A SOFTWARE FOR SIMULATION OF FERMENTATION PROCESSES

By

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Abstract

Fermentation is a key process stage in ethanol production and has a high incidence in the overall process efficiency in distillery. For improving this efficiency, a rigorous analysis for defining the best choice in operation variables is needed. Simulation of the process is a recognised way for doing such an analysis. In this paper, the software tool FERMENTA® for simulation of fermentation processes is presented. This software tool allows simulation of continuous and discontinuous modes of fermentation. In the continuous mode of operation, several stages can be considered. In the batch mode, alternatives for feeding (batch, fed batch with different politics) can be analysed. The mathematical model includes non-stationary balances for biomass, substrate, ethanol, oxygen, energy. The kinetic expression can be selected by the user among more than 30 possibilities, taking or not into account inhibition factors in the process. Parameters in this equation can be estimated from experimental data. The result is a useful software tool that can be used for analysis of fermentation processes in industry and also for academic purposes. In this paper, the software is described and some case studies used for showing its possibilities.

Introduction

The technology of ethanol production by anaerobic fermentation of sugarcane molasses is well known. This process, used initially in the production of alcoholic drinks, reached high generalisation grade during the Second World War with the purpose of having an alternative source of fuel. Nowadays, ethanol with different purity grades is still produced from molasses and sugar juices. Their uses go from the pharmaceutical and cosmetics industry to the production of alcohol used as fuels by vehicles.

Keeping in mind the multiple uses of ethanol, there is an intensified interest in the study of all steps involved in ethanol production in order to reduce costs. Mathematical modelling reduces research costs by eliminating part of the experimental work, because it allows the study of process parameter interactions through simulation. Besides, it provides understanding of the process, which is helpful for operational policy definitions and can be applied for later optimisation and control.

In this paper, the mathematical model of alcohol fermentation process and FERMENTA 4.0 software, designed for simulating this process, is presented. This software could be useful in other fermentative processes because it reduces costs by eliminating part of the experimental work and allows the study of process parameter interactions through simulation.

Finally, we present a case study where the influence of some operational conditions on the yield and productivity of fermentation processes was assessed. We compare the simulation results with typical industrial data.

Process description

The conversion of sugar to ethanol is an anaerobic biological reaction by *Saccharomyces cerevisiae* yeast cells. This yeast is used commercially and classified as heterotrophics, the necessities of coal and energy are satisfied by monosaccharides (simple sugars) like glucose. Disaccharides like sucrose can also be used after the hydrolysis of the yeast in fructose and glucose (inverted sugar).

Raw materials used for ethanol production include sugary ones: juices of different fruits, beet juices, juices of cane sugar, intermediate streams of the process like molasses, etc. Sugarcane is a raw material of easy transformation and the agronomic aspects of cultivation do not present limitations, and has been cultivated for centuries. For this reason, it is the most economical raw material.

Ethanol production is divided into two phases: respiration (yeast propagation under aerobic conditions) and ethanol fermentation under anaerobic conditions. You can calculate the theoretical yield of glucose conversion to ethanol in the fermentation stage using the Gay Lussac equation, including an estimate of the heat evolved (Q) during the reaction.

Respiration

Aerobic conditions

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O \tag{1}$$

Anaerobic conditions

$$\begin{aligned} &Glu\cos e \rightarrow Carbon\ Dioxide\ +\ Ethanol \\ &C_6H_{12}O_6 \rightarrow \quad 2CO_2 \quad + \quad 2C_2H_5OH \ + \ Q \\ &100\ g \quad \rightarrow \quad 49\ g \quad + \quad 51\ g \ - \ 23.7Kcal \end{aligned} \tag{2}$$

Beginning with equation (2), the theoretical yields of ethanol and CO_2 can be calculated.

$$\begin{aligned} &y^T_{p/s} = 0.51 \qquad \text{g ethanol/ g glucose} \\ &y^T_{CO_2} = 0.49 \qquad \text{g } CO_2 \text{ / g glucose} \end{aligned} \tag{3}$$

The real yields, however, are smaller because part of the glucose is used in the biomass generation and maintenance energy.

There are different operating fermentation modes. The first one is the batch mode where the fermenter is inoculated and fed with substrate until its working volume is reached and the process is carried out until the biotechnological conversion of sugars to ethanol finishes. Second one is the fed-batch mode with any number of feeding streams and the last is continuous fermentation, with continuous feed of fresh medium and continuous extraction of fermented medium; the fermentation can last stably for weeks and months.

Mathematical modelling

Mathematical models have been used to predict the influence of operating parameters on cell concentration, substrate utilisation rate and ethanol production rate (Sabadí *et al*., 1990, Rosso *et al*., 1995, Corsano *et al*., 2004, Rivera *et al*., 2006 and Ribas *et al*., 2006). These models may lead to the development of better strategies for the optimisation of the fermentation process to ensure its economic viability.

Microbial growth

The growth of microorganisms can be best imagined as the increase of cell material expressed in terms of mass (or cell numbers). It is the result of a highly coordinated series of

enzymatically catalysed biological steps. Optimal expression of growth kinetics depends on optimal maintenance of the transport of the necessary nutrients in the medium to cell surface, rate of mass transfer from the medium into the cells, and environmental parameters (such as temperature and pH).

In technical literature, different models describing the growth kinetics of cells are reported. The Monod (1942) model, based on Michaelis-Menten kinetics, describes the relationship between the specific growth rate (μ) and the substrate concentration limit (S).

$$\mu = \mu_{\max} \frac{s}{k_s + s} \tag{4}$$

where:

μ: specific cell growth rate $\left(h^{-1}\right)$

$\mu_{\max}$: maximum specific cell growth rate $\left(h^{-1}\right)$

k_s: saturation parameter (g/L). Maximum substrate concentration that can use the cells for their growth. It is defined as the substrate concentration at half the maximum specific cell growth rate.

S: Substrate concentration (g/L)

A high substrate concentration inhibits cell growth due to the effect of osmotic pressure. The model of Andrews (1968) takes this effect into consideration.

$$\mu = \mu_{\max} \frac{s}{k_s + s + \frac{s^2}{k_{is}}} \tag{5}$$

$k_{is} > \left(\frac{s^2}{2 * k_s}\right)$: substrate inhibition parameter (g/L)

Microorganisms have a certain tolerance to the concentration of alcohol in the growth medium. Increasing alcohol concentration will gradually retard microbial growth and may finally completely inhibit it. This phenomenon is included in the Levenspiel (1980) model.

$$\mu = \mu_{\max} \frac{s}{k_s + s}\left(1 - \frac{p}{p_{\max}}\right)^n \tag{6}$$

Parameter value $n \in [0.3; 2.0]$

Maiorella (1984) developed a model that describes the specific production rate of ethanol and biomass considering the yeast *Saccharomyces cerevisiae*, very often used in ethanol production.

$$\mu_p = \left[\mu_{p\max} \frac{s}{k_s + s}\right]\left[1 - \frac{p}{p_{\max}}\right]^n \tag{7}$$

where:

μ_p:	Specific ethanol production rate. .	(g ethanol/ g cell h)
$\mu_{p\max}$:	Maximum specific ethanol production rate.	(g ethanol/ g cell h)
K_s:	Saturation parameter.	(g/L)
$P_{\max}$:	Ethanol inhibition term.	(g/L)

The specific cell growth rate can be calculated as:

$$\mu = E * \mu_p \tag{8}$$

where:

E : Efficiency in the use of sugar for the cells production. (g. cell / g sugar).

Wijtzes *et al.* (1993) and Ratkowsky *et al.* (1982) develop a model with environmental parameters such as temperature (T) and (pH).

$$\mu_{\max} = \mu_{opt} * f(pH) * g(T) \tag{9}$$

μ_{opt} : Value of $\mu_{\max}$ under optimal conditions (pH_{opt}, T_{opt}). $\left(\frac{1}{h}\right)$

In software FERMENTA 4.0, more than 30 kinetics growth models are implemented among which are the previous ones. These models are classified in four categories:

- Models without substrate inhibition.
- Models with substrate inhibition.
- Models with ethanol inhibition.
- Models with mixed inhibition.

A fermentation system, in a general way, can be represented schematically as Figure 1. Starting from here, it is possible to write the mass balance and determine how cells, substrate and ethanol concentrations change over time.

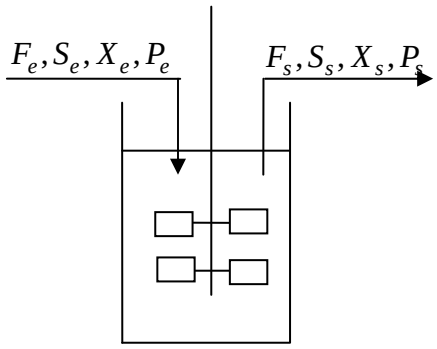


Fig. 1— Schematic representation of ethanol fermentation system.

Cell concentration (g/L)

S : Substrate concentration (g/L)

P : Ethanol Concentration (g/L)

V : Fermentation Volume (L)

F : Flow rate (L/h)

Indices: e: input.

s: output

Total balance

The mass balance equation depends on operation mode. In batch fermentation, there are no inlet and outlet flows in the fermenter and the volume remains constant. In the case of a semi-continuous fermentation, an inlet flow exists to the fermenter that can be operated in different ways.

- Feeding flow for stages: The fermenter is partially fed until sugar is exhausted. It is fed again and so forth until the vessel is filled.

- Constant feeding flow.

$$\frac{dV}{dt} = F \tag{10}$$

- Incremental feeding.

$$\frac{dV}{dt} = F_0 e^{kt} \qquad k > 0 \tag{11}$$

- Increment or decrement feeding with exponential law.

$$\frac{dV}{dt} = F_0 e^{kt} \qquad k < 0 \tag{12}$$

Lastly, in continuous fermentation, there are inlet and outlet flows.

$$\begin{bmatrix}\text{Rate of change}\\ \text{in Volume}\end{bmatrix} = \begin{bmatrix}\text{Volume}\\ \text{input}\\ \text{rate}\end{bmatrix} - \begin{bmatrix}\text{Volume}\\ \text{output}\\ \text{rate}\end{bmatrix}$$

$$\frac{dV}{dt} = F_e - F_s \tag{13}$$

Cell mass

Keeping in mind the same considerations mentioned, a cell mass balance can be written as follows:

$$\begin{bmatrix}\text{Rate of change}\\ \text{in cells}\end{bmatrix} = \begin{bmatrix}\text{Cell}\\ \text{input}\\ \text{rate}\end{bmatrix} - \begin{bmatrix}\text{Cell}\\ \text{output}\\ \text{rate}\end{bmatrix} + \begin{bmatrix}\text{Cell}\\ \text{growth}\\ \text{rate}\end{bmatrix} - \begin{bmatrix}\text{Cell}\\ \text{death}\\ \text{rate}\end{bmatrix}$$

$$\frac{d(xV)}{dt} = F_e x_e - F_s x_s + V(u - k_d)x \tag{14}$$

μ: Specific growth rate $(1/h)$

k_d: Specific death rate $(1/h)$

Substrate

A substrate mass balance can be written as follows:

$$\begin{bmatrix}\text{Substrate}\\ \text{utilization}\\ \text{rate}\end{bmatrix} = \begin{bmatrix}\text{Substrate}\\ \text{input}\\ \text{rate}\end{bmatrix} - \begin{bmatrix}\text{Substrate}\\ \text{output}\\ \text{rate}\end{bmatrix} - \begin{bmatrix}\text{Substrate uptake}\\ \text{rate for}\\ \text{growth}\end{bmatrix} - \begin{bmatrix}\text{Substrate uptake}\\ \text{rate for}\\ \text{product formation}\end{bmatrix} - \begin{bmatrix}\text{Substrate uptake}\\ \text{rate for}\\ \text{maintenance}\end{bmatrix}$$

$$\frac{d(sV)}{dt} = F_e s_e - F_s s_s - V\left[\frac{\mu x}{Y_{x/s}} + \frac{r_p}{Y_{p/s}} + mx\right] \tag{15}$$

where:

$Y_{x/s}$: Growth yield coefficient $(\text{g cells / g substrate})$.

$Y_{p/s}$: Product yield coefficient $(\text{g product formed / g substrate})$.

m: Maintenance coefficient $(\text{g substrate/g cells per h})$.

$r_p = \frac{dp}{dt}$ Product formation rate.

Product formation

The product may be obtained from the beginning of process fermentation, together with cell growth. It can also begin to be formed during the stationary phase or begin in the growth stage and

continue in the stationary phase. In dependence of this, the kinetics of product formation is defined like:

- Product associated with growth

$$r_p = Y_{p/x}\mu x = \alpha\mu x \tag{16}$$

$Y_{p/x} = \alpha$: Product yield coefficient (g product formed / g cells)

- Product not associated with growth

$$r_p = \beta x \tag{17}$$

- Product partially associated with growth

$$r_p = \alpha\mu x + \beta x \tag{18}$$

$$\begin{bmatrix}\text{Rate of change}\\ \text{in product}\end{bmatrix} = \begin{bmatrix}\text{Product}\\ \text{input}\\ \text{rate}\end{bmatrix} - \begin{bmatrix}\text{Product}\\ \text{output}\\ \text{rate}\end{bmatrix} + \begin{bmatrix}\text{Product}\\ \text{formation}\\ \text{rate}\end{bmatrix} - \begin{bmatrix}\text{Product}\\ \text{degraded}\\ \text{rate}\end{bmatrix}$$

$$\frac{d(pV)}{dt} = F_e p_e - F_s p_s + V(r_p - k_{dp} p) \tag{19}$$

k_{dp} : Specific product degraded rate $(1/h)$

Energy balance

An energy balance for the fermentation process can be written as follows:

$$\begin{bmatrix}\text{Rate of change}\\ \text{of heat}\end{bmatrix} = \begin{bmatrix}\text{Heat}\\ \text{input}\end{bmatrix} - \begin{bmatrix}\text{Heat}\\ \text{output}\end{bmatrix} + \begin{bmatrix}\text{Heat}\\ \text{evolved}\end{bmatrix} - \begin{bmatrix}\text{Heat}\\ \text{transfer}\end{bmatrix}$$

$$\frac{dQ}{dt} = Q^E - Q^S + Q_g - Q_{tranf} \tag{20}$$

where:

Q: Heat accumulation rate. $\left(\frac{Kcal}{h}\right)$.

Q^E: Total heat accumulated by the inlet flow until time t. $(Kcal)$

Q^S: Total heat in outlet flow until time t. $(Kcal)$

Q_g: Total heat evolved by the process during the reaction. $(Kcal)$

Q_{tranf}: Heat transfer by the wall. $(Kcal)$

The set of coupled relationship equations 15, 16, 20 and 21, describing the fermentation kinetics, together with equations of microbial growth, was solved numerically by employing Runge Kutta method of five order with variable step (Dormand *et al*., 1980). This algorithm was included in FERMENTA 4.0 software.

Software FERMENTA 4.0

The software FERMENTA 4.0 has been designed and implemented to be used on Microsoft Windows platforms. It has a friendly user interface and a great variety of graphical reports. The main menu of the system is shown in Figure 2, representing the fermentation process according to the configuration analysed by the end user.

The system is configurable to simulate different types of fermentation (batch, fed-batch, continuous with tanks connected in series and recycling cells with one or two stages).

For simulating the process, it is necessary to input data related to the initial conditions and several parameters of yields previously adjusted if necessary to match given fermentation results.

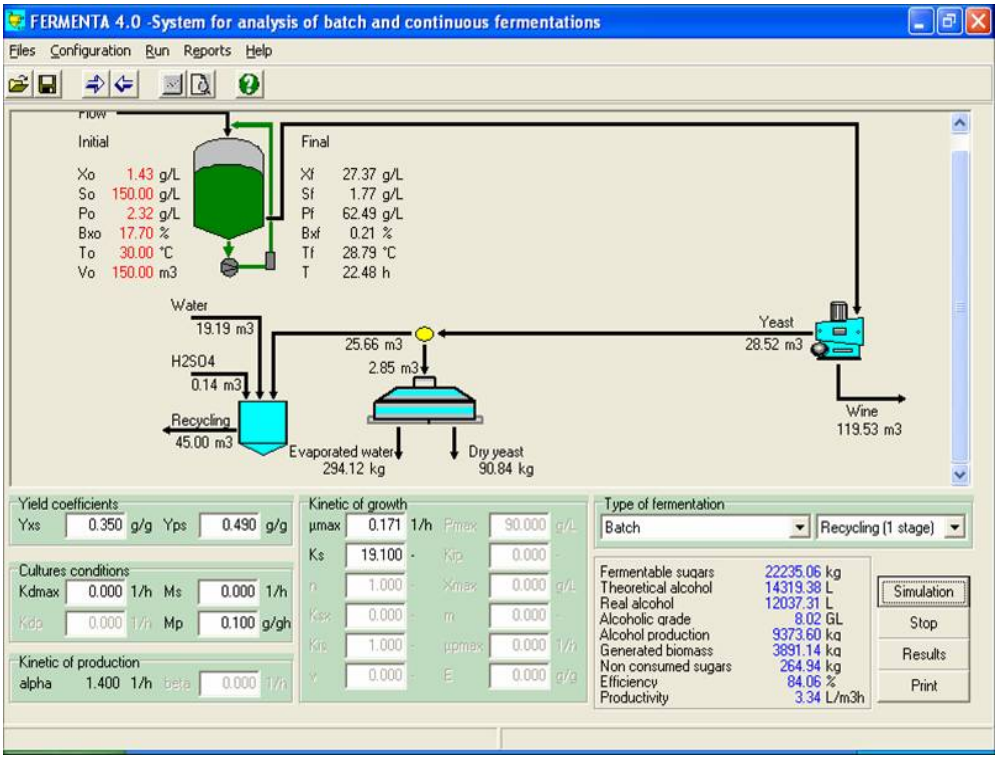


Fig. 2—FERMENTA 4.0 Main view.

In Figure 2, a simulation of an alcohol fermentation is shown, operated in batch mode with a fermenter capacity of 150 m³ and biomass recycle. The initial conditions of this fermentation are typical of most industrial alcohol fermentation.

- Initial substrate concentration 150 g/L
- Initial ethanol concentration 2.32 g/L
- Initial biomass concentration 1.43 g/L

The software also allows simulating the biomass recycle by the Mellé-Boinot batch process. To do this, it is necessary to give the information about the centrifuges used, number of nozzles and the efficiency (Figure 3).

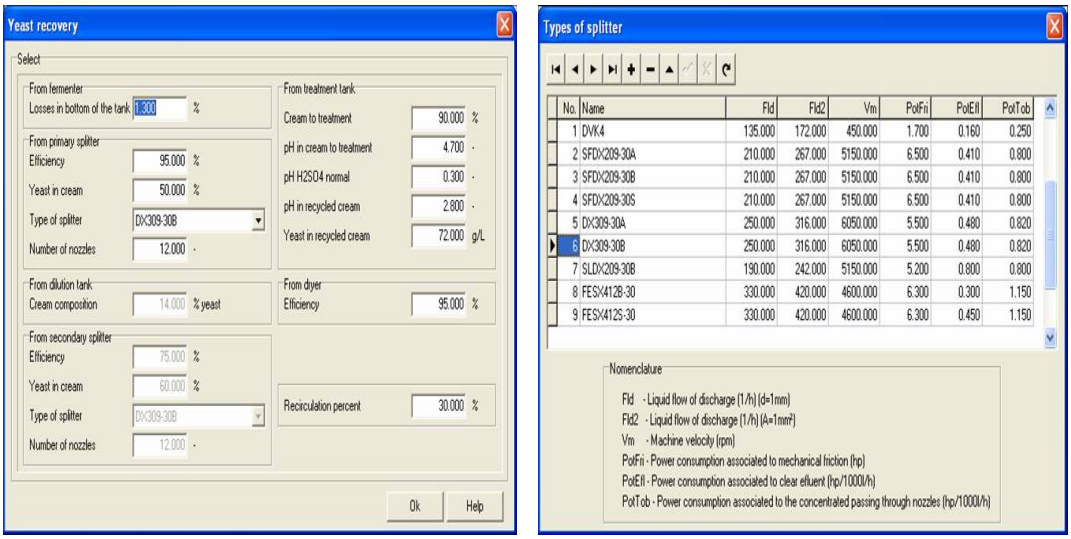


Fig. 3—Yeast recovery.

The fundamental characteristics of most used centrifuges in the ethanol industry are in a database. The calculations are carried out to obtain yeast with 92% dry matter, considering the drying efficiency in the balance.

Another important step before the simulation is to select the kinetics growth equation (Figure 4). You can see the equation of the selected growth model.

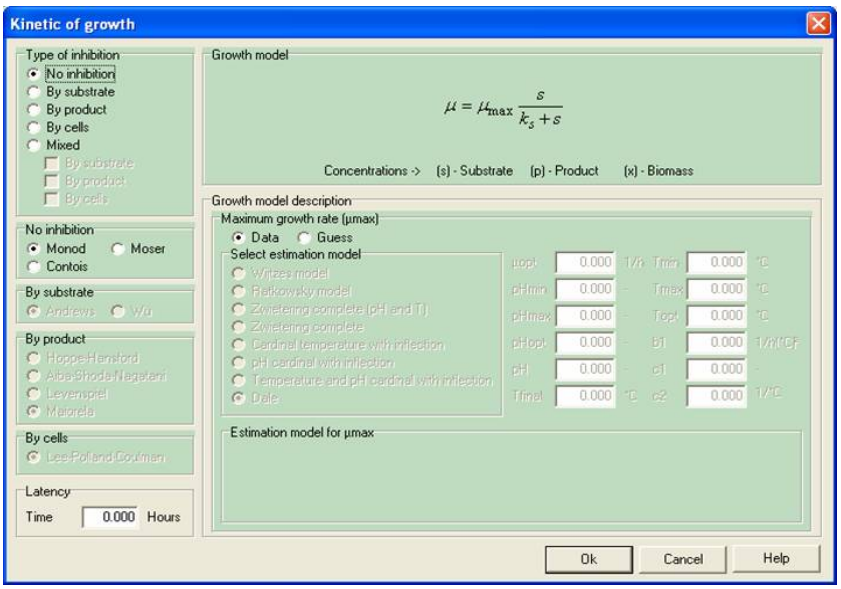


Fig. 4—Selection of growth kinetics.

In Figure 5, you can appreciate the kinetic behaviour of the fermentation. The results predict a fermentation time of 22 hours; in industrial alcohol fermentation, this parameter is estimated to be from 20 hours to 25 hours as mean. The concentration of substrate decreased from 150 g/L to less than 3 g/L. This value is very close to the final condition of many industrial alcohol fermentations. On the other hand, the ethanol concentration increased from 2.32 g/L to 62.5 g/L, which represents an ethanol content of wine equal to 8.06° GL. This parameter varies between 8°GL and 8.5° GL in real processes. The ethanol yields were 84.06% of the theoretical values and present a good productivity of 3.34 L/m^{3}h.

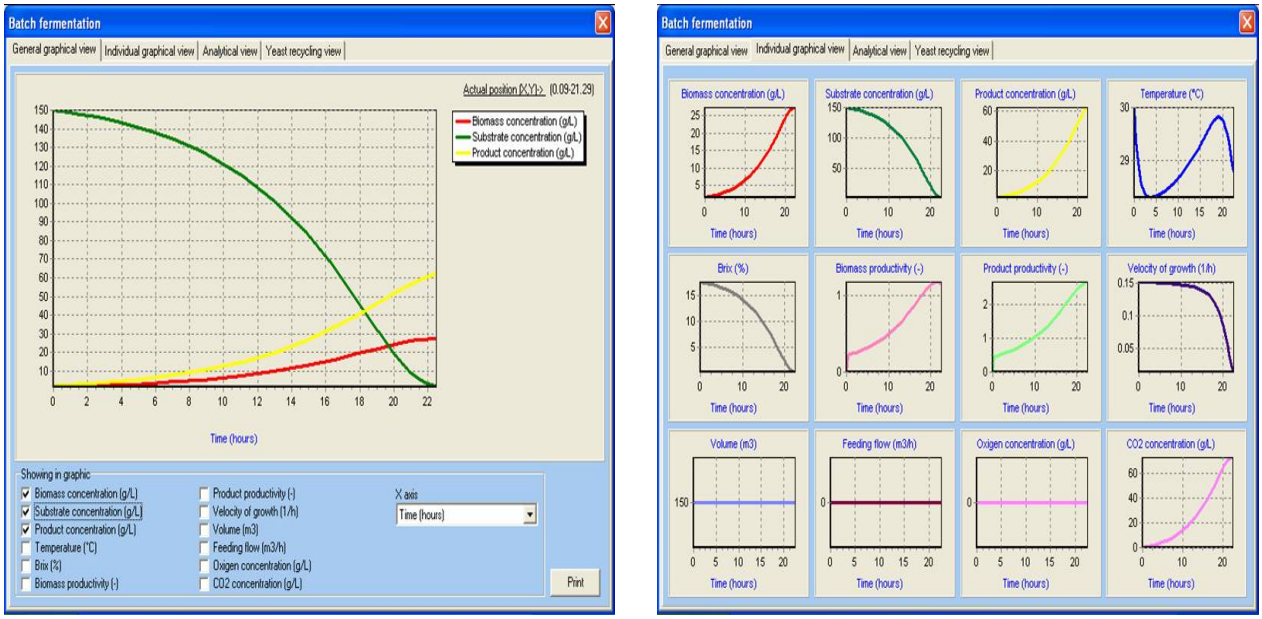
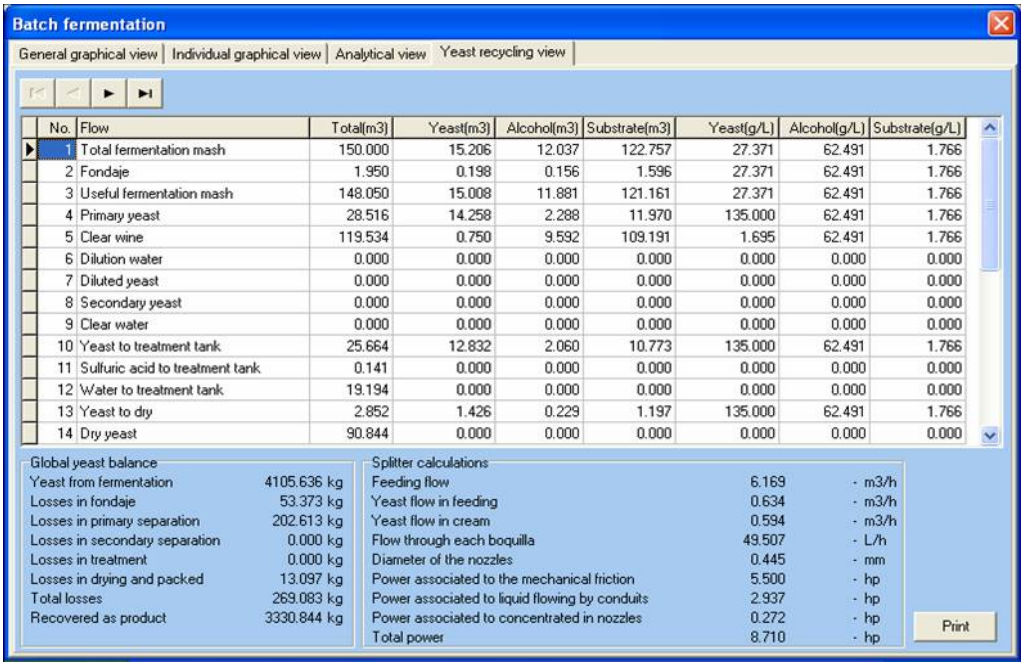


Fig. 5—Batch fermentation results, graphically.

The results of the yeast recovery scheme (Figure 6) indicate that a quantity of 4105.6 kg of yeast is produced by fermentation, recovering as product around 81%. The total power consumption of centrifuges was 8.7 HP.



No.	Flow	Total(m3)	Yeast(m3)	Alcohol(m3)	Substrate(m3)	Yeast(g/L)	Alcohol(g/L)	Substrate(g/L)
1	Total fermentation mash	150.000	15.206	12.037	122.757	27.371	62.491	1.766
2	Fondaje	1.950	0.198	0.156	1.596	27.371	62.491	1.766
3	Useful fermentation mash	148.050	15.008	11.881	121.161	27.371	62.491	1.766
4	Primary yeast	28.516	14.258	2.288	11.970	135.000	62.491	1.766
5	Clear wine	119.534	0.750	9.592	109.191	1.695	62.491	1.766
6	Dilution water	0.000	0.000	0.000	0.000	0.000	0.000	0.000
7	Diluted yeast	0.000	0.000	0.000	0.000	0.000	0.000	0.000
8	Secondary yeast	0.000	0.000	0.000	0.000	0.000	0.000	0.000
9	Clear water	0.000	0.000	0.000	0.000	0.000	0.000	0.000
10	Yeast to treatment tank	25.664	12.832	2.060	10.773	135.000	62.491	1.766
11	Sulfuric acid to treatment tank	0.141	0.000	0.000	0.000	0.000	0.000	0.000
12	Water to treatment tank	19.194	0.000	0.000	0.000	0.000	0.000	0.000
13	Yeast to dry	2.852	1.426	0.229	1.197	135.000	62.491	1.766
14	Dry yeast	90.844	0.000	0.000	0.000	0.000	0.000	0.000

Fig. 6—Batch fermentation results, table.

Sensitivity analysis.

With the help of FERMENTA 4.0 software, we decided to carry out an experimental factorial design with two controllable factors, the initial concentration of substrate (S_0) at three levels and the fermentation temperature (T) at two levels. The results are given in Table 1.

Table 1—Sensitivity analysis results.

Input value for model		Output values of model			
S_o (g/L)	T $\left({}^oC\right)$	*Efic.* (%)	Prod. $\left(L/m^3h\right)$	*Ethanol content.* $\left({}^oGL\right)$	A $\left(m^2\right)$
150	32	84.17	3.35	8.03	90
130	32	86.23	2.97	7.17	65
110	32	89.07	2.59	6.22	55
150	35	84.03	3.33	8.00	45
130	35	86.47	2.98	7.14	35
110	35	89.24	2.59	6.22	25

The sensitivity analysis shows the effect of the initial concentration of substrate on the productivity of the fermentation. When the initial substrate concentration decreased from 150 g/L to 110 g/L, the process productivity (Prod.), the ethanol content of wine (Ethanol content) and the necessary area heat transfer (A) decreased, too.

The industrial fermentations are very sensitive to the variation of media composition, presenting a similar tendency to the one described by the simulation.

Conclusions

Dynamic models for simulation of fermentation processes were developed and implemented in FERMENTA 4.0 software, which includes different equations representing cell growth rate. They are sufficiently general and can be applied to different practical scenarios.

FERMENTA 4.0 software allows process engineers and managers to perform different analyses, in order to select the best alternatives to operate the fermentation process.

In the case study, the sensitivity analysis option allowed studying the effect of initial substrate concentration variations on the efficiency, productivity and final alcoholic grade, as well as the temperature of fermentation on the necessary area of heat exchange.

The simulation results agree closely with typical data obtained on an industrial scale.

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UN LOGICIEL DE SIMULATION DES PROCEDES DE FERMENTATION

Par

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MOTS-clés : Procédé de Fermentation, Modélisation Mathématique, Production d'Éthanol, Logiciel de Simulation.

Résumé

LA FERMENTATION est une étape clé dans la production d'éthanol et a une incidence élevée dans l'efficacité du processus de la distillerie . Pour améliorer cette efficacité, une analyse rigoureuse pour définir le meilleur choix dans les variables de l'opération est indispensable. La simulation du processus est un moyen reconnu pour faire une telle analyse. Dans cette étude, le logiciel FERMENTA ® pour la simulation des procédés de fermentation est présenté. Ce logiciel permet la simulation des modes de fermentation continus et discontinus. Dans le mode de fonctionnement continu, plusieurs étapes peuvent être considérées. Dans le mode de traitement discontinu, des solutions alternatives d'alimentation peuvent être etudiées (batch, alimentation par batch selon differents protocoles). Le modèle mathématique comporte des equilibres transitoires pour la biomasse, le substrat, l'éthanol, l'oxygène et l'énergie. L'expression cinétique peut être sélectionnée par l'utilisateur parmi plus de 30 possibilités, en prenant ou pas en compte les facteurs d'inhibition dans le processus. Les paramètres dans cette équation peuvent être estimés à partir des données expérimentales. Le résultat est un logiciel utile qui peut être utilisé pour l'analyse de processus de fermentation de l'industrie et également à des fins académiques. Dans cette communication, le logiciel est décrit et certaines études de cas utilisées pour démontrer ses possibilités.

UN SOFTWARE PARA SIMULACIÓN DE PROCESOS DE FERMENTACIÓN

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PALABRAS CLAVE: Procesos Fermentativos, Modelación Matemática, Producción de Etanol, Software de Simulación.

Resumen

LA FERMENTACIÓN es la etapa clave en la producción de etanol y tiene una alta incidencia en la eficiencia total del proceso de la destilería. Para mejorar esta eficiencia se precisa un riguroso análisis para definir la mejor selección de las variables de operación. La simulación del proceso es una vía reconocida para realizar este análisis. En este trabajo se presenta la herramienta software FERMENTA® para simular el proceso de fermentación. Este software herramienta permite simular modos continuos y discontinuos de fermentación. En el modo continuo de operación se pueden considerar varias etapas. En el modo discontinuo se pueden analizar alternativas de alimentación (batch, batch incrementado con diferentes políticas). El modelo matemático incluye balances no-estacionarios para biomasa, sustrato, etanol, oxígeno, energía. La expresión cinética pude seleccionarse por el usuario entre más de 30 posibilidades, tomando ó no en cuenta factores inhibidores en el proceso. Los parámetros en esta ecuación pueden estimarse de los datos experimentales. El resultado es un útil 'software herramienta' que puede utilizarse para analizar el proceso de fermentación en la industria y también para propósitos académicos. En este artículo se describe el software y se emplean algunos estudios para mostrar sus posibilidades.