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INVESTIGATION OF ZINC BIOSORPTION BY BREWER’S YEAST CELLS

ABSTRACT: The highest amount of zinc (= 90%) is bound after 3 hrs of contact at low initial (total) concentrations of zinc in suspension of yeast, 10—100 mg/l at 10—30°C. The equilibrium between bound and free zinc ions is established after 6 hrs of contact time, independently on the total zinc concentration in yeast milk. No bigger changes of content of zinc bound to brewer’s yeast cells was determined at temperatures 10°C and 30°C. 40% of bound zinc in the equilibrium state is bound during the first 15 min of contact of zinc ions and brewer’s yeast cells at all initial (total) zinc concentrations in suspension of yeast both at 10°C and 30°C. The “KEKAM” equation can be used for the description of kinetics of zinc biosorption by waste brewer’s yeast cells, for the ranges of zinc concentration 10—100 mg/l at 30°C (mean correlation coefficient 0,96) and 60,0—100 mg/l at 10°C (mean correlation coefficient 0,95).

KEY WORDS: Biosorption, “KEKAM”, waste brewer’s yeast, zinc

INTRODUCTION

Biosorption is the capability of dead biomass, mostly of microbiological origin, to bind and accumulate metals from relatively dilute solutions. Of special interest are “industrial” heavy metals for their toxicity in the environment or metals of technological interest. Regarding the microbiologic biomass, the ones available in bigger amounts are more important. The activities of industrial fermentation are often connected with the problem of waste removal, i.d. use of biomass. The biosorption involves the phenomena connected with the biomass and metal ions, when the concentration of those ions in the media is higher than physiologically necessary for certain microorganism. Biosorption is the accumulation of metals without the active binding by cells (Volesky, 1994). The conditions of culture growth may affect the metal uptake capacity which is for non-living and live yeast cells Zn > (Cd) > U > Cu and Zn > Cu > (Cd) > U, respectively (Volesky, May-Philips, 1995). Due to the complex structure of microorganisms there is a number of ways of metal binding to the cells (Veglio, Beolchini, 1997). Two widely accepted mo-
models of absorption isothermes can be linearized, as recommended by Langmuir and Freundlich (Volenskyy, 1994). Both models while capable of describing many biosorption isotherms can hardly have a meaningful physical interpretation in biosorption. However, the parameters of these models are not acceptable for the accurate physical interpretation. In addition to equilibrium studies, the kinetics of biosorption has to be determined in order to establish the time course of the metal uptake. Rapid uptake of the metal by the biosorbent is desirable providing for a short solution-biosorbent contact time in the actual process. The type and dimensions of the contact equipment depend on the contact time, further directly affecting the total and processing price of the process. The equilibrium and kinetic characterization of biosorbent material are also important for the quantitative investigation of its characteristics and for the process design (Volenskyy, 1991). Topochemical reactions are localized at the surface of active centres of solid career. A group of Russian scientists has developed a global kinetic equation known as “KEKAM” (Kolmogorov-Erofeev-Kozeeva-Avrami-Mampel), which can describe the topochemical reactions (Avrami, 1939, 1940). The paper presents the investigation of kinetics model of zinc ion biosorption by waste yeast from brewery (waste brewer’s yeast), in the range of zinc concentration 1,0—15,0 g/l at 10°C and 30°C. The global kinetic equation which can describe the topochemical reactions “KEKAM” was used.

MATERIALS AND METHODS

Waste brewer’s yeast, purchased directly in a national brewery, kept at +4°C, was used for the preparation suspension of yeast. The content of dry biomass of the yeast was determined by drying till constant mass and was 30,8%. The final content of dry biomass in the prepared suspension of yeast was 20 g/l. Zinc solution, was added to suspension of yeast till the final zinc concentration amounting 10,0; 20,0; 30,0; 40,0; 60,0; 80,0 and 100,0 mg/l. ZnSO₄ x 7 H₂O (Merck) and bidistilled water were used for the preparation of the solution, which was sterilized by filtration (0,22 ssnm). The percentage of non-living brewer’s yeast cells was determined by microscoping of cells in 0,01% solution of methylene blue. The non-living cells were dark blue coloured, while the live ones were non-dyed or very light blue. The percentage of dead cells was 1,5%, meaning that live cells of brewer’s yeast were used. The pH value of the prepared yeast milk was 4,4—4,6, and was not corrected. This value enables the presence of zinc in the form of Zn²⁺ ions in the suspension of yeast. Aliquots of 50 ml of prepared suspension of yeast with known zinc concentrations were poured into 100 ml plastic Erlenmayer flasks. Incubation was performed on the rotary shaker at 30°C and 150 rpm. Lower temperature of incubation, 10°C, was used for suspension of yeast samples containing 60,0; 80,0 and 100,0 mg/l of total zinc. 5 ml of suspension of yeast samples were taken for every zinc concentration and incubated 0,25; 0,5; 1; 3 and 24 hrs. Each sample was analyzed in three replications. The samples were filtered (0,45 ssmm) and the obtained filtrates analyzed for residual zinc content. Wet
digestion method was used for the preparation of samples in mixture of cc H₂SO₄ and cc HNO₃ (Górsuch, 1970). The zinc content was determined by atomic absorption spectrophotometry (Varian AA10, Australia). All chemicals used for atomic absorption spectrophotometry were ultra-pure grade ("Suprapur", Merck). The content of zinc bound by brewer’s yeast cells (Veglio et al., 1997) is expressed by the equation: \( q = \frac{(C_i - C_f)}{X} \), where:

- \( q \) — content of bound zinc (mg/g d.m.),
- \( C_0 \) — content of total zinc (mg/l),
- \( C_e \) — content of free zinc (mg/l),
- \( X \) — content of dry biomass (g/l).

The degree of biosorption of zinc by yeast cells is expressed as (Knežević et al., 1998): \( \alpha = \frac{q}{q_{\text{max}}} \), where: \( \alpha \) — degree of biosorption, \( q \) — content of bound zinc (mg/g d.m.), \( q_{\text{max}} \) — maximal content of bound zinc to yeast cells as the value after 24 h incubation (mg/g dm). The linear form of "KEKAM" equation was used for the processing of experimental data:

\[
\ln (-\ln (1-\alpha)) = \ln k + n \ln t
\]

In the \( -\ln (1-\alpha) \) — Int system "KEKAM" equation represents a straight line. The parameters \( \ln k \) and \( n \) can be determined from the cut and slope and of the given function, respectively.

RESULTS AND DISCUSSION

The kinetics of zinc ions binding by yeast cells is presented in Figure 1, at different total (initial) content of zinc in suspension of yeast, at 30°C and 10°C. The kinetic curves can be divided into three parts: the first part is linear,
the amount of bound zinc is increasing proportionally with time, the second part, where the increase of bound zinc amount is very slight and the third — equilibrium part, when the biosorption is not time-dependent.

The largest amount of zinc ions (= 90%) is bound within the first 3 hrs of contact, at all initial (total) zinc concentrations in the suspension of yeast. The equilibrium between the bound and free zinc ions was established after 6 hrs of contact, independently on the total zinc concentration in suspension of yeast. The change of total zinc content for yeast biomass in the last 24 hrs of contact was insignificant. The biosorption was also investigated at 10°C for total zinc concentrations 60, 80 and 100 mg/l in suspension of yeast. The aim was to determine the influence of temperature on the biosorption of zinc ions by yeast cells. The results show that the difference between trials at 10°C and 30°C was rather insignificant. This finding is in accordance with literature data (Failla et al., 1976), which state that temperature range 4—40°C has no significant influence on biosorption, i.e. binding of metal ions to yeast cells surface. At all initial (total) zinc concentrations in suspension of yeast, both at 10°C and 30°C, 40% of final uptake of zinc (equilibrium) was bound within the first 15 min of contact. Figure 2 presents the kinetic curves $\alpha = f(t)$, obtained by using the experimental results, degree of biosorption and time of contact at 30°C and 10°C, respectively. The kinetic curves are of sigmoidal character, and belong to the family of curves with zero initial rate. The presented kinetic curves are the initial bases for the investigation of topochemical reactions.

![Figure 2. Dependence of biosorption level on incubation time of suspension of yeast at 30°C](image)

The results presented in Figure 3, and the kinetic parameters and correlation coefficients given in Table 1, lead to the conclusion that the linearization of “KEKAM” equation is acceptable for the experimental results. This means
that the theory of topochemical reactions can be used for the description of flow of zinc ions biosorption by brewer’s yeast cells, in experimental conditions used during this work. Reproducibility of the “0” samples analyses, expressed as relative standard deviation (average value) was 3.9%. During the biosorption the analyses reproducibility was not changed significantly.

Table 1. Kinetic parameters, correlation coefficient and standard deviation for “KEKAM” equation

<table>
<thead>
<tr>
<th>Range content of zinc [mg/l]</th>
<th>Temperature of incubation [°C]</th>
<th>k</th>
<th>n</th>
<th>Correlation coefficient</th>
<th>Standard deviation [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,0—100,0</td>
<td>30</td>
<td>1,38</td>
<td>0,588</td>
<td>0,96</td>
<td>3,9</td>
</tr>
<tr>
<td>60,0—100,0</td>
<td>10</td>
<td>1,38</td>
<td>0,586</td>
<td>0,95</td>
<td>3,8</td>
</tr>
</tbody>
</table>

Evaluation of the equilibrium sorption performance needs to be supplemented by process-oriented studies of its kinetics and eventually by dynamic continuous-flow tests. The rate of the sorption process, together with the hydrodynamic parameters, determines the size of the contact equipment. Reaction engineering concepts apply for the experimental approach leading to expressing the values of key process parameters used for comparative, process design and scale-up purposes. The most widely used contacting device for sorption processes is the fixed-bed reactor configuration and its modifications. The principles and methodology of deriving and evaluating the key process parameters have been dealt with extensively in the chemical engineering literature (Voílesky, 1994). The values of kinetic parameters for “KEKAM” equation, for both incubation temperatures, confirm that temperature does not affect the kinetic of zinc ions biosorption by waste brewer’s yeast cells, in the

![Graph](image)

**Figure 3.** Linearized “KEKAM” equation for the range of zinc concentrations 10—100 mg/l at 30°C
mentioned experimental conditions. It is important to mention that the conditions (medium) of yeast culture growth (obtaining of biomass), significantly affect the “physiological state” of yeast cells influencing further the possibility of heavy metals binding. Therefore, it is difficult to compare the “yield of biosorption” with the literature data, since it is hardly possible to perform the trial under the same conditions, as well as to find biomass in the appropriate “physiological state”.

CONCLUSION

The dynamic equilibrium between the free zinc ions in suspension of yeast and ions bound by brewer’s yeast cells is established after 6 hrs of incubation in all zinc concentrations investigated. After 3 h of incubation, for all total zinc concentrations in suspension of yeast, the (≈ 90%) of maximal amount of bound zinc is bound by brewer’s yeast cells. No significant change of bound zinc ions content was estimated in the temperature range 10—30°C, e.g. in these trial conditions the temperature does not affect the biosorption. The linearization of “KEKAM” equation is satisfactory for the obtained experimental results (mean correlation coefficient 0.96). This means that the theory of topochemical reactions can be applied (used) for the description of zinc ions adsorption to brewer’s yeast cells in zinc concentration range 10—100 mg/l, at incubation temperature 30°C. The “KEKAM” equation can be used as well for the description of kinetics of biosorption for the zinc concentration interval 60—100 mg/l in brewer’s yeast cell at 10°C.

REFERENCES

ИСПИТИВАЊЕ БИОСОРПЦИЈЕ ЈОНА ЦИНКА 
ЋЕЛИЈАМА ПИВСКОГ КВАСЦА

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Резиме

У раду је испитивана биосорпција јона цинка ћелијама пивског квасца (отпадни пивски квасац из једне домаће пиваре) у интервалу концентрације јона цинка од 10 до 100 mg/l у суспензији пивског квасца са концентрацијом суве биомасе пивског квасца 20 g/l (вредност pH 4,5), на температурама 10°C и 30°C. Количина слободног цинка у суспензији пивског квасца одређивана је атомским апсорпцијом спектрофотометром (Varian A10, Australia). Након 3 h контакта ћелија пивског квасца и јонова цинка, око 90% укупне количине цинка се веже за ћелије пивског квасца и то на обе испитиване температуре. Равнотежно стање, између везаног цинка за ћелије пивског квасца и слободних јонова цинка у суспензији, успоставља се након 6 h контакта. У погледу количине везаног цинка за ћелије пивског квасца нема значајне разлике на температури 10°C и 30°C. Након 15 минута контакта за ћелије пивског квасца веже се око 40% укупне количине цинка присутне у суспензији, и то на обе испитиване температуре. Кинетика биосорпције јона цинка ћелијама пивског квасца, под условима примењеним у овом раду, може се описати „КЕКАМ” једначином: \( \ln (-\ln (1- \alpha)) = \ln k + n \ln t \); где је \( \alpha \) — степен биосорпције јона цинка (однос количине везаног цинка изражене по граму суве материје пивског квасца у неком времену \( t \) и максималне количине везаног цинка изражене по граму суве материје пивског квасца која се остварује након 24 h контакта), \( t \) — време контакта, \( k \) и \( n \) — кинетички параметри биосорпције. Применом наведене једначине, у испитиваном интервалу концентрација јона цинка у суспензији пивског квасца, на температури од 30°C остварује се коefficient корелације 0,96, а на температури од 10°C остварује се коefficient корелације 0,95.