Application of fluorescence technique in pollution monitoring

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Abstract. Plant cell wall has the composition, structure and properties that make it suitable for many uses, such as composite and paper manufacture. Cell wall has many active sites on its surface which are involved in interaction with its environment (solvent). We investigate its capacity for purification of water pollutants.

In this study, we investigated capacity of plant cell walls isolated from milled maize stem to remove different concentrations of nickel from polluted water. By combining fluorescence technique with spectrophotometry, complementary results were obtained. Autoflorescence spectra of plant cell walls were recorded and their spectral changes during interaction between cell wall and pollutants were examined. Emission spectra were analyzed by fitting with multiple log-normal distribution curves, whose form resembles to real experimental spectra of individual fluorophores.

INTRODUCTION

The industrial activities alter the natural flow of materials and introduce novel chemicals into the environment (Faisal and Hasnain, 2004). The effluents are increasing in the environment especially waters as a result of urbanization. Most of these effluents contain toxic substances especially heavy metals. The presence of heavy metals in the environment is of major concern because of their toxicity, bio-accumulating tendency, threat to human life and the surroundings (Igwe and Abia, 2003). Their accumulation in the environment and in food chains, can damage biological processes. The anthropogenic sources of heavy metals include wastes from the electroplating and metal finishing industries, metallurgical industries, chemical manufacturing, mine drainage, battery manufacturing, fertilizer industries, pigment manufacturing industries,

contaminated ground water from hazardous waste sites (Faisal and Hasnain, 2004).

Adsorptive removal of heavy metals from aqueous effluents which have received much attention in recent years is usually achieved by using activated carbon or activated alumina (Shim et al., 2001; Monser and Adhoun, 2002). Biosorption or bioremediations consists of a group of applications which involve the detoxification of hazardous substances instead of transferring them from one medium to another by means of microbes and plants. Many other biosorbents of algal, fungal and bacteria biomass have been utilized. These includes among others certain bacterial strains (Ioannis and Zouboulis, 2004).

In this work we studied possibility to use plant cell walls, for removing nickel from its solution in water. As a model we used the cell walls isolated from maize stems. Autoflorescence spectra of plant cell walls were recorded and their spectral changes during interaction between cell wall and nickel ions were examined.

EXPERIMENTAL

The cell walls were incubated in the ... $NiSO_4$ solution for one hour, without stirring. The cell wall samples were taken for the fluorescence measurement in the 15 min intervals.

Fluorescence spectra were collected using a Fluorolog-3 spectrofluorimeter (Jobin Yvon Horiba, Paris, France) equipped with a 450 W xenon lamp and a photomultiplier tube. The cell walls were measured in a wet state, in a front-face configuration of the measuring cell. The slits for the excitation and emission beams were fixed at 4 and 1 nm, respectively. The spectra were corrected for the dark current. In each measurement, five scans were averaged. All measurements were performed at 25 ^{o}C and controlled by a Peltier element.

In order to determine number of components in an integral emission spectrum, which correspond to the number of different fluorophores in the structure, we applied a specific measuring procedure. Emission spectra of each sample were obtained by excitation at different wavelengths, starting from the excitation maximum at 360 nm, with a 5 nm-step. These were deconvoluted into

a varying number of log–normal components, from three to seven, using the log–normal method (Radotić et al. 2006; Kalauzi et al. 2007) in order to find the optimal number of components for emission spectral analysis. Non-linear fitting of all fluorescence spectra was performed using the Nelder–Mead simplex algorithm implemented in Matlab 6.5. For each sample, positions of component maxima, obtained for a series of spectra measured with a 5 nm step, were treated as random variables. In order to calculate an approximate distribution of the probability density for positions of all the components, we constructed corresponding histograms of component maxima positions. However, since histogram profiles (positions and relative amplitudes of histogram maxima) depended on the number of histogram abscissa intervals, we calculated the corresponding approximate probability density distribution (APD) by weighed averaging of histogram values for a set of histograms with varying abscissa intervals (Radotić et al. 2006; Kalauzi et al. 2007). The summation spectrum based on the four-component deconvolution showed the best fit to the recorded emission spectra. Attempts with more components did not reduce error significantly. Therefore we adopted four component Log-n model as the most appropriate.

RESULTS AND DISCUSSION

Figure 1a shows emission spectra of the maize stem cell walls after 15 min incubation in the solution of $NiSO_4$. The spectra were excited at various wavelengths, starting from 360 nm, with 5 nm steps. It is known that the emission of the cell wall originates mainly from the lignin polymer (Donaldson et al. 2010), one of the cell wall constituents. It is obvious red shift of the spectrum with increasing excitation wavelength, due to the stepwise response of the cell wall fluorophores to the excitation light. Figure 1b shows the main cell wall emission band (maximum at 440 nm) after 360 nm excitation, of the cell wall treated with 12 mM $NiSO_4$ concentration. The spectrum was recorded

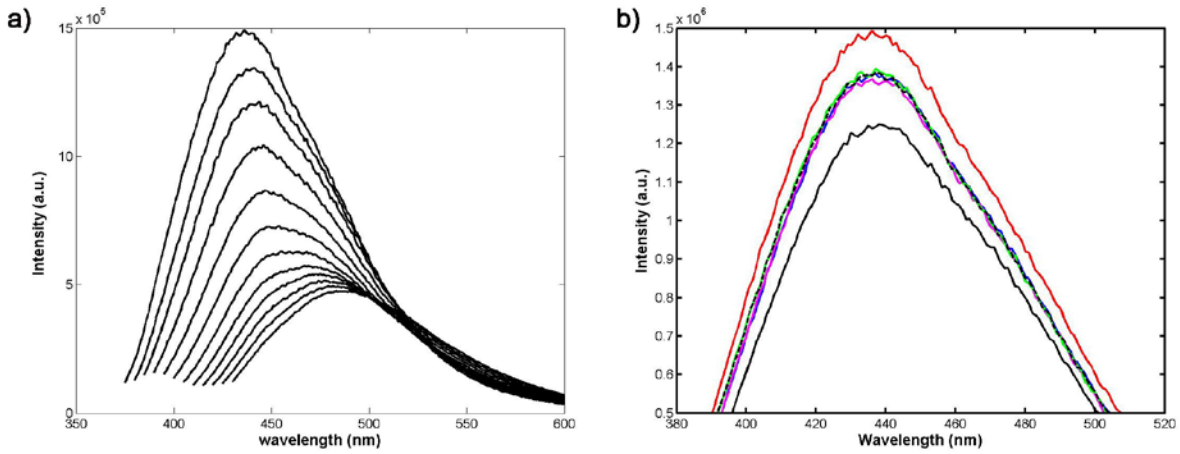

Figure 1. a) Emission spectra of the maize cell walls after 15 min incubation in the solution of $NiSO_4$. The spectra were excited at various wavelengths, starting from 360 nm, with 5 nm steps. b) The main cell wall emission band (maximum at 440 nm) after 360 nm excitation, of the cell wall treated with 12 mM $NiSO_4$, after 0 min (black), 15 min (red), 30 min (blue), 45 min (pink), 60 min (green) incubation.

every 15 min during one hour duration of cell wall incubation in the nickel solution. Nickel induced an increase in fluorescence intensity, possibly due to the energy transfer between cell wall and bound nickel ions (ref). The change in the intensity of the 440 nm band is due to the binding of the nickel ions on the cell wall. The most pronounced change in the intensity of this spectral band was after 15 min incubation of the cell walls in the nickel solution. This shows that 15 min is an optimal time period for removing nickel ions of the given concentration from the solution by using cell walls. For longer incubations there was a change in the band intensity in the opposite direction, indicating that nickel binding is a reversible process.

Figure 2 shows APDs for the cell walls incubated 15 min and 60 min in the $NiSO_4$ solution. Both APDs contain five components. After 15 min incubation, i.e. when the nickel binding on the cell wall is most pronounced, there is a blue shift of the APD components at 520 nm, 480 nm and 450 nm. This indicates that nickel binding induces redistribution of the charges on a part of the molecular groups. This leads to the changes of the emitting fluorophores and consequent shift of their corresponding APD components. After 60 min incubation most APD components shift back on their positions before the

treatment, indicating reversible reaction of nickel binding. The component at 441 nm may originate from a newly formed fluorophore after nickel unbinding. This may be due to the polymer conformation changes induced by the temporary binding of nickel ions.

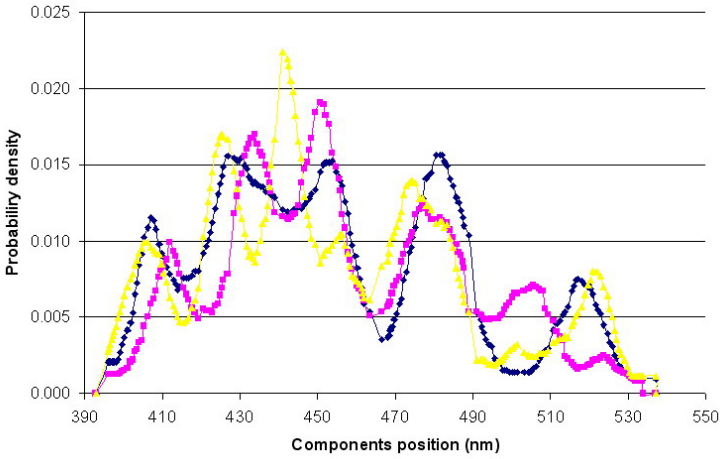

Figure 2. APDs for the cell walls incubated 15 min (pink) and 60 min (yellow) in the 12 mM $NiSO_4$ solution

The results show that the cell walls isolated from the maize stems are a reliable and promising substrate for removing nickel ions from polluted water. After collection of the nickel by using cell walls, the obtained composite could be recycled by burning and subsequently the produced ash can be used as a raw material.

Acknowledgements. This work was supported by the grant 173017 from the Ministry of Education and Science of the Republic of Serbia.

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