

# Kinetic Models for Citric Acid Production

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Although the citric acid is one of the oldest biotechnology products, the abundance of possible raw materials and microorganisms which could process those substrates made very difficult the possibility of finding a general kinetic for this biological process. Furthermore, the cross inhibition of both substrate and product and the capacity of the microorganisms to switch from regular carbon-based substrates to citric acid as substrate, ceasing its production, when the former vanished from the bioreactor add great challenges to finding a useful kinetic model. The aim of the paper is to propose and analyse several improved kinetic models for the process of citric acid fermentation, based upon published experimental data, on one hand, and the qualitative opinions related to inhibition found in literature, on the other. According to the considered substrat, the best model minimizing the usual sum of squared residuals model – experiment was chosen.

Keywords: kinetic models, citric acid fermentation, molasses, whey

Citric acid is one of the most used substances synthesized in biotechnology. The current world production is estimated around 1.1 mil tones/year. Industrial production of citric acid started in 19<sup>th</sup> century. After Wehmer discovery that *Penicillium* can synthesize citric acid from sugars [1], many microorganisms on different substrates were tested. The first industrial process using fermentation by a microorganism (*Aspergillus niger*) started in the second decade of the 20<sup>th</sup> century. *A. niger*, able to grow on various substrates, was the dominating microorganism in the industrial processes for an important period of time [1, 2]. Subsequently, yeasts (*Candida*) were also found to be efficient producers [1, 3]. As a result, more alternative carbon sources were considered, taking advantage of the smaller sensitivity of the yeast to some environmental conditions (i.e. metal ions) compared to *A. niger*.

One advantage of *A. niger* is its ability of fermenting substrates which come as waste products from other process, e.g. whey [4, 5], a carbohydrate reservoir of lactose [6]. This is an important characteristic considering the tendency of reducing production costs and increasing processes sustainability. Cost reduction was achieved also by improving the microbial producer strain by mutagenesis [7-9].

However, the production of citric acid by *A. niger* is strongly influenced by the type of the processes used (i.e. surface, submerged) and characteristics of the substrates [10]. The type and concentration of the carbon source are two of the important factors shown to play key role in the overall efficiency of the industrial process [11].

In this paper we propose and validate some kinetic models for the citric acid fermentation on two substrates, molasses and whey supplemented with glucose, starting from experimental data reported in literature [4, 12]. These are representative substrates, for which many experimental data exist [5, 12-15], but few mathematical models were developed [16-18], especially in the case of whey. The models are obtained to be further used for simulations and optimal control of the process with consideration of the sustainability component.

## Mathematical models

Considering the biochemistry of citric acid accumulation by *A. niger*, three types of models with different complexity

have been proposed to describe the process. The core of the models is of Monod type, with no inhibition on  $\mu$  and  $\mu_p$ :

$$\mu = \mu_{\max} \cdot \frac{S}{K_S + S} \quad (1)$$

$$\mu_p = \mu_{p,\max} \cdot \frac{P}{K_P + P} \quad (2)$$

where:

$\mu_{\max}$  and  $\mu_{p,\max}$  represent the maximum specific growth rate on substrate and maximum consumption rate for the product when substrate vanishes, respectively ( $\text{h}^{-1}$ );

$S$  and  $P$ , - substrate and product concentrations (g/L);

$K_S$  and  $K_P$  - the Monod saturation constants (g/L).

First group of three models adds asymptotic inhibition separately on  $\mu$ , then,  $\mu_p$  and finally on both of them. Maximum specific growth rate is inhibited by product concentration, while  $\mu_p$  is affected by substrate concentration. In the second group of models the inhibition was of logistic type, according to the generic relationship,

$\frac{1}{1 + \beta_M \cdot \exp(-\alpha_M \cdot M)}$  which is applied separately on  $\mu$  and  $\mu_p$  ( $\alpha_M$  and  $\beta_M$  generic model parameters,  $M$  – product or substrate concentration, depending upon the inhibition type). The third group of models considers that the inhibition

obeys a power law relationship of the type  $1 - \left(\frac{M}{K_M}\right)^{nM}$

and  $K_M$  are model parameters,  $M$  – product or substrate concentration). In a compact writing, the specific rates were:

$$\mu = \mu_{\max} \cdot \frac{S}{K_S + S} \cdot \left\{ \frac{\frac{K_{SP}}{K_{SP} + P}}{1 + \beta_p \cdot \exp(\alpha_p \cdot P)} \cdot \frac{1}{1 - \left(\frac{P}{K_{SP}}\right)^{nP}} \right\} \quad (3)$$

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$$\mu_p = \mu_{p,\max} \cdot \frac{P}{K_p + P} \cdot \left\{ \frac{\frac{K_{PS}}{K_{PS} + S}}{1 + \beta_S \cdot \exp(\alpha_S \cdot S)} \right. \quad (4)$$

$$\left. 1 - \left( \frac{S}{K_{PS}} \right)^{n_S} \right\}$$

Since the data available from literature consists of time series obtained from batch experiments, the complete mathematical model used for parameter estimation is given by the following equations:

$$\frac{dS}{dt} = -r_s \quad (5)$$

$$\frac{dX}{dt} = r_x + r_p^* - k_d \cdot X \quad (6)$$

$$\frac{dP}{dt} = r_p - r_p^* \quad (7)$$

where the reaction rates take into account the substrate consumption for maintenance, the death of microorganisms due to external factors and the use of the citric acid as secondary substrate for growth. The latter assumption was made considering the decrease of citric acid concentration in batch experiments in various fermentation systems on whey [4]. The reaction rates in eqs. (5)-(7) have the following expressions:

$$r_x = \mu \cdot X \quad (8)$$

$$r_s = \frac{r_x}{Y_{XS}} + m \cdot X \quad (9)$$

$$r_p = r_x \cdot Y_{PX} \quad (10)$$

$$r_p^* = \mu_p \cdot X \quad (11)$$

where  $X$  is biomass concentration (g/L).

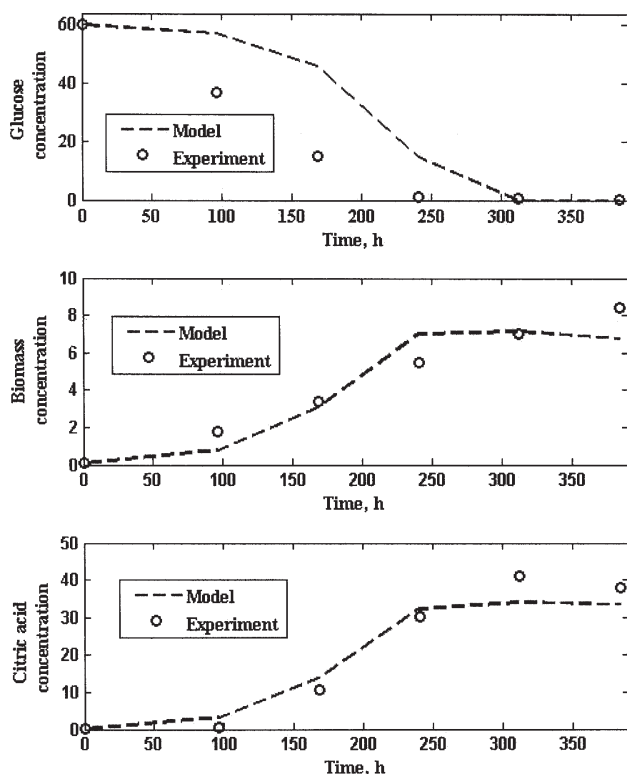


Fig. 1. The kinetic model considering power law inhibition (dashed line) when the substrate is whey supplemented with glucose [4] – concentrations are expressed in g L<sup>-1</sup>

## Parameter estimation

Two solving methods were employed for estimating the kinetic parameter based on the experimental data [4, 12]: genetic algorithms (GAs) and direct search algorithm of Luus and Jaakola [19] modified as in [20]. GAs use a representation of parameters in a chromosome-like structure, each parameter being a gene whose allele are seek in the allowed interval. The optimal chromosome is searched within a pool of candidate solutions, randomly generated initially. Several probabilistic rules, known as genetic operators (selection, crossover, mutation and cloning), are used to create new offspring from individuals complying with a fitness threshold based on the objective function value [21]. In Luus-Jaakola algorithm, a random search takes place in the feasible region of parameters, in discs with given radius, starting from an initial point. When a new optimum point is reached, the centres of discs are moved accordingly. After each jump, a contraction of the discs radii is imposed, till either the minimum value for the objective function is found or the allowed number of iterations is reached.

## Results and discussions

Between eight and twelve parameters were regressed for the aforementioned models of citric acid production, function of their complexity. The regression was realized using a weighted function of deviation between calculated and experimental values of substrate, biomass and product concentrations. The type of models best fitting the experimental data were different for the two substrates considered.

It is important to note that in the case of fermentation on whey, the substrate is supplemented with glucose [4, 5, 22]. The data used for regression were obtained for a media containing 60 g/L glucose, fermented at 30°C with an initial pH = 4.28 [4]. The evolution of residual glucose concentration, which is measured, gives the information on substrate consumption. However, the lactose from whey is not properly quantified in this measurement (figs. 1 - 2). On the other hand, the kinetic model describing microorganism growth and product formation is based on the carbon source in its entirety, especially that the

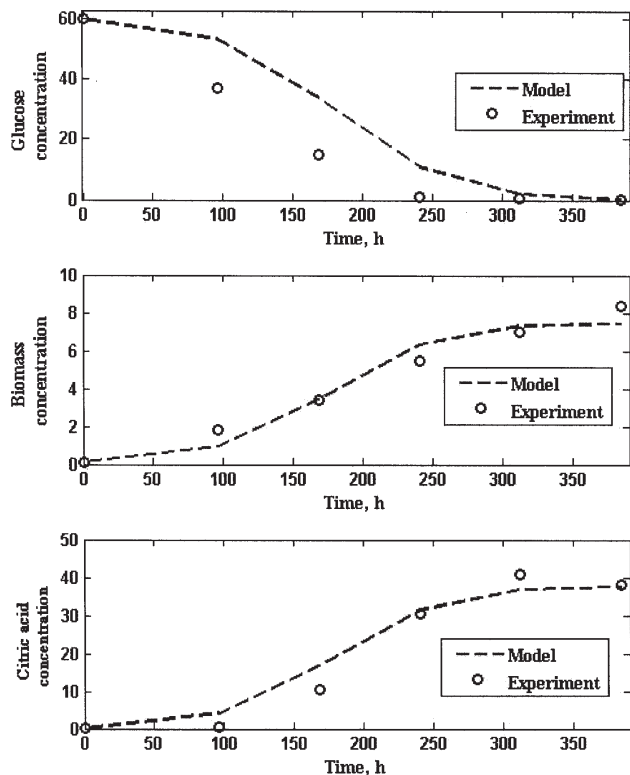


Fig. 2. The kinetic model considering logistic inhibition (dashed line) when the substrate is whey supplemented with glucose [4] – concentrations are expressed in  $g\ L^{-1}$

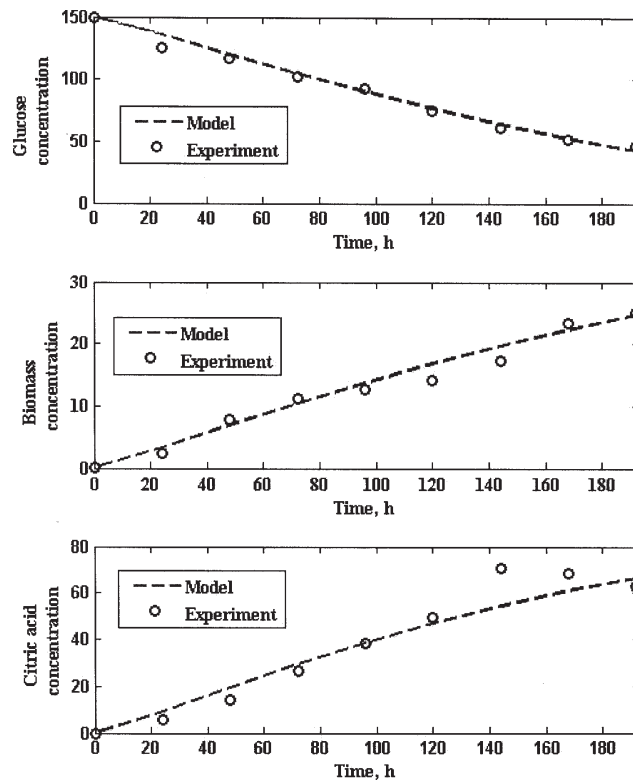


Fig. 3. Kinetic model regressed for the experimental data [12] obtained for molasses as substrate – concentrations are expressed in  $g\ L^{-1}$

Parameter name	Parameter symbol	Units	Value	
			Molasses	Whey
Maximum specific growth rate	$\mu_{max}$	$h^{-1}$	0.97494	1.9366
Monod saturation constant for substrate	$K_S$	$g/l$	115.3839	4968.75
Monod inhibition constant for substrate	$K_{SP}$	$g/l$	0.93285	-
Maximum consumption rate for the product when substrate	$\mu_{p,max}$	$h^{-1}$	0.22882	0.1019
Monod saturation constant for product	$K_P$	$g/l$	0.38945	6875.18
Monod inhibition constant for product	$K_{PS}$	$g/l$	0.1006	-
Logistic coefficient for substrate inhibition by product	$\alpha_P$	$g/l$	-	0.0348
Logistic coefficient for substrate inhibition by product	$\beta_P$	-	-	0.0022
Logistic coefficient for product inhibition by substrate	$\alpha_S$	$g/l$	-	5.7732
Logistic coefficient for product inhibition by substrate	$\beta_S$	-	-	2.8186
Biomass yield on substrate	$Y_{XS}$	$g/g$	0.22882	0.1309
Product yield on biomass	$Y_{PX}$	$g/g$	2.8841	4.991
Endogen substrate consumption	$m$	$h^{-1}$	0.0011674	0.0016
Death rate	$k_d$	$h^{-1}$	-	0.00012

**Table 1**  
PARAMETERS VALUES FOR THE  
BEST REGRESSIONS

product variation is the important aspect for further optimal control of the process. As a consequence, in all regressed cases considered, there was a deviation of the model from the residual glucose measured experimentally that we consider coming from the uncounted lactose.

For cheese whey supplemented with glucose, the models with asymptotic inhibition were not able to describe the lag period at the beginning of the process and the transition between whey and citric acid as substrates for the biomass. Power law inhibition on  $\mu$  and  $\mu_p$  had apparently a good chance to give a satisfactorily representation of the process (fig. 1), but it was abandoned as a viable option because of unrealistic values of the parameters (e.g.  $\mu_{max} = 1.33 \cdot 10^3 \text{ h}^{-1}$  and  $K_s = 3.5 \cdot 10^6 \text{ g/L}$ ). The best fitting was obtained for the model having logistic inhibition on both  $\mu$  and  $\mu_p$  (fig. 2).

Unlike for the whey substrate case, for molasses the glucose concentration variation and the resulted model were in a good correlation (fig. 3). In this case, the model with asymptotic inhibition on both  $\mu$  and  $\mu_p$  was chosen for the best representation of the experimental data. The experimental data used for regression were obtained for a fermentation medium clarified cane molasses 15% sugar, pH = 6, temperature 30° and a constant aeration of 1.01 l<sup>1</sup>min<sup>-1</sup> [12].

In table 1 are presented the regressed values for the parameters; they will be further used in simulations and optimal control.

## Conclusions

This paper aimed to propose, analyse and fit kinetic models for the process of acid citric fermentation on two different substrates. Three generic types of models were proposed, taking into account the biochemistry of the process. For whey supplemented with glucose, a Monod based model with logistic inhibition on the maximum specific growth rate and the maximum consumption rate for the product used as substrate was found to give the best correlation with literature experimental data. For molasses, an asymptotic inhibition model was selected.

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