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ACCUMULATION OF CADMIUM BY HAIRY-ROOT CULTURES OF *SOLANUM NIGRUM*

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SUMMARY

Cadmium uptake by cultures of transformed hairy-roots of *Solanum nigrum* was studied. The effect of pH, buffer type, temperature, exposure time and Cd^{2+} content ranging from 0.2 to 2000 ppm was measured. Cd^{2+} uptake was dependent on increasing metal concentration and it was time dependent. From the variety of buffers tested, MES buffer and borate ions were found to be beneficial for the Cd^{2+} uptake. The high effectivness of Cd^{2+} accumulation in the roots decreased significantly after increasing the Cd^{2+} content in the buffer over 2 ppm.

INTRODUCTION

Heavy metals significantly contribute to the pollution of the environment in industrial regions. One of the harmful heavy metals that is found in the soil, wastewaters, sewage sludge and in the aquatic environment is cadmium. Uptake of cadmium by plants and its subsequent accumulation in the food chain is a potential health hazard (Nriagu et al., 1988). For example cadmium ability to induce carcinogenesis has been described (Waalkes, 1992).

Recently biological methods for removing heavy metals or for their recovery using biosorption and bioaccumulation by microbial and also plant cells have been intensively studied (Gadd and White, 1993, Ornes and Sajwan, 1993). Compared with microorganisms fewer results have been published on the uptake of metal ions by plant cells and especially by roots and almost no data are available about bioaccumulation or biosorption by transformed root cultures. Metzger et al (1993) described transformed root cultures of tobacco and morning glory as models for evaluating the availability of cadmium from sewage sludge, but the authors did not published in detail the experimental physico-chemical factors (pH, cadmium concentration, temperature etc.) influencing metal accumulation by roots.

In our paper we demonstrate the ability of transformed hairy roots of *Solanum nigrum* to accumulate cadmium from aqueous solutions under different conditions.

MATERIALS AND METHODS

All experiments described in this paper were performed using hairy-root culture of *Solanum nigrum*, clone SNC-90, transformed by *Agrobacterium rhizogenes* bearing RI-plasmid C58ci. The roots were cultivated in the dark at 27°C in liquid medium according to Macek (1989), without any exogeneous growth regulators added.

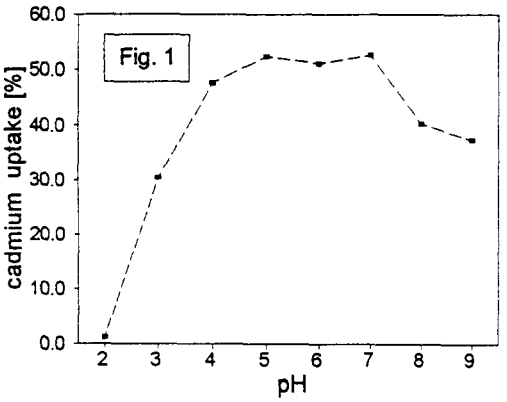
Roots, after 20 day cultivation, were harvested from the medium by filtration, rinsed with water, and 1g (fresh weight) of the cells was transfered to the 50ml of the test solution (diluted HNO_3, pH of which was adjusted with 0.05M sodium borate) containing 1ppm or 2ppm of cadmium, prepared by addition of analytical grade $Cd(NO_3)_2$. After exposure for 2 hours carried out at 26°C under shaking, the cells were withdrawn and washed with 2x20ml of the test solution without cadmium. Metal content in the roots was determined after drying (to the constant weight at 95°C) and mineralization with concentrated HNO_3 (4 hours, 170°C) by differential pulse anodic stripping voltametry (Laresen et al, 1973). All cadmium uptake readings were an verage of two runs and metal content in plant material was calculated on dry weight basis. The results show the amount of cadmium accumulated by the roots, expressed as the percentage of the initial cadmium content in the test solution.

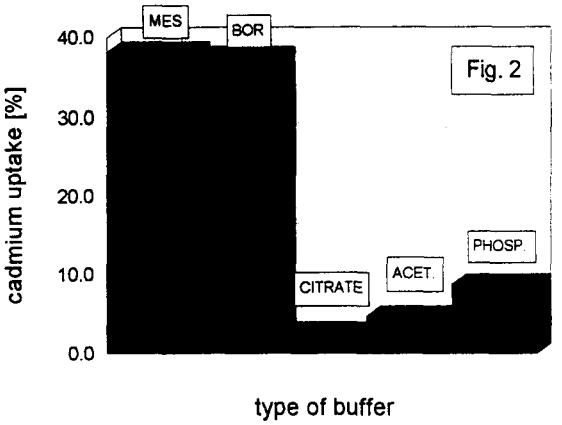
RESULTS AND DISCUSSION

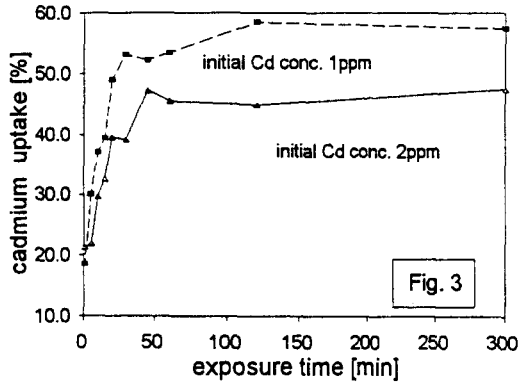
Cadmium uptake as a function of various pH conditions (pH 2-9) is shown in Figure 1. The highest cadmium removal efficiencies appeared over the pH 5-7 range of the exposition solution, with initial content 2ppm of cadmium. pH 5.6 was chosen for all further experiments because it is also the value optimal for the growth of roots.

To study the effect of different composition of the test solutions on cadmium uptake, we performed experiments in 0.1M MES, 0.1M acetate, 0.1M citrate, 0.1M phosphate buffers and solution containing HNO_3 and sodium borate. From the variety of buffers tested, MES buffer and borate ions were found to be optimal for cadmium uptake. The use of phosphate buffer resulted in the lower accumulation of cadmium which was probably caused by precipitation of cadmium with phosphate ions, as described also by Gadd and White (1993).

`Figure 3 shows cadmium uptake over an exposure period of 5 hours at concentrations of cadmium in the test solutions 1ppm and 2ppm. First rapid stage of accumulation can indicate physical adsorption, followed by slower uptake suggesting that accumulation is in part metabolically sponsored.







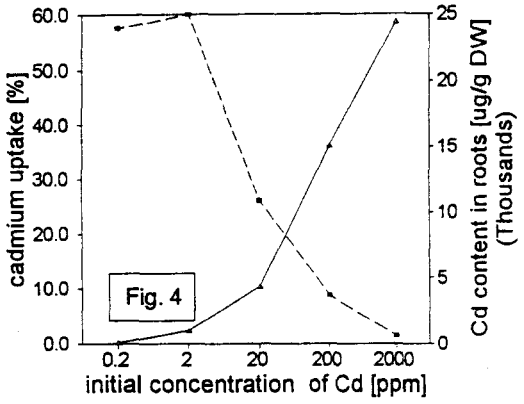


Fig.1: Dependence of Cd^{2+} uptake on pH of test solution , (initial Cd^{2+} concentration 2ppm)

Fig 2: The effect of the composition of the test solution on Cd^{2+} uptake (MES-MES buffer, BOR-HNO_3 solution containing borate ions, CITRATE-citrate buffer, ACET.-acetate buffer, PHOSP.-phosphate buffer, initial Cd^{2+} concentration 2ppm)

Fig 3: Cadmium uptake as the function of exposure time at two initial Cd^{2+} concentrations 1 ppm -■-, 2 ppm -▲-

Fig 4: Cd^{2+} content in the roots -▲- and the percentage -■-of Cd^{2+} accumulated by the roots as a function of initial Cd^{2+} concentration in the test solution

The dependence of cadmium uptake on its initial concentration in the exposition solution was followed in the concentration range from 0.2 to 2000 ppm of cadmium (see Fig.4). The increased content of cadmium in the exposition solution was reflected by the increase of cadmium accumulated in the plant tissue. The cellular level of cadmium reached from 96.5ug/g to 24 455ug/g of dry weight after 2 hours exposure in the solutions containing initial concentrations from 0.2ppm to 2000ppm of cadmium respectively. Nevertheless the efficiency of accumulation expressed as percentage of cadmium accumulation related to the initial cadmium content in the test solution was significantly decreased at concentrations of cadmium over 2ppm.

Finally, the effect of lower temperature on cadmium uptake by transformed roots was studied. Cadmium removal from the test solution initially containing 2ppm of cadmium decreased at 4°C to 29% comparing to 60% at 26°C after 2 hours of exposure.

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