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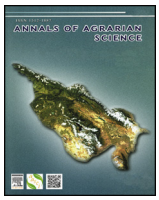

About possibility of alga Spirulina application for phytoremediation of water polluted with 2,4,6-trinitrotoluene

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ABSTRACT

The aim of presented work is to estimate remediation potential of the blue-green alga Spirulina (*Spirulina platensis*), in particular, its ability to uptake 2,4,6-trinitrotoluene (TNT) that is one of the most widely used military high explosive and a dangerous pollutant of the environment. The obtained results show that Spirulina has high ability to adsorb TNT, and the uptake of TNT and/or its metabolites by Spirulina carried out via adsorption on surface of cellular hydrocarbons coat and then by their moving into vacuoles. The model experiments for testing the feasibility of the alga-based approach of phytoremediation technology were performed in reservoir with a volume of 40 L, with permanent air barbotage and illumination, at temperature 25C. The results of experiments indicate that Spirulina uptakes about 87% of toxicant from water polluted with 22.5 ppm TNT during 15 days, and its biomass accumulation decreases only by 38% compared to the reference variant, where the algae was cultivated in an uncontaminated medium. The results of model experiments signify that application of *Spirulina Platensis* as phytoremediator is an efficient tool for cleaning TNT-polluted water.

Introduction

Hundreds of millions of tons of chemicals are produced in the world annually. These compounds, most often toxicants, are concentrated in biosphere in huge amounts by different ways, and considerably affect the ecological balance. Plants may be successfully used for long-term protection of environment and disposal of chemical toxicants from the environment or their conversion into common non-toxic compounds. Nowadays, phytoremediation technologies successfully replace non-biological methods of remediation of environment. Special phytoremediation technologies have been developed recently, based on the unique abilities of plants and microorganisms to assimilate a wide range of toxic compounds: aliphatic, aromatic and polycyclic hydrocarbons, phenols, pesticides, organochlorine compounds, explosives based on nitro compounds, heavy metals, radionuclides, etc. [1–3].

Modern phytoremediation technologies may include different methodological approaches, depending on their purpose. The chemical nature and concentration of a toxicant, an object needing remediation (soil patch, water reservoir, wastewater, groundwater, etc.), climate, etc. determine the type and diversity of possible phytoremediation technologies. One of the methods to clean chemically polluted waters is Phycoremediation based on application of algae [4]. There are some examples of algae usage for cleaning water polluted with heavy metals, petroleum hydrocarbons, pesticides, etc [4–8].

Spirulina (*Spirulina platensis*) should have prospects for phytoremediation of waters polluted by different toxic compounds. According to unique chemical content and biological properties, besides biologists Spirulina is an object of interest for pharmacists and food industry workers [9,10]. Our interest in Spirulina as potential phytoremediator is caused by the following factors:

- Spirulina is characterized by reproduction and fast biomass formation in extreme conditions (alkali area, high mineralization of medium) that are unfavorable for growing of most microorganisms.
- Spirulina has rich content of proteins and peptides not only from algae, but also from whole plant kingdom. Supposedly, these compounds should be immobilizing heavy metals as well as conjugating metabolites of organic pollutants.

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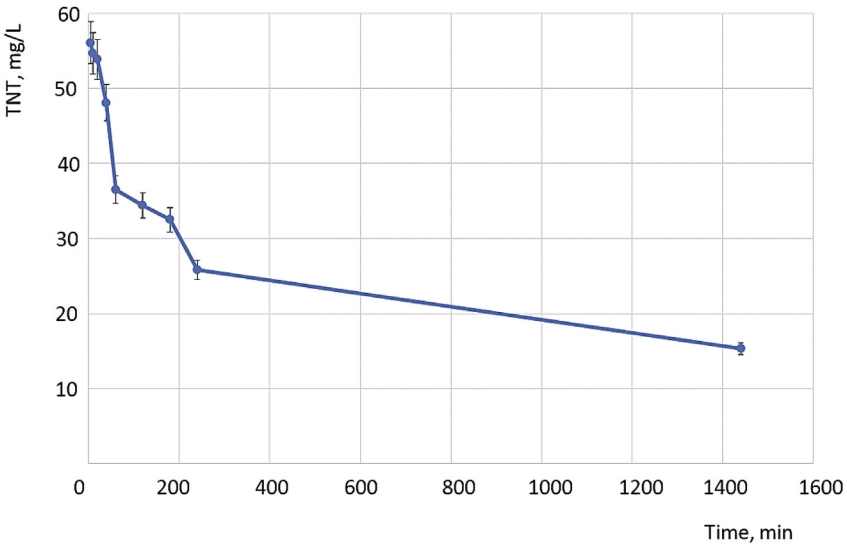

Fig. 1. The dynamics of TNT uptake by Spirulina. Incubation medium (volume – 1 L): pH – 8.7; content in g/L: TNT – 0.056, $NaHCO_3$ – 8.4, K_2HPO_4 - 0.25, $NaNO_3$ – 1.25, K_2SO_4 – 0.5, NaCl – 0.5, $MgSO_4{\cdot}7H_2O$ – 0.1, $CaCl_2{\cdot}2H_2O$ – 0.02, $FeSO_4{\cdot}7H_2O$ – 0.005, EDTA – 0.04; and microelements kit A5 – 0.5 mL; the initial biomass of Spirulina – 5 g/L. The incubation was carried out in a glass beaker with volume 1.5 L, at temperature 25 °C, under following illumination conditions: a photoperiod of lighting 16 L/8D, PPFD »15 μmol m^{-2} s^{-1}. The number of replicates – 3.

- During the aging, the vacuoles of Spirulina cells inflate with air bubbles and as a result, Spirulina colonies float onto the water surface. Therefore, after remediation, Spirulina biomass will be easily (mechanically) separated from cleared water body.

However, there is only little information concerning using alga Spirulina in phytoremediation technologies. Among them are researches about heavy metals uptake processes by Spirulina [5,7]. Recently some data has shown using Spirulina for removal of some estrogens from wastewater [11]. Thus, evaluation of ecological potential of Spirulina, in particular, its tolerance and detoxification ability towards organic ecotoxicants, is valuable for xenobiochemistry. The aim of presented work is to estimate ability of Spirulina to uptake 2,4,6-trinitrotoluene (TNT) that is one of the most widely used military high explosive and a dangerous pollutant of the environment [12].

Materials and methods

In the experiments were used the biomass *Spirulina platensis* obtained via cultivation of the alga in standard Zarrouk's medium (pH – 8.7; content in g/L: $NaHCO_3$ – 16.8, K_2HPO_4 - 0.5, $NaNO_3$ – 2.5, K_2SO_4 – 1.0, NaCl – 1.0, $MgSO_4{\cdot}7H_2O$ – 0.2, $CaCl_2{\cdot}2H_2O$ – 0.04, $FeSO_4{\cdot}7H_2O$ – 0.01, EDTA – 0.08; and microelements kit A5 – 1 mL). The incubation was carried out with permanent air barbotage (rate of air flow 2 L/min), at temperature 25 °C, and under following illumination conditions: a photoperiod of lighting 16 L/8D (16 h of light: 8 h of dark), a total photosynthetic photon flux density (PPFD) of »15 μmol m^{-2} s^{-1}.

Experiments for determining the ability of Spirulina to assimilate of TNT from polluted water were carried out in different conditions. The details of separate experiments are given on corresponding legends of the figures.

The biomass of Spirulina was measured spectrophotometrically at 750 nm [13].

TNT content in incubation medium was measured by a spectrophotometric method using 447 nm extinction in a highly alkaline area (pH > 12.2) [14].

For determination of ultrastructural localization of assimilated TNT in Spirulina cells has been used a modified autoradiography method

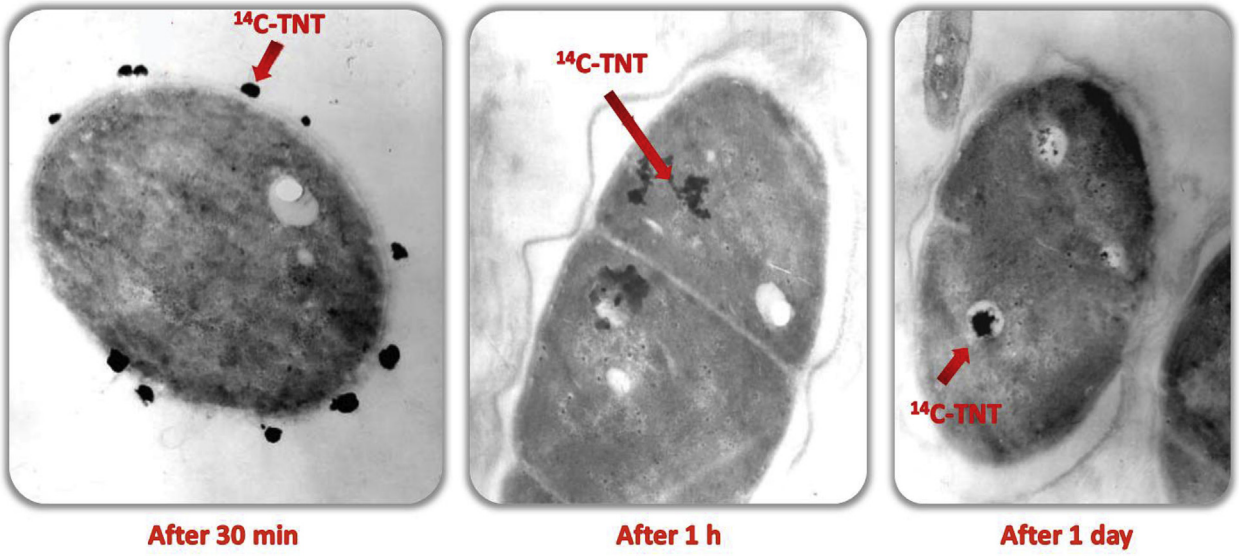

Fig. 2. Localization of [1–^{14}C] TNT in Spirulina cells. Incubation medium (volume – 0.5 L): pH – 8.7; content in g/L: [1–^{14}C] TNT – 0.056, $NaHCO_3$ – 8.4, K_2HPO_4 - 0.25, $NaNO_3$ – 1.25, K_2SO_4 – 0.5, NaCl – 0.5, $MgSO_4{\cdot}7H_2O$ – 0.1, $CaCl_2{\cdot}2H_2O$ – 0.02, $FeSO_4{\cdot}7H_2O$ – 0.005, EDTA – 0.04; and microelements kit A5 – 0.5 mL, the initial biomass of Spirulina – 5 g/L. The incubation was carried out in a glass flacks with volume 1 L, at temperature 25 °C, under following illumination conditions: a photoperiod of lighting 16 L/8D, PPFD »15 μmol m^{-2} s^{-1}.