NUCLEAR AND RELATED ANALYTICAL TECHNIQUES FOR BIO-NANO-TECHNOLOGY

Frontasyeva Marina.V.*

Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research,
str. Joliot-Curie, 6, 141980 Dubna, Moscow Region, Russian Federation

ABSTRACT

Some results from applying nuclear and related analytical techniques in medical, environmental and industrial biotechnologies are presented. In the biomedical experiments biomass from the blue-green alga *Spirulina platensis* (*S. platensis*) has been used as a matrix for the development of pharmaceutical substances containing such essential trace elements as selenium, chromium and iodine. The feasibility of target-oriented introduction of these elements into *S. platensis* biocomplexes retaining its protein composition and natural beneficial properties was shown. The negative influence of mercury on growth dynamics of *S. platensis* was observed. Detoxification of Cr and Hg by *Arthrobacter globiformis* 151B was demonstrated. Microbial synthesis of technologically important silver nanoparticles by the novel actinomycete strain *Streptomyces glaucus* 71 MD and *S. platensis* was characterized by a combined use of scanning electron microscopy (SEM) and epithermal neutron activation analysis (ENAA).

Key words: biotechnology, nanotechnology, ENAA, SEM, Spirulina platensis, Arthrobacter globiformis 151B, Streptomyces glaucus 71 MD

INTRODUCTION

Investigation in the field of biotechnology by using various microorganisms is one of actual trends in life sciences and industry. Owing to their small size, relatively simple morphology, and reproduction modes, microorganisms can be easily cultivated under laboratory conditions by microbiological methods in artificial mineral or organic solid or liquid nutrient media.

Neutron activation analysis with epithermal neutrons (ENAA) was used as a well-proven analytical technique for the determination of elemental composition of biological objects. Due to activation with resonance neutrons, the technique makes it possible to minimize matrix effects of biological samples and at the same time to determine concentrations of over 40 major, minor and trace elements. Biochemical investigations for substantiation of the experimental technique were carried out at the Andronikashvili Institute of Physics (Tbilisi, Georgia). Analytical research was conducted at the IBR-2 pulsed fast reactor of the FLNP JINR (Dubna, Russia). Using ENAA, significant results were ob-

* e-mail: marina@nf.jinr.ru, tel: +7(49621)65609, fax: +7(49621)65085

tained in the following three directions – medical biotechnology [1], environmental biotechnology [2–4] and industrial biotechnology [5]. The fourth promising direction of ENAA application in nano-biotechnologies using microorganisms is under development [6]. These studies are reviewed in the given paper.

MEDICAL BIOTECHNOLOGY

The blue-green algae *S. platensis* is a living microorganism and in the process of cell cultivation it is capable to assimilate certain amounts of some essential microelements (Se, Cr, and I) from a nutrient medium and to incorporate them into the composition of its biological macromolecules.

The analytical control of accumulation process makes it possible to establish a unique dependence between the element concentration in the nutrient medium and its content in the obtained *S. platensis* biomass. This dependence determined by means of ENAA serves as a basis for substantiation of biotechnology for the production of substances for pharmaceutical preparations with required doses of a given essential element.

Based on the results of ENAA, the curves of concentrations of elements in question in the obtained *S. platensis* substance *versus* concentrations of these elements in the nutrient medium were built. These curves served as the basis for biotechnology of cultivation of Se, Cr, I- containing biomass of *S. platensis* and they allow a precise choice of doses depending of the purposes these medications (therapeutic or prophylactic) are created for [1].

Methods for obtaining selenium and chromium-containing substances of spirulina are protected by patents of the Russian Federation. Visual microscopic observation of the state of culture, determination of total protein content in the biomass as well as investigation of its electrophoregrams revealed natural properties of the obtained Se-, Cr-, I-containing biomass.

ENVIRONMENTAL BIOTECHNOLOGY

Spirulina platensis as a sorbent of mercury. The possibility to use spirulina in remediation of sewage waters from toxic metal – mercury was studied by means of ENAA at the reactor IBR-2, FLNP JINR. The accumulation of metals in the cells of microorganisms goes at different rates. The binding time of a metal depends on the accumulation mechanism. If a metal is adsorbed on the cell surface, which is caused by ionic interaction, that does not depend on temperature and does not take energy, the process goes fast and takes from several seconds to an hour. The process of active transport of metals inside the cell goes more slowly and takes more than one hour. In the long-term experiments to study the Hg accumulation by the *S. platensis* cells the concentrations of Hg in nutrient medium loading by mercury glycinate constituted 100, 50, 5 μg Hg/L. Cultivation of the *S. platensis* cells was conducted for 6 days. Samples in all the series were taken every 24 hours.

The results of ENAA show the exponential character of the dependence of Hg concentrations in *S. platensis* biomass on time. Such character of dependence seems to be clear, as the number of *S. platensis* cells grows exponentially, the number of sites of Hg(II) ion binding surpasses considerably the number of Hg(II) ions in nutrient medium [2]. In the short-term experiments the Hg adsorption by *S. platensis* cells was studied. Concentration of nutrient medium loading of mercury glycinate was 500 μg Hg/L. Dynamics of the adsorption process, usually taking place during 1–2 hours, was observed during 1 hour.

Theoretical calculations of the adsorption isotherm on the basis of the obtained experimental data were performed in accordance with the Langmuir-Freindlich model, which takes into account both physical adsorption and chemisorption. Thus, at relatively low Hg concentrations (of the order of 100 μg/L) in the medium *S. platensis* can be used in the remediation of industrial and sewage waters from mercury.

*Biotechnology of Cr(VI) and Hg(II) transformation***.** The mechanisms of toxic Cr(VI) transformation into non-toxicCr(III) by Cr(VI)-reducer bacteria belonging to *Arthrobacter* genera isolated from the polluted basalts from the Republic of Georgia have been studied. In the experiments the dose-dependent formation of Cr(III) complexes and uptake of chromium by *Arthrobacter oxydans* – a gram-positive bacterium from these rocks were studied along with the testing under aerobic conditions of two bacterial strains of *Arthrobacter* genera [3]. ENAA in Dubna was used to track accumulation of chromium in the bacterial cells. To monitor and identify Cr(III) complexes in these bacteria, the electron spin resonance (ESR) spectrometry was employed. It was shown that the tested bacteria of *Arthrobacter genera* could efficiently detoxify high concentrations of Cr(VI). It was revealed that under aerobic conditions accumulation of chromium in bacteria is dose-dependent and its character is changing significantly at higher concentrations of Cr(VI). The chromium accumulation process fits well with the Langmuir-Freundlich model.

Several types of bacteria (*Arthrobacter* genera) can reduce of cationic mercury Hg(II) to the elemental state Hg(0). INAA was applied to study accumulation of Hg(II) in *A. globiformis* 151B and accumulation of Cr(VI) in bacterial cells in the presence of Hg(II) [4]. Experiments were carried out using the facilities of the 2 MW nuclear research reactor ''Hoger Onderwijs Reactor'' of the Reactor Institute Delft, Delft University of Technology, the Netherlands [4]. In experiments the ability of *A. globiformis* 151B to accumulate Hg(II) was investigated at different (50–5000 μg/L) Hg(II) concentrations. The strong accumulation of mercury by bacteria suggests that this undesirable element might be removed from the environment by bacterial trapping and sequestration. Addition of Hg(II) to *A. globiformis* 151B cells enhanced significantly the accumulation and toxicity of other heavy metals, namely Cr(VI) in our case.

INDUSTRIAL BIOTECHNOLOGY

An attempt to study the technological process of bacterial leaching of a wide range of rare and scattered elements contained at low concentrations in lean ores, rocks and industrial wastes in Georgia was undertaken by instrumental epithermal neutron activation analysis at the IBR-2 reactor, FLNP JINR, Dubna. As the source of microorganisms the natural organic mass of vegetal origin – peat – was used. Abundance of peat bogs in West Georgia and thus the opportunity of obtaining a cheap source of microorganisms with their inherent optimal bacterial content predetermined our choice of peat as the natural source of microorganisms.

The study of behaviour of different elements during the bacterial leaching was conducted on the basis of neutron-activation analysis of 30 elements of initial and leached metals also.

The results of NAA showed that bacterial leaching may be used for extraction such elements as Au, Se, Rh, In, Cd, Ir, Ru, Hf, Ta, Zr, as well as the radioactive elements Sr, U, Th [5].

MICROBIAL SYNTHESIS OF NANOPARTICLES

In our study a novel strain of actinomycetes, *Streptomyces glaucus* 71MD, isolated in Georgia, was used to produce silver nanoparticles [6]. In addition, synthesis of silver nanoparticles by *S. platensis* was investigated.

Scanning electron microscopy (SEM), transmission electron microscopy (TEM) and energy-dispersive analysis of X-rays (EDAX) were used as the key techniques. According to our experiments, the tested actinomycete *Streptomyces glaucus* 71 MD produces silver nanoparticles extracellularly when acted upon by the silver nitrate solution ($AgNO_3$), which offers a great advantage over an intracellular process of synthesis from the point of view of applications. Production of silver nanoparticles proceeded extracellularly by blue-green microalgae *S. platensis*; however, in this study we have shown that this process depends on the experimental conditions. Specifically, it is different under the short-term and long-term silver action.

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