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Simple Kinetic Model of Cellulase Production by *Trichoderma Reesei* for Productivity or Yield Maximization

Etienne Jourdier[a], Fadhel Ben Chaabane*[a], Laurent Poughon[b], Christian Larroche[b], Frédéric Monot[a]

[a]IFP Energies nouvelles, 1 et 4 avenue de Bois-Préau, 92852 Rueil-Malmaison, France.
[b]Clermont Université, Université Blaise Pascal, Institut Pascal, Polytech Clermont-Ferrand, 24 av. des Landais, BP 20206, 63174 Aubière cedex, France.
fadhel.ben-chaabane@ifpen.fr

A simple kinetic model of cellulase production by *Trichoderma reesei* on lactose was developed and parameters identified using bibliographic and personal data. Assuming a mean representation of industrial hyperproducer strains, this model was used to simulate and compare several cultivation strategies regarding three criteria: in addition to usual productivity and yield calculation, oxygen demand was studied and used as a constraint for protocol design.
Results showed that none of the protocols maximizes both productivity and yield, requiring to find a compromise for these parameters. Moreover, substrate concentration in the feed was the main criterion for the choice of the protocol, while oxygen demand and biomass concentration were the main issues to reach high productivities. The model will be useful for an economic study of cultivation strategies.

1. Introduction

Biological conversion of ligno-cellulosic materials into biofuels or chemicals requires the use of cellulolytic (or cellulases) and hemicellulolytic enzymes to hydrolyse cellulose and hemicellulose into fermentable sugars. Cellulases represent a large array of enzymes with activity against plant cell wall, and are naturally produced by plant pathogens (Gibson et al., 2011). Among these microorganisms, *Trichoderma reesei* is currently recognized as the best industrial producer (Philippidis, 1994), owing to its very high protein secretion capacity, and it has been successfully cultivated at industrial scale up to at least 30 m^3 (Ballerini et al., 1994).
Since cellulase production is repressed by the presence of readily-metabolized sugars, *T. reesei* cultivation must be conducted under carbon limitation, in fed-batch or continuous modes (Tolan and Foody, 1999) to maximize enzyme production. As a substitute to the natural inducer cellulose, which is hard to handle and control at an industial scale, lactose is the most usual soluble inducer and carbon source used for industrial cellulase production (Warzywoda et al., 1988).
Although industrial fed-batch protocols produce high protein levels, cellulase production is still the main economic bottleneck in biological lignocellulose conversion processes. In the case of a cellulosic ethanol plant from corn stover with in-situ cellulase production using *T. reesei*, the two major ways to reduce the ethanol production cost are (after feedstock cost) enzyme production cost and enzyme loading for hydrolysis (Kazi et al., 2010).
Three criteria are important for the economic study of a biological process: productivity affects investment costs (fermentor cost), yield affects operating costs (substrate cost), and oxygen demand

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affects both investment costs (motor and compressor) and operating costs (electric power for stirring and aeration)
In this study, a simple *T. reesei* kinetic model was built based on bibliography review and accurate kinetic study, then used to compare different cultivation strategies regarding productivity, yield and oxygen demand.

2. Kinetic model

An identical growth phase was assumed for all strategies and only the protein production period was studied.

2.1 Protein production model

Since protein production was known to be maximum under carbon limitation, this study assumed production in fed-batch or continuous mode with low feeding rate so that residual substrate concentration remained null in the bioreactor. The fed substrate was directly consumed by cells, giving Eq (1).

$$\frac{dSV}{dt} = 0 = F_{in}S_{in} - qS.X.V \quad (1)$$

A generalized expression of Pirt law was assumed for substrate utilization. It fictitiously divided substrate uptake into three metabolic processes: growth, protein production and maintenance (Muthuvelayudham and Viruthagiri, 2007).

$$qS = \frac{\mu}{Yx^{\circ}} + \frac{qP}{Yp^{\circ}} + m \quad (2)$$

In chemostat studies, (Allen and Andreotti, 1982) as well as (Pakula et al., 2005) observed an almost constant and maximum specific protein production rate for dilution rates between 0.02 and about 0.04 h^{-1}. (Tolan and Foody, 1999) assumed it was also constant for growth rates from 0 to 0.02 h^{-1}.
Therefore most studies considered an uncoupled production model (Lo et al., 2010; Velkovska et al., 1997), which was assumed valid here for low growth rates between 0 and 0.03 h^{-1} (Eq. 3).

$$qP = \beta \quad (3)$$

2.2 Parameters identification

During growth on lactose, a maximal yield Yx° of 0.6 $g.g^{-1}$ was generally observed both at high growth rates (Lo et al. 2010), and at low growth rates (Pakula et al., 2005), so this value was assumed to be valid for this work. Bibliographic and personal data (submitted for publication) for cellulase production by *T. reesei* on lactose were used for the identification of the other parameters (Table 1). Since parameters were consistent between strains, a mean strain representative of hyperproducing strains was hypothesized and used for simulations.

Table 1: parameters identification, and values chosen for this study, assuming Yx° = 0.6 $g.g^{-1}$.

strain	cultivation mode	Yp° $g_P.g_S^{-1}$	m $mg_S.g_X^{-1}.h^{-1}$	qP $mg_P.g_X^{-1}.h^{-1}$	references
MG80	continuous	0.60	10.8	16.9	(Nicholson et al. 1989)
VTT D-99676	continuous	0.60	8.3	19.9	(Bailey and Tahtiharju 2003)
CL 847	fed-batch	0.58-0.62	8-12	12-18	(submitted for publication)
mean strain		0.6	10	15	hypothesis for simulations

2.3 Oxygen transfer

The minimal oxygen transfer coefficient K_La needed to keep a constant dissolved oxygen concentration C_{O2}, could be estimated by Eq. 4:

$$K_L a = \frac{rO_2}{C_{O2} - C*} \quad (4)$$

where C* was oxygen concentration at saturation (8 $mg.L^{-1}$) and the oxygen uptake rate rO_2 was calculated using mass balance owing to mean biomass and protein concentration (data submitted for publication). To avoid oxygen limitation, a minimal and constant dissolved oxygen concentration of 20 % saturation was assumed.

2.4 Simulations

Simulations were performed using Matlab® v7.1 or Microsoft® Office Excel 2003, with 0.24 h time step.

3. Results and discussion

3.1 Continuous mode – steady state

Simulations were performed for dilution rates from 0.01 to 0.03 h^{-1} and sugar concentrations in feed from 20 to 100 $g{\cdot}L^{-1}$. As expected, productivity depended only on the biomass concentration in the bioreactor (with proportional factor qP) but since minimal K_La was also almost proportional to biomass, high productivities in continuous mode will be limited by oxygen transfer (Figure 1A). Interestingly, since specific protein production rate was assumed to be constant, yields depended only on the dilution rate (Figure 1B), with protein yields ranging from 0.18 to 0.29 $g_P{\cdot}g_S^{-1}$.

3.2 Fed-batch mode

Simulations of fed-batch mode with constant feed rate showed that the physiology approached an equilibrium for which specific substrate uptake rate was stabilized and specific growth rate was null (Figure 2A). Biomass was constant in mass, and protein mass increased linearly. Accordingly this protocol was quite suitable for new strains with unknown specific protein production rate since biomass will automatically adapt to the substrate flux to transform it in proteins. Thereby protein yield was maximized, as expected for fed-batch mode.

However, due to dilution by the feed, the total working volume of the bioreactor was not used. The increase in volume led to a decrease in the concentration of producing biomass and, as a result, the protein concentration was not linear (Figure 2B). Moreover, the less concentrated the substrate feed, the more pronounced this effect. Therefore, productivity was much lower than in continuous mode, especially when substrate concentration was low (Figure 3 black).

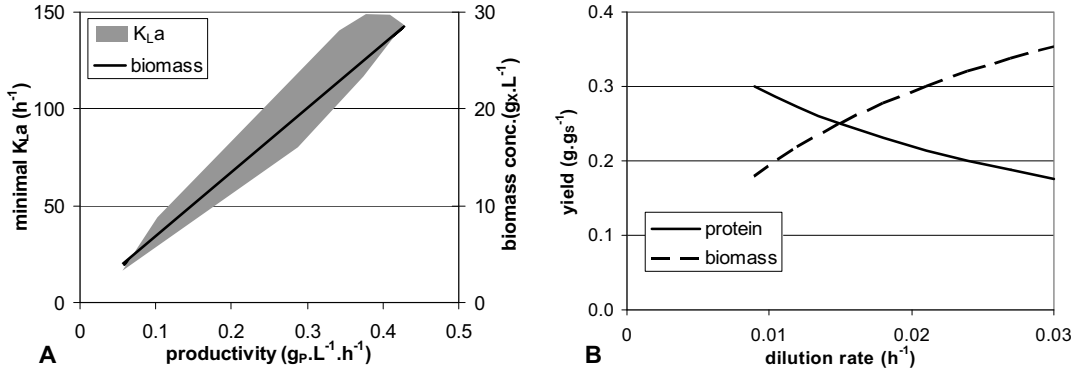

Figure 1: Continuous mode simulation at steady state: (left) biomass concentration (black line) and minimal Kla (gray area) – (right) yields for biomass (dashed line) and protein (plain line)

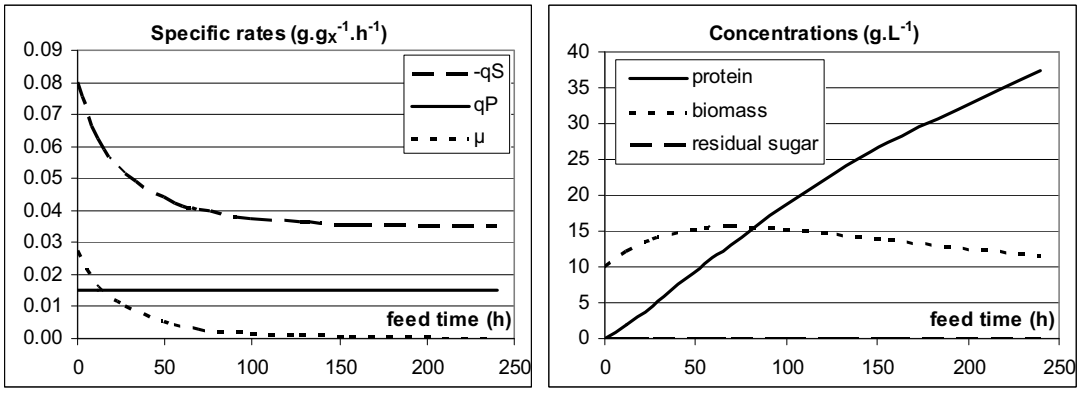

Figure 2: Fed-batch mode simulation. Specific rates (left) and concentration (right) for protein (plain line), biomass (dotted line) and substrate (dashed line).

One possibility to obtain better productivities in fed-batch mode was to achieve a constant biomass concentration by using an exponential feed rate. However, since substrate was used for growth, protein yield would be lower. In our study case (15 g·L^{-1} initial biomass), this strategy resulted in around15 % higher productivity, with near 10 % lower yield (Figure 3). However, it should be pointed out that with this strategy the difference between initial and final volumes was even higher than before, which might be an issue for stirring design.

3.3 Strategies comparison under constraints

This model was used to compare the 3 previous protocols under some typical constraints to find out which protocol would be best suited for industrialisation. Since a high biomass concentration could lead to high viscosity, cell dry weight concentration was limited to 20 g·L^{-1}. In order to avoid high volume change during fed-batch, the initial volume was at least 50 % of the final volume. Since oxygen transfer might be costly in an aerobic process (for stirring and aeration), a maximum oxygen transfer coefficient K_La of 80 h^{-1} was assumed.

Under these hypothesis, simulations were performed to compare continuous mode, constant fed-batch and exponential fed-batch at different substrate feed concentrations. Results for the maximum productivity and yield obtained under these constraints were presented in Figure 4.

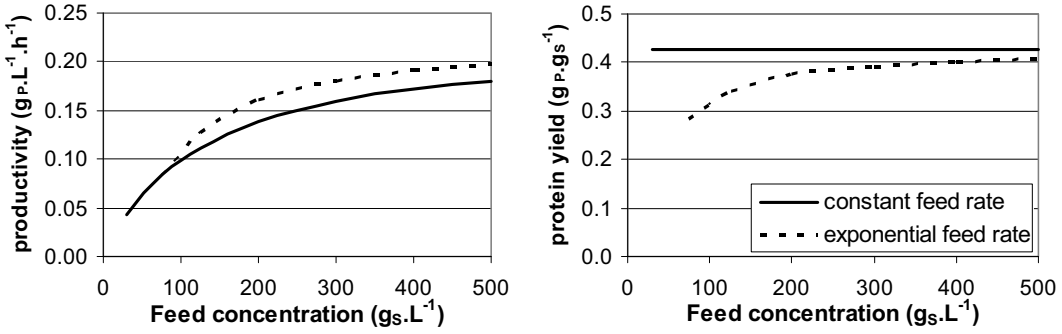

Figure 3: 240 h fed-batch phase productivity (left) and yield (right) for constant feed rate maximizing yield (plain line) or exponential feed rate for constant biomass concentration (dotted line). Initial biomass concentration X_0 = 15 g·L^{-1}.

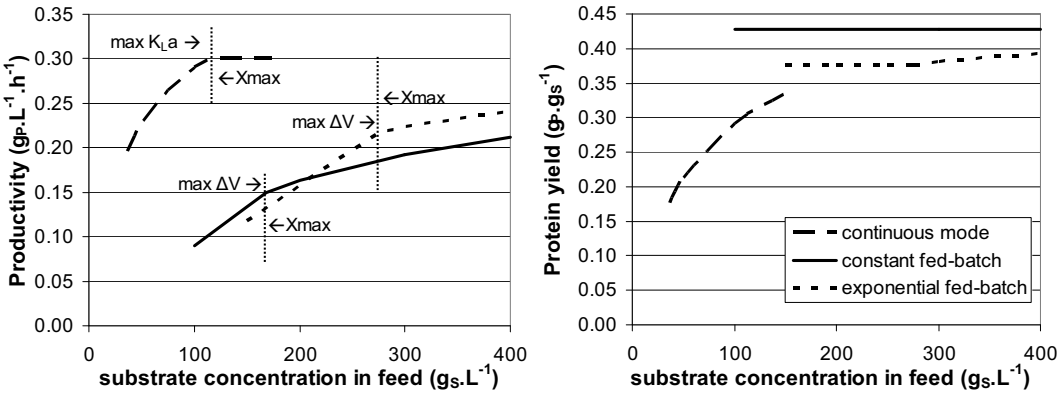

Figure 4: Maximal productivity (left) and yield (right) for three different cultivation modes: constant rate fed-batch (plain line), exponential rate fed-batch (dotted line), and continuous mode (dashed line) under constraints ($X < 20\ g{\cdot}L^{-1}$; $K_La < 80{\cdot}h^{-1}$; $\Delta V < 100\ \%$).

In the fed-batch mode, the productivity was limited by maximum biomass (at high substrate concentration) or volume change (at low substrate concentration). Protein yield was always maximized when applying a constant rate, and slightly lower for an exponential feed. For both protocols, substrate feed below 150 g·L-1 led to very low productivities.
The continuous mode was more suitable for low feed concentration, and led to high productivities. However it was limited by maximum biomass at high substrate concentration in feed (and low dilution rate), and by oxygen transfer at low substrate concentration (and higher dilution rate). Protein yield was lower than in fed-batch mode and dropped at high dilution rate (low substrate concentration).
Interestingly, industrial constraints chosen here had an impact almost only on productivity. Indeed, protein yield depended only on growth rate (see Figure 1B), which is determined by outlet rate, so did not depend on volume change, biomass or oxygen demand. In particular oxygen transfer might limit productivity in continuous mode but had no impact on protein yield.

4. Conclusion

A study of the bibliographic data showed that the specific cellulase production rate was constant at low growth rate for highly inducing soluble sugars like lactose. Then a simple kinetic model was developed to describe cellulase production by *T. reesei* and its parameters were determined using bibliography and personal results. This model was used to simulate and compare different cultivation strategies under industrial constraints. Even though some results were expected (fed-batch maximizes yield, continuous mode maximizes productivity), other ones were new and specific to *T.reesei* cellulase production. In particular in continuous mode, the yield depended only on the dilution rate, and oxygen transfer was a very important issue since it might limit the productivity. In fed-batch mode, productivity was much lower and could be limited by volume increase.
As a result, there is no optimal strategy for the three criteria of main importance for industrial design, namely productivity, yield and oxygen demand. Therefore the cheaper approach for cellulase production by *T. reesei* is not intuitive and this model will be used for an economic comparison of the different strategies.

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Nomenclature

V: Bioreactor volume (L)
X: Biomass concentration in bioreactor ($g_X{\cdot}L^{-1}$)

S: Residual substrate concentration in bioreactor ($g_S \cdot L^{-1}$)
S_{in}: Substrate concentration in feed ($g_S \cdot L^{-1}$)
F_{in}: Inlet flow rate ($L \cdot h^{-1}$)
D: Dilution rate (h^{-1})
μ: Biomass growth rate (h^{-1})
qP: Specific protein production rate ($mg_P \cdot g_X^{-1} \cdot h^{-1}$)
qS: Specific substrate uptake rate ($mg_S \cdot g_X^{-1} \cdot h^{-1}$)
m: Maintenance coefficient ($mg_S \cdot g_X^{-1} \cdot h^{-1}$)
Yx°: Maximal biomass yield ($g_X \cdot g_S^{-1}$)
Yp°: Maximal protein yield ($g_P \cdot g_S^{-1}$)
K_La: Oxygen transfer coefficient (h^{-1})
rO_2: Volumetric oxygen uptake rate ($g \cdot L^{-1} \cdot h^{-1}$)
C_{O2}: Dissolved oxygen concentration in bioreactor ($g \cdot L^{-1}$)
C*: Dissolved oxygen concentration at saturation ($g \cdot L^{-1}$)

References

Allen A.L., Andreotti R.E., 1982, Cellulase Production in Continuous and Fed-Batch Culture by Trichoderma-Reesei Mcg80, Biotechnol. Bioeng., 451-459.

Bailey M.J., Tahtiharju J., 2003, Efficient cellulase production by Trichoderma reesei in continuous cultivation on lactose medium with a computer-controlled feeding strategy, Appl. Microbiol. Biotechnol., 62(2-3), 156-162.

Ballerini D., Desmarquest J.P., Pourquie J., Nativel F., Rebeller, M., 1994, Ethanol Production From Lignocellulosics - Large-scale Experimentation and Economics, Bioresour. Technol., 50(1), 17-23.

Gibson D.M., King B.C., Hayes M.L., Bergstrom G.C., 2011, Plant pathogens as a source of diverse enzymes for lignocellulose digestion, Curr. Opin. Microbiol., 14(3), 264-270.

Kazi F.K., Fortman J.A., Anex R.P., Hsu D.D., Aden A., Dutta A., Kothandaraman G., 2010, Techno-economic comparison of process technologies for biochemical ethanol production from corn stover, Fuel, 89, S20-S28.

Lo C.M., Zhang Q., Callow N.V., Ju L.K., 2010, Roles of extracellular lactose hydrolysis in cellulase production by Trichoderma reesei Rut C30 using lactose as inducing substrate, Process Biochem., 45(9), 1494-1503.

Muthuvelayudham R., Viruthagiri T., 2007. Optimization and modelling of cellulase protein from Trichoderma reesei Rut C30 using mixed substrate, Afr. J. Biotechnol., 6, 41-46.

Nicholson C., Foody B., Foody P., Malloch E., Malloch L., Balon J., 1989, Proceedings 7th Canadian Bioenergy Seminar, Conseil national de recherches du Canada.

Pakula T.M., Salonen K., Uusitalo J., Penttila M., 2005, The effect of specific growth rate on protein synthesis and secretion in the filamentous fungus Trichoderma reesei, Microbiology-Sgm., 151, 135-143.

Philippidis G.P. 1994, Cellulase Production Technology, In: Enzymatic Conversion of Biomass for Fuels Production, 566 ed. American Chemical Society, 188-217.

Tolan J., Foody B., 1999, Cellulase from Submerged Fermentation, In: Recent Progress in Bioconversion of Lignocellulosics, 65 ed. Eds. Tsao G. et al., Springer Berlin / Heidelberg, Germany, 41-67.

Velkovska S., Marten M.R., Ollis D.F., 1997, Kinetic model for batch cellulase production by Trichoderma reesei RUT C30, J. Biotechnol., 54(2), 83-94.

Warzywoda M., Ferre V., Pourquie J., 1988, Process for producing cellulolytic enzymes, US4762788, Institut Français du Pétrole, Rueil-Malmaison France, (patent).