Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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To cite this Article Fisel, S. , Dulman, V. and Burcoveanu, C.(1978) 'Co(II) and Ni(II) Concentration, and Co(II) Purification with Microbiological Collectors (*Saccharomyces cerevisiae*)', Separation Science and Technology, 13: 10, 835 — 842
To link to this Article: DOI: 10.1080/01496397808057133
URL: http://dx.doi.org/10.1080/01496397808057133

SEPARATION SCIENCE AND TECHNOLOGY, 13(10), pp. 835–842, 1978

Co(II) and Ni(II) Concentration, and Co(II) Purification with Microbiological Collectors *(Saccharomyces cerevisiae)*

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Abstract

Co(II) and Ni(II) can be concentrated quantitatively using a microbiological collector consisting of a *Saccharomyces cerevisiae* strain suspended in a glucose containing phosphate buffer. Optimal conditions for such accumulation as regards pH, time, and concentration have been studied. The influence of some complexing agents on the accumulation of a mixture of Co(II) and Ni(II) has also been investigated. By adapting the *Saccharomyces cerevisiae* strain to Co(II), separation of Co(II) from Ni(II) in dilute solution has been achieved.

INTRODUCTION

Microorganisms capable of accumulating and concentrating elements have been called "microbiological collectors" (*1*). The capacity of yeasts toward accumulating various metallic ions, and adapting themselves to different media concentrations of such ions, is well-known (*2–13*).

By cultivating the *Saccharomyces ellipsoideus* yeast on media containing $CdCl_2$, strains have been obtained which are resistant to cadmium. The contents of RNA and total nitrogen in these Cd-resistant cells have been compared with similar data for strains resistant to Cu(II), Co(II), and

Ni(II). These comparisons have shown that strains with a developed resistance to Cd(II) or Co(II) have higher contents of RNA and total nitrogen than those resistant to Ni(II) and Cu(II) (*9*).

It has been shown that Ni(II), Co(II), and Zn(II) can bind reversibly to surface anionic sites of yeast, but they can also be transported intracellularly. Cells of commercial fresh baker's yeast pretreated with glucose and phosphate stimulate this transport. A series of affinities of yeast for bivalent metallic ions has been established: Mg(II), Co(II), Zn(II) > Mn(II) > Ni(II) > Ca(II) > Sr(II) (*14*).

Along with a study of the effects of ferric ions on *Candida utilis*, the accumulation dependence of Co(II) and other elements has also been investigated (*15*).

This paper presents a study of Co(II) and Ni(II) concentration with a microbiological collector in the form of a culture of *Saccharomyces cerevisiae* which was suspended in a glucose-containing phosphate buffer. The action of some complexing agents on the concentration of Co(II) and Ni(II) in the mixture has also been studied, along with the possibility of separating Co(II) and Ni(II) by using a strain adapted to cobalt.

EXPERIMENTAL

A strain of *Saccharomyces cerevisiae* isolated from commercial fresh baker's yeast and cultivated on a Sabouraud solid medium has been employed in the experiment. The strain was maintained by repetition of culturing at 3-day intervals. The 48-hr culture obtained on a solid medium distributed in Petri dishes was harvested by washing with physiological saline solution, thus yielding a dense suspension that was standardized by determining the weight of a certain aliquot which had been dried at 105°C to constant weight.

Under sterile conditions the standardized suspension was introduced into Erlenmeyer flasks containing 25 ml glucose-phosphate buffer with a given content of the ion under study. These cultures were kept at room temperature (20 ± 2°C) without stirring.

The Co(II) and Ni(II) solutions were made up from the corresponding nitrates.

After the required time interval, the samples were centrifuged at 13,000 rpm (Janetzki K 24) for 30 min. The deposit thus obtained was calcinated (700°C), dissolved in aqua regia, and evaporated. The remaining residue was then dissolved in demineralized water, and Co(II) and Ni(II) were determined by atomic absorption spectroscopy (S.P-90 Pay-Unicam).

For determination of the Co(II) and Ni(II) accumulation capacity, the K_D distribution coefficient was calculated

$$K_D = \frac{\text{amount accumulated in 1 g dry weight of cells}}{\text{amount left in 1 ml of medium}}$$

as well as the percent accumulation $E\%$. Between K_D and $E\%$ there is the relation

$$K_D = \frac{(E\%)(V)}{(100 - E\%)m}$$

where V is the sample volume (ml) and m is the dry weight of cells at 105°C (g).

RESULTS AND DISCUSSION

Influence of the Presence of Glucose

A series of growth media was prepared based on a phosphate buffer of pH 6.9. To this buffer was added either Co(II) (2.9 μg/ml) or Ni(II) (3 μg/ml) and glucose in concentrations ranging from 1 to 6% w/v. To identical volumes of each of these media was added a 2-ml aliquot of cellular suspension. The accumulation of both elements increases with the glucose concentration up to 2% and then stays constant.

pH Influence

A standardized amount of yeast suspension in physiological saline solution was introduced in phosphase buffers (pH = 3.8 to 8.3) containing 2% glucose and 2.9 μg/ml Co(II) or 3 μg/ml Ni(II). There is a correlation between the pH of the medium and the accumulation of these ions. In the case of Co(II) there is an increase in the accumulated amount up to pH = 6.3 where a maximal accumulation is reached; between pH 6.3 and 7.7 it remains constant and after pH 7.7 it decreases. In the case of Ni(II) the maximal accumulation is obtained at pH = 6.3.

Time Influence

The cellular suspension (m = 0.0575 g), introduced in a glucose-phosphate buffer of pH 6.3 with the above-mentioned Co(II) and Ni(II) concentrations, was separated from the medium at various time intervals

and analyzed with respect to its content of Co(II) or Ni(II). After 21 hr a maximal accumulation of 86% is attained for Co(II) ($\log K_D = 3.47$); the accumulation then remains constant during the investigated time interval (93 hr). Ni(II) is more slowly accumulated and a maximal percent is attained at 45 hr (83%, $\log K_D = 3.37$), after which it decreases. The different behavior of the *Saccharomyces cerevisiae* strain in this case may be ascribed to the stronger toxic effect of Ni(II) as compared with Co(II).

The Influence of Element Concentration

The accumulation of Co(II), and Ni(II) ions depends on their concentration as shown in Fig. 1. In dilute solutions of 0.46 μg/ml Co(II) and 0.48 μg/ml Ni(II), the yeast ($m = 0.0562$ g) accumulates under the above-mentioned conditions ~99% Co(II) and ~98% Ni(II) (respectively $\log K_D = 4.75$ and $\log K_D = 4.74$). The percent accumulation of the two ions decreases with an increase of their concentration. The results we have obtained point to the possibility of concentrating Co(II) and Ni(II) from dilute solutions which contain less than 0.5 μg/ml of the two elements.

The Influence of Stirring

A percent accumulation ($E\%$) in samples mixed with a magnetic stirrer, as compared with static samples kept under otherwise identical conditions,

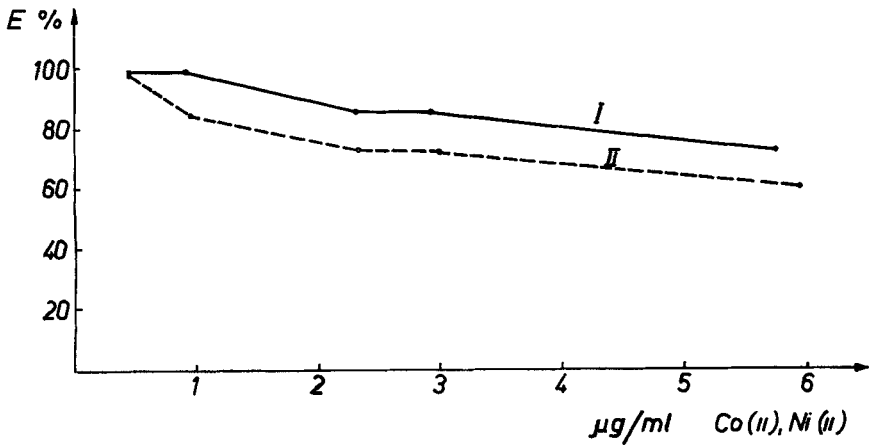

FIG. 1. Concentration influence. I: Co(II). II: Ni(II).

has been determined. The static samples lead to accumulations that are ~6% higher for Co(II) and ~3% higher for Ni(II).

The Influence of Cell Washing on the Removal of Accumulated Ions

Samples kept under the above-mentioned optimal conditions have been filtered through sintered glass filter crucibles G_5 and the deposit washed three times with 10 ml physiological saline solution and finally with 10 ml demineralized water. It was found that washing the cells in this manner removes ~1% Co(II) and Ni(II). This proves that the accumulated elements are closely linked either to the cellular wall or to the inside of the cell.

The Influence of Some Complexing Agents on Co(II) and Ni(II) Concentration

At a pH of 6.3 and a metal ion concentration of 0.5 μg/ml, 0.067 g (dry weight) of cells was shown to accumulate 99% Co(II) and 98% Ni(II) over a 48-hr time interval. Under otherwise identical conditions, but with both elements present simultaneously in the mixture, an accumulation of 92% Co(II) and 86% Ni(II) is observed. It follows that the percent accumulation of both the ions decreases when they are mixed.

Various concentrations of complexing agents such as SCN^-, nitroso-R-salt, dimethylglyoxime, and EDTA were added to the above-mentioned mixture. The results are shown in Fig. 2. It is not possible to separate Co(II) from Ni(II) in the mixture in this way.

Saccharomyces cerevisiae Strain Adaptation to Different Contents of Co(II) or Ni(II) in the Growth Medium

A yeast strain was inoculated in 25 ml Sabouraud medium with 0.46 μg/ml Co(II) or 0.48 μg/ml Ni(II), $m = 0.0813$ g. Every other day for 6 days, additions were made of increasing amounts of Co(II) or Ni(II) in 5 ml Sabouraud medium. Before each addition an analysis was made of the contents of Co(II) and Ni(II) in the *Saccharomyces cerevisiae* mass. Curves I and II in Fig. 3 show that in the growth medium the accumulation is very small compared with what is obtained by suspending the cells in phosphate buffer. In 6 days the percent accumulation decreases from 47 to 24% for Co(II) and from 15 to 7% for Ni(II) with the increase of the medium concentration of the elements.

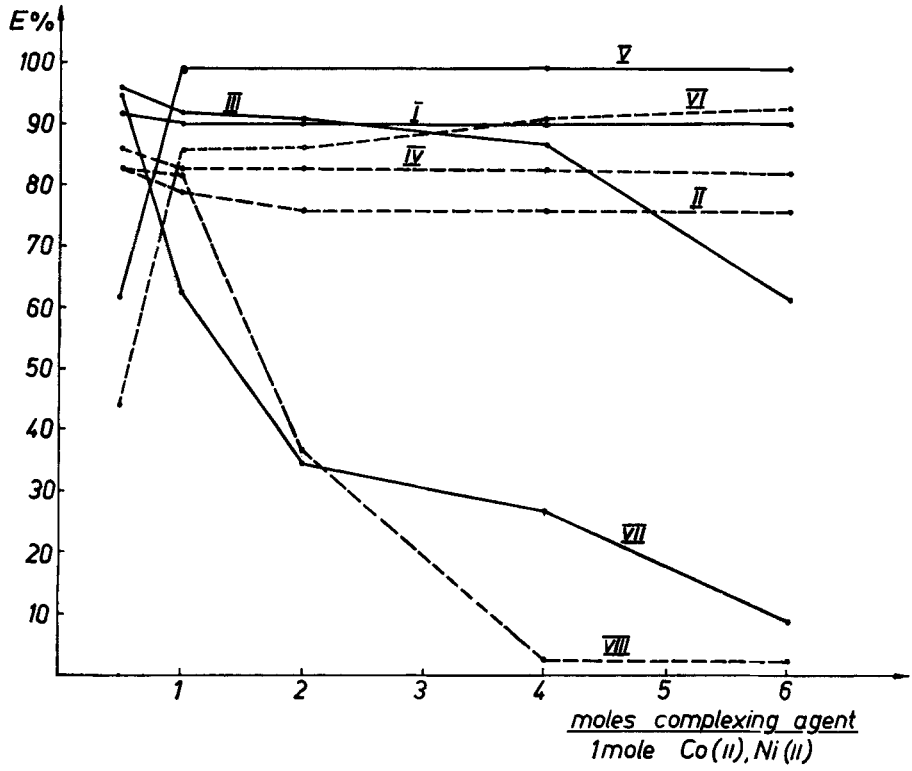

FIG. 2. The influence of the complexing agents on the accumulation of Co(II) (Curves I, III, V, VII) and Ni(II) (Curves II, IV, VI, VIII). Curves I and II: In the presence of SCN^-. Curves III and IV: In the presence of nitroso-R-salt. Curves V and VI: In the presence of dimethylglyoxime. Curves VII and VIII: In the presence of EDTA.

The mass of yeast grown and adapted after this time interval was separated by centrifugation, washed three times with physiological saline solution, and subsequently suspended in glucose-phosphate buffer (2%, pH 6.3) for 48 hr in the presence of a mixture of 0.46 μg/ml Co(II) and 0.48 μg/ml Ni(II). The yeast adapted to Co(II) accumulates 15% Co(II) and 100% Ni(II), while the one adapted to Ni(II) accumulates 86% Co(II) and 79% Ni(II). Thus the strain adapted to cobalt displays a higher affinity for nickel.

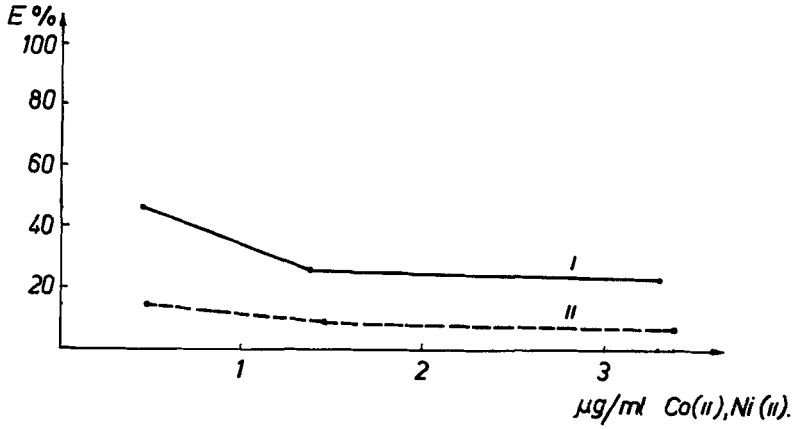

FIG. 3. The *saccharomyces cerevisiae* strain adaptation to Co(II) or Ni(II) in the growing medium. I: Adaptation to Co(II) [Co(II) addition every other day for 6 days]. II: Adaptation to Ni(II) [Ni(II) addition every other day for 6 days].

Co(II) Purification from Ni(II)

To enhance the separation factor, attempts have been made at daily strain adaptation to cobalt that was added in ever-increasing amounts [from 0.46 to 3.75 μg/ml Co(II)] in 5 ml Sabouraud medium. The yeast mass (m = 0.0945 g) was analyzed after 2, 4, and 6 days for its Co(II)

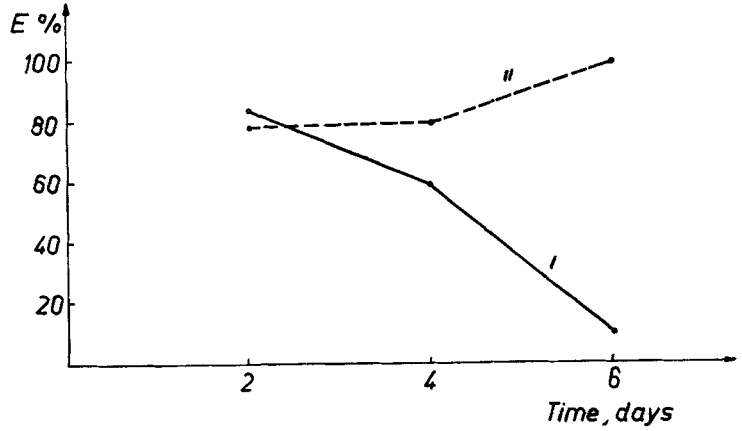

FIG. 4. Co(II) purification from Ni(II). I: Co(II). II: Ni(II).

content. In 6 days the percent accumulation decreased from 67 to 15%. In conjunction with the determination of the Co(II) content, we also tested the capacity of the yeast to separate a mixture of Co(II) and Ni(II). The procedure is the one mentioned above and the results are shown in Fig. 4.

It can be seen that the strain adapted to cobalt accumulated with time preferably Ni(II). After 6 days, 100% Ni(II) and 11% Co(II) from the mixture can be found in the yeast mass. This finding leads to the possibility of advanced purification of Co(II) from Ni(II) in some dilute solutions.

CONCLUSIONS

With a *Saccharomyces cerevisiae* strain a quantitative concentration of Co(II) or Ni(II) from diluted solutions (<0.5 μg/ml) can be achieved by suspending the yeast in glucose-phosphate buffer under optimal conditions of pH, glucose concentration, and time.

By adding various complexing agents (SCN^-, nitroso-R-salt, dimethylglyoxime, EDTA) to the mixture, the Co(II)/Ni(II) accumulation rate is changed but no separation may be achieved.

A purification of Co(II) from Ni(II) in dilute solutions can be achieved by using a *Saccharomyces cerevisiae* strain adapted to Co(II).

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Received by editor March 3, 1978