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A New Process for Acrylic Acid Synthesis by Fermentative Process

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Abstract

With the synthesis of chemical products through biotechnological processes, it is possible to discover and to explore innumerable routes that can be used to obtain products of high added value. Each route may have particular advantages in obtaining a desired product, compared with others, especially in terms of yield, productivity, easiness to separate the product, economy, and environmental impact. The purpose of this work is the development of a deterministic model for the biochemical synthesis of acrylic acid in order to explore an alternative process. The model is built-up with the tubular reactor equations together with the kinetic representation based on the structured model. The proposed process makes possible to obtain acrylic acid continuously from the sugar cane fermentation.

Index Entries: Acrylic acid; biotechnological processes; bidimensional model; dynamic reduced model; modeling; *Saccharomyces cerevisiae*; tubular bioreactor.

Introduction

Biological sciences are likely to make the same impact in the formation of new industries in the present and the next centuries, as the physical and chemical sciences have had on industrial development throughout the last century. In fact, the knowledge from biological sciences, when combined with recent and future advances in process engineering, can become the foundation for producing a wide variety of industrial products from renewable plant resources *(1)*.

The present capabilities of genetic engineering for the transfer of specific catalytic functions between organisms can only provide increased opportunities for the future manufacture of chemical commodities from biomass *(2)*. Biotechnological processes, generally, occur under mild conditions.

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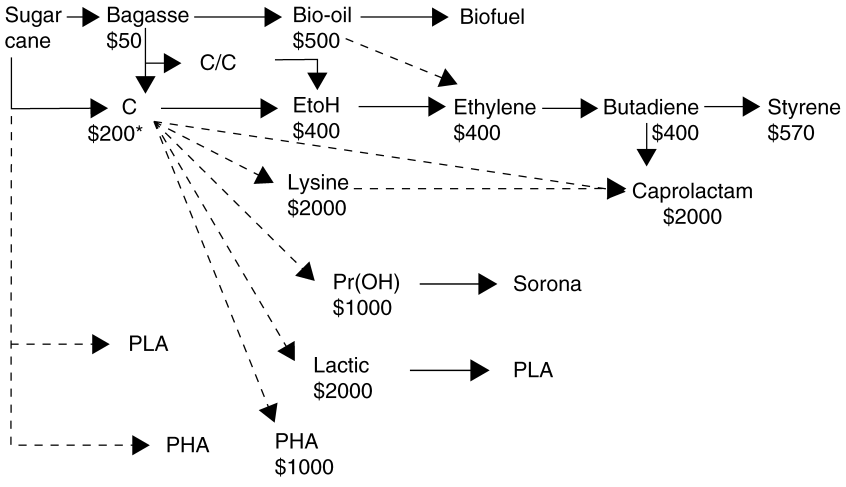

Fig. 1. Pathways of several chemical using sugar cane as a feedstock, prices are in USD per ton of compound (*see* ref. *4*).

Biocatalysts, substrates, intermediates, and byproducts, as well as the product itself, are biodegradable. In most cases, water is used as the solvent *(3)*.

With the synthesis of chemical products through biotechnological processes, it is possible to discover and to explore innumerable routes that can be used to obtain products of high added value. Such routes have to be investigated to evaluate their potential in terms of yield, productivity, easiness to recover the product, economy, and environmental impact. A possible feedstock is the sugar cane glucose which is readily available in several countries. Figure 1 depicts possible routes to obtain chemicals from sugar cane, including the use of bagasse *(4)*.

Among these products, acrylic acid is an interesting one. In fact, from the industrial point of view, the acrylic acid production by fermentative process is presented as an innovative process of great importance, because of the possibility of low cost for its production and because of a renewable raw material. This is an important point to be considered, because acrylic acid is used worldwide and its production by fermentation is through environmentally friendly process. In fact, this biochemical route has very low environmental impact, when compared with the conventional petrochemical process. Acrylic acid, known as 2-propenoic acid, is one of the most important industrial chemicals, with an annual production of approx 4.2 mt *(5)*.

Currently, 100% of acrylic acid is produced from fossil fuel. Production from renewable resources is propagated through lactic acid fermentation and subsequent chemical conversion to acrylic acid *(6)*. Bearing this in mind, the purpose of this work is the development of a deterministic model for a biochemical synthesis of acrylic acid, aiming to propose a new methodology for its production. The proposed process makes possible to obtain acrylic acid continuously from the sugar cane fermentation. The reactor is tubular,

continuously operated, and the challenge is to define operating strategy and conditions to achieve the product with the desired specifications.

A deterministic model built-up coupling the reactor and the kinetic equations is developed to study the process. The kinetic model is based on the concepts of structured representation, and adapted from a structured growth model developed by Lei et al. *(7)*, and a structured model for ethanol production developed by Stremel *(10)*, so that the main phenomena taking place in the system is considered. The mathematical models describing the dynamic behavior of the reactor lead to a nonlinear distributed parameter problem requiring excessive computational time. This may be a restriction for control and optimization of online applications. In order to overcome this problem, through the use of reduction techniques, a simplified model is derived. The results show how the model may be used to find out suitable operating conditions and to analyze the effect of kinetic parameters to obtain acrylic acid.

Structured models describing culture kinetics are powerful tools in the control of bioreactors, as they are able to provide a mathematical description of the cellular fermentation mechanism of the process. This is important to help in the optimization and control decisions. The simplest representation of microbial kinetics is the unstructured model, which describes biomass growth, substrate consumption, and extracellular metabolic product formation in a macro balance approach. The unstructured model includes the most fundamental microbial processes: the rate of cell mass production is proportional to biomass concentration; saturation limit growth rate on each substrate is taken into account; and the use of substrate for cell maintenance and the property of the cells to synthesize products even when they do not grow. However, this type of model does not recognize any internal structure of the cell, nor diversity between cell forms, which may be an important feature of certain cell cultures *(8)*.

The application of unstructured models is quite satisfactory in many situations, but there are a large number of applications where such models tend to fail. This is the case when the composition of the system changes drastically, as in any batch process or when the molasses sugar contents change because of the sugar production. In fact, changes in composition affect the initial step of growth as well as in situation where one specific component (protein and RNA in single-cell protein [SCP] production) must be modeled to better use the substrate to a particular pathway. In these cases, a structured model is necessary.

An alternative to simplify the modeling of the bioreactor, even taking into account the cell internal structure is to reduce the dimension of the system of partial differential equations. This can be done taking a mean along a certain position of the reactor, eliminating the dependent variables on radial position, and by formulating an approximation of the variables along that dimension. This dimensionality reduction may be made by application of reduction techniques.

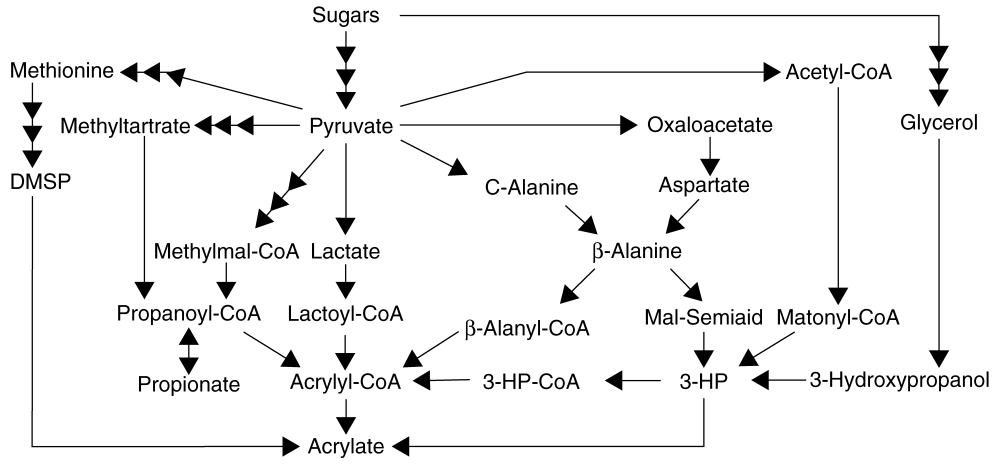

Fig. 2. Overview of existing and hypothetical metabolic pathways for biosynthesis of acrylate from sugars (*see* ref. *5*).

Methods

Metabolic Route

Figure 2 shows the different routes for converting sugars into acrylate. The most direct route is through lactate.

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow[\text{dehydrogenase}]{\text{Lactate}} \text{Lactate} + \text{NAD}^+$$

Several studies have focused on blocking the enzyme that converts acrylyl-CoA to propanoyl-CoA during the aforementioned lactate fermentation by *Clostridium propionicum*, for example, by using 3-butynoic acid as an inhibitor, to obtain conversion of lactate into acrylate. However, acrylate concentrations never exceed 1% of the initial substrate concentration *(9)*.

There are several problems with production of acrylate through this pathway. First, one-third of the lactate does not lead to acrylate, because it is converted into acetate and CO_2. Without this conversion to acetate, no adenosine triphosphate (ATP) for growth and maintenance is generated. The only driving force for the pathway from lactate toward acrylate seems to be the fact that acrylyl-CoA can be used as an electron acceptor for the reducing equivalents produced on formation of acetate and CO_2 *(5)*. A method for direct conversion of complex substrate for propionic acid production, with the cultivation of *Lactobacillus* and *Propionibacterium shermanii* and conversion of propionate for acrylate with *C. propionicum* was investigated *(2)*. This route of conversion of propionate to acrylic acid claims to obtain yield more than 18.5%. However, so far it is not clear how to obtain high yields of acrylate from sugars *(5)*. For an economically competitive fermentation process, the molar yield of acrylate on sugar should preferably be almost quantitative. Taking glucose as the sugar, the desired stoichiometry is *(5)*:

$$\text{Glucose} \rightarrow 2\ \text{Acrylate} + 2\ \text{Water}$$

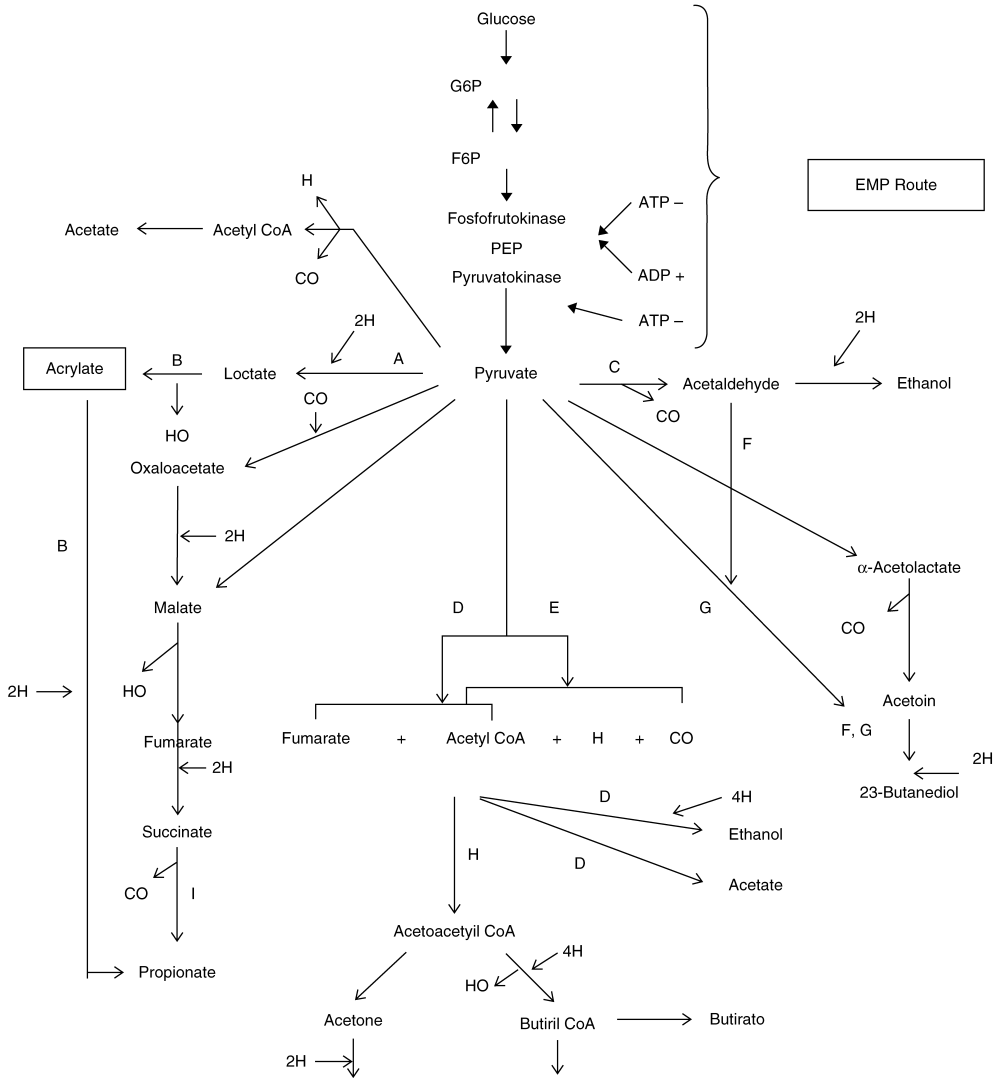

Fig. 3. Glycolytic route (*see* ref. *11*).

Proposed Process

As there is no definitive knowledge on the potential of biotechnological routes for acrylic acid production, and also, because of the few number of structured kinetic models until now developed, in this work, a structured kinetic model for production of acrylic acid by fermentation of sugar cane glucose is developed. The model is based on a structured growth model developed by Lei et al. *(7)*, and on a bioethanol production model developed by Stremel *(10)*. Figure 3 shows the glycolytic route used for the development of the model for acrylic acid and bioethanol production, as they share the same initial route.

The process occurs with the degradation of glucose, which undergoes successive phosphorylations, consuming ATP in the Embden-Meyerhoff-Parnass route, until the pyruvate production. This process is called glycolysis. The pathway of glycolysis can be seen as consisting of two separate phases. In the first phase, two equivalents of ATP are used to convert glucose to fructose-1,6-bisphosphate. In the second phase fructose-1,6-bisphosphate is degraded to pyruvate, with the production of four equivalents of ATP and two equivalents of NADH. Because of action of metabolization through the tricarboxylic acid (TCA) cycle it is converted to lactate the enzyme *lactate dehydrogenase*. The lactate undergoes dehydration, generating acrylate.

Bioreactor Mathematical Model

A mathematical model for the dynamic simulation of a tubular bioreactor that uses *Saccharomyces cerevisiae* immobilized in pellets with 4% of citric pectin for production of bioethanol was developed by Stremel *(10)*, and posteriorly adapted to investigate the acrylate production. As both process share some pathways and the reactor and kinetic structured models are general, the deterministic representation is valid to explore all the possible routes, as the kinetic parameters are available. For the case of bioethanol production, in order to prevent the CO_2 accumulation caused for the fermentation process, a type tower fixed-bed bioreactor with gas separator was used. It is important to take this into account as CO_2 accumulation may happen in different rates depending on how the process is operated. A scheme of the system is shown in Fig. 4.

The differential balances in the axial direction of the bioreactor take into account the convective terms, assume constant axial dispersion, and the mass interphase transfer and reaction are evaluated in terms of the effectiveness factor. Bearing this in mind the model can be written as:

Substrate in the phase fluid:

$$\frac{\partial S_f}{\partial t} = \frac{D_{ax}}{L^2}\left(\frac{\partial^2 S_f}{\partial z^2}\right) - \frac{u}{L}\left(\frac{\partial S_f}{\partial z}\right) - \frac{1-\varepsilon}{\varepsilon}\eta V_{sup} \quad (1)$$

Ethanol in the phase fluid:

$$\frac{\partial E_f}{\partial t} = \frac{D_{ax}}{L^2}\left(\frac{\partial^2 E_f}{\partial z^2}\right) - \frac{u}{L}\left(\frac{\partial E_f}{\partial z^2}\right) - \frac{1-\varepsilon}{\varepsilon}\eta Y_{ES}\left(V_{sup}\right) \quad (2)$$

Kinetics for Chemicals Synthesis

A simplification of the glycolytic and respiratory routes (TCA) that was considered in the model, to represent bioethanol synthesis is shown below by stoichiometric expressions.

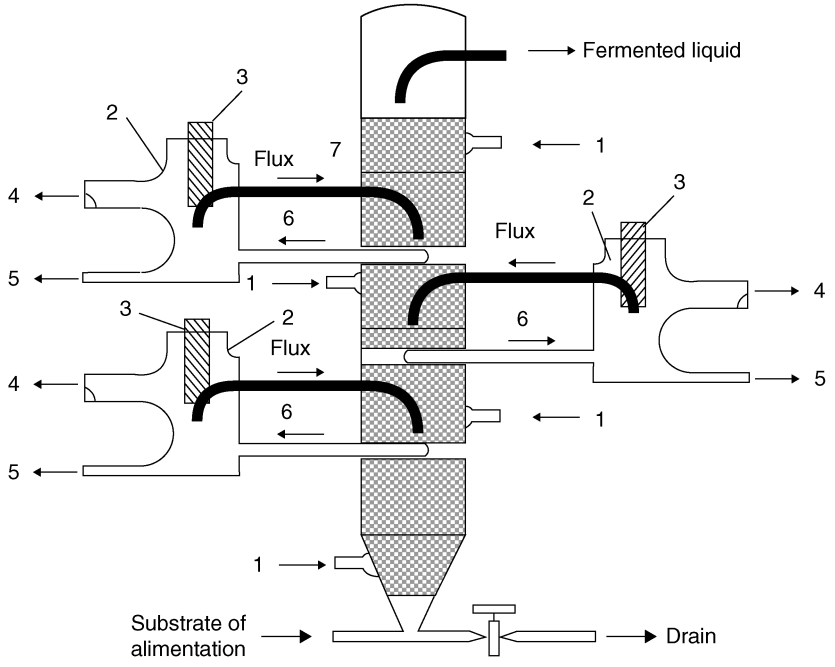

Fig. 4. Type tower bioreactor. (1) Pellets of evacuation the alimentation; (2) separator gas-liquid; (3) indutive sensor; (4) exit CO_2; (5) evacuation of sample; (6) fermented liquid with flux of CO_2; and (7) fermented liquid without flux of CO_2.

$$
\begin{aligned}
F_1[\mathrm{S}] &\xrightarrow{R_1} F_1[\mathrm{P}] \\
F_6[\mathrm{S}] &\xrightarrow{R_6} 0.732\, X_{[\mathrm{S}]} \xrightarrow{0.732R_6X_{[\mathrm{S}]}} X_{[\mathrm{T}]} \\
F_4[\mathrm{P}] + \mathrm{O}_2 &\xrightarrow{R_2} \mathrm{TCA} + \mathrm{CO}_2 \\
F_4[\mathrm{P}] &\xrightarrow{R_3} F_3[\mathrm{A}] \\
F_4[\mathrm{A}] + \mathrm{O}_2 + X_{[\mathrm{r}]} &\xrightarrow{R_4} \mathrm{TCA} + X_{[\mathrm{r}]} + \mathrm{CO}_2 \\
F_4[\mathrm{A}] + X_{[\mathrm{fe}]} &\xrightarrow{R_5} F_5[\mathrm{E}] + X_{[\mathrm{fe}]} + \mathrm{CO}_2 \\
F_4[\mathrm{A}] &\xrightarrow{R_7} 0.850\, X_{[\mathrm{S}]} \xrightarrow{0.852R_7X_{[\mathrm{S}]}} X_{[\mathrm{T}]} \\
X_{[\mathrm{S}]} &\xrightarrow{R_8} X_{[\mathrm{P}]} \xrightarrow{(0.732R_6 + 0.850R_7)X_{[\mathrm{P}]}} X_{[\mathrm{T}]} \\
X_{[\mathrm{S}]} &\xrightarrow{R_9} X_{[\mathrm{r}]} \xrightarrow{(0.732R_6 + 0.850R_7)X_{[\mathrm{r}]}} X_{[\mathrm{T}]} \\
X_{[\mathrm{S}]} &\xrightarrow{R_{10}} X_{[\mathrm{fe}]} \xrightarrow{(0.732R_6 + 0.850R_7)X_{[\mathrm{fe}]}} X_{[\mathrm{T}]} \\
X_{[\mathrm{T}]} &\xrightarrow{k_\mathrm{d}} X_{[\mathrm{nv}]}
\end{aligned}
\tag{3}
$$

For the biotechnological production of acrylic acid, three substrates involved in the process are verified. In the first step the glucose is converted into pyruvate, and this into lactate, which is metabolized to produce acrylate. The generic reaction proceeds from the following form:

$$\mathrm{G} \xrightarrow{R_\mathrm{P}} \mathrm{P} \rightarrow \mathrm{La} \xrightarrow{R_\mathrm{a}} \mathrm{Acryl} \tag{4}$$

Balance of mass for glucose in the fluid phase:

$$\frac{d(VG)}{dt} = \mathrm{G_i F_i} - \mathrm{GF} - \frac{\mu_\mathrm{L} X_\mathrm{L}}{Y_{\mathrm{XL/G}}} V \tag{5}$$

Reaction rate of glucose conversion in pyruvate:

$$R_\mathrm{P} = \frac{\mu_\mathrm{L} X_\mathrm{L}}{Y_{\mathrm{XL/G}}} \tag{6}$$

Balance of mass for lactate:

$$\frac{d(VLa)}{dt} = \mathrm{La_i F_i} - \mathrm{LaF} - \frac{\mu_\mathrm{L} X_\mathrm{L}}{Y_{\mathrm{XL/G}}} V \tag{7}$$

Reaction rate of pyruvate conversion in lactate:

$$R_\mathrm{a} = \frac{\mu_\mathrm{L} X_\mathrm{L}}{Y_{\mathrm{XL/L}}} \tag{8}$$

Balance of mass for acrylate:

$$\frac{d(VAcryl)}{dt} = \mathrm{Acryl_i F_i} - \mathrm{AcrylF} + \frac{\mu_\mathrm{c} X_\mathrm{c}}{Y_{\mathrm{XC/A}}} V \tag{9}$$

Reduction Techniques

The solution for diffusion and reaction multidimensional problems present difficulties associated with a large analytic involvement and also request considerable computational effort. Thus, for practical applications in engineering, online optimization and control is useful to obtain models with lower dimensionality compared with original system of partial differential equations. This may be achieved through the reduction of the number of model independent variables. Therefore, one or more independent variables can be integrated, leading to approximate formulations that retain detailed local information in the remaining variable as well as mean information in the eliminated directions by the integration. The techniques investigated generate models that describe the axial profiles as a function of the time for the convenient explicit elimination of the dependence in the radial variable, in case of the fixed-bed catalytic reactor. The techniques utilized are:

Classic Reduction Technique

This technique is based on the mean value theorem, i.e., each radial mean value is defined for each variable *(12–14)*

$$\left[\ \right]_{\mathrm{m}} = 3\int_0^1 \left[\ \right] r^2 dr \tag{10}$$

where r = particle radius and [ ] = radial mean value.

Reduction Technique Based on the Hermite Integrations Formulas

Hermite procedure allows the model order reduction by approaching an integral on the values of the integrating and their derivatives on the limits of the integration, as follow:

$$H_{\alpha,\beta} = \int_{x_{i-1}}^{x_i} y(x)dx = \sum_{v=0}^{\alpha} C_v y^{(v)}\left(x_{i-1}\right) + \sum_{v=0}^{\beta} D_v y^{(v)}\left(x_i\right) \tag{11}$$

The technique makes use of $H_{0,0}$, $H_{1,1}$ definitions and simultaneously of the spherical coordinates transformation, facilitating the generation of the radial medium variables *(13,15,16)*.

$$H_{0,0} = \int_0^1 y(x)dx \cong \frac{1}{2}\left[y(0) + y(0)\right] \tag{12}$$

$$H_{1,1} = \int_0^1 y(x)dx \cong \frac{1}{2}\left[y(0) + y(1)\right] + \frac{1}{12}\left[y'(0) + y'(1)\right] \tag{13}$$

General Reduction Technique

This technique is a generic mathematical representation, obtained when the Eq. 10 is used with a quadratic equation for the inside particle concentration in function of the mean radial concentration

$$C(\mathrm{r}) = C_{\mathrm{m}} b - br^2 \tag{14}$$

Results

Figure 5 shows the microorganism (*S. cerevisiae*), ethanol (C_2H_6O), acetaldehyde (C_2H_4O), pyruvate ($C_3H_3O_3$), and substrate ($C_6H_{12}O_6$) concentration at the reactor exit when the steady state is reached. The experimental operation was conducted at pH 4.0 and 30°C using 161.4 g/L initial glucose concentration. The acetaldehyde and pyruvate concentration, intermediary components, appear in low concentration because they are formed and consumed rapidly in the course of respiratory and glycolytic process. At the end of 140 h, 73.0 g/L of ethanol was formed along with 43.0 g/L of cell mass. As can be seen the glucose is used not only to achieve the desired product but also for the maintenance and growth of the microorganism.

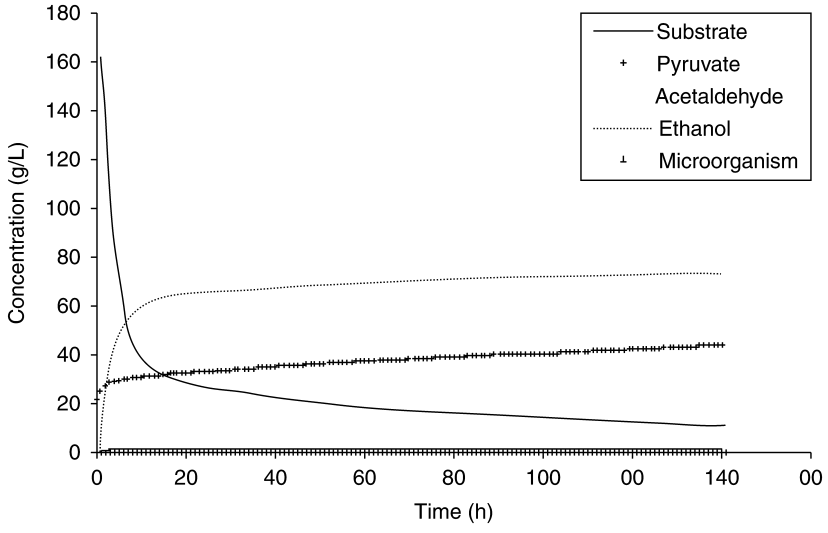

Fig. 5. Microorganism, ethanol, acetic acid, pyruvate, and substrate concentration in the reactor exit.

A possible competitive route to obtain the acrylic acid by fermentation through *S. cerevisiae*, is shown in Fig. 6. Acrylate ($C_3H_4O_2$), lactate ($C_3H_5O_3$), pyruvate ($C_3H_3O_3$), and substrate ($C_6H_{12}O_6$) are obtained and their concentrations at the reactor exit when the steady state is established depict the potential to produce acrylic acid. The dynamic behavior follows a system of first order with asymptotic shape for all the species. In fact, an inverse response was not observed because the temperature is constant along the reactor length. This is expected to occur when tubular reactors are used, and it could be a drawback to use this type of design because of difficulties in process control. The steady state operation is achieved in about 140 h.

The Fig. 7 shows the concentration profiles of acrylate and microorganisms concentration at the reactor exit. The product and cell yields obtained from the glucose fermentation were 0.46 g acrylate per gram of glucose and 0.10 g dry cell per gram of glucose, respectively. Changes in the kinetic values alter significantly the specific values of desired product concentration, but what is interesting to realize is that the acrylate production is associated with the microbial growth, i.e., the acrylate production is directly related with energetic metabolic route. This is important information for reactor design and operation because a suitable residence time should be chosen to allow the reactor to operate near the steady state. However, because of the fact that the production is associated with the microbial growth, it is not possible to determine the necessary amount of glucose for the synthesis of the product. This means that it is necessary to find out operating conditions

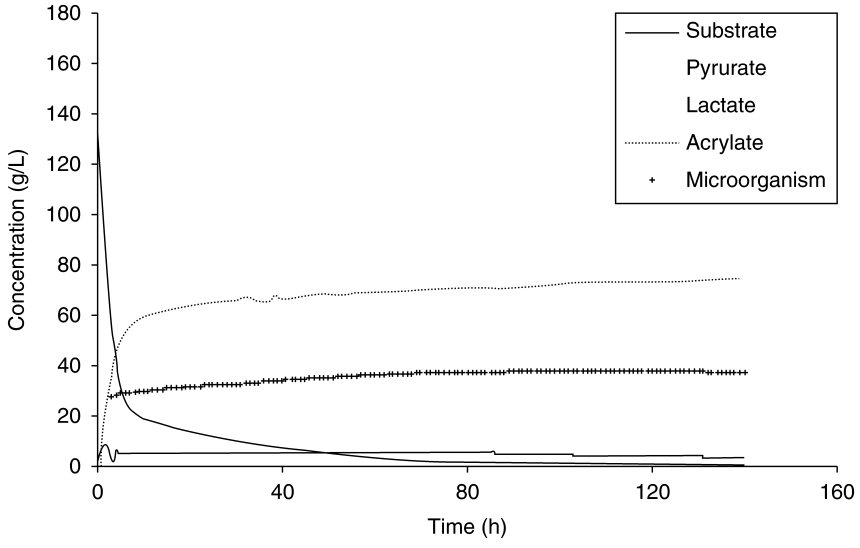

Fig. 6. Microorganism, acrylate, lactate, pyruvate, and substrate concentration in the reactor exit.

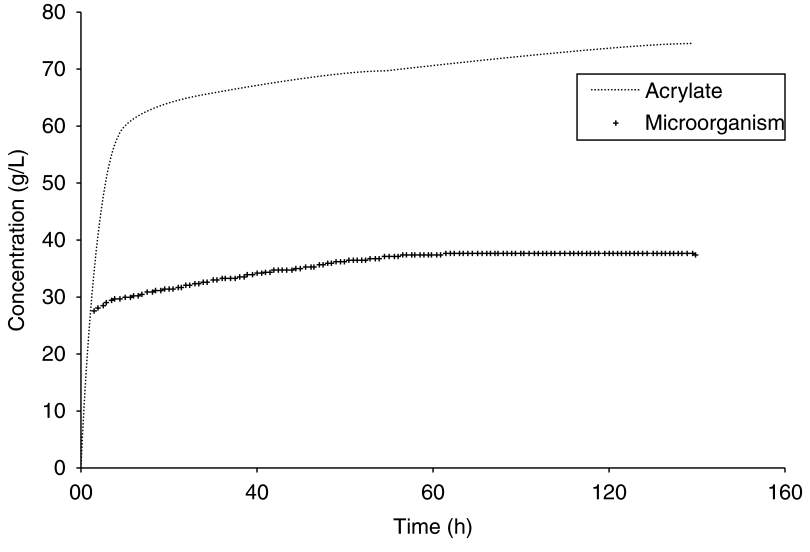

Fig. 7. Microorganism, acrylate concentration in the reactor exit.

in such a way that it is possible to achieve desired amount of the product (acrylic acid) with the required amount of substrate for the maintenance and growth of the microorganism.

The kinetic values for a given microorganism, in this case *S. cerevisae*, is dependent on the system operating conditions including substrate

composition. This information may be used to define operating strategies that may drive the system to obtain a desired product. The acrylic acid production by fermentation occurs from glucose degradation and might be expressive depending on the operational conditions, although its conversion as shown in the literature is significantly smaller when compared with the conventional production, i.e., petrochemical via, where the conversion is of approx 90%.

Conclusions

In this work, a structured deterministic model for the process of production of acrylic acid is proposed. It allowed the understanding of the modeling problem of biochemical processes taking into account the metabolic routes. In this case it is possible to consider the competition among the several product to be formed so that it is possible to foresee operational strategies, which take into account for instance the feed of different substrate compositions as they impact the route that the microorganism will follow. Also it is important to have dynamic information about the time to reach operating conditions around the steady state, so that suitable design can meet required production levels. This is important, as the conversion for biochemical processes is usually low compared with the petrochemical via. For the specific case of acrylic acid production, it is observed that the acid production is strictly related to the microbial growth and this should be considered in the reactor design. Through the application of the reduction techniques it was possible to reduce significantly the number of differential equations to be solved, thus reducing the complexity of the modeling as well as the computer time and burden, whereas still keeping important process information.

Nomenclature

η	Effectiveness factor
μ	growth specific velocity (h^{-1})
ε	Porosity of the bed
A	Acetaldehyde
Acryl	Acrylate
C	Concentration (g/L)
C_m	Mean radial concentration (g/L)
D_{ax}	Axial dispersion of coefficient (m^2/h)
E	Ethanol
$F_1, \ldots, F_6$	Adjustment of constant
G	Glucose
L	Length of the bioreactor (m)
La	Lactate
P	Pyruvate
R	Inside radius (m)

R	Particle of the radius (m)
$R_{1,\ldots,}R_6$	Metabolic reaction rate (g/Lh)
R_a, R_p	Reaction rate (g/h)
S	Substrate
Sh	Sherwood number
t	Time (h)
u	Fluid interstitial velocity (m/h)
V_{sup}	Superficial reaction rate (g/Lh)
X	Total mass concentration (g/L)
Y	Yield coefficient (g/g [%])
Z	Axial length of the bioreactor (m)

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References

1. Dale, B. E. (2003), *J. Chem. Technol. Biotechnol.* **78,** 1093–1103.
2. O'Brien, D. J., Panzer, C. C., and Eisele, W. P. (1990), *Biotechnol. Prog.* **6,** 237–342.
3. Willke, T. and Vorlop, K. D. (2004), *App. Microbiol. Biotechnol.* **66,** 131–142.
4. Nossin, P., Joosten, J., and Bruggink, A. (2002), Future Feedstocks for Commodity Polymers: EcotheneTM—Sustainability in the 21st century. DSM Research BV, Geleen
5. Straathof, A. J. J, Sie, S., Franco, T. T., and Van der Wielen, L. A. M. (2005), *Appl. Microbiol. Biotechnol.* **67,** 727–734.
6. Danner, H. and Braun, R. (1999), *Chem. Soc. Rev.* **28,** 395–405.
7. Lei, F., Rotboll, M., and Jorgensen, S. B. (2001), *J. Biotechnol.* **88,** 205–221.
8. Birol, G., Kirdar, B., and Onsan, Z. (2002), *J. Ind. Microbiol. Biotechnol.* **29,** 111–116.
9. Akedo, M. Cooney, C. L., and Sinskey, A. J. (1983), *Biotechnol.* **1,** 791–794.
10. Stremel, D. P (2001), In: *Desenvolvimento de Modelos Estruturados Alternativos Para o Processo de Produção de Etanol*, Tese de doutorado, *FEQ/UNICAMP*, Campinas.
11. Bailey, J. E. and Ollis, D. F. (1986), *Biochemical Engineering Fundamentals*, McGraw Hill, New York: pp. 276.
12. Mcgreavy, C. and Naim, H. (1977), *Can. J. Chem. Eng.* **55,** 326–332.
13. Toledo, E. C. V. and Maciel Filho, R. (1997), In: *Modelos reduzidos para controle avançado de reatores de leito fixo*. ELAIQ'94, Chile, pp. 36–41.
14. Stremel, D. P. and Maciel Filho, R. (1998), In: *Aplicação de um modelo estruturado no processo fermentativo em biorreator de alta produtividade para produção de etanol*. *12° Congresso Brasileiro de Engenharia Química (COBEQ).* Porto Alegre, RS.
15. Corrêa, E. J. and Cotta, R. M. (1996), Appl. Mathem. Model. **22,** 137–152.
16. Vasco de Toledo, E. C. (1999), In: *Modelagem, simulação e controle de reatores catalíticos de leito fixo*. Tese de doutorado, FEQ/UNICAMP, Campinas.