NUKLEONIKA 2003;48(3):121–125

ORIGINAL PAPER

Saccharomyces cerevisiae as uranium bioaccumulating material: the influence of contact time, pH and anion nature

**Karin Popa,
Alexandru Cecal,
Gabi Drochioiu,
Aurel Pui,
Doina Humelnicu**

Abstract The possibility of bioaccumulation of uranium species in beer yeast was investigated. The behaviour of the *Saccharomyces cerevisiae*–UO_2^{2+} system was studied *vs.* contact time, pH and anion nature with no ionic competition. Analysis of the data revealed the following optimal working conditions: contact time = 1 h, pH = 6.5 and 10^{-1} M $UO_2(CH_3COO)_2$ solution as uranyl source; as a result, the maximum degree of bioaccumulation attends a value nearly 8.75 mmol UO_2^{2+}/g yeast. Both, a scanning electron microscope (SEM) and amino acid determinations lead to the conclusion that the uranyl nitrate solution may devastate the yeast cells provoking membrane damage and the release of the cell constituents (including the bioaccumulated uranium species). The results suggest the possible use of *Saccharomyces cerevisiae* as a biological decontaminant of uranium containing wastewaters.

Key words bioaccumulation • radioactive wastewaters • *Saccharomyces cerevisiae* • uranium

K. Popa✉, A. Cecal, A. Pui, D. Humelnicu
Department of Inorganic Chemistry,
Faculty of Chemistry,
"Al. I. Cuza" University,
11 Carol I Blvd, 6600 – Iasi, Romania,
Tel.: +40/ 232 272478, Fax: +40/ 232 201313,
e-mail: kpopa@ch.tuiasi.ro

G. Drochioiu
Department of Organic Chemistry and Biochemistry,
Faculty of Chemistry,
"Al. I. Cuza" University,
11 Carol I Blvd, 6600 – Iasi, Romania

Received: 20 February 2003, Accepted: 7 May 2003

Introduction

Although uranium represents a normal constituent of the environment, the concentration of this element in waters and soils increased dramatically after 1945, as a result of both energetic and nuclear weapon development. According to recent estimations 231,000 tons of uranium was produced in the former GRD from 1945 until 1989 [14]. South Africa extracted 75,000 tons of uranium in the Witwatersrand gold mining area [7].

Under the natural conditions uranium occurs in multiple positive oxidation states (from III to VI), and in two valence states: U(IV) (as U^{4+} aqua-cation) and U(VI) (which hydrolyzes in solution to form the aqua-cationic complex UO_2^{2+}). Under conditions of a natural aqueous systems, the insoluble uranium rapidly corrodes forming yellow uranyl compounds, where the linear $[O = U = O]^{2+}$ entity forms characteristic structural elements [3]. Most notably, the solution chemistry of U(VI) is relatively complex, with numerous mono- and polynuclear uranyl–hydroxide and uranyl–carbonate complexes being formed.

The dissolved uranium concentration in river water ranges from 0.2 μg/L to 0.6 μg/L [14], depending on the river, time of sampling and sample treatment; seasonal variations were also noted. The origin of these variations is the mobilization/immobilization equilibrium processes between the soluble and insoluble uranium. The micro-organisms contained in the natural water play a very important role in establishing the above-mentioned equilibrium. A recent study [11] has demonstrated that sorption of uranium onto biomass surface can extract nearly 100% of aqueous U(VI) from mining wastewater.

These studies led us to the idea of the utilization of a cheap and easily obtainable microorganism, namely the

beer yeast *Saccharomyces cerevisiae*, as a biological decontaminant. The *Saccharomyces cerevisiae*' hyperaccumulating capacity was already signalized for the Ca [6, 8], Cd [6, 16, 22], Zn [12] and Cr [16] ions. Few studies regarding the *Saccharomyces cerevisiae*–uranium system are available too [18, 21].

The present study is focused on the explanation of the influence of contact time, pH and anion nature on the *Saccharomyces cerevisiae*–uranium system. All the experimental determinations were carried out using radioactive solutions simulating the natural wastewaters, with no ionic competition.

Materials and methods

All reagents used in the work (of analytical reagent grade) were obtained from Fluka Company. As radioactive effluents, 10^{-1} M $UO_2(NO_3)_2$ (pH = 5.5) and 10^{-1} M $UO_2(CH_3COO)_2$ (pH = 6.5) were employed. The commercial reagents $UO_2(NO_3)_2$ and $UO_2(CH_3COO)_2$ as uranium sources were purified by the decay products of uranium by precipitation with NaOH, filtration of liquid phase and resolubilization of $Na_2U_2O_7$ in HNO_3 and CH_3COOH, respectively.

The *Saccharomyces cerevisiae* bioaccumulating material was a commercial one (Pangran Company).

The influence of pH, contact time and anion nature in the *Saccharomyces cerevisiae*–uranium system was studied under the conditions of continuous illumination, with intermittent agitation, at high concentration of the radioactive ion. Preliminary studies indicated that these conditions yield a high degree of bioaccumulation. In addition, the high concentration of the radioactive ion led to a rapid biological response of the studied system.

Parallel cultures were set up by suspending 0.1 g of yeast in 10 mL of 0.1 M UO_2^{2+} solution (acetate or nitrate) and placed in a thermostatic "Myton" room at 22°C, constant light (3.6×10^{-3} J·cm^{-2}·s^{-1}), humidity and ventilation. The suitable pH was 5.5 and 6.5 for nitrate and acetate solutions, respectively. With the view to establish the optimal value of pH (between 4.0 and 7.0), 0.1 M U(VI) solutions was prepared by solving $UO_2(NO_3)_2$ and $UO_2(CH_3COO)_2$ in acid buffer. The values of pH (measured by a "Radelkis" model OP-208/1 pH-meter fitted with a calomel electrode) after 24 h incubation time in the *Saccharomyces cerevisiae* –uranium system, did not change dramatically.

The quantitative determinations of total soluble uranium species in the respective supernatant resulting by centrifugation an aliquot of the sample at 3000 rpm was carried out spectrophotometrically ("CECIL 1020" spectrophotometer) with arsenazo III at 660 nm.

The bioaccumulation capacity was expressed in terms of fractional equilibrium (F), defined as the concentration of uranium in the sorbent at time t (c_t) divided by the concentration of uranium in sorbent at equilibrium (c_∞)

$$K_d = \frac{c_t}{c_\infty}. \quad (1)$$

For the uranium bioaccumulation degree as a function of the pH, a description in mmol UO_2^{2+} retained per gram of dry sorbent was done.

The morphology and the yeast cell size was evaluated by scanning electron microscopy (SEM) on a "MICROSPEC WDX-2A" microscope at 10 kV as follows: 0.1 g of yeast was suspended in 10 mL of 0.1 M UO_2^{2+} solution (acetate and nitrate, respectively). After one hour contact time, the bioaccumulating material was filtered ("Robu-Glass" G_4 filtering crucibles) and washed with small volumes of bidistilled water (3–5 mL). In order to obtain a good resolution, the biological samples were made conductible by covering them with a monolayer of gold atoms.

The FT-IR spectra were taken on a "Jasco 660-Plus" Fourier transform infrared spectrometer using KBr-diluted samples against a KBr standard (1–2 per cent of analysing sample). The measured wave number range was 350–1400 cm^{-1} with a resolution of 4 cm^{-1} and a scanning speed of 2 mm/s.

To identify the cationic species appearing in the aqueous phase after the bioaccumulation process, a "SHIMADZU AA-660" elemental chemical analyser was employed.

Amino acids in the supernatant were determined with ninhydrin (1,2,3-indantrione) as reported elsewhere [9]. 1 mL of extraction solution containing amino acids was pipetted into a test-tube, into which 1 mL of a ninhydrin reagent was added. The mixture was stirred vigorously. The test-tube was kept for 30 min in a boiling water-bath at 100°C, then cooled to room temperature and 5 mL of alcohol solution (80%, v/v) was added. A "Carl Zeiss Spekol" spectrophotometer with 1 cm matched cells was used for all spectral measurements, at 540 nm. In the amino acid assay, the nitrate ions interfered seriously with the determinations, therefore their positive errors were eliminated using another blank with uranyl nitrate.

Results and discussion

The dependence of distribution coefficient with time is presented in Fig. 1, while the variation of the bioaccumulation degree on pH of the suspension used is shown in Fig. 2.

As shown in Fig. 1, bioaccumulation occurs at a very high rate during the first 15 min, followed by a process of much lower rate. Under the employed experimental

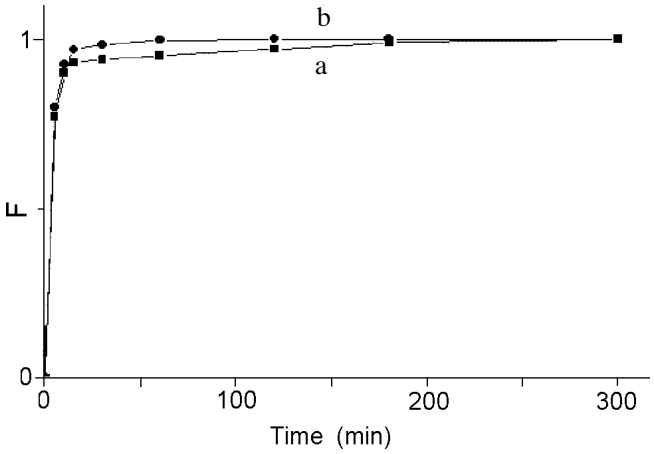


Fig. 1. Variation of fractional equilibrium (F) for UO_2^{2+} from nitrate (a) and acetate (b) solutions *vs.* contact time.

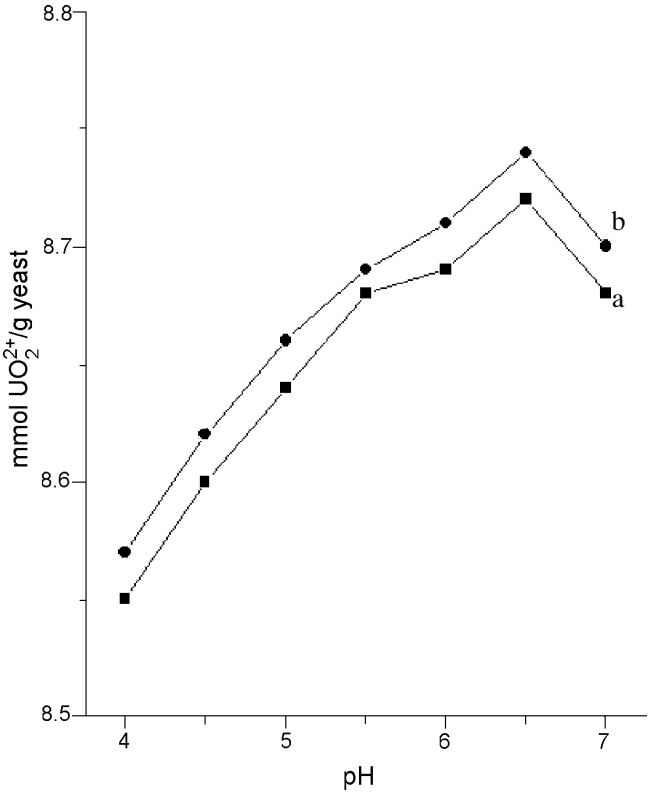


Fig. 2. Dependence of bioaccumulation degree for UO_2^{2+} from nitrate (a) and acetate (b) solutions on pH (contact time: 1 h, V/M = 100 mL/g).

conditions, the uptake of uranium species from aqueous solution attended its maximum after one hour. The obtained maximum values of bioaccumulation degree (about 8.5 mmol UO_2^{2+} per gram of dry yeast) are 2÷18 times higher that the values reported previously [18, 21]. Nevertheless, the absence of ionic competition and the stimulative growing condition of yeast conducted to these huge values.

The U(VI) bioaccumulation can proceed in two distinct mechanisms. Firstly, the O- (e.g. -OH, -COOH, $>PO_3H$, $-SO_3H$, >N-OH, >CO), N- (e.g. $-NH_2$, >NH, -N=) and S- (e.g. -SH, -S-) donor groups on the cell surface are able to complex UO_2^{2+} [1, 2]. Secondly, the radioactive ions penetrate the cell membrane and get inside the cytoplasm; responsible for this phenomenon can be a protein similar to YOR316c *COT*1, with an important role in accumulation and transport of metallic ions [17].

The bioaccumulation degree of $UO_2(CH_3COO)_2$ (pH = 6.5) is greater than that of nitrate. This is due to increased $[(UO_2)_2CO_3(OH)_3]^-$ hydrate species concentration on the expense of $[(UO_2)_3(OH)_5]^+$ species [10], most stable at pH = 5.5 (specific value for 10^{-1} M $UO_2(NO_3)_2$ solution).

However, the differences between the maximum values of bioaccumulation degree cannot exclusively result from the pH value established in the system: within the pH = 4.0–7.0 studied interval, the values of bioaccumulation degree are constantly lower for $UO_2(NO_3)_2$ than for $UO_2(CH_3COO)_2$ (Fig. 2).

This has its origin in the strongly oxidative character of NO_3^- species; the presence of these species in the considered bioaccumulation systems represents an internal obstruction on the reducing of U(VI) to U(IV) and the equilibrium:

$$(2) \qquad UO_2^{2+} \underset{-2e^-}{\overset{+2e^-}{\rightleftharpoons}} UO_2$$

is hardly moved towards the soluble U(VI) oxidation state.

A linear increase of bioaccumulation degree in the pH range of 4.0 and 6.5 was observed, for both the acetate and nitrate solution sources. After a maximal values obtained at pH = 6.5, a decrease of the values of bioaccumulation degree was noted. For the pH > 7, new insoluble anionic uranate and diuranate species appear in the system [5]. Consequently, the decrease of uranium concentration in aqueous phase is not exclusively due to the bioaccumulating capacity of the yeast, but also to a second process of precipitation of soluble uranium [4].

As a result of the bioaccumulation process, after 24 h contact time in the considered systems, in the aqueous phase only traces of Zn (0.015 μg/mL) and Fe (0.055 μg/mL) ions were detected. Fe(II) can be involved in the mobilization/immobilization equilibrium process [13] as follows:

$$(3) \qquad UO_2^{2+} + 2Fe^{2+} \rightleftharpoons UO_2 + 2Fe^{3+}.$$

As shown in Fig. 3, FT-IR spectra of *Saccharomyces cerevisiae* (without uranium) are complex, reflecting the complex biochemical nature of the accumulator. In spite

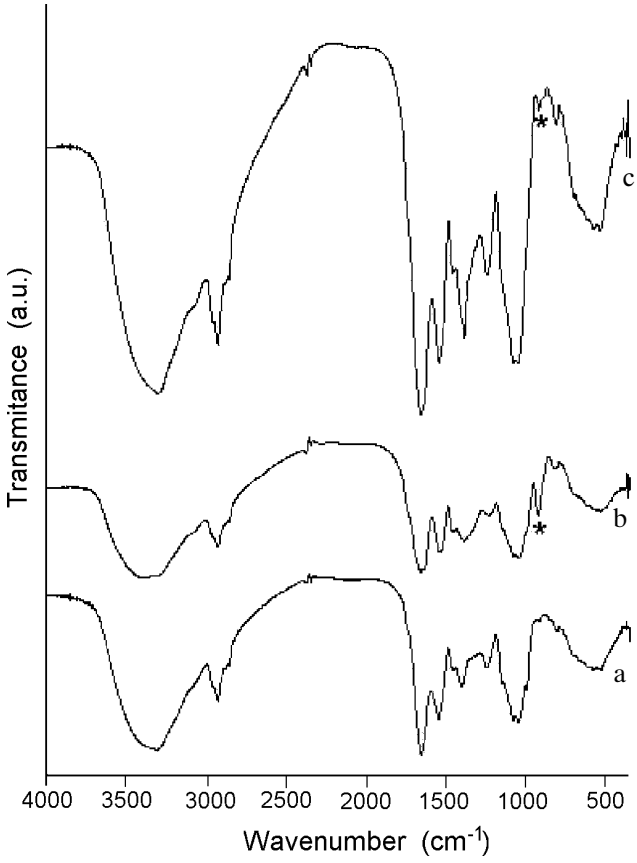
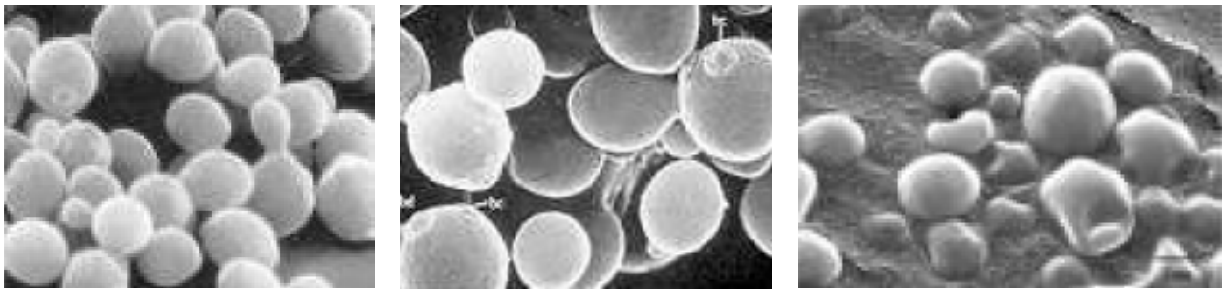


Fig. 3. FT-IR spectra of *Saccharomyces cerevisiae* (a) and *Saccharomyces cerevisiae*–UO_2^{2+} systems from acetate (b) and nitrate (c) sources.



a b c

Fig. 4. SEM images of *Saccharomyces cerevisiae* (a) and *Saccharomyces cerevisiae*–UO_2^{2+} systems from acetate (b) and nitrate (c) sources.

of this complexity, some characteristic peaks can be assigned as listed in Table 1 [19].

In the case of the interaction product between *Saccharomyces cerevisiae* a new band appears at 925–915 cm^{-1} (*). This absorption band originates both from the asymmetric stretching vibration of the uranyl unit [15] and from the interactions between UO_2^{2+} ions and the groups belonging to various cellular components, such as peptides, phospholipids, peptidoglycan, etc. The decrease of the wave number specific to UO_2^{2+} entity coming from acetate solution (916.0 cm^{-1}) compared with the nitrate solution (921.8 cm^{-1}) as well as the peak intensity (superior in the first case) indicate a stronger metal–ligand bond corresponding to pH = 6.5.

Through SEM electronic microscopy one can notice that the general morphology of yeast cells is spherical (Fig. 4a). Following the bioaccumulation of uranium from 0.1 M $UO_2(CH_3COO)_2$ solution, an attachment of cells to the bioaccumulating material can be observed (Fig. 4b). The nature of the solid phase is not known. It can be supposed that it formed as a result of the interaction between uranium and the exopolysaccharides produced by the yeast [20]. For an acidic pH (as in the case of 0.1 M $UO_2(NO_3)_2$ solution), part of the *Saccharomyces cerevisiae* cells broke down (Fig. 4c). Consequently, the bioaccumulation process is obstructed, and bioaccumulation degree values are lower.

Under the action of uranyl acetate, the yeast produced lower amounts of amino acids than the untreated sample. Thus, the free amino acid concentration of the supernatant in the case of yeast treated with uranyl acetate, expressed as μg alanine/mL, was 41.48 per cent, which is lower (10.30 μg/mL) than the untreated yeast. On the contrary, uranyl nitrate treatment caused a 9-time drastically greater release of amino acids from the yeast (157.00 μg of free amino acids *vs.* 17.60 μg in the each mL supernatant of the untreated samples). Also, small traces of proteins were present in the supernatant of the yeast treated with uranyl nitrate.

It can be concluded that nitrate ions may devastate the yeast cells provoking membrane damage and the release of the cell constituents.

Table 1. Assignment of some characteristic peaks from *Saccharomyces cerevisiae* biomass FT-IR spectra.

Wave number (cm^{-1})	Groups
3600–3200	-OH from water
3000–2800	CH stretching modes of hydrocarbon chains
1660–1640	>C=O
1530	>NH from amide II
1200–1000	$>PO_3H$ and/or polisugars

Conclusions

The results regarding the uranium bioaccumulation in yeast with no ionic competition indicate a high capacity of radioactive wastewater depollution. The results of our analyses are in good agreement with one another and reveal that a 1 h contact time at pH = 6.5 and 10^{-1} M $UO_2(CH_3COO)_2$ solution as uranyl source are the optimal conditions for gaining the best degree of bioaccumulation.

The uranium bioaccumulation originated from two distinguished phenomena: the complexation via the O-, N- and S- donors groups existing to the cell surface as well as the enzymatic transport by membrane, inside the living cell.

The results of this study indicate the possible utilization of *Saccharomyces cerevisiae* as decontaminant biomaterial for uranium containing wastewaters, resulting from uranium mining activities. The main advantages of this biotechnology are: environment friendliness, self-reproducibility, adaptability, recyclisation of bioproducts, specificity, and good cost/benefit ratio. The disadvantages include the slowness of the processes and the difficulty to control it. However, due to the increasing need for the safe removal of radionuclides and the continued public interest in environmental problems, this kind of microbial processes may play an important role in the future of waste management. The development of these topics of biotechnology is desirable on both environmental and economic aspects.

References

1. Al Saraj M, Abdel Latif MS, El Nahal I, Baraka R (1999) Bioaccumulation of some hazardous metals by sol-gel entrapped microorganisms. J Non-Cryst Solids 248:137–140
2. Anderes Y, Redercher S, Thouand G (1999) Microorganisms as potential vectors of the migration of radionuclides? Czech J Phys 49;S1:197–204
3. Blackman WC Jr (ed.) (2001) Basic hazardous waste management, 3rd ed. Lewis Publishers, Boca Raton
4. Cecal A, Palamaru I, Popa K, Caraus I, Rudic V, Gulea A (1999) Accumulation of $^{60}Co^{2+}$ and UO_2^{2+} ions on hydrophytae plants. Isot Environ Health Stud 35:213–219

5. Choppin GR, Rydberg J, Liljenzin JO (1995) Radiochemistry and nuclear chemistry. Butterwoth–Heinemann, New York
6. Clemens S, Antosiewicz DM, Ward JM, Schachtman DP, Schroeder JI (1998) The plant cDNA *LCT*1 mediates the uptake of calcium and cadmium in yeast. Proc Natl Acad Sci USA 95:12034–12048
7. Coetzee H, Chervel S, Cottard F (2002) Inter-disciplinary studies of the impact of gold and uranium mining in the Witwatersrand goldfield. In: Merkel BJ, Planer-Friedrich B, Wolkersdorfer C (eds) Uranium in the aquatic environment. Springer, Berlin, pp 552–559
8. Cunningham KW, Fink GR (1994) Ca^{2+} transport in *Saccharomyces cerevisiae*. J Exp Biol 196:157–166
9. Drochioiu G, Sunel V, Oniscu C, Basu C, Murariu M (2001) The breakdown of plant biostructure followed by amino acids determination. Roum Biotechnol Lett 6;2:155–165
10. Haas JR, Bayley EH, Purvis OW (1998) Bioaccumulation of metals by lichens: uptake of aqueous uranium by *Petligera membranacea* as a function of time and pH. Am Mineral 83:1494–1502
11. Haas JR, Dichristina TJ, Wade R Jr (2001) Thermodynamics of U(VI) sorption onto *Shewanella putrefaciens*. Chem Geol 180:33–54
12. Kamizono A, Nishizawa M, Teranishi T, Murata K, Kimura A (1998) Identification of a gene conferring resistance to zinc and cadmium ions in the yeast *Saccharomyces cerevisiae*. Mol Gen Genet 219:161–167
13. Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Environ Qual 31:109–120
14. Meinrath A, Schneider P, Meinrath G (2003) Uranium ores and depleted uranium in the environment, with a reference to uranium in the biosphere from the Erzgebirge/Sachsen, Germany. J Environ Radioact 64:175–193
15. Merroun M, Henning C, Rossberg A, Reich T, Nicolai R, Heise K-H, Selenska-Pobell S (2002) Characterization of uranium(VI) complexes formed by different bacteria relevant to uranium mining waste piles In: Merkel BJ, Planer-Friedrich B, Wolkersdorfer C (eds) Uranium in the aquatic environment. Springer, Berlin, pp 505–511
16. Mirsky N, Weiss A, Dori Z (1980) Chromium in biological systems I. Some observations on glucose tolerance factor in yeast. J Inorg Biochem 13:11–21
17. Pearson BM, Hernando Y, Payne J, Wolf SS, Kalogeropoulos A, Schweizer M (1996) Sequencing of a 35.71 kb DNA segment on the right arm of yeast chromosome XV reveals regions of similarity to chromosomes I and XIII. Yeast 12;10B:1021–1031
18. Poole RK, Gadd GM (eds) (1989) Metal–microbe interaction. IRL Press, Oxford
19. Popa K, Cecal A, Grigoriu G, Palamaru MN, Craciun II, Paraschiv G (2002) On clearing up the mechanisms of radiocations bioaccumulation on *Lemna minor* by IR spectrometry. Anal St Univ Al I Cuza-Iasi 10;1:15–21
20. Simionescu C, Rusan V, Popa V (1974) The chemistry of seaweeds. Romanian Academy, Bucharest
21. Strandberg GW, Shumate SE, Parrot JR (1981) Microbial cells as biosorbents for heavy metals: accumulation of uranium by *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa.* Appl Environ Microbiol 41:237–245
22. Volesky B, May-Phillips HA (1995) Biosorption of heavy metals by *Saccharomyces cerevisiae*. J Appl Microbiol Biotechnol 42:797–806