Advanced Fuzzy Modeling of Integrated Bio-systems

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Abstract: - Philosophy of integrated bio-systems springs from the holistic approach to the processes in the nature and society. The term “integrated bio-system” is introduced to describe the role of resources and information signals of mutually connected phenomena in complex biological systems. Various examples from the living nature are given in the introduction, connected with the applications in biomedicine, biotechnology, and ecology. The short survey on modeling method is presented, followed by own research results in field of biotechnology and microbial ecology by implementing advanced fuzzy modeling methods as well as adaptive neuro-fuzzy inference systems and fuzzy networks for the design of an Inferential Composition Model. The advanced fuzzy modeling approaches were applied for hard to measure biochemical and microbial growth parameters determination in both studied integrated bio-systems.

Key-Words: - Integrated bio-systems, advanced fuzzy modeling, fuzzy networks, inferential composition model, biotechnology, microbial ecology

1 Introduction

Philosophy of integrated bio-systems springs from the holistic approach to the processes in the nature and society. The term “integrated bio-system” is introduced to describe the role of resources and information signals of mutually connected phenomena in complex biological systems. Various examples from the living nature can be given. Living matter is built by cells, which accept, conduct, transform, save and transfer information concerning the irritant (stimulus) from internal or external environment. The living matter owns property of excitability and conductivity; realizes coordination of many processes in the living organisms. During excitation of neurons in the cell membrane, spring up neuronal impulses, which are reproduced as well as electrical current [5, 14, 22, 29]. The neurons are mutually connected in chains [6]. Under the influence of internal and external irritants neuronal impulses appear in the receptor cells, which are strongly specialized modified neurons. These impulses are conducted by the neuronal chains from one to the other neuron to the muscle fibers and glandular. As a result muscle shortening is carried out after which the secretion is observed.

The other example, characteristic of living matter, is homeostasis; its name appears from Greek words ομοιος, hómoios, which means "similar" and στάσις which means stasis or "standing still". Homeostasis is the property of a system that regulates its internal environment and tends to maintain a stable, constant condition of properties like temperature or pH. It is a system capable of supporting a stable state of dynamic balance, realizing efficiently certain functions regarding the environmental changes. After establishing a disturbance the biological system usually reacts on the basis of the negative feedback. This leads to the stabilization of conditions by regulating the activity of certain organ or system. For example, when the level of blood sugar is low, glucagon is secreted to provoke the transformation of glycogen to the glucose and recovery of the initial level of blood sugar.

The Adenosine triphosphate (ATP) transports chemical energy within cells for metabolism and supports the motion function of muscle fibres. Biochemical reaction of ATP synthesis is highly powerful but has small energetic capacity – only for 2-3 seconds. Without re-synthesizing ATP by other bio-energetic processes the muscle function or metabolism will be stopped.

Living organism’s survival depends on continued power flow. Chemical reactions, responsible for the structure and the organism’s functions are directed to utilization of power from substrates, supplied by food. For example, chemotaxis is the phenomenon, specific for the somatic cells, bacteria and other single-cell or multi-cellular organisms, in which they direct their movements according to certain chemicals in their environment. This is important for bacteria to find food (for example, glucose) by swimming towards the highest concentration of food molecules, or to flee from poisons (for example, phenol or furfural, as shown in the following Example 2).

In the microbial populations, chemotaxis is a means of coordinating cellular aggregation, of locating favorable concentrations of oxygen and nutrients and of avoiding toxic conditions. In predacious bacteria and fungi, chemotaxis may function in locating and maintaining proximity to the host organism [2].

This energy, obtained from substrates, serves for biochemical reactions of organisms and as a material substance for synthesis of new molecules, cell structures and cells [13, 17, 20, 21, 23]. Great part of the power is wasted in form of heat or is emitted in form of waste metabolite products.

Plants, other phototrophes and chemotrophes bring power from the sun and high energetic substances (methane, sulphuretted hydrogen, etc.) in the live world and they are basis of food pyramid. A part of the obtained power is implemented for biomass synthesis, the other part – for supporting life functions, growth and development.

The most important processes of power transformation are incorporated by the metabolism and cell respiration. The metabolism means all chemical reactions which occur in the living organism, including digestion and transportation of substances into and between different cells. Metabolism is usually divided into two categories: catabolism which breaks down organic matter to gather energy in cellular respiration and anabolism, which uses energy to construct components of cells such as proteins and nucleic acids. The metabolite chemical reactions are organized into metabolic pathways, in each of them the chemical is transformed through a series of steps into another chemical, by a sequence of enzymes.

Enzymes are most important to the metabolism because they allow organisms to drive desirable reactions that require energy and will not occur by themselves.

Integrated bio-systems in ecology are oriented to the sustainable development of modern industry [3, 7, 15, 30]. They make functional connections between agriculture, aquaculture, food processing, waste management, water use, and fuel generation. They encourage the dynamic flows of material and energy by treating wastes and by-products of one operation as inputs for another. In this way food, fertilizer, animal feed and fuel can be produced with the minimum input of nutrients, water and other resources.

The idea of integrated systems changes the research philosophy, which can lead to innovative technological solutions with high economical and social effect [16, 24].

2 Modeling of the integrated bio-systems

Integrated bio-systems are a special class of complex processes, which usually consist of interacting sub-processes characterized by different types of uncertainty. This fact represents a serious challenge to the modeling of such multi-factorial processes with unknown interconnections between input/output variables.

Many strategies have been proposed in the literature to model real systems depending on the level of a priori knowledge of the process. Models can be obtained either on the basis of first principles analysis (also known as mechanistic models) or by using gray- or black-box identification approaches. Models implementing first principle require preliminary knowledge regarding the relations between input and output variables, certain limitations. Usually they are in form of a nonlinear differential equations system. The gray-box approach can lead to very accurate models because it exploits any available source of information to refine the model. Black-box identification approaches (as well as ANN, fuzzy-logic, neuro-fuzzy, evolution algorithms) show high accuracy in the desired output prediction and often they help to discover new knowledge about the studied phenomena by extracting new rules from the data-bases [16, 24, 26, 28, 29, 31, 32, 33].

Following parts show some examples in modeling several integrated bio-systems.

2.1 Modeling in biomedicine

Homeostasis control mechanisms – positive or negative feedback, is first recognized as broadly applicable by Norbert Wiener in his 1948 work on cybernetics. Negative feedback mechanisms consist of reducing the output or activity of any organ or system back to its normal range of functioning. An example of this is regulating blood pressure. One positive feedback example event in the body is blood thrombocytes accumulations, which, in turn, causes blood clothing in response to a break or tear in the lining of blood vessels.

Quantitative description of membrane current and its application to conduction and excitation in nerve is subject of the research of the Nobel laureates Hodgkin and Huxley [14]. The well known mathematical models of the bio-impulses influence on the cells excitability in the human organism are also the models of Connor–Stevens [8], Morris–Lecar [22], FitzHugh–Nagumo [10, 23], Traub [29]. The evaluation of hard-to-measure process variables (usually electrical current) can be realized by inferential measurement methods (Kalman filter, Luenberger observer, etc.) and by designing residual-based generator for improving the predicted output [18, 19]. Modeling of neuronal chains or neurons connection is subject of [6].

2.2. Modeling of integrated biotechnological and ecological systems

Biotechnological and ecological systems are multi-dimensional, non-linear and non-stationary systems. Its internal structure involves many complicated mutual relationships due to self-organization and self-regulation of living matter and because these processes are governed by nonlinear forces. Often it may be impossible to create an accurate, manageable differential equations-based model. Moreover information uncertainties combined with the lack of analytical methods causes different problems in modelling and optimal control design [1, 4, 9, 13, 15, 17, 20, 21, 25, 31, 32, 33, 34].

The first mathematical models of the fermentation process are created on the basis of a comparison between catalytic reactions in chemistry and fermentation processes, where the enzymes (proteins) synthesized by biomass play role of organic catalyst converting substrate into a bio-product [1, 9, 13]. Enzymes are used in food processing; dairy production; pharmacy, cosmetics, paper and bio-fuel industry; rubber and photography and molecular biology.

Major transitions in history and future prospects of mathematical modeling and analysis in biochemical engineering were driven by the appearance of the Hinshelwood, Monod and Pirt, Aiba, Humphrey, and Millis texts, Fredrickson's guidance on conceptualizing mathematical representations of cell populations, and Ramkrishna's development of the cybernetic modeling approach [1, 13, 20, 25].

In bio-system integration, the management of wastes and residues is treated as a central design feature. Thus, in contrast to other production systems where waste disposal and remediation are essentially treated as externalities, sustainable design features are intrinsic to integrated bio-systems. Such design features include the following: minimize resource inputs by redirecting "waste" outputs within the system; contain material flows within the system; treat production and consumption as a continuous cyclical process, rather than a linear one; tighten production-consumption loops to minimize losses, transport costs etc; maximize efficiency of natural conversion processes (e.g., microbial decomposition and trophic links) and of nutrient/water retention. These design features make for increased system efficiency.

Further, integrated bio-systems take advantage of natural ecological processes, and as a result some components of such systems can be low technology, requiring less management, less maintenance and less capital expense [3, 7, 15, 34].

3 Examples from biotechnology

Two examples of integrated bio-systems are shown in this part: Example 1 is devoted to a detailed study on the mutual influence between composition parameters of base red wine and sparkling wine and Example 2 deals with the poison influence on microbial population growth. Both modeling methods implement advanced fuzzy logic approaches.

3.1. Example 1

Quality of the final product plays a significant role in industry. Wine manufacturing is known for many years but until now it remains as well as an art than as a usual production process. Wine composition as a final quality evaluation is of great importance in wine manufacturing. The final concentration of aldehydes, acetoin and diacetyl are obligatory analyzed [12]. Diacetyl and acetoin occur naturally in alcoholic beverages and are added to some foods to impart a buttery flavor. Aldehydes are common in organic chemistry. Many fragrances are aldehydes.

The accelerate introduction of high quality biotechnology’s requires development of products with a higher constant quality and different taste qualities. It is connected with time consuming and expensive scientific researches. New computer aided methods and means based on the numeric processing of various by kind, linguistic and numeric, incomplete and inexact information, on the formalized human skill and long years experience are developed nowadays as a helpful scientific tool. A natural way of coping with quantitative complexity is to use the concept of a general network. The latter is composed of nodes and connections whereby the nodes represent the elements of an entity and the connections reflect the interactions among these elements. The scale of entity is reflected by the overall size of the network whereas the number of elements is given by the number of nodes [11].

An obvious way of dealing with qualitative complexity is to use the concept of fuzzy network. The concept of fuzzy network is based on the structure consisting of nodes and connections. The nodes in this structure are fuzzy systems and the connections reflect the interactions among these fuzzy sub-systems. In this case, the uncertainties about the data or knowledge to an entity are reflected by the rule bases of the corresponding fuzzy sub-systems and underlying fuzzy logic.

Advanced modeling method by Fuzzy Networks implementing Inferential Composition Model [11] is used for modeling the sparkling wine manufacturing which typically consists of three different fermentation processes. First fermentation is obtaining of base red wine. The final product of first fermentation – base red wine, is used also as medium for starter culture of *Saccharomyces bayanus* yeasts batch cultivation in conditions similar to the conditions for the third fermentation process - sparkling wine manufacturing, in order to adapt its metabolism to the low temperature anaerobic fermentation [4, 25, 27]. The third fermentation or pressurizing of the red wine medium is carried out by yeast strain *Saccharomyces bayanus* in bottles, following the primary alcoholic and malolactic fermentation, converting the carbohydrates into ethanol and carbon dioxide, CO_2, which leads to the specific taste, flavor and quality of the sparkling wine [25, 26, 27]. It was found that a significant relation between aeration conditions, fermentation performance and the sparkling wine composition exist. The final concentration of wine quality factors as well as acetaldehyde (AL1), acetoin (AC1) and diacetil (D1) as products of oxidative metabolism in the starter culture are often analyzed.

The principal scheme of advanced fuzzy modeling approach which implements the Inferential Composition Model for sparkling wine manufacturing is depicted on Fig.1.

The effect of temperature (T,ºC), aeration volume (O_2, l/l.min), agitation speed (n, l/min) and the amount of additional ammonium nitrogen (N_2, mg/l) was studied. The levels of input attributes change are as follows: low, middle and high. The temperature was changed at the corresponding levels: 19, 16 and 13ºC; the aeration volume – at 1, 0.5 and 0 [l/l/min]; the agitation speed – at 800, 400 and 0 [1/min] and the ammonium nitrogen – at 1, 0.5 and 0 [mg/l].

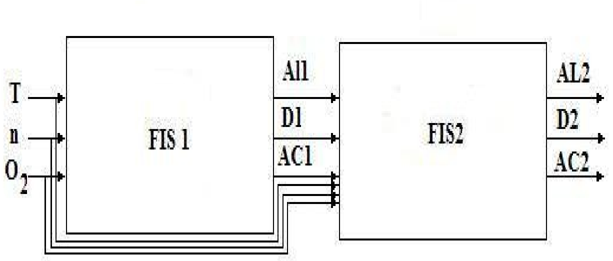

Fig. 1 Principal scheme of Fuzzy Networks design implementing Inferential Composition Model for sparkling wine manufacturing.

The yeast strain *Saccharomyces bayanus* was injected into a microprocessor-controlled laboratory fermentor ABR 01 (designed in the former Central Laboratory of Bioinstrumentation and Automation, Bulgarian Academy of Sciences), equipped with the automation system (Fig.2), containing commercially prepared filter-sterilized red base wine (11.6 [vol.%] alcohol, 56 [mg/l] total SO_2, 8 [mg/l] ammonium nitrogen, and 30 [g/l] sugar). Sufficient yeast culture was added to give 2.10^6 cells /ml. The ammonium nitrogen was added as a mixture of ammonium sulfate and ammonium phosphate in a ratio of 2:1.

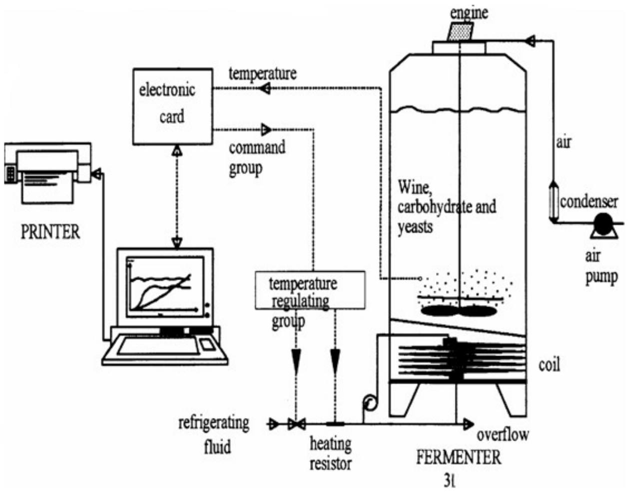

Fig.2 Instrumentation of the yeast starter culture production system

During the propagation process, periodic determinations were made of the sugar content, total cell count and the number of budding and dead cells.

After the sugars in the culture were reduced to 5-6 g/l, aldehydes (AL), diacetyl (D) and acetoin (AC) were analyzed chemically. It was found that ammonium nitrogen is mostly important for yeast population growth [27, 28] and is not directly responsible for the wine composition.

Bottle champagnisation at 15.5-16.0 deg.C was conducted with each resultant yeast variant, using the same wine material but with the sugar content adjusted to 22.5-23.0 g/l. The initial cell concentration in the tirage [20] was 2.10^6 cells /ml.

During the secondary fermentation, the sugar content and total cell count were determined at regular intervals. The fermentation activity was estimated as the number of grams of fermented sugar from 1-gram yeast (dry weight) per hour over the first 60 hours after tiraging [20]. The yeast cultures were assessed for sugar, aldehydes, diacetyl and acetoin by the following methods: sugar-Luff-Schoorl method after clarification with neutral lead acetate, without ion exchange, according to the official OIV method (1990); aldehydes-iodometric bisulphite method of Ribereau-Gayon and Peynaud [26]; diacetyl and acetoin-colorimetric method of Guymon and Crowell [12]. The cell concentration was determined by triplicate counting in a Thoma chamber.

The data involved in the data base, implemented for fuzzy sub-systems design, are acquired from a full plane-experiment with four factors on two levels or 16 experiments and 5 additional experiments in the centre of the experimental plan or 21 experiments. Each experiment is three-time repeated to eliminate rough errors of the measurements and the biochemical analysis. Selected data instances, are presented in Table 1.

Table 1. Selected data instances

T	O_2	n	AL1	D1	AC1	AL2	D2	AC2
19	1	800	492.8	23.2	750	89.6	1.60	1.70
13	1	800	200.2	19.9	470	83.6	1.12	4.80
19	0	800	110.0	4.5	174	77.0	0.90	6.00
13	0	800	149.6	15.5	265	79.2	0.68	13.2
19	1	0	248.6	23.3	770	79.2	0.43	3.60
13	1	0	398.2	34.6	640	74.8	0.36	3.40
19	0	0	173.8	3.1	56	74.8	1.30	2.30
13	0	0	169.4	7.7	68	77.0	0.90	3.40
16	0.5	400	297.0	23.0	690	79.2	0.50	1.80
16	0.5	400	299.8	26.0	860	79.2	0.76	1.80
16	0.5	400	255.4	28.6	790	79.2	0.72	1.30

Using an advanced modeling system with a two-level hierarchical structure performed neuro-fuzzy modeling of wine composition. In two levels of the system were solved following problems:

- modeling of red base wine composition parameters (aldehydes, AL1, acetoin, AC1 and diacetil, D1) in the stage of yeast starter culture batch cultivation at the constant inoculum content (Xo) and under different aeration conditions by using two input attributes – the dissolved oxygen content (O_2) and agitation speed (n), selected by sensitivity analysis as best predictors;
- modeling of sparkling wine composition parameters (aldehydes, AL2, acetoin, AC2 and diacetil, D2) in the second wine fermentation, depending on five input attributes - the dissolved oxygen content (O_2), agitation speed (n), aldehydes (AL1), acetoin (AC1) and diacetil (D1).

In this case, the composition is applied to the sub-models such that each of them is subject to fuzzification, inference and defuzzification.

Two fuzzy inference systems (FIS1 and FIS2) were developed for solving both tasks. The associated model of the hierarchical system is based on inferential composition of both fuzzy sub-models, which are interacting on the basis of preliminary known fact about the mutual influence between the composition parameters of red wine (AL1, AC1, D1) and the sparkling wine composition parameters (AL2, AC2, D2).

The studied problem belongs to the non-linear multivariate regression analysis problems. For such a case with three inputs and three output variable we need approximately 10^6 points that is too large data size. The disadvantage of the collected experimental information is the non-stationary and non-linear character of the fermentation processes that leads to the obtaining of uncertain and inaccurate data. Analyzed experimental data were obtained from 63 (21x3) experiments with a batch propagation of the studied yeast strain. After removing the missing values the size of used data set was 378 or it corresponds to $(378)^{0.33}$=2.7 points for single input data fitting. The problem of the data scarcity is ubiquitous in modeling of biotechnological processes. To cope with this problem the data set was divided into equal by size training and checking data sets. In some cases, comparison of the size of input database and the size of linear fitting parameters of FIS shows that the resultant model is not reliable toward unseen inputs. Two approaches solve such a problem: the reduction of the input dimension or the implementation of the scatter partition [28].

The first sub-model FIS1 is based on using data from the full factorial experiment as shown in Table1. The prediction of output variables AL1, AC1 and D1 is realized by using four fuzzy rules per each variable prediction or rule base consists of twelve fuzzy rules. The membership functions used for presenting linguistic values of input attributes of the first sub-model FIS1 are two Bellman shaped membership functions. The output variables were represented by linear functions.

For illustration of this influence, in Table 2 and Table 3 are shown obtained fuzzy sub-models with the corresponding minimal root mean square error (RMSE).

Table 2 FIS1: Red wine composition for starter culture obtaining

Fuzzy model	Min RMSE
AL1 = f(O_2, n)	2,1268
AC1 = f(O_2, n)	2,1062
D1 = f(O_2, n)	0,5345

Table 3 FIS2: Sparkling wine composition

Fuzzy model	Min RMSE
AL2 = f(O_2, n, AL1, AC1, D1)	6,1268
AC2 = f(O_2, n, AL1, AC1, D1)	2,6048
D2 = f(O_2, n, AL1, AC1, D1)	0,0369

For consistency, all inputs and outputs in the fuzzy sub-models FIS1 and FIS2 are considered in the same variation range. The fuzzy rules in the each sub-model are extracted from the data-base by implementing adaptive neuro-fuzzy inference system (anfis) [28].

Rule base connects both fuzzy inference sub-systems as a sequence of two models, whereby the outputs of FIS1 – AL1, D1 and AC1 are fed as input attributes into the second sub-model – FIS2.

The rule base of each fuzzy model in FIS2 consists of 2^5=32 rules. In the Fig.3 and Fig.4 are presented two of the predicted composition parameters of the final product AC2 and D2 of the second fuzzy sub-model FIS2.

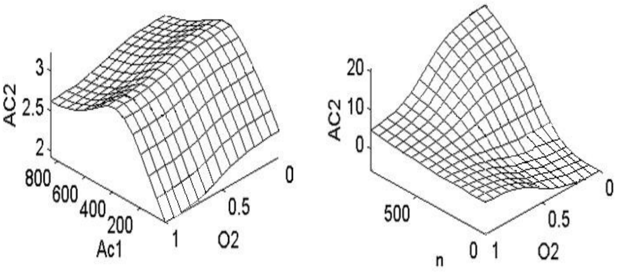

Fig.3 FIS2-optimal 3-D surface of AC2 fuzzy model

The biochemical analysis of volatile substances as well as aldehydes (AL), acetoin (AC) and diacetil (D) leads to high errors in the observed data, which influences the model accuracy, independently on the sophisticated modeling techniques applied for its prediction. On Fig. 5 and Fig.6 are depicted comparisons of the training and checking root mean square errors (RMSE) for prediction of AC2 and D2.

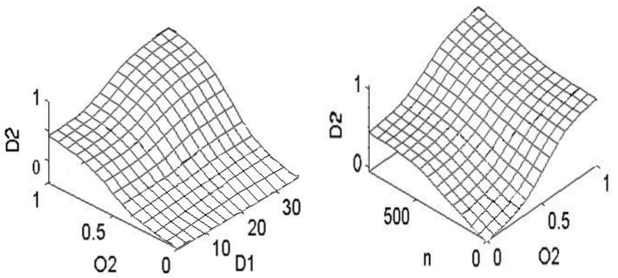

Fig.4 FIS2-optimal 3-D surface of D2 fuzzy model

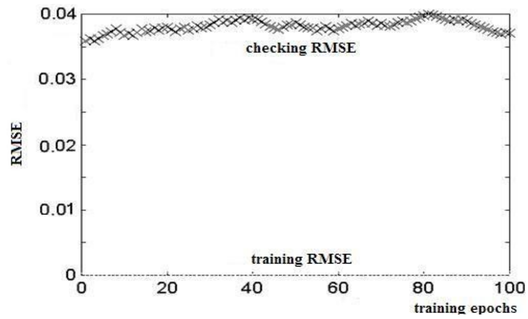

Fig.5 RMSE of D2 fuzzy model: comparison between the training and the checking error.

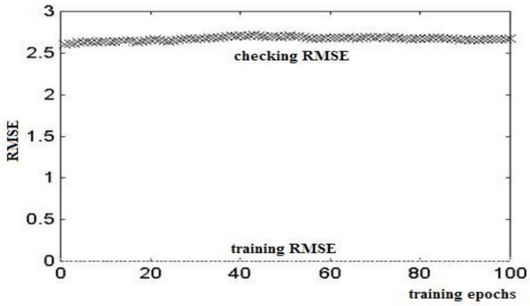

Fig.6 RMSE of AC2 fuzzy model: comparison between the training and the checking error.

The accuracy is acceptable proven [26] that these wine constituents are highly reactive and volatile.

3.1. **Example 2**

Furfural is one of the main growth inhibitors for yeasts cultivated on wood hydrolyzates media. It was found that it affects negatively yeast viability, the specific growth rate, the biomass yield of *Candida tropicalis* and the fermentation rate of *Saccharomyces cerevisiae*. It has been proved that some enzymes are sensitive to furfural and the glycolytic enzymes glyceraldehydes–3–phosphate-dehydrogenase, alcohol dehydrogenase and hexokinase are among the most sensitive ones. The strong inhibiting effect of furfural is diminished when it is reduced to the less inhibitory compound furfurilic alcohol.

A detailed study on inhibitory effect of furfural on the bioproductivity of lactose-assimilating strain *Candida blankii* 35 in chemostat is carried out by different dilution rates D [h^{-1}] [28]. Fuzzy modeling was preferred in our work for its approximation capability to cope with uncertain experimental data set and to explain the complex and ill-defined character of furfural inhibitory effect on continuous fermentation processes.

To establish the data base necessary for fuzzy model parameter identification the original data set was interpolated by implementing cubic splines. As a result the database consists of 810 data points for each input/output attribute, divided in the equal training and the checking data set.

The most important predictors were selected with the help of statistic methods. For the studied process they are input model attributes: furfural concentration, residual lactose concentration, protein content and RNA. The predicted output attributes are biomass (X) and protein-synthesizing ability (A), obtained by the formulae:

$$A = \frac{\text{Protein content (\%)}}{\text{RNA(\%)}} D\left[h^{-1}\right] \qquad (1),$$

where D is the dilution rate of the studied chemostat, carried out by different dilution rates D (D=0.1 and 0.25 h^{-1}).

The suitable linguistic presentation of the input variables is by implementing 3 Bellman-shaped membership functions. The fuzzy outputs are defuzzyfied by centroid of area (COA) method. The adaptive neural network technique is used to identify the fuzzy rule (FRi) parameters

$$FR_i : \text{If } x_i \text{ is } A_i \text{ then } y \text{ is } B \qquad (2),$$

where x_i, i=1,2,3,4 corresponds to input variables in the premise part of FRi and y is the consequent part of the rule. The starting parameters of the antecedents are adapting during training the equivalent to our fuzzy inference system artificial neural network which implements back propagation and gradient descent. Obtained for each steady state rule base of FIS2 in our case consists of 81 fuzzy rules. By using adaptive neural network learning capability of parameter identification technique the fuzzy recognition model parameters were calculated with a high accuracy. The corresponding RMSE reached for the experiments with D=0.1[h^{-1}] are 0.01 for X and 0.03 for A, and for D=0.25[h^{-1}] – 0.06 for X and 0.05 for A prediction.

The mutual influence of furfural and biomass (X) content on the protein-synthesizing ability (A) is shown in Fig.7 for D=0.1[h^{-1}], and D=0.25[h^{-1}] . The two time higher maximum of protein-synthesizing ability (A) is expected for higher value of D=0.25 [h^{-1}]. In agreement with the experimental data the maximal value of A can be reach for furfural content about 0.3 [g/dm^3]. The different biomass content, residual lactose and RNA show the tendency of maximal A for both dilution rates. When the dilution rate D=0.1, the maximal A is obtained for biomass content about 6 [g/dm^3], residual lactose about 1.25[g/dm^3] and RNA approximately 6 [%]. These values are different for the highest D, when maximal A is obtained for biomass content about 4 [g/dm^3], residual lactose about 3 [g/dm^3] and RNA approximately 10 [%]. The simulation results show microbial culture is influenced by the furfural content, but also by the dilution rate D, which as it is known fulfills the requirement $D \leq \mu_{max}$.

On the same principle is investigated the complex influence of four input variables as well as furfural, residual lactose, protein content and RNA on the biomass growth.

From the fuzzy recognition model it was found a maximal biomass content is reached for the lowest level of furfural and residual lactose.

Complex influence impact of the dilution rate, furfural, protein and RNA on the biomass content for both chemostats show different tendencies. The highest biomass content is observed for the fermentation process with the lowest dilution rate. When the protein content is about 45 [g/dm^3] and furfural is about zero, the expected biomass content for D=0.1[h^{-1}] is approximately 2 time higher then for the fermentation carried out with D=0.25[h^{-1}]. The 10 time highest biomass content can be reached by D=0.1[h^{-1}] in comparison with the fermentation process carried out by D=0.25[h^{-1}], furfural content approximately about 0.3 [g/dm^3] and RNA about 6 [%] in both cases. Obtained simulation results allow to conclude concerning the permitted furfural value. A key value of furfural in both chemostats is about 0.3 [g/dm^3]. In accordance with the experimental results it can be seen fiurfural content, higher than 0.4 [g/dm^3], lead to irreversible changes in the culture or a new steady state can not be reached for D=0.25[h^{-1}] (Fig.8). Moreover the studied yeast culture, cultivated under higher D, is more sensitive to the inhibitory effect of furfural, compared to the effect of the same furfural content at a lowest D. Simulation results present the inhibitory effect of furfural has a non-linear correlation with the dilution rate D.

In conclusion of Example 2, neuro-fuzzy recognition models of high accuracy were developed for yeast culture steady state investigations, which can be efficiently implemented for process optimization in conditions of uncertainty knowledge and information.

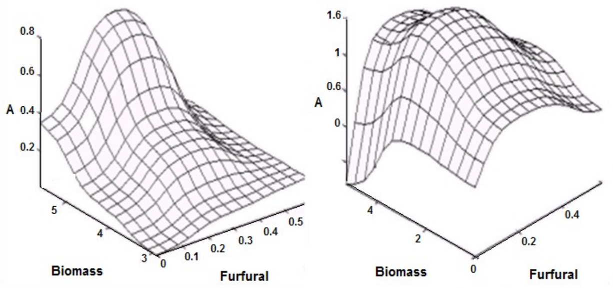

Fig.7 Influence of furfural and biomass (X) content on the protein-synthesizing ability (A) for $D=0.1[h^{1}]$ and $D=0.25[h^{-1}]$.

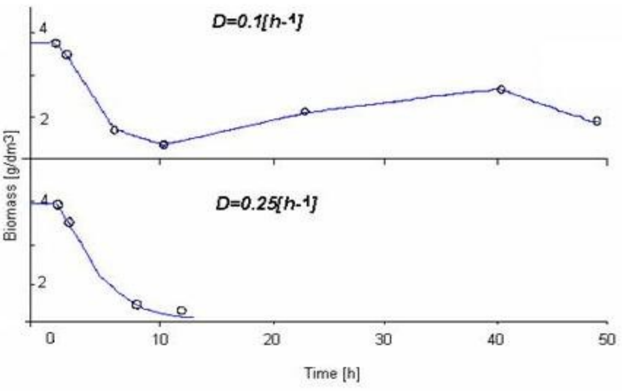

Fig.8 Biomass growth adaptation after adding 0.8 g/dm^3 furfural (o original data, – simulation data) at the dilution rates $D=0.1[h^{1}]$ and $D=0.25[h^{-1}]$

4 Conclusion

Integration mechanisms in the complex systems are not well studied. Because of that in last years are carried out investigations and are developed new branches of science as “collective or collaborative intelligence”, “symbol intelligence”, „quorum sensing”, “quorum quenching “, which are related to the collective decision making not only in the biological systems (cells behavior in living organisms regarding to the viruses, microbial population relation to the competitive microbial populations, etc.), but also in the social groups, in communication and computer nets, in industrial complex processes and ecology.

In connection with the explanation of the integration mechanism in wine manufacturing, the first example in this paper presents a detailed study on the mutual influence between composition parameters of red wine and the sparkling wine. An only fermentation process from the described in Example 1 main stages of sparkling wine manufacturing is carried out in aerobic conditions – the starter culture obtaining. It is well known that starter culture play a significant role on following anaerobic fermentation processes – sparkling wine production and pressurisation of wine in bottles. An interesting idea was to show the effect of the aerobe fermentation (aerobic growth of the starter culture) on the wine composition and wine quality which creates a multi-factorial integrated bio-system.

The applied advanced modeling method by fuzzy networks for the Inferential Composition Model design shows that oxygen content and agitation speed influence the wine composition parameters as well as aldehydes, acetoin and diacetyl in both bio-subsystems – aerobic and anaerobic. The obtained result is important in wine industry for improving quality of the trade products. The implemented method for modeling complex bio-system by fuzzy networks represents a natural counterpart of the artificial neural networks [11]. Both, neural networks and fuzzy networks are computational intelligence based networks with nodes and connections. However, the nodes in the artificial neural networks are represented by neurons whereas the nodes in the fuzzy network are represented by the fuzzy rules which built the rule base of the integrate bio-system.

In connection with the explanation of the integration mechanism in the bio-systems of microbial ecology, the influence of poisoned environment at various cultivation conditions of a continuous microbial growth are studied in Example 2. The idea has come up to study the growth and bioproductivity of yeast populations cultivated in mixed substrates consisting of wood hydrolyzates and whey [30]. Cultivating in such mixed substrates would bring to reducing the inhibitor content in the media, as well as to more complete and effective uptake of sugars from both substrates. It was found that strain *Candida blankii* 35 effectively assimilates lactose from whey [31].

Both examples, developed in this study, show that the effectiveness of systems management depends on the way in which the outputs in an integrated system are influenced by its inputs. These influences may be described by nonlinear functional mappings that are usually referred to as nonlinearity, which is specific feature of the mentioned in the paper bio-systems.

Integrated bio-systems are often connected with the problem of high dimensionality. The nonlinearity together with the relevance of the data, information and knowledge seriously influence system management due to the difficulties in handling nonlinear mappings. The imprecision, incompleteness, vagueness or ambiguity of the data and knowledge, known as uncertainty, are crucial in solving safety-critical cases.

New methods for integrated bio-systems modeling, simulation and control, based on knowledge processing as well as fuzzy logic, will help to discover the unknown mechanisms of bacteria quorum sensing for mutual coordination; function's detection of signaling molecules, explanation of regulation mechanisms of unwell studied bio-processes.

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