Developments in Fuzzy Sets, Intuitionistic Fuzzy Sets, Generalized Nets and Related Topics Volume II: Applications

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Generalized net model of the process of the prognosis biomass accumulation with neural network

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Abstract

The proposed generalized net will give us a possibility for using a feed-forward neural network based on backpropagation algorithm used for prognosis biomass accumulation. The prognosis of the data is very important for many real processes.

Keywords: Neural networks, biomass accumulation, prognosis.

1   Introduction

1.1 The family Enterobacteriaceae

Enterobacteriaceae are Gram-negative, oxidase-negative, rod-shaped bacteria, 0.3-1.0 x 1.0-6.0 um. Typically, they are motile by peritrichous flagella. They are facultative anaerobes, being chemoorganotrophs that exhibit both respiratory and fermentative metabolism. Most grow well between 22 and 35°C on media containing peptone or beef extract. Most grow on glucose as a sole carbon source, although some require vitamins and/or amino acids for growth. They produce mixed acids and often gas from fermentation of sugars. With very few exceptions they are catalase-positive, and most strains reduce nitrate to nitrite.

Escherichia coli is the type species. *E. coli* is considered the most thoroughly studied of all species of bacteria, and the family *Enterobacteriaceae,* as a whole, is the best studied group of microorganisms. Among the reasons for their popularity are their medical and economic importance, ease of isolation and cultivation, rapid generation time, and their ability to be genetically manipulated.

Enterobacteriaceae are distributed worldwide. They are found in water and soil and as normal intestinal flora in humans and many animals. They live as saprophytically, as symbionts, epiphytes, and parasites. Their host range includes animals ranging from insects to humans, as well as fruits, vegetables, grains, flowering plants, and trees.

1.2 Economic and medical importance

As stated above, one of the reasons that the *Enterobacteriaceae* have ben so widely studied is due to their obvious impact on human and animal health and on agricultural practice. The *Enterobacteriaceae* include of food poisoning and gastroenteritis, hospital-acquired infections, enteric fevers (e.g. typhoid fever) and plague. They also cause infections in domestic farm and zoo animals and include an important group of plant pathogens. Some of these bacteria are discussed below.

1.3 Physiology of E. coli

Physiologically, *E. coli* is versatile and well-adapted to its characteristic habitats. In the laboratory it can grow in media with glucose as the sole organic constituent. Wild-type *E. coli* has no growth factor requirements, and metabolically it can transform glucose into all of the macromolecular components that make up the cell. The bacterium can grow in the presence or absence of O_2. Under anaerobic conditions it will grow by means of fermentation, producing characteristic "mixed acids and gas" as end products. However, it can also grow by means of anaerobic respiration, since it is able to utilize NO_3 or fumarate as final electron acceptors for respiratory electron transport processes. In part, this adapts *E. coli* to its intestinal (anaerobic) and its extraintestinal (aerobic or anaerobic) habitats.

In the ecological niches that *E. coli* occupies, its abilities to grow both aerobically and anaerobically are important. *E. coli* is well adapted to its intestinal environment as it is able to survive on a relatively limited number of low-molecular weight substances, which may only be available transiently, and at relatively low concentrations. The generation time for *E. coli* in the intestine is thought to be about 12 hours. The type of nutrients available there to *E. coli* consists of mucus, desquamated cells, intestinal enzyme secretions, and incompletely digested food. Given the absorption capacity and efficiency of the intestine, there are probably only small amounts free carbohydrates or other easily absorbable forms of nutrients, and there is competition from hundreds of other type's pf bacteria. A similar situation probably also applies to sources of nitrogen.

In its natural environment, as well as the laboratory, *E. coli* can respond to environmental signals such as chemicals, pH, temperature, osmolarity, etc., in a number of very remarkable ways considering it is a single-celled organism.

For example, it can sense the presence or absence of chemicals and gases in its environment and swim towards or away from them. Or it can stop swimming and grow fimbriae that will specifically attach it to a cell or surface receptor. In response to changes in temperature and osmolarity, it can vary the pore diameter of its outer membrane porins to accommodate larger molecules (nutrients) or to exclude inhibitory substances (e.g. bile salts). With its complex mechanisms for regulation of metabolism the bacterium can survey the chemical content its environment in advance of synthesizing any enzymes necessary to use these compounds. It does not wastefully produce enzymes for degradation of carbon sources unless they are available, and it does not produce enzymes for synthesis of metabolites if they are available as nutrients or growth factors in the environment.

1.4 Biotechnological applications of E. coli

The advances in molecular biology, genetics and biochemistry during the past four decades have led to an enormous development in the field of biotechnology. Studies with *E. coli* have played a major role in these developments, and the bacterium has been in the forefront of many technological advances.

In the early days of biotechnology (1960s), emphasis was placed on improvements of established procedures of bioprocessing, such as the production of yeasts, vaccines, and antibiotics. These investigations stimulated genetic research of microbes to increase their potential to produce a wide variety of products in the service of humanity. Although much was being learned about *E. coli* and its genetics, the direct use of the bacterium in the industry was limited. The industrial production of the ammo acid threonine by *E. coli* mutants, begun in 1961, is an exception.

At this time, organisms were generally subjected to mutagenic agents, which produced a series of random mutations, from which the specifically required mutants were selected.

In the last two decades, procedures have evolved which permit the preparation of strains that have very specific productive capabilities. As the genetic structure of *E. coli* was well known, and it is an organism which can grow on simple media (mineral salts and glucose) under aerobic and anaerobic conditions, the bacterium became the basis for most developments in genetic manipulations leading to genetic engineering.

The basic principle of these genetic manipulations is gene cloning, which enables the isolation and replication of individual DNA fragments. This consists of a series of linked steps, involving the isolation of the desired gene as double-stranded DNA (dsDNA), insertion of the gene into a suitable vector, and using the vector to introduce the DNA into a cell which will express the desired genetic information. In the case of cloning a gene in *E. coli,* first the DNA

of suitable character is isolated, and then it is joined to the DNA of a suitable vector producing a series of recombinant molecules. Then the recombinant molecules are introduced into the bacterium in which the target gene becomes established. Recombinants are selected in various ways with the purpose of expressing the desired genetic information.

The source for DNA cloning can be genomic DNA fragments, cDNA fragments produced by the action of reverse transcriptase on mRNA molecules, chemically synthesized oligonucleotides, or amplified DNA from the products of the polymerase chain reaction (PCR). Plasmids, phages, and cosmids have all been successfully used as vectors, and transformation, transfection, and transduction have all been used to introduce the foreign DNA into the *E. coli* cell. Plasmids are among the most widely used vectors for the insertion of foreign DNA into an *E. coli*. Plasmids lend themselves very well as vectors since they are independent replicons which are stably inherited in an extrachromosomal state and can be made to carry easily identifiable phenotypic markers such as antibiotic resistance or sugar fermentation.

An example of the use of plasmids to introduce a foreign gene into *E. coli* in order to produce a useful product is illustrated by the use of the *E. coli* plasmid pBR322 to clone the gene for production of the human growth hormone, somatostatin. In this case, the gene for the small polypeptide hormone was produced by synthetic means. The double-stranded DNA coding for the 15 amino acids of somatostatin was synthesized with the addition of a translation a stop signal at the end. The synthetic gene was then recombined with the plasmid within the beta-galactosidase structural gene and introduced into *E. coli*. In this way, the production of the somatostatin peptide could be controlled by the lac operon. In a similar manner, the genes for human insulin production were inserted into *E. coli* which was then able to synthesize the human hormone.

Such general techniques of molecular biology and bacterial genetics are now being applied within research laboratories and industry to produce a wide variety of strains of genetically engineered *E. coli* from which a number of useful products can be produced. Likewise, the problems associated with the expression of eukaryotic DNA by a prokaryotic promoter in *E. coli* were solved by construction of a fusion gene. In this system, the control region and the N-terminal coding sequence of an *E. coli* gene are ligated to a eukaryotic sequence so that translation of the chimeric protein can occur. The only condition is that the eukaryotic sequence must be in the correct reading frame. The desired protein is then enzymatically or chemically cleaved from the *E. coli* product.

E. coli strains have been genetically engineered to produce a variety of mammalian proteins, especially products of medical or veterinary interest including enzymes and vaccine components. *E. coli* has also been used to manufacture other substances including enzymes that are useful in the degradation of cellulose and aromatic compounds and enzymes for ethanol

production. There may be no limit to what *E. coli* can produce through recombinant DNA technology as long as the substance is a natural product for which a genetic sequence can be found.

1.5 Microorganisms and nutrient media

Strain *Escherichia coli-K12* was used, obtained from the National ank for industrial microorganisms and cell cultures - Sofia. The strain was laintained on Endo at temperature of 4°C.

The bacteria were cultivated in LB broth with the following omposition: bacto tryptone 10 g, bacto yeast extract 5 g, sodium chloride 0 g (concentration g/1 in distilled water). The nutrient medium was terilized at 1 .MPa for 20 min.

1.6 Revitalization of bacteria Escherichia coli-K12

The bacteria delivered in anabiosis were suspended in 1 ml ihysiological solution. Then, 100 ml LB broth was inoculated with the uspension and then cultivated for 12 h.

The revitalized culture was subjected to nephelometric studies on uspension optical density at A= 420 nm (using Specol 11) to determine the lumber of bacterial colonies by the method of Koch.

1.7 Growth curve of E. coli in presence of 0.2 mg/100ml L- arginine

Liquid nutrient medium LB was placed in cultivation flask and noculated with a single colony of the biological material. The data on iptical density and number of cells were registered hourly for 12 h. lie growth curve of E. coli in presence of 0.5mg/100ml L-arginine and the complexes were obtained by the same method.

2 Prognosis with multilayer neural network of the Escherichia coli

In a series of papers the process of functioning and the results of the work of different types of neural networks (NN) [4] are described by Generalized Nets [1,2]. Here, we shall discuss the process for prognosis with trained of feed-forward Neural Networks.

The different types of NNs can be implemented in different ways [6] and can be learned by different algorithms [3, 9, 10, 11, 12].

Prognosis with NN allows prognosis for one-dimensional and n-dimensional function and as a base for the base we use neural network learned with BackPropagarion algorithm [3].

In the multilayer NNs, one layer exit yeld the entries for the next one. The equations describing this operation are:

$$a^3 = f^3(w^3 f^2(w^2 f^1(w^1 p + b^1) + b^2) + b^3),$$ (1)

where:

- a^m is the exit of the m-th layer of the NN for $m = 1, 2, 3$;
- w is a matrix of the weight coefficients of each of the entries;
- b is the neuron's entry bias;
- f^m is the transfer function of the m-th layer.

The neuron in the first layer receives p outside entries.

The neurons' exits from the last layer determine the number a of NN's exits.

A couple of numbers is submitted (an entry value and an achieving aim – on the network's exit) to the algorithm, since it belongs to the training methods with teacher:

$$<p_1, t_1>, <p_2, t_2>, ..., <p_Q, t_Q>,$$ (2)

where $Q \in \{1,...,n\}$, n – numbers of learning couple, where p_Q is the entry value (on the network entry), and t_Q is the exit's value corresponding to the aim. Every network's entry is preliminary established and constant, and the exit has to correspond to the aim. The difference between the entry values and the aim is the error: $e = t$-a.

The "backpropagation" algorithm [9] uses least-quarter error:

$$\hat{F} = (t - a)^2 = e^2.$$ (3)

In the process of training the NN, the algorithm recalculates the network's parameters (W and b) in order to achieve least-mean square error.

The "backpropagation" algorithm for the i-th neuron, for $k+1$-th iteration uses equations:

$$w_i^m(k+1) = w_i^m(k) - \alpha \frac{\partial \hat{F}}{\partial w_i^m};$$ (4)

$$b_i^m(k+1) = b_i^m(k) - \alpha \frac{\partial \hat{F}}{\partial b_i^m},$$ (5)

where:

α - learning rate for neural network;

$\frac{\partial \hat{F}}{\partial w_i^m}$ - relation between the changes of the mean square error and the changes of the weights;

$\dfrac{\partial \hat{F}}{\partial b_i^m}$ - relation between the changes of the mean square error and the changes of the biases.

The network is trained when

$$e^2 < E\text{max},\qquad\qquad\qquad\qquad (6)$$

where Emax is the maximum mean square error.

During the process of the prognosis with NN we can train it with data that has been onse saved. This type of learning data is used as a part of the data provided in the input of the NN and other data – to the output.

The process of learning can be represented in the following order:

-   from the series of the data $x_1, x_2, x_3, \ldots, x_N$, to the input we put m values, where N – number measuring, m – number of inputs of the NN. Let the values be: $P = x_{i+1}, x_{i+2}, x_{i+3}, \ldots, x_{i+m}$, and the output has next value from the series of measurement $T = x_{i+m+1}$ (for $i = 0, 1, 2, 3, 4, 5\ldots, N\text{-}m\text{-}1$);

-   series from the measurements from the inputs P and the next value from the series T constructed learning couples (P, T), where T is a target. We use the BackPropagation algorithm;

-   i begin from zero and increase with 1 to $N\text{-}m\text{-}1$.

Process of the prognosis use follow algorithm:

-   in the input of the NN we put next m values from the series. The result value come from the output;

-   this result value is added to the learning series P with number x_{N+1};

-   the next prognosis is based on the series with $N+1$ elements.

In Table 1 we present data for biomass accumulation. Different column present different metals added to solution. In first 13 rows are data for real values. The next 2 rows (number 14 and 15) are prognosis data each type of solution, based on all first eleven values.

As can see the error are small percent. In column II, III, IV, V, VI and VIII we collect data and after that we made prognosis and calculate error. Only in column VII we used prognosis.

Based on this data we can see that:

1. The results indicate that the predicted values are identical with the values obtained experimentally for concentration of biomass.

2. Using a mathematical model one can predict the next values from the kinetic curve.

3. Using the "Artificial neural networks" method one can predict the bacteriostatic effect of complexes of heavy metals on bacteria E.Coli.

Table1

Time, h	OD, 420nm	OD^{420} Arg (0.2)	OD^{420} Arg (0.5)	OD^{420} Arg-Cu (0.1)	OD^{420} Arg-VO (0.1)	OD^{420} Arg-Mo (0.1)	OD^{420} Arg-Fe (0.1)
I	II	III	IV	V	VI	VII	VIII
1	0.03	0.10	0.015	0.023	0.02	0.02	0.008
2	0.045	0.022	0.012	0.035	0.036	0.04	0.04
3	0.06	0.053	0.014	0.043	0.044	0.056	0.06
4	0.1	0.154	0.013	0.043	0.061	0.072	0.108
5	0.234	0.235	0.059	0.063	0.105	0.123	0.171
6	0.581	0.316	0.155	0.149	0.16	0.266	0.215
7	0.827	0.595	0.3	0.379	0.259	0.536	0.355
8	0.991	0.738	0.453	0.646	0.485	0.787	0.486
9	1.1	0.843	0.676	0.805	0.78	0.91	0.606
10	1.114	0.908	0.96	0.989	0.92	0.97	0.76
11	1.13	0.941	1.185	1.012	0.986	0.986	0.877
12	1.13	0.941	1.341	1.005	1.014		1.021
13	1.13	0.949	1.483	0.989	1.022		1.019
14*	1.1298	0.9410	1.3717	0.9824	1.0131	0.9587	0.9440
15*	1.1240	0.9410	1.5692	1.0500	1.0367	0.9463	0.9827
Error1 %	1.8	0	2.24	2.3	0.02		8.1
Error2 %	5.34	0.85	5.5	5.9	1.5		3.68

3 A GN-model

The model describing the work and learning of the multilayer perceptron is proposed in Fig. 1.

Initially the following tokens enter in the GN:
- in place S_{data} - β-token with characteristic "biomass data for training of the neural network";
- in place S_{ez} - γ-token with characteristic "least square error for learning";
- in place S_{wb} - δ-token with characteristic "initial value of the weight coefficients and biases".

Initially the following token stays in the GN:
- in place S_{Str} stays α-token with characteristic "available architectures of the feedforward neural networks and transfer functions".
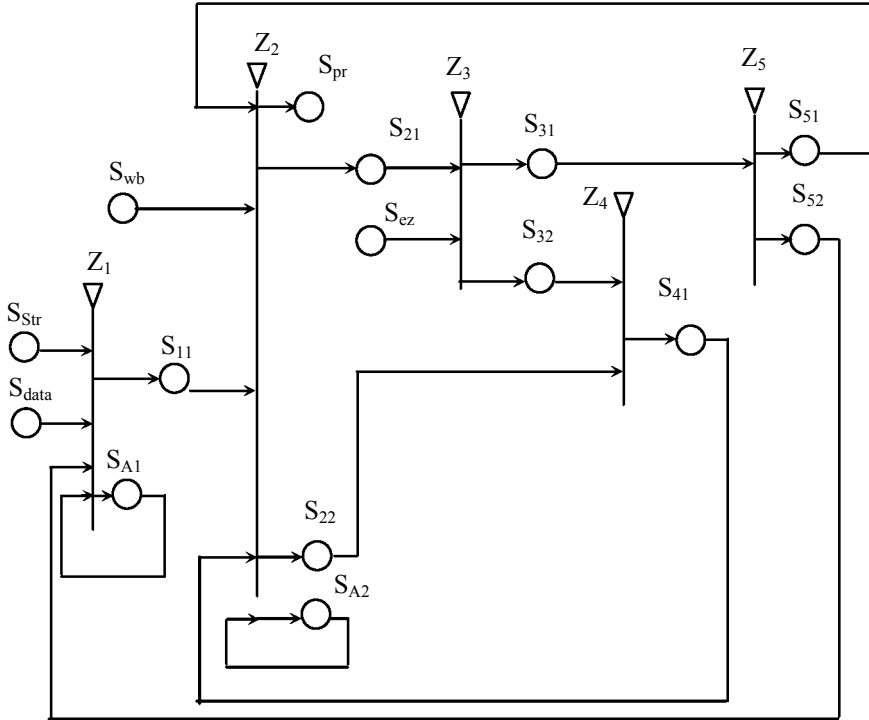
Figure 1: GM model of the process of the prognosis biomass accumulation with neural network

The GN is presented by a set of transitions [1, 2]:

$$A = \{ Z_1, Z_2, Z_3, Z_4, Z_5 \},$$

where transitions describe the following processes:

Z_1- Defining of the learning couples $\{p_1, t_1\}, \{p_2, t_2\}, ..., \{p_Q, t_Q\}$;

Z_2- Calculating the outputs of the neural network (feed forward);

Z_3- Determination $e^2 < E_{max}$;

Z_4- Learning the neural network (backward)

Z_5- Determination learning for the all learning couples.

Transitions of the GN-model have the following forms.

$$Z_1 = < \{S_{Str}, S_{data}, S_{52}, S_{A1}\}, \{S_{11}, S_{12}\}, R_1, M_1, \wedge (S_{Str}, S_{data}, S_{52}, S_{A1}) >$$

	S_{21}	S_{A2}
S_{Str}	True	False
R₁= S_{data}	False	True
S_{52}	True	False
S_{A1}	True	True

In position S_{21} enter token with characteristic - "the next learning couples (p,t)".

In position S_{A2} enter token with characteristic - "learning couples $\{p_1, t_1\}$, $\{p_2, t_2\}$, ..., $\{p_Q, t_Q\}$,".

$Z_2 = < \{S_{51}, S_{wb}, S_{11}, S_{41}, S_{A2}\}, \{S_{pr}, S_{21}, S_{22}, S_{A2}\}, R_2, M_2, \wedge (S_{a2}, \vee (S_{41}, S_{51}), \vee (S_{11}, S_{wb})) >$

	S_{pr}	S_{21}	S_{22}	S_{A2}
S_{51}	True	False	False	False
S_{wb}	False	False	False	True
R₂= S_{11}	False	False	False	True
S_{41}	False	False	False	True
S_{A2}	False	True	True	False

In positions S_{21} and S_{22} enter tokens with characteristics " least square error on the output of the neural network".

In position S_{pr} enter token with characteristic "prognosis value from the neural network based on the experimental data".

In position S_{A2} enter token with characteristic "(w,b)".

$Z_3 = < \{S_{21}, S_{ez}\}, \{S_{31}, S_{32}\}, R_3, M_3, \wedge (S_{31}, S_{ez}) >$

	S_{31}	S_{32}
R₃= S_{21}	$W_{21,21}$	$W_{21,32}$
S_{ez}	$W_{ez,31}$	$W_{ez,32}$

where:
$W_{21,31}$ = "$e^2 < E$max";
$W_{ez,31}$ = "$e^2 < E$max";
$W_{21,32}$ = "$e^2 > E$max";
$W_{ez,32}$ = "$e^2 > E$max".

The neural network is full learned where $e^2 > E$max

In position S_{31} enter token with characteristic "learned neural network".

In position S_{32} enter token with characteristic " least square error on the output of the neural network".

$$Z_4 = < \{S_{32,} S_{22}\}, \{S_{41}\}, R_4, M_4, \wedge (S_{32,} S_{22}) >$$

$$R_4 = \begin{array}{c|c}
 & S_{41} \\
\hline
S_{22} & True \\
S_{22} & True
\end{array}$$

In position S_{41} enter token with characteristic "W(k+1);b(k+1)".

$$Z_5 = < \{S_{31}\}, \{S_{51,} S_{52}\}, R_5, M_5, \wedge (S_{31}) >$$

$$R_5 = \begin{array}{c|cc}
 & S_{51} & S_{52} \\
\hline
S_{31} & W_{31,51} & W_{31,52}
\end{array}$$

where
$W_{31,51} =$" $i = N$-m-1";
$W_{31,52} =$" $i \neq N$-m-1";

In positions S_{51} and S_{52} enters token with characteristics "New weight coefficients and biases (W(k+1);b(k+1)) for all learning couples".

4   Conclusions

Prognosis process with neural network allows prognosis of the one and multиfunction based on the feedforward neural network. In this case usually uses algorithm with backward propagation of the error – BackPropagation. For constructing a model of Feedforward Neural Network are used Generalized Nets because they allow their simulation and tracing their behavior in future, their management and respectively a selection of proper structure for solving the set problem.

Also we proof that using a mathematical model one can predict the next values from the kinetic curve. Using the "Artificial neural networks" method one can predict the bacteriostatic effect of complexes of heavy metals on bacteria E.Coli.

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The papers presented in this Volume 2 constitute a collection of contributions, both of a foundational and applied type, by both well-known experts and young researchers in various fields of broadly perceived intelligent systems.
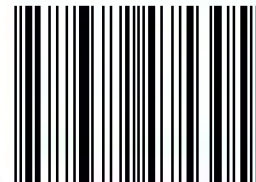
It may be viewed as a result of fruitful discussions held during the Eighth International Workshop on Intuitionistic Fuzzy Sets and Generalized Nets (IWIFSGN-2009) organized in Warsaw on October 16, 2009 by the Systems Research Institute, Polish Academy of Sciences, in Warsaw, Poland, Centre for Biomedical Engineering, Bulgarian Academy of Sciences in Sofia, Bulgaria, and WIT – Warsaw School of Information Technology in Warsaw, Poland, and co-organized by: the Matej Bel University, Banska Bistrica, Slovakia, Universidad Publica de Navarra, Pamplona, Spain, Universidade de Tras-Os-Montes e Alto Douro, Vila Real, Portugal, and the University of Westminster, Harrow, UK:

http://www.ibspan.waw.pl/ifs2009

The Eighth International Workshop on Intuitionistic Fuzzy Sets and Generalized Nets (IWIFSGN-2009) has been meant to commence a new series of scientific events primarily focused on new developments in foundations and applications of intuitionistic fuzzy sets and generalized nets pioneered by Professor Krassimir T. Atanassov. Moreover, other topics related to broadly perceived representation and processing of uncertain and imprecise information and intelligent systems are discussed.

We hope that a collection of main contributions presented at the Workshop, completed with many papers by leading experts who have not been able to participate, will provide a source of much needed information on recent trends in the topics considered.