

Effect of Co(II) ions on frozen at -80°C and -20°C and with Cs^{137} irradiated *Spirulina* after cultivation

Abstract

Influence of Co(II) ions on frozen at -80°C and -20°C temperature and high dose irradiated Cs^{137} (400kGy) and then recultivated on *spirulina*, when after cultivation all samples were placed in 4°C temperature during three weeks were studied using UV-Visible spectrometer. The absorption processes was relatively fast in the small concentrations for Co(II) in the case of interaction with irradiated *spirulina*, but for frozen *spirulina* absorption decrease was regarded very slow.

It was shown, that effect of Co(II) ions on *spirulina* constituents ((Chlorophyll a, phycocyanin, carotenoids) at various conditions are different.

Keywords: *Spirulina*, Co(II) ions

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Introduction

Blue-green algae (cyanobacteria) *spirulina*, is an alternative source of protein for human food and feed purposes. It involves polysaccharides, lipids, and vitamins.^{1,2} Currently, *spirulina* is commercialized and sold for therapeutic purposes. It has immune protective, anti-diabetic, antiviral, etc. properties, has the ability to dramatically reduce the chance of developing cancer, heart disease or stroke. *Spirulina* is known to ameliorate organ toxicity caused by heavy metals.

Some studies have shown that conventional drying systems may be ineffective in preserving the nutritional quality of microalgae. Therefore, alternative methods that better preserve its functional and nutritional properties, especially the content of bioactive compounds, should be considered and evaluated.^{3,4} In works^{5,6} was shown that all sample versions of *spirulina* is radioresistant, when the algae is irradiated with high doses of Cs^{137} gamma irradiation. Therefore, the use of high dose of radiation can be an interesting alternative for processing *spirulina* biomass due to its advantages over other conventional methods. In cyanobacteria, metals are exerting toxic action mostly by damaging chloroplast and disturbing photosynthesis.⁷ In works^{8,9} effect of toxic metal ions Cd(II) and Pb(II) on *spirulina* intact cells have been studied with optical and differential scanning microcalorimetry (DSC) methods after 7.2 kGy ^{137}Cs gamma irradiation and without irradiation.⁸ It was shown that the addition of metal ions causes a decrease in optical absorption spectra band intensities. In the case of irradiation, the absorption band intensity decreases higher than without irradiation. In work¹⁰ effect high dose of Cs^{137} and Co(II), Cd(II), Ag(I) ions on *spirulina* after cultivation was study. It was shown that possible use of a high dose of gamma irradiation does not change the nature of the interaction of these metal ions for intact cells *spirulina* and its constituents. Although consumption of *spirulina* is increasing worldwide, relatively few studies have reported the potential health effects of heavy metals in populations across different settings on *spirulina*.

In this work, using UV-visible spectrometry were studied the effect of Co(II) ions on those frozen at -80°C and -20°C and irradiated with high doses (400kGy) of Cs^{137} , and then re-cultivated on *spirulina*. In contrast to the studies in work,¹⁰ where Co(II) effects on *spirulina* were performed immediately after removal of high-dose irradiation and subsequent 2-week cultivation, in this work, after irradiation and

after 2-week cultivation *spirulina* were placed on temperature 4°C and influence Co(II) ions on *spirulina* was studied after 3 weeks. Similar conditions were considered for frozen cases.

Materials and Methods

Spirulina IPPAS B-256 strain was cultivated in a standard Zarrouk¹¹ alkaline water-salt medium at 34°C , illumination $\sim 5000\text{lux}$, at constant mixing in batch cultures.¹² The cultivation of the *spirulina* intact cells was conducted for 7 days. The growth was measured by optical density by monitoring of changes in absorption at wavelength 560 nm using the UV-Visible spectrometer Cintra 10e. *Spirulina* grows optimally in pH range of 9-11.

The intact cells suspension of *spirulina* at pH 10.1 in the nutrition medium was used for scanning the absorption spectra from 400 to 800 nm. The concentration of *spirulina* was determined by the instrumental data. *Spirulina* suspension (100 ml) after 7 days of cultivation have been irradiated with 400 kGy gamma radiation using Cs^{137} as a gamma radiation source at the Applied Research Center, E. Andronikashvili Institute of Physics (Dose rate -1.1Gy during one minute). By analogue after 7 days of cultivation *spirulina* was frozen at -20°C in first case and -80°C in other case during 48 hours and then suspension after the irradiation (400 kGy) and frozen were filled up with Zarrouk medium and they were recultivated during 14 days. Melting of frozen *spirulina* was performed by step wise at -20°C , 4°C , 23°C , and -80°C , 4°C , 23°C . After cultivation samples were placed in 4°C temperature during three weeks, as in the case after irradiation. The solutions of metal ions were prepared in deionized water with appropriate amounts of inorganic salt.

Results and Discussions

Influence of Co(II) ions on frozen at -80°C and -20°C and high dose irradiated Cs^{137} and then recultivated on *spirulina* were studied as a function of metal concentration. Fig.1 shows the absorption characteristics after irradiation, frozen and re-cultivation of cells *spirulina*. The peak at 681 nm is due to the absorption of Chl a peak. At 621 nm is due to the absorption of phycocyanin (PC). At 500 nm is due to the absorption of carotenoides. A peak at 440 nm is due to soret band of Chl a.¹³ In Figure 1 (A, B,C) are shown effect of Co(II) ions on the absorption of the irradiation (400kGy), frozen at -80°C and -20°C temperatures and thawed and after that re-cultivation

cells of *spirulina*. (It is seen from Figure 1), that with increasing metal concentrations absorption intensity decreased for all cases. As can be seen from this figure 1-5, the uptake processes were relatively fast at low concentrations of Co(II) in the case of interaction with irradiated *spirulina*, but for frozen *spirulina* the effect is considered to be very slow. In this case, when irradiated *spirulina* was placed at 4°C for 3 weeks after 2 weeks of cultivation, there was no difference in the effect of Co(II) interaction on irradiated *spirulina* after cultivation and immediately measurements.¹⁰

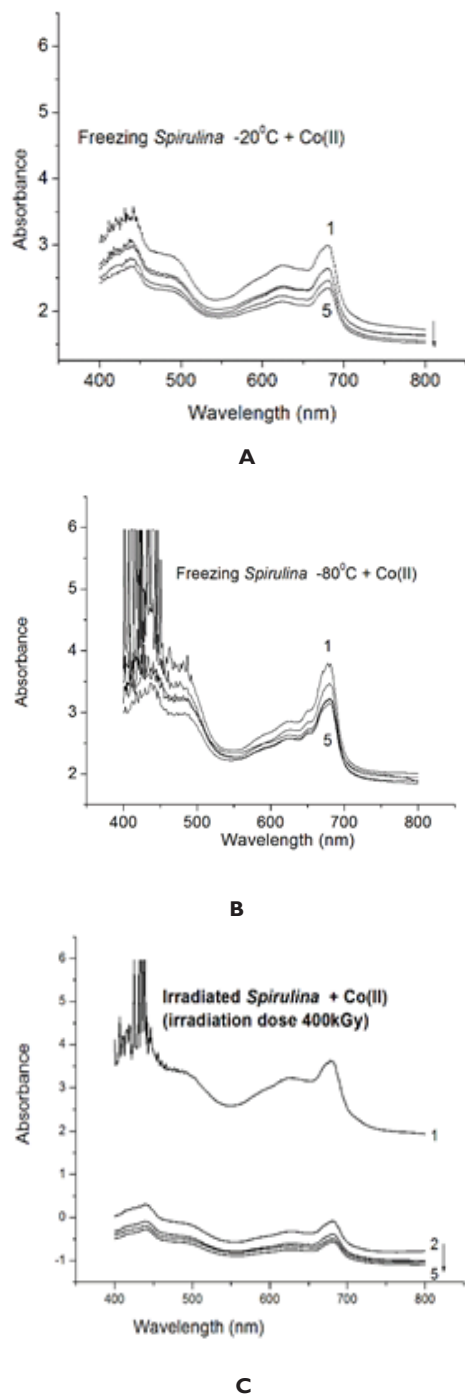


Figure 1(A,B,C) Absorption spectra of *spirulina* after frozen at -80°C , at -20°C, at irradiated and re-cultivation-(1) + Co(II) ions (2-5). 1→5 [Co(II)]=0 +5 mM; (after 2-week cultivation in all cases *spirulina* were placed on temperature 4°C during three weeks).

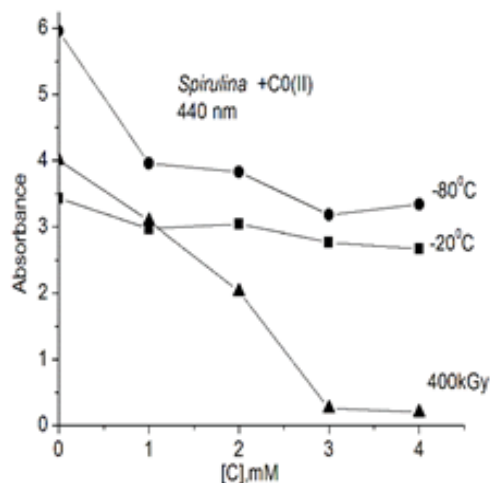


Figure 2 Influence of Co(II) ions after irradiation (400kGy) and after frozen -20°C and -80°C on temperature and then re-cultivation of *spirulina* (changes in the soret band of Chl a (440nm)).

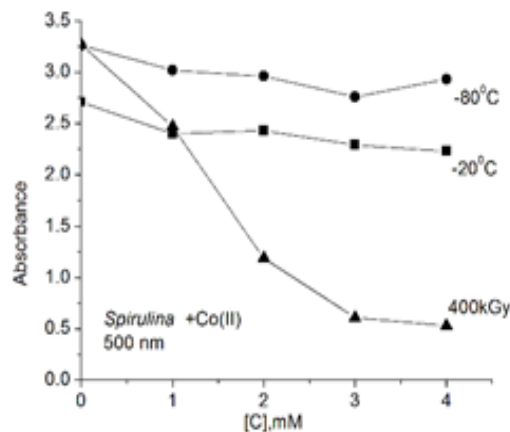


Figure 3 Influence of Co(II) ions after irradiation (400kGy) and after frozen -20°C and -80°C on temperature and then re-cultivation of *spirulina* (changes in the carotenoids(500nm)).

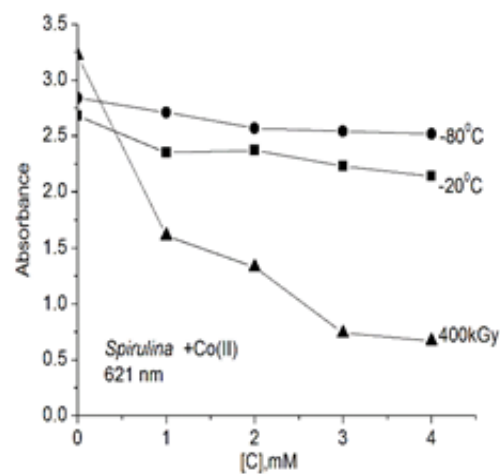


Figure 4 Influence of Co(II) ions after irradiation (400kGy) and after frozen -20°C and -80°C on temperature and then re-cultivation of *spirulina* (changes in the basic protein -the phycocyanin(621nm)).

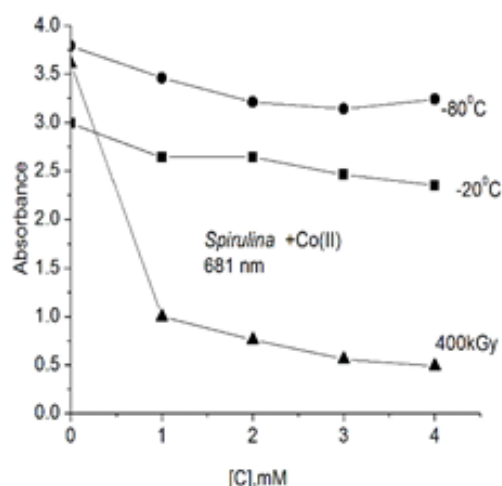


Figure 5 Influence of Co(II) ions after irradiation (400kGy) and after frozen -20°C and -80°C on temperature and then re-cultivation of *spirulina* (changes to the constituent- chlorophyll a(681nm)).

Influence of Co(II) ions after irradiation (400kGy) and after frozen -20°C and -80°C on temperature and then re-cultivation of *spirulina* constituents (Chl a, PC, carotenoids) are shown in fig.2-5. It is clear, that effect of Co(II) ions on the absorption intensity maximums for wavelength 440nm, 500 nm, 621 nm and 681nm of *spirulina* constituents are different. In particular, at 440 nm (changes to the constituent- solet band into the chlorophyll a) with increasing Co(II) concentration the intensity of absorption decrease at high dose of radiation, decreases less after frozen -80°C, but almost does not change for the case -20°C on temperature. As for the change in absorption intensity at 621 nm, which is the peak of absorption of the major protein-phycoyanin of *spirulina*, the absorption intensity very efficiently decreases at high dose of radiation, but almost does not change for the case after frozen -20°C and -80°C on temperature. Analogue results were received for carotenoids at 500 nm wavelength and for the chlorophyll a at 681nm wavelength.

If we compare the results obtained after irradiation with the results obtained in our work,¹⁰ we can see that a three weeks delay at 4°C temperatures of *spirulina* did not affect the influence of Co(II) on *spirulina*. In¹⁴ reported that the viability of marine unicellular algae under cryopreservation did not correlate to the age of culture, but related to the size and specific growth rate. Using the optical and DSC studies, it was shown that *spirulina* irradiated with Cs¹³⁷ γ 400 kGy and then stored under anaerobic conditions in dark at 4 °C remained viable despite 96.9 % denaturation of its whole biomass.^{5,6} The DSC data showed that PBSc in the recultivated samples, which were preliminarily irradiated with 10–400 kGy doses, melted cooperatively.^{5,6} The high subzero preservation of *spirulina* CMU2 and *spirulina* GDI was performed by step wise freezing at 25, 4, and -20°C for 30 min. Then they were preserved at -80°C for seven months with four cryoprotectants: dimethyl sulfoxide, horse serum, calf serum and glycerol.¹⁵ The studies on equilibrium isotherm experiments showed highest maximum metal loading by living cells (181.0 mg Co(II)/g¹⁶ followed by lyophilized (79.7 mg Co(II)/g and oven-dried (25.9 mg Co(II)/g, biomass of *spirulina* on a dry weight basis. *Spirulina* contain a variety of functional groups such as carboxyl, hydroxyl, sulphate and other charged groups which are responsible for metal binding.¹⁶ The employment of this blue-green alga to uptake heavy metals in contaminated sites has been explored.¹⁷ Recent studies have

shown that *spirulina* can be used for treating wastewater, including effluent from fish culture activities, as it can efficiently metabolize the nutrients and remove the heavy metals contained in aquaculture effluent.¹⁸ In work¹⁹ provides insight into the potential of algal biomass as agricultural fertilizers, when coupled with aquaculture wastewater remediation.

Thus, based on our results, it can be assumed that a large dose of radiation and freezing at different temperatures does not affect the viability of *spirulina* and, despite extreme changes in external conditions, can also interact with Co(II) ions as without these conditions.

Acknowledgments

None.

Conflicts of interest

Authors declare no conflict of interest.

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