is influenced by the light scattering by bacterial cells. In order to reduce the effect of scattering optimal dilution of the initial suspension was chosen. This dilution was 75 times. The amount of the cells in the analyzed object was $8 * 10^7$ CFU.

To determine the nature of the luminescence signal (fluorescence or phosphorescence) the study of strobe parameters affecting the signal was done. It was established that decreasing the delay time leads to increasing the signal intensity. It proves that the nature of the analyzed signal is fluorescence.

It was determined that a necessary condition for the analysis is a full correction of the received signal. This correction removes the influence of the device's noise on the NADH luminescence signal.

Acknowledgments

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