INTERCRITERIA ANALYSIS FOR IDENTIFICATION OF *ESCHERICHIA COLI* FED-BATCH MATHEMATICAL MODEL

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Abstract

Escherichia coli produces insulin, interferons, growth factors, exo- and endo- products, e.g. enzymes, etc. In this paper we present a new approach for multicriteria decision making – InterCriteria Analysis to mathematical modelling of a fermentation process. It is based on the apparatus of index matrices and intuitionistic fuzzy sets. The approach for multicriteria analysis makes it possible to compare certain criteria or estimated by them objects. In this paper we apply the ideas in an E. coli fed-batch laboratory process. We explore the basic dependencies between different criteria in fed-batch fermentation – biomass, substrate, oxygen and carbon dioxide. As a result, we develop structural and parametric identification of the process.

Key words: *intercriteria analysis, e. coli, fed-batch process, mathematical modelling*

1. INTRODUCTION

Cultivation of recombinant micro-organisms, e.g., *Escherichia coli*, in many cases is the only economical way to produce pharmaceutical biochemical's such as interleukins, insulin, interferon's, enzymes and growth factors. Simple bacteria like *E. coli* are manipulated to produce these chemicals so that they are easily harvested in vast quantities for use in medicine. *E. coli* is still the most important host organism for recombinant protein production. Scientists probably know more about *E. coli* than they do about any other species on Earth. Research on *E. coli* accelerated even more after 1997, when its entire genome was published. Scientists were able to survey all 4,288 of its genes and to discover how groups of them worked together to break down food, make new copies of DNA and do other tasks. But, despite decades of research, there is a lot more we need to know about *E. coli*, and in order to research it further, *E. coli* experts have been joining forces. In 2002, they formed the International E-coli Alliance to organize projects that many laboratories could carry out together. As knowledge of E. coli keeps growing, scientists are starting to build models of the microbe that capture certain aspects of its behaviour. It is important to be able to predict how fast the microbe will grow on various sources of food, as well as how its growth changes if individual genes are knocked out. Here is the place of mathematical modelling (Roeva et al. 2012).

Bioprocesses such as *Escherichia coli* cultivation have advanced tremendously in recent years. Due to their multidisciplinarity, they have attracted significant interest from microbiologists, biochemists, molecular biologists, bioengineers, chemical engineers, food and pharmaceutical chemists, etc. (Pencheva et al. 2006). Cultivation processes are characterized by a complicated structure of organization and independent characteristics which determine their nonlinearity and non-stationary. The model formulation for a bioprocess is traditionally performed under conditions of a well-defined medium with single-substrate limitations, conditions that are not applied to most industrial cultivations, typically running in a complex medium. In many cases, the globally valid conventional numeric models which describe the overall process behaviour cannot be used in on-line monitoring and control, either because they do not describe the process well enough or contain too many poorly known parameters (Roeva et al. 2007).

Atanassov et al. (2014) introduced a new approach for multicriteria decision making, namely *InterCriteria Analysis for decision making*. It is based on the apparatus of index matrices (IMs) (Atanassov 1987, 2010a, 2010b) and intuitionistic fuzzy sets (IFs) (Atanassov 2012, Atanassov et al. 2013) and can be applied for decision making in different areas of science and practice. The new approach for multicriteria decision making makes it possible to compare certain criteria or objects estimated by them. Atanassova et al. (2014a, b, c) applied InterCriteria approach in an EU member states competitiveness analysis. They carried out a temporal and threshold analysis.

In this paper we apply the ideas of InterCriteria Analysis to the modelling of a fed-batch fermentation laboratory process.

2. MATERIALS AND METHODS

The experimental scale-up of the *E. coli* laboratory fed-batch fermentation process is shown in **Fig. 1** (Hitzmann et al. 2011).

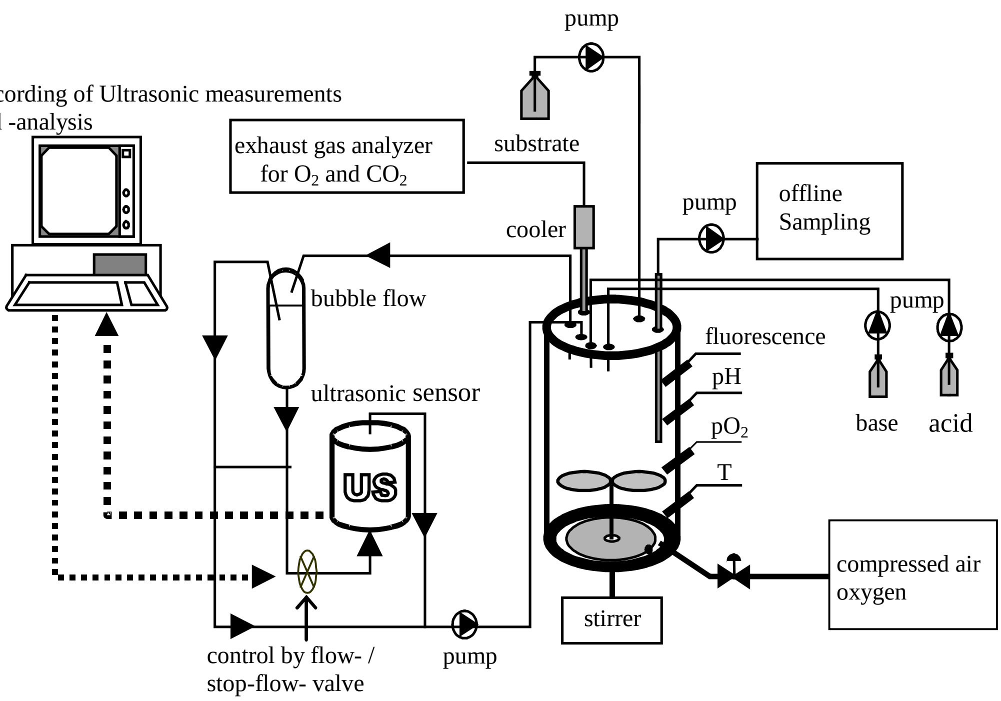

Fig. 1. Scheme of the experimental process

The cultivation characteristics of *E. coli* fermentation are as follows:

- *Mezophile regime;*
- *Medium for the batch phase – glucose and mineral salts;*
- *Bioreactor type – a laboratory stirred tank bioreactor;*
- *Cultivation type – non-limited feeding, there is no substrate limitation* (*glucose*).

The fermentation parameters are:

- *Temperature* 35°C;
- *pH* 6.8;
- *Gas flow rate* 275 l/h;
- *Impeller speed at the beginning*; 900 rpm
- *Oxygen concentration* 35%;
- *Impeller speed at the end of the process* 1500 rpm;

- *Glucose concentration in the batch process* 2.5 g/l;
- *Bioreactor volume* 1.5 l;
- *Glucose concentration in the feeding solution* 100 g/l.

3. INTERCRITERIA DECISION MAKING ANALYSIS

This method for decision making, based on IMs (Atanassov 1987, 2010, 2010b) and IFs (see Atanassov 2012) is introduced. The IMs are essentially new and not widely known mathematical objects, that are extensions of the ordinary matrices. The concept of an Intuitionistic Fuzzy Pairs (IFPs) is examined in this paper (Atanassov, 2013, Atanassova et. al. (2014)).

Remarks on Intuitionistic Fuzzy Pairs (Atanassov, 2013). The IFPs is an object in the form of an ordered pair $\langle a + b \rangle \leq 1$, where $a, b \in [0,\ 1]$ and $a + b \leq 1$, that is used as an evaluation of some object or process, and which components (a and b) are interpreted, respectively, as degrees of membership and non-membership to a given set, or degrees of validity and nonvalidity, or degree of correctness and non-correctness, etc.

Let us have two IFPs $x = \langle a, b \rangle$ and $y = \langle c, d \rangle$.

Atanassov et al. (2013) defined the relations:

$x < u$ iff $a < c$ and $b < d$

$x \leq u$ iff $a \leq c$ and $b \geq d$

$x = u$ iff $a = c$ and $b = d$

$x \geq u$ iff $a \geq c$ and $b \leq d$

$x > u$ iff $a > c$ and $b < d$

Remarks on index matrices. Atanassov (1987, 2010a, 2010b) presented the concept of IMs and given the basic definitions and properties.

Let I be a fixed set of indices and $\mathcal{R}$ be the set of all numbers. But IMs with index sets K and L $(K, L \subset I)$, we mean the object:

$$\left[K, L, \ \left\{a_{k_i,l_j}\right\}\right] \equiv \begin{array}{c|cccc} & l_1 & l_2 & \dots & l_n \\ \hline k_1 & a_{k_1,l_1} & a_{k_1,l_2} & \dots & a_{k_1,l_n} \\ k_2 & a_{k_2,l_1} & a_{k_2,l_2} & \dots & a_{k_2,l_n} \\ \vdots & & & & \\ k_m & a_{k_n,l_1} & a_{k_m,l_2} & \dots & a_{k_m,l_n} \end{array}$$

where $= \{k_1, k_2, \dots, k_m\}$, $L = \{l_1, l_2, \dots, l_m\}$, and for $1 \leq i \leq m$, and $1 \leq j \leq n$: $a_{k_i,l_j} \in \mathcal{R}$.

On the basis of the above definition, Atanassov (2010b) introduced the new object – the Intuitionistic Fuzzy IM (IFIM) in the form:

$$\left[K, L, \left\{\langle \mu_{k_i,l_j}, \nu_{k_i,l_j} \rangle\right\}\right]$$

$$\equiv \begin{array}{c|cccc} & l_1 & l_2 & \dots & l_n \\ \hline k_1 & \langle \mu_{k_1,l_1}, \nu_{k_1,l_1} \rangle & \langle \mu_{k_1,l_2}, \nu_{k_1,l_2} \rangle & \dots & \langle \mu_{k_1,l_n}, \nu_{\mu_{k_1,l_n}} \rangle \\ k_2 & \langle \mu_{k_2,l_1}, \nu_{k_2,l_1} \rangle & \langle \mu_{k_2,l_2}, \nu_{k_2,l_2} \rangle & \dots & \langle \mu_{k_2,l_n}, \nu_{k_2,l_n} \rangle \\ \vdots & & & & \\ k_m & \langle \mu_{k_m,l_1}, \nu_{k_m,l_1} \rangle & \langle \mu_{k_m,l_2}, \nu_{k_m,l_2} \rangle & \dots & \langle \mu_{k_m,l_n}, \nu_{k_m,l_n} \rangle \end{array}$$

where for every for $1 \le i \le m$, $1 \le j \le n$: $\mu_{k_i,l_j}, \nu_{k_i,l_j}$, $\mu_{k_i,l_j} + \nu_{k_i,l_j} \le 1, i.e.$ $\langle \mu_{k_i,l_j}, \nu_{k_i,l_j} \rangle$ is an IFPs.

The proposed InterCriteria analysis for decision making method. Let us have an IMs:

$$A \equiv \begin{array}{c|ccccccc} & O_1 & \cdots & O_k & \dots & O_l & \dots & O_n \\ \hline C_1 & a_{C_1,O_1} & \cdots & a_{C_1,O_k} & \dots & a_{C_1,O_l} & \dots & a_{C_1,O_n} \\ \vdots & \vdots & \vdots & \vdots & \vdots & & \vdots & \vdots \\ C_i & a_{C_i,O_1} & \cdots & a_{C_i,O_k} & \dots & a_{C_i,O_l} & \dots & a_{C_i,O_n} \\ \vdots & \vdots & \vdots & \vdots & \vdots & & \vdots & \vdots \\ C_j & a_{C_j,O_1} & \cdots & a_{C_j,O_k} & \dots & a_{C_j,O_l} & \dots & a_{C_j,O_n} \\ \vdots & \vdots & \vdots & \vdots & \vdots & & \vdots & \vdots \\ C_m & a_{C_m,O_1} & \cdots & a_{C_m,O_k} & \cdots & a_{C_m,O_l} & \dots & a_{C_m,O_n} \end{array}$$

where for every $p,\ q\ (1 \le p \le m, 1 \le q \le n)$:

- C_p is a criterion, taking part in the evaluation.
- O_q is an object, being evaluted
- a_{C_p,O_q} is a real number or another object, that is comparable about relation R with other α – object, so that for each $i,\ j,k$: $R\left(a_{C_k,O_i}, a_{C_k,O_j}\right)$ is defined. Let $\bar{R}$ be the dual relation of R in the sense that if R satisfied, then $\bar{R}$ is not satisfied and vice versa. For example, if "R" is the relation "<", then $\bar{R}$ is the relation ">", and vice versa

Let $S_{l,k}^{\mu}$ be the number of cases in which $R\left(a_{C_k,O_i}, a_{C_k,O_j}\right)$ and $R\left(a_{C_l,O_i}, a_{C_l,O_j}\right)$are simultaneously satisfied. Let $S_{k,l}^{\nu}$ be the number of cases is which $R\left(a_{C_k,O_i}, a_{C_k,O_j}\right)$ and $\bar{R}\left(a_{C_l,O_i}, a_{C_l,O_j}\right)$ are simultaneously satisfied.

Obviously,

$$S_{l,k}^{\mu} + S_{l,k}^{\nu} \le \frac{n(n-1)}{2}$$

Now, for every $k,\ l$ such that $1 \le k < l \le m$, and for $n \ge 2$, it can be defined

$$\mu_{C_k,C_l} = 2\frac{S_{k,l}^{\mu}}{n(n-1)}, \qquad \nu_{C_k,C_l} = 2\frac{S_{k,l}^{\nu}}{n(n-1)}$$

Therefore, $\langle \mu_{C_k,C_l}, \nu_{C_k,C_l} \rangle$is an IFPs. The IMs can construct:

	C_1	$\cdots$	C_k
C_1	$\langle \mu_{C_1,C_1}, \nu_{C_1,C_1} \rangle$	$\cdots$	$\langle \mu_{C_1,C_m}, \nu_{C_1,C_m} \rangle$
$\vdots$	$\vdots$	$\vdots$	$\vdots$
C_m	$\langle \mu_{C_m,C_1}, \nu_{C_m,C_1} \rangle$	$\cdots$	$\langle \mu_{C_m,C_m}, \nu_{C_m,C_m} \rangle$

That determined the degrees of correspondence between criteria $C_1, \ldots, C_m$.

Let $\alpha, \beta \in [0,1]$ be given, so that $\alpha + \beta \leq 1.$. We say that criteria C_k and C_l are in:

- (α, β) - positive consonance, if $\mu_{C_k,C_l} > \alpha$ and $\nu_{C_k,C_l} < \beta$
- (α, β) - negative consonance, if $\mu_{C_k,C_l} < \alpha$ and $\nu_{C_k,C_l} > \beta$
- (α, β) – dissonance, otherwise.

4. APPLICATION OF INTERCRITERIA METHOD TO A FED-BATCH PROCESSES

We have experimental data for the examined process. This is a fed-batch with the following means: X - biomass concentration, g.L^{-1}; S - substrate concentration, g.L^{-1}; $C_L^{O_2}$ - oxygen concentration in the liquid phase, g.L^{-1}; $C_G^{O_2}$ - oxygen concentration in the gas phase; $C_L^{CO_2}$ - carbon dioxide concentration, g.L^{-1}; r_X - specific growth rate, g.L^{-1}, η - specific consummation rate of substrate, g.L^{-1}/h, F – feeding rate, g.L^{-1}.h^{-1}. Our colleagues have developed a program for the purpose of applying the method. The results are shown in **Tables 1** and **2**.

Table 1. Comparison of the calculated values of $\mu_{C_i C_j}$ for 0th to 14th hour

μ	X	S	$C_L^{O_2}$	$C_G^{O_2}$	$C_L^{CO_2}$	Q	r_X
X		0.88324	0.91610	0.03560	0.03590	0.80501	0.98213
S	0.88324		0.90357	0.03560	0.09371	0.13659	0.87412
$C_L^{O_2}$	0.91610	0.90357		0.93904	0.94531	0.14784	0.98241
$C_G^{O_2}$	0.03560	0.0356	0.93904		0.98903	0.14314	0.60021
$C_L^{CO_2}$	0.03590	0.09371	0.94531	0.98903		0.82238	0.30021
Q	0.80501	0.13659	0.14784	0.14314	0.82238		0.28364
r_X	0.98213	0.87412	0.98241	0.60021	0.30021	0.28364	

Table 2. Comparison of the calculated values of $\nu_{C_i C_j}$ for 0th to 14th hour

ν	X	S	$C_L^{O_2}$	$C_G^{O_2}$	$C_L^{CO_2}$	Q	r_X
X		0.113516	0.08390	0.91440	0.96410	0.15895	0.01345
S	0.113516		0.096430	0.91440	0.90485	0.82780	0.00000
$C_L^{O_2}$	0.08390	0.096430		0.05569	0.05469	0.817405	0.02774
$C_G^{O_2}$	0.91440	0.91440	0.05569		0.00569	0.820253	0.00382
$C_L^{CO_2}$	0.96410	0.90485	0.05469	0.00569		0.14285	0.45272
Q	0.15895	0.82780	0.8174	0.82025	0.14285		0.60921
r_X	0.01345	0.00000	0.02774	0.00382	0.45272	0.60921	

In Fig. 2 we present the dependencies between μ and ν.

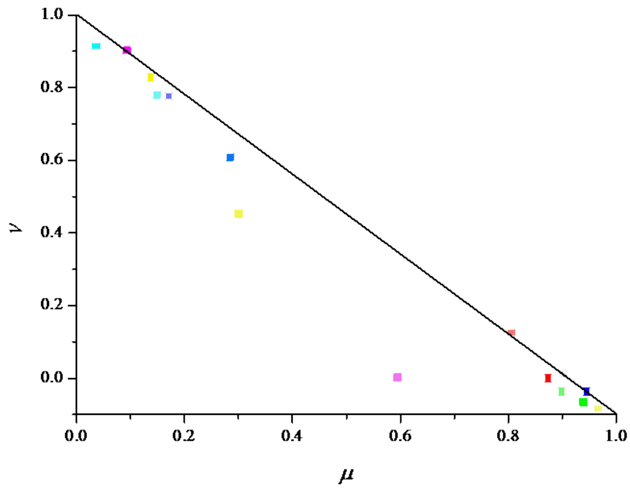

Fig. 2. Relations between μ and ν for the different criteria

With the help of these tables we will investigate the dependencies between the basic kinetic variables of the process. The basic kinetic variables include biomass concentration, substrate concentration, oxygen concentration in the liquid and the gas phases and dissolved oxygen. We will also investigate the dependencies between them and their specific growth/consumption rates.

Biomass

- Dependencies of the biomass: to substrate the pair $(\mu; \nu)$ is (0.88324; 0.113516) and has some small uncertainty – 0,0032, because of that the substrate will be present in the equation of the biomass.
- Biomass to oxygen in the liquid phase – the pair $(\mu; \nu)$ is (0.91610; 0.0839) and the uncertainty is 0, because of that the oxygen in the liquid phase will be present in the equation of the biomass.
- Biomass to oxygen in the gas phase – $(\mu; \nu)$ is (0.03560; 0.9144), the uncertainty is 0.05, the oxygen in the gas phase will not be present in the equation of the biomass .

- Biomass to carbon dioxide – the pair *(μ; ν)* is (0.0359, 0.96410;), the uncertainty is 0, the carbon dioxide will not be present in the equation of biomass.
- Biomass to a specific growth rate of biomass – the pair *(μ; ν)* is (0.98213; 0.01345), *π*=0.00442, because of that the specific growth rate of the biomass will consist in the equation of the biomass.

Substrate

- Substrate to biomass was discussed above.
- Substrate to oxygen in the liquid phase. The pair *(μ; ν)* is (0.90357; 0.09643), and the uncertainty is 1. In this way, emphatically the oxygen in liquid phase will not be present in the equation for the substrate.
- Substrate to oxygen in the gas phase. The pair *(μ; ν)* is (0.03560; 0.9144), but the uncertainty is 0.05. In this case we can assume that the oxygen in the gas phase will not have place in the equation of the substrate.
- Substrate to carbon dioxide. The pair *(μ; ν)* is (0.09371; 0.90485), the uncertainty is 0.00144. We can assume that the carbon dioxide will not be present in the equation of substrate;
- Substrate to a specific growth rate of the biomass. The pair (*μ; ν*) is (0.696667; 0), *π*=0.12588. We can accept that the specific growth rate of the biomass will be in equation of the substrate.

Oxygen in the liquid phase

- Oxygen in the liquid phase to oxygen in the gas phase. The pair (*μ; ν*) is (0.93904; 0.05569), *π*=0.11665. We can conclude that the oxygen in the gas phase depends by the oxygen in the liquid phase.
- Oxygen in the liquid phase to carbon dioxide. The pair (*μ; ν*) is (0.94530; 0.05569), *π*=0. We can conclude that the oxygen in the gas phase will be present in the equation of carbon dioxide.
- Oxygen in the liquid phase to a specific growth rate of the biomass. The pair (*μ; ν*) is (0.956667; 0.02774), *π*=0.01559. We can conclude that the oxygen depends by the specific growth rate of the biomass.

Oxygen in the gas phase

- Oxygen in the gas phase to carbon dioxide. The pair (*μ; ν*) is (0,00569; 0,98903), *π*=0.00507. We can conclude that the carbon dioxide depends by the oxygen in the gas phase.
- Oxygen in the gas phase to a specific growth rate. The pair (*μ; ν*) is (0.30021; 0.45272), *π*=0.39597. We cannot make conclusions about the oxygen dependence because there is a high degree of uncertainty.

Carbon dioxide

- The specific growth rate depends on the substrate and the dissolved oxygen in the liquid phase.

We can generalize all these results and interpret the basic kinetic dependencies of the bioprocesses. The results are shown in Table 3.

Table 3. Dependencies between the basic kinetic criteria

Criteria	**Depended on**
Biomass	Substrate, Oxygen in the liquid phase, Specific growth rate
Substrate	Biomass, Oxygen in the liquid phase, Specific growth rate
Oxygen in the liquid phase	Biomass, Oxygen in the liquid phase, Specific growth rate
Oxygen in the gas phase	Biomass, Oxygen in the gas phase, Specific growth rate

Carbon dioxide	Biomass, Oxygen in the liquid phase, Specific growth rate
Specific growth rate of biomass	Substrate, Oxygen in the liquid phase

We defined the following dependency for the specific growth rate of the biomass and the specific consumption rate of the substrate:

$$r_X(S, C_L^{O_2}) = r_{X\max} \frac{S^2}{(K_S + S^2)} \frac{C_L^{O_2\,2}}{(K_C + C_L^{O_2\,2})}$$, and

$$\eta = \frac{r_X(S, C_L^{O_2})}{Y} + k_m$$

In this case the model of the process has the following form:

$$\frac{dX}{dt} = r_X(S, C_L^{O_2})\,X - \frac{F}{V} X$$
$$\frac{dS}{dt} = \frac{F}{V}(S_0 - S) - K_1\,\eta(\mu)\,X$$
$$\frac{dC_L^{O_2}}{dt} = K_L^{O_2}a(C_G^{O_2} - C_L^{O_2}) - K_2\,r_X(S, C_L^{O_2})\,X - \frac{F}{V}\,C_L^{O_2}$$
$$\frac{dC_G^{O_2}}{dt} = K_G^{O_2}a(C_L^{O_2} - C_G^{O_2})$$
$$\frac{dC_L^{CO_2}}{dt} = K_L^{CO_2}a(C_L^{CO_2*} - C_L^{CO_2}) + K_3\,r_X(S, C_L^{O_2})\,X - \frac{F}{V}\,C_L^{CO_2}$$
$$\frac{dV}{dt} = F.$$

The initial process conditions are:

$S(0)$=2.6 g.L^{-1}, $X(0)$=0.1 g.L^{-1}; $C_L^{O_2}$ (0)=96.1%, $C_G^{O_2}$ (0)=21%;
$C_L^{CO_2}$ (0)=0.2%; $C_G^{CO_2*}$ =21.10%, $V(0)$=1.1 L.

This is the structural identification.

Based on the real and model data, we developed an identification of the parameters with the help of the Method of least square. With this program we defined the coefficients in the model. They have the following values:

Coefficient	$\mu_{\max}$	K_1	K_2	K_3	K_s	K_c
Value	0.510	0.312	0.0248	3.2354	0.321	0.00921
Coefficient	K_m	Y	$K_L^{O_2}a$	$K_L^{CO_2}a$	$K_G^{O_2}a$	-
Value	0.461	0.539	128.34	265.132	93.083	-

The following figures present the modelling and experimental data.

In the figure X_e and X_m denote the model and the experimental data for the biomass, S_e and S_m are the experimental and model data for the substrate; $C_L^{O_2}e$ and $C_L^{O_2}m$ – the experimental and model oxygen data in the liquid phase; $C_G^{O_2}e$ and $C_G^{O_2}m$ – the experimental and model oxygen data in the gas phase; $C_L^{CO_2}e$ and $C_L^{CO_2}m$ – the experimental and model data carbon dioxide data.

We developed a validation of the model by using a correlation coefficient and Fisher function. The results and figures showed that the model predicted experimental data. Thus, the models are adequate.

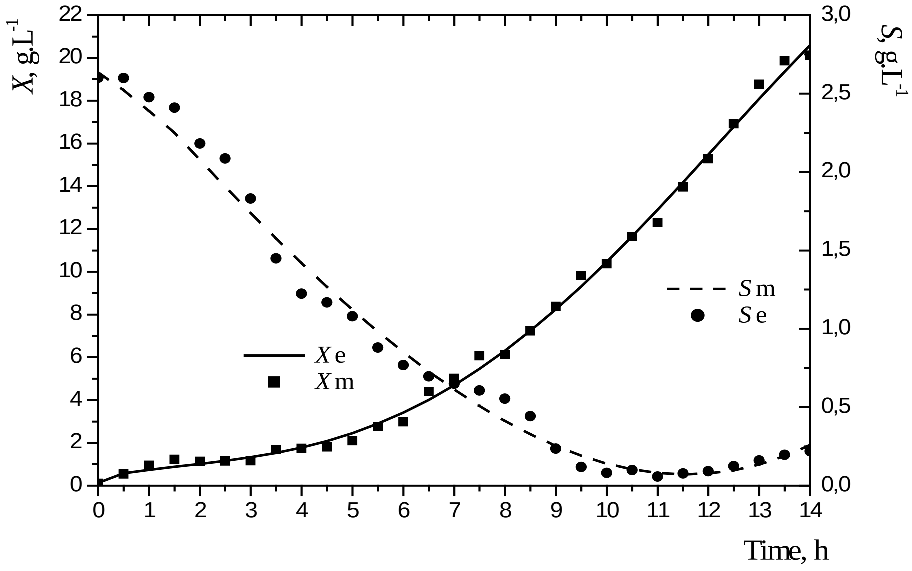

Fig. 3. Experimental and model data for the biomass and substrate concentration

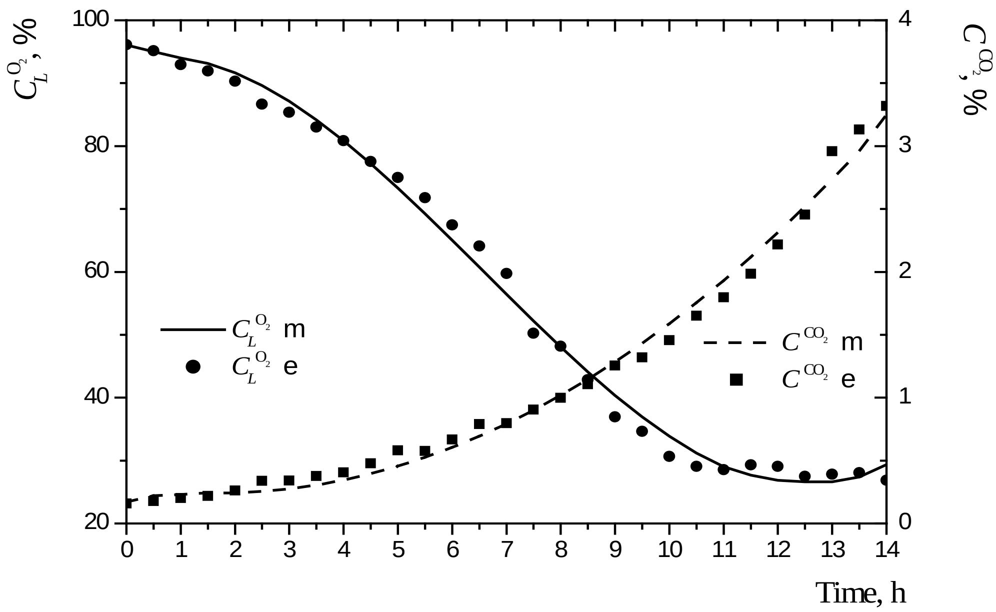

Fig. 4. Experimental and model data for the carbon dioxide and oxygen concentration in the liquid phase

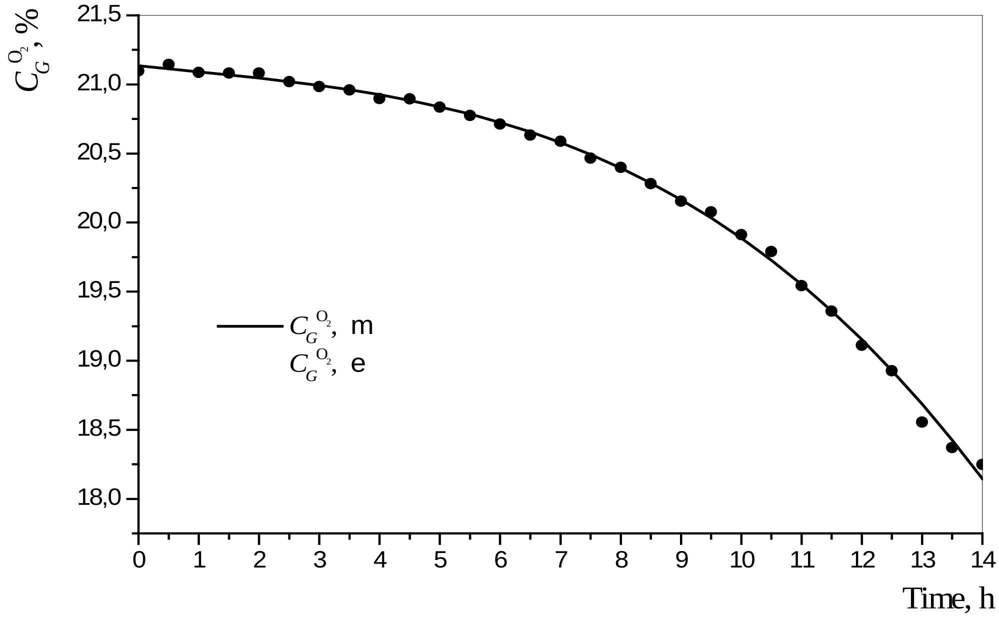

Fig. 5. Experimental and model data for the oxygen concentration in the gas phase

CONCLUSIONS

Cultivation of recombinant micro-organisms, e.g *Escherichia coli*, in many cases is the only economical way to produce pharmaceutical biochemical's such as interleukins, insulin, interferon's, enzymes and growth factors. Simple bacteria like *E. coli* are manipulated to produce these chemicals so that they are easily harvested in vast quantities for use in medicine. *E. coli* is still the most important host organism for recombinant protein production.

We investigated InterCriteria Analysis and a method for decision making in the modelling of the process. We examined three processes for lysine. The aim was to determine the basic dependencies between the different criteria in the process. After that the desired modelling of the process is achieved. With InterCriteria Analysis we found the dependencies between the process criteria. We developed a model of the process. The model predicted the experimental data. This was assessed with a correlation coefficient and Fisher function. The models proved to be adequate.

The model has shown good statistical indices and can be used for InterCriteria and Multi-objective process optimization.

In this way we have shown another application of InterCriteria Analysis – in the modelling of and complex fermentation fed-batch process.

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