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APPLICATION OF LASER BIOTECHNOLOGY FOR MORE EFFICIENT PHYTOREMEDIATION OF BIOGENIC ELEMENTS

ZASTOSOWANIE BIOTECHNOLOGII LASEROWEJ DO ZWIĘKSZENIA FITOREMEDIACJI PIERWIASTKÓW BIOGENNYCH

Abstract: Some species of aquatic plants have capability to remove biogenic elements from contaminated water and therefore are used for water treatment. Some hydrophytes (like eg duckweed, yellow iris, reed-mace, common reed) are used in small household hydrobotanical treatment plants.

The aim of experiment was to increase the efficiency of hydrobotanical sewage treatment plant by the stimulation of the duckweed (*Lemna minor*) and yellow iris (*Iris pseudoacorus*) with the argon laser and two laser diodes. The groups of experimental plants were exposed to different parameters of laser stimulation. The results of experiments showed that photostimulation by laser light significantly speeds up cell divisions and causes a significant growth of biomass stimulating quicker and more efficient uptake of biogenic elements contained in sewage. The beneficial influence of laser biotechnology was extension of the vegetation season of plants.

Keywords: laser stimulation, phytoremediation, hydrophytes, biogenic elements, eutrophication, hydrobotanical treatment plant

Eutrophication is an increase of the natural fertility in water reservoirs caused by the inflow of biogenic compounds. The main sources of water pollution are sewage effluents, waste dumps, septic tanks and agricultural flows. Significant quantity of nutrients disturb the naturally balance in water ecosystems and accelerate the primary production. The effective solution for improving water quality is to use in small household hydrobotanical treatment plants, based on hydrophytes' ability to bioremediation of biogenic elements from water.

Cleanup technologies based on phytoremediation process are cheap, effective, comfortable in use and friendly to the environment. The efficiency of purification depends on vegetation season, plants sensitivity on a large range of pollutants

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concentration and slow biomass increase. Attempts of increase phytoremediation efficiency rely mostly on genetic engineering, agrotechnical measures and use of nontoxic chelation compounds (EC Regulation No. 16702007 of 19th February 2007).

The application of the laser stimulation in environmental biotechnology to the optimization of natural processes (eg to sewage treatment, soil reclamation and sewage sludge management) was introduced by Dobrowolski in the last decades of 20th century [1]. Effects of photostimulation by monochromatic, coherent and polarized light on biological material can increase the base for the activation of biological mechanisms.

The laser biostimulation of plants increase their ecological resistance on unfavorable environmental factors: low temperatures, a short growth season and environmental pollution. [2–5]. Specific parameters of laser stimulation (the type of diode, wave length, time and power of irradiation) of hydrophytes may prolong their growth season and increase the biomass production in experimental groups. A very interesting issue is a biostimulation effect on phytoremediation abilities. Laser biotechnology is also promising for more efficient bioremediation of trace elements and biogenic from contaminated soil and sewage [1, 6, 7].

Material and methods

The purpose of research was to increase the efficiency of hydrobotanical method of sewage treatment by the photostimulation of some species of hydrophytes.

Duckweed (*Lemna minor*) and additionally yellow iris (*Iris pseudoacorus*) were chosen as an experimental material. These plant species are used in hydrobotanical and municipal sewage treatment plants. The experimental samples were exposed to variable parameters of laser stimulation: the type of diode, wave length, time and power of irradiation (four parameters optimization). Each of experimental plant groups were irradiated with argon laser and laser diodes. These coherent light sources emit light wave corresponding to blue and red colours.

Following light sources were used in the experiment:

– red light emitting laser diode with a wavelength of 660 nanometers and output power of 20 mW,

– blue light emitting laser diode with a wavelength of 473 nanometers and output power of 20 mW, produced by Changchun New Industries Optoelectronics Tech Co.,

– argon gas laser with a wavelength of 514 nanometers (celadon) and output power of 20 mW, type ILA-120, produced by Carl Zeiss Jena (Ar).

Parameters of laser stimulation were optimized from the point of plants biomass increase. The experimental samples were exposed to variable parameters of laser stimulation: the type of diode, wavelength, time and power of irradiation. It was twenty centimeters distance between coherent light source and irradiated plants. Parameters of stimulation were chosen individually for each plant species. Incidence angle of radiation beam was perpendicular. Parameters of stimulation were chosen in laboratory conditions and after them experiment was continued in natural conditions. One part of experimental groups was left without exposure on laser light as control groups. All groups were planted in ponds filled with partially treated sewage that was previously

subjected to mechanical treatment process. Initially, all plants in each group were in similar condition. Total number of duckweed in each group was the same. Rootstocks of yellow iris in groups were selected according to their weight.

During cultivation, after each vegetation season, observations in equal time intervals were made on growth rate, health condition and changes in the number of plants in experimental groups.

For measurement of biomass increase microscope Nikon Eclipse e6000 with experimental setting to visualization and picture analyze, digital video camera Nikon DXM 1200, digital camera Nikon Coolpix 995 and image analysis software Aphelion, version 3.0 were use. Biomass of plants was also measured with a laboratory scale.

Biogenic elements (N, K) content in the dry plant biomass was measured by atomic absorption spectrometry method (AAS) after each of the growth seasons.

Results and discussion

The research objective was finding optimal laser stimulation parameters fitting selected hydrophytes species that could be use in hydrobotanical treatment plants. The results of experiments can make a base to create optimal parameters of the preparation of plant material for sewage treatment process. The laser stimulation caused faster growth of selected plants species in experimental groups.

Optimal parameters of laser stimulation (the type of coherent light source, radiation power, time and irradiation method) of duckweed (*Lemna minor*) were obtained for the experimental group irradiated with laser diode with the following parameters: wavelength of light emitting: λ = 660 nm, power of radiation 20 mW and irradiation time: 3 times for 3 seconds (Fig. 1). The biggest increase of biomass, in comparison with control group, was observed in this experimental group (300 %) already after at the end

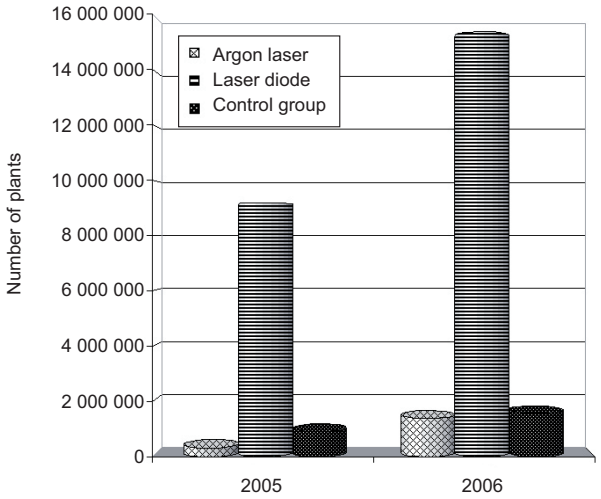

Fig. 1. Comparison of duckweed biomass increase in experimental groups, years 2005–2006

of the first growth season. This effect lasted during subsequent vegetation seasons without repetition of laser stimulation because generative propagation of duckweed causes biostimulation effects in filial plants.

The group of experimental plants irradiated with laser diode was also characterized by better survival rate and resistance to unfavorable environmental conditions such as temperature decrease. This group was also characterized by a small number of plants with chlorosis symptoms.

It was observed that there were considerable differences between plants from experimental groups planted in similar environmental conditions. The duckweed after irradiation with argon laser emits light at $\lambda = 514$ nm (during 3 times for seconds) have the bigger surface of leafs recounted per one plant.

The increase of nitrogen and phosphorus concentration in dry duckweed biomass after chemical analysis in experimental groups irradiated with argon laser was found (twice in comparison with control groups) and laser diode ($\lambda = 660$ nm) (Figs. 2, 3).

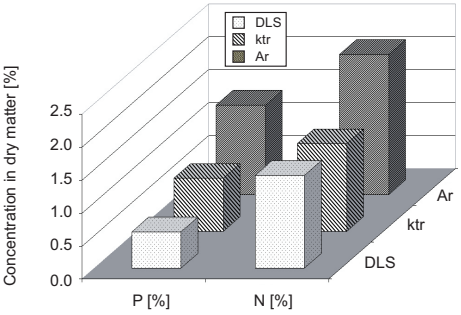

Fig. 2. Comparison of biogenic elements concentration in dry mass of duckweed (*Lemna minor*) in experimental groups in 2006 (ktr – control group, Ar – plants irradiated with an argon laser $\lambda = 514$ nm., DLS – plants irradiated with a laser diode $\lambda = 660$ nm)

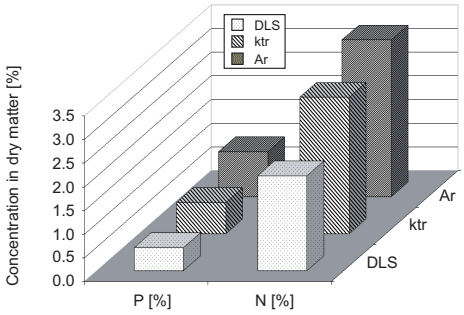

Fig. 3. Comparison of biogenic elements concentration in dry mass of duckweed (*Lemna minor*) in experimental groups in 2007 (ktr – control group, Ar – plants irradiated with an argon laser $\lambda = 514$ nm., DLS – plants irradiated with a laser diode $\lambda = 660$ nm)

Each of hydrophytes species has a different uptake ability of biogenic elements from contaminated water. The duckweed (*Lemna minor*) has specific ability to nutrients remediation. Nitrogen and phosphorus content in duckweed biomass are not a linear function of these elements concentration in water, but confirm cumulative properties of the plant. The ability of the duckweed to store high nutrient levels in tissues is called the "luxury uptake" [6].

The largest biomass increase of the yellow iris (*Iris pseudoacorus*) was in experimental group which was exposed to argon laser (wavelength $\lambda = 514$ nm) for 3 times for 30 seconds (Fig. 4). Yellow iris is not universally used in sewage treatment process, but it may become a valuable element of garden sewage treatment plant for its aesthetic values. The laser stimulation of iris rootstocks caused a significant increase of leaf biomass, number of flowers and plants regeneration after autumn cutting.

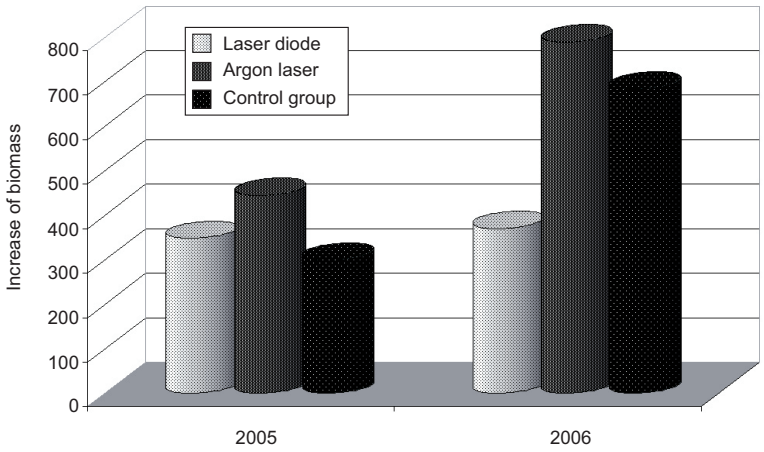

Fig. 4. Comparison of yellow iris biomass increase in experimental groups, years 2005–2006

Differences of biogenic elements concentration in the yellow iris dry biomass from experimental samples were not observed.

It has to be asserted that a choice of adequate hydrophyte species characterized by luxury uptake enables to remove biogenic elements from sewage in a quantity proportional to the size of duckweed biomass in a sewage pond. Optimization of laser stimulation parameters should carry on increase biogenic elements uptake, not only by biomass increase but also by nutrients concentration in dry mass of plants. Removing of nutrients from sewage is a very important part of sewage treatment process because surplus of biogenic compounds may cause surface waters eutrophication [5, 6, 11].

The results of the research showed that stimulation by coherent, monochromatic and polarized light significantly speeds up cell divisions and causes a significant growth of plants.

Application of laser stimulation enables to optimize natural biological processes and use them in environmental biotechnology without changes in plants genotype, but by optimal phenotype expression. The biggest differences in stimulation effects were observed between experimental groups planted in a polluted environment [7].

Conclusions

It was observed that photostimulation by laser light caused a significant growth of biomass and increase plants ability to the remediation of pollutants.

This thesis showed:

– statistically important increase of irradiated plant biomass in comparison with control group,

– increased plants resistance to environmental pollutions and unfavorable environmental factors: low temperatures, short growth season and environmental pollution,

– increased concentration of biogenic elements in plants tissues and slowed down eutrophication process,

– irradiation of plants with a coherent light caused reduction of trace elements concentration (Pb, Zn, Ni, Cd) in duckweed tissues and protected plants against phytotoxic effects [8, 12],

– biostimulation effects in duckweed groups kept during subsequent vegetation seasons without the repetition of irradiation.

It was stated additionally that biostimulation effects after irradiation with argon laser (λ = 514 nm) and blue laser diode (λ = 473nm) were very similar. These light sources had a similar physical characteristic, but they were significantly different in costs of capital expenditure and exploitation. Application of a laser diode in laser biotechnology was more economical.

Acknowledgements

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ZASTOSOWANIE BIOTECHNOLOGII LASEROWEJ DO ZWIĘKSZENIA FITOREMEDIACJI PIERWIASTKÓW BIOGENNYCH

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Abstrakt: Fitoremediacja to metoda, w której do oczyszczania m.in. wód powierzchniowych, gruntowych oraz gleb wykorzystuje się zdolności różnych gatunków roślin do usuwania, stabilizowania i unieszkodliwiania zanieczyszczeń. Pewne gatunki hydrofitów są wykorzystywane powszechnie w hydrobotanicznych oczyszczalniach ścieków.

Celem przeprowadzonych doświadczeń była próba zwiększenia skuteczności hydrofitowej oczyszczalni ścieków poprzez stymulację rzęsy drobnej (*Lemna minor*) i kosaćca żółtego (*Iris pseudoacorus*) przy użyciu diod laserowych oraz lasera argonowego. Grupy roślin doświadczalnych poddano ekspozycji na działanie światła spójnego, stosując różne algorytmy naświetlania. Uzyskane wyniki potwierdziły statystycznie wpływ światła spójnego na przyspieszenie podziału komórek, co prowadziło do zwiększenia przyrostu biomasy oraz szybszego wychwytu pierwiastków biogennych. Dodatkowo zaobserwowano wydłużenie wegetacji roślin w grupach doświadczalnych.

Słowa kluczowe: stymulacja laserowa, fitoremadiacja, hydrofity, pierwiastki biogenne, eutrofizacja, roślinne oczyszczalnie ścieków