# PROBIOTIC MICROORGANISMS AGAINST A POSSIBLE RADIUM CONTAMINATION OF DRINKING WATER

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## ABSTRACT

Many anthropogenic activities have resulted in contamination of hydrosphere with heavy metals and radionuclides, which endanger human health. Radium is the heaviest alkaline earth metal, which occurs naturally in the form of four radioisotopes resulting from the decay chains of primordial radionuclides. Although these radioisotopes usually occur in foodstuffs and in drinking water at low concentrations, extraordinary high concentrations may be correlated with industries extracting or processing Naturally Occurring Radioactive Materials (NORM). It is also reported that the greatest potential health risk for human exposure to radium is through drinking water, as radium is considered as a potent human carcinogen.

It is also known that different microorganisms have the capability to bind heavy metals. According to FAO and WHO, probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host.” Because they do not colonize the human intestinal track, probiotic microorganisms should be consumed “in adequate amounts as part of food”.

The present study aims to contribute to a dispute of scientific community, if and under what conditions, probiotic microorganisms could become effective detoxification means. More specifically, this study is an attempt to explore the possible selectivity of two probiotic microorganisms, namely *Lactobacillus bulgaricus* and *Saccharomyces bulardii* to bind barium, which is considered as a good analogue for radium. The barium ion solution used was spiked with a radiotracer ($^{133}$Ba), so as the barium concentration to be determined easily and accurately by measuring the radioactivity of a certain volume.

For comparison, activated carbon (Merck 1. 02186) was tested in parallel experiments under identical conditions.

Batch sorption experiments were carried out, bringing in contact 0.02 g of lyophilized cells or material for comparison with 4 mL of $^{133}$Ba solution of known initial concentration and of constant ionic strength ($I$=0.1 M $NaClO_4$).

The suspension was agitated on a rotary shaker for 24 h at 37 $^{o}$C. Then, the supernatant was separated by centrifugation and the final metal concentration was determined measuring the radioactivity. The barium uptake onto adsorbents was determined by the difference between initial and final metal concentration.

The interaction of barium ions with lyophilized cells of *L. bulgaricus or S. bulardii* was studied under different pH values, ionic strength solutions and metal ion concentrations.
It was found that the metal uptake by both microorganisms was strongly dependent upon pH and it was always higher than the uptake attained by activated carbon.

**Keywords:** Radium, probiotics, *Lactobacillus bulgaricus*, *Saccharomyces bulardii,* activated charcoal, metal uptake.

## 1. Introduction

Many anthropogenic activities have resulted in contamination of hydrosphere with heavy metals and radionuclides, which endanger human health (Naja *et al.*, 2009 ; Goulet *et al.* 2011). Radium is the heaviest alkaline earth metal, which occurs naturally in the form of four radioisotopes resulting from the decay chains of primordial radionuclides $^{232}$Th, and $^{235/238}$U. Although these radioisotopes usually occur in foodstuffs and in drinking water at low concentrations, extraordinary high concentrations may be correlated with industries extracting or processing Naturally Occurring Radioactive Materials (NORM) (Vandenhove *et al.*, 2005). The greatest potential health risk for human exposure to radium is through drinking water, as radium is considered as a potent human carcinogen (ATSDR, 1990).

The known capability of different microorganisms to bind heavy metals and radionuclides has often been studied for decontamination reasons. According to FAO and WHO, probiotics are defined as “live microorganisms, which when administered in adequate amounts, confer a health benefit on the host.” Because they do not colonize the human intestinal track, probiotic microorganisms should be consumed “in adequate amounts as part of food” (FAO/WHO, 2001).

In an attempt to reply to a general issue, if and under what conditions, probiotic microorganisms could become effective detoxification means, we tried to explore the uptake of radium by two probiotic microorganisms like *Lactobacillus bulgaricus* (*L. bulgaricus)* and *Saccharomyces bulardii* (*S. bulardii*).

Instead of radium, we used barium, which is considered as a chemical analogue for it. More specifically, we investigated the dependence of barium uptake on the following parameters:

1) growth phase from which the cells were isolated
2) pH and initial metal concentration

Additionally, we compared the metal capacity of both microorganisms with that of a classical adsorbent, like activated carbon.

## 2. Experimental part

### 2.1. Barium test solutions

A barium chloride ($^{133}BaCl_2$) solution, with known metal content was purchased from POLATOM (Poland). By appropriate dilutions, a series of solutions was prepared and their specific radioactivity (due to $^{133}Ba^{2+}$) was determined by counting a certain volume by a sodium iodide detector. The solutions of known barium concentration were checked as the correlation between counted specific activity and barium content (mol/L) produced straight lines with excellent fitting. By linear interpolation of radioactivity in the obtained calibration curves (straight lines), determination of barium concentration was done easily and accurately.

### 2.2. Growth of biomass

*L. bulgaricus* (*Lactobacillus delbrueckii* subsp. *bulgaricus,* DSMZ 20081) and *S. bulardii* (been isolated from a pharmaceutical product) were grown at 37 °C, under anaerobic conditions. For growth of the first microorganism, MRS broth (from Lab M. Limited) was used. The second microorganism was grown in a mixture containing 2% glucose, 0.4% yeast extract, 0.1% $KH_2PO_4$, 0.1% $(NH_4)_2SO_4$ and 1% $MgSO_4 \cdot 7H_2O$.

The progress of cell cultures was assessed by optical density measurements and so, the exponential and stationary phase of growth were determined. Biomass from individual cell cultures of both microorganisms was isolated from the middle of exponential and the beginning of stationary phase. The isolated cells were washed with water and then were lyophilized and stored at -20 °C.

### 2.3. Barium uptake experiments

Batch sorption experiments were carried out, bringing in contact 0.02 g of lyophilized cells with 4 mL of $^{133}Ba^{2+}$ solution of known initial concentration and constant ionic strength (*I*), by adding $NaClO_4$, as background electrolyte. In each experiment, three vials were used. Two duplicate vials (denoted as test vials) contained the metal solution and the adsorbent and a third one (denoted as blank), contained only the metal solution. All the vials were agitated on a rotary shaker for 24 h at 37 ± 0.5 $^oC$. After centrifugation, a certain volume of the supernatant was isolated. The initial ($C_{in}$) and the final metal concentration ($C_{fin}$) in the supernatant, were directly proportional to radioactivity of the blank and test solution respectively. The metal concentration (mol/L) in each sample was determined by interpolation of the counted radioactivity using the calibration curves mentioned above.

For comparison, activated carbon (Merck 1.02186) was tested in parallel experiments under identical conditions.

The percentage of barium uptake R (%) was determined by the difference between initial and final metal concentration, using the equation:

$$R\,(\%)=\frac{(C_{in}-C_{fin})}{C_{in}}\cdot 100 \qquad (1)$$

while the uptake q (mol/g), by the equation:

$$q=(C_{in}-C_{fin})\cdot\frac{V}{m} \qquad (2)$$

where:

$C_{in}$ and $C_{fin}$ , were expressed in mol/L
$V$, the volume (L) of barium solution used in each experiment and
$m$, is the accurate dry weight of the adsorbent (g).

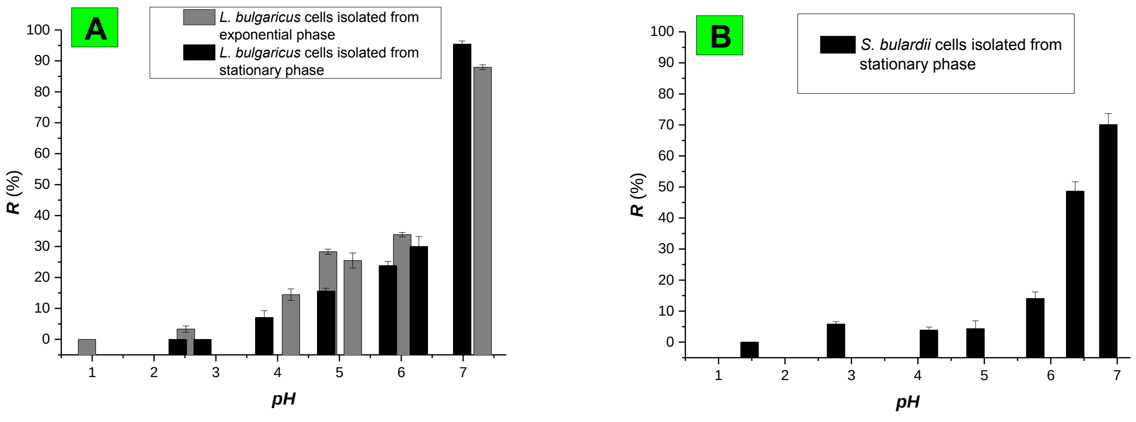
## 3. Results and discussion

### 3.1. The effect of pH

The effect of pH on barium uptake is shown in figure 1. It is evident that barium uptake by all adsorbents studied, increased significantly with increasing pH, while in the acidic region (pH<4), a minimum uptake (less than 10 %) was observed.

It can also be seen in figure 1A, that exponential cells of *L. bulgaricus* indicated a little higher capacity than stationary ones, which is consistent with literature reports e.g. Anagnostopoulos *et al.* (2011).

Moreover, it is clear that in the pH region studied, probiotic cells indicated higher metal uptake than activated carbon. Specifically, barium uptake by *L. bulgaricus* cells reached a maximum (nearly 90%) at pH≈7, whereas at the same pH the metal uptake by *S. bulardii* and activated carbon were found almost ~ 70 and 50 % respectively.



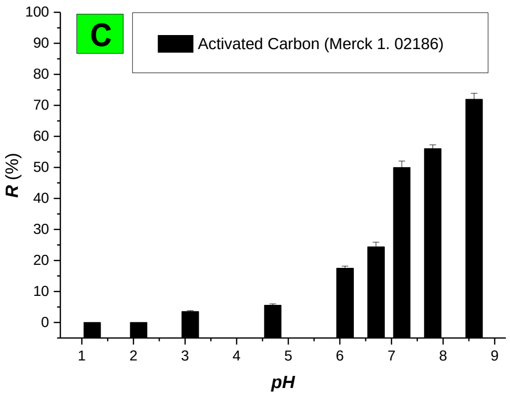


**Figure 1:** Effect of pH on uptake of barium by: A) *L. bulgaricus* cells. Black and gray columns represent the phase (exponential or stationary) from which the cells were isolated. B) *S. bulardii* cells isolated from stationary phase. C) Activated carbon (Merck 1. 02186). Initial metal concentration $5 \cdot 10^{-5}$ mol barium/L, *I* = 0.1 M $NaClO_4$, contact time 24 hours at (37 ± 0.5) °C.

### 3.2. The effect of metal ion concentration

Preliminary experiments indicated that a contact time of 24 hours was enough, so as equilibrium to be attained. Experimental data of metal uptake at pH = 6 ± 0.1 and at (37 ± 0.5) °C were fitted to Langmuir, Freundlich and Dubinin-Radushkevich (D-R) models, using the linear form of equation (3), (4) and (5) respectively.

$$q=\frac{q_{max} \cdot K_L \cdot C_{fin}}{1+(K_L \cdot C_{fin})}, \quad \text{or its linear form,} \quad \frac{C_{fin}}{q}=\frac{1}{q_{max} \cdot K_L}+\frac{C_{fin}}{q_{max}} \tag{3}$$

$$q=K_f \cdot C_{fin}^{\,1/n}, \quad \text{or its linear form,} \quad \ln q = \ln K_f+\frac{1}{n}\ln C_{fin} \tag{4}$$

$$q=q_{max} \cdot e^{(-K' \cdot \varepsilon^2)}, \text{ or its linear form,} \quad \ln q = \ln q_{max}-K' \cdot \varepsilon^2 \tag{5}$$

where $\varepsilon$ is Polanyi's adsorption potential, which is defined as $\varepsilon = RT \cdot \ln(1+\frac{1}{C_{fin}})$.

For the evaluation of the best fitting adsorption model, the correlation coefficients ($R^2$) of linear plots were considered, as well as the difference between the experimental ($q_{exp}$) and the estimated values ($q_{th}$) by each isotherm model. Equation (6) calculates the difference in terms of normalized standard deviation, $\Delta q$ (%), where $n$ is the number of experimental points (Sun *et al.*, 2003).

$$\Delta q(\%)=100 \cdot \sqrt{\frac{\Sigma\,[(q_{exp}-q_{th})/q_{exp})^2}{n-1}} \tag{6}$$

The estimated parameters of the studied models were compiled in table 1.

From table 1, it is obvious that none of the studied models could describe accurately all the experimental data. Langmuir model, one the most often used adsorption model, failed completely. In most cases, D-R model is likely to provide the best fitting. The estimated values of binding energy (*E*) by this model, suggest chemi-sorption of barium ions.

**Table 1:** Fitting of experimental data to three widely used adsorption models and determination of their parameters by linear regression.

| Barium adsorption onto | Model | Regression parameters | $R^2$ | $\Delta q$ (%) |
|---|---|---|---|---|
| *L. bulgaricus*, $I$ = 0.025 M $NaClO_4$ | **Freundlich** | $n = 1.1$ *<br>$K_F = 0.13$ L/g | $0.90_3$ | 35 |
| | **Dubinin-Radushkevich** | $K' = 5.7 \times 10^{-3}$ $mol^2/kJ^2$<br>$q_{max} = 8.1 \times 10^{-4}$ mol/g<br>$E = 9.4$ kJ/mol | $0.90_1$ | 35 |
| *L. bulgaricus*, $I$ = 0.1 M $NaClO_4$ | **Langmuir** | $K_L = 82 \times 10^3$ L/mol<br>$q_{max} = 7.4 \times 10^{-6}$ mol/g | $0.87_2$ | 47 |
| | **Freundlich** | $n = 2.1$ *<br>$K_F = 7.8 \times 10^{-4}$ L/g | $0.71_5$ | 45 |
| | **Dubinin-Radushkevich** | $K' = 3.1 \times 10^{-3}$ $mol^2/kJ^2$<br>$q_{max} = 4.6 \times 10^{-5}$ mol/g<br>$E = 13$ kJ/mol | $0.71_9$ | 47 |
| *L. bulgaricus*, $I$ = 1.0 M $NaClO_4$ | **Freundlich** | $n = 0.98$ *<br>$K_F = 0.029$ L/g | $0.90_7$ | 56 |
| | **Dubinin-Radushkevich** | $K' = 7.3 \times 10^{-3}$ $mol^2/kJ^2$<br>$q_{max} = 1.5 \times 10^{-4}$ mol/g<br>$E = 8.3$ kJ/mol | $0.91_9$ | 52 |
| *S. bulardii*, $I$ = 0.1 M $NaClO_4$ | **Freundlich** | $n = 1.1$ *<br>$K_F = 0.067$ L/g | $0.95_6$ | 48 |
| | **Dubinin-Radushkevich** | $K' = 6.2 \times 10^{-3}$ $mol^2/kJ^2$<br>$q_{max} = 4.0 \times 10^{-4}$ mol/g<br>$E = 9.0$ kJ/mol | $0.98_8$ | 31 |

**Parameters of Langmuir model were omitted whenever fitting was impossible ($R^2$<0.1)**

*** Value of $n$, when $q$ and $C_{fin}$ are expressed in mol/g and mol/L respectively.**

### 3.3. The effect of ionic strength

From figure 2, it is clear that barium uptake decreased with increasing ionic strength. This dependence is a strong evidence of a predominantly electrostatic interaction (Soupioni *et al.*, 1996), because the higher the ionic strength of the solution, the more the compression of the electrical double layer around the suspended cell.

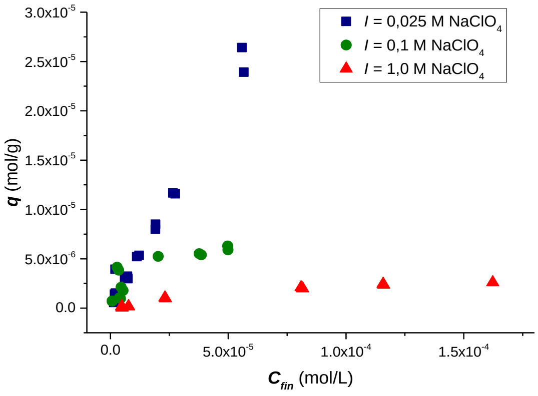


**Figure 2:** Isotherms of barium adsorption by *L. bulgaricus* cells isolated from stationary phase, at three different values of ionic strength.

## 4. Conclusions

Our results reveal that:

- The uptake of $Ba^{2+}$ by all adsorbent studied, is strongly dependent upon pH.
- The uptake of $Ba^{2+}$ by *L. bulgaricus,* is higher at lower ionic strength, indicating a predominantly electrostatic interaction. These data do not fit the widely used Langmuir and Freundlich adsorption models, whereas D-R model seems to provide the best fitting, suggesting chemi-sorption.
- Undoubtedly, both probiotic microorganisms were better adsorbents of $Ba^{2+}$ than a classical one, such as activated carbon.

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## REFERENCES

1. Agency for Toxic Substances and Disease Registry (ATSDR) (1990) Toxicological Profile for Radium, Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA.
2. Anagnostopoulos V.A., Bekatorou A. and Symeopoulos B.D. (2011) Contribution to interpretation of metal uptake dependence upon the growth phase of microorganisms. The case of uranium(VI) uptake by common yeasts, cultivated at different temperatures, with or without aeration, Journal of Radioanalytical and Nuclear Chemistry, 287(2), 665-671.
3. FAO/WHO (2001) Report on Joint FAO/ WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria.
4. Goulet R.R., Fortin C. and Spry DJ. (2011) Uranium, Fish Physiology, 31, 391-428.
5. Naja G.M. and Volesky B. (2009) Metals in the environment: toxicity and sources, Taylor & Francis and CRC Press, Boca Raton FL.
6. Soupioni M., Symeopoulos B., Athanasiou J., Gioulis, A., Koutsoukos P. and Tsolis-Katagas P. (1999) A preliminary study of mercury uptake by a Greek zeoliferous rock. Natural Microporous Materials in Environmental Technology, 362, 365–369.
7. Sun, Q. and Yang, L. (2003) The adsorption of basic dyes from aqueous solution on modified peat-resin particle, Water Research., 37,15, 35-44.
8. Vandenhove H., Eyckmans T. and Van Hees M. (2005) Can barium and strontium be used as tracers for radium in soil-plant transfer studies? Journal of environmental radioactivity, 81 (2-3), 255–67.