



A fundamental analysis of continuous flow bioreactor models governed by Contois kinetics. II. Reactor cascades

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ABSTRACT

The rate determining step of a number of biological processes is now known to be described by Contois growth kinetics. In particular this growth rate has been found to describe the treatment of contaminated wastewaters containing biodegradable organic materials from a variety of industrial processes. The efficient treatment of such waste materials is of ever growing environmental concern. This contribution is the first steady-state analysis for the treatment of industrial wastewaters, obeying Contois kinetics, in a cascade of continuous flow bioreactors without recycle. The steady-states of the model are found and their stability determined as a function of the residence time in each reactor of the cascade.

Asymptotic solutions are obtained for the effluent concentration leaving a cascade of n reactors for two scenarios, in which it is assumed that the reactors in the cascade have the same residence time. In the first scenario the limiting case of large total residence time (τ_n^*) is considered. The effluent concentration leaving the reactor (S_n^*) is found to be given by $S_n^* \approx (1/\tau_n^{*n})$, when $n = 1, 2, 3$ and 4 . It is conjectured that this relationship holds for all n . Thus, for a fixed total residence time increasing the number of reactors in the cascade has a dramatic effect on the quality of the wastewater leaving the cascade. In the second scenario, the limiting case when the total residence time is slightly larger than the washout point is considered. In this region, a small increase in the total residence time leads to a large decrease in the effluent concentration.

These results are illustrated by considering the anaerobic digestion of ice-cream wastewater.

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1. Introduction

We analyse the steady-state behaviour of a reactor cascade, without recycle, containing N reactors. We find the steady-state solutions, determine their stability and obtain asymptotic solutions in the limit of high residence times. The principle application that we have in mind is the treatment of industrial wastewaters. Although there exists detailed models for wastewater treatment kinetics, such as the IWA ASM model [1], we use a simple two-variable empirical kinetic model in which the degradation of a biodegradable organic material is given by the Contois expression [2]. It is not the intention of this paper to suggest that the Contois expression is *per se* superior to the Monod expression. Instead our choice of the Contois expression is motivated by a number of experimental investigations, detailed in Section 1.1, in which this kinetic model was found to accurately describe the processing of certain industrial wastewaters.

In an earlier paper we investigated the behaviour of a single reactor, possible including recycle, in which the degradation of the

substrate was controlled by a Contois expression [3]. The objectives of the current paper are to extend the process model to a cascade of reactors without recycle. Specifically we consider cascades having either two, three or four reactors. In Section 1.3 we provide an overview of the mathematical analysis of cascade reactors. It should be noted that the emphasis in this paper is the determination of the steady-state solutions, and their stability, of a cascade without recycle. The emphasis is *not* on the optimisation of a cascade. Of course, explicit formulae for the steady-state solutions of a cascade provides the foundations from which optimisation problems can be investigated. Such an investigation is outside the purview of the present contribution.

1.1. Contois growth kinetics

Many industrial processes, particularly in the food industry, produce slurries or wastewaters containing high concentrations of biodegradable organic materials (pollutants). For example, the production of slurries is a feature of large pig and poultry farms and other operations involving animal production. Before the slurry/wastewater can be discharged the concentration of the biodegradable organic pollutant must be reduced. One way to achieve this is to pass the wastewater through a bioreactor

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containing biomass which grows through consumption of the pollutant. Anaerobic conditions are often favoured for the processing of waste materials with high levels of biodegradable organic pollutants as these can be removed with low investment and operational costs [4].

The Contois growth model, Eq. (3), has been found to describe the aerobic degradation of wastewater originating from the industrial treatment of black olives [5], the aerobic biodegradation of solid municipal organic waste [6], the anaerobic treatment of dairy manure [7–9], the anaerobic digestion of ice-cream wastewater [10], and the anaerobic treatment of textile wastewater [11].

Within the confines of a more detailed kinetic model it has been shown that experimental data for the processing of cattle manure, swine waste, sewage sludge and cellulose (the last two in anaerobic digester's) can be accurately simulated by assuming that the hydrolysis kinetics are governed by Contois kinetics [12]. In fact, the Contois growth rate has been used as a default growth-rate model in simulations of the cleaning of wastewaters by microorganisms [13].

The Contois growth expression has also been found to model the anaerobic reduction of sulphate by a sulphate-reducing bacteria [14]. This procedure has application in the cleaning of sulphate-containing industrial effluents and in the cleaning of acid mine drainage.

In [7,8,10–12,14] the Contois growth model gave predictions that were in excellent agreement with experimental measurements. In some cases the Contois model was shown to give better agreement with data than other growth rate expressions [7,10,11,9,14]. What types of processes can be accurately modelled by Contois kinetics? Harmand and Godon [15] have proposed that the macroscopic behaviour of attached biomass bioreactors will be better described by growth rate laws that are a function of the ratio of substrate to biomass (S/X), such as the Contois expression, than growth rate laws that depend only upon the substrate concentration, such as the Monod expression. To support this position they provide a mixture of qualitative reasoning, literature data and numerical simulations. It is proposed that in compartmental models ratio dependent growth laws, rather than biomass-independent growth laws, better 'reflect' the underlying environmental heterogeneity. Accordingly, they are better able to mimic the effect of mass-transfer limitations, such as diffusion into biofilms and circumstances in which substrate consumption is controlled by biomass diffusion.

Finally, we note that there is a similarity between the consumption of a resource by an organism (microbiology) and the consumption of a prey by a predator (population ecology). Just [16] explored the use of 'growth-rate' functions containing organism/predator dependence in microbiology and population ecology. The equivalent of the Contois model in population ecology is the ratio-dependent model introduced by Arditi and Ginzburg [17].

1.2. Model assumptions

Wastewater from the food industries contains a complex mixture of biodegradable organic materials, such as fresh and partially decomposed food scraps and crop-residues, that may be in suspension or dissolved. Lumping these into a single substrate species, and the variety of microorganisms existing in the biological reactor into a single microorganism, is a convenient mathematical approximation. Formally, the use of a model containing a single substrate and a single microorganism can be justified if the overall process kinetics are controlled by a process-rate limiting step. The work cited in Section 1.1 suggests that in some cases this provides a reasonable approximation to an undoubtedly more complex process. In [12] the slowest step in the anaerobic digestion with suspended or dissolved wastes is identified as the hydrolysis of solids or the methanogenesis.

It has been suggested that the Contois specific growth rate accurately describes experimental data when mass-transfer limitations ensure that the underlying kinetic process is restricted by the available surface area. The Contois specific growth rate, Eq. (3), can be written in the equivalent form

$$\mu = \mu_{max} \left(\frac{S/X}{K_S + S/X} \right).$$

This reformulation shows that as the population density of biomass increases the growth rate of any particular microbe decreases, due to an increasing obstruction to substrate uptake. In the limit of large biomass concentration the Contois growth rate reduces to

$$\mu \approx \mu_{max} S / (K_S X),$$

indicating that the limiting factor is the surface area of the particulate substrate. Examples where the Contois model is interpreted as a surface limiting process include [18,19,12].

In Eq. (2) the term $-k_d X$ represents a combination of first-order processes. These include endogenous respiration, predation, and cell death and lysis [20].

1.3. Cascade reactors

In this section we provide a brief discussion of the literature relating to the mathematical analysis of cascade reactors. The problem of optimising the design of a cascade of bioreactors has been of interest since the early 1960s. In earlier papers the emphasis was on investigating the steady-state solutions of the system [21,22]. In latter papers the emphasis has been on the optimisation of the cascade design [23–29]. In the papers discussed in this section, unless otherwise stated, the biological process was modelled using Monod kinetics.

Herbert [21] comprehensively analysed the behaviour of two-reactor single-stream and multiple-stream cascades assuming that there was no death of the microorganisms. For both reactor configurations he found expressions for the steady-state solutions of a n reactor cascade. Regarding single-stream cascades he stated that

"there will seldom be much practical advantage in using more than two stages, so far as quantity production of cells is concerned. On the other hand, further stages might be important in obtaining cells of a desired quality. . ." ([21, p. 33])

Regarding multi-stream systems he stated that

"The great flexibility of multi-stream systems, combined with their stability and the ease of operation at high flow-rates make them superior to single-stream systems in all respects." ([21, page 41])

In particular, he showed that at identical residence times, a higher product yield was achieved and a more efficient use of substrate was seen in a multistage continuous system. Herbert's perspective was that of a microbiologist and his 'objective functions' were the reactor productivity and the effective yield. In his analysis he assumed a zero death-rate.

Powell and Lowe [22] investigated the behaviour of a cascade of N reactors of equal volume with recycle between the final and first reactors of the cascade. They showed that N is increased, the behaviour of the system approached that of an ideal tubular fermenter with plug flow. The critical value of the residence time, above which washout does not occur was found. It was assumed that there was a zero death-rate.

Bischoff [23] investigated the optimisation of a single-stream reactor cascade containing two reactors. The objective function was to minimise the total residence time for a specified conversion. It was assumed that there was a zero death-rate.

Erickson and Fan [24] considered the optimisation of a N -reactor cascade ($N = 2$ or 3) with recycle with a non-zero death rate. They considered two objective functions. The first was to minimise the total reactor volume of the cascade given a desired effluent concentration. The second function included two terms: one accounting for the cost of the organic waste being discharged and one accounting for the cost of the total reactor volume of the cascade.

Erickson et al. [25] considered the optimisation of a N -reactor multi-stream cascade with recycle and a zero death rate. Each reactor contained two stages: a mixing stage and an aeration stage. The influent entered into the mixing stage, it was assumed that there was no reaction in this stage. Thus the cascade contained $2N$ reactors. The objective function was, given specified concentrations of the organisms and substrate in the recycle stream, to find the allocation of the influent into the mixing tanks and the distribution of reactor volumes so that the total volume of the reactor cascade is minimised. This process was carried out for $N = 2, 3, 4$ and 5 .

Grieves and Kao [26] considered a multi-stream reactor cascade with three reactors and a non-zero death rate. The objective function was to maximise substrate utilisation by varying the reactor volumes and the feed input distributions.

Grady and Lim reviewed the theory of reactor cascades without recycle [30], and with recycle [31] from the perspective of biological wastewater treatment. Their review included the use of a single feed stream and multiple feed streams. They investigated the optimisation of reactor design from two points of view: the minimum effluent concentration that can be obtained from a specified total volume and the minimum reactor volume that is required to deliver a specified effluent concentration.

Braha and Hafner [32] develop a graphical technique that can be used to analyse data obtained from a cascade containing upto four reactors, possible including recycle from the fourth to first reactor. Assuming that the death rate is zero, the technique provides estimates of the biokinetic parameters in the Monod expression. More detailed, and accurate, approaches to estimating the biokinetic parameters, and other relevant parameters, are outlined by Grady and Lim [30,31].

Hill and Robinson [27] investigated the minimum residence time required to achieve a specified substrate conversion as a function of the number of tanks in series for systems governed by Monod kinetics, substrate inhibition and product inhibition. For the majority of cases considered, little reduction in the minimum residence time was achieved by increasing the number of tanks over three. Three reactors in series, when optimised, gave a performance at least comparable to, and often superior to, a plug flow reactor. The benefit obtained by optimising the system was lower for systems subject to substrate inhibition, compared to systems subject to Monod kinetics, and higher for systems subject to product inhibition. However, in both cases the change was "not all that great" ([27, p. 822]). Relative to the case of Monod kinetics both substrate and product inhibition increases the minimum residence time required to achieve a specified conversion. Finally, it was shown that the use of reactors with equal size generally causes the total residence time required to achieve a specified conversion to increase sharply; this is most noticeable when the cascade contains three, or more, reactors.

Scuras et al. [28] present a general procedure to determine the optimum reactor configuration for a range of influent and effluent substrate concentrations, half-saturation coefficients, and number of tanks in series for both inhibitory substrates, obeying Andrews kinetics, and non-inhibitory substrates, obeying Monod kinetics. The objective function is to minimise the quantity of biomass in the cascade to achieve a specified effluent concentration. The justification for this choice of objective function is that reducing the biomass concentration lowers the biomass flux into the sedimentation basin which in turn reduces the required settling area. The

benefits of staging increased when there is either a high influent substrate concentrations and/or stringent discharge requirements. It is concluded that three tanks in series is generally best and that optimal tank sizing is significantly better than using tanks of equal size. Relative to the requirements of a single reactor, staging can reduce the biomass requirement by upto 20%. It is assumed that the death-rate is zero.

Harmand et al. [29] investigated the optimisation of a two-stage reactor cascade in which the design configuration may include two feed streams (multi-stream flow) and/or a recirculation loop. The objective function is to minimise the total volume of the cascade given a specified conversion. The steady-state design problem was solved for a generalised growth rate law. Special cases of this generalised growth rate law include Monod kinetics, substrate inhibition (Andrews kinetics) and product inhibition. (Only results for the Monod case and substrate inhibition are presented.) It is assumed that the death-rate is zero.

It was shown that the optimisation problem gives one of two generic cases. In the first case the optimal reactor design is unique. It does not use a recycle loop and all the feed stream enters the first reactor. In the second case the optimal reactor design is non-unique but consists of a class of systems having the same total volume. The optimal solutions differ in the way in which the functionality of the two feed streams and the recirculation loop are utilised. A simple criteria identifies which case is appropriate to a given circumstance. Conditions for which the optimised reactor design are locally stable are established.

In their generalised formulation the problem considered by Hill and Robertson [27] appears as a special case. As the specified degree of conversion *decreases* the approach of Harman et al. produces optimised cascades with significantly lower volumes than that of Hill and Robinson [27].

2. Model equations and summary of results for a single reactor

In Sections 2.1 and 2.2 we provide the dimensional and dimensionless model equations for a n reactor cascade. In Section 2.3 we state some results for a Contois process in a single reactor [3].

2.1. The dimensional model

The model equations for a n reactor cascade without recycle are

$$V_i \frac{dS_i}{dt} = F(S_{i-1} - S_i) - \frac{1}{\alpha} \mu(S_i, X_i) V_i X_i, \quad (1)$$

$$V_i \frac{dX_i}{dt} = F(X_{i-1} - X_i) + \mu(S_i, X_i) V_i X_i - V_i k_d X_i, \quad (2)$$

Specific growth rate

$$\mu(S_i, X_i) = \frac{\mu_{\max} S_i}{K_s X_i + S_i}, \quad (3)$$

Residence time

$$\tau_i = \frac{V_i}{F}, \quad (4)$$

where i ($1 \leq i \leq n$) denotes the i th reactor in the cascade. The units that the concentrations of the substrate species, S , and the microorganisms, X , are measured in are denoted by $|S|$ and $|X|$ respectively. The parameters in the model are: F , the flowrate through the reactor cascade ($\text{dm}^3 \text{h}^{-1}$); K_s , the saturation constant ($|S||X|^{-1}$); S_i , the substrate concentration within the i th reactor of the cascade ($|S|$); S_0 , the concentration of substrate flowing into the first reactor of the cascade ($|S|$); V_i , the volume of the i th reactor of the cascade (dm^3); X_i , the concentration of cell-mass within the i th reactor of the cascade ($|X|$); X_0 , the concentration of cell-mass flowing into

the first reactor of the cascade ($|X|$); k_d , the death coefficient (h^{-1}); t , time (h); α , the yield factor ($|X||S|^{-1}$); μ , the specific growth rate model (h^{-1}); μ_{max} , the maximum specific growth rate (h^{-1}); and τ_i , the residence time within the i th reactor of the cascade (h).

For a specific wastewater, a given biological community and a particular set of environmental conditions the parameters K_s , k_d , α and μ_{max} are fixed. The parameters that can be varied are S_0 , X_0 and τ_i .

In our numerical simulations we use parameter values for the anaerobic digestion of ice-cream wastewater [10]. These are: $K_s = 0.4818$ (g COD) (g VSS) $^{-1}$, $k_d = 0.0131$ (day $^{-1}$), $\alpha = 0.2116$ (g VSS) (g COD) $^{-1}$, $\mu_{max} = 0.9297$ (day $^{-1}$).

In Eqs. (1) and (2) it is assumed that the yield coefficient is constant. Extensions of this model in which the yield coefficient is assumed to be a linear function of the substrate concentration ($\alpha = \alpha_0 + \beta S$) have recently been investigated [33,34].

2.2. The dimensionless model

By introducing dimensionless variables for the substrate concentration in the i th reactor of the cascade [$S_i^* = S_i/S_0$], the cell mass concentration in the i th reactor of the cascade [$X_i^* = K_s X_i/S_0$] and time [$t^* = \mu_{max} t$] the dimensional model, Eqs. (1) and (2), can be written in the dimensionless form

$$\frac{dS_i^*}{dt^*} = \frac{1}{\tau_i^*} (S_{i-1}^* - S_i^*) - \frac{1}{\alpha^*} \frac{X_i^* S_i^*}{X_i^* + S_i^*}, \quad (5)$$

$$\frac{dX_i^*}{dt^*} = \frac{1}{\tau_i^*} (X_{i-1}^* - X_i^*) + \frac{X_i^* S_i^*}{X_i^* + S_i^*} - k_d^* X_i^*, \quad (6)$$

where the parameter groups are: the dimensionless substrate concentration in the feed [$S_0^* = 1$]; the dimensionless biomass concentration in the feed [$X_0^* = X_0 K_s/S_0$]; the dimensionless decay rate [$k_d^* = k_d/\mu_{max}$], the dimensionless yield coefficient [$\alpha^* = K_s \alpha$] and the dimensionless residence time [$\tau_i^* = V_i \mu_{max}/F$]. All parameters in the model are strictly non-negative.

From now on we assume that the growth medium fed into the bioreactor is sterile, i.e. there are no microorganisms in the influent ($X_0 = X_0^* = 0$), and that $S_0^* > 0$.

For the anaerobic digestion of ice-cream wastewater we have $\alpha^* = 0.1019$ and $k_d^* = 0.0141$.

2.3. Summary of results for a single reactor

The steady-state solutions in the first reactor of the cascade, $i = 1$ in Eqs. (5) and (6), are given by

$$\begin{aligned} &\text{Washout branch} \\ &(S_1^*, X_1^*) = (1, 0). \end{aligned} \quad (7)$$

No-washout branch,

$$(S_1^*, X_1^*) = \frac{\alpha^*}{A} (1 + k_d^* \tau_1^*, -1 + (1 - k_d^*) \tau_1^*), \quad (8)$$

$$A = (1 + k_d^* \tau_1^*) [\alpha^* - 1 + (1 - k_d^*) \tau_1^*].$$

The no-washout branch is only physically meaningful when the substrate and cell-mass concentrations are positive ($S_1^* > 0$, $X_1^* > 0$). This happens when

$$-1 + (1 - k_d^*) \tau_1^* > 0,$$

which requires that $0 < k_d^* < 1$. Henceforth we assume that this condition holds. The washout branch is stable when the no-washout branch is not physically meaningful ($\tau_1^* < 1/(1 - k_d^*)$). The washout branch is unstable when the no-washout branch is physically meaningful ($\tau_1^* > 1/(1 - k_d^*)$). The no-washout branch is stable when it is physically meaningful.

A transcritical bifurcation occurs when the residence time takes the value

$$\tau_1^* = \tau_{tr}^* = \frac{1}{1 - k_d^*}.$$

At this value of the residence time the washout solution branch intersects the no-washout solution branch [$(S^*, X^*) = (1, 0)$]. For the anaerobic digestion of ice-cream wastewater we have $\tau_{tr}^* = 1.01$, which corresponds to $\tau_{tr} = 1.091$ day = 26.164 h.

Consider a cascade of n reactors of equal residence time having a total residence time of τ_t^* . Then the residence time in each reactor is τ_i^*/n . It follows that when

$$\tau_t^* < \frac{n}{1 - k_d^*}$$

washout occurs throughout the cascade. We call the value

$$\tau_t^* = \frac{n}{1 - k_d^*}$$

the washout point.

3. Results

The assumption that there is no recycling in the cascade means that the process variables in the first reactor (S_1, X_1) are independent of the process variables in all other reactors and that the process variables in the i th reactor ($S_i, X_i, i \neq 1$) depend only upon those in the preceding reactor (S_{i-1}, X_{i-1}). This significantly simplifies the study of system (5) and (6).

In Section 3.1 we show that, provided that washout does not occur in the first reactor, the system has a unique steady-state solution and give the steady-state values of the substrate and cell-mass concentrations in the i th reactor of the cascade. In Section 3.2 the steady-state solution is shown to be locally stable.

In Sections 3.1 and 3.2 the residence time's in each reactor are arbitrary. In Sections 3.3–3.5 we assume that the residence time in each reactor of the cascade is equal and treat the total residence time of the reactor as the primary bifurcation parameter. In Section 3.3 we provide asymptotic solutions for the substrate and cell-mass concentrations in the first four reactors of a cascade at large residence times. In Section 3.4 asymptotic solutions are presented for residence times just higher than the washout point ($\tau_t^* = (n/(1 - k_d^*))$).

In Section 3.5 we discuss steady-state diagrams for the effluent concentration leaving the cascade. We investigate the performance of a cascade of two, three and four reactors as a function of the total residence time inside the cascade. We also consider the performance of a cascade of two and three reactors in which the residence times inside each reactor are optimised to minimise the effluent concentration at a specified total residence time. The performance of these optimised cascades is compared against that of reactor cascades in which the residence times in each reactor are equal.

3.1. Steady-state solution

The steady-state solutions in the i th reactor of the cascade ($i > 1$) are found by solving the algebraic equations

$$f(S_i^*, X_i^*) = \frac{1}{\tau_i^*} (S_{i-1}^* - S_i^*) - \frac{1}{\alpha^*} \frac{X_i^* S_i^*}{X_i^* + S_i^*} = 0, \quad (9)$$

$$g(S_i^*, X_i^*) = \frac{1}{\tau_i^*} (X_{i-1}^* - X_i^*) + \frac{X_i^* S_i^*}{X_i^* + S_i^*} - k_d^* X_i^* = 0.$$

By considering the expression

$$f(S_i^*, X_i^*) + \frac{g(S_i^*, X_i^*)}{\alpha^*} = 0$$

we deduce that

$$S_i^* = A_i - B_i X_i^*,$$

where the coefficients A_i and B_i are defined below. Substitution of this expression into either of the equations in (9) shows that the cellmass concentration X_i^* satisfies the quadratic equation

$$0 = a_i X_i^{*2} + b_i X_i^* + c_i, \quad (10)$$

and is given by

$$X_i^* = \frac{-b_i \pm \sqrt{b_i^2 - 4a_i c_i}}{2a_i}. \quad (11)$$

The coefficients in the preceding three equations are

$$\begin{aligned} A_i &= S_{i-1}^* + \frac{X_{i-1}^*}{\alpha^*}, \\ B_i &= \frac{1 + k_d^* \tau_i^*}{\alpha^*}, \\ a_i &= (B_i - 1)k_d^* \tau_i^* + B_i(1 - \tau_i^*) - 1, \\ b_i &= (1 - B_i)X_{i-1}^* + A_i[(1 - k_d^*)\tau_i^* - 1], \\ c_i &= A_i X_{i-1}^*. \end{aligned}$$

Thus the solution of the steady-state Eq. (9) is given by

$$(S_i^*, X_i^*) = (A_i - B_i X_i^*, X_i^*). \quad (12)$$

We show in Appendix A that the solution corresponding to the choice of the positive square root in Eq. (11) is not physically meaningful as either the substrate concentration (S_i^*) or the cell mass concentration (X_i^*) are negative. However, the solution corresponding to the choice of the negative square root is physically meaningful; both the cell mass and substrate concentrations are positive. Thus, given that washout does not occur in the first reactor, the solution in each reactor in the cascade is unique.

Note that even if the residence time in the i th reactor is smaller than the washout condition ($\tau_i^* < \tau_{ir}^*$) then washout does not occur in the i th reactor because biomass from the $(i - 1)$ th reactor flows into the i th reactor. And by assumption washout does not occur in the first reactor.

3.2. Stability of solutions

The Jacobian matrix for a n -reactor cascade is given by

$$J = \begin{pmatrix} J_1 & \mathbf{0} & \mathbf{0} & \mathbf{0} & \dots \\ B_2 & J_2 & \mathbf{0} & \mathbf{0} & \dots \\ \mathbf{0} & B_3 & J_3 & \mathbf{0} & \dots \\ \mathbf{0} & \ddots & \ddots & \ddots & \ddots \end{pmatrix}.$$

This matrix has size $2n \times 2n$. The blocks B_i and J_i each have size 2×2 and are defined by

$$\begin{aligned} B_i &= \begin{pmatrix} \frac{1}{\tau_i^*} & 0 \\ 0 & \frac{1}{\tau_i^*} \end{pmatrix}, \\ J_i &= \begin{pmatrix} \frac{1}{\tau_i^*} - \frac{1}{\alpha^*} \frac{X_i^{*2}}{(X_i^* + S_i^*)^2} & -\frac{1}{\alpha^*} \frac{S_i^{*2}}{(X_i^* + S_i^*)^2} \\ \frac{X_i^{*2}}{(X_i^* + S_i^*)^2} & -1 - \frac{k_d^* \tau_i^*}{\tau_i^*} + \frac{S_i^{*2}}{(X_i^* + S_i^*)^2} \end{pmatrix}, \end{aligned}$$

and $\mathbf{0}$ represents the zero matrix of size 2×2 . We establish in Appendix B that the Jacobian matrix has two eigenvalues associated with each block J_i . The block J_1 has two eigenvalues with negative real parts when (Section 2.3)

$$\tau_1^* > 1/(1 - k_d^*).$$

The Jacobian matrix J_i ($i > 2$) can be re-written as

$$J_i = \begin{pmatrix} -\frac{1}{\tau_i^*} - \frac{1}{\alpha^*} \frac{X_i^{*2}}{(X_i^* + S_i^*)^2} & -\frac{1}{\alpha^*} \frac{S_i^{*2}}{(X_i^* + S_i^*)^2} \\ \frac{X_i^{*2}}{(X_i^* + S_i^*)^2} & -\frac{S_i^* X_i^*}{(X_i^* + S_i^*)^2} - \frac{X_{i-1}^*}{X_i^*} \frac{1}{\tau_i^*} \end{pmatrix};$$

in obtaining this expression for $J_i(2, 2)$ we have used the fact that along the no-washout branch

$$\frac{-1 - k_d^* \tau_i^*}{\tau_i^*} = -\frac{S_i^*}{X_i^* + S_i^*} - \frac{X_{i-1}^*}{X_i^*} \frac{1}{\tau_i^*},$$

which follows from Eq. (6). The eigenvalues of the 2×2 matrix J_i have negative real parts when the determinant ($\det J$) and trace of J_i ($\text{trace} J$) are positive and negative respectively. We have

$$\begin{aligned} \det J &= \left(\frac{1}{\tau_i^*} + \frac{1}{\alpha^*} \frac{X_i^{*2}}{(X_i^* + S_i^*)^2} \right) \left(\frac{X_i^* S_i^*}{(X_i^* + S_i^*)^2} + \frac{X_{i-1}^*}{X_i^*} \frac{1}{\tau_i^*} \right) \\ &\quad + \frac{X_i^{*2}}{(X_i^* + S_i^*)^2} \frac{1}{\alpha^*} \frac{S_i^{*2}}{(X_i^* + S_i^*)^2}, \\ \text{trace} J &= -\frac{1}{\tau_i^*} - \frac{1}{\alpha^*} \frac{X_i^{*2}}{(X_i^* + S_i^*)^2} - \frac{S_i^* X_i^*}{(X_i^* + S_i^*)^2} - \frac{X_{i-1}^*}{X_i^*} \frac{1}{\tau_i^*}. \end{aligned}$$

The trace of the Jacobian is negative and the determinant of the Jacobian is positive for all physically meaningful solutions ($X_i^* > 0$ and $S_i^* > 0$). Consequently, provided that washout does not occur in the first reactor, the steady-state solution of the cascade is locally stable.

3.3. Large residence time approximations

In this section we provide large residence approximations to the concentrations of substrate and microorganisms inside the first four reactors of a cascade of n reactors. We denote by τ_t^* the total residence time in the cascade. We assume that the residence time in each reactor of the cascade is equal, therefore $\tau_i^* = \tau_t^*/n$.

At large values of the cascade residence time (τ_t^*) we have the approximations

$$S_1^* \approx \frac{n\alpha^*}{1 - k_d^*} \frac{1}{\tau_t^*} + O\left(\frac{1}{\tau_t^{*2}}\right), \quad X_1^* \approx \alpha \frac{n}{k_d^*} \frac{1}{\tau_t^*} + O\left(\frac{1}{\tau_t^{*2}}\right), \quad (13)$$

$$S_2^* \approx c_2 \left(\frac{n\alpha^*}{1 - k_d^*} \right)^2 \frac{1}{\tau_t^{*2}} + O\left(\frac{1}{\tau_t^{*3}}\right),$$

$$X_2^* \approx \alpha d_2 \left(\frac{n}{k_d^*} \right)^2 \frac{1}{\tau_t^{*2}} + O\left(\frac{1}{\tau_t^{*3}}\right), \quad (14)$$

$$S_3^* \approx c_3 c_2 \left(\frac{n\alpha^*}{1 - k_d^*} \right)^3 \frac{1}{\tau_t^{*3}} + O\left(\frac{1}{\tau_t^{*4}}\right),$$

$$X_3^* \approx \alpha d_3 d_2 \left(\frac{n}{k_d^*} \right)^3 \frac{1}{\tau_t^{*3}} + O\left(\frac{1}{\tau_t^{*4}}\right), \quad (15)$$

$$S_4^* \approx c_4 c_3 c_2 \left(\frac{n\alpha^*}{1 - k_d^*} \right)^4 \frac{1}{\tau_t^{*4}} + O\left(\frac{1}{\tau_t^{*5}}\right),$$

$$X_4^* \approx \alpha d_4 d_3 d_2 \left(\frac{n}{k_d^*} \right)^4 \frac{1}{\tau_t^{*4}} + O\left(\frac{1}{\tau_t^{*5}}\right), \quad (16)$$

where the coefficients c_i and d_i are given by

$$c_2 = \frac{\alpha^* k_d^* + 1 - k_d^*}{\alpha^* k_d^* + 1}$$

$$c_3 = \frac{\alpha^{*2} k_d^{*2} + \alpha^* k_d^* (1 - k_d^*) + 1 - k_d^*}{\alpha^{*2} k_d^{*2} + \alpha^* k_d^* + 1}$$

$$c_4 = \frac{\alpha^{*3} k_d^{*3} + \alpha^{*2} k_d^{*2} (1 - k_d^*) + \alpha^* k_d^* (1 - k_d^*) + 1 - k_d^*}{(1 + \alpha^* k_d^*) (1 + \alpha^{*2} k_d^{*2})}$$

$$d_2 = \frac{\alpha^* k_d^* + 1 - k_d^*}{1 - k_d^*}$$

$$d_3 = \frac{\alpha^{*2} k_d^{*2} + \alpha^* k_d^* (1 - k_d^*) + 1 - k_d^*}{(\alpha^* k_d^* + 1) (1 - k_d^*)}$$

$$d_4 = \frac{\alpha^{*3} k_d^{*3} + \alpha^{*2} k_d^{*2} (1 - k_d^*) + \alpha^* k_d^* (1 - k_d^*) + 1 - k_d^*}{(1 - k_d^*) (\alpha^{*2} k_d^{*2} + \alpha^* k_d^* + 1)}$$

Eqs. (13)–(16) show that the substrate concentration leaving a n -reactor cascade (S_n^*) is proportional to $(1/\tau_t^{*n})$ ($n = 1, 2, 3, 4$). It is conjectured that this holds for any n . Should this be true then, at sufficiently large total residence times, the addition of an extra reactor reduces the effluent concentration by a multiplicative factor of $(1/\tau_t^*)$. Eqs. (13)–(16) give the dimensionless substrate and cell-mass concentrations in the first four reactors of a cascade. These expressions hold for any value of n . Thus, for example, substitution $n = 10$ in these gives the concentrations in the first through to fourth reactor of a ten-reactor cascade. As n increases the substrate concentration leaving a particular reactor increases as the residence time in that reactor decreases.

3.4. Residence time approximations near the washout point

In this section we provide approximations for the concentrations of substrate and cell mass inside each reactor in a cascade of n reactors ($n = 1, 2, 3$ and 4) when the total residence time is slightly higher than the washout point ($\tau_t^* = (n/(1 - k_d^*))$). Thus we seek an approximation near the value

$$\tau_t^* = \frac{1}{1 - k_d^*} + \frac{\epsilon}{n}, \quad \epsilon > 0.$$

For small values of the parameter ϵ we have the approximations

$$S_1^* \approx 1 - \frac{(1 - k_d^*) \epsilon}{\alpha^* n} + O\left(\frac{\epsilon}{n}\right)^2, \quad X_1^* \approx (1 - k_d^*)^2 \frac{\epsilon}{n} + O\left(\frac{\epsilon}{n}\right)^2, \quad (17)$$

$$S_2^* \approx 1 - \frac{(1 - k_d^*)^{1/2}}{\alpha^*} \left(\frac{\epsilon}{n}\right)^{1/2} + O\left(\frac{\epsilon}{n}\right),$$

$$X_2^* \approx (1 - k_d^*)^{1+1/2} \left(\frac{\epsilon}{n}\right)^{1/2} + O\left(\frac{\epsilon}{n}\right), \quad (18)$$

$$S_3^* \approx 1 - \frac{(1 - k_d^*)^{1/4}}{\alpha^*} \left(\frac{\epsilon}{n}\right)^{1/4} + O\left(\frac{\epsilon}{n}\right)^{1/2},$$

$$X_3^* \approx (1 - k_d^*)^{1+1/4} \left(\frac{\epsilon}{n}\right)^{1/4} + O\left(\frac{\epsilon}{n}\right)^{1/2}, \quad (19)$$

$$S_4^* \approx 1 - \frac{(1 - k_d^*)^{1/8}}{\alpha^*} \left(\frac{\epsilon}{n}\right)^{1/8} + O\left(\frac{\epsilon}{n}\right)^{1/4},$$

$$X_4^* \approx (1 - k_d^*)^{1+1/8} \left(\frac{\epsilon}{n}\right)^{1/8} + O\left(\frac{\epsilon}{n}\right)^{1/4}. \quad (20)$$

Consider a cascade of n reactors operating at a residence time slightly higher than that corresponding to washout through the cascade ($\tau_t^* = 1/(1 - k_d^*) + \epsilon/n$). Expressions (17)–(20) show that as ϵ increases from zero the effluent concentration decreases much

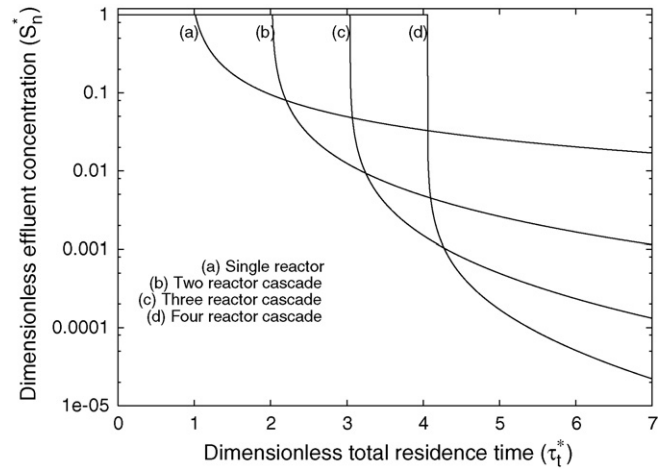


Fig. 1. Effluent concentration (S_n^*) in a cascade of n reactors of equal residence time.

more sharply as the number of reactors increases. This can be seen visually in Fig. 1. As in Section 3.3 these expressions hold for any value of n .

3.5. Steady-state diagrams

Fig. 1 shows the effluent concentration (S_n^*) leaving a cascade of n equal reactors as a function of the total residence time in the cascade for the cases $n = 1, 2, 3$ and 4 . In each case three regions of behaviour can be identified. When the total residence is lower than the washout point ($\tau_t^* = (n/(1 - k_d^*))$) washout occurs in each reactor in the cascade. The effluent concentration is therefore the same as the pollutant concentration entering the first reactor in the cascade ($S_n^* = 1$). In the second region, in which $0 < \tau_t^* - (n/(1 - k_d^*)) \ll 1$, the effluent concentration decreases rapidly with small increases in the total residence time. This region is increasingly noticeable as the number of reactors in the cascade increases. In the third region the substrate concentration decreases much more slowly as the residence time increases. In this region comparatively small decreases in effluent concentration require considerable increases in total residence time. If the required degree of process efficiency lays in the third region, large residence times will be required. The figure shows that at a total residence time $\tau_t^* = 7$ the effluent concentration leaving a single reactor can be several orders of magnitude higher than that leaving a reactor cascade consisting of two, three and four reactors.

The behaviour at sufficiently large residence times is governed by the equations given in Section 3.3. These equations indicate why the performance of the reactor only increases marginally with increases with total residence time in this region. For instance, for a single reactor the effluent concentration at large residence times is inversely proportional to the residence time. For a four-reactor cascade the effluent concentration, at large residence times, is inversely proportional to the fourth power of the residence time. The effluent concentration leaving a four-reactor cascade at a total residence time $\tau_t^* = 7$ is $S_4^* = 2.21 \times 10^{-5}$. To achieve the same effluent concentration in a cascade of three, two and one reactors requires total residence times of $\tau_t^* = 11.69$, $\tau_t^* = 44.51$ and $\tau_t^* = 4678.9$ respectively.

Fig. 1 shows that there is a range of residence times over which the performance of a cascade decreases as the number of reactors in the cascade is increased. Table 1 shows the residence time above which a cascade with i reactors has a lower effluent concentration than a reactor cascade with j reactors ($i > j$). Thus, for example, the effluent concentration leaving a three-reactor cascade is lower than that leaving a two-reactor cascade provided that the total residence

Table 1

The residence time above which a reactor cascade of i reactors has a lower effluent concentration than a reactor cascade of j reactors ($i > j$).

	1	2	3	4
2	2.20	×	–	–
3	3.07	3.25	×	–
4	4.06	4.10	4.27	×

time satisfies $\tau_1^* > 3.25$. The existence of such a ‘transition point’ is a consequence of the fact that the washout point of a cascade increases with the number of reactors in the cascade.

In Fig. 1 the residence time in each reactor of the cascade was assumed to be the same. However, for a fixed total residence time such a reactor configuration may not minimise the effluent concentration. In Fig. 2 the effluent concentration leaving a double reactor cascade is determined as a function of the residence time in the first reactor; the total residence time in the cascade is fixed to $\tau_t^* = 7$. In this figure the limits $\tau_1^* = 0$ and $\tau_1^* = 7$ correspond to the ‘degenerate’ case in which the two reactor cascade consists of a single reactor having a residence time $\tau_1^* = 7$. As the residence time in the first reactor increases from zero we see that the effluent concentration initially increases: the performance of the cascade is inferior to that of the single reactor. The reason for this is that whilst the value of the residence time in the first reactor is lower than the washout condition for a single reactor ($\tau_1^* < \tau_{tr}^* = 1.01$) an increase in the residence time in the first reactor reduces the operational residence time of the second reactor. As soon as the residence time in the first reactor exceeds the washout value a small increase in its value causes the effluent concentration to rapidly decrease.

Around the optimal design point of a double-reactor cascade the effluent concentration is a very flat function of the design parameter (τ_1^*). Small variations in the value of the design parameter around the optimal value therefore have a very small influence upon the effluent concentration. The reactor with equal residence times in each reactor ($\tau_1^* = 3.5$) is within the ‘flat’ region. Consequently there is very little improvement to be had, at a total residence $\tau_t^* = 7$, by optimising the reactor design compared to having two reactors of equal residence time. In Fig. 2 the effluent concentration is minimised when $\tau_1^* = 4.007$, which gives $\tau_2^* = 2.993$ and is $S_2^* = 1.11 \times 10^{-3}$. However, when there is a equal residence time distribution, $\tau_1^* = \tau_2^* = 3.5$, the effluent concentration is $S_2^* = 1.14 \times 10^{-3}$.

We have determined, for a fixed total residence time, the minimum effluent concentration that can be obtained by optimising

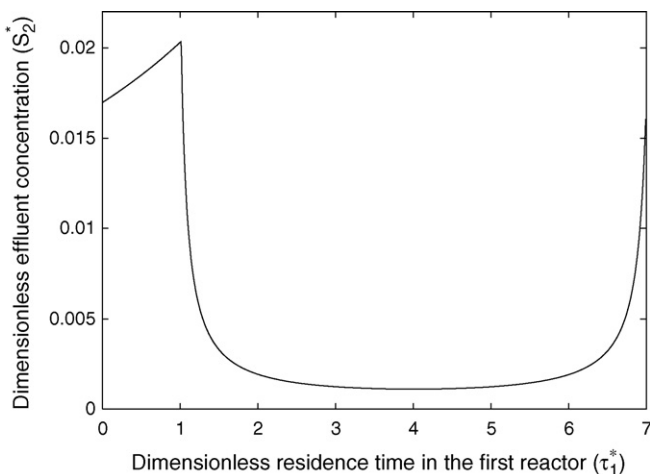


Fig. 2. Effluent concentration (S_2^*) as a function of the residence time in the first reactor (τ_1^*) of a two reactor cascade. Parameter value: total residence time, $\tau_t^* = 7$.

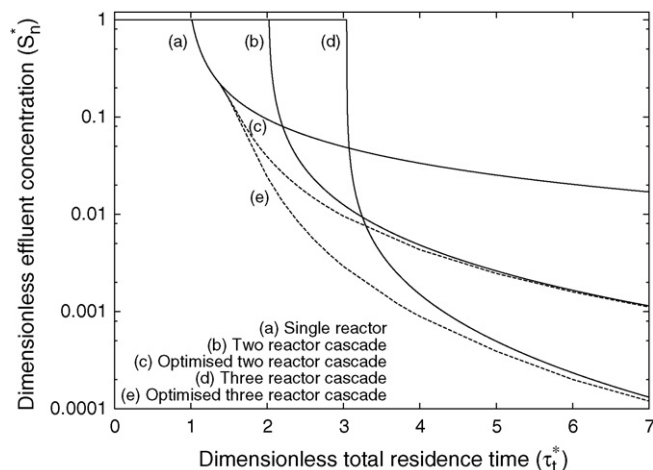


Fig. 3. Effluent concentration (S_n^*) in a cascade of n reactors of equal residence time (τ_i^*) (curves a, b and d) and in an optimised reactor of n reactors of unequal residence time (curves c and e).

the design of a cascade of two or three reactors. This data is shown in Fig. 3 where it is compared against the effluent concentration leaving a cascade of one, two and three reactors having equal residence times in each reactor. For example, at a fixed total residence $\tau_t^* = 7$ the effluent concentration from a triple cascade is minimised when $\tau_1^* = 3.02564$, $\tau_2^* = 2.01023$ and $\tau_3^* = 1.96413$: $S_3^* = 1.19 \times 10^{-4}$. The effluent concentration from a triple reactor with equal residence times is $S_3^* = 1.31 \times 10^{-4}$. At small residence times, the effluent concentration leaving an optimised reactor cascade can be substantially lower than that leaving a cascade with equal residence times. This is most noticeable in parameter regions in which the reactor configuration with equal residence times operates in a state of washout whereas the optimised configuration operates in a state of no-washout. Fig. 3 shows that as the total residence time becomes larger the reactor configuration with equal residence times converges towards the optimised configuration.

4. Discussion

There are a number of definitions which are used to characterise the steady-state performance of a continuous flow bioreactor processing industrial wastewaters [35]. The results stated in this section only apply when the no-washout branch is physically meaningful, that is when

$$\tau^* > \tau_{tr}^* = \frac{1}{1 - k_d^*} > 0.$$

The specific utilisation (\mathcal{U}), which is also known as the process loading factor, the substrate removal velocity and the food to microorganism ratio, is the rate of substrate utilisation per unit mass of microorganisms. For the first reactor in a cascade, or for a single reactor, it is defined by

$$\mathcal{U}_1 = \frac{S_0 - S}{X} \frac{1}{\tau}.$$

The dimensionless specific utilisation (\mathcal{U}^*) is given by

$$\mathcal{U}_1^* = \frac{1 - S^*}{X^*} \frac{1}{\tau^*}.$$

The dimensionless specific utilisation in the i th reactor of a cascade is given by

$$\mathcal{U}_i^* = \frac{S_{i-1} - S_i^*}{X_i^*} \frac{1}{\tau_i^*}.$$

Table 2

The total residence time in a cascade of n reactors to achieve an efficiency of 99%, 99.9% and 99.99%.

\mathcal{E}^*	$n = 1$	$n = 2$	$n = 3$	$n = 4$
99%	11.25	3.19	3.23	4.07
99.9%	104.27	7.41	4.31	4.28
99.99%	1034.48	21.37	7.55	5.38

Using system (9) this expression can be simplified to

$$\mathcal{U}_i^* = \frac{k_d^*}{\alpha^*} + \frac{1}{\alpha^* \tau_i^*} \left(1 - \frac{X_{i-1}^*}{X_i^*} \right).$$

We now assume that the residence times in each tank are equal and given by $\tau_i^* = \tau^*/n$, where n is the total number of tanks in the cascade. For the first reactor we have the exact expression

$$\mathcal{U}_1^* = \frac{k_d^*}{\alpha^*} + \frac{n}{\alpha^* \tau_1^*}.$$

For the second and third tanks in the cascade we have the asymptotic expressions

$$\begin{aligned} \mathcal{U}_2^* &= \frac{k_d^{*2}}{\alpha^* k_d^* + 1 - k_d^*} + \frac{u_2}{\tau_i^*} + O\left(\frac{1}{\tau_i^*}\right)^2, \\ \mathcal{U}_3^* &= \frac{\alpha^* k_d^{*3}}{\alpha^{*2} k_d^{*2} + (1 - k_d^*)k_d^* \alpha^* + 1 - k_d^*} + \frac{u_3}{\tau_i^*} + O\left(\frac{1}{\tau_i^*}\right)^2, \\ u_2 &= \frac{n(2 - k_d^* + 2k_d^* \alpha^*)k_d^*}{(1 + k_d^* \alpha^*)(1 - k_d^* + k_d^* \alpha^*)^2}, \\ u_3 &= \frac{[(1 - k_d^*)(2k_d^{*2} \alpha^{*2} + 3\alpha^* k_d^* + 2) + 3k_d^{*3} \alpha^{*3} + 4k_d^{*2} \alpha^{*2} + 3k_d^* \alpha^* + 1]n\alpha^* k_d^{*2}}{(1 + \alpha^* k_d^*)(\alpha^{*2} k_d^{*2} + (1 - k_d^*)\alpha^* k_d^* + 1 - k_d^*)^2 (1 + \alpha^* k_d^* + \alpha^{*2} k_d^{*2})}. \end{aligned}$$

Along the no-washout branch we have the requirement that $0 < k_d^* < 1$ and thus the coefficients u_2 and u_3 are positive. It follows that the specific utilization in the second and third reactor is asymptotically a decreasing function of the residence time. Furthermore, in the limit of infinite residence times it follows that

$$\mathcal{U}_1(\tau_i^* = \infty) > \mathcal{U}_2(\tau_i^* = \infty) > \mathcal{U}_3(\tau_i^* = \infty).$$

The treatment, or process, efficiency (\mathcal{E}_n) of a n -reactor cascade measures the percentage of substrate that has been removed by the cascade. It is defined by

$$\mathcal{E}_n = 100 \frac{S_0 - S_n}{S_0}.$$

In dimensionless variables this is

$$\mathcal{E}_n^* = 100(1 - S_n^*)$$

Using Eqs. (13)–(16) we see that at large residence times the efficiency is given by

$$\mathcal{E}_n^* = 100 \left(1 - \prod_{i=1}^n c_i \left(\frac{n\alpha^*}{1 - k_d^*} \right)^n \frac{1}{\tau_i^{*n}} \right),$$

where $n = 1, 2, 3, 4$. Thus as the residence time approaches infinity the efficiency of the process approaches 100. However, the approach to 100 is faster as the number of reactors in the cascade (n) increases.

To illustrate the rapid increase in efficiency with the number of reactors in a cascade Table 2 shows the total residence time required to achieve efficiencies of 99%, 99.9% and 99.99%. At first sight the results for an efficiency of 99% seem surprising as the two reactor cascade outperforms both the single reactor and all other cascades. The reason for this can be gleaned from Fig. 1. The required value

of the total residence time for a double-reactor cascade, $\tau_2^*(\mathcal{E}^* = 99)$ satisfies

$$2\tau_{tr}^* < \tau_2^*(\mathcal{E}^* = 99) < 3\tau_{tr}^*,$$

where τ_{tr}^* is the value of the residence time at the transcritical bifurcation in a single reactor. For a cascade of n reactors the no-washout branch is only stable if $\tau^* > n\tau_{tr}^*$. Thus, with the efficiency set to 99%, the two-reactor cascade will outperform any cascade with three, or more, reactors.

The remaining results shown in Table 2 show that the total residence time to achieve a given efficiency decreases as the number of reactors in the cascade increases. However, at an efficiency of 99.9% the decrease in total residence time obtained going from a three reactor cascade to a four reactor cascade is insignificant: the difference in dimensionless residence time of 0.03 corresponds to a decrease of residence time of approximately 46 min.

The rate of waste treatment in a n -reactor cascade is defined by

$$\mathcal{W}_n = \frac{S_0 - S_n}{\tau_t}.$$

In dimensionless variables this is

$$\mathcal{W}_n^* = \frac{1 - S_n^*}{\tau_t^*}.$$

Using Eqs. (13)–(16) we see that at large residence times the efficiency is given by

$$\mathcal{W}_n^* \approx \frac{1}{\tau_t^*} + O\left(\frac{1}{\tau_t^{*1+n}}\right),$$

i.e. it is independent of the number of reactors in the cascade.

5. Conclusion

We have investigated a bioreactor model for the interaction between a microorganism and a rate-controlling substrate in a reactor cascade. The specific growth rate used was the Contois expression with the addition of a microorganism decay coefficient. In recent years this biochemical model has found application in describing the treatment of wastewater from a variety of industrial processes. This is the first study to analyse the treatment of such wastewaters in a reactor cascade. The results obtained here were illustrated by considering the anaerobic digestion of ice-cream wastewater, using kinetic values from [10].

Provided that washout does not occur in the first reactor of the cascade then there is a unique steady-state solution in each reactor of the cascade. An expression for the steady-state biomass and substrate concentration in reactor i of a cascade was found, it depends upon the corresponding values in the previous reactor. This solution was shown to be stable. The steady-state solution and stability were determined for an arbitrary residence-time in each reactor. For a cascade in which the residence time in each reactor are identical washout occurs throughout the cascade whenever the total residence time, τ_t^* , satisfies the inequality

$$\tau_t^* < \frac{n}{1 - k_d^*}.$$

Asymptotic solutions were obtained for the effluent concentration for values of the residence time slightly larger than $\tau^* = (n/(1 - k_d^*))$. These show that in this region of parameter space small increases in the total residence time lead to a dramatic decrease in the value of the effluent concentration. Asymptotic solutions were determined in the limit of high residence times. These suggest that at sufficiently high residence times the effluent concentration in

a n -reactor cascade decay like $1/\tau^{*n}$. This result shows the striking decrease in effluent concentration obtained by adding an extra reactor onto a cascade whilst fixing the total residence.

We have investigated minimising the effluent concentration by, at a fixed total residence time, optimising the residence time in each reactor. At sufficiently high total residence times the performance of a cascade with equal residence times in reactor converges towards that of an optimised reactor cascade. However, at lower residence times there can be a substantial difference in effluent concentration leaving an optimised reactor configuration and one with equal residence times in each reactor. This difference is more noticeable as the number of reactors in the cascade increases.

Assuming that the residence time in each reactor was equal we evaluated three performance characterisations of continuous flow bioreactors: specific utilisation, treatment efficiency and the rate of waste treatment. At a fixed total residence time the use of a reactor cascade generally improves the treatment efficiency, an improvement of several orders of magnitude is possible. However, as shown in Table 2, it is possible that increasing the number of reactors in a cascade does not decrease the total residence time required to achieve a specified treatment efficiency.

As we were able to obtain an explicit representation of the steady-state solution, our results can be used to quickly determined the total residence time required in a cascade of n reactors to obtain a pre-determined treatment efficiency.

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Appendix A. Steady-state analysis

Here we show that the solution of Eq. (11) in the second reactor of a cascade ($i = 2$) is only physically meaningful ($S_2^* > 0$ and $X_2^* > 0$) when the negative square root sign is taken. In doing so the only property of S_1^* and X_1^* that we use is that they are positive.

The steady-state equations for the concentrations inside the third reactor of a cascade ($i = 3$) are identical to those of the second reactor in the cascade except that the all indices have increased by one. It immediately follows that the physically meaningful steady-state solution in the third reactor, and hence any reactor in the cascade, is given by the negative square root in Eq. (11).

In Section A.1 we state some preliminary observations. In Section A.2 we establish the desired result for the non-degenerate case with $a_i = 0$. In Section A.3 we show that the steady-state solution is physically meaningful in the degenerate case that $a_i = 0$.

A.1. Preliminaries

From Eqs. (12) and (11) we have

$$X_i^* = \frac{-b_i \pm \sqrt{b_i^2 - 4a_i c_i}}{2a_i}, \quad (21)$$

$$S_i^* = A_i + \frac{b_i B_i}{2a_i} \mp \frac{B_i \sqrt{b_i^2 - 4a_i c_i}}{2a_i}. \quad (22)$$

To establish the positivity of the solutions it is useful to reformulate Eq. (11) as a quadratic equation in the substrate concentration (S_i^*) rather than the cellmass concentration (X_i^*). The steady-state

solutions can then be written in the equivalent form

$$X_i^* = \frac{A_i - S_i^*}{B_i},$$

$$0 = a_i S_i^{*2} + q_i S_i^* + r_i,$$

$$S_i^* = \frac{-q_i \pm \sqrt{q_i^2 - 4a_i r_i}}{2a_i}, \quad (23)$$

$$X_i^* = \frac{1}{B_i} \left[A_i + \frac{q_i}{2a_i} \mp \frac{\sqrt{q_i^2 - 4a_i r_i}}{2a_i} \right], \quad (24)$$

The coefficients in the preceding equations are

$$\begin{aligned} A_i &= S_{i-1}^* + \frac{X_{i-1}^*}{\alpha^*}, \\ B_i &= \frac{1 + k_d^* \tau_i^*}{\alpha^*}, \\ a_i &= (B_i - 1)k_d^* \tau_i^* + B_i(1 - \tau_i^*) - 1, \\ q_i &= -2A_i(1 + k_d^* \tau_i^*) - B_i(1 - B_i)X_{i-1}^* + A_i B_i [(1 - k_d^* \tau_i^*) - 1] \\ r_i &= A_i B_i X_{i-1}^* - A_i^2 (1 + k_d^* \tau_i^*) X \end{aligned}$$

Comparing solution (21) with solution (24) we establish that taking the positive (negative) square root sign in Eq. (21) corresponds to taking the negative (positive) square root sign in Eq. (23).

A.2. Positivity of the steady-state solution ($a_i \neq 0$)

Consider the coefficient c_i . We have

$$\begin{aligned} c_i &= A_i X_{i-1}^* = \left(1 + \frac{X_{i-1}^*}{\alpha^*}\right) X_{i-1}^*, \\ c_2 &= \left(1 + \frac{1}{\alpha^*}\right) X_1^* > 0, \end{aligned}$$

as by definition $\alpha^* > 0$ and by assumption $X_1^* > 0$. Thus when $a_i < 0$ Eq. (21) has two real solutions: a positive solution, corresponding to the choice of the negative sign in Eq. (21), and a negative solution, corresponding to the choice of the positive sign in Eq. (21). We show shortly that when $a_i > 0$ Eq. (21) has two positive solutions and that the solution of interest corresponds to the choice of the negative sign in Eq. (21).

Consider the coefficient r_i . We have

$$\begin{aligned} r_i &= A_i B_i X_{i-1}^* - A_i^2 (1 + k_d^* \tau_i^*) = -(S_{i-1}^* + \frac{X_{i-1}^*}{\alpha^*})(1 + k_d^* \tau_i^*), \\ r_2 &= -(S_1^* + \frac{X_1^*}{\alpha^*})(1 + k_d^* \tau_2^*) < 0, \end{aligned}$$

as by definition $\alpha^* > 0$, $k_d^* > 0$, $\tau_i^* > 0$ and by assumption $S_1^* > 0$ and $X_1^* > 0$. It now follows that when $a_i > 0$ Eq. (23) has two real solutions: a positive solution, corresponding to the choice of the positive sign in Eq. (23), and a negative solution, corresponding to the choice of the negative sign in Eq. (23).

We now eliminate the possibility that either Eq. (10) or Eq. (23) has complex solutions. Consider Eq. (10). It appears that complex solutions may occur when $a_i > 0$. However, when $a_i > 0$ we know that Eq. (23) has two real solutions for S_i^* and through Eq. (24) there are two real solutions for X_i^* . Hence Eq. (10) always has two real solutions and similarly for Eq. (23).

The question now arises as to the physical interpretation of the two positive solutions for Eq. (10) when $a_i > 0$. We know that when $a_i > 0$ Eq. (23) has two solutions, one negative and one positive. The negative (positive) solution corresponds to the negative (positive) square root sign in Eq. (23). Therefore when $a_i > 0$ the physically meaningful solution corresponds to the choice of the positive square root sign in Eq. (23) which, by the comment at the end

of Section A.1, corresponds to the choice of the negative square root sign in Eq. (21).

We therefore conclude that, for any value of a_i , $a_i \neq 0$, the choice of the negative square root in Eq. (11) gives a physical meaningful solution ($S_i^* > 0, X_i^* > 0$) whilst the choice of the positive square root in Eq. (11) gives a physical unmeaningful solution with one negative concentration.

A.3. Positivity of the steady-state solution ($a_i = 0$)

In this section we show that the solution of Eq. (11) is positive in the degenerate case when $a_i = 0$.

After some algebra the coefficient a_i can be written in the form

$$a_i = -\frac{1}{\alpha^*} (k_d^* \tau_i^* + 1) [(1 - k_d^*) \tau_i^* - (1 - \alpha^*)].$$

The coefficient a_i is therefore equal to zero when either $\tau_i^* = -1/k_d^* < 0$ or $\tau_i^* = (1 - \alpha^*) / (1 - k_d^*)$. From now on we assume that $0 < \alpha^* < 1$. After some algebra the steady-state solution pair is given by

$$S_i^* = \frac{\alpha^* (1 - k_d^*) (\alpha^* S_{i-1}^* + X_{i-1}^*) S_{i-1}^*}{(1 - \alpha^* k_d^*) X_{i-1}^* + (1 - k_d^*) \alpha^{*2} S_{i-1}^*} > 0,$$

$$X_i^* = \frac{1 - k_d^*}{(1 - \alpha^* k_d^*) X_{i-1}^* + (1 - k_d^*) \alpha^{*2} S_{i-1}^*} (\alpha^* S_{i-1}^* + X_{i-1}^*) > 0,$$

as by definition $0 < k_d^* < 1$ and by assumption $0 < \alpha^* < 1, 0 < S_{i-1}^*$ and $0 < X_{i-1}^*$.

Appendix B. The eigenvalues of the Jacobian matrix J

Here we establish the result that the Jacobian matrix J has two eigenvalues associated with each block J_i . To show this we write the Jacobian block J_i in the form

$$J_i = \begin{pmatrix} J_i(1, 1) & J_i(1, 2) \\ J_i(2, 1) & J_i(2, 2) \end{pmatrix}$$

The Jacobian matrix for a n -reactor cascade can be written as

$$J = \begin{pmatrix} J_1(1, 1) & J_1(2, 1) & 0 & 0 & 0 & 0 & 0 & \dots \\ J_1(2, 1) & J_1(2, 2) & 0 & 0 & 0 & 0 & 0 & \dots \\ \frac{1}{\tau_2^*} & 0 & J_2(1, 1) & J_2(2, 1) & 0 & 0 & 0 & \dots \\ 0 & \frac{1}{\tau_2^*} & J_2(2, 1) & J_2(2, 2) & 0 & 0 & 0 & \dots \\ 0 & 0 & \frac{1}{\tau_3^*} & 0 & J_3(1, 1) & J_3(1, 2) & 0 & \dots \\ 0 & 0 & 0 & \frac{1}{\tau_3^*} & J_3(2, 1) & J_3(2, 2) & 0 & \dots \\ 0 & \ddots & \ddots & \ddots & \ddots & \ddots & \ddots & \ddots \end{pmatrix}.$$

The eigenvalues of the Jacobian J are found by solving the equation

$$\det J = \begin{vmatrix} J_1(1, 1) - \lambda & J_1(2, 1) & 0 & 0 & 0 & 0 & 0 & \dots \\ J_1(2, 1) & J_1(2, 2) - \lambda & 0 & 0 & 0 & 0 & 0 & \dots \\ \frac{1}{\tau_2^*} & 0 & J_2(1, 1) - \lambda & J_2(2, 1) & 0 & 0 & 0 & \dots \\ 0 & \frac{1}{\tau_2^*} & J_2(2, 1) & J_2(2, 2) - \lambda & 0 & 0 & 0 & \dots \\ 0 & 0 & \frac{1}{\tau_3^*} & 0 & J_3(1, 1) - \lambda & J_3(1, 2) & 0 & \dots \\ 0 & 0 & 0 & \frac{1}{\tau_3^*} & J_3(2, 1) & J_3(2, 2) - \lambda & 0 & \dots \\ 0 & \ddots & \ddots & \ddots & \ddots & \ddots & \ddots & \ddots \end{vmatrix} = 0. \tag{25}$$

Expanding along the front row we obtain

$$0 = [J_1(1, 1) - \lambda] \begin{vmatrix} J_1(2, 2) - \lambda & 0 & 0 & 0 & 0 & 0 & \dots \\ 0 & J_2(1, 1) - \lambda & J_2(2, 1) & 0 & 0 & 0 & \dots \\ \frac{1}{\tau_2^*} & J_2(2, 1) & J_2(2, 2) - \lambda & 0 & 0 & 0 & \dots \\ 0 & \frac{1}{\tau_3^*} & 0 & J_3(1, 1) - \lambda & J_3(1, 2) & 0 & \dots \\ 0 & 0 & \frac{1}{\tau_3^*} & J_3(2, 1) & J_3(2, 2) - \lambda & 0 & \dots \\ 0 & \ddots & \ddots & \ddots & \ddots & \ddots & \ddots \end{vmatrix} \\ \times \begin{vmatrix} J_1(2, 1) & 0 & 0 & 0 & 0 & 0 & \dots \\ \frac{1}{\tau_2^*} & J_2(1, 1) - \lambda & J_2(2, 1) & 0 & 0 & 0 & \dots \\ 0 & J_2(2, 1) & J_2(2, 2) - \lambda & 0 & 0 & 0 & \dots \\ -J_1(1, 2) & 0 & \frac{1}{\tau_3^*} & 0 & J_3(1, 1) - \lambda & J_3(1, 2) & 0 & \dots \\ 0 & 0 & \frac{1}{\tau_3^*} & J_3(2, 1) & J_3(2, 2) - \lambda & 0 & \dots \\ 0 & \ddots & \ddots & \ddots & \ddots & \ddots & \ddots \end{vmatrix}.$$

We now expand both of the determinants in the previous equation along the first row. Factorising the resulting expression we obtain

$$0 = \{ [J_1(1, 1) - \lambda] [J_1(2, 2) - \lambda] - J_1(1, 2) J_1(2, 1) \} \begin{vmatrix} J_2(1, 1) - \lambda & J_2(2, 1) & 0 & 0 & 0 & \dots \\ J_2(2, 1) & J_2(2, 2) - \lambda & 0 & 0 & 0 & \dots \\ \frac{1}{\tau_3^*} & 0 & J_3(1, 1) - \lambda & J_3(1, 2) & 0 & \dots \\ 0 & \frac{1}{\tau_3^*} & J_3(2, 1) & J_3(2, 2) - \lambda & 0 & \dots \\ 0 & \ddots & \ddots & \ddots & \ddots & \dots \end{vmatrix}. \tag{26}$$

The equation

$$0 = [J_1(1, 1) - \lambda] [J_1(2, 2) - \lambda] - J_1(1, 2) J_1(2, 1)$$

is the characteristic equation for the Jacobian block J_1 . Observe that the structure of the determinant in Eq. (26) is identical to that in Eq. (26): all that has changed is that the indices have increased by one. It follows by induction that the eigenvalues of the Jacobian matrix J are those of the constituent blocks J_i .

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