A Mathematical Model for End-Product Toxicity

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Abstract
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A Mathematical Model for End-Product Toxicity

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Abstract:
Alcohol based biofuels, such as bio-butanol, have considerable potential to reduce the demand for petrochemical fuels. However, one of the main obstacles to the commercial development of biological based production processes of biofuels is end-product toxicity to the biocatalyst. We investigate the effect of end-product toxicity upon the steady-state production of a biofuel produced through the growth of microorganisms in a continuous flow bioreactor. The novelty of the model formulation is that the product is assumed to be toxic to the biomass. The increase in the per-capita decay rate due to the presence of the product is assumed to be proportional to the the concentration of the product. The steady-state solutions for the model are obtained, and their stability determined as a function of the residence time. These solutions are used to investigate how the maximum yield and the reactor productivity depend upon system parameters. Unlike systems which do not exhibit toxicity there is a value of the feed concentration which maximises the product yield. The maximum reactor productivity is shown to be a sharply decreasing function of both the feed concentration and the toxicity parameter.

Keywords: biofuel, bioreactor, end-product toxicity, fermentation, stress tolerance

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1 Introduction

There has been ongoing interest in recent decades in the production of biofuels from renewable biomass. As plant biomass is both abundant and renewable, energy generated from it provides an environmentally friendly mechanism to reduce reliance upon the use of gasoline, diesel and aviation fuels derived from petrochemicals [1, 11]. In some applications biofuels have the potential to completely replace existing petroleum fuels whilst in others they may reduce demand by blending. The range of fuels that can currently be made biologically includes acetone, biodiesel, n-butanol, ethanol, hydrogen, methane, and methanol [1].

The fermentation of biomass is a promising route to either directly obtain fuels, such as bio-alcohols, or to obtain precursor chemicals which can then be chemically converted to fuels after additional processing steps. An example of a biologically obtained precursor is monoterpene, which upon hydrogenation gives saturated paraffins which can be used as the light-fraction components of jet fuels. A formidable hurdle to that must be overcome in the microbial synthesis of most biofuels is end-product toxicity which adversely effects production parameters such as rate, titer, and yield [5, 6]. Indeed, production of many biofuels and precursor compounds will remain commercially unviable until the problem of end-product toxicity is overcome.

Examples of processes subject to end-product toxicity include the microbial production of butanol [4–12] and monoterpene [2, 3]. In both cases the products are highly toxic to all current biocatalysts. Incidentally, bio-butanol is by no means a ‘new’ fuel as it has been almost continuous production through the ABE fermentation process since 1916 [1].

Here the effect of product toxicity upon the yield and productivity for a biologically controlled process being carried out in a continuously stirred tank reactor are investigated. We extend the standard biochemical model by including the effect of product toxicity. It is assumed that product toxicity increases the specific decay rate of the microorganisms. This is achieved by adding a term that is linear in the product concentration to the specific decay rate. This is the simplest model for product toxicity.

Two ways to measure the performance of a biological reactor are to determine the yield of the performance and/or the maximum reactor productivity. In a continuously stirred biological reactor the yield is the concentration of the product in the stream leaving the reactor divided by the concentration of feed in the stream entering the reactor. The reactor productivity is the concentration of the product in the exit stream multiplied by the flow rate. This is the mass of product leaving the reactor per unit time. We use our steady-state results
to investigate how these characterisations depend upon the degree of product toxicity and the concentration of the feed.

By the end of this paper we demonstrate that highly toxic products lead to significant decreases in yield and reactor productivity compared against equivalent systems with no toxicity. Thus, conventional reactor configurations must be adapted to reduce the toxicity of the environment faced by biocatalysts.

2 Equations

In this section we write down the model equations for the concentration of microorganisms, substrate and product within bioreactor. A generalised bioreactor model is used in which the parameter $\beta$, eq. (2), defines the reactor model. Values of $\beta$ over the range $0 \leq \beta \leq 1$ give different reactor configurations [10]. Figure 1 provides an elementary schematic flow diagram of the reactor geometry.

In practice microbial fermentation produces a range of products. For example, although the main product from the fermentation of *C. acetobutylicum* ATCC 824 is butanol, ethanol and acetone are also produced [12]. In principle all end-products could contribute to the overall solvent stress experience by the microorganism. We simplify matters by assuming that only the main product is the cause of end-product toxicity.

2.1 The dimensional model

The model equations are

\[
\frac{dS}{dt} = \frac{(S_0 - S)}{\tau} - \frac{\mu(S)}{a_s} \cdot X, \tag{1}
\]

\[
\frac{dX}{dt} = -\frac{\beta X}{\tau} + \frac{\mu(S)}{a_s} \cdot X - \left( k_d + k_p P \right) X. \tag{2}
\]

\[
\frac{dP}{dt} = -\frac{P}{\tau} + \frac{\mu(S)}{a_p} \cdot X. \tag{3}
\]

The specific growth rate is given by Michaelis-Menten/Monod kinetics

\[
\mu(S) = \frac{\mu_m S}{K_s + S}. \tag{4}
\]

The nomenclature is defined in Appendix C.

Note that the product toxicity is modelled by the term $-k_p X P$ in eq. (2). When $k_p = 0$ the system eqs (1)–(3) reduces to a simple cellmass-substrate system where the growth kinetics are Monod [9].

In eqs (1)–(3) the main experimental control parameter is the residence time ($\tau$).

The yield ($Y$) is defined as the ratio of the product concentration leaving the reactor to the feed concentration entering the reactor,

\[
Y = \frac{P}{S_0}. \tag{5}
\]

2.1.1 The dimensionless model

The dimensionless model is given by

\[
\frac{dS^*}{dt^*} = \frac{1}{\tau^*} \left( S_{0}^* - S^* \right) - \frac{S^* X^*}{1 + S^*}. \tag{5}
\]
\[
\frac{dX^*}{dt^*} = -\frac{\beta X^*}{\tau^*} + \frac{S^* X^*}{1 + S^*} - \left( k_p^* + k_d^* \right) X^*, \tag{6}
\]

\[
\frac{dP^*}{dt^*} = -\frac{P^*}{\tau^*} + \frac{S^* X^*}{1 + S^*} \tag{7}
\]

where the parameter groups are defined in Appendix C. The standard cellmass-substrate model with Monod growth kinetics is obtained in the limit as \( k_p^* \) approaches zero.

The dimensionless yield (\( Y^* \)) is defined by

\[
Y^* = \frac{P}{S_0^*}.
\]

### 2.2 Reduction to a planar system

The scaled system eqs (5)–(7) consists of three non-linear differential equations.

By adding eqs (5) and (7) we find that in the limit of large dimensionless time

\[
P^* = S_0^* - S^*.
\]

Thus the system eqs (5)–(7) reduces to the planar system

\[
\frac{dS^*}{dt^*} = \frac{1}{\tau^*} \left( S_0^* - S^* \right) - \frac{S^* X^*}{1 + S^*}, \tag{8}
\]

\[
\frac{dX^*}{dt^*} = \frac{\beta}{\tau^*} \left( X_0^* - X^* \right) + \frac{S^* X^*}{1 + S^*} - \left( S_0^* - S^* \right) \left( k_p^* + k_d^* \right) X^*. \tag{9}
\]

This reduction arises as a consequence of the biochemistry. Product is only produced by a single biochemical mechanism: consumption of the substrate by the micro-organisms. Substrate is only removed by a single biochemical mechanism: consumption by micro-organisms. It follows that the product concentration can be estimated by measuring the difference between the concentration of substrate in the feed and in the exit stream.

### 3 Results

#### 3.1 Global results

In Appendix A we show that the region

\[
0 \leq S^* \leq S_0^*, \quad 0 \leq X^* \leq \frac{S_0^*}{\beta} - S^*, \quad 0 \leq P^* \leq S_0^* - S^*.
\]

is positively invariant.

Furthermore, we also establish that this region is ‘attracting’ for any physically meaningful initial condition, i.e. the solution corresponding to any non-negative initial coordinates outside the invariant region eventually enters the invariant region.

Using Dulac’s test [8] it is possible to show that the system eqs (8) and (9) can not have limit-cycles. This is achieved using the test function \( \rho = X^{-1} \).
3.2 Steady-state solution branches

The steady-state solution branches consists of a washout branch

$$\langle S^*, X^* \rangle = (S^*_0, 0).$$  \hfill (10)

and a no-washout branch

$$\langle S^*, X^* \rangle = \left( S^*, \frac{S^*_0 - S^*}{S^* \tau^*} (1 + S^*) \right).$$ \hfill (11)

The steady-state substrate concentration along the no-washout branch ($\tilde{S}^*$) solves the quadratic equation

$$\mathcal{G} \left( \tilde{S}^* \right) = a \tilde{S}^*^2 + b \tilde{S}^* + c = 0,$$ \hfill (12)

where

$$a = k^*_p \tau^*,$$

$$b = \left[ (1 - S^*_0) k^*_p + (1 - k^*_d) \right] \tau^* - \beta,$$

$$c = - \left[ (k^*_d + k^*_p S^*_0) \tau^* + \beta \right].$$

Equation (12) has one positive and one negative root. The physically meaningful root is

$$\tilde{S}^* = -\frac{b + \sqrt{b^2 - 4ac}}{2a}.$$ \hfill (13)

The primary focus of our work is to investigate how the degree of product toxicity, given by the value of the parameter $k^*_p$, effects the steady-state substrate concentration, given by eq. (13).

Differentiating eq. (13) we obtain

$$\frac{d S^*}{d \tau} = \begin{cases} 0, & \beta = 0, \\ \frac{\beta}{2k_p \tau^*} \left[ -\frac{b}{\sqrt{b^2 - 4ac}} - 1 - \frac{2k_p \tau^*}{\sqrt{b^2 - 4ac}} \right] < 0, & 0 < \beta \leq 1. \end{cases}$$ \hfill (14)

When $\beta = 0$ (an idealised membrane bioreactor [9]), neither the substrate concentration nor the product concentration depend upon the residence time. When $0 < \beta \leq 1$ the the concentration of substrate and product within the bioreactor decrease and increase respectively as the value of the residence time is increased. Consequently, both the product concentration and the yield are maximised at an infinite residence time.

The no-washout branch is only physically meaningful all concentrations are positive. We have already established that $\tilde{S}^* > 0$. From eq. (11) we require $\tilde{S}^* < S^*_0$. This condition is met when

$$\tau^* > \tau^*_{cr} = \frac{(1 + S^*_0) \beta}{S^*_0 (1 - k^*_d) - k^*_d}$$

and $$0 < k^*_d < \frac{S^*_0}{1 + S^*_0}.$$ \hfill (15)

When $\tau^* = \tau^*_{cr}$ the two solution branches intersect at a transcritical bifurcation.

3.3 Stability of the steady-state solutions

3.3.1 Stability of the washout solution

Along the washout steady-state solution the Jacobian matrix is

$$J \left( S^*_0, 0 \right) = \begin{pmatrix} -\frac{1}{\tau^*} & -\frac{S^*_0}{1 + S^*_0} \\ 0 & -\frac{2}{\tau^*} + \frac{S^*_0}{1 + S^*_0} - k^*_d \end{pmatrix}.$$

with eigenvalues
\[ \lambda_1 = -\frac{1}{\tau^*} < 0, \]
\[ \lambda_2 = -\beta - \frac{S_0^*}{1 + S_0^*} - k_d^*. \]

If the decay rate is sufficiently high,
\[ k_d^* > \frac{S_0^*}{1 + S_0^*}, \]
then the washout branch is always locally stable regardless of the reactor configuration \((0 \leq \beta \leq 1)\). In fact, it can be shown that in this case the washout branch is globally stable. As this case is not of practical interest the proof is not included.

If the decay rate is sufficiently low,
\[ k_d^* < \frac{S_0^*}{1 + S_0^*}, \]
then the washout branch is stable \((0 < \beta \leq 1)\) when the residence time is sufficiently low,
\[ \tau^* < \tau^{\text{cr}} = \frac{(1 + S_0^*) \beta}{S_0^* - k_d^* (1 + S_0^*)}. \]

Note that residence time the value for \(\tau^{\text{cr}}\) is independent of the dimensionless toxicity parameter \((k_p^*)\).

### 3.3.2 Local stability of the no-washout solution

Along the no-washout solution branch the Jacobian matrix can be written in the form
\[
J (S^*, X^*) = \begin{pmatrix}
-\frac{1}{\tau^*} & -\frac{X^*}{S^*} - \frac{S^*}{1 + S^*} \\
\frac{X^*}{S^*} + k_p^* X^* & 0
\end{pmatrix}.
\]

This solution branch is stable if \(\det J > 0\) and \(\text{trace} J < 0\). We have
\[
\det J = \frac{S^*}{1 + S^*} \left( \frac{X^*}{(1 + S^*)^2} + k_p^* X^* \right),
\]
\[
\text{trace} J = -\frac{1}{\tau^*} - \frac{X^*}{(1 + S^*)^2}.
\]

It therefore follows that \(\det J > 0\) and \(\text{trace} J < 0\) whenever \(X^* > 0\) and \(S^* > 0\). Thus the no-washout branch is stable whenever it is physically meaningful.

Figure 2 shows how the dimensionless steady-state product concentration \((P^*)\) as a function of the dimensionless residence time. The figure indicates a dramatic decrease in the steady-state product concentration when \(k_p^* = 10\).

### 3.4 Small dimensionless product toxicity approximations

For small values of product toxicity coefficient \((k_p^* \ll 1)\) the no-washout branch solution can be approximated by an asymptotic solution
\[
S^* \approx \frac{\beta + k_d^* \tau^*}{(1 - k_d^*) \tau^*} - \beta - a_1 \cdot k_p^* + O \left( k_p^* \right),
\]

\[(17)\]
\[ X^* \approx \frac{(1 + S_0^*) \beta + [k_d^* - (1 - k_d^*) S_0^*] \tau^*}{[\beta - (1 - k_d^*) \tau^*][\beta + k_d^* \tau^*]} + b_1 \cdot k_p^* + O\left(\frac{1}{k_p^*}\right), \tag{18} \]

\[ a_1 = \frac{\left(\beta + [k_d^* - (1 - k_d^*) S_0^*] \tau^*\right) \tau^*}{\left[\beta - (1 - k_d^*) \tau^* - \beta\right]^3} < 0 \quad \text{using inequality (15),} \tag{19} \]

\[ b_1 = \frac{\left[\beta - (1 - k_d^*) \tau^* + \beta + k_d^* \tau^*\right]^2 - \tau^* c_1 c_2}{\left[\beta - (1 - k_d^*) \tau^*\right]^3} \tag{20} \]

\[ c_1 = \beta + [k_d^* - (1 - k_d^*) S_0^*] \tau^*, \tag{21} \]

\[ c_2 = \left(\beta + k_d^* \tau^*\right)^2 - S_0^* \left(\tau^* - \beta\right)^2 + S_0^* \left[k_d^* \tau^* + 2(\beta - \tau^*)\right]. \tag{22} \]

Equation (17) indicates the extent to which product toxicity, when the toxicity coefficient is small, increases the substrate concentration inside the reactor. The corresponding decrease in the product concentration is given by

\[ P^* = S_0^* - \frac{\beta + k_d^* \tau^*}{(1 - k_d^*) \tau^* - \beta} + a_1 \cdot k_p^*. \tag{26} \]

### 3.5 Large dimensionless product toxicity approximation

For large values of the product toxicity constant \((k_p^* \gg 1)\) the no-washout branch solution can be approximated by an asymptotic solution

\[ S^* \approx S_0^* - d_1 \cdot \frac{1}{k_p^*} + O\left(\frac{1}{k_p^*}\right)^2, \tag{23} \]

\[ X^* \approx \frac{1}{S_0^* \tau^*} \left\{ - (1 + S_0^*) \beta + \left[(1 - k_d^*) S_0^* - k_d^* \tau^*\right]\right\} + O\left(\frac{1}{k_p^*}\right)^2, \tag{24} \]

\[ d_1 = -\frac{1}{2} \left[\frac{2\beta}{\tau^*} - (1 - 2k_d^*) + \frac{1 - S_0^*}{1 + S_0^*}\right] > 0 \quad \text{using inequality (15).} \tag{25} \]

Equation (23) indicates the extent to which large product toxicity reduces the substrate concentration inside the reactor. The corresponding product concentration is given by

\[ P^* = d_1 \cdot \frac{1}{k_p^*} + O\left(\frac{1}{k_p^*}\right)^2. \tag{26} \]

This shows that for fixed residence time, which is not large compared to the toxicity constant, that the product concentration is reduced towards zero if the toxicity constant is sufficiently large. Line (d) in Figure 2 illustrates the reduction in product concentration due to large toxicity.

### 3.6 Large residence time approximations

In 3.2 we noted that the product concentration \((P^* = S_0^* - S^*)\), is maximised in the limit of infinitely large residence time. In the case of no-product toxicity, \(k_p^* = 0\), the maximum possible product concentration is given by [9]
\[ P^* \left( \tau^* = \infty, k_p^* = 0 \right) = S_0^* - \frac{k_d^*}{1 + k_d^*}. \]

In practice the yield may be close to 100% as \( k_d^* \ll 1 \).

For comparison with systems exhibiting product toxicity it is instructive to note that when there is no product toxicity the maximum yield increases as the feed concentration \( (S_0^*)^2 \) increases.

It is important to know how the presence of product toxicity changes the maximum obtainable yield. As this is obtained at infinite values of the residence this question can be investigated by obtaining an asymptotic approximation along the no-washout solution branch for large values of the residence time. We have

\[ S^* \approx \frac{1}{2k_p^*} \left[ - \left( 1 - k_d^* \right) + (S_0^* - 1) k_p^* + \sqrt{A} \right] \]

\[ = \frac{\beta}{2k_p^*} \left\{ 1 - \frac{\left[ (1 - k_d^*) - (1 + S_0^*) k_p^* \right]}{\sqrt{A}} \right\} \cdot \frac{1}{\tau^*} + O \left( \frac{1}{\tau^{*2}} \right), \]

\[ X^* \approx \frac{1}{2k_p^*} \left[ \frac{(1 - k_d^* - k_p^*) k_p^* + k_p^* S_0^* (2 - k_d^*) - k_d^* \sqrt{A}}{k_d^* + k_p^* S_0^*} \right] \cdot \frac{1}{\tau^*} + O \left( \frac{1}{\tau^{*2}} \right), \]

\[ A = (S_0^* k_p^* - 1)^2 + 2 \left( k_d^* - k_p^* \right) + (k_d^* + k_p^*) \left[ (1 + 2S_0^*) k_p^* + k_d^* \right] \]

We now investigate further the maximum product concentration in the limits of small and large dimensionless product toxicity.

### 3.6.1 Small dimensionless product toxicity

For small values of the product toxicity parameter we obtain the following approximation along the no-washout solution branch:

\[ S^* \approx \frac{k_d^*}{1 - k_d^*} + \frac{\left( 1 - k_d^* \right) S_0^* - k_d^*}{(1 - k_d^*)^3} \cdot k_p^* \]

\[ \approx + \frac{\left[ (1 - k_d^*)^2 - \left( 1 + 2k_d^* - 2(1 - k_d^*) S_0^* \right) k_p^* \right]}{(1 - k_d^*^2)} \cdot \frac{\beta}{\tau^*}, \]

\[ X^* \approx \frac{\left( k_d^* (1 - k_d^*)^2 - \left( 1 + S_0^* \right) k_p^* - 2S_0^* k_d^* + S_0^* \right) \left[ (1 - k_d^*) S_0^* - k_d^* \right]}{(1 - k_d^*^2) k_p^*} \cdot \frac{1}{\tau^*}. \]

For small values of the product toxicity we have

\[ P^* \left( \tau^* = \infty, k_p^* \ll 1 \right) = P^* \left( \tau^* = \infty, k_p^* = 0 \right) = \frac{(1 - k_d^*) S_0^* - k_d^*}{(1 - k_d^*^2)} \cdot k_p^* \]

i.e. a small amount of toxicity decreases the maximum product concentration by a small amount.

For large values of the feed concentration the maximum yield no longer approaches 100%, as is the case when the product is not toxic, but rather the value

\[ Y^* \left( \tau^* = \infty, k_p^* \ll 1, S_0^* \gg 1 \right) \approx 1 - \frac{1}{(1 - k_d^*^2)} \cdot k_p^*. \]

(This formula is not valid in the limit that the feed concentration goes to infinity because terms of order \( S_0^*/\tau^* \) are not negligible.)
The effect of toxicity upon the maximum product concentration can also be gauged by comparing its value against that in the absence of toxicity. We have

\[
P^\tau(\tau^* = \infty, k_p^* \ll 1) = 1 - \frac{1}{(1 - k_d^*)^2} \cdot k_p^*.
\]

Thus the proportional reduction in product concentration at high residence times, compared to a system without product inhibition, is independent of the substrate concentration in the feed.

### 3.6.2 Large dimensionless product toxicity

For large values of the product toxicity parameter we obtain the following approximation along the no-washout solution branch

\[
S^* \approx S_0^* - \frac{e_1}{k_p^*} + \beta \cdot \frac{1}{k_p^* \tau^*},
\]

\[
X^* \approx \left(1 - k_d^*\right) \frac{S_0^* - k_d^*}{S_0^*} \cdot \frac{1}{k_p^* \tau^*},
\]

\[
e_1 = \left(1 - k_d^*\right) \cdot \frac{S_0^*}{1 + S_0^*}.
\]

For large values of the product toxicity we have

\[
P^\tau(\tau^* = \infty, k_p^* \gg 1) = \frac{e_1}{k_p^*},
\]

i.e. a large degree of product toxicity has a significant effect on the maximum product concentration. The yield is now given by

\[
Y^* \left(\tau^* = \infty, k_p^* \gg 1\right) \approx \frac{1 - k_d^*}{(1 + S_0^* \cdot k_p^*)}.
\]

Thus the yield is a decreasing function of the feed concentration.

Comparing the maximum product concentration against that in the absence of toxicity we have for large values of the feed concentration

\[
P^\tau(\tau^* = \infty, k_p^* \ll 1, S_0^* \gg 1) \approx \frac{1 + k_d^*}{S_0^* \cdot k_p^*}.
\]

Thus the proportional reduction in product concentration at high residence times increases as the feed concentration increases.

### 4 Discussion

The maximum yield and the maximum reactor productivity are key factors in determining whether the production of biofuels is commercially viable. In Sections 4.1 and 4.2 we investigate the effect of the toxicity constant \((k_p^*)\) upon the maximum yield and maximum reactor productivity respectively. In particular, for specified operating conditions, we determine the value of the toxicity constant at which the values for the maximum yield and productivity are 1\% of the values in a system without product toxicity.
4.1 Maximum yield

In 3.6 we obtained an asymptotic formula for the substrate concentration at high residence time, eq. (27). Simplications of this formula were obtained for the cases of low and high product toxicity, eqs (30) and (30), respectively. These were used to investigate how the product yield varies as a function of the feed concentration for the two limiting cases.

Figure 3 (a) and (b) show the variation of the maximum product concentration and the maximum yield as a function of the feed concentration for three values of the toxicity parameter. In all three cases the product concentration, Figure 3 (a), is an increasing function of the feed concentration. However, the behaviour of the yield is very different. This increases to a maximum value before decreasing towards zero. Thus the effects of product toxicity become more pronounced as the feed concentration increases.

In line (a) the yield increases from 87.8%, when $S_0^* = 1$, to 91.1%, when $S_0^* = 7$. Over this range of feed concentrations the asymptotic solution for small product toxicity, using eq. (30), is almost indistinguishable from the exact solution, using eq. (27). The asymptotic and exact formulae eventually diverge, when $S_0^* = 22.1$ the exact value of the yield reaches a maximum value, $Y_{\max} = 97.9%$. Thereafter the yield decreases towards zero.

In line (b) the yield increases to a maximum, $Y_{\max} = 82.4%$, when the feed concentration is $S_0^* = 2.62$, and then decreasing towards zero in the limit that the feed concentration approaches infinity.

In line (c) the yield increases to a barely perceptible maximum, $Y_{\max} = 53.9%$, when the feed concentration is $S_0^* = 1.003$, and then decreasing towards zero in the limit that the feed concentration approaches infinity.

From eq. (27) we deduce that for high values of the feed concentration that the maximum yield is given

$$Y(\tau^* = \infty, S_0^* \gg 1) \approx \frac{1-k_p^*}{k_p^*} \cdot \frac{1}{S_0^*}.$$  

This shows that regardless of the toxicity constant the yield asymptotes towards zero at sufficiently high feed concentrations.

We show in Appendix B that the yield ($Y(\tau^* = \infty)$) is maximised when the feed concentration takes the value

$$S_{0,\text{max}}^* = \frac{(k_p^* \sqrt{k_p^*})}{(1-k_p^*)} + \frac{(1-k_p^* \sqrt{k_p^*})}{k_p^*}.$$  

We finish by calculating the value of the toxicity constant ($k_p^*$) required to reduce the value of the yield to 1% of its value in the absence of product toxicity. For feed concentrations $S_0^* = 1$, $S_0^* = 10$ and $S_0^* = 100$ these values are $k_p^* = 43.8$, $k_p^* = 8.15$ and $k_p^* = 0.691$ respectively. Thus the larger the feed concentration, the lower the degree of toxicity required to reduce the yield to 1% of the value in the absence of toxicity.

4.2 Reactor productivity

The dimensionless reactor productivity is defined by

$$Pr^* = \frac{P^*}{\tau^*},$$  

where $P^*$ is the steady-state dimensionless product concentration and $\tau^*$ is the dimensionless residence time. For sufficiently low values of the dimensionless residence time ($\tau^* < \tau_{tr}$) only the washout steady-state solution is stable ($P^* = 0$). The reactor productivity is zero in this range. Clearly the reactor productivity decreases towards zero in the limit of large residence time. Consequently, there exists a value of the residence time which maximises the productivity. In this section we investigate the effect of product toxicity and feed concentration upon the maximum reactor productivity.

Figure 4 shows the steady-state reactor productivity as a function of the residence time. Line (a) corresponds to the case when the product is not toxic ($k_p^* = 0$) whereas lines (b–d) correspond to increasing product toxicity. The square box denotes both the location of the maximum reactor productivity. The maximum productivity decreases as the toxicity parameter increases: it is 81%, 27% and 3.3% of the maximum productivity in the absence of toxicity in lines (b), (c) and (d) respectively.

To see how the maximum productivity varies as the toxicity parameter is varied we introduce the maximum relative reactor productivity. For a fixed feed concentration this is the ratio of the maximum reactor productivity
for a given value of the toxicity parameter to the maximum productivity in the absence of toxicity expressed as a percentage.

Figure 5 shows the maximum relative reactor productivity as a function of the toxicity parameter for three values of the feed concentration. This value rapidly decreases as either the toxicity constant or the feed concentration is increased. The value of the toxicity constant required to reduce the maximum relative reactor productivity to 1% is: $k_p^* = 34.1$ ($S^*_0 = 1$, line a), $k_p^* = 3.54$, ($S^*_0 = 10$, line b) and $k_p^* = 0.272$ ($S^*_0 = 100$, line c). Thus as the feed concentration increases, increasingly ‘milder’ toxicity has a pronounced effect on the reactor productivity.

5 Conclusion

We have extended a commonly used bioprocess engineering model for the production of a chemical by microbial cells to handle situations in which the micro-organism is subject to product toxicity. The motivation for this work is the end-product toxicity exhibited by certain biofuels, which is restricting their commercial development. Product toxicity was modelled by allowing the specific death rate of the microorganism to increase linearly with the product concentration.

The expression for the no-washout steady-state solution was used to quantify the effect of product toxicity upon two key reactor outputs: the yield and the maximum reactor productivity. When there is no product toxicity ($k_p^* = 0$), the product yield is an increasing function of the feed concentration ($S^*_0$). Increasing the feed concentration increases the yield. When the product is toxic ($k_p^* > 0$), there is a value of the feed concentration that maximises the yield ($S^*_0 = S^*_0,_{\text{max}}$). For values of the feed concentration that are higher than this value the yield is a decreasing function of the feed concentration.

In particular we calculated the values of the toxicity constant at which either the yield or productivity is reduced to 1% of its value in the absence of product toxicity. We showed that for high concentrations of the feed, only very small degrees of toxicity are required to reduce the maximum value of the yield to 1% of the corresponding value in the absence of toxicity.

Product toxicity was modelled as a linear function of the product concentration ($k_p P$). A ‘Monod’-type expression may be appropriate if the increase in decay rate due to the presence of the product plateaus ($\mu_{d,P}/(K_p + P)$). Other plausible formulations exist. Thus suggests the need for toxicity studies that quantify the effect of product concentration upon the specific decay rate.

Successful fermentations to produce biofuels must overcome the limited tolerance of the biomass to the fermentation product. A variety of techniques have been developed to reduce the effects of end-product toxicity. These include two-phase solvent extraction expanded bed adsorption extractive fermentation and the use of polystyrenic resins It is our intention to develop the model analysed here to include these techniques.

![Figure 1: Schematic flow diagram for the reactor geometry. A feed containing substrate ($S_0$) flows into the reactor (rate $F$) where micro-organisms ($X$) grow producing product ($P$). The vertical line intersecting the effluent stream indicates the potential use of a membrane or a settling unit to retain micro-organisms within the reactor.](image-url)
Figure 2: Steady-state diagram showing the variation of dimensionless product concentration ($P^*$) as a function of the dimensionless residence time ($\tau^*$). Only physically meaningful solutions are plotted, i.e. the stable solution for sufficiently low residence times ($\tau^* < \tau^*_{tr}$) is the washout branch $P^* = 0$. Parameter values: dimensionless death rate, $k^*_d = 0.1$; dimensionless feed concentration, $S^*_0 = 1$; reactor parameter, $\beta = 1$. The value of the dimensionless product toxicity constant ($k^*_p$) is as given.

Figure 3: Variation of maximum product concentration, $P^* (\tau^* = \infty)$, and maximum yield, $Y^* (\tau^* = \infty)$, as a function of the feed concentration ($S^*_0$). The values of the toxicity parameter are: $k^*_p = 0.01$ (a), $k^*_p = 0.1$ (b) and $k^*_p = 0.4$ (c). Parameter value: $k^*_d = 0.1$. 
Figure 4: Steady-state diagram showing the variation of dimensionless product productivity ($Pr^*$) as a function of the dimensionless residence time ($\tau^*$). The boxes denote the location of the maximum productivity. Parameter values as in Figure 2.

Figure 5: The dimensionless relative reactor productivity as a function of the toxicity parameter for three values of the feed concentration. Parameter values: (a) $S_0^* = 1$, (b) $S_0^* = 10$ and (c) $S_0^* = 100$. Other parameter values as in Figure 2.

Appendix

A Attracting Region

In this appendix we show that the region $R$ is both positively invariant and attracting.

A.1 Solution components may not become negative

We first show that if the initial conditions are non-negative that the solution remains non-negative. In order for the solution to become negative it’s value must reduce in value to zero. We have

$$\frac{dS^*}{dt^*} \bigg|_{S^*=0} = \frac{S_0^*}{\tau^*} \geq 0,$$

$$\frac{dX^*}{dt^*} \bigg|_{X^*=0} = 0,$$

$$\frac{dP^*}{dt^*} \bigg|_{P^*=0} = \frac{S^*X^*}{1+S^*} \geq 0.$$

Note that second of these derivatives shows that the line $X^* = 0$ is itself (positively) invariant.

A.2 The substrate component is bounded

We now show that the region $0 \leq S^* \leq S_0^*$ is both (positively) invariant and exponentially attracting. We have

$$\frac{dS^*}{dt^*} \leq \frac{1}{\tau^*} (S_0^* - S^*), \quad \text{(as } X^* \geq 0 \text{ and } S^* \geq 0\text{)}.$$

Let $Z_1$ be the solution of the differential equation

$$\frac{dZ_1}{dt^*} = \frac{1}{\tau^*} (S_0^* - Z_1).$$
with initial condition $Z_1(0) = S(0)$. It follows from the classical scalar comparison theorem for ordinary differential equations that $S^*(t^*) \leq Z^*(t^*)$. Hence

$$S^*(t^*) \leq S_0^* - [S_0^* - S^*(0)] \exp \left[ - \frac{t^*}{\tau^*} \right].$$

This inequality shows that the region $0 \leq S^* \leq S_0^*$ is invariant, because if the initial condition is within the invariant region, i.e. $S^*(0) \leq S_0^*$, then the solution remains within the invariant region, i.e. $S^* \leq S_0^*$. Furthermore, if the initial condition is outside the invariant region, $S^*(0) > S_0^*$, then the solution must eventually enter the invariant region, i.e. $S^* \leq S_0^*$.

**A.3 The biomass component is bounded**

Let $Z = S^* + X^*$ with initial condition $Z(0) = S^*(0) + X^*(0)$. Then adding eqs (5) we have and (6)

$$\frac{dZ}{dt^*} \leq \frac{S_0^*}{\tau} - \frac{\beta}{\tau} Z,$$

(as $X^* \geq 0, P^* \geq 0$, and $0 \leq \beta \leq 1$).

Applying the scalar comparison theorem, as in A.2, we have

$$Z = X^* (t^*) + S^* (t^*) \leq \frac{S_0^*}{\beta} - \left[ \frac{S_0^*}{\beta} - S^* ((0) - X^* (0)) \right] \exp \left[ - \frac{\beta}{\tau} t^* \right].$$

Combining this result with our earlier bound on the scaled substrate concentration it follows that the region

$$0 \leq S^* \leq S_0^*,$$

$$0 \leq X^* \leq \frac{S_0^*}{\beta} - S^*$$

is bounded and exponentially attracting.

**A.4 The product component is bounded**

Let $Z = S^* + P^*$ with initial condition $Z(0) = S^*(0) + P^*(0)$. Then adding eqs (5) we have and (7)

$$\frac{dZ}{dt^*} = \frac{S_0^*}{\tau} - \frac{P^*}{\tau}.$$

Applying the scalar comparison theorem, as in A.2, we have

$$Z = P^* (t^*) + S^* (t^*) = S_0^* - \left[ S_0^* - S^* ((0) - P^* (0)) \right] \exp \left[ - \frac{P^*}{\tau} t^* \right].$$

Combining this result with our earlier bound on the scaled substrate concentration it follows that the region

$$0 \leq S^* \leq S_0^*,$$

$$0 \leq P^* \leq S_0^* - S^*$$

is bounded and exponentially attracting.

Furthermore, taking the limit $t \to \infty$ we obtain

$$P^* = S_0^* + P_0^* - S^*.$$
B The optimal value for the feed concentration to maximise the yield

After some algebra we find that

\[ \frac{d}{dS_0^*} Y (\tau^* = \infty) = 0, \tag{37} \]

\[ \Rightarrow \mathcal{G}(S_0^*) = a_1 S_0^{*i} + b_1 S_0^* + c_1 = 0, \tag{38} \]

where the coefficients are given by

\[ a_1 = (1 - k_d^*) k_p^*, \tag{39} \]

\[ b_1 = -2 k_d^* k_p^* \left[ k_p^* - (1 - k_d^*) \right], \tag{40} \]

\[ c_1 = -\left[ (1 + k_p^* + k_d^*)^2 - 4 k_d^* \right] k_d^*. \tag{41} \]

The discriminant of the quadratic eq. (38) is positive

\[ b_1^2 - 4 a_1 c_1 = 4 k_d^* k_p^* \left( k_d^* - k_p^* - 1 \right)^2. \tag{42} \]

Consequently eq. (38) has two solutions. As the coefficient \( a_1 \) is strictly positive we have

\[ S_{0,+}^* > S_{0,-}^*, \tag{43} \]

where

\[ S_{0,+}^* = \frac{-b_1 + \sqrt{b_1^2 - 4 a_1 c_1}}{2 a_1}, \tag{44} \]

\[ S_{0,-}^* = \frac{-b_1 - \sqrt{b_1^2 - 4 a_1 c_1}}{2 a_1}. \tag{45} \]

The condition for the no-washout branch to be physically meaningful, eq. (15), can be written in the equivalent form

\[ S_0^* > S_{0,cr} = \frac{k_d^*}{1 - k_d^*}, \quad 0 < k_d^* < 1. \tag{46} \]

We demonstrate that the solution \( S_{0,-}^* \) is not physically meaningful because

\[ S_{0,-}^* < S_{0,cr}. \tag{47} \]

We have

\[ S_{0,-}^* = \frac{(k_d^* - \sqrt{k_d^*})}{(1 - k_d^*)} - \frac{(k_d^* + \sqrt{k_d^*})}{k_p^*}, \tag{48} \]

\[ < \frac{k_d^*}{(1 - k_d^*)}, \quad \text{as } 0 < k_d^* < 1 \text{ and } k_d^* > 0, \tag{49} \]
\[ S^*_0 = S^*_{0,cr}. \] (50)

It similarly follows that
\[ S^*_{0, cr} > S^*_0. \] (51)

Thus the yield is maximised when the feed concentration is given by \[ S^*_0 = S^*_{0, max} = S^*_{0, cr} \] where
\[ S^*_{0, cr} = \frac{(k_d^* + \sqrt{k_d^*})}{(1-k_d^*)} + \frac{(1-\sqrt{k_d^*})\sqrt{k_d^*}}{k_p^*}. \] (52)

**C Symbols used**

- \( D \) : Specific decay rate. (hr\(^{-1}\))
- \( F \) : Flowrate through the bioreactor. (dm\(^3\) hr\(^{-1}\))
- \( g \) : Singularity equation. (—)
- \( I \) : Jacobian matrix. (—)
- \( K_s \) : Monod constant. (g dm\(^{-1}\))
- \( P \) : Product concentration within the bioreactor. (g dm\(^{-1}\))
- \( P^* \) : Dimensionless product concentration. (—)
  
  \[ P^* = \frac{a_p P}{(a_s K_s)} \]

- \( S \) : Substrate concentration within the bioreactor. (g dm\(^{-1}\))
- \( S^* \) : Dimensionless substrate concentration. (—)
  
  \[ S^* = \frac{S}{K_s} \]

- \( S^* (0) \) : The scaled substrate concentration inside the reactor at time \( t^* = 0 \). (—)
- \( S^*_0 \) : Substrate concentration in the feed (\( S^*_0 > 0 \)). (g dm\(^{-1}\))
- \( S^*_0 \) : Dimensionless substrate concentration in the feed (\( S^*_0 > 0 \)). (—)
  
  \[ S^*_0 = \frac{S_0}{K_s} \]

- \( S^*_{0, cr} \) : The no-washout solution is only physically meaningful for \( S^*_0 > S^*_{0, cr} \). (—)
  
  \[ S^*_{0, cr} = \frac{k_d^*}{1-k_d^*} \]

- \( S^*_{0, max} \) : The value of the feed concentration which maximises the yield. Defined by eq. (35). (—)

- \( V \) : Volume of the bioreactor. (dm\(^3\))
- \( X \) : Concentration of microorganisms within the bioreactor. (g dm\(^{-1}\))
- \( X^* \) : Dimensionless microorganism concentration. (—)
  
  \[ X^* = \frac{X}{(a_s K_s)} \]

- \( Y \) : Yield. (—)
  
  \[ Y = \frac{P}{S} \]

- \( Y^* \) : Dimensionless yield. (—)
  
  \[ Y^* = \frac{P^*}{S^*} \]

- \( k_d \) : Decay coefficient, representing a combination of endogenous respiration, predation, and cell death followed by subsequent lysis \( k_d^* > 0 \). (hr\(^{-1}\))

- \( k_d^* \) : Dimensionless decay coefficient \( (k_d^* > 0) \). (—)
  
  \[ k_d^* = \frac{k_d}{\mu_m} \]

- \( k_p \) : Product toxicity constant. (hr\(^{-1}\) g\(^{-1}\) dm\(^3\))
- \( k_p^* \) : Dimensionless product toxicity constant. (—)
  
  \[ k_p^* = \frac{k_p}{\mu_m} \cdot \frac{a_s K_s}{a_p} \]

- \( t \) : Time. (hr\(^{-1}\))
- \( t^* \) : Dimensionless time. (—)
  
  \[ t^* = \frac{\mu_m t}{k_p^*} \]
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Units</th>
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<tr>
<td>$\alpha_p$</td>
<td>Product yield factor, the ratio of the weight of product produced to the weight of substrate consumed.</td>
<td>(—)</td>
</tr>
<tr>
<td>$\alpha_s$</td>
<td>Substrate yield factor, the ratio of the weight of product produced to the weight of substrate consumed.</td>
<td>(—)</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Reactor parameter model.</td>
<td>(—)</td>
</tr>
<tr>
<td>$\mu(S)$</td>
<td>Specific growth rate model.</td>
<td>(hr$^{-1}$)</td>
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<td>$\mu_m$</td>
<td>Maximum specific growth rate.</td>
<td>(hr$^{-1}$)</td>
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<td>$\tau$</td>
<td>Residence time ($\tau &gt; 0$).</td>
<td>(hr)</td>
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<tr>
<td>$\tau^*$</td>
<td>Dimensionless residence time ($\tau^* &gt; 0$).</td>
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<td>$\tau^*_{cr}$</td>
<td>The value of the dimensionless residence time at the transcritical bifurcation.</td>
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**References**