Hydrolysis Process of Collagen Protein from Tannery Waste Materials for Production of Biostimulator and its Mathematical Model

H. Vaskova, K. Kolomaznik, V. Vasek

***Abstract*—** The paper is focused on an area of secondary processing of leather waste materials, which belongs together with a problem of waste formation and its disposal among the actual global issues. The emphasis is given to chrome-tanned waste which can represent a threat to the environment and human health because of the content of hexavalent chromium. This agent is classified as a carcinogen and its repeated exposure to human organism have hazardous impact. Secondary waste processing is a feasible solution against adverse effects resulting from waste materials. Economic and environment-friendly requirements are cardinal aspects of these ways of waste processing. The authors introduce the complex process of collagen protein hydrolysis gained from tannery wastes for the purpose of production inducers of resistance for agricultural plants. Along the treatment the chromium tanned shavings are three times hydrolyzed under different conditions. The intermediate products of the process find further applications. The process can be rank among closed-loop processes by its nature. Mathematical model for the third step of the whole process, the acid hydrolysis of enzymatic hydrolyzate based on balance equations for hydrolysis is presented.

***Keywords*—**Hexavalent chromium, inducers of resistance, leather, mathematical model, protein hydrolysis, tannery waste.

I. INTRODUCTION

PRODUCTION and disposal of waste are as old as human society. People are surrounded by waste materials for centuries, but only in several last decades waste has become a problem that should be systematically solved and be organized. This long-standing situation logically resulted into global problem that require attention. The issue of waste reduction and ways of their safe, environmentally and economically favorable disposal is one of the most current and pressing problems worldwide.

A production of different types of waste has the upward trend for decades. A significant share of waste arises from manufacturing sector. The authors deal in this contribution with an area of leather industry. Leather products such as footwear, clothing, wallets, handbags, fashion accessories, upholstery, etc. are among the objects of our everyday needs. Achieving the final products comprise a lot of waste arising from the raw material processing and manufacturing specific leather items. Just about twenty percent of raw hide is transformed into final products the rest is waste in different forms [1]. Only in tanneries millions of tons of waste are generated every year.

At the end of the 19th century it was discovered that using complex salts of trivalent chromium (CrIII) in the process of tanning effectively influenced the desired functional properties of leather. In present time chrome-tanning is the most common type of tanning in the world. It seems improbably that chromium will be replaced from the tanning process in a near future hence arises an urgent need to resolve the issue of effective disposal of waste containing chromium or better recycling chromium from the waste.

The fibril-forming collagens are the major structural proteins of hides and skins. Trivalent chromium stabilizes a hide by crosslinking the collagen fibers and supply required qualities. However, CrIII contained in leather can be under various conditions in small amounts oxidized to another form – hexavalent chromium (CrVI). Hexavalent chromium belongs among toxic elements and is classified as carcinogen in the contrast to the trivalent form, which is benign, safe and largely beneficial [2, 3]. Although knowledge of CrIII to CrVI conversion is well described in literature, the precise mechanism is complex and details are not clearly explained [4].

The threat arising from a chrome-tanned waste may be therefore related both to the environment and to human health. We can speak about three categories of chrome-tanned waste. The first is tannery waste comprising solid (shavings, trims and splits) and liquid form (the waste water containing chromium). The issue of this type of waste treatment has been successfully solved. The second is manipulation waste such as leather scraps in the manufacture of footwear or leather goods.

This work is supported by the Ministry of Education, Youth and Sports of the Czech Republic, by European Regional Development Fund under the project CEBIA-Tech No. CZ.1.05/2.1.00/03.0089. The work is also supported by Internal Grant Agency of Tomas Bata University in Zlin under the project No. IGA/FAI/2012/056.

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The third group is represented by the used leather goods, such as old shoes, or hide-upholstered furniture, which are often a part of municipal waste at the dumps. A considerable amount of CrIII contained in these discarded leather items may succumb to uncontrolled oxidation and make the waste hazardous. Not to mention the possibility of leakage into the soil and groundwater.

Issue of processing of various types of waste and the possibility of using innovative method Raman spectroscopy for detection the traces of chromium in leather goods is dealt in [5, 6]

Leather shavings – a tannery waste material contain collagen protein that can find further use after a specific treatment. For instance synthetic polymers, nowadays so widespread in packaging technology, are a source of problems because of their limited recycling possibilities. In [7] chrome-tanned leather waste hydrolyzate is suggested to be utilized as a biodegradable packing material, what is based on cross-linking hydrolyzate with epichlorhydrin.

The authors of this paper propose to utilize a processed collagen protein as an inducer of resistance for plant protection.

Plant diseases are annually responsible for large financial and agricultural losses worldwide. The current methods for protecting crop plants mainly include application of toxic chemicals for eliminating pests and pathogens. To reduce chemical control more environmentally suitable alternatives should be applied. The use of genetic potential of plants and the development of natural plant defense mechanism could contribute [8]. The induced resistance of plant is an enhanced ability of natural defense mechanisms of plants against different pathogens [9].

In this paper the complex process of chrome-tanned shavings (the first tannery waste material) treatment is introduces. Possible further utilization of intermediate and final products is referred. Also the mathematical model for one of the steps of the process, the acidolysis of enzymatic hydrolyzate, is presented.

II. PROCESS OF LEATHER WASTE TREATMENT

Chromium tanned shavings are used as an initial matter for obtaining protein hydrolyzate. Three steps have to be made to achieve the protein with proper properties needful for the inducers of resistance – a biostimulator.

In the first stage the collagen protein undergo to liquefaction and is separated from the chromium sludge. Chromium sludge can be subjected to revitalization for obtaining chromium in a form of salts for further leather tanning [10].

This first stage - the process of dechromation of chrome-tanned waste was already resolved in out institute and the technology for this process was realized in laboratory conditions. In [1, 11] is described a whole computer system for chromium recycling with focus on the control structure of the most important parts of the technology.

The part of resulting gelatinous protein a relatively expensive high-quality gelatin finds many applications e. g. in food industry, pharmacy or cosmetic, and therefore can be used cost-effectively. It is needful to realize, that the current problem with recycling processes is not at the level of technological solutions, but at the economical level. If any new method is to be successfully implemented in industrial processes it is necessary to be optimized in the means of investments and operating costs.

The rest of gelatinous protein is used for the purpose of further processing. Enzyme activity leads in alkaline conditions to molar mass decrease of gelatinous hydrolyzate. Enzymatic hydrolyzate, however, is still inefficient due to very low diffusion of collagen protein so the splitting of protein chain continues by the act of acid [12]. The scheme of the whole dechromation process is displayed in Fig. 1.

The key emphasis is laid on the molar mass of the final protein hydrolyzates as is shown in Fig. 2. Table 1 shows the molar masses of the initial and final substances including intermediated proteins obtained in the process. Due to the low

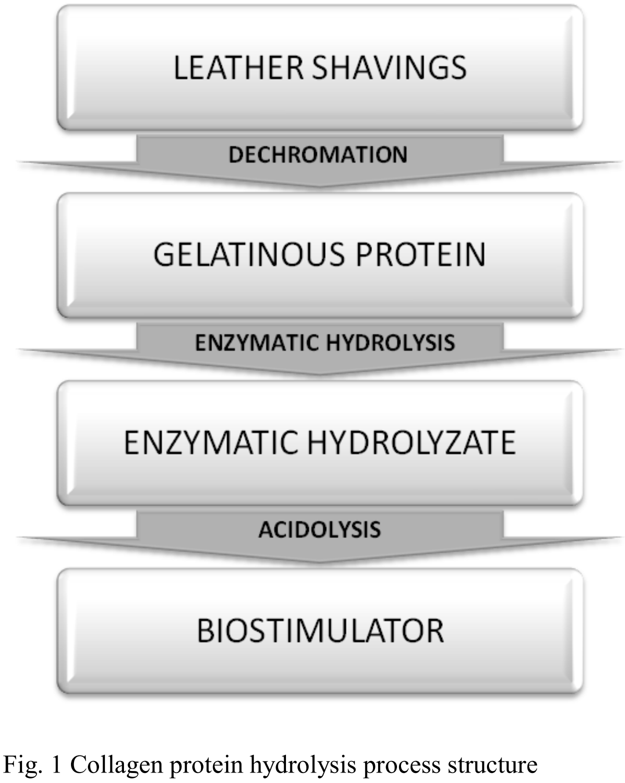

Fig. 1 Collagen protein hydrolysis process structure

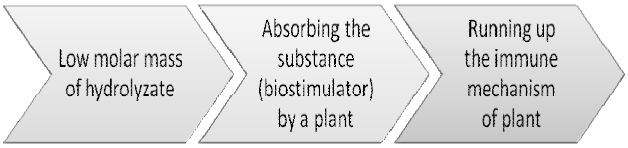

Fig. 2 Consequence of a low molar mass of hydrolyzate

molar mass of biostimulator a plant is able to absorb this substance what can run its immune mechanism.

Table 1 The molar masses of proteins during the process

SUBSTANCE	**MOLAR MASS [kg/kmol]**
Collagen protein in shavings	300 000 – 500 000
Gelatinous protein	100 000 – 150 000
Enzyme hydrolyzate	10 000 – 30 000
Biostimulator	2 000 – 5 000

III. MATHEMATICAL MODEL

Linearized state mathematical model including input, output and inner-state variables is used for the description of the final stage in the process of protein hydrolysis for the purpose of producing inducers of resistance.

A. State model of dynamic system

Diagram of the used system is shown in Fig. 3. Analogue nonlinear system is described by (1) and (2):

$$\dot{X} = F(X,U) \tag{1}$$

$$Y = G(X,U) \tag{2}$$

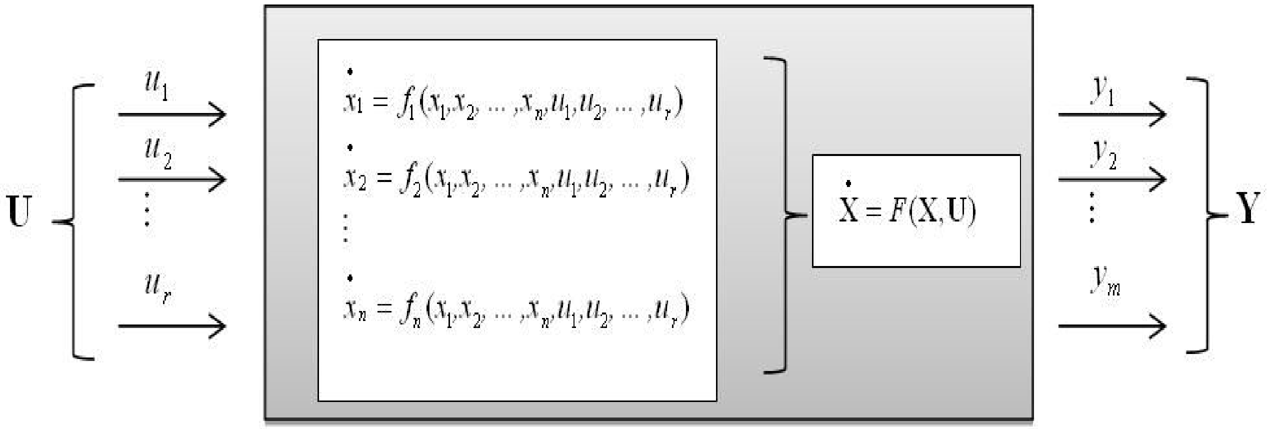

Fig. 3 Mathematical model of the system

Where:
$\mathbf{X} = (x_1, x_2, \ldots, x_n)$ is the state vector,
$\mathbf{U} = (u_1, u_2, \ldots, u_r)$ is the input vector,
$\mathbf{Y} = (y_1, y_2, \ldots, y_m)$ is the output vector,
$F = (f_1, f_2, \ldots, f_n)$ and $G = (g_1, g_2, \ldots, g_m)$ are vector functions and $\dot{\mathbf{X}}$ is a time derivative of state variables

$$\dot{\mathbf{X}} = \frac{\mathrm{d}X}{\mathrm{d}t}. \tag{3}$$

B. Model linearization

Linearized model is obtained by introducing deviations of state and input variables from their stationary states and then linearized using Taylor series.

$$\Delta\mathbf{X} = \mathbf{X} - \mathbf{X^0} \tag{4}$$

$$\Delta\mathbf{U} = \mathbf{U} - \mathbf{U^0} \tag{5}$$

$\mathbf{X^0}$ and $\mathbf{U^0}$ are stationary states values, the time derivative of state variables gives zero as shows (6).

$$\Delta \dot{X}^{\mathbf{0}} = \Delta\mathbf{F}(\mathbf{X^0}, \mathbf{U^0}) = 0 \tag{6}$$

Analogue linear system is described by the state equation (7) and output status (8) [9]

$$\Delta\dot{\mathbf{X}} = \mathbf{A}\Delta\mathbf{X} + \mathbf{B}\Delta\mathbf{U} \tag{7}$$

$$\Delta\mathbf{Y} = \mathbf{C}\Delta\mathbf{X} + \mathbf{D}\Delta\mathbf{U}. \tag{8}$$

Where:
A is state matrix, $\dim \mathbf{A} = n \times n$,
B is the input matrix, $\dim \mathbf{B} = n \times r$,
C is the output matrix, $\dim \mathbf{C} = m \times n$,
D is the zero matrix, $\dim \mathbf{D} = m \times r$.

The state matrixes A to D are given by (9) – (12).

$$\mathbf{A} = \begin{bmatrix} \frac{\partial f_1^0}{\partial x_1} & \frac{\partial f_1^0}{\partial x_2} & \cdots & \frac{\partial f_1^0}{\partial x_n} \\ \frac{\partial f_2^0}{\partial x_1} & \frac{\partial f_2^0}{\partial x_2} & \cdots & \frac{\partial f_2^0}{\partial x_n} \\ \vdots & \vdots & \vdots & \vdots \\ \frac{\partial f_n^0}{\partial x_1} & \cdots & \cdots & \frac{\partial f_n^0}{\partial x_n} \end{bmatrix} \quad (9)$$

$$\mathbf{B} = \begin{bmatrix} \frac{\partial f_1^0}{\partial u_1} & \frac{\partial f_1^0}{\partial u_2} & \cdots & \frac{\partial f_1^0}{\partial u_r} \\ \frac{\partial f_2^0}{\partial u_1} & \frac{\partial f_2^0}{\partial u_2} & \cdots & \frac{\partial f_2^0}{\partial u_r} \\ \vdots & \vdots & \vdots & \vdots \\ \frac{\partial f_n^0}{\partial u_1} & \cdots & \cdots & \frac{\partial f_n^0}{\partial u_r} \end{bmatrix} \quad (10)$$

$$\mathbf{C} = \begin{bmatrix} \frac{\partial g_1^0}{\partial x_1} & \frac{\partial g_1^0}{\partial x_2} & \cdots & \frac{\partial g_1^0}{\partial x_n} \\ \frac{\partial g_2^0}{\partial x_1} & \frac{\partial g_2^0}{\partial x_2} & \cdots & \frac{\partial g_2^0}{\partial x_n} \\ \vdots & \vdots & \vdots & \vdots \\ \frac{\partial g_m^0}{\partial x_1} & \cdots & \cdots & \frac{\partial g_m^0}{\partial x_n} \end{bmatrix} \quad (11)$$

$$\mathbf{D} = \begin{bmatrix} \frac{\partial g_1^0}{\partial u_1} & \frac{\partial g_1^0}{\partial u_2} & \cdots & \frac{\partial g_1^0}{\partial u_r} \\ \frac{\partial g_2^0}{\partial u_1} & \frac{\partial g_2^0}{\partial u_2} & \cdots & \frac{\partial g_2^0}{\partial u_r} \\ \vdots & \vdots & \vdots & \vdots \\ \frac{\partial g_m^0}{\partial u_1} & \cdots & \cdots & \frac{\partial g_m^0}{\partial u_r} \end{bmatrix} \quad (12)$$

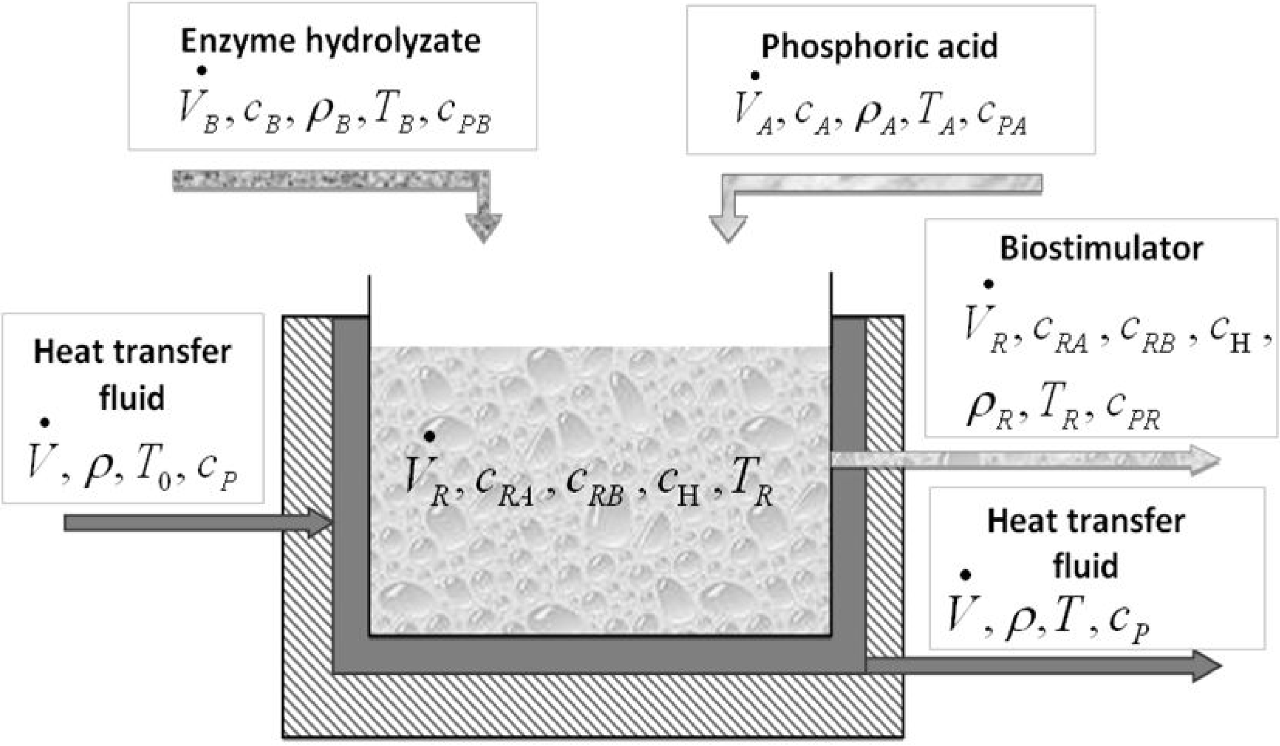

Fig. 4 The scheme of protein hydrolysis process with input and output variables

This system as a real system fulfils the strong physical condition of the feasibility, i.e. the outputs are functions only of the state variables. This means

$$\Delta\mathbf{Y} = \Delta\mathbf{X}\,. \qquad (13)$$

Equation (8) is then simplified, because matrix **C** is the unit matrix and D is the zero matrix.

IV. PRODUCTION OF INDUCER OF RESISTANT

The chemical process of a collagen protein hydrolysis for producing inducer of resistance takes place in an open flow reactor. The scheme of reactor is shown in Fig. 4. Enzymatic hydrolyzate and phosphoric acid are the feedstock, the reaction mixture is stirred while the reactor is heated by a heat transfer fluid and is thermally insulated from surrounding.

Mathematical model for the third step of the complex protein hydrolysis, the acid hydrolysis, is given by the balance equations (14) – (18).

Mass balance:

a) Substance A – protein/ enzymatic hydrolyzate

$$\dot{V}_A\, c_A = \dot{V}_R\, c_{RA} + k c_{RA} c_{RB} V + \frac{\mathrm{d}(V c_{RA})}{\mathrm{d}t} \qquad (14)$$

b) Substance B – phosphoric acid

$$\dot{V}_B\, c_B = \dot{V}_R\, c_{RB} + k c_{RA} c_{RB} V + \frac{\mathrm{d}(V c_{RB})}{\mathrm{d}t} \qquad (15)$$

c) Hydrolyzate – biostimulator

$$0 = \dot{V}_R\, c_H - k c_{RA} c_{RB} V + \frac{\mathrm{d}(V c_H)}{\mathrm{d}t} \qquad (16)$$

Enthalpy balance

d) Heat transfer fluid

$$\dot{V}\, \rho c_P T_0 = \dot{V}\, \rho c_P T + K(T - T_R)S + \frac{\mathrm{d}(V \rho c_P T)}{\mathrm{d}t} \qquad (17)$$

e) Reaction mixture

$$\begin{aligned} \dot{V}_A\, \rho_A c_{PA} T_A + \dot{V}_B\, \rho_B c_{PB} T_B + + K(T - T_R)S &= \\ = \dot{V}_R\, \rho_R c_{PR} T_R + \frac{\mathrm{d}(V_R \rho_R c_{PR} T_R)}{\mathrm{d}t} & \end{aligned} \qquad (18)$$

The condition for volumetric flow rates is

$$\dot{V}_R = \dot{V}_A + \dot{V}_B \qquad (19)$$

Symbols used in mathematical relations:

c_i is concentration,
ρ_i is density,
$\mathrm{T_i}$ is temperature,
c_{pi} is specific heat,
k is reaction rate constant,
K is heat transfer coefficient,
S is area of the reactor heat transport,
V_i is volume,
$\dot{V} =: \frac{\mathrm{d}}{\mathrm{d}t} V$ is the volumetric flow rate.

Indexes used are:

$_\mathrm{A}$ as the input substance A,
$_\mathrm{B}$ as the input substance B,
$_\mathrm{R}$ is reaction mixture,
$_\mathrm{H}$ represents output product of the hydrolysis the biostimulator,
$_0$ – initial value for heat transfer fluid.

A. Input and state variables

Two assumptions were taken into consideration**:**

- Deviations around the desired quantity are small, in order of hundredths so the density dependence on temperature and the specific heat dependence on temperature can be neglected.
- The volume of the reactor remains constant. The changes in the volume generated by a chemical reaction and resulting from evaporation can be neglected.

Input and state variables for the system are listed in Table 2.

Table 2 The list of state and input variables

INPUT VARIABLES	**STATE VARIABLES**
$u_1 = \dot{V}_A$	$x_1 = c_{RA}$
$u_2 = T_A$	$x_2 = c_{RB}$
$u_3 = c_A$	$x_3 = c_H$
$u_4 = \dot{V}_B$	$x_4 = T$
$u_5 = T_B$	$x_5 = T_R$
$u_6 = c_B$	
$u_7 = \dot{V}$	
$u_8 = T_1$	

Then the mathematical model is given by following functions:

$$\dot{x}_1 = f_1 = \frac{\dot{V}_A c_A - \dot{V}_R c_{RA} - kc_{RA}c_{RB}V}{V} \tag{20}$$

$$\dot{x}_2 = f_2 = \frac{\dot{V}_B c_B - \dot{V}_R c_{RB} - kc_{RA}c_{RB}V}{V} \tag{21}$$

$$\dot{x}_3 = f_3 = \frac{kc_{RA}c_{RB}V - \dot{V}_R c_H}{V} \tag{22}$$

$$\dot{x}_4 = f_4 = \frac{\dot{V}\rho c_P T_0 - \dot{V}\rho c_P T - K(T-T_R)S}{V\rho c_P} \tag{23}$$

$$\dot{x}_5 = f_5 = \frac{\dot{V}_A \rho_A c_{PA} T_A + \dot{V}_B \rho_B c_{PB} T_B + K(T-T_R)S - \dot{V}_R \rho_R c_{PR} T_R}{V_R \rho_R c_{PR}} \tag{24}$$

B. Transfer function

Taking the Laplace transform of (6) and (7) yields

$$s\Delta X(s) = A\Delta X(s) + B\Delta U(s) \tag{25}$$

$$\Delta Y(s) = C\Delta X(s) + D\Delta U(s) \tag{26}$$

$$\Delta X(s) = (sI-A)^{-1} B\Delta U(s) \tag{27}$$

After the substitution for ΔX(s) in the output equation (22) we get

$$\Delta Y(s) = (C(sI-A)^{-1}B + D)\Delta U(s). \tag{28}$$

Than the transfer function is

$$G(s) = \frac{\Delta Y(s)}{\Delta U(s)} = (C(sI-A)^{-1}B + D) \tag{29}$$

Together with (9) it is

$$G(s) = (sI-A)^{-1}B \tag{30}$$

Matrixes A (31) nad B (32) are then based on them mathematical model (20) – (24).

$$\mathbf{A} = \begin{bmatrix} -\frac{\dot{V}_A+\dot{V}_B}{V} - kc_{RB} & -kc_{RA} & 0 & 0 & 0 \\ -kc_{RB} & -\frac{\dot{V}_A+\dot{V}_B}{V} - kc_{RA} & 0 & 0 & 0 \\ kc_{RB} & kc_{RB} & -\frac{\dot{V}_A+\dot{V}_B}{V} & 0 & 0 \\ 0 & 0 & 0 & -\frac{\dot{V}}{V} - \frac{KS}{V\rho c_P} & \frac{KS}{V\rho c_P} \\ 0 & 0 & 0 & \frac{KS}{V_R\rho_R c_{PR}} & -\frac{KS}{V_R\rho_R c_{PR}} - \frac{\dot{V}_A+\dot{V}_B}{V_R} \end{bmatrix} \tag{31}$$

he transfer function G(s) represents the ratio of the output to

$$\mathbf{B}=\begin{bmatrix} \frac{c_A-c_{RA}}{V} & 0 & \frac{\dot{V}_A}{V} & -\frac{c_{RA}}{V} & 0 & 0 & 0 & 0 \\ -\frac{c_{RB}}{V} & 0 & 0 & \frac{c_B-c_{RB}}{V} & 0 & \frac{\dot{V}_B}{V} & 0 & 0 \\ -\frac{c_H}{V} & 0 & 0 & -\frac{c_H}{V} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \frac{T_0-T}{V} & \frac{\dot{V}}{V} \\ \frac{\rho_A c_{PA} T_A}{V_R \rho_R c_{PR}}-\frac{T_R}{V_R} & \frac{\dot{V}_A \rho_A c_{PA}}{V_R \rho_R c_{PR}} & 0 & \frac{\rho_B c_{PB} T_B}{V_R \rho_R c_{PR}}-\frac{T_R}{V_R} & \frac{\dot{V}_B \rho_B c_{PB}}{V_R \rho_R c_{PR}} & 0 & 0 & 0 \end{bmatrix} \qquad (32)$$

V. CONCLUSION

A disposal and possible secondary processing of waste is an actual problem worldwide. The ways of this processing should meet economic and environment-friendly requirements what is not always possible to adjust. It is necessary for people begin to think forward. Non-utilization of secondary materials is not economical and may bring series of difficult problems in the future.

Chromium still remains the most used agent for hide tanning in leather/tanning industry. These industries produce (and will produce) a great amount of waste contaminated among other agents by the chromium. Unfortunately chromium can exist except of trivalent form that is used in tanning process also as hexavalent variant, which is highly toxic and is hazardous to the human health and the environment. In the interest of not deteriorating situation even hazardous waste should be recycled or better secondary processed.

The process of collagen protein hydrolysis gained from tannery wastes for the purpose of production inducers of resistance for crop plants was introduced. Mathematical models for the third step of the whole process, the acid hydrolysis of enzyme hydrolyzate is presented. The Model comes out of analogue non-linear system through the linearization and with consideration of physical conditions of feasibility. Mathematical model is based on balance equations arising from the physical and chemical background of the hydrolysis process. Using this model concentration of biostimulator, the final product of the tannery waste material treatment on the basis of low molar mass protein, will be processed.

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Karel Kolomazník was born in Kromeriz, May 5, 1938. He took up an academic position in Zlín in 1970 at the Faculty of Technology of Brno University of Technology (which was later changed to Faculty of Technology of Tomas Bata University in Zlín). Since 2006 he has been working at the newly established Faculty of Applied Informatics. His research group deals with optimization of technological processes particularly in the field of processing waste generated by tanning, leather and food processing industries into valuable products.

He has led and participated in many successful projects funded e.g. by the Ministry of Education, Youth and Sports of the Czech Republic, Ministry of Industry and Trade or the Ministry of Agriculture, the Czech Science Foundation, the US-Asia Environmental Partnership program, etc. In addition to that, he has put most of his results into industrial practice in cooperation with commercial partners from both the Czech Republic and abroad. He has had a long-term cooperation with the Eastern Regional Research Center of the U.S. Department of Agriculture, Wyndmoor, PA where he successfully applied the ammonia-free deliming of white hide. In the NIKE Inc. (Ho Chi Minh City, Vietnam) he implemented a patented technology for the processing of manipulation waste from leather industry including the application of the products. He has been also active in industrial applications within the Czech Republic, e.g. implementation of a patented technology of a protein hydrolyzate production from animal-based organic waste in the Kortan, s.r.o. in Hrádek nad Nisou.

Prof. Kolomaznik was awarded in 1998 the “Rolex Award for Enterprise”, for the technology of processing and recycling of potentially hazardous chrome-tanned waste produced by the leather industry. In 2009 he received the ALSOP Award of the American Leather Chemists Association for his long-term contribution to the association and the leather industry.

Vladimir Vasek was born in Zlin, December 5, 1948. He took up an academic position in Zlín in 1973 at the Faculty of Technology of Brno University of Technology. In 2006 he participated at the establishment of a new faculty – Faculty of Applied Informatics at Tomas Bata University in Zlin and is its first dean. His research group deals with ….

Prof. Vasek deals in his research activities with the discrete deterministic control especially with the use of microcomputers, in a recent time in a form of implemented embedded systems.