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RESEARCH ARTICLE

Biosorption of strontium ions from simulated high-level liquid waste by living *Saccharomyces cerevisiae*

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Abstract

In this study, the *Saccharomyces cerevisiae* (*S. cerevisiae)* was modified by γ-ray. The RNA-seq results reflect that the high γ-ray energies could change some gene fragments, such as deletion, recombination, and mutation. The biosorption of strontium ions (Sr^{2+}) to different types of *S. cerevisiae* (*S. cerevisiae* (K-0), modified *S. cerevisiae* (Y-7), and non-living *S. cerevisiae* (H-K)) from the simulated high-level liquid waste (S-HLLW) was assessed at different experimental conditions. The sorption experimental results show that, under an appropriate condition, γ-ray radiation can enhance its biosorption capacity slightly of Sr^{2+} to *S. cerevisiae*. The maximum metal uptake and efficiency of Y-7 under S-HLLW were 11.656 mg g^{-1} and 37.91% at 32 h (wet weight), respectively. They decreased to 9.46 mg g^{-1} and 30.76% under radiation conditions. SEM-EDX and TEM analysis indicates that Sr^{2+} was adsorbed both on the cellular surface and the inner parts of the cells. Our experimental results fit well to the Langmuir and Freundlich model isotherms ($r^2 > 0.94$), and the maximum biosorption capacity values reached $q_{max} > 24.74$ mg g^{-1} at 32 °C. Negative values of ΔG^0 and positive values of ΔH^0 were observed, indicating the spontaneous and endothermic nature of Sr^{2+} biosorption on modified *S. cerevisiae*. The biosorption kinetics follow a pseudo-second-order equation at 32 °C ($r^2 > 0.94$). The desorption efficiency of Sr^{2+} adsorbed onto Y-7 was 7.65 ± 0.52%, 76.51 ± 2.13%, and 65.62 ± 2.42% by deionized water, 1 M HCl, and 0.1 M EDTA-Na, respectively. However, they were lower than H-K (18.82, 83.32, and 73.32%). Our findings demonstrate that living *S. cerevisiae* (Y-7) is a promising sorbent material for the treatment of radioactive process streams.

Keywords *Saccharomyces cerevisiae* · RNA-seq · Biosorption · Strontium · Simulated high-level liquid waste · Kinetics

Introduction

With a worldwide increased demand for power generation, nuclear power development has presented new solutions to address energy shortage. For a sustainable development of nuclear energy, the safety management of high-level liquid waste (HLLW) has become one of the most important research topics (Shozugawa et al. 2012). Among the fission products present in HLLW, strontium isotope such as ^{90}Sr or ^{89}Sr is the most hazardous and radioactive contaminant in the environment, which causes a wide range of environmental pollution and exists as a serious threat to human health (Bailly du Bois et al. 2012). In fact, the purified strontium can be reused as radiation and heat sources in the field of industry. To minimize the long-term radiological risk and facilitate the management of HLLW, developing effective approaches to separate and recycle strontium is highly desirable.

The conventional methods employed for recycling strontium ion from HLLW include precipitation (Luo et al. 2004; Nilchi et al. 2009), ion exchange (Dabbagh et al. 2007; Ho et al. 1996), solvent extraction, chromatography, and the membrane separation. Those methods require high cost but give low radiation stability (Chen and Wang 2008; Eccles 1995). On the other hand, economic biomaterials have demonstrated the advantages of good selectivity, low cost, and easy implementation for wastewater treatment, ever since the

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first report on biosorption (Clarke P H. 1985). In particular, microorganisms are able to decrease the concentration of radionuclides solution from milligrams per liter to micrograms per liter level (environmentally relevant level), which is highly efficient and environment-friendly (Farooq et al. 2010; Sud et al. 2008).

Saccharomyces cerevisiae is an inexpensive and eco-friendly biomaterial for heavy metal removal from wastewater, which is readily available from the source of biomass. Our previous study showed that the uptake of strontium by living *S. cerevisiae* reached 92% under laboratory conditions, and the biosorption capacity can be enhanced using denatured yeast cells (Qiu et al. 2017). The living *S. cerevisiae* can adsorb up to 26.12 mg g^{-1} uranium in the initial 600 mg L^{-1} (Liu et al. 2010). Compared to yeast, the other biosorbents were of less concern. The removal ratios of strontium ions were 44 and 39% for *Rhizopus arrhizus* and *Penicillium chrysogenum*, respectively (Marešová et al. 2011). The algae showed higher biosorption capacity approximately 220 mg g^{-1} for strontium ions after special treatment (Tu et al. 2015). Moreover, living *S. cerevisiae* is an ideal model organism to investigate the interactions of the metal-microbe at the molecular level (Wang et al. 2017). Therefore, living *S. cerevisiae* is a promising biological adsorbent for metal removal.

The biosorption of strontium by growing or living microorganism involves three processes: an initial rapid, passive adsorption followed by a much slower active bioaccumulation process, and further sorption stage. The first and last stages are metabolism-independent processes, while the second step is metabolism-dependent (Wang et al. 2017). The majority of studies on the bioaccumulation of heavy metal ions by microorganisms only consider the final concentration of metal ions, and a comprehensive study on the interaction between strontium ion and other radionuclides during the process of biosorption is still lacking. The HLLW has many hazardous components including toxic metals, other potentially damaging radionuclides/radiation, extreme pH ranges, and other chemical factors (e.g., high salinity, solvents, etc). Furthermore, although the radiation resistance is a critical factor that can determine whether *S. cerevisiae* can be used as a biosorbent for removing radioactive nuclear waste (Parab et al. 2016), no study has been conducted on the effect of γ-ray radiation on the biosorption of Sr^{2+} to *S. cerevisiae*. Thus, it is timely important to conduct a comprehensive study on the adsorption and bioaccumulation processes from HLLW by living *S. cerevisiae*, as well as the underlying mechanisms, which will promote the application of these techniques in wastewater treatment.

In this work, we carried out an investigation of the radiation effects on the *S. cerevisiae*. The strontium biosorption capacity of different *S. cerevisiae* was compared under S-HLLW condition, from which we investigated the strontium biosorption conditions of living *S. cerevisiae*. Based on these experimental results, we further determined the Sr^{2+} biosorption isotherm and kinetic model. The biosorption mechanism was also investigated through comparison of the desorption efficiencies of the living and non-living cells.

Materials and methods

Yeast and reagents

In our previous studies, we had used cyclic irradiation method to culturing an irradiated *S. cerevisiae*: K-4000 and Y-7 (Qiu et al. 2017). In this paper, we investigated the reason why *S. cerevisiae* can survive after continuous exposure to irradiation and observed whether it can still survive under S-HLLW conditions.

RNA extraction, library preparation, and RNA-seq

After K-4000 and K-0 reached the beginning of the exponential growth phase, the total RNA was extracted by TRIZOL reagent (Invitrogen, San Diego, USA) following the manufacturer's protocol. Then, purified using oligo (dT) magnetic beads. The integrity of the RNA was verified with an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, USA) and lodged in Basepair Technology Company (Suzhou, China). The paired-end library preparation and sequencing was achieved by using a TruSeq RNA sample preparation kit (Illumina Inc., San Diego, USA), respectively. The libraries were sequenced by HiSeqTM 2000 device (Illumina).

Biosorption experiments

The components of S-HLLW are listed in Table 1 (Izumida T 1990; R.L. Smith 1997; Zhang A 2006). To simulate the environment of real HLLW (Kubota M 1980), all batch sorption experiments were carried out with the initial conditions of pH = 3–4, C_0 = 615 mg L^{-1}, T was room temperature (25–32 °C), and the initial biomass was 25 g L^{-1} (wet weight). The different *S. cerevisiae* strains (K-0, Y-7, H-K) were observed for sorption capacity, respectively (Dai et al. 2014). (H-K: The cell was killed by heating, 100 °C for 10 min.) The experiments were conducted in duplicate, and control experiments without biosorbents were performed.

To investigate the effects of environmental factors on biosorption capacity, batch experiments were studied in 100-mL Erlenmeyer flasks. Cell suspensions were mixed well by shaking and incubated on a rotary shaker (120 rpm). A sample of the suspension liquid (5 mL) was taken and centrifuged (4000 rpm; 10 min; 4 °C) to obtain concentrated samples for atomic absorption spectrometry (AAS). The percentage adsorption R (%) and adsorption capacity q_t (mg g^{-1}) were determined at different experimental conditions, respectively.

Table 1 Chemical composition and compounds for preparation of simulated HLLW

Element	Contents (g L^{-1})	Compounds
Ce	0.581	$Ce(NO_3)_3{\cdot}6H_2O$
Cs	0.762	$Cs\ NO_3$
Fe	0.743	$Fe(NO_3)_3{\cdot}6H_2O$
K	0.462	KNO_3
La	0.593	$La(NO_3)_2{\cdot}6H_2O$
Mo	0.122	$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$
Nd	0.112	$Nd(NO3)_3{\cdot}6H_2O$
Ni	0.127	$Ni(NO_3)_2\ {\cdot}6H_2O$
Nb	0.123	$NbCl_5$
Mn	0.235	$MnCl_2$
Sr	0.615	$Sr(NO_3)_2$
Rh	0.142	$RhCl_3{\cdot}3H_2O$
Ru	0.235	$RuCl_3$
Sm	0.0371	$Sm(NO_3)_3{\cdot}6H_2O$
Ti	0.0931	$K_2TiO(C_2O_4)_2$
Y	0.0422	$YCl_3{\cdot}6H_2O$
Zr	0.0211	$Zr(NO_3)_4{\cdot}5H_2O$
Ba	0.0172	$Ba(NO_3)_2$
Pb	0.0333	$PbCl_2$
Al	1.59	$Al(NO_3)_3$
Na	5.12	NaCl
H	According to demand	

Metal uptake (q_t) was determined as follows:

$$q_t = \frac{(C_o - C_t)V}{m} \quad (1)$$

$$R = \frac{C_o - C_t}{C_0} \times 100\% \quad (2)$$

C_t was the residual Sr^{2+} concentration at any time (mg L^{-1}), C_0 was initial Sr^{2+} concentration (mg L^{-1}), m was adsorbent dosage (g), and V was the volume of the experimental system.

Effect of radiation

Sorption experiments under radiation environment (RE) studies were performed at the same conditions by different *S. cerevisiae* (Y-7, K-00, H-K). The *S. cerevisiae* (20 g L^{-1}) was suspended in S-HLLW and the samples of suspension liquid were separated from the solution by centrifugation (4000 rpm, 10 min, 4 °C) after 32 h. The total dose was 4000 Gy and the dose was 2.08 Gy min^{-1}.

Effect of *S. cerevisiae* (Y-7) concentration

Similar batch experiments were carried out (mentioned in 2.3). The initial biomass (Y-7) was studied from 10 to 250 mg (wet weight). The samples of suspension liquid were taken out and determined at the time of 32 h, respectively.

Effect of temperature

The sorption experiments were carried out with the initial conditions of pH = 3–4, C_0 = 615 mg L^{-1}, and C_m = 20 g L^{-1} at different temperatures of 10, 20, 27, 32, 38, and 40 °C. The samples of suspension liquid were taken out and determined at the time of 32 h, respectively.

Effect of contact time

Similar batch experiments were carried out (mentioned in 2.3) and the biomass (Y-7) was 20 g L^{-1} at different times of 4, 8, 12, 16, 20, 24, 30, and 36 h, respectively.

The staining experiment after biosorption process

We used staining experiment to detect the survival of *S. cerevisiae* after biosorption.

The staining experiments under S-HLLW

Sorption experiments with different *S. cerevisiae* (Y-7, K-0) were carried out and the samples of *S. cerevisiae* were separated from the solution by centrifugation (4000 rpm, 10 min, 4 °C) at different times of 14, 24, 30, and 36 h. The *S. cerevisiae* was washed (by PBS for 3 times) and stained (with methyl blue), then observed by high power microscope (Olympus CX22, Japan).

The staining experiment under RE + S-HLLW

The *S. cerevisiae* was separated from the samples of suspension liquid of 2.3.2. The staining experiment was the same as 2.4.1.

Characterization

The cellular localization of strontium complexes formed by cells of Y-7 was performed by using transmission electron microscopy (TEM) (JEM-1011 JEOL Japan) and energy dispersive X-ray spectroscopy (EDX) for elemental information analyses. Cells of Y-7 and strontium-adsorbed were treated under the optimal conditions, fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) at 4 °C for 4 h, and washed three times with the same phosphate buffer. The cell pellets were treated with 1% OsO_4 for 30 min at 4 °C, and then dehydrated through a graded ethanol (70, 80, 90, and 95%) series for 10 min. The dehydrated samples were embedded in epoxy resins and sectioned into ultra-thin specimens. Thin

sections were supported on copper grids and examined after staining with lead citrate.

The isotherm biosorption and kinetic studies of Sr^{2+} to Y-7

Isotherm biosorption studies

Biosorption equilibrium was described two isotherm models: the Langmuir and Freundlich models. The Langmuir model is the most commonly used isotherm for sorption, and it assumes that a reversible sorption process occurs on a homogeneous surface forming a monolayer sorption without interactions between the adsorbed species, in addition to uniform energies of the sorption onto the surface, and is expressed as the following equation:

$$\frac{1}{q_e} = \frac{1}{q_m} + \frac{1}{bC_e q_m} \tag{3}$$

q_e is the metal uptake capacity (mg g^{-1}) and C_e is the equilibrium concentration of metal ions in the solution (mg L^{-1}); q_m (mg g^{-1}) is the biosorption capacity when the surface is covered with metal ions completely (maximum biosorption capacity); and b is a constant that represents the affinity between the biosorbent and the metal ion. (Ho et al. 1996).

The Freundlich isotherm model considers the heterogeneity of the surface and multilayer biosorption to the binding sites located on the surface of the biosorbent. It is empirically expressed as follows:

$$\ln q_e = \ln K_F + \frac{1}{n} \ln C_e \tag{4}$$

K_F (Lm g^{-1}) is the Freundlich constant indicating biosorbent capacity and n is the Freundlich adsorption constant known as biosorbent intensity. The model parameters of Freundlich isotherm can be determined from plotting $\ln q_e$ versus $\ln C_e$. (Ho et al. 1996).

Kinetic studies

In order to investigate the biosorption mechanism of heavy metals, pseudo-second order was used to fit the kinetic experimental data according to Ho's method. The sorption data was analyzed in terms of a pseudo-second-order mechanism:

$$\frac{dq_t}{dt} = K_2 (q_e - q_t)^2 \tag{5}$$

$t = 0$; $q_t = 0$; the integral form:

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^{\,2}} + \frac{1}{q_e} t \tag{6}$$

K_2 is the second order rate constant (g/mg h) and q_t is the amount of biosorption at time "t" (mg g^{-1}); q_e is adsorption Sr^{2+} concentration on the adsorbent at equilibrium (mg g^{-1}).

The studies of thermodynamic parameters

In environmental engineering practice, both energy and entropy factors must be considered in order to determine what processes will spontaneously occur. The Gibbs free energy change ΔG^0 is the fundamental criterion of spontaneity. Reactions occur spontaneously at a given temperature if $\Delta G^0 < 0$. The biosorption process of metal ions can be summarized by the following reversible process, which represents a heterogeneous equilibrium. The apparent equilibrium constant (K_c') of the biosorption is defined as:

$$K_c' = \frac{C_{ad,e}}{C_e} \tag{7}$$

$C_{ad,\,e}$ adsorption Sr^{2+} concentration on the adsorbent at equilibrium (mg L^{-1}).

The value of K_c' in the lowest experimental concentration can be obtained. The K_c' value is used in the following equation to determine the Gibbs free energy of biosorption:

$$\Delta G^0 = -RT \ln K_c' \tag{8}$$

The enthalpy (ΔH^0) and entropy (ΔS^0) can be obtained from the slope and intercept of a van's Hoff equation of ΔG^0 versus T:

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \tag{9}$$

Desorption experiments

After biosorption experiment conducted as described above, the bacterial suspension was centrifuged and the biomass was resuspended in one of three desorption reagents: deionized water, 1.0 mol L^{-1} HCl (AR grade, Sigma, USA) and 0.1 mol L^{-1} EDTA-Na_2 (AR grade, Sigma, USA). The suspension was kept a shaker for 24 h (32 °C, 160 rpm) and then centrifuged at 10000 rpm for 5 min. The Sr^{2+} concentration in the supernatant was analyzed by AAS (Persee TA5-990, China). The desorption efficiency (n_d) of $Sr^{2+}$was calculated as follows:

$$n_d(\%) = \frac{m_d}{m_a} = 100\% \times \frac{C_d \times V_d}{m_a} \tag{10}$$

where C_d was the concentration of strontium in the desorption solution (mg Sr/L); V_d is the volume of solution for desorption (mL); m_a is the adsorbed amount of strontium (mg); m_d is the desorbed amount of strontium from the cell (mg).

Results and discussion

Differentially expressed genes (DEGs) analysis

Through our pretreatment, we finally got a total of 30 million valid RNA-sep data. The data quantity is 23.8 G and the average length is 126 bp. The sample data is more than 5 G and it could meet the needs of analysis (Chepelev et al. 2009).

In order to analyze the expressed genes after irradiation, the filtered sequence was screened and assembled. Those results were compared to the standard database and screened the differential expression genes through statistical comparison (Fig. 1) (Anders and Huber 2010). (cufflinks: *t* test, fold change test, *p* value.) From Table S.1, we found that only 613 of the total unigene appeared to be differentially transcribed between K-0 and K-4000. For all of those DEGs, 316 were up- and 297 were down-regulated in K-4000.

Gene ontology analysis

For the further understanding of functions and metabolic pathways involved for these stages-specific genes and differentially expressed genes, GO analysis and pathway enrichment analysis were performed (Wang et al. 2010).

From the results (Table S.2), there were 970 genes could be noted in Go database and total of DEGs were 357. Two hundred thirteen DEGs were attributed to molecular function (59.67%), 90 DEGs belonged to biological process (25.21%), and the rest of DEGs were about cellular component (15.12%).

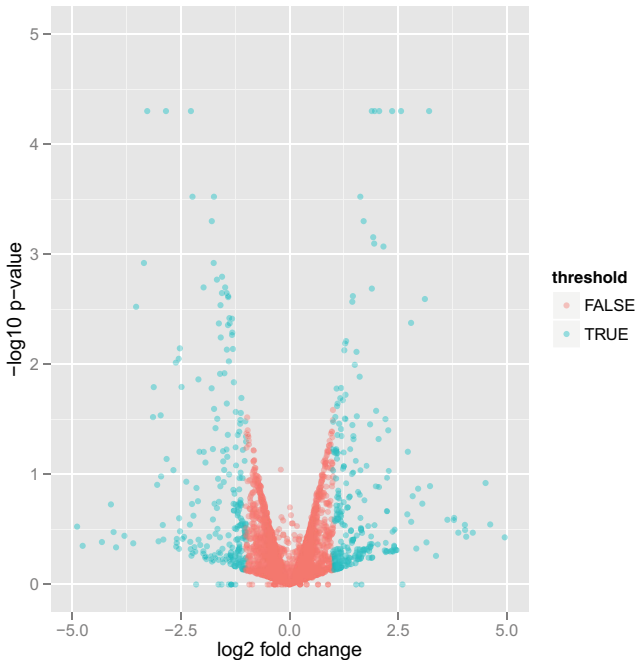

Fig. 1 Differential gene map (the expression of genes with significant differences in blue; abscissa represents the change of gene expression and ordinate represents the statistical significance of difference in gene)

From the results (Table S.3), there were 451 genes could be noted in pathway base and the total of DEGs were 130. Forty DGEs were attributed to metabolic pathway (30.77%); the DEGs belonged to signaling pathways are 7 (5.38%) and the rest of DEGs (63.85%) were about genetic information.

Our RNA-seq results reflected that under the pressure of radiation, some genes could be evolved. The DEGs could be classified for three main categories, "cellular component," "molecular function," and "biological process" category, respectively. The most important components (Table 2) were "developmental maturation" (Go:0021700, etc) and "cell death" (Go:0022415, etc) subgroups of the "biological process" category and the "endoribonuclease activity activity (Go:0016891, etc)", "cellular assembly (Go:0019069, etc)" of subgroups of the "molecular function". From the results of pathway significant enrichment analysis (Table S.3), most of the DEGs belonged to "genetic information" (2.7.7.49, 2.7.7.7, etc). This phenomenon indicated that the "deletion" (sce03040), "recombination" (2.7.7.49), and other events of gene fragments were the critical factor that helped the modified *S. cerevisiae* adapt to the radiation environment (Bahieldin et al. 2015).

Yeast strains *S. cerevisiae* (K-4000) were modified from radioactive environment; genome of radioactive (K-4000) and non-radioactive (K-0) were evaluated. Information related to the radiation resistance of some yeasts has been reported upon; their D_{10} values fall in the range 0.1–0.5 k Gy, the absolute lethal dose of *S. cerevisiae* is about 3.5 k Gy (Yaochuan Wang 1994). Previous studies had also showed that the proportion of primary radiation damage would increase after repeated exposures and the cells were not able to recover (Petin et al. 2014). However, in this study, we found that radiation resistance could be induced by gradient recycle irradiation and the modified yeast (K-4000) had a higher radiation resistance than reference yeast strain that has been reported (Deák and Beuchat 1996; Li et al. 2015). Furthermore, K-4000 could keep living under irradiation environment. Perhaps microorganisms have developed mechanisms of resistance that may lead to the selection of resistant variants that can tolerate radiation (Petin and Kim 2014).

Biosorption studies

Biosorption by *S. cerevisiae*

In environmental engineering practice, both energy and entropy factors must be considered in order to determine what processes will spontaneously occur. In our previous study, we had tested the resistance to radiation and strontium of

Table 2 Go functional categorization of DEGs: K-0—K-4000 (top 10)

GO[a]	DEGs with GO annotation[b]	*p* value[c]	All genes with GO annotation[d]	*Q* value[e]	GO term[f]
GO:0016891	22	1.15E-08	61	1.68E-05	Endoribonuclease activity, producing 5′-phosphomonoesters
GO:0016893	23	2.57E-08	69	3.76E-05	Endonuclease activity, active with either ribo- or deoxyribonucleic acids and producing 5′-phosphomonoesters
GO:0019067	18	3.02E-08	43	4.96E-05	Cellular assembly, maturation, egress, and release
GO:0019068	18	3.02E-08	43	4.96E-05	Cell assembly
GO:0021700	18	3.02E-08	43	4.96E-05	Developmental maturation
GO:0022415	18	3.02E-08	43	4.96E-05	Cellular reproductive process
GO:0046797	18	3.02E-08	43	4.96E-05	Cellular procapsid maturation
GO:0010927	18	3.02E-08	43	4.96E-05	Cellular component assembly involved in morphogenesis
GO:0019069	18	3.02E-08	43	4.96E-05	Cellular capsid assembly
GO:0019058	18	3.02E-08	43	4.96E-05	Cellular infectious cycle

[a] The ID of Gene Ontology

[b] DEGs with GO annotation: total number of DEGs annotations on Go term

[c] *p* value: the significant level of enrichment analysis

[d] All genes with GO annotation: total number of genes annotations on Go term

[e] *Q* value: the significant level of FDR correction

[f] GO term: functional descriptions of Go term

Y-7, respectively (Qiu et al. 2017). Here, we tested the sorption capacity of Y-7 under HLLW or RE conditions. The results (Fig. 2) showed that the bioremoval of strontium ions from simulated wastewater was different compared to that from a simple solution (Wang et al. 2013b). In the S-HLLW, the hydrolysis condition, redox reactions, and precipitation are strongly influenced by those heavy metal, and conversely, strongly influence the biosorption availability. The highest metal uptake and efficiency were 11.656 mg g^{-1} and 37.91% at 32 h by Y-7. A similar result was obtained for Dicratetia inornata and *Platymonas subcordiformis*, which can adsorp strontium ion concentrations greater than 1.44 mmol L^{-1}(Mei et al. 2004). The *D. inornata* biosorption capacity was severely inhibited when the initial strontium concentration reached 5.76 mmol L^{-1} (approximately 500 mg L^{-1}) (Liu et al. 2008).

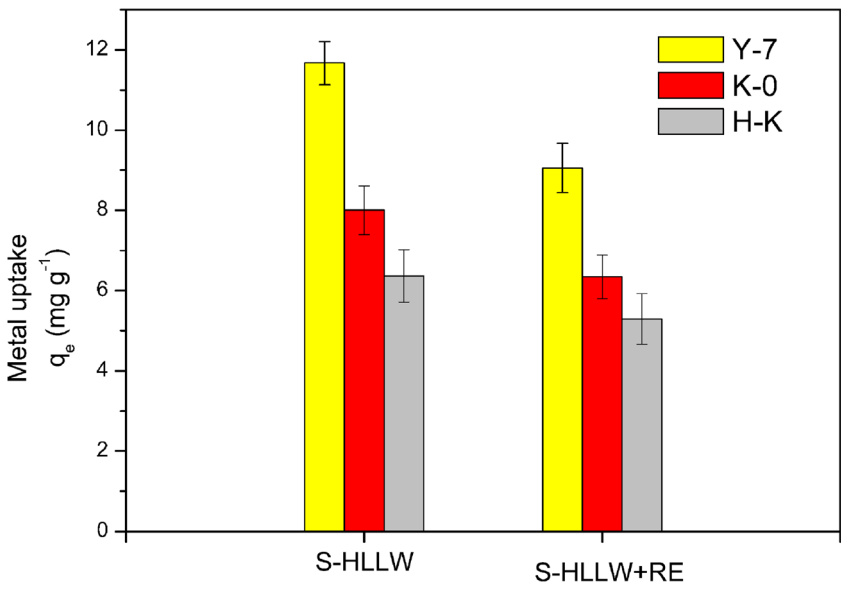

Fig. 2 Biosorption of strontium under different conditions (C_m = 20 g L^{-1}; pH 3–4; room temperature 27–32 °C; C_0 615 mg L^{-1}; contacting time 32 h)

The different adsorption rates of H-K, K-0, and Y-7 may due to their biosorption mechanisms being different (Liu et al. 2014). From that, we inferred that the dead *S. cerevisiae* might adsorb Sr^{2+} only owing to electrostatic interaction caused by the functional groups of the cell surface including carboxyl, phenyl, hydroxyl, amino, etc. (Wang et al. 2013b). Those results suggest that strontium by living *S. cerevisiae* (Y-7) in HLLW is feasible and could be an efficient means for treating nuclear wastewater. Figure 2 also presents the biosorption efficiency of Sr^{2+} by Y-7 and other strains at 4000 Gy with S-HLLW, respectively. From the results, the sorption capacity of Y-7 improved when compared with the other *S. cerevisiae*. The uptake and biosorption efficiency of Sr^{2+} by Y-7 strain was 9.46 mg g^{-1} and 30.76% at 32 h, respectively. However, compared with the results of no-RE, the uptake and efficiency of Y-7 had decreased in the radiation environment.

Effect of *S. cerevisiae* (Y-7) concentration

Biosorption experiments were performed with using solutions containing 10–25 g L^{-1} biomass for 32 h. From Fig. 3, the biosorption efficiency of Sr^{2+} by Y-7 increased and the sorption capacity decreased with increasing of initial biomass. The maximum sorption rate was found about 37.125% at 250 mg, with the sorption capacity results, the most favorable initial concentration range was 20 g L^{-1}. When increasing the initial biomass after 20 g L^{-1}, the uptakes of Sr^{2+} would not increase. This appears to be because of the increase the number of biomass competing for the available binding sites in the S-HLLW and also due to the saturated binding sites for complexation of Sr^{2+} higher concentration levels. (Tan Y et al. 2017).

Effect of contact time

The uptake of Sr^{2+} by Y-7 was studied in the time range of 4–40 h. From Fig. 4, it showed that strontium biosorption was closely related to contact time. The maximum uptakes were found about 10.05 mg g^{-1} at 30 h and the theoretical maximum should be about 32 h. The biosorption rate increased quickly in the first 12 h and then gradually came up to 32.68%. It suggested that the activity sites of Sr^{2+} would increase in the S-HLLW at longer time, which could provide more the effective occupation degree of Sr^{2+} at the active sites of Y-7. After 30 h, those active sites might be saturated and from our straining experiment, the cells begin to die which means the living cells cannot adsorb Sr anymore (Chen and Wang 2016). This could explain why the maximum biosorption efficiency was observed at 32 h.

Effect of temperature

From our previous studies, the biosorption experiments were performed with using solution containing 10–40 °C. From Fig. 5, the maximum uptake was found about 11.5 mg g^{-1} at 32 h at 32 °C. In addition, the activity of Sr^{2+} increased in the aqueous phase at higher temperature, which may improve the effective occupation degree of Sr^{2+} at the active sites of yeast cells. But at higher temperature (> 32 °C), Sr^{2+} might not interact with the binding sites may be because of the result of self-regulation by Y-7. It was the same as our previous reported (Qiu et al. 2017).

Those results suggested that factors that affected biosorption include ion mixing, time, temperature, pretreatment, and biomass quantity. The optimal conditions for strontium biosorption for living *S. cerevisiae* were cell concentration 20 g L^{-1}, contacting time 32 h, and temperature 32 °C. Some results were different from traditional studies; this is

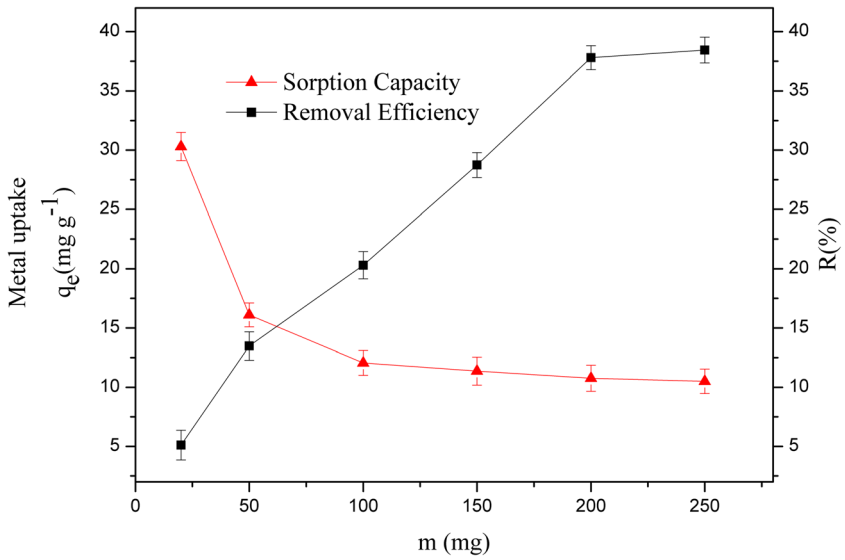

Fig. 3 Effect of *S. cerevisiae* (Y-7) concentration on strontium biosorption (pH 3–4; *T* room temperature 27–32 °C; C_0 615 mg L^{-1}; contacting time 32 h)

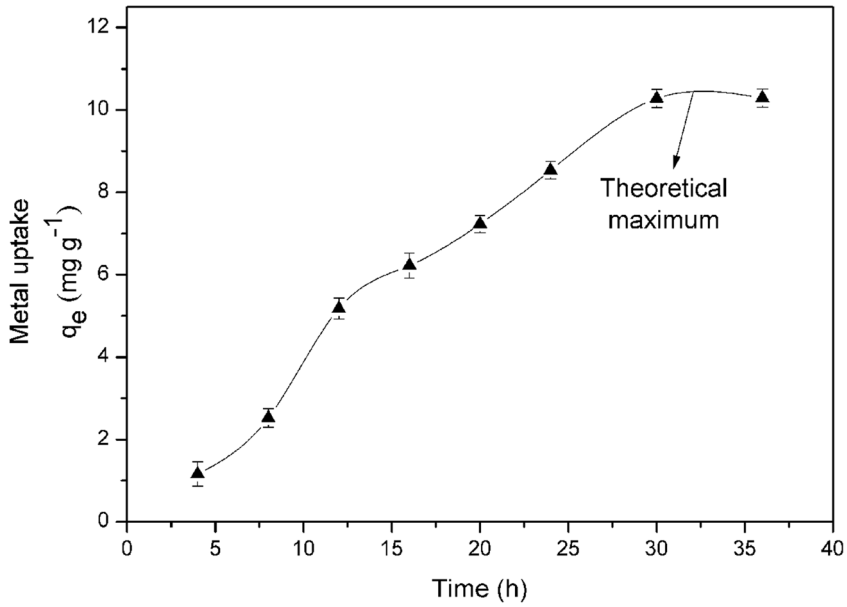

Fig. 4 Effect of contacting time on strontium by Y-7 (pH 3–4; room temperature 27–32 °C; C_0 615 mg L^{-1}; C_m = 20 g L^{-1})

probably because the *S. cerevisiae* was living, when the conditions increased, the biosorption efficiency increased rapidly and then remained. For the biosorption system of living cells, each living cell is not utilized to adsorb strontium ions. (Coexistent metal ions mainly affect the biosorption of strontium ions from wastewater.) With the increased of biomass concentration, there were many strontium ions for every cell to absorb and the live cell system always stayed in the excess strontium ions state. But in this system, the biomass concentration was constant, it reflected that for living cells, the saturated binding sites were limited (Liu et al. 2014). The contacting time result suggested that when living cells was in a new culture environment that it broke the ionic equilibrium. Thus, additional time was necessary for the cell to regenerate its balance and survive in such an environment. The result was similarly reported in other studies (Gipps and Coller 1980).

Some studies had shown that the maximum adsorption or bioaccumulation quality of strontium by a non-living or living microorganism in the traditional adsorption method varies from 4.05 to 7.35 mg g^{-1} (Hu et al. 2017; Nie et al. 2016). In this study, we found that maximum strontium biosorption efficiency and quality of living *S. cerevisiae* under HLLW were 37.91% and 11.656 mg g^{-1}. Those results are higher than the adsorption quantity reported in previous studies (Marešová et al. 2011; Weerasekara et al. 2013), but also those

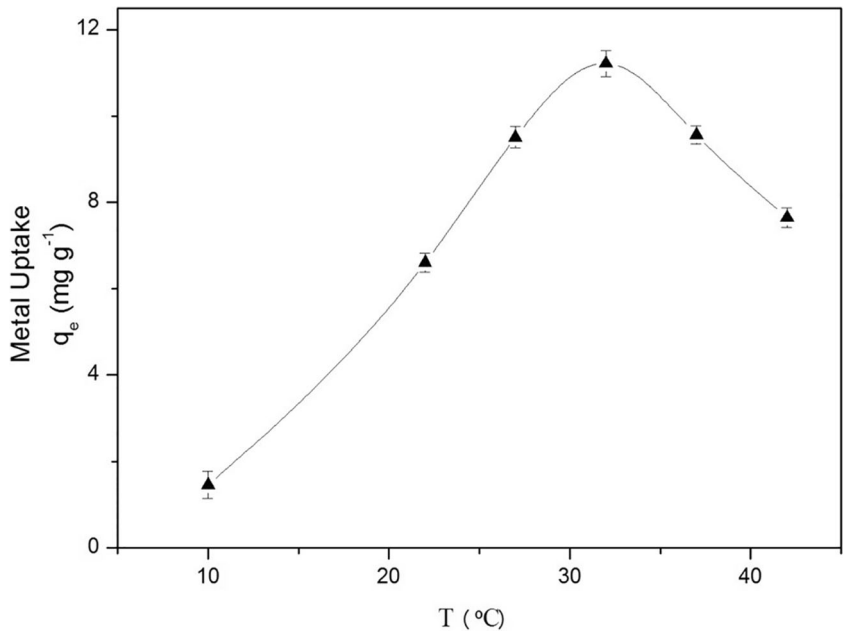

Fig. 5 Effect of temperature on strontium by Y-7 (pH 3–4; C_0 615 mg L^{-1}; C_m = 20 g L^{-1}; contacting *t* time 32 h)

results indicate that radiation and the toxicity of heavy meals could decrease the sorption capacity. Therefore, it is necessary to study the behavior of these biosorbents for removing the potential radionuclides as a multicomponent system.

Results of staining experiment

In this study, we used methylene blue to verify whether the *S. cerevisiae* survived after biosorption. For living cells, their cell membranes are integrity which can exclude the methylene blue into the cell; if the cell loses its activity, it can increase the permeability of cell membrane, so methylene blue can enter the cell and bind the nucleic acid, making it become blue. Figure S.4 reflected the survival of *S. cerevisiae* after biosorption. Y-7 could remain active for 24 h in simulated condition (Fig. S.4(A)), but some dead cells were observed when the sorption time prolonged 32 h. For K-0 (Fig. S.4(B)), the staining result reflected that it could not remain active for 12 h and most of the cells were already dead when the sorption time prolonged 24 h. After biosorption for 32 h under RE, Y-7 retained its activity but K-0 was dead (Fig. S.5).

Results of TEM and EDX

In our previous studies, we used TEM-EDX to analyze the elemental information after biosorption. But from the result (S.7), we could find that despite the emergence of strontium, there are still too many energy peaks of other heavy metals. In order to avoid the error caused by other heavy metals in the EDX test, we decided to use SEM-EDX to the analysis of Y-7 after biosorption.

As shown in Fig. S.7(A), before biosorption, it was obvious that we can observe some organelles like vacuole, mitochondria, and nucleus. After biosorption, we only could observe part of vacuole and mitochondria from Fig. S.7(B), but it was obvious that the cell internal structure changed significantly and irreversible damage formed. From EDX results, before biosorption, it was not detected by TEM-EDX for Y-7 (Fig. S.9). From SEM-EDX (Fig. S.8), strontium was detected in one area (upper, spot1) after biosorption. Combined with the desorption results, those findings indicated that strontium ions entered into Y-7 by some mechanism, and were not merely fixed on the cell surface. As reported before (Lan et al. 2014; Merroun et al. 2003), metal binding to anionic functional groups or micro precipitated on the cell surface are general mechanisms. In response to metal pollution, microorganisms immobilize the toxic (heavy) metals using extracellular materials and prevent them into the cells. However, in this study, we had exposed the tolerance of irradiated *S.cerevisiae* (K-4000) and our previous studies had showed that Y-7 could survive after continuous exposure to strontium and continue to grow under culture conditions. Thus, we could find that after biosorption, the volume of vacuole had decreased and the cytoplasm was destroyed, this might because that the heavy metal entered into the cell. Therefore, we speculate that living microorganism biosorption can occur by metabolism-dependent and metabolism-independent processes (Kapoor and Viraraghavan 1995; Tomioka et al. 1998). The adsorption mechanism must be very complicated; it includes physical adsorption; chemisorption, and biological accumulation (Robalds et al. 2016).

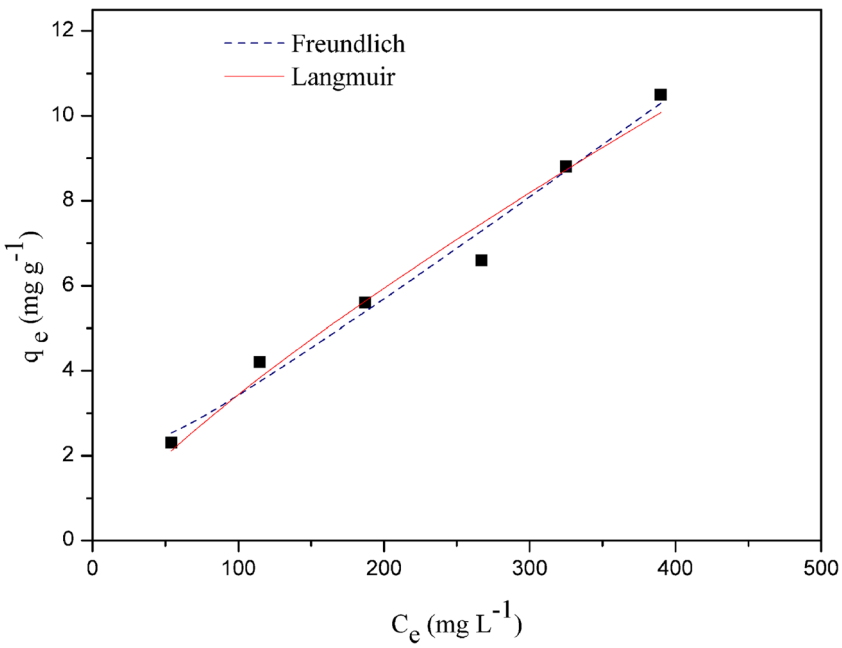

Fig. 6 Isotherm curves for Sr^{2+} biosorption on to 20 g L^{-1} of Y-7 (pH 3–4; contacting time 32 h)

Table 3 The Langmuir, Freundlich isotherm, and pseudo-second order kinetics constants obtained for the biosorption of strontium ions onto Y-7

Langmuir constants			Freundlich constants			Pseudo-second-order kinetics		
q_{max} (mg g^{-1})	K_L (L mg^{-1})	r^2	K_F (L mg^{-1})	n	r^2	q_e (mg g^{-1})	K_2 (g mg^{-1} h^{-1})	r^2
37.4	0.0351	0.98	1.38	1.25	0.97	22.6	0.0442	0.94

Equilibrium of strontium biosorption to Y-7

To analyze the effect of pretreatment and HLLW on the isotherm biosorption of Sr^{2+} to Y-7, two isotherms of the Langmuir and Freundlich models were discussed in the present study. The non-linearized Langmuir and Freundlich isotherms of Sr^{2+} are shown Fig. 6, the experimental data were analyzed by non-linear regression analysis, and then, the isotherm constants and correlation coefficients are given in Table 3. From those results, it can be seen that the coefficient of determination (r^2) decreased in the following order: Langmuir > Freundlich models and it should be noted that in Langmuir results, the q_e was found smaller to be smaller than q_{max} (24.74 mg L^{-1}) indicating that the biosorption of Sr^{2+} to Y-7 is occurred by a monolayer-type adsorption in which the surface of the microorganism was not fully covered.

Kinetics of strontium biosorption by Y-7

The correlation coefficients (r^2) for the pseudo-second-order kinetic model at different initial Sr^{2+} concentrations were found to be more than 0.9 (Table 3). It indicated that the biosorption of Sr^{2+} ions to Y-7 follows a pseudo-second-order model. This suggests that chemical biosorption involves valence forces through the sharing or exchange of electrons between the biosorbents. However, it should be noted that biosorption of Sr^{2+} to Y-7 under S-HLLW is not a rapid process (Fig. 7), this is probably due to the other metal ions in the conditions which may decrease the biosorption capacity of Sr^{2+}. The adsorption may be the rate-limiting step of biosorption process (Liu et al. 2014).

Thermodynamic parameters

The values of the Gibbs free energy change (ΔG^0), enthalpy change (ΔH^0), and entropy change (ΔS^0) for the biosorption process were obtained from Eqs. The negative value (Table 5) of ΔG^0 confirms the feasibility of the process and the spontaneous nature of biosorption of Sr^{2+} to Y-7. The ΔG^0 values indicate the degree of spontaneity of the adsorption process, where more negative values reflect a more energetically favorable adsorption process. The values (Table 4) of ΔH^0 the biosorption of Sr^{2+} to Y-7 were positive which indicated that the biosorption processes are endothermic and the positive of ΔS^0 reflected the increase of randomness at the solid/solution interface and the good affinity of Sr^{2+} for Y-7. The thermodynamic results showed that all the biosorption reactions were spontaneous process and the temperature

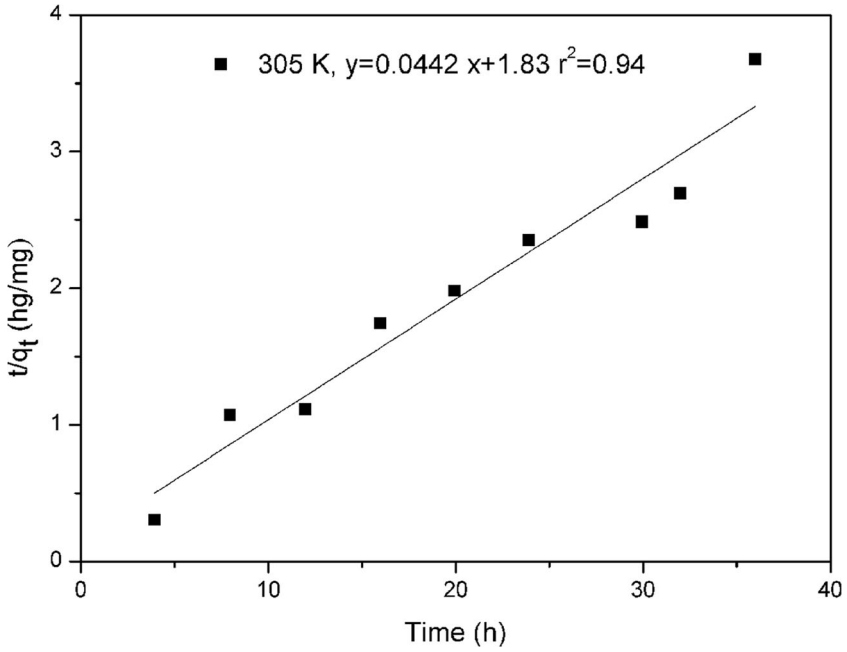

Fig. 7 Pseudo-second order kinetic model curves

Table 4 Thermodynamic parameters for the sorption of strontium ion by Y-7

Thermodynamic parameters	10 °C	20 °C	32 °C
ΔG^0 (kJ mol^{-1})	− 0.456	− 0.530	− 0.710
ΔH^0 (kJ mol^{-1})		2.86	
ΔS^0 (kJ mol^{-1} K^{-1})		117	

increasing were favorable for the biosorption of Sr^{2+} to Y-7 for the experimental conditions considered.

Desorption results

Several mechanisms responsible for the binding of metal ions to bacterial cells can be quantitatively assessed using appropriate desorption reagents. Desorption of the adsorbed strontium on Y-7 was investigated in the presence of deionized water, HCl, and EDTA-Na_2 in solution. As depicted in Table 5, the HCl and EATA-Na are high-efficiency desorbing agents, capable of recovering 60.62 and 76.51% of strontium adsorbed by Y-7 and H-K for 0.1 M EDTA-Na_2, respectively. The desorption efficiency of strontium adsorbed onto Y-7 and H-K was 76.51 and 83.32% by 1 M HCl. However, the desorption efficiency was only 7.65 and 18.82% for Y-7 and H-K by deionized water, respectively. When metal ions come into contact with bacterial cells, some ions enter into the cell wall mesh structure and are adsorbed by physical entrapment. This part is bound weakly and can be desorbed by water (Wang et al. 2013a), it could be called "microprecipitation" (Robalds et al. 2016). Another fraction that complexes with cell wall functional groups such as carboxyl and phosphate groups can be desorbed by EDTA but not by water, this part include chemisorption (Robalds et al. 2016). Also, the adsorbed strontium can be desorbed from various biomaterials by H^+, because it includes not only the functional group, but also interaction (redox) and ion exchange (Mg^{2+}, Ca^{2+}) (Wang et al. 2013a), and this part could be attributed as "electrostatic attraction" (Robalds et al. 2016).

Also, from the results, we could observe that the desorption rate of H-K was much higher than that of Y-7. This result was similar to previous studies (Chen and Wang 2016; Wang et al. 2013a) and it indicated that the adsorption of non-living cells mainly concentrated on the cell surface, for living cell strontium was considered to be accumulated inside the cell. Considering cost, safety, and efficiency, HCl was suggested to be applied for strontium recovery from Y-7.

Table 5 The percentage of strontium released from Y-7 after treatment with various desorbents

Desorbents		Desorption rate (%)		
	Conc	V (mL)	Y-7	H-K
Deionized water		15	7.65 ± 0.52	18.82 ± 1.82
HCl	1.0 M	15	76.51 ± 2.13	83.32 ± 1.73
EDTA-Na_2	0.1 M	15	60.62 ± 2.42	73.32 ± 1.81

Conclusion

Strontium sorption by *S. cerevisiae* was increased after pre-exposure to irradiation. The significant changes observed in RNA-seq results and the structural degradation of biomass may be due to the radiolysis from the marginal increment of the sorption capacity of *S. cerevisiae* towards strontium.

The biosorption of strontium to living cells (Y-7) from S-HLLW is a complicated process controlled by many environment variables, including time, cell concentrations, and temperature. The biosorption capacity of living cells (Y-7) under S-HLLW conditions could reach 11.656 mg g^{-1}. From our biosorption process analysis, the strontium ions were firstly adsorbed to the surface of cells, and then moved into the *S. cerevisiae* across the cell membrane, which were not merely fixed on the cell surface. We propose that the absorption of strontium by living *S. cerevisiae* has two steps: the first step is passive biosorption, during which strontium ions are adsorbed to the cell surface due to chemical and physical sorption. The second step is the penetration of the cell membrane into cytoplasm or on organelle, which can be regarded as initiative biosorption. Initiative biosorption may depend on energy and is related to the metal transport in the living cells. Our work indicates that living *S. cerevisiae* (Y-7), as a sorbent material, is promising for applications in the treatment of radioactive process streams.

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