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Modelling, Simulation and Dynamic Analysis of the L-lysine Production Process

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Abstract

L-Lysine is an essential amino acid which can be produced by chemical processes from fossil raw materials, as well as by microbial fermentation, the latter being a more economical procedure. In the present work the fermentation process of L-lysine from sugarcane molasses is studied, using modelling and dynamic simulation tools to compare batch and fed-batch process, as well as to determine feasible operating regions in terms of productivity, product yield and fermentation time. These results will be used in the future for economic analysis, optimization studies and environmental impact.

Keywords: Modelling, Simulation, Biorefinery.

1. Introduction

Biorefineries are facilities that integrate biomass conversion processes and equipment to produce fuels, power and commodity chemicals (Luo et al., 2010). These can be classified by a number of features, such as the type of raw materials like starch crops (i.e. wheat and corn), sugar crops (i.e. sugar beet and cane), lignocellulosic crops (i.e. firewood forest and short rotation coppice), lignocellulosic residues (i.e. bagasse and straw), oil crops (i.e. palm and rapeseed), aquatic biomass (i.e. algae and seaweed) and organic waste (i.e. industrial, commercial and post-consumer) (Jong et al., 2012). Some benefits of biorefineries are: compensation of the cost of biofuels, improvement of process economics, minimization of waste discharge, reduction of dependence on oil products, and also providing new economic opportunities for agriculture and chemical industry (Fitzpatrick et al., 2010).

Among the basic chemicals obtained through biorefineries are the amino acids, and among these is the L-lysine that cannot be synthesized by the body, and is of great importance in human and animal; so it must be supplied in sufficient quantities by the daily diet (Eggeling and Sahm, 1999). The worldwide production volume of L-lysine is 850,000 t/y. It is estimated that in the future there will be high competition in the fermentative production of L-Lysine. For example, the global market for L-lysine has increased more than 20 times in the past two decades. The estimates assume that the market is currently increasing by 10 % ± 15 % per year (Leuchtenberger et al., 2005).

During the last decades the efficiency of the industrial production of L-lysine has been progressively increased isolation of high producing mutant strains and the development

of processes (Anastassiadis, 2007). Recently some studies about design, operation and sustainability have been report (Taras and Woinaroschy, 2012). However, optimization, monitoring, control activities including process engineering are still open research issues for this by-product. To carry out these studies is necessary to have a model and a dynamic analysis of the process. Moreover, the promotion of biotechnology and the (re) use of biomass have generated a broad field for the production of L-lysine from biomass.

The aim of this work is to study the production process of L-lysine from sugarcane molasses using modelling and dynamic simulation tools to compare batch and fed-batch process, as well as to determine feasible operating regions in terms of productivity, product yield and fermentation time. These results will be used in the future for optimization studies, economic analysis and environmental impact.

2. Process Model

The production process of L-lysine from glucose has been previously studied by Heinzle et al. (2007), where simulations were performed using an adaptable stoichiometric reaction based on the product yield and productivity. In the other hand, the kinetics and modelling of fermentation reactor for lysine has been reported by Büchs (1994). The production process has two sections: reaction section (which includes steps of pre-mixing, sterilization and fermentation) and purification section (which includes biomass filtration, water evaporation and product drying). In this work first the fermentation step of sugarcane molasses is studied, and then the complete production process is analyzed.

A dynamic model is developed based on mass and energy balances, describing the biological reaction activities and two types of fermentation operation (batch or fed-batch). Fermentation is carried out under aerobic conditions, using potassium phosphate (KH_2PO4) as phosphate source, ammonium hydroxide (NH_4OH) as a nitrogen source, *Corynebacterium glutamicum* as bacterium, and threonine is supplemented to the culture media in case of auxotrophic strains. The biological reaction is as follows:

$$\begin{aligned} &a\ C_6H_{12}O_6 + b\ KH_2PO_4 + c\ NH_4OH + d\ C_4H_9NO_3 + e\ O_2 \longrightarrow \\ &\quad f\ CH_{1.9}O_{0.3}N_{0.24}P_{0.02}K_{0.01} + g\ C_6H_{14}N_2O_2 + h\ H_2O + i\ CO_2 + j\ K \end{aligned} \tag{1}$$

The dynamic reactor model is given by the following six differential equations:

$$\frac{dc_s}{dt} = -\frac{1}{Y_{x/s}}\mu c_x - \frac{1}{Y_{p/s}} r_p c_x - m_s c_x + \frac{F}{V} c_{SF}, \quad \mu = \mu_{\max} \frac{c_s}{c_s + K_s} \cdot \frac{c_L}{c_L + K_o} \cdot \frac{c_{Thr}}{c_{Thr} + K_{Thr}} \tag{2}$$

$$\frac{dc_p}{dt} = r_p\ c_x, \qquad r_p = (\alpha_p \cdot \mu + \beta_p) \cdot \frac{C_S}{C_S + K_{ps}} \cdot \frac{C_L}{C_L + K_O} \tag{3}$$

$$\frac{dc_L}{dt} = -\frac{1}{Y_{x/o}}\mu\, c_x - \frac{1}{Y_{p/o}} r_p\ c_x - m_o\ c_x + OTR, \qquad OTR = k_{La}\left(L_{O2} p\ y_{O_2} - C_L\right) \tag{4}$$

$$\frac{dc_x}{dt} = (\mu - k_d)\, c_x\,; \qquad \frac{dc_{Thr}}{dt} = -\frac{1}{Y_{x/Thr}}\mu\, c_x\,; \qquad \frac{dV}{dt} = F \tag{5}$$

Table 1. Parameters for the dynamic model of L-lysine fermentation

Parameter	Value	Units	Parameter	Value	Units	Parameter	Value	Units
$Y_{x/s}$	0.52	g/g	k_d	0.0028	g/L	m_o	0.036	g/L
$Y_{p/s}$	0.6	g/g	K_o	6.4×10^{-6}	g/L	m_s	0.034	g/L
$Y_{x/Thr}$	0.33	g/g	K_s	0.01	g/L	α	0.2	g/g)
$Y_{p/o}$	4.11	g/g	K_{Thr}	0.01	g/L	β	0.043	g/g h
$Y_{x/o}$	1.29	g/g	K_{ps}	0.072	g/L	y_o	0.2095	mol/mol
μ_{max}	0.28	1/h	L_{O2}	0.00118	mol/L/bar			

Where c_S, c_{SF}, c_x, c_{Thr} ,c_L are concentrations of substrate in the reactor, substrate in the feed, biomass, threonine and oxygen in the reactor, respectively; μ is the specific growth rate, r_p is the rate of lysine production, m_s and m_o are specific consumption of substrate and oxygen for maintenance, respectively; $Y_{p/o}$, $Y_{p/s}$, $Y_{x/s}$, $Y_{x/o}$, $Y_{x/Thr}$ are the product and biomass yields; *OTR* is the oxygen transfer rate, V is the fermentation volume, and F is the feedflow. The corresponding parameter values are given in Table 1.

3. Simulation results for the fermentation section

As mentioned before, we are interested in the comparison of batch and fed-batch cases, and also in determining regions of operation leading to high product yields (equivalent to high product concentrations) and high productivities. For simulations purposes, the operation conditions were: $V_{reactor}$ = 300,000L, K_{La} = 1,000 s^{-1}, P = 1 atm. The initial conditions for batch operation mode were set at: $C_{S,0}$ = 200 g /L, $C_{P,0}$ = 0, $C_{L,0} = C_{L,max}$, $C_{x,0}$ = 0.1 g/L, $V_0 = V_{reactor}$. For fed-batch operation conditions were: $C_{S,0}$ = 35 g/L, $C_{P,0}$ = 0, $C_{L,0} = C_{L,max}$, $C_{x,0}$ = 0.1 g/L, $V_0 = 0.75 V_{reactor}$ (the reactor is initially filled to 75% volume, then a glucose solution with C_{SF} = 700 g/L is added up to 100%). The initial threonine concentration (C_{Thr}) was varied in the range 0.6-2 g/L.

Some results are shown in Figure 1, where dynamic behaviour of concentrations for batch and fed-batch are compared using C_{Thr} = 1.6 g/L. The final fermentation time (θ_R) for batch operation was determined when product concentration achieved a maximum value. While for fed-batch operation, the reactor volume was constant (at 75 % filling) until the substrate (glucose) is exhausted, then a glucose solution is added until the reactor is filled to 100 % (this point defines θ_R). In both operation modes, the same amount of glucose was added, corresponding to a total glucose concentration of $C_{S,T}$ = 200 g/L. In Figure 1, it can be seen that the final Lysine concentrations, C_P (θ_R), are similar in both cases: 44 g/L for batch and 38.3 g/L for fed-batch; while θ_R are quite different: 35.3 h for batch and 62.5 h for fed-batch.

The overall product yield ($Y_{p/s}$) and the productivity (i.e. space time yield, STY) were calculated as: $Y_{p/s} = C_P(\theta_R)/C_{S,T}$ and STY = $C_P(\theta_R)/\theta_R$. Values of these two parameters are shown in Figure 2. Maximum productivity for batch operation (1.25 g $h^{-1}L^{-1}$) is achieved when C_{Thr} = 1.6 g/L, while fed-batch case achieves the maximum (0.87 g $h^{-1}L^{-1}$) at C_{Thr} = 1 g/L. However at the maximum point, the product yield for batch operation (0.218 g/g) is minor than fed-batch operation (0.31 g/g). This means that there is a trade-off between product yield and productivity.

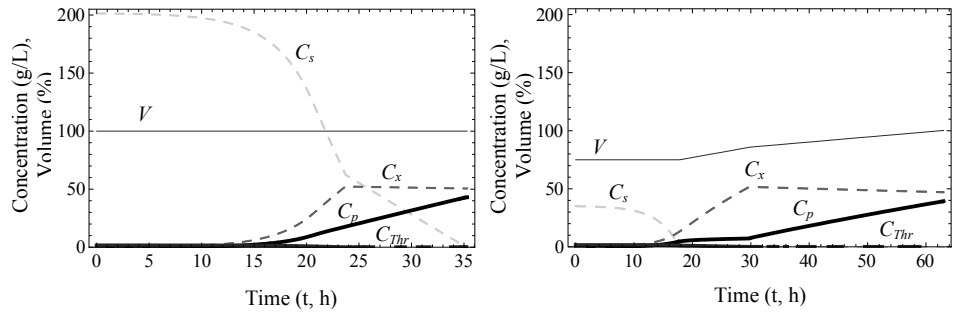

Figure 1. Dynamic behaviour of the fermentation reactor to produce L-lysine: (a) batch operation and (b) fed-batch operation, under similar conditions ($c_{Thr,0}$ = 1.6 g/L).

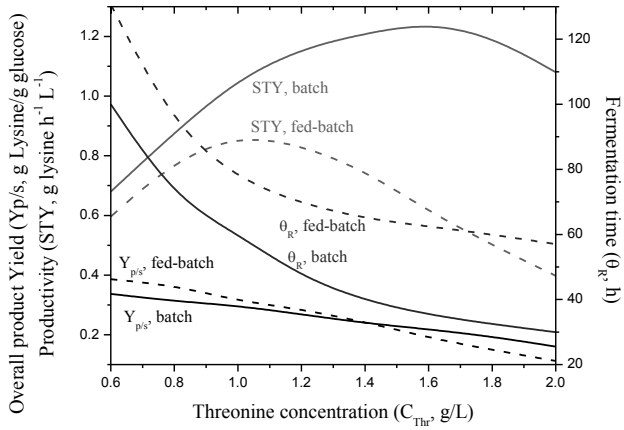

Figure 2. Overall product yield, productivity and reaction time for batch and fed-batch operations.

4. Simulation results for the production process

The production process was divided into four sections, as shown in Figure 3: (a) preparation of molasses, (b) preparation of the culture medium, (c) fermentation, and (d) purification. The process simulation was implemented in SuperPro Designer ®. In the first section, the molasses is diluted in water, then part of the impurities is removed by filtration and another is eliminated using a column of ion exchange chromatography. The resulting solution will be added to the reactor after being heat sterilized. In the section of the culture medium preparation, the nutrients are dissolved in water and heat sterilized. Then they are also transferred to the fermentation vessel. The fermentation time, yield and productivity depends on the strain of *Corynebacterium glutamicum* and threonine concentration, as shown in previous section. So that the stoichiometric reaction is derived for different values of C_{thr}, using the results obtained in by dynamic simulation. Molar stoichiometric coefficients of reaction (1) are given in Table 2 for some cases. The fermentation reaction is achieved at 35 °C, so that cooling water is used to remove heat from exothermic fermentation processes and to maintain a constant temperature. Once fermentation is complete, the broth is discharged into a tank acting as a buffer between the fermentation section and the purification section. Purification begins with the removal of the biomass by a rotary filter. The clarified liquor is then

concentrated in an evaporator and cooled to 35 °C using a heat exchanger. This is then sent to a neutralization vessel and crystallization. There, the solution is neutralized with HCI solution and cooled to 15 °C in order to crystallize the L-lysine salt. The slurry solids are retained by a rotary vacuum filter and rinsed with water to remove remaining impurities. The wet crystals are dried, and the final product has about 99.8 % pure crystals of lysine-HCl.

From mass and energy balances, production rate and energy consumption was analyzed in order to understand the operation conditions. It is important to mention that due space limitations only some insights are presented. For instance, energy consumption in terms of utility amounts and costs are shown in Table 3, while Table 4 reports production rate, recipe time and energy cost. According these results, it can be seen that: (i) in a batch process, higher threonine concentrations requires minor energy, but also minor production rate (due to low product yields); (ii) a fed-batch operation requires more energy than a batch operation, and production rate is slightly higher. So that for these preliminary results, it can be concluded that the batch operation mode with C_{thr} = 1 g/L is the best case analyzed in terms of production rate and energy consumption.

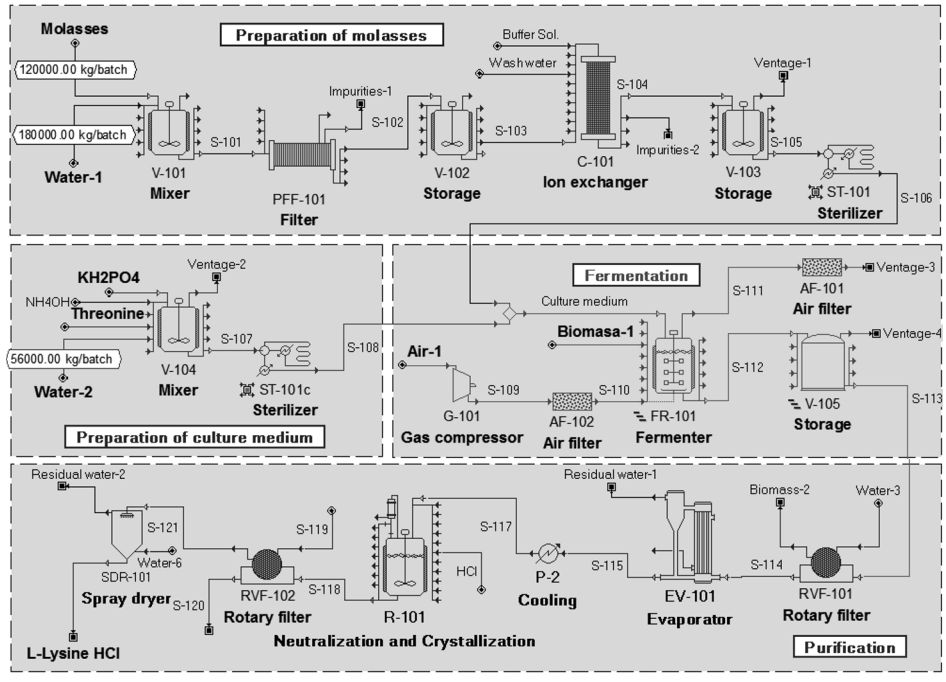

Figure 3. Flowsheet for the production process of L-lysine .

Table 2. Molar stoichiometric coefficients of fermentation reaction (1).

Operation mode	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*
Batch (C_{Thr} = 1.6 g/L)	1.000	0.039	0.996	0.012	1.863	1.957	0.269	4.840	2.475	0.020
Batch (C_{Thr} = 1 g/L)	1.000	0.022	0.985	0.007	2.193	1.080	0.367	4.925	2.749	0.011
Fed-batch (C_{Thr} = 1 g/L)	1.000	0.020	1.013	0.007	2.116	1.025	0.387	4.902	2.683	0.010

Table 3. Energy consumption in terms of utility amounts and costs.

	Batch (C_{Thr} = 1.6 g/L)		Batch (C_{Thr} = 1 g/L)		Fed-batch (C_{Thr} = 1 g/L)	
Utility	Amount	Cost ($)	Amount	Cost ($)	Amount	Cost ($)
Standard Power	23,805 kW-h	2,380	40,326 kW-h	4,033	51,590 kW-h	5,159
Steam	632 MT	177	647 MT	181	647 MT	181
Chilled Water	156 MT	27	171 MT	30	173 MT	30
Cooled water	37,897 MT	947	39,535 MT	988	40,654 MT	1,016
TOTAL		3,532		5,232		6,387

Table 4. Production rate, recipe time and energy cost.

Operation mode	*Production rate (Kg/batch)*	*Recipe Time (h/batch)*	*Energy Cost ($/Kg)*
Batch (C_{Thr} = 1.6 g/L)	16,097	111.9	0.219
Batch (C_{Thr} = 1 g/L)	21,940	136.6	0.238
Fed-batch (C_{Thr} = 1 g/L)	23,161	153.6	0.276

5. Conclusions

The presented methodology allows understanding the effect of parameters and operating conditions in the Lysine production process. As future work is planned to perform the process simulations with rigorous kinetics, and analyze the economic evaluation of the whole plant. The modelling and simulations can be used for: synthesis and process optimization, monitoring and controlling the process to maintain the level of production and product quality, fault detection, etc.

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