Mathematical model of protein sorption and evaluation of its validity in deproteination of chrome-tanned wastes

M. Bařinová, J. Pecha, and K. Kolomazník

***Abstract*—** Leather industry is a prominent producer of large amount of waste. Some of this waste, especially chrome-tanned waste, is classified as hazardous. Disposal of this waste in landfills is expensive and brings both health and environmental risks. However, this waste also represents interesting source of valuable raw materials and it is advantageous to search for possibilities of its processing. Two-step enzyme hydrolysis seems the most attractive processing technology so far; however, it is not completely zero-waste due to its by-product, so-called chromium sludge. Relatively high protein content in the sludge represents technological obstruction and limits its further treatment and applications. The paper focuses on deproteination (removal of proteins) of chromium cake through a combination of mathematical and experimental methods. Mathematical model has been proposed for desorption of the protein fraction into the washing bath for various soaking numbers and decantation washing. The reliability of the model to predict the course of the desorption process was evaluated by means of experimental desorption of the protein fraction into the washing solution. The results of mathematical modeling showed that appropriate choice of conditions can lead to practically total deproteination of chromium cake. The mathematical model also serves as a basis for optimization of the process.

***Keywords*—**Chrome-tanned waste, mathematical modeling, protein sorption, sorption isotherm.

I. Introduction

IMPORTANCE of the processing of wastes generated by the leather industry is apparent particularly from the mass balance of the final products (leather). One ton of the raw material (salt-cured wet raw hides) gives only about 200 kg of the final leather and is accompanied by the production of 250 kg of chrome-tanned solid waste, 350 kg of non-tanned waste and considerable amount of waste water.

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Processing of solid chrome-tanned waste generated by the leather industry, the typical example of which are chromium shavings, represents a long-term technological challenge. Their disposal at open landfills is not suitable since there is a risk of oxidation of trivalent chromium present in this waste into carcinogenic hexavalent chromium salts [1]. However, this waste also represents interesting source of valuable raw materials. For this reason, several methods have been proposed to exploit the waste potential.

The most promising approach so far seems to be a two-step enzyme hydrolysis, the description of which, including automatic control of the process, can be found in e.g. [2, 3, 4, 5], particularly with respect to the economic aspects of the process. Simplified scheme of thy hydrolysis technology is depicted in Fig. 1:

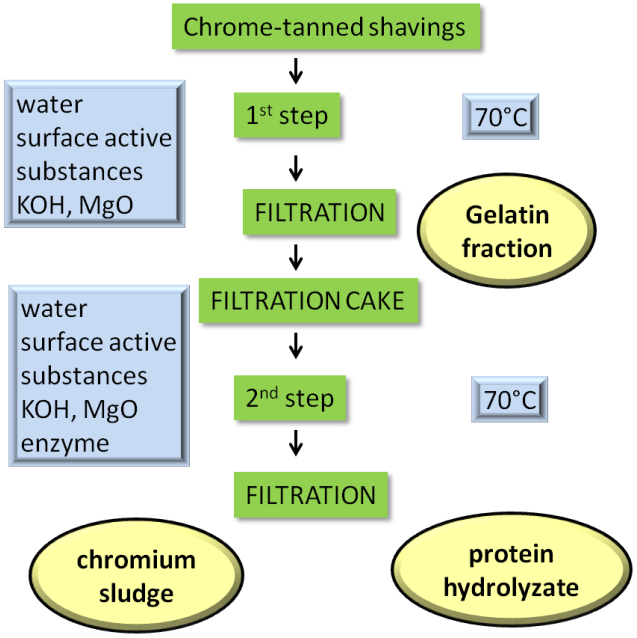

Fig. 1 Two-step hydrolysis of chromium shavings, modified according to [4]

Advantage of two-step hydrolysis is gaining two valuable products, namely gelatin (in the first step) and protein hydrolyzate (in the second step). At the same time, the process increases the degree of deproteination. Although this degree

could reach up to 95 % in laboratory conditions, industrially obtained deproteination levels have not exceeded 60 %.

The other product of enzymatic hydrolysis, so-called chromium sludge (or chromium cake) is on the other hand quite resistant to degradation; processing of chromium cake then remains the major problem in the hydrolytic treatment of chromium shavings. Intensive research has been carried out to find economically viable and environmentally friendly solutions, involving mainly chemical treatment, both direct and indirect [for example 4, 5, 6], or biotechnological methods [7]. Some of the cited solutions also include direct re-use of chromium cake in the manufacturing cycle.

Desorption of proteins into the washing bath after the second step of hydrolysis represents potentially suitable way of further reduction of the protein components in the chromium cake, with simultaneous desirable increase in the purity of chromium present in the cake. However, existing efforts dealing with this problem are limited either by very low efficiency of deproteination [8], or quite complex and energetically demanding reaction conditions, moreover connected with oxidation of part of trivalent chromium to its carcinogenic hexavalent form [9].

The aim of our contribution was to propose a mathematical model of desorption of protein fraction from chromium cake into the washing bath and experimentally evaluate the ability of the model to reproduce the experimental data at acceptable level, and therefore to predict the course of deproteination (desorption) beyond the measured range of conditions. Further it is possible to determine whether this way of deproteination is suitable for the processing of this kind of waste material, i.e. whether the method leads to sufficient reduction in the protein content in the filter cake and at the same time does not increase the operating costs of the treatment. In other words, whether it is possible on the basis of accurate mathematical model to optimize the deproteination process.

II. Mathematical modeling

A. Theory

Mathematical model of deproteination of chromium cake through its washing in water is based on the assumption that the protein part (collagen) is adsorbed onto chromium complex via coordination bonds with the glutamic and/or aspartic acids [8]. The modeling of desorption proceeds from the mass balance of the process. Not all the protein fraction is involved in the coordination bonds (this part is called unbound). This part passes into the washing bath by diffusion. However, part of the bound protein is also washed out into the bath through desorption – the level of desorption predicted by the model is closely dependent on the selected adsorption isotherm. Deproteination can be performed repeatedly, with lower amount of water in each step than in one-step washing; the process is then called decantation washing. This leads to reduction in the consumption of washing water, or higher efficiency of deproteination at the same water consumption [9].

B. Adsorption isotherm

Modeling of adsorption is often based "merely" in the selection of suitable isotherm, which most accurately reflects the experimental data. Several types of adsorption isotherms can be found in the literature, which more or less accurately depict individual cases. They are often parts of empiric mathematical models describing only the relationships between equilibrium concentrations of the adsorbate on the adsorbent which do not reflect real physical-chemical processes taking place during adsorption [10]. On the other hand, rare mathematical models based on descriptions of the processes during adsorption and mutual interactions between the adsorbent and adsorbate [e.g. 11] are relatively complicated and therefore have limited application in mathematical models of processes where other phenomena take place in addition to adsorption. If real experimental data and the course of the process can be accurately described by a simpler model, it is inefficient to use a complex one.

For the case of deproteination of chromium cake we used Langmuir isotherm that is based on the idea that the adsorbed substance forms only monomolecular layer on the adsorbent, the probability of adsorption is equal anywhere on the surface and there are no interactions between adsorbed molecules [12]. Langmuir isotherm can be expressed as:

$$c_A = \frac{A \cdot c}{1 + B \cdot c} \tag{1}$$

Where c_A is concentration of bound protein, A stands for bond strength, B is for sorption capacity, and c is equilibrium concentration of unbound protein. For determination of the course of deproteination it is useful to find out in which part of the isotherm the system is. In the area where c_A reaches its maximal value of a_m, only unbound component is washed out; then (1) can be simplified as ($Bc >> 1$):

$$c_A = \frac{A}{B} = a_m \tag{2}$$

For very low concentrations we can assume linear dependence of c_A on c ($Bc << 1$):

$$c_A = A \cdot c \tag{3}$$

We assume that at first the bound protein is desorbed and subsequently released as unbound protein which can be then transferred into the washing bath.

C. Mathematical model of deproteination using Langmuir isotherm

Mathematical description of deproteination is based on mass balance of individual components of the system. The overall content of the component to be washed out (i.e. protein fraction) can be expressed as the sum of weights of bound and non-bound protein and the part of protein in the washing bath:

$$c_s V = c_A V + cV + c_0 V_0 \tag{4}$$

c_s is total protein concentration in the cake, c is concentration of unbound protein c_A concentration of bound protein, c_0 is protein concentration in the bath, V is the chromium cake volume and V_0 the bath volume. Then:

$$c_s = c_A + c + c_0 Na \tag{5}$$

Where Na is soaking number for which it holds:

$$Na = \frac{V_0}{V} \tag{6}$$

The amount of unbound protein in the cake is (7):

$$cV = c_0 V_f \rightarrow c = c_0 \frac{V_f}{V} = c_0 \varepsilon \tag{7}$$

By expressing c from (7) and c_A from (1) in (5), we get:

$$c_s = \varepsilon c_0 + \frac{A\varepsilon c_0}{1 + B\varepsilon c_0} + c_0 Na \tag{8}$$

Concentration of protein in the washing bath is then:

$$c_0 = \frac{-(\varepsilon + Na + A\varepsilon - c_s B\varepsilon) + \sqrt{(\varepsilon + Na + A\varepsilon - c_s B\varepsilon)^2 + 4(B\varepsilon^2 + NaB\varepsilon)c_s}}{2(B\varepsilon^2 + NaB\varepsilon)} \tag{9}$$

The efficiency y of the deproteination can be expressed as:

$$y = \frac{c_0 V_0}{c_s V} = \frac{c_0}{c_s} Na \tag{10}$$

After rearrangement, we get:

$$y = \left(\frac{-(\varepsilon + Na + A\varepsilon - c_s B\varepsilon) + \sqrt{(\varepsilon + Na + A\varepsilon - c_s B\varepsilon)^2 + 4(B\varepsilon^2 + NaB\varepsilon)c_s}}{2(B\varepsilon^2 + NaB\varepsilon)}\right) \cdot \frac{Na}{c_s} \tag{11}$$

For more comfortable calculation of the bond strength and sorption capacity from experimental data, the following rearrangement of (8) was made:

$$\frac{c_0}{c_s - c_0(\varepsilon + Na)} = \frac{1}{A\varepsilon} + c_0 \frac{B}{A} \tag{12}$$

Where V_f is volume of the pores in the cake and ε stands for porosity. Deproteination can be easily alternated by the change of soaking number. The range of efficiency at the porosity ε = 0.5, sorption capacity B = 1 g/l and initial concentration c_s = 20 g/l is depicted in Fig. 2. It is interesting to compare the general prediction presented in Fig. 2 with the same prediction, but with changed sorption capacity B (see Fig. 3). It is apparent that the washing efficiency increases with higher sorption capacity. Therefore, sorption capacity is an important parameter and has to be determined for accurate prediction of the actual course of deproteination.

D. Mathematical model of deproteination in linear area

For the linear area of deproteination, the Langmuir isotherm can be simplified to (3) or possibly (2). By integration of (3) into the rearranged mass balance equation (5), we get the relation describing the system in linear area for low concentration of protein in the bath:

$$c_s = c_0 \varepsilon + c_0 A\varepsilon + c_0 Na \tag{13}$$

The concentration of protein in the bath is then:

$$c_0 = \frac{c_s}{Na + \varepsilon(A+1)} \tag{14}$$

Analogously for the deproteination efficiency:

$$y = \frac{Na}{Na + \varepsilon(A+1)} \tag{15}$$

E. Mathematical model of decantation washing

By decantation washing we understand multiple repetition of the washing process. Mathematical model of decantation washing is from major part derived in publication [13]. It holds for the deproteination efficiency in the *n-th* step of decantation (y_n):

$$y_n = \frac{Na}{Na + \varepsilon(A+1)} \frac{\frac{[\varepsilon(A+1)]^n}{[Na + \varepsilon(A+1)]^n} - 1}{\frac{\varepsilon(A+1)}{Na + \varepsilon(A+1)} - 1} \tag{16}$$

The rearrangement of (16) gives:

$$y_n = 1 - \left[\frac{\varepsilon(A+1)}{Na + \varepsilon(A+1)}\right]^n \tag{17}$$

The bond strength A can be expressed as (18):

$$A = \frac{(1 - y_n)^{\frac{1}{n}}(Na + \varepsilon) - \varepsilon}{\varepsilon\left[1 - \left((1 - y_n)^{\frac{1}{n}}\right)\right]} \tag{18}$$

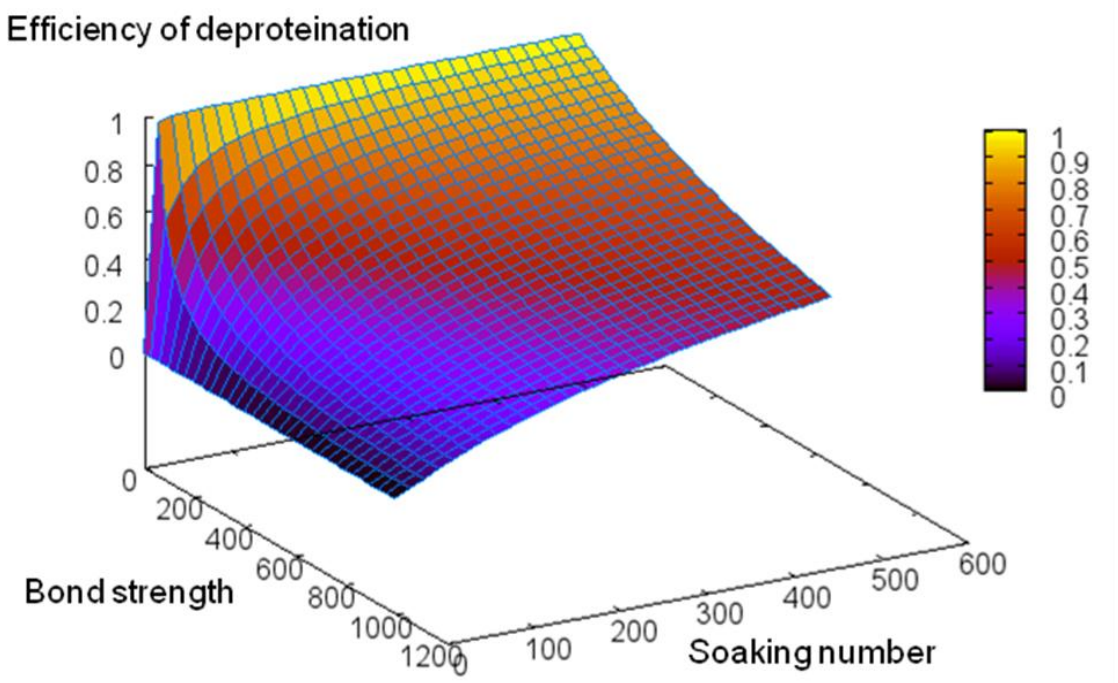

Fig. 2 Dependence of washing efficiency on soaking number and bond strength at $\varepsilon = 0.5$; $B = 1$ l/g and $c_s = 20$ g/l

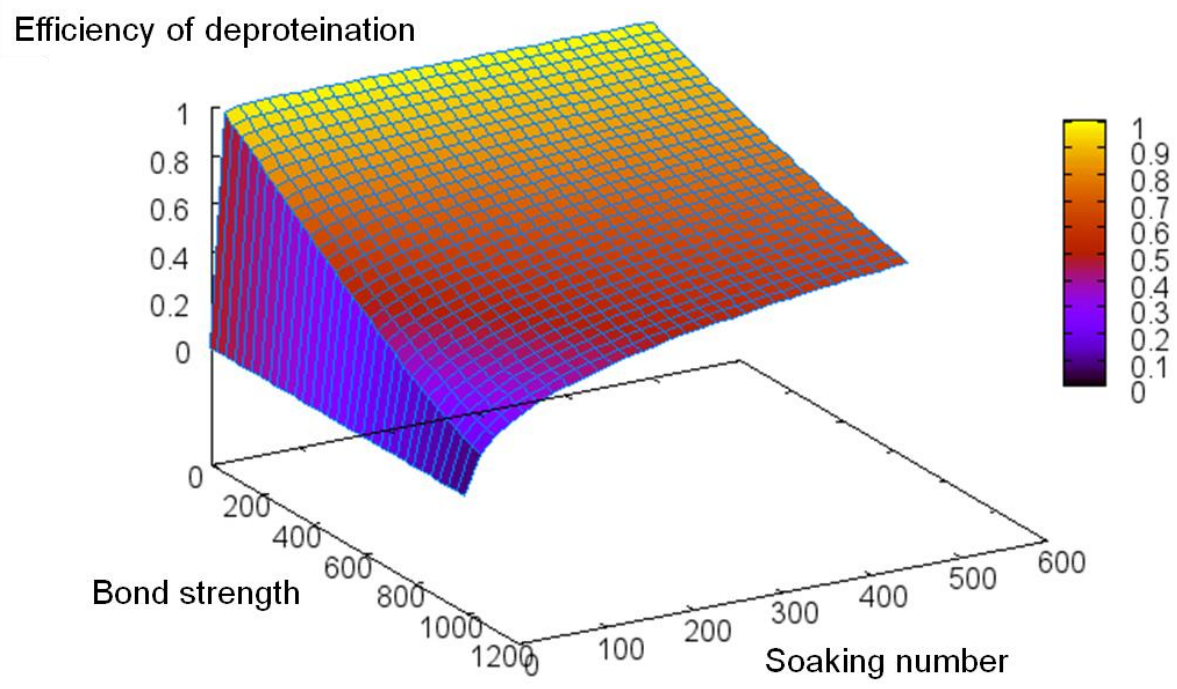

Fig. 3 Dependence of washing efficiency on soaking number and bond strength at $\varepsilon = 0.5$; $B = 50$ l/g and $c_s = 20$ g/l

F. Application of simplified model for the linear area in non-linear area

Due to significant simplification in mathematical description of decantation washing, it could be comfortable to use the model valid for the linear area in also non-linear area. However, it is necessary then to evaluate to which extent the prediction given by the simplified model differs from the prediction of the model valid also in non-linear area.

The deviation between the linear and non-linear area models proceeds from simplification of the Langmuir isotherm. Both equations ((1) and (3)) are equal only if $B \cdot c$ is equal to 0. The deviation could be calculated from the following equation (19) for the relative error:

$$\delta = \frac{X - x}{X} = \frac{\Delta X}{X} \tag{19},$$

where δ is relative error, X stands for the correct value and x is approximated value. Relative error is often expressed in percents. Since relative error can be both positive and negative, we rearranged (19) to:

$$\delta = \left| \frac{\Delta X}{X} \right| \tag{20},$$

The correct value in our case is concentration of bound protein c_A determined by the Langmuir isotherm and the approximated value is concentration of bound protein calculated according to (3). Then:

$$\delta = \left| \frac{\frac{A \cdot c}{1 + B \cdot c} - A \cdot c}{\frac{A \cdot c}{1 + B \cdot c}} \right| \tag{21},$$

Since the values of bound protein concentration determined by Langmuir isotherm cannot be negative, (21) can be simplified to:

$$\delta = \frac{\left| \frac{A \cdot c}{1 + B \cdot c} - A \cdot c \right|}{\frac{A \cdot c}{1 + B \cdot c}} \tag{22},$$

This represents a linear equation with absolute value. Since sorption capacity B and bond strength A are positive constants and the concentration cannot be negative, the solution of (22) within the interval $<0, \infty$ is:

$$\delta = B \cdot c \tag{23},$$

Relative error of the Langmuir isotherm approximation is then linear function of the concentration of the non-bound component. Assuming the validity of the conditions used for deriving of (7), the relative error of the Langmuir isotherm approximation can be expressed as a linear function of the concentration of protein in the washing bath:

$$\delta = B \cdot \varepsilon \cdot c_0 \tag{24},$$

Further, it is necessary to find out how error in the prediction of concentration of the bound protein c_A affects the error in the prediction of the protein concentration in the washing bath c_0. For this case, let us assume that the protein concentration in the washing bath is only a function of c_A, because we only know the relative error of the approximation for the concentration of the bound protein. Relative error for the protein concentration in the washing bath can be then (based on the probability theory) expressed as:

$$\delta(c_0) = \left| \frac{dc_0}{dc_A} \right| \cdot \delta(c_A) \tag{25},$$

where $\delta(c_0)$ is relative error of the protein concentration in the washing bath and $\delta(c_A)$ is relative error of approximation (given by (24)). For the derivation in (25) we use for the c_0 the rearranged (5):

$$c_0 = \frac{c_S - c_A}{\varepsilon + Na} \tag{26},$$

By subsequent integration of (26) into (25), relative error of the protein concentration in the washing bath c_0 can be calculated according to:

$$\delta(c_0) = \frac{B \cdot \varepsilon \cdot c_0}{\varepsilon + Na} \tag{27},$$

In the same way we derive relative error of the deproteination efficiency, by rearrangement of (26) by the same method as in (11), or more precisely in (10).

$$y = \frac{(c_S - c_A) \cdot Na}{(\varepsilon + Na) \cdot c_S} \tag{28},$$

Analogously as for the relative error of protein concentration in the washing bath (25), the following equation holds for the relative error of deproteination efficiency (let us note that again we know only the relative error of the approximation of the bound protein):

$$\delta(y) = \left| \frac{dy}{dc_A} \right| \cdot \delta(c_A) \tag{29},$$

where $\delta(y)$ is relative error of deproteination efficiency. By derivation of (29) we get:

$$\delta(y) = \frac{B \cdot \varepsilon \cdot c_0 \cdot Na}{(\varepsilon + Na) \cdot c_S} \quad (30),$$

With the help of equations (27) and (30) it is possible to determine the range of concentrations for which we can use the simplified model of Langmuir isotherm (3) while keeping the desired accuracy.

III. Experimental

A. Preparation of the chromium cake

The chromium cake was prepared by two-step alkaline-enzymatic hydrolysis from chromium. 3 kg of the shavings were put into a stirred reactor with a thermostat together with 17 l of water. 90 g of magnesium oxide (MgO) and 60 g of sodium carbonate (Na_2CO_3) were added. The pH of the reaction mixture was 11. The mixture was heated up to 70 °C and this temperature was kept for 4 h. As a results of acidity of the input shavings, decrease of pH was observed during the reaction. The pH was kept at desired levels between 9 and 10 (optimal conditions for the enzyme) by means of addition of Na_2CO_3. After that, 100 ml 1% solution of the enzyme Alcalase (NovoNordisk, Norway) was added and the reaction mixture was stirred at the same temperature for another 1 h. The mixture was then filtered through linen. The results of analyses of the input chrome-tanned shavings, as well as of the output chromium sludge and protein hydrolysate, are given in the following Table I:

Table I Analyses of chromium the input shavings and the obtained filtrate and filter cake, respectively.

	Chromium shavings	Filter cake (chromium sludge)
Dry matter	85.80 %	18.42 %
Ash*	9.58 %	26.83 %
Nitrogen*	14.04 %	10.63 %
Cr_2O_3*	4.19 %	7.18 %
Mg*	-	2.42 %

*related to the dry matter
All the per cent data mean w/w

B. Analytical methods

Standards analytical methods were used for analyses of the input materials and products. Protein content is represented by the value of TNK (Total Kjeldahl Nitrogen). Higher protein concentrations in the washing bath were measured spectrophotometrically with the use of biuret reaction. Lower protein concentrations were determined through the Kjeldahl method (TNK) with subsequent determination of ammonium ions via coulometric titration. Dry matter content was determined by 12-hour drying of the samples at 103 °C. The ash content was measured by combustion of the sample for 0.5 hour and subsequent annealing of the ash in muffle furnace at 800 °C. The amount of chromium in the ash was measured iodometrically as content of Cr_2O_3. Magnesium content was determined via atomic absorption spectrometer GBC 933AA, GBC Scientific Equipment Pty Ltd.

C. Washing of the chromium cake at various soaking numbers

Chromium cake was placed in wash-bottles and proportional amount of water was added to achieve the required soaking number of 1, 10, 100, 250 and 500. The samples were washed for 7.5 hours in nitrogen atmosphere, and then left overnight in the wash-bottles. After that the samples were washed another 1 hours; the total time of washing was 8.5 hours. The suspension was then filtered under mild pressure through Buchner funnel equipped with filtration paper with fast rate of filtration. The protein content was measured as described in the previous sub-chapter.

D. Decantation washing of the chromium cake

Chromium cake and water in the volume ratio of 1:1 (i.e. *Na* = 1) were placed in a beaker. Deproteination was carried out for 1 hour under intensive stirring. The suspension was then filtered under mild pressure through Buchner funnel equipped with filtration paper with fast rate of filtration. The protein content in the filtrate was determined by biuret reaction. The filtration cake was again mixed with water to the ratio of 1:1 and the washing process was repeated in the same way. Five decantation cycles were carried out in total.

IV. Results and Discussion

A. Washing of the chromium cake at various soaking numbers

The results are shown in Table II.

Table II The results of washing of chromium cake at various soaking numbers.

Na	***c_0* [g/l]**	***y_i***	$\frac{c_0}{c_S - c_0(\varepsilon + Na)}$
1	3.638	0.19	0.258
10	0.578	0.30	0.043
100	0.138	0.70	0.024
250	0.056	0.72	0.010
500	0.033	0.85	0.012

The table shows protein concentrations in the washing baths at various soaking numbers, washing efficiencies and the data necessary for calculations of the bond strength *A* and sorption capacity *B* from (17). It can be seen that there is significant increase in washing efficiency up to the soaking number of approximately 100; then the efficiency rises more slowly.

B. Decantation washing

The results after decantation washing of the chromium cake are shown in Table III.

Table III Decantation washing of the chromium cake.

Decantation cycle	c_0 **[g/l]**	y_i	$\sum y_i$
1	7.72	0.394	0.394
2	2.55	0.130	0.524
3	0.78	0.040	0.564
4	0.62	0.032	0.596
5	0.14	0.007	0.603

The table shows protein concentrations in the washing baths in individual steps of decantation, deproteination efficiencies and also the sums of efficiencies in individual steps of decantation. It is apparent from the results that from the efficiency point of view it is reasonable to perform two, maximally three decantation steps at the given soaking number (Na = 1).

The graph of linearized deproteination model (Fig. 2) confirms the assumed linear dependence of the left side of (12) on the concentration of washing bath. The value of Pearson correlation coefficient suggests very good prediction of the concentration of protein in the washing bath for a wide range of soaking numbers. The graph of the prediction of the bath concentration (Fig. 3) further affirms this conclusion. The values of bond strength of A = 231.5 and sorption capacity B = 15.8 g/l were calculated from the linear regression of experimental data (Fig. 4) and from (12).

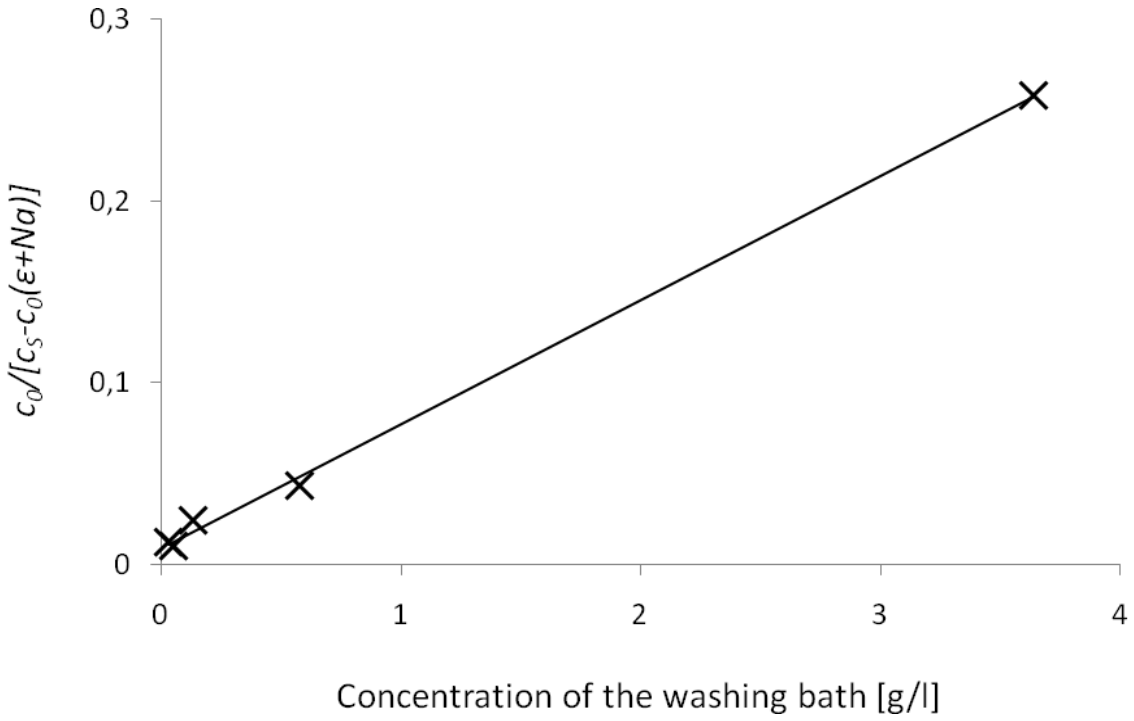

Fig. 4 Linearized deproteination model, Pearson correlation coefficient r^2 = 0.998.

Closer examination of the prediction of deproteination efficiency (Fig. 6) leads to the conclusion that the model quite accurately reflects the course of deproteination efficiency; nevertheless it slightly differs from the experimental data. This is caused by high soaking numbers, at which even very small change of c_0 leads to significant change in the entire process efficiency. For the prediction of the decantation washing we used (17); however, this relationship was derived for the linear area of deproteination. The average bond strength A calculated from (18) is 8.85.

The prediction of the protein concentration in the washing bath during decantation washing shown in Fig. 7 suggests that the prediction is a very rough reflection of the actually measured data. That implies that deproteination does not take place in the linear area for which the model was derived.

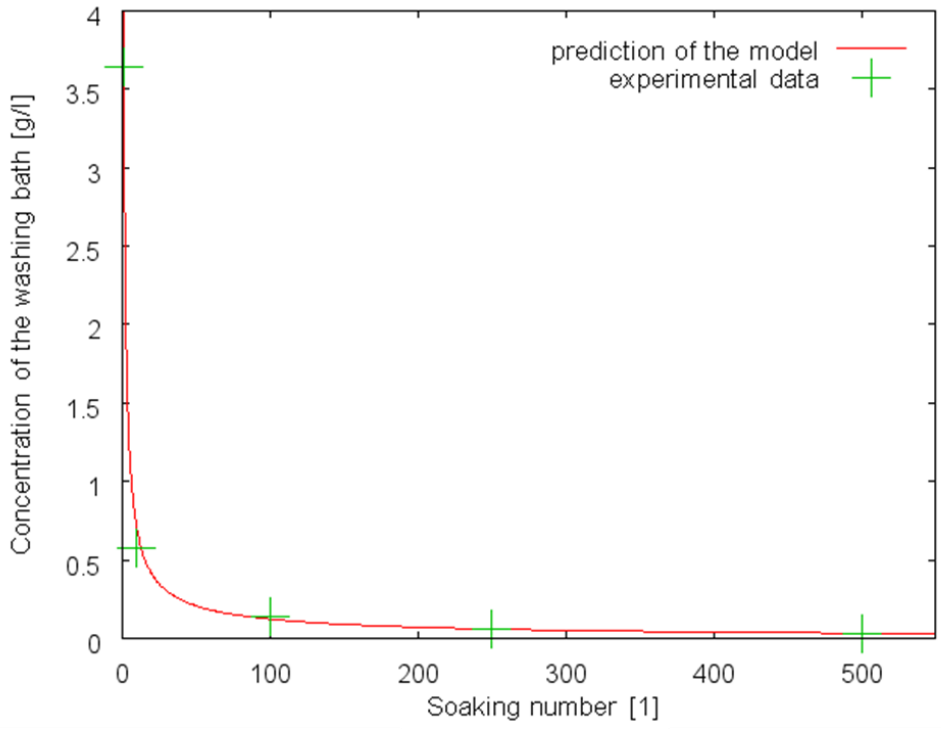

Fig. 5 Concentration of the washing bath predicted by the model in comparison with experimental data.

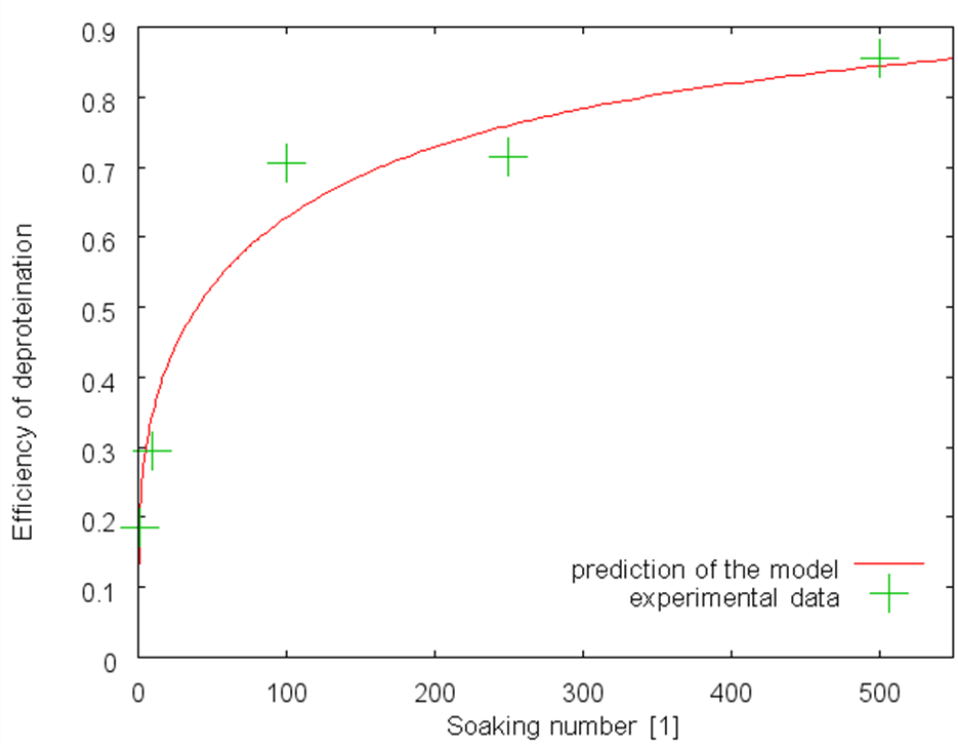

Fig. 6 Efficiency of deproteination predicted by the model for various soaking numbers in comparison with experimental data.

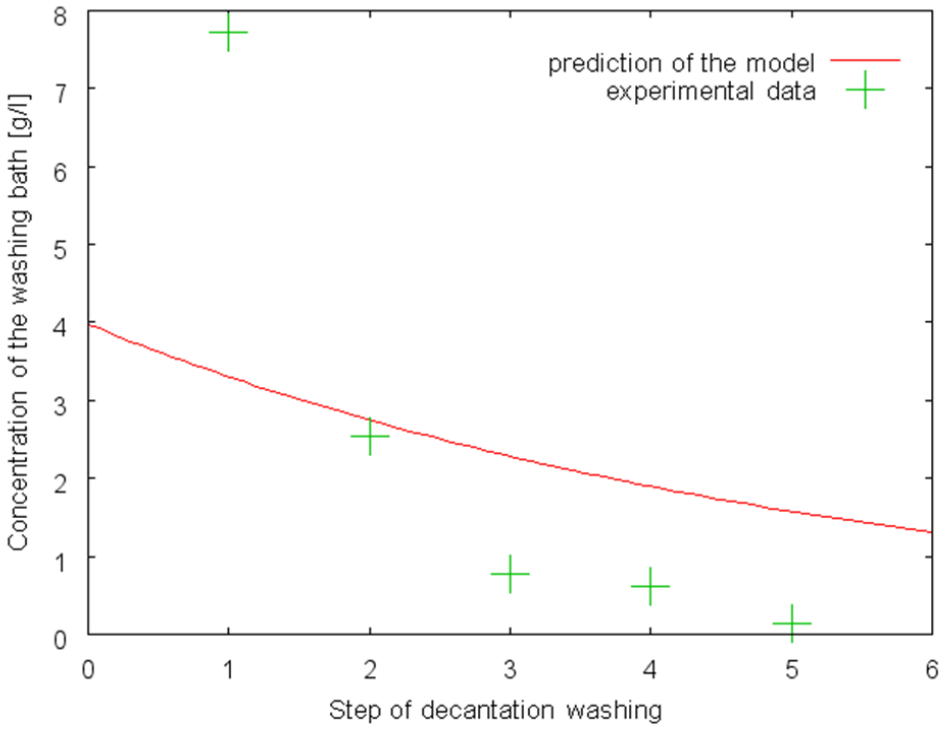

Fig. 7 Concentration of the washing bath predicted by the model in comparison with experimental data.

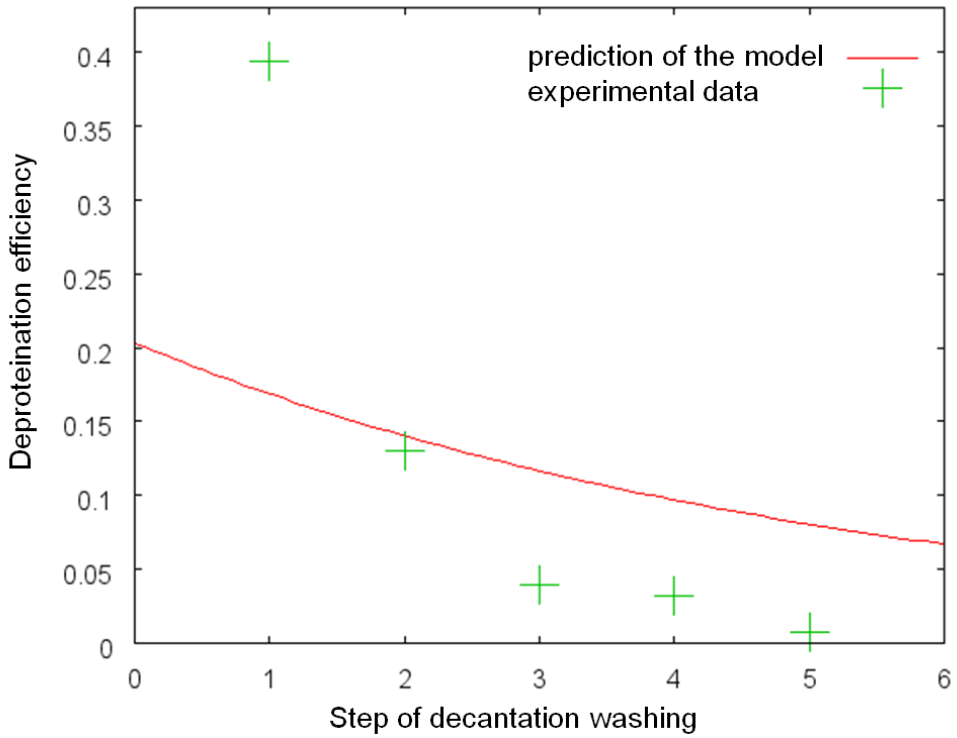

Fig. 8 Deproteination efficiency of the washing bath predicted by the model in comparison with experimental data.

V. Conclusion

Deproteination of chromium sludge by washing can be considered a perspective way of the processing of this kind of waste. The experiments showed that the efficiency of deproteination could reach up to 85 % at soaking number of 500. The most suitable technical design of deproteination in particular cases depends mainly on economic conditions and can be selected through optimization of the entire process. It can be concluded from the comparison of the predictions given by the proposed mathematical model with experimentally measured data that the model describes with good accuracy the real course of deproteination. Therefore it can be used for simulation of the process within wide range of soaking numbers. For reliable prediction it is necessary to know the values of the bond strength (A) and sorption capacity (B), which can be determined experimentally.

Simplified mathematical model applicable for the linear part of the isotherm was used for deproteination by decantation washing. The experiment showed that the model did not reflect to acceptable extent the real process. This could probably be caused by carrying out the experiment outside the range of the simplified linear model applicability. Practical utilization of the full model also in non-linear area encounters mathematical limitations coming from the fact that the series describing the sum of individual efficiencies is a general function series for which it the sum of n first members is more complicated than in the case of the geometric series of the simplified model.

Suitable design of deproteination for individual cases is highly dependent on the economic conditions of the processing. Favorable economic balance can be achieved through optimization of the process. For the sake of completeness let us note that the waste water from the washings bath can be further processed without specific problems by standard method for waste water treatment used in sewage treatment plants. This results from the fact that practically only the non-toxic protein fraction from the shavings comes into the washing bath.

VI. List of symbols

A	Bond strength	[1]
B	Sorption capacity	$[g \cdot l^{-1}]$
Na	Soaking number	[1]
V	Volume of chromium cake	$[m^3]$
V_f	Volume of pores in chromium cake	$[m^3]$
V_0	Volume of the washing bath	$[m^3]$
c	Equilibrium concentration of unbound protein	$[g \cdot l^{-1}]$
c_A	Concentration of bound protein	$[g \cdot l^{-1}]$
c_S	Total protein concentration in chromium cake	$[g \cdot l^{-1}]$
c_0	Protein concentration in the bath	$[g \cdot l^{-1}]$
y	Efficiency of deproteination	[%]
ε	Porosity	[1]

Other symbols are explained directly in the text.

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