MODELLING AND OPTIMIZATION OF FED-BATCH FILAMENTOUS FUNGAL FERMENTATION

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Abstract: This paper presents a model-based optimization study for enzyme production in a filamentous fungal fermentation. An existing morphologically structured model is amended by including the effect of low levels of oxygen on growth and production, as well as the effect of medium rheological properties on the oxygen transfer. These changes are needed to extend the applicability of the model to fed-batch operation. Based on this model, optimal feeding strategies are proposed for improving the performance of the process compared to its current operation in industry. The main result is that it is better to reduce the initial substrate concentration (and also the amount of biomass formed), which goes against expected intuition that having more catalyst (biomass) available will improve the performance optimization. Yet, with this strategy, less dead biomass is formed and there is no oxygen limitation problem.

Keywords: hybrid modelling, rheological properties, numerical optimization, filamentous fungal fermentation, enzyme production

1. INTRODUCTION

Filamentous fungi are extensively used in the fermentation industry for the production of a large number of products including primary metabolites, antibiotics, industrials enzymes and proteins (McIntyre *et al.*, 2001).

Filamentous fungal fermentations are traditionally carried out in fed-batch mode, operated at moderate to high biomass concentrations in order to increase production. The growth of filamentous microorganisms is achieved by tip extension and branch formation. The result is microscopic morphology characterized by a network of entangled filaments, forming clumps of different degree of compactness. The operation from moderate to high biomass concentrations induces high viscosity and non-Newtonian behaviour in the fermentation medium (Li *et al.*, 2000). This leads to problems of poor mixing as well as heat and mass transfer limitations, including oxygen limitation.

The filamentous fungal (α-amylaze producing strain of *Aspergillus Oryzae*) fermentation process studied in this paper was modeled in (Agger *et al.*, 1998). This model is a morphologically structured one, providing a description of biomass, glucose and enzyme concentrations of submerged

cultures of filamentous fungi, under no limiting oxygen conditions, for continuous reactors.

In order to increase the range of applicability of this model for optimization purposes of fed-batch cultivations, the influence of the dissolved oxygen limitation on the biomass and enzyme production has to be incorporated. Thus, the first objective of this paper is to include these effects and to fit and validate the model on experimental data provided by Novozymes, Bagsvaerd, Denmark.

The performance of fed-batch fermentation processes can typically be increased by the use of model-based optimization techniques. Using a fairly general model structure, many useful results have been derived in (Modak *et al.*, 1986; Van Impe *et al.*, 1994; Claes *et al.*, 1999). One key result is that, in many cases, the final product yield (ratio of the amount of product formed and the amount of substrate consumed) can be maximized simply by maximizing the instantaneous yield. This can be achieved by maintaining the substrate concentration at an optimal level s^*.

It was shown, however, in (Bodizs *et al.*, 2004) that this is not true for the filamentous fungal fermentation process, modeled in (Agger *et al.*, 1998). In this case, s^* that maximizes the instantaneous yield is very low and requires accordingly a very low feed flow rate. This leads, in turn, to a very long operation time, that exceeds the time limit allowed for production. In this case, the optimal operation consists of a time-varying substrate concentration that arises from the compromise between improving the product yield and respecting the time limit.

So, another objective of this paper is to check whether the same is true for the new model by optimizing the process, i.e. finding numerically the optimal open-loop feeding strategy.

The paper is organized as follows. Section 2 presents the amended dynamic model of filamentous fungal fermentation. The model-based optimization study is developed in Section 3, where simulation results are discussed. Some conclusions and perspectives close the paper.

2. FILAMENTOUS FUNGAL FERMENTATION MODEL

The influence of dissolved oxygen has been incorporated within the kinetic equations using Monod expressions and an additional state – dissolved oxygen – has been included in the model. In the following subsections only those equations that were modified or added compared to (Agger *et al.*, 1998; Bodizs *et al.*, 2004) are presented.

2.1 Model description

Growth kinetics. The morphologically structured model proposed by (Agger *et al.*, 1998) is based on the division of the biomass into three different compartments:
- *Active region (X_a)*, responsible for the uptake of substrate and growth of the hyphal element. It is assumed also that only the active region is responsible for the enzyme production.
- *Extension region (X_e)*, where building of new cell wall and extension zone take place.
- *Hyphal region (X_h)* is the degenerated part of the hyphal elements, which are inactive.

$$\begin{aligned} S + DO &\xrightarrow{X_a} X_e \\ S + DO &\xrightarrow{X_e} X_a \longrightarrow X_h \\ S + DO &\xrightarrow{X_a} P \end{aligned} \qquad (1)$$

where S stands for the substrate (glucose), DO for the disolved oxygen and P for the product (α-amylaze).

The corresponding kinetic expressions read:
Branching:

$$q_1 = \frac{k_1 s}{a(s + K_{S1})} x_a \frac{DO}{DO + K_{DO}} \qquad (2)$$

Differentiation:

$$q_2 = k_2 x_a \qquad (3)$$

The growth of the active region is given by:

$$q_3 = \frac{k_3 s}{s + K_{S3}} a x_e \frac{DO}{DO + K_{DO}} \qquad (4)$$

The specific growth rate of total biomass is:

$$\mu = \frac{q_3}{x_t} \quad (5)$$

where $x_t = x_e + x_a + x_h$ represents the total biomass concentration. x_e, x_a and x_h are the concentrations of the extension, active and hyphal zones, respectively. *s* and *DO* are the substrate and dissolved oxygen concentrations. *a* represents the number of tips per unit mass of the extension zones; this parameter is described as a function of μ. For details concerning the morphological model, the reader is referred to (Agger *et al.*, 1998).

Specific rate of enzyme production. Enzyme production in filamentous fungi is a classical example of growth associated product formation. The enzyme production is subject to glucose (substrate) inhibition and oxygen limitation, as given in (6):

$$r_{ps} = \left(\frac{\mu_0 s}{K_S + s + \frac{s^2}{K_I}} + k_c \frac{s}{s + K_{cor}} \right) \frac{DO}{DO + K_{DO}} \quad (6)$$

The expression used in (Agger *et al.*, 1998) describes the substrate inhibition by an exponential decrease in the specific growth rate of the enzyme when the substrate concentration exceeds a certain threshold value. In this study, the expression of the specific rate of enzyme production is modified by using a Haldane expression to describe the substrate inhibition. This modification is motivated in order to be able to apply an extremum-seeking controller to this process in a straightforward manner (Titica *et al.*, 2004). The parameter k_c quantifies the constitutive level of the enzyme production at high glucose concentrations (during the batch phase).

The specific rate of dissolved oxygen consumption is expressed as:

$$r_{DO} = Y_{XO} \frac{q_3}{x_t} + Y_{PO} r_{ps} \frac{x_a}{x_t} + m_o \frac{DO}{DO + K_{DO}} \quad (7)$$

where Y_{XO} and Y_{PO} are the yield of dissolved oxygen consumption for growth and enzyme production, respectively. m_o is the maintenance coefficient that stands for the oxygen consumption of the biomass.

2.2 Mass balance equations

The model proposed in (Agger *et al.*, 1998) consists of a set of five balance equations for the three regions of biomass, substrate and product concentrations, as represented in (8) - (12). A supplementary mass balance is added here to describe the dissolved oxygen concentration in the bioreactor (13). Finally, (14) describes the evolution of the bioreactor volume, which is time varying during the fed-batch operation and defines the end of the fermentation process.

Morphological states

$$\frac{dx_e}{dt} = q_1 - \frac{F}{V} x_e \quad (8)$$

$$\frac{dx_a}{dt} = q_3 - q_1 - q_2 - \frac{F}{V} x_a \quad (9)$$

$$\frac{dx_h}{dt} = q_2 - \frac{F}{V} x_h \quad (10)$$

Glucose (*s*)

$$\frac{ds}{dt} = -\left(Y_{XS} q_3 + Y_{PS} r_{PS} x_a + m_S x_t \frac{DO}{DO + K_{DO}} \right) \frac{F}{V} (s_f - s) \quad (11)$$

Enzyme (*p*)

$$\frac{dp}{dt} = r_{ps} x_a - \frac{F}{V} p \quad (12)$$

Dissolved O_2 concentration (*DO*)

$$\frac{dDO}{dt} = -r_{DO} x_t + k_L a (DO^* - DO) - \frac{F}{V} DO \quad (13)$$

Volume (V)

$$\frac{dV}{dt} = F - F_{evap} \quad (14)$$

In these equations, *F* represents the feeding rate. In (11), Y_{XS} and Y_{PS} are the yield of substrate consumption for growth and enzyme production, respectively. m_S is the maintenance coefficient (based on the total amount of biomass), s_f is the substrate concentration in the feed. In the (13), $k_L a$ represents the mass transfer coefficient for

the oxygen, DO^* is the saturation concentration of the oxygen.
The model parameters and their numerical values are reported in Table 1, at the end of Section 2.

The parameters related to the microscopic morphology (2)-(4) were taken from (Agger *et al.*, 1998). The kinetic parameters for the Haldane expression (6) were identified by fitting the model to simulated data provided by the Agger model. The yield coefficients Y_{XS}, Y_{PS}, Y_{XO}, Y_{PO} were identified from experimental data provided by Novozymes.

2.3 Modelling of gas-liquid mass transfer coefficient for oxygen (k_La)

The k_La value in stirred bioreactors depends on impeller characteristics and physical properties of the fermentation medium, mainly the rheological properties. Typically, the value of k_La in bioreactors is determined experimentally by the dynamic method of OTR (oxygen transfer rate measurement) (Moser, 1988), giving an average k_La value for the whole bioreactor that is constant in time. Since the variation of viscosity during a fed-batch operation is important, it leads to important variations in k_La. Consequently, modelling k_La as a function of rheological properties is needed for optimization purposes.

The estimation of rheological properties is summarised in the literature (e.g. Moser, 1988). Typically, rheological considerations were incorporated in the model by using empirical correlations with the aid of dimensionless quantities (analogously to the dimensionless numbers used in chemical engineering). For the mass transfer coefficient, the following relationship has been used (Moser, 1988):

$$k_L a = \alpha P^{\beta} v_s^{\gamma} \left(\frac{\nu}{\rho}\right)^{\varpi} \qquad (15)$$

where α, β, γ and ϖ are experimentally determined coefficients, P is the power per unit volume required for stirring, v_s is the superficial gas viscosity, ν is the kinematic viscosity and ρ the liquid density.

In this study, the relationship k_La vs. rheological properties has been determined based on an experimental analysis of a data set provided by Novozymes. The experiment was performed at varying feed rate, in such a way that oxygen limitation occurs. The maximum air flow rate and constant agitation speed has been applied. On-line measurements of oxygen transfer rate (OTR), viscosity and DO were available, as well as off-line measurement of the total biomass. The k_La value was calculated from OTR as follows:

$$k_L a = \frac{OTR}{(DO^* - DO)V} \qquad (16)$$

Based on (16) and given the experimental conditions for the fermentation system considered here, the main parameter inducing k_La variations during the process is the viscosity.

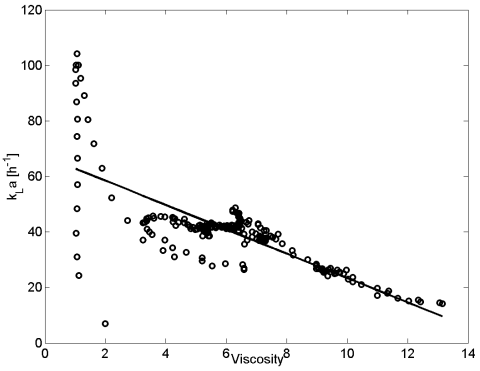

Figure 1: Relation between k_La and viscosity during a fed-batch fermentation process. As expected, k_La decreases with increasing viscosity.

As illustrated in Figure 1, the relationship k_La vs. viscosity during the fed-batch (at high viscosity levels) can be approximated by a linear equation:

$$k_L a = c_0 - c_1 \eta \qquad (17)$$

where η represents the on-line measurement of viscosity and c_0 and c_1 are linear regression coefficients (R^2=*0.94*).

Using (17) in simulation or for optimization purposes requires knowledge of the viscosity. For a given stirring rate, the viscosity of the filamentous suspension is influenced mainly by two factors: the biomass concentration, and the fibrous structure of the biomass. Due to the lack of

quantification of the fibrous structure, the viscosity was described based upon linear regression from the available experimental data. Linear correlation between viscosity and biomass is adequate for experimental observations (data not shown). This is in agreement with literature data for fermentations of *Penicillium chrysogenum* and *Aspergillus niger* (e.g. Moser, 1988). This correlation can be improved by introducing the dissolved oxygen as follows:

$$\eta = a_X x_t - a_{DO} DO + \eta_0 \qquad (18)$$

where a_X, a_{DO} and η_0 are linear regression coefficients (R^2=*0.84*). A negative effect of DO on the viscosity was obtained. This can be interpreted as follows: high dissolved oxygen levels in the medium reduce the increase in viscosity as a result of biomass growth. Since (18) is empirically established, the structure is not based on physical knowledge.

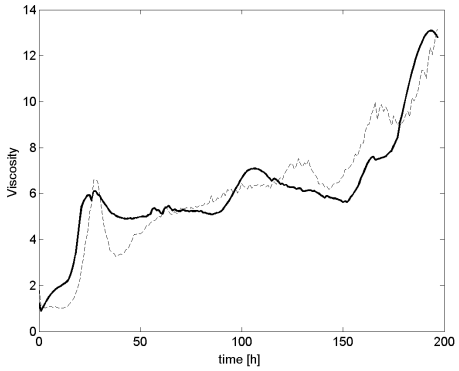

Figure 2: Evolution of the viscosity during fed-batch operation. Measured (--) and calculated using (18) (—).

2.4 Summary of modeling study

A mechanistic model of enzyme production by filamentous fungal fermentation was extended, by including the rheological considerations into the process modelling. Namely, the relationship between oxygen transfer and viscosity has been incorporated in the model, based upon an analysis of experimental data. As illustrated in Fig. 3, the model predictions show good agreement with the measurements of the dissolved oxygen during fed-batch operation.

Model dynamics were validated satisfactorily against industrial data of on-line measurements of the dissolved oxygen (data not shown).

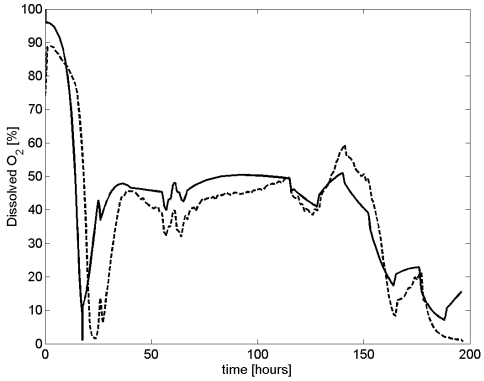

Figure 3: Dissolved oxygen evolution during fed-batch operation. Measured (--) and predicted curve (—) as a function of time.

The incorporation of dissolved oxygen and rheological considerations via k_La increases the applicability of the morphologically structured models for optimization and control purposes as well as for on-line estimation of other process variables.

Table 1: Model parameter values

Parameter	*Value*	*Measurement unit*
α	0.57	
f	80	%
k_2	0.08	h^{-1}
k_{bran}	0.0017	tip/(μm h)
k_c	8	FAU/(g active DW h)
K_{cor}	10^{-6}	g/L
K_{cor1}	10^{-3}	%
K_I	1.5 10^{-3}	g/L
K_{DO}	2.5	%
K_s	0.0211	g/L
K_{s1}	0.003	g/L
K_{s3}	0.006	g/L
$k_{tip,max}$	49	g active DW/(tip h)
m_0	0.01	%/(g DW h)
m_s	0.01	g glucose/(g DW h)
μ_0	227	FAU/(g active DW h)
ρ	1	g/cm^3
s_f	430	g/L
w	0.67	g/g DW
Y_{pO2}	37	% /FAU
Y_{sp}	5316	FAU/g
Y_{xO2}	35	%/ g active DW
Y_{xCO2}	57	%/ g active DW
c_0	67.2	h^{-1}
c_1	-4.3816	
a_X	0.094	
a_{DO}	0.04	
η_0	4.185	

3. NUMERICAL OPTIMIZATION

The aim of this section is to study the possibilities of improving the performance of the process compared to its current operation at Novozymes (constant feed profile and initial conditions, as given below in Section 3.1) by:

a) using a time-varying feed profile
b) adjusting the initial substrate concentration.

The problem of production time (t_f) minimization was formulated and solved numerically (Section 3.2).

The common practice at Novozymes is to use a high initial substrate concentration, since it produces a lot of biomass that is expected to increase process performance due to its catalyzing role.

However, it was shown in (Bodizs *et al.*, 2004) that the product yield is maximized by keeping the substrate concentration at very low values (i.e. very low feed rate and low biomass concentration). This is because substrate is consumed to grow biomass and make product. Thus, all the substrate that is consumed to grow biomass is in some sense a waste since it has been taken away from the potential product.

Yet, since the production time is typically limited and the reactor has to be filled within this time, the substrate concentration will have to be higher than its optimal value from the yield point of view. So, the optimal solution is determined by the compromise between improving the yield (low feed) and reducing the time for production (high feed). The optimal substrate concentration and feed rate profile corresponding to this compromise are time varying. This compromise was shown for Agger's model in (Bodizs *et al.*, 2004).

It will be shown in the following subsections that this compromise is also present for the amended model.
In order to elucidate the role of each manipulated variable in the optimal solution and to allow easier comparison with the current operation, the cases presented in Table 2 were studied.

Table 2 Optimization cases according to the available decision variables

	Feed profile	Initial conditions
Common Practice	*fixed*	*fixed*
Case A	*free*	*fixed*
Case B	*fixed*	*free*
Case C	*free*	*free*

The optimization study was carried out based on the model presented in Section 3.1, using the following parameters and initial conditions : V_0 = 1300 L, V_{max} = 1738.6 L, F_{max} = 10 L/h, F_{evap} = 1.25 L/h, t_f = 196 h, s_0 = 45 g/L. The software used for numerical optimization was MATLAB. The input was parameterized using piecewise-linear, non equidistant elements and optimized using a gradient-based search algorithm implemented in the function `fmincon`.

3.1 Common practice at Novozymes

The feed profile that is used today at Novozymes and the response of the system are presented in Figure 4. The current feeding policy consists of:

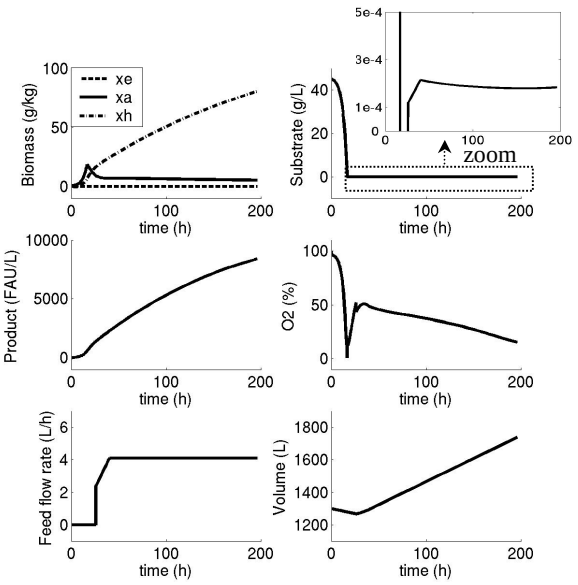

Fig. 4. Current operation at Novozymes

1. a batch phase, during which the substrate concentration is reduced

from its very high initial value to its operational domain
2. a linearly increasing feed rate whose role is to avoid oxygen limitation in the early phase of the fed-batch
3. a constant feed rate that is chosen in order to exactly fill the reactor in the remaining operation time.

3.2 Minimizing production time

Table 2 Optimization problem for Cases A-C (where $A(t_f) = p(t_f)V(t_f)$)

Case A	***Case B***	***Case C***
$\min_{F(t),\,t_f} J = t_f$	$\min_{s(0),\,t_f} J = t_f$	$\min_{F(t),s(0),\,t_f} J = t_f$
s.t. (model)	*s.t.* (model)	*s.t.* (model)
$A(t_f) \geq A(t_f)_{today}$	$A(t_f) \geq A(t_f)_{today}$	$A(t_f) \geq A(t_f)_{today}$
$V(t_f) = V_{\max}$	$V(t_f) = V_{\max}$	$V(t_f) = V_{\max}$
$F_{\min} \leq F(t) \leq F_{\max}$	$F(t) = kF_{fixed}(t)$	$F_{\min} \leq F(t) \leq F_{\max}$
$s(0) = s_{0\,fixed}$	$s(0) \geq 0$	$s(0) \geq 0$

Case A

The best numerical solution, using a piecewise-linear input parameterization, is presented in Figure 5.A and it contains three arcs:

1. Initially, there is a batch phase whose role is to reduce the substrate concentration. Oxygen limitation in the batch phase is unavoidable since it depends only on the initial substrate concentration that is fixed.
2. Then, the optimal substrate concentration s^* (Bodizs *et al.*, 2004) cannot be applied because of the oxygen limitation. Thus, in the short second arc, the feed is chosen in order to avoid oxygen limitation.
3. In the third arc, the oxygen is not limiting any more and it is possible to operate the process at the optimal time-varying substrate profile.

The reduction in production time compared to current operation is 6.15%.

Case B

It can be observed in Figure 5.B that the initial substrate concentration is reduced considerably since, from the point of view of yield maximization as explained in (Bodizs *et al.*, 2004), it is optimal to operate at a very low substrate concentration.

Note that oxygen limitation does not occur since the substrate and biomass concentrations are low. The reduction in production time compared to current operation is 23.92%.

In this case the feed rate profile (both its amplitude and the switching times) given in Section 3.1 was scaled, by the scaling factor *k*, according to the final time in order to be able to fill the reactor.

Case C

Combining the positive effects of *Cases A* and *B*, an even greater gain in production time can be obtained: 31.84% (Figure 5.C).

Table 3 Comparison of results.

Case A - free feed profile - fixed initial conditions	t_f = 183.20 h s_0 = 45 g/L Gain = **6.15%** (Figure 5.A)
Case B - fixed/scaled feed profile - free initial conditions	t_f = 146.25 h s_0 = 4.44 g/L Gain = **23.92%** (Figure 5.B)
Case C - free feed profile - free initial conditions	t_f = 129.78 h s_0 = 0 g/L Gain = **31.84%** (Figure 5.C)

Beside minimizing the production time, the problem of maximizing production ($A(t_f)$) was also solved numerically. The obtained results are esentially the same and reducing the initial substrate concentration yields a more significant gain than using a the time varying feed profile.

4. CONCLUSIONS

A model-based optimisation study of the enzyme production in a filamentous fungal fermentation process has been presented. First, a mechanistic model of enzyme fermentation was taken as the basis for a hybrid model, developed for accounting for

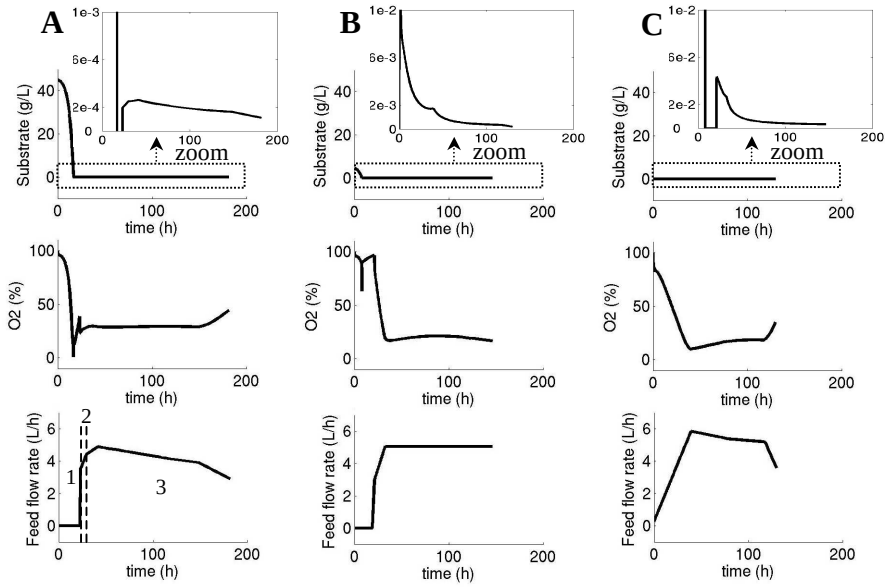

Fig. 5. Solutions of Cases A-C

the effects of low levels of dissolved oxygen on growth and production. Furthermore, the effect of rheological properties of the medium on the oxygen dynamics was successfully described by empirically modeling the dependence of the mass transfer coefficient ($k_L a$) on viscosity, and of the viscosity on biomass and dissolved oxygen concentration.

Based on this model, which was validated using DO measurements, the aim of the optimisation study was to propose optimal feeding strategies for improving the performance of the process compared to its current operation at Novozymes.

There are two main messages that come out of this optimization study.

1. Though biomass acts as a catalyst, its formation consumes substrate, which is a potential product. Thus, more biomass growth means less product at the end. Also, more biomass means more dead biomass, which in turn reduces yield and causes oxygen to be limited (higher viscosity – poorer transfer). Hence, it is better to reduce the initial substrate concentration (and also the amount of biomass formed), which goes against the normal intuition that having more catalyst (biomass) is better. Also, with this strategy, less dead biomass is formed and there is no problem with oxygen limitation. A large improvement is possible with this approach.

2. The bell-shaped feed rate trajectory is optimal since it considers all compromises such as biomass growth, production of enzyme, and allowing more death than growth towards the end. However, the gain with such a fine shaping of the feed rate profile is not significant. With simple profiles, it is possible to get very close to the optimal solution.

However, since the optimization results are based only on computer simulations, they need to be confirmed in practice in the future.

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