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2 Biofilms and the plasmid maintenance question [☆]

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7 Abstract

8 Can a conjugative plasmid encoding enhanced biofilm forming abilities for its bacterial host facilitate the
9 persistence of the plasmid in a bacterial population despite conferring diminished growth rate and segrega-
10 tive plasmid loss on its bearers? We construct a mathematical model in a chemostat and in a plug flow envi-
11 ronment to answer this question. Explicit conditions for an affirmative answer are derived. Numerical
12 simulations support the conclusion.

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14 *Keywords:* Plasmid; Segregation; Conjugation; Biofilm; Chemostat; Plug flow; Bacterial wall growth; Model

16 1. Introduction

17 Plasmids abound in natural bacterial populations. They often carry genes for such beneficial
18 factors as resistance to antibiotics and heavy metals, the ability to ferment sugars, or to produce
19 toxins. Some carry genes for F-pili production and mating pair formation that allow the horizon-
20 tal transfer of the plasmid to other bacteria and still others exploit this ability by coding for mobil-
21 ity factors that allow them to hitch hike along. However, many plasmids have no known function
22 in bacteria and may simply be parasitic. Vertical transmission of plasmids occurs during cell

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23 division; rarely, however, one daughter cell may end up without plasmid while the other daughter
24 cell receives multiple copies. This loss of plasmid is referred to as segregative loss. Furthermore,
25 there is some cost to an organism carrying plasmids since the cell may produce plasmid gene prod-
26 ucts (which may interfere with cellular metabolism) and must duplicate it during cell division
27 which leads to a reduced reproductive rate. We should note however that recent work suggests
28 these costs may diminish over a number of generations due to the effects of natural selection,
29 [2,11]. See [12] for a readable review, particularly of modeling aspects, and [27] for a general re-
30 view. Because the benefits of plasmid carriage, if any, depend on ever-changing environmental
31 conditions while the costs are always present, a longstanding focus of theoretical studies has been
32 to determine conditions under which plasmids can be maintained in bacterial populations. See
33 [13,17,26] for modeling results related to this problem.

34 In a recent paper Ghigo [7] established that several natural conjugative plasmids express factors
35 that induce some bacteria to form biofilms. Experimental studies showed that a strain of *E. coli*
36 bearing a certain plasmid formed a thick biofilm within one day while those not carrying the plas-
37 mid produced no macroscopically observable biofilm. Interestingly, Ghigo's results suggest that
38 the pili responsible for the horizontal transfer of the plasmid, may also act as an adhesion factor
39 for cell-to-surface contact (see also [18,20]). Ghigo points out the many beneficial aspects for bac-
40 teria in biofilms relative to the fluid environment and speculates that such factors 'may provide a
41 rationale for the unexplained vertical maintenance of the numerous uninfected cryptic plasmids
42 found in natural populations'. He also observes that by inducing bacteria to form the denser com-
43 munities characteristic of biofilms the plasmid increases the likelihood of its own horizontal trans-
44 fer via conjugation. Reisner et al. [21] noted the enhancing effect of transfer constitutive IncF
45 plasmid carriage on biofilm formation in an *E. coli* strain.

46 In this paper, we explore the suggested link between plasmid maintenance and biofilms by con-
47 structing a mathematical model of a bacterial population consisting of plasmid-bearing and plas-
48 mid-free organisms. We assume that the plasmid codes for enhanced biofilm forming ability in its
49 bacterial host which in its absence can form only a macroscopically unobservable biofilm. The
50 question we address is under what circumstances can the ability to form a robust biofilm commu-
51 nity in which conjugative transfer of the plasmid may occur be sufficiently advantageous for the
52 plasmid-bearing organism to compensate for the energetic cost of bearing the plasmid and the seg-
53 regative loss of the plasmid. Our model is built on the plasmid model of Stephanopoulos and Lap-
54 idus [25] including conjugation terms used by Stewart and Levin [26].

55 We examine this issue in two settings. For maximum simplicity and mathematical tractability,
56 we first examine the model in the chemostat based on a wall-growth model of Pilyugin and Walt-
57 man [19]. We define the basic reproductive number R_0^+ , giving the sum of the expected number of
58 plasmid-bearing daughter cells and the number of conjugative transfers of plasmid to plasmid-free
59 cells made by a single hypothetical plasmid-bearing cell, introduced into the plasmid-free steady
60 state (all other cells are plasmid-free), before it is washed out of the chemostat. An explicit for-
61 mula for R_0^+ is derived and the condition for plasmid maintenance is shown to be intuitive:
62 $R_0^+ > 1$. In case that the plasmid confers enhanced biofilm formation abilities (as measured by
63 lower sloughing rate or higher surface-affinity), the plasmid-bearing cell's advantages of infectious
64 transfer of plasmid and greater residence time in the chemostat in which to leave both horizontal
65 and vertical transmissions must overcome the twin disadvantages of segregative loss of plasmid
66 and the cost of plasmid carriage. In case that the plasmid is parasitic (confers no biofilm forma-

tion advantage), then a plasmid-bearing cell does not have a greater residence time than the plasmid-free cell. Plasmid maintenance is only possible if the infectious transfer rate times the generation time of a cell exceeds the sum of the cost of plasmid carriage and the probability of plasmid loss during cell division.

In order to examine a more realistic spatially inhomogeneous environment where advection plays a role, we consider the model in the setting of a fully three-dimensional flow reactor based on the Freter model of wall-growth [4], as extended by Jones et al. [8,10]. Due to the complexity of the model in this setting, we are unable to provide useful analytical estimates for the key invasion eigenvalue as we could for the chemostat setting. However, numerical simulations of the invasion of a plasmid-free steady state by an inoculum of plasmid-bearing organism are qualitatively similar to those obtained for the simpler chemostat-based model.

Due to substantial uncertainty in key parameters of the model, our result must be interpreted with great caution. We show the potential for a conjugative plasmid containing genes coding for enhanced biofilm formation abilities to be maintained in bacterial populations. Our work suggests that the conjugative transfer of plasmid is an insignificant advantage to plasmid-bearing organism when compared to the advantage of the biofilm refuge created by enhanced affinity for the wall.

We provide a quantitative expression of a potential mechanism which may be significant in plasmid maintenance. The availability of colonizable surfaces that provide a selective advantage for an organism carrying a biofilm-enhancing conjugative plasmid may be less episodic in natural environments than are those selecting for other advantages conferred by plasmids. Not all plasmids are conjugative, nor do all plasmids confer biofilm forming advantages on their host, so we cannot claim to have settled the plasmid maintenance question.

The same models developed in this paper could also be used to study the important phenomena of horizontal spread of antibiotic resistance in the gut. Rather than assuming the plasmid codes for enhanced biofilm forming ability one would assume that it codes for antibiotic resistance. Selection for the resistant strain could, of course, be arranged by adding antibiotic. Ingestion of bacteria containing plasmid coding for antibiotic resistance could lead to the spread of resistance to the gut microflora. This phenomena may play a significant role in the proliferation of antibiotic resistant pathogens [27].

2. Chemostat model

Let u (u_+) denote the density of planktonic plasmid-free (plasmid-bearing) organism and w (w_+) denote the areal density of wall-adherent plasmid-free (plasmid-bearing) organism. These populations are supported by the substrate S in continuous culture. Model parameters are described in Table 1 and a schematic diagram of the model is depicted in Fig. 1. Bacterial variables and parameters without the '+' sign refer to plasmid-free cells while those with subscript '+' refer to plasmid-bearing cells.

The model equations in the setting of a CSTR of volume V , colonizable surface area A and flow rate Φ takes the form ($D = \Phi/V$, $\delta = A/V$):

Table 1
Model parameters for the chemostat: t = time, m = mass, l = length

Symbol	Description	Dimension
u, u_+	Biomass concentration of planktonic bacteria	ml^{-3}
w, w_+	Areal biomass density of adherent bacteria	ml^{-2}
β, β_+	Sloughing rate	t^{-1}
α, α_+	Rate constant of adhesion	t^{-1}
S	Concentration of limiting substrate	ml^{-3}
S^0	Concentration of the substrate in the feed	ml^{-3}
γ	Yield constant	–
a	Half saturation constant	ml^{-3}
m	Maximum growth rate of plasmid-free organism	t^{-1}
c	Fractional energetic cost of plasmid carriage, $0 < c < 1$	–
q	Fractional segregation loss factor, $0 < q < 1$	–
D	Dilution rate	t^{-1}
μ	Biofilm conjugational transfer parameter	$l^2(mt)^{-1}$
$\bar{\mu}$	Planktonic conjugational transfer parameter	$l^3(mt)^{-1}$

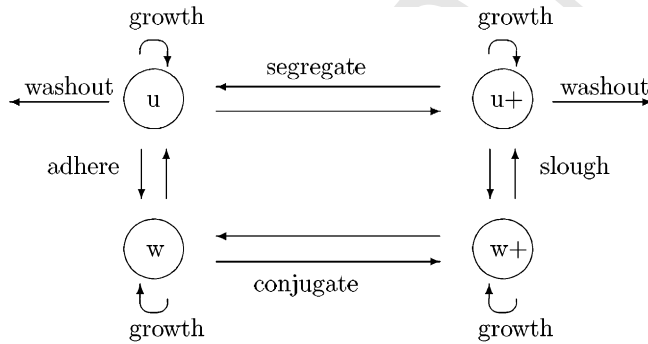


Fig. 1. Flow chart of biomass flow between model compartments, u, u_+, w, w_+ .

$$\begin{aligned}
 S' &= D(S^0 - S) - \gamma^{-1}[f_u(S)u + f_u(S)u_+] - \gamma^{-1}\delta[f_w(S)w + f_w(S)w_+], \\
 u' &= (f_u(S) - D)u + qf_u(S)(1 - c)u_+ - \alpha u + \beta\delta w - \bar{\mu}uu_+, \\
 w' &= f_w(S)w + qf_w(S)(1 - c)w_+ + \alpha\delta^{-1}u - \beta w - \mu ww_+, \\
 u'_+ &= [f_u(S)(1 - c)(1 - q) - D]u_+ - \alpha_+u_+ + \beta_+\delta w_+ + \bar{\mu}uu_+, \\
 w'_+ &= f_w(S)(1 - c)(1 - q)w_+ + \alpha_+\delta^{-1}u_+ - \beta_+w_+ + \mu ww_+.
 \end{aligned}
 \tag{2.1}$$

108 Although it is not crucial for our results, we assume that the specific growth rates of both plank-
109 tonic and wall adherent plasmid-free cells are Monod functions

$$f_i(S) = m_i S / (a_i + S), \quad i = u, w. \tag{2.2}$$

113 The specific growth rates of planktonic and adherent plasmid-bearing organism are reduced by a
114 factor $1 - c$ over that of the plasmid-free organism although uptake rates are the same. Such a

115 cost of plasmid carriage is well documented in the literature, see [11] although it may decline over
116 many generations due to the effects of natural selection as observed by Dahlberg and Chao [2].

117 Planktonic plasmid-free organisms are attracted to a portion of the surface of the chemostat
118 with rate constant α while plasmid-free wall-adhering cells are sloughed off the surface with rate
119 constant β . Similarly, for plasmid-bearing cells with rates α_+ , β_+ . Our model equations (2.1) are an
120 amalgam of the plasmid model of Stephanopoulos and Lapidus [25] and the wall-growth model of
121 Pilyugin and Waltman [19]. Stephanopoulos and Lapidus [25] assume that a fraction q of the
122 daughter cells of the planktonic plasmid-bearing population produced in the time interval
123 $[t, t + dt]$, given by $f(S)(1 - c)(u_+)dt$, acquire no plasmid during cell division, and therefore con-
124 tribute to the plasmid-free population, while the fraction $1 - q$ acquire one or more plasmid
125 and thus contribute to the plasmid-bearing population. Similarly, for the wall-adherent plas-
126 mid-bearing organism. This treatment of segregation seems to us more natural than a linear trans-
127 fer rate relation $\pm\tau u_+$ between the u and u_+ compartments assumed by Stewart and Levin [26].
128 The latter approach appears to treat segregation as the loss of plasmid from a mature cell rather
129 than as a loss associated with cell division. See also [14,15] for a similar modeling of segregative
130 loss in their model of competition between plasmid-bearing and plasmid-free organisms in selec-
131 tive media.

132 Horizontal transmission of the plasmid between the wall-adherent sub-populations is assumed
133 to occur at a rate proportional to the product of their areal densities: $\mu w w_+$. Similarly, horizontal
134 transmission of plasmid in the fluid environment is modeled by the term $\bar{\mu} u u_+$. This mass-action-
135 like rate requires some defense since we view wall-adherent organisms as relatively sessile. It seems
136 clear that the transfer rate must increase (and vanish) with each of the densities w and w_+ and
137 mass action captures this property while being relatively standard. The mass action rate is used
138 by Stewart and Levin [26] in considering planktonic bacteria. An alternative might include divi-
139 sion of the mass action term by an affine term $1 + ew + fw_+$; for simplicity, the mass action term
140 will be used. We do not include terms such as $\mu u w_+$ or $\mu u_+ w$ primarily for simplicities sake
141 although we view such terms to be of less importance than those included since the bulk of the
142 biofilm cells are likely to be inaccessible to planktonic cells.

143 The mechanisms employed in the initial attachment of bacteria to surfaces and subsequent for-
144 mation of a mature biofilm are under active investigation and may depend on species and sub-
145 strates (see e.g. [16,18,20,21]). Based on genetic studies of *E. coli*, Pratt and Kolter developed a
146 conceptual model of the initial formation of biofilm by *E. coli* in nutrient rich media: ‘Motility,
147 but not chemotaxis, is important for cells to first come into contact with an abiotic surface. This
148 requirement may reflect a necessity to overcome repulsive forces present at abiotic surface to be
149 colonized. Once a surface is reached, type I pili are required to achieve stable cell-to-surface
150 attachment. The presence of the FimH adhesion, when it is not bound to mannose, promotes such
151 stable adherence to abiotic surfaces. Finally, we hypothesize that motility facilitates the develop-
152 ment of a mature biofilm by allowing movement along a surface, thereby promoting spread of the
153 biofilm’. The more recent studies of Reisner et al. [21] and Klausen et al. [16] have shown different
154 modes of biofilm establishment, that do not necessarily require cell motility, and which lead the
155 latter authors to conclude that: ‘biofilm development occurs differently under different nutritional
156 and environmental conditions, and that biofilm models with present knowledge can only be
157 contextual.’

158 By contrast with this complexity, the Pilyugin and Waltman model treats bacterial attachment
159 and detachment in a very simple way using a first order attachment rate α and detachment rate β .
160 We will have more to say of these parameters below.

161 Parameter values are chosen as in Freter [4], as used in Jones et al. [8]. In particular, $\gamma = .5$,
162 $a = 9 \times 10^{-7}$ g/ml, $m = 1.66$ h⁻¹, $S^0 = 2.09 \times 10^{-6}$ g/ml, $D = .23$ h⁻¹, $V = 1$ cm³, $A = 6$ cm².
163 Simonsen [12] suggests $c = .01$ and $q = .0001$. He also points out that the value of μ is highly
164 uncertain. Gordon [6] finds the transfer rate μ for natural *E. coli* populations to lie between
165 10^{-11} and 10^{-18} , the average being $\mu_{\text{cell}} = 10^{-15}$ ml/cell h in a fluid environment. We may convert
166 these numbers to biomass by assuming 1.8×10^{12} cells/gm so $\bar{\mu} = 1.8 \times 10^{12} \mu_{\text{cell}}$ ml/g h = .001 ml/
167 g h where we have taken only the order of magnitude since the precise values have no significant
168 digits. A milliliter, equal to a cubic centimeter, is equivalent to the product of a typical biofilm
169 thickness h cm times an area $1/h$ cm². As an appropriate measure for us is a volume of thickness
170 h over a square centimeter, we finally obtain $\mu = (1.8/h)10^{12} \mu_{\text{cell}}$ cm²/g h. Ghigo [7] gives a figure
171 of $h = 500$ $\mu\text{m} = 5 \times 10^{-4}$ for a thick biofilm. Thus, using only the order of magnitude, $\mu = 1$.
172 Parameters α , α_+ , β , β_+ are taken from Freter [4] whose focus on the gut wall may give values that
173 are inappropriate in our setting. Indeed, he does not cite any references supporting his values. For
174 the chemostat we use values that lie in a range between 10^{-1} and 10^{-2} and will be further de-
175 scribed below.

176 The initial value problem for the model system (2.1) is shown to be well-posed in Appendix A.
177 In addition, positivity of solutions is established and the system is shown to be dissipative.

178 In the absence of the plasmid-bearing organism, the system takes the form

$$\begin{aligned} S' &= D(S^0 - S) - \gamma^{-1}[f_u(S)u + \delta f_w(S)w], \\ u' &= (f_u(S) - D - \alpha)u + \beta\delta w, \\ w' &= (f_w(S) - \beta)w + \alpha\delta^{-1}u, \end{aligned} \tag{2.3}$$

182 which we refer to hereafter as the plasmid-free system. It was studied by Pilyugin and Waltman
183 [19] as a simple wall-growth model. Their main results are as follows.

184 **Theorem 2.1** (Pilyugin and Waltman). *The following hold for (2.3):*

- 185 (a) *There is a positive, ‘plasmid-free steady state’ $(\bar{S}, \bar{u}, \bar{w})$ ($\bar{u}, \bar{w} > 0$) if and only if the washout state $(S^0, 0, 0)$ is unstable in the linear approximation. When it exists, the plasmid-free state is unique and asymptotically stable in the linear approximation.*
- (b) *If the washout steady is unstable, then the bacterial population persists. More precisely, there exists $\epsilon > 0$, independent of initial data, such that for all solutions of (2.3) satisfying $u(0) + \delta w(0) > 0$, there is $T > 0$ such that*

$$u(t) + \delta w(t) > \epsilon, \quad t > T.$$
- 193 (c) *If $f_u = f_w$, then the plasmid-free steady state $(\bar{S}, \bar{u}, \bar{w})$ attracts all solutions with $u(0) + \delta w(0) > 0$ when it exists.*

195

196 The washout state is asymptotically stable (respectively, unstable) if and only if the largest
197 eigenvalue of the matrix

$$B(S^0) = \begin{pmatrix} f_u(S^0)(1-c)(1-q) - D - \alpha & \beta \\ \alpha & f_w(S^0)(1-c)(1-q) - \beta \end{pmatrix} \quad (2.4)$$

200 is negative (respectively, positive). We denote by $s(B(S^0))$ the largest of the two real eigenvalues of
 201 this matrix; it is referred to as the stability modulus of $B(S^0)$. If $s(B(S^0)) > 0$, the unique positive
 202 plasmid-free steady state $(\bar{S}, \bar{u}, \bar{w})$ exists although a simple expression for it is lacking. \bar{S} is the un-
 203 unique value of $S \in (0, S^0)$ such that $s(B(\bar{S})) = 0$ and $(\bar{u}, \delta\bar{w})^T$ is the positive right eigenvector corre-
 204 sponding to the zero eigenvalue of $B(\bar{S})$ which satisfies

$$D(S^0 - \bar{S}) = \gamma^{-1}[f_u(\bar{S})\bar{u} + \delta f_w(\bar{S})\bar{w}].$$

207 Pilyugin and Waltman conjectured that the positive steady state attracts all solutions starting
 208 with organism present ($u(0) + \delta w(0) > 0$) but were only able to establish this in the special case
 209 (c) that wall adherent organisms and planktonic cells have the same growth rates. Abstract results
 210 ([23]) imply the conjecture holds as well if the latter is only approximately true, $f_u \approx f_w$. Hereafter,
 211 we assume that the washout state is unstable and that the plasmid-free state attracts all solutions
 212 of (2.3) when plasmid-free cells are initially present, i.e., when $u(0) + \delta w(0) > 0$.

213 The key question is whether or not the plasmid-bearing population can invade the plasmid-free
 214 steady state and thereby establish a biofilm community. The answer comes from the linearization
 215 of (2.1) about the plasmid-free state. This 5×5 matrix decomposes into a 3×3 block and a 2×2
 216 block on the diagonal with zeros below the two blocks. As shown in Theorem 5.1 of Pilyugin and
 217 Waltman [19], the 3×3 block is a stable matrix reflecting the fact that the plasmid-free steady
 218 state is locally asymptotically stable for the system (2.3). Thus, the portion of the full linearization
 219 about the plasmid-free steady state which determines stability is the 2×2 block:

$$A = \begin{pmatrix} f_u(\bar{S})(1-c)(1-q) - D - \alpha_+ + \bar{\mu}\bar{u} & \beta_+ \\ \alpha_+ & f_w(\bar{S})(1-c)(1-q) - \beta_+ + \mu\bar{w} \end{pmatrix}. \quad (2.5)$$

222 If the stability modulus, $s(A)$, of A (the largest eigenvalue) is negative then the plasmid-free state is
 223 locally attracting for (2.1); if $s(A) > 0$ then the plasmid-free state is unstable. If $s(A) > 0$, the plas-
 224 mid is maintained.

225 **Theorem 2.2.** *If $s(A) > 0$ then the plasmid-bearing population persists. More precisely, there exists*
 226 *$\epsilon > 0$, independent of initial data, such that for all solutions of (2.1) satisfying $u_+(0) + \delta w_+(0) > 0$, we*
 227 *have*

$$u_+(t) + \delta w_+(t) > \epsilon \quad (2.6)$$

230 *for all sufficiently large t . In addition, there exists at least one coexistence steady state satisfying $u,$*
 231 *$u_+, w, w_+ > 0$.*

232 The proof is deferred to an [Appendix A](#). There can be no steady state at which only the plas-
 233 mid-bearing organism is present due to the effects of segregation.

234 The stability modulus $s(A)$ does not easily lend itself to biological interpretation. Diekmann
 235 and Heesterbeek [3] establish that $s(A) > 0$ if and only if the spectral radius (largest positive eigen-
 236 value), $\rho(TQ_+)$, of the product of the diagonal matrix

$$T = \text{diag}[f_u(\bar{S})(1-c)(1-q) + \bar{\mu}\bar{u}, f_w(\bar{S})(1-c)(1-q) + \mu\bar{w}]$$

239 with matrix

$$Q_+ = \begin{pmatrix} 1/D & 1/D \\ \alpha_+/D\beta_+ & (\alpha_+ + D)/D\beta_+ \end{pmatrix}$$

242 satisfies

$$R_0^+ := \rho(TQ_+) > 1, \tag{2.7}$$

246 R_0^+ is the basic reproductive number for plasmid-bearing cells in the environment determined by
 247 the plasmid-free steady state. It represents the number of plasmid-bearing progeny that a hypo-
 248 theoretical ‘single plasmid-bearing cell’ would leave before being washed out of the chemostat. Eq.
 249 (2.7) says that a single plasmid-bearing cell must leave more than one plasmid-bearing progeny
 250 for plasmid maintenance.

251 In order to support this interpretation, we examine the matrices T and Q_+ separately. Each
 252 diagonal entry of T represents the sum of two rates; $f_u(\bar{S})(1 - c)(1 - q)$ is the rate at which plank-
 253 tonic plasmid-bearing daughter cells are produced by a single planktonic plasmid-bearing cell
 254 (technically, the rate of production of plasmid-bearing biomass by a unit biomass of plasmid-
 255 bearing cells, but we prefer our more prosaic interpretation) and $\bar{\mu}\bar{u}$ is the rate of infectious trans-
 256 fer of plasmid to planktonic plasmid-free cells by a single planktonic plasmid-bearing cell. The
 257 second diagonal entry has a similar interpretation for wall adherent cells instead of planktonic
 258 cells. It must be stressed that these rates are relative to the environment imposed by the plas-
 259 mid-free steady state (note the argument \bar{S} of f_u and f_w); all other cells are plasmid-free. The entries
 260 of Q_+ represent units of time. For example, $1/D$ represents the mean time a cell spends in the
 261 planktonic state given that it started in the planktonic (or adherent) state; α_+/β_+D represents
 262 the mean time a plasmid-bearing cell spends in the wall adherent state if it starts in the planktonic
 263 state and $(\alpha_+ + D)/\beta_+D$ represents the mean time a cell spends in the wall adherent state if it starts
 264 in the wall adherent state. See Appendix A for a detailed argument.

265 Therefore, the entries of the product

$$TQ_+ = \begin{pmatrix} (f_u(\bar{S})(1 - c)(1 - q) + \bar{\mu}\bar{u})/D & (f_u(\bar{S})(1 - c)(1 - q) + \bar{\mu}\bar{u})/D \\ (f_w(\bar{S})(1 - c)(1 - q) + \mu\bar{w})\frac{\alpha_+}{D\beta_+} & (f_w(\bar{S})(1 - c)(1 - q) + \mu\bar{w})\frac{\alpha_++D}{D\beta_+} \end{pmatrix}$$

268 are readily interpreted. The upper left entry gives the mean number of planktonic plasmid-bearing
 269 progeny, from both horizontal and vertical transmission, that a single plasmid-bearing, plank-
 270 tonic cell leaves if it started out a planktonic cell, the upper right entry gives the mean number
 271 of planktonic plasmid-bearing progeny that a single plasmid-bearing planktonic cell leaves if it
 272 started out as an adherent cell. The lower left entry gives the mean number of adherent plas-
 273 mid-bearing progeny that a single adherent plasmid-bearing cell leaves if it started out as a plank-
 274 tonic cell and the lower right entry gives the mean number of adherent plasmid-bearing progeny
 275 that a single adherent plasmid-bearing cell leaves if it started out as an adherent cell. The spectral
 276 radius, $\rho(TQ_+)$ of TQ_+ , monotone increasing in each entry by the Perron-Frobenius theory (see
 277 [1]), summarizes in a single scalar quantity the basic reproductive number. Exploiting this mono-
 278 tonicity property, we observe that

$$\min\{f_u(\bar{S}) dp + \bar{\mu}\bar{u}, f_w(\bar{S}) dp + \mu\bar{w}\}\rho(Q_+) \leq R_0^+ \leq \max\{f_u(\bar{S}) dp + \bar{\mu}\bar{u}, f_w(\bar{S}) dp + \mu\bar{w}\}\rho(Q_+),$$

281 where $d := 1 - c$ and $p := 1 - q$. But $\rho(Q_+)$ is readily seen to be the *mean residence time* (MRT⁺)
282 of a plasmid-bearing cell in the chemostat (see Appendix A). Therefore, we may write

$$R_0^+ = [F^+(f_u(\bar{S})(1 - c)(1 - q) + \bar{\mu}\bar{u}) + (1 - F^+)(f_w(\bar{S})(1 - c)(1 - q) + \mu\bar{w})] \cdot \text{MRT}^+, \quad (2.8)$$

286 where $0 \leq F^+ \leq 1$. It is tempting to view F^+ as the fraction of time that our single plasmid-bearing
287 cell spends in the planktonic state (regardless of its initial state) and $1 - F^+$ as the fraction of time
288 it spends in the wall adherent state although these interpretations are difficult to support due to
289 the fact that F^+ may depend on $f_u(\bar{S}) dp + \bar{\mu}\bar{u}$ and $f_w(\bar{S}) dp + \mu\bar{w}$. Formula (2.8) clearly establishes
290 that R_0^+ is a measure of the sum of plasmid-bearing daughter cells and plasmid-infected cells cre-
291 ated by a hypothetical single plasmid-bearing cell, introduced into the plasmid-free steady state,
292 before being washed out of the chemostat. The condition $R_0^+ > 1$ for plasmid maintenance is then
293 intuitive.

294 Of course, the quadratic formula readily yields an unilluminating expression for $R_0^+ = \rho(TQ^+)$,
295 see Appendix A.

296 As our main interest is the case that the plasmid-bearing organism can form a healthy biofilm
297 while the plasmid-free organism cannot, it is reasonable to assume that the plasmid-bearing
298 organism's sloughing rate does not exceed that of the plasmid-free organism and that its adhesion
299 rate constant is not less than that of the plasmid-free organism:

$$\beta_+ \leq \beta \quad \text{and} \quad \alpha \leq \alpha_+. \quad (2.9)$$

302 Strict inequality is assumed to hold in at least one of these inequalities. These assumptions im-
303 ply that

$$Q < Q^+,$$

306 where Q is identical to Q^+ except that α, β replace α_+, β_+ . Thus, not surprisingly, the mean res-
307 idence time of a plasmid-bearing organism in the chemostat strictly exceeds the mean residence
308 time of the plasmid-free organism in case the plasmid confers an advantage in attaching and/or
309 resisting sloughing:

$$\text{MRT} = \rho(Q) < \rho(Q^+) = \text{MRT}^+$$

312 giving the plasmid-bearing organism more time to leave progeny via infection and cell division
313 before washout than the plasmid-free cell has to leave daughter cells. In summary, the plasmid
314 survives if (2.7) holds: $R_0^+ > 1$; the advantage of infectious transfer of plasmid and greater resi-
315 dence time to infect and leave plasmid-bearing progeny must out weigh the disadvantages of re-
316 duced growth and segregative loss.

317 Fig. 2 depicts the invasion of the plasmid-free state by a tiny inoculum of plasmid-bearing
318 planktonic cells that have a smaller sloughing rate than plasmid-free cells. The output has been
319 scaled by $S/a, v/(a\gamma)$, $v = u, w, u_+, w_+$, where a is the half-saturation constant in (2.2) (we assume
320 that $f_u = f_w$). Parameters are as described above and $\alpha = .1, \alpha_+ = .1, \beta = .4, \beta_+ = .1, \mu = 1,$
321 $\bar{\mu} = .001$ but note that μ, \bar{u} get multiplied by $a\gamma$ due to the scaling. Initial data are chosen to be
322 near the plasmid-free steady state $(\bar{S}, \bar{u}, \bar{w}) = (.16, 2.21, .93)$ with S, u, w exactly at steady state
323 and $u_+ = .001, w_+ = 0$. Observe that the simulation tracks the plasmid-free steady state for the
324 first 100 h then makes a transition to a coexistence state dominated by wall-adherent, plasmid-

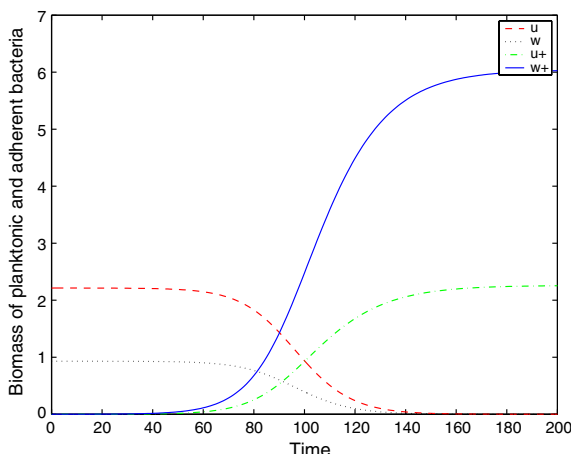


Fig. 2. Time series (h) of the invasion of the plasmid-free steady state by inoculum of plasmid-bearing bacteria that are superior wall-adherers.

325 bearing cells. We also reran the simulation setting $\mu = \bar{\mu} = 0$ noting no change in the time series.
326 Therefore, the conjugation terms play no role in this simulation.

327 It is instructive to ask whether the plasmid could survive if it confers no advantage in biofilm
328 forming ability on its host: $\alpha = \alpha_+$ and $\beta = \beta_+$. That is, can the plasmid survive as a parasite. This
329 question was considered by Stewart and Levin [26] for a simple chemostat-based model ignoring
330 wall growth. The question boils down to whether or not its advantage in horizontal spread out-
331 weighs its growth and segregation disadvantages. In this case, the plasmid-bearing cell has no res-
332 idence time advantage since $Q = Q^+$. The condition for plasmid survival is again (2.7)

$$R_0^+ = \rho(TQ) > 1.$$

335 In relation to this formula, it is useful to recall that at the plasmid-free steady state, each plasmid-
336 free cell leaves exactly one daughter cell before washout so

$$\rho \begin{pmatrix} f_u(\bar{S})/D & f_u(\bar{S})/D \\ f_w(\bar{S})\frac{\alpha}{D\beta} & f_w(\bar{S})\frac{\alpha+D}{D\beta} \end{pmatrix} = 1.$$

339 Indeed, by the above-mentioned result of Diekmann and Heesterbeek [3] $s(B(\bar{S})) = 0$ if and
340 only if the spectral radius of the matrix is unity. Therefore, monotonicity of the spectral radius
341 in the entries of a non-negative matrix leads to the estimates

$$\min \left\{ \frac{f_u(\bar{S}) dp + \bar{\mu}\bar{u}}{f_u(\bar{S})}, \frac{f_w(\bar{S}) dp + \mu\bar{w}}{f_w(\bar{S})} \right\} \leq \rho(TQ) \leq \max \left\{ \frac{f_u(\bar{S}) dp + \bar{\mu}\bar{u}}{f_u(\bar{S})}, \frac{f_w(\bar{S}) dp + \mu\bar{w}}{f_w(\bar{S})} \right\}$$

344 and consequently,

$$R_0^+ = \rho(TQ) = (1-c)(1-q) + F \frac{\bar{\mu}\bar{u}}{f_u(\bar{S})} + (1-F) \frac{\mu\bar{w}}{f_w(\bar{S})}$$

347 for some F satisfying $0 \leq F \leq 1$. So $R_0^+ > 1$ if and only if

$$F \frac{\bar{\mu}\bar{u}}{f_u(\bar{S})} + (1 - F) \frac{\mu\bar{w}}{f_w(\bar{S})} > 1 - (1 - c)(1 - q) = c + q - cq. \quad (2.10)$$

351 This condition for parasitic plasmid maintenance makes evident that the rate of infectious
 352 transfer of plasmid must exceed a threshold value depending on the cost c of plasmid carriage
 353 and the probability q of plasmid loss during cell division. Obviously, the planktonic cell density
 354 \bar{u} and wall adherent density \bar{w} play a role as well as the infectious transfer rates $\bar{\mu}$ and μ . Interpret-
 355 ing $1/f_u(\bar{S})$ as a generation time for plasmid-free planktonic cells, the inequality (2.10) says
 356 roughly that

Infection rate \times generation time $>$ cost of carriage + probability of segregative loss

359 is sufficient for parasitic plasmid maintenance.

360 Eq. (2.10) indicates that the conjugation terms must exceed a threshold to maintain a parasitic
 361 plasmid. In order to see this more clearly, we fix $\alpha = \alpha_+ = .1$ and $\beta = \beta_+ = .4$, with all other
 362 parameters as in Fig. 2, except that $\bar{\mu} = \mu \times 10^{-3}$. We then vary μ and plot the resulting stable
 363 steady state value of $u_+ + \delta w_+$ in Fig. 3. This was done by integrating the differential equations,
 364 starting with a tiny inoculum of plasmid-bearing cells, to steady state. If $u_+ + \delta w_+ = 0$, as it does
 365 for small μ , that means the stable steady state is the plasmid-free state; if $u_+ + \delta w_+ > 0$, then we
 366 are plotting the coexistence steady state value of $u_+ + \delta w_+$. This bifurcation diagram shows that
 367 the critical value $\mu_c \approx 6 \times 10^4$ at which the coexistence steady state appears is much larger than our
 368 order of magnitude estimate of a biologically reasonable value of $\mu \approx 1$.

369 3. Flow reactor model

370 In this section, we replace the chemostat by the flow reactor and the Pilyugin and Waltman
 371 model by the Freter Model, developed by Jones et al. [8,9]. We follow [9] except that we ignore

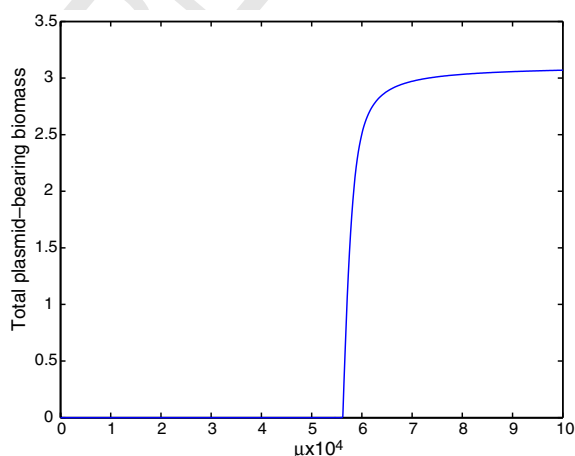


Fig. 3. Bifurcation of coexistence steady state from the plasmid-free steady state at a critical value of μ . Vertical axis is the coexistence steady state value of total plasmid-bearing biomass $u_+ + \delta w_+$. Horizontal axis is the conjugation rate μ with $\bar{\mu} = \mu \times 10^{-3}$.

372 cell death in conformity with the chemostat model. A flow reactor amounts to a section of a cylin-
373 drical tube $\Omega \equiv \{0 < x < L, 0 \leq r^2 = y^2 + z^2 < R^2\}$ in which a steady flow with velocity
374 $v(r) = V_{\max}[1 - (r/R)^2]$ of fluid in the direction of increasing x is imposed. The flow carries fresh
375 nutrient at concentration S^0 into the reactor across the $x = 0$ interface and carries unused nutrient
376 and bacteria out of the reactor across the $x = L$ interface. The equations describing nutrient den-
377 sity $S = S(x, y, z, t)$ and biomass density $u = u(x, y, z, t)$ of plasmid-free bacteria and u^+ of plasmid-
378 bearing organism in the fluid (i.e., in Ω) are given by:

$$\begin{aligned} S_t &= d_x^S S_{xx} + d_r^S [S_{yy} + S_{zz}] - v(r)S_x - \gamma^{-1} u f_u(S) - \gamma^{-1} u^+ f_u(S), \\ u_t &= d_x u_{xx} + d_r [u_{yy} + u_{zz}] - v(r)u_x + u f_u(S) + q u^+ f_u(S)(1 - c) - \bar{\mu} u u_+, \\ u_t^+ &= d_x u_{xx}^+ + d_r [u_{yy}^+ + u_{zz}^+] - v(r)u_x^+ + u^+ f_u(S)(1 - c)(1 - q) + \bar{\mu} u u_+, \end{aligned} \quad (3.1)$$

382 for $(x, y, z) \in \Omega$.

383 The areal density of wall-attached cells on the radial boundary $r = R$ of Ω , denoted by
384 $w = w(x, y, z, t)$ (plasmid-free) and w^+ (plasmid-bearing), satisfy the equations

$$\begin{aligned} w_t &= w[f_w(S)G(W) - \beta] + \alpha u(1 - W) + q f_w(S)(1 - c)G(W)w^+ - \mu w w^+, \\ w_t^+ &= w^+[f_w(S)(1 - c)(1 - q)G(W) - \beta_+] + \alpha_+ u^+(1 - W) + \mu w w^+. \end{aligned} \quad (3.2)$$

388 Note that we assume the biofilm is infinitesimally thin so it does not influence the fluid flow.
389 Terms in the model are described below.

390 An important difference between the Freter model and the Pilyugin–Waltman model is that the
391 former assumes a maximal attainable areal density of wall-attached bacteria w_∞ while the latter
392 does not. The quantity

$$W = (w + w^+)/w_\infty$$

395 represents the occupation fraction.

396 Danckwerts' boundary conditions describe the interface conditions between the up-stream and
397 down-stream flow and the reactor. They are as follows: at $x = 0$:

$$\begin{aligned} v(r)S^0 &= -d_x^S S_x + v(r)S, \\ 0 &= -d_x u_x + v(r)u, \\ v(r)u^{+0}(t) &= -d_x u_x^+ + v(r)u^+, \end{aligned} \quad (3.3)$$

401 where S^0 is substrate concentration in the feed, $u^{+0}(t) \equiv U^+ \geq 0$ on $0 \leq t \leq t_0$ and $u^{+0}(t) = 0$ for
402 $t > t_0$. The latter allows us to pulse plasmid-bearing organisms into the inflow to simulate an inva-
403 sion of plasmid-bearing organisms.

404 at $x = L$:

$$S_x = u_x = u_x^+ = 0. \quad (3.4)$$

408 The radial boundary conditions at $r = R$ provide the only coupling between the wall-adherent
409 and planktonic sub-populations of plasmid-bearing and plasmid-free cells:

$$\begin{aligned} 0 &= d_r^S S_r + \gamma^{-1} w f_w(S) + \gamma^{-1} w^+ f_w(S), \\ 0 &= d_r u_r + \alpha u(1 - W) - w[f_w(S)(1 - G(W)) + \beta], \\ 0 &= d_r u_r^+ + \alpha_+ u^+(1 - W) - w^+[f_w(S)(1 - c)(1 - G(W)) + \beta_+] \end{aligned} \quad (3.5)$$

413 see [8] for a detailed discussion of these. Here we point out that the important attachment and
 414 detachment terms involving α and β , appearing in (3.2), are communicated to the planktonic pop-
 415 ulations u and u_+ through the second and third of these boundary conditions. From the second of
 416 these, the term $-d_r u_r = \alpha u(1 - W)$ reflects the flux of planktonic bacteria to the surface. Note that
 417 the motility coefficient d_r plays a role as well as α in this attachment term. The factor $1 - W$, the
 418 fraction of unoccupied wall sites, takes account of the level of saturation of wall-attachment sites.
 419 Finally, daughter cells of wall-attached bacteria compete for space on the wall. A fraction G of the
 420 daughter cells find attachment sites and the fraction $1 - G$ do not and are forced into the fluid.
 421 $G = G(W)$ is a decreasing function of the occupation fraction W because a more fully saturated
 422 wall provides less chance for a daughter cell to find space on it. Freter [4,5] employs:

$$G(W) = \frac{1 - W}{1.1 - W}.$$

425 In addition, S , u , w satisfy (non-negative) initial conditions at $t = 0$:

$$\begin{aligned} S(x, y, z, 0) &= S_0(x, y, z), \\ u(x, y, z, 0) &= u_0(x, y, z), \\ u^+(x, y, z, 0) &= u_0^+(x, y, z), \\ w(x, y, z, 0) &= w_0(x, y, z), \\ w^+(x, y, z, 0) &= w_0^+(x, y, z). \end{aligned} \tag{3.6}$$

429 If $u_0^+ \equiv 0$, $w_0^+ \equiv 0$, and $U^{+0} = 0$, then $u^+ = 0$ and $w^+ = 0$ for all $t \geq 0$ while S, u satisfy the plas-
 430 mid-free system below (this is the analog of (2.3)):

$$\begin{aligned} S_t &= d_x^S S_{xx} + d_r^S [S_{yy} + S_{zz}] - v(r)S_x - \gamma^{-1} u f_u(S), \\ u_t &= d_x u_{xx} + d_r [u_{yy} + u_{zz}] - v(r)u_x + u f_u(S), \end{aligned} \tag{3.7}$$

434 while w satisfies

$$w_t = w[f_w(S)G(W) - \beta] + \alpha u(1 - W) \tag{3.8}$$

438 and the boundary conditions are the appropriate subset of (3.3) and (3.5), the latter with $w^+ = 0$ in
 439 the S boundary condition.

440 System (3.7) and (3.8) was studied in Jones et al. [8,10] as a simple model of biofilm formation.
 441 There it was shown that the system behavior largely depends on the stability of the washout steady
 442 state

$$S \equiv S^0, \quad u \equiv 0, \quad w = 0.$$

445 The stability of the washout steady state depends on a single real eigenvalue of an associated ellip-
 446 tic-algebraic eigenvalue problem. If the dominant eigenvalue is negative, then the washout steady
 447 state is stable in the linear approximation; if it is positive, then the washout state is unstable. See
 448 Theorem 3.3 of [8]. The main analytical result of that paper (Theorem 3.5) established the
 449 following:

450 **Theorem 3.1.** [8] Eqs. (3.7) and (3.8) has a radially symmetric positive (plasmid-free) steady state
 451 $(\bar{S}, \bar{u}, \bar{w})$ if the washout state $(S^0, 0, 0)$ is unstable in the linear approximation.

452 The plasmid-free steady state $(\bar{S}, \bar{u}, \bar{w})$ satisfies

$$S^0 > \bar{S}(x, r) > 0, \quad \bar{u}(x, r) > 0, \quad 0 < \bar{w}(x) \leq w_\infty. \quad (3.9)$$

456 Unlike Theorem 2.1, neither the uniqueness nor local stability properties of such a steady state
457 could be determined analytically. However, extensive numerical simulations suggest that it is un-
458 unique and attracts all solutions satisfying $(u_0, w_0) \neq (0, 0)$.

459 A sufficient condition for instability of the washout state is

$$f_w(S^0)G(0) \geq \beta \quad (3.10)$$

462 see [8,10] for additional information.

463 We will proceed on the assumption that the steady state (3.9) exists and is unique. Our focus
464 will be on whether the plasmid-bearing organism can invade the plasmid-free steady state. We fol-
465 low Jones et al. [9], see especially Eq. (4.10) in that paper, in our formal linearized stability anal-
466 ysis of the plasmid-free state. We assume further that the plasmid-free state is ‘internally stable’ in
467 the sense that it is stable in the linear approximation for the system (3.7) and (3.8) without plas-
468 mid-bearing organism. This allows us to focus attention on that part of the variational equations
469 which determine whether the plasmid-bearing organism can invade the plasmid-free state if intro-
470 duced in infinitesimal amount. More precisely, we obtain the variational equations by setting

$$S = \bar{S} + \epsilon \tilde{S}, \quad u = \bar{u} + \epsilon \tilde{u}, \quad w = \bar{w} + \epsilon \tilde{w}, \quad u^+ = \epsilon U^+, \quad w^+ = \epsilon W^+$$

473 into (3.1)–(3.6), letting $\epsilon \rightarrow 0$, and keeping only first order terms in ϵ . The $U^+ - W^+$ equations
474 decouple from the remainder of the variational equation (compare with [9, (4.10)]:

$$\begin{aligned} U_t^+ &= L^u U^+ + U^+ f_u(\bar{S})(1-c)(1-q) + \bar{\mu} \bar{u} U^+, \\ W_t^+ &= W^+ [f_w(\bar{S})(1-c)(1-q)G(\bar{W}) - \beta_+ + \mu \bar{w}] + \alpha U^+(1 - \bar{W}), \end{aligned} \quad (3.11)$$

477 where U^+, W^+ denote the variational counterparts of u^+, w^+ , L^u denotes the elliptic differential
478 operator and $\bar{W} \equiv \bar{w}/w_\infty$. Corresponding boundary conditions for U^+ at $x = 0$ and $x = L$ are gi-
479 ven in (3.3) and (3.4) while at $r = R$ they are given by

$$0 = d_r U_r^+ + \alpha U^+(1 - \bar{W}) - W^+ [f_w(\bar{S})(1-c)(1-q)G(\bar{W}) + \beta_+]. \quad (3.12)$$

482 According to Theorem 4.4 in [9], a sufficient condition for the plasmid-free steady state to be
483 unstable is given by

$$\sup_x [f_w(\bar{S}(x, R))(1-c)(1-q)G(\bar{W}(x)) - \beta_+ + \mu \bar{w}(x)] > 0. \quad (3.13)$$

487 Simulations as well as intuition suggest that (fresh nutrient enters at $x = 0$) both $\bar{S}(x, R)$ and $\bar{W}(x)$
488 attain their maxima at $x = 0$. Using this and the crude estimates (3.9), we obtain the following
489 sufficient condition for (3.13) to hold:

$$\beta_+ < f_w(S^0)(1-c)(1-q)G(0) + \mu w_\infty.$$

492 In summary, our results for the flow reactor are mainly suggestive and backed by simulations to
493 be presented below. There exists at least one plasmid-free steady state when the washout state is
494 unstable. However, in contrast to the ODE model of the previous section, we do not know
495 whether there is exactly one or several plasmid-free steady states. Simulations suggest it is unique

Table 2
Parameter values for the plug flow model

w_∞	$2.78 \times 10^{-6} \text{ g/cm}^2$
γ	.5
β	1 h^{-1}
β_+	$.01 \text{ h}^{-1}$
α	.5 cm/h
α_+	.5 cm/h
S^0	$2.09 \times 10^{-6} \text{ g/ml}$
a	$9 \times 10^{-7} \text{ g/ml}$
m	1.66 h^{-1}
q	1×10^{-4}
c	.01
$\mu, \bar{\mu}$	$1.0 \text{ cm}^2/(\text{g h}), .001 \text{ ml}/(\text{g h})$
V_{\max}	5.0 cm/h
$d_x^S = d_r^S$.04 cm^2/h
$d_x^u = d_r^u$.2 cm^2/h
Tube size	4 cm \times 1 cm
Grid resolution	$\Delta r = \Delta x = 1/60 \text{ cm}$

496 and globally attracting for the plasmid-free system. Assuming uniqueness and conditional global
 497 stability, we have provided a crude sufficient condition for the plasmid-free state to be unstable to
 498 invasion by the plasmid-bearing organism. Unfortunately, we do not currently have an appropri-
 499 ate dynamical systems theory for the plug flow system that would permit us to justify a persistence
 500 result analogous to Theorem 2.2 (Table 2).

501 4. Simulations for flow reactor

502 The governing PDE for the PFR, (3.1) and (3.2), is discretized using a standard second-order
 503 finite difference scheme with an explicit temporal discretization. We assume the solution is radially
 504 symmetric making the physical domain equivalent to the rectangle $\{(x,r):0 \leq x \leq L, 0 \leq r \leq R\}$.
 505 Here $L=4$ and $R=1$. The symmetry assumption implies the artificial boundary condition
 506 $\nabla S \cdot \hat{r} = \nabla u \cdot \hat{r} = 0$ at $r=0$. The domain is divided into 240 cells in the x direction and 60 in the
 507 r to give a uniform mesh size of $\Delta x = \Delta r = 1/60$.

508 To determine S and u on the boundary, the boundary conditions are employed. The condition
 509 (3.5) at $r=R$, $0 = d_r^S \partial S / \partial r + \gamma^{-1} w f_w(S) + \gamma^{-1} w^+ f_w(S)$, is non-linear in S since $f_w(\cdot)$ is non-linear.
 510 To solve for S on the boundary, the derivative is approximated using a second-order, one-sided
 511 differencing, and a non-linear system is formed for S on the boundary. The system is solved using
 512 a Newton–Raphson scheme. The value for u on the $x=L$ boundary as well as S and u on the
 513 other boundaries are then found by approximating the derivatives in the boundary conditions
 514 by second-order, one-sided differences.

515 The initial conditions for the plasmid-free bacteria are found by running $S_0 = S^0$, $w_0 = 0$, and
 516 $u_0 = 1 \times 10^{-7} \text{ g/ml}$, $u^+ = .0$ and $w^+ = .0$ to steady state (plasmid-free steady state), depicted in
 517 Fig. 3 by contour plots of \bar{S} and \bar{u} and the graph of $\bar{w}(x)$. This plasmid-free steady state provides

518 the initial conditions for S , u and w in the main computation of the invasion of this plasmid-free
 519 steady state by an inoculum of planktonic plasmid-bearing cells, depicted in Figs. 4 and 5. The
 520 reactor is charged with plasmid bearing bacteria by taking $u^{+0}(t) = 10^{-10}U_5(t)$, where $U_5(t)$ is
 521 the step function of unit height turning to zero at $T = 5$. In order to provide a steady state profile,
 522 the equations were integrated to time $T = 4000$ h at which point no further change could be de-
 523 tected, as can be seen in the left-most Fig. 4. The approximate coexistence steady state profiles of
 524 are depicted in the right-most Fig. 4 (w, w_+) and Fig. 5 (S, u, u_+) (Fig. 6).

525 **Appendix A.** In this appendix we derive assertions made in Section 2 regarding various matrices
 526 and the biological meaning of their entries and we prove Theorem 2.2.

527 First consider the problem of how to define the mean residence time of a cell in the chemostat.
 528 The relevant differential equations for a plasmid-free cell are $x' = Ax$ where $x = (u, \delta w)^T$ and

$$A = \begin{pmatrix} -D - \alpha & \beta \\ \alpha & -\beta \end{pmatrix}.$$

531 Note that A contains the inter-compartmental flow rates. A is a stable, quasi-positive (off-diagonal
 532 entries non-negative), irreducible matrix. As such, the Perron–Frobenius theory (or direct compu-

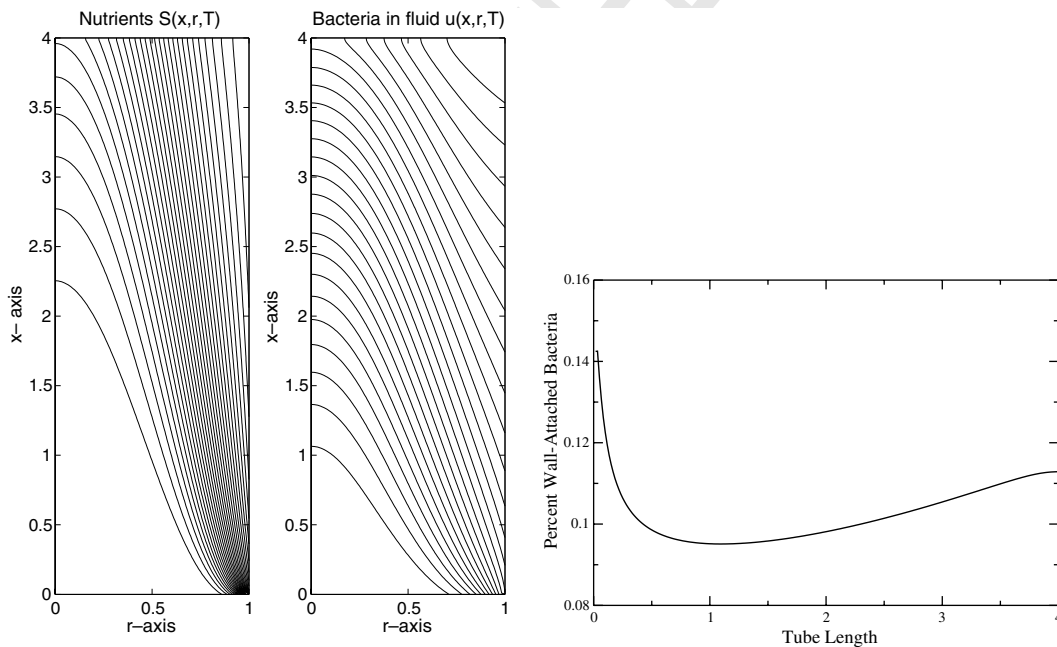


Fig. 4. The plasmid-free steady state in plug-flow. (Left) Contour plot of the plasmid-free steady state nutrient density \bar{S} . Contours of the nutrient have a maximum at the entrance of 2.0×10^{-6} g/ml and diminish to 5×10^{-7} g/ml towards the upper-right corner. (Center) Contour plot of the plasmid-free steady state planktonic bacteria density \bar{u} has a minimum of 1×10^{-7} g/ml near the entrance and close to the wall and increase to 6×10^{-7} g/ml towards the upper-right corner. (Right) Plot of the plasmid-free steady state profile of wall-adherent bacteria, \bar{w} .

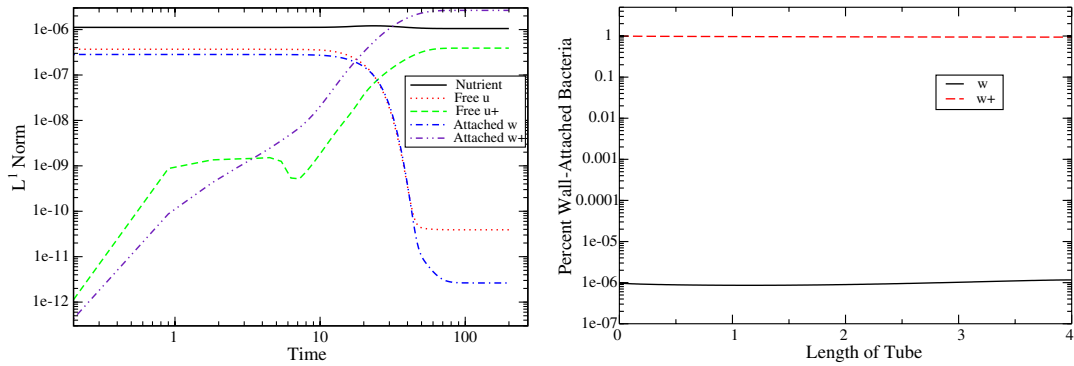


Fig. 5. (Left) time series plot of the invasion of the plug-flow plasmid-free steady state by an inoculum of planktonic plasmid-bearing cells pulsed into the reactor at $x = 0$ for 5 h. Total amounts of S , u , u_+ , w , w_+ over relevant domain is plotted versus time. (Right) Coexistence steady-state plot of wall-adherent bacteria w , w_+ versus x ultimately reached in simulation depicted on left.

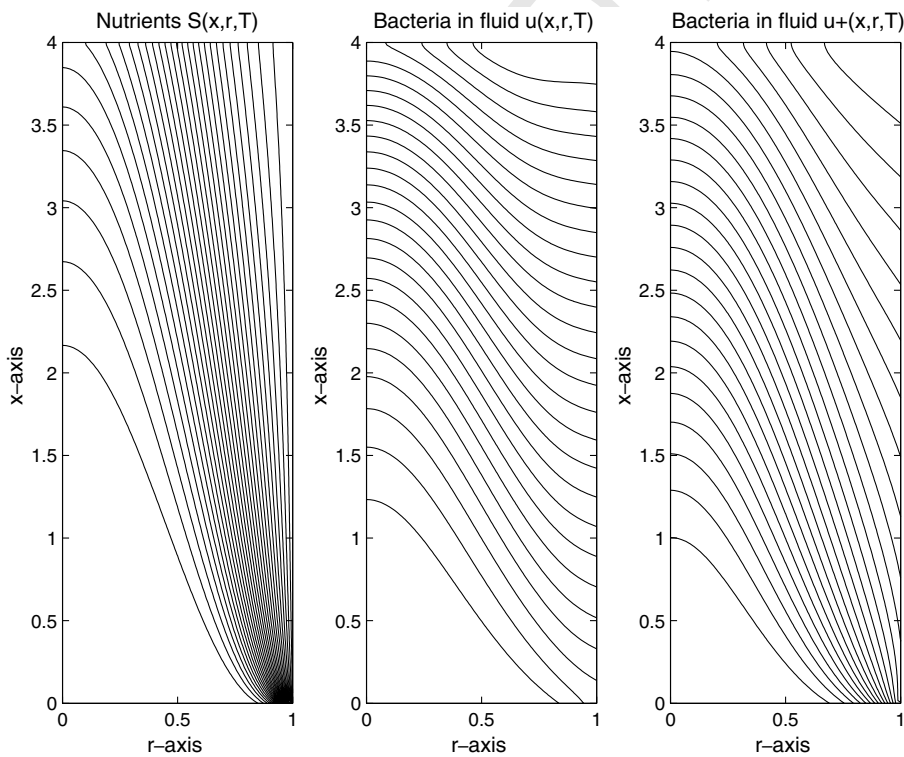


Fig. 6. Contour plots of S , u , u_+ at coexistence steady state reached in the simulation depicted in Fig. 5 (left). (Left) Contour plots of nutrient density S at the coexistence steady state. (Center) Contours of the planktonic plasmid-free density u at coexistence steady state has a minimum of 2×10^{-11} g/ml near the entrance and close to the wall, and increases to 1×10^{-10} g/ml. Similarly, (right) u^+ has a minimum of 1×10^{-7} g/ml and a maximum of 6×10^{-7} g/ml.

533 tation) implies that it has a dominant simple eigenvalue, $s(A)$, with a corresponding eigenvector v
 534 that has positive components: $Av = s(A)v$. Thus, all non-negative solutions of $x' = Ax$ are asymp-
 535 totic to a positive multiple of $x(t) = e^{s(A)t}v$, which decays exponentially to zero since $s(A) < 0$. We
 536 are therefore lead to take as our definition of *mean residence time (MRT)*

$$\text{MRT} := -1/s(A).$$

539 A simple calculation gives

$$s(A) = - \left[\frac{D + \alpha + \beta - \sqrt{(D + \alpha + \beta)^2 - 4\beta D}}{2} \right]. \quad (\text{A.1})$$

542 It is easily verified that MRT is the spectral radius of the matrix Q :

$$\text{MRT} = \rho(Q),$$

545 where

$$Q = \begin{pmatrix} 1/D & 1/D \\ \alpha/D\beta & (\alpha + D)/D\beta \end{pmatrix}.$$

548 Monotonicity of the spectral radius in the entries of Q shows that

$$\frac{d}{d\beta} \text{MRT} < 0, \quad \frac{d}{d\alpha} \text{MRT} > 0.$$

551 It is intuitive that the mean residence time increases with the wall affinity α and decreases with the
 552 sloughing rate β .

553 Now lets view the system $x' = Ax$ as the Kolmogorov differential equations for the finite, con-
 554 tinuous time Markov chain depicted in Fig. 7. The authors are grateful to Sebastian Schrieber for
 555 help with the following calculations. Washout is an absorbing state, u and w are transient states
 556 and α, β, D are rate constants. It is convenient to use numerals for states so we let u be state 1 and
 557 w be state 2. For $i, j, k \in \{1, 2\}$, let T_i denote the mean time a cell spends in state i before jumping
 558 to another state, T_{ij} denote the mean time spent in j before washout given that the cell starts in
 559 state i , and p_{ik} be the probability a cell goes from state i to k upon exiting from state i . Then

$$T_{ij} = \delta_{ij}T_i + \sum_k p_{ik}T_{kj}$$

562 or, in matrix language, $T - PT = \text{diag}[T_1, T_2]$ so $T = (I - P)^{-1} \text{diag}[T_1, T_2]$. In our case,

$$P = \begin{pmatrix} 0 & \frac{\alpha}{D+\alpha} \\ 1 & 0 \end{pmatrix}$$

565 and $T_1 = 1/(\alpha + D)$, $T_2 = 1/\beta$. Thus

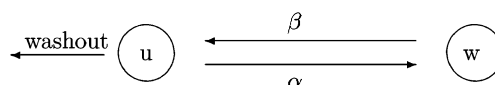


Fig. 7. Simple continuous time Markov chain with absorbing state 'washout'.

$$T = Q^T = \begin{pmatrix} 1/D & \alpha/\beta D \\ 1/D & \frac{D+\alpha}{\beta D} \end{pmatrix},$$

568 where superscript T denotes transpose. It follows that the entries of Q^+ have the meanings as-
569 signed in Section 2.

570 We now turn to the proof of Theorem 2.2. Local existence and positivity of solutions of (2.1)
571 are standard (see [22]). We begin by establishing a uniform ultimate upper bound on solutions.

572 **Lemma A.1.** *All non-negative solutions of (2.1) are ultimately uniformly bounded in forward time,*
573 *and thus they exist for all positive time. In fact,*

$$\limsup_{t \rightarrow \infty} \left(S + \frac{u}{\gamma} + \frac{\delta w}{\gamma} + \frac{u_+}{\gamma} + \frac{\delta w_+}{\gamma} \right) \leq S^0/b, \tag{A.2}$$

576 where $\bar{\beta} = \min\{\beta, \beta_+\}$, $d = \max\{D + \alpha + \bar{\beta} + f_w(S^0), f_u(S^0)c + D + \alpha_+ + \bar{\beta} + f_w(S^0)\}$, $e = f_u(S^0)$,
577 and $b = \frac{-d + \sqrt{d^2 + 4\bar{\beta}e}}{2e}$.

578 **Proof of Lemma.** From the inequality

$$S' \leq D(S^0 - S)$$

581 we conclude that $\limsup_{t \rightarrow \infty} S \leq S^0$. Monotonicity of f_u and f_w imply that, for given $\epsilon > 0$ we have
582 $f_u(S(t)) \leq f_u(S^0) + \epsilon$ and $f_w(S(t)) \leq f_w(S^0) + \epsilon$, for $t \geq T$

583 For given a solution we define

$$M(t) = \frac{u + u_+}{u + u_+ + \delta w + \delta w_+}.$$

586 Then

$$M' = \left[\frac{(u + u_+)' }{(u + u_+ + \delta w + \delta w_+)} \right] - \left[\frac{(u + u_+ + \delta w + \delta w_+)'(u + u_+)}{(u + u_+ + \delta w + \delta w_+)^2} \right] =: l + n.$$

589 The first square bracket, l , is

$$l = \frac{(f_u u - Du - \alpha u + \beta \delta w + f_u(1 - c)u_+ - Du_+ - \alpha_+ u_+ + \beta_+ \delta w_+)}{(u + u_+ + \delta w + \delta w_+)},$$

592 where $f_u = f_u(S(t))$ and $f_w = f_w(S(t))$. If $a = a(t) := \min\{(f_u - D - \alpha), (f_u(1 - c) - D - \alpha_+)\}$, then

$$l \geq aM + \bar{\beta}(1 - M).$$

595 The second square bracket, n , in M' is

$$n = - \frac{(f_u u - Du + f_w \delta w + f_u(1 - c)u_+ - Du_+ + (f_w(1 - c)\delta w_+)(u + u_+)}{(u + u_+ + \delta w + \delta w_+)^2},$$

$$n \geq \frac{(-f_u u - f_u u_+ - f_w \delta w - f_w \delta w_+)}{(u + u_+ + \delta w + \delta w_+)} M + DM^2,$$

$$n \geq -f_w M - f_u M^2 + f_w M^2 + DM^2.$$

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M. Imran et al. / Mathematical Biosciences xxx (2005) xxx-xxx

598 So

$$M' \geq \bar{\beta} + (a - \bar{\beta} - f_w)M + M^2(D + f_w - f_u).$$

601 Using the result of the first paragraph of the proof, and considering both cases one by one for
602 a , given $\epsilon > 0$, there is $T > 0$ such that

$$a - \bar{\beta} - f_w \geq -d - \epsilon + f_u \geq -d - \epsilon$$

605 for all $t \geq T$. So $M' \geq \bar{\beta} - M(d + \epsilon) - M^2e/2$. The right hand side of this inequality is a parabola
606 opening down wards. Inside the positive region there is only one stable rest point. Consequently,

$$\frac{-(d + \epsilon) + \sqrt{(d + \epsilon)^2 + 2\bar{\beta}e}}{e} \leq \liminf_{t \rightarrow \infty} M$$

609 and since $\epsilon > 0$ is arbitrary,

$$\frac{-d + \sqrt{d^2 + 2\bar{\beta}e}}{e} \leq \liminf_{t \rightarrow \infty} M.$$

612 Let $z = S + \frac{u}{\gamma} + \frac{\delta w}{\gamma} + \frac{u_+}{\gamma} + \frac{\delta w_+}{\gamma}$. Adding the five equations of (2.1) we find that,

$$z' = D \left(S^0 - S - \frac{u}{\gamma} - \frac{u_+}{\gamma} \right) - \gamma^{-1} c f_u(S) u_+ - \gamma^{-1} c f_w(S) w_+.$$

615 For ϵ satisfying $b = \frac{-d + \sqrt{d^2 + 2\bar{\beta}e}}{e} > \epsilon > 0$, there exists $T > 0$ such that for $t \geq T$

$$u + u_+ \geq [b - \epsilon](u + u_+ + \delta w + \delta w_+).$$

618 Therefore, for $t \geq T$

$$\begin{aligned} z' &\leq D(S^0 - S) - D\gamma^{-1}(b - \epsilon)(u + u_+ + \delta w + \delta w_+) - \gamma^{-1} c f_u(S) u_+ - \gamma^{-1} c f_w(S) w_+ \\ &\leq D[S^0 - (b - \epsilon)z] \end{aligned}$$

621 implying that $\limsup_{t \rightarrow \infty} z \leq S^0/b$. \square

622 Note that it is critical for the proof that $\beta, \beta_+ > 0$. If, for example, $\beta = 0$, the wall population
623 may grow unboundedly. \square

624 **Proof of Theorem 2.2.** We follow a similar argument used in Theorem 5.3 of [24], applying The-
625 orem 4.6 in [28]. Lemma A.1 establishes that (2.1) has a compact attractor so that the dissipative-
626 ness requirement of Theorem 4.6 holds.

627 Using the notation of that result, we set $X = R_+^5$, $X_2 = \{(S, u, w, u_+, w_+) \in X : u_+ = 0 \text{ or } w_+ = 0\}$,
628 and $X_1 = X \setminus X_2$. Observe that solutions of (2.1) starting in X_2 immediately enter X_1 , where u_+ ,
629 $w_+ > 0$, unless $u_+(0) = w_+(0) = 0$. We want to show that solutions which start in X_1 are eventually
630 bounded away from X_2 . Using the notation $x(t) = (S(t), u(t), w(t), u_+(t), w_+(t))$ for a solution of
631 (2.1), define

$$Y_2 = \{x(0) \in X_2 : x(t) \in X_2, t \geq 0\} = \{x(0) \in X : u_+(0) = w_+(0) = 0\}$$

634 and Ω_2 , the union of omega limit sets of solutions starting in X_2 , is, by our hypotheses, the set
635 $\{E_0, E_1\}$ where $E_0 := (S^0, 0, 0, 0, 0)$ and $E_1 := (\bar{S}, \bar{u}, \bar{w}, 0, 0)$. We will show that if $M_0 = \{E_0\}$ and

636 $M_1 = \{E_1\}$, then $\{M_0, M_1\}$ is an isolated acyclic covering of Ω_2 in Y_2 and each M_i is a weak repel-
 637 ler. All solutions starting in Y_2 but not on the S -axis converge to E_1 while those on the axis con-
 638 verge to E_0 . E_1 , being locally asymptotically stable relative to Y_2 , cannot belong to the alpha limit
 639 set of any full orbit in X_2 different from E_1 itself. Similar arguments apply to E_0 ; the only solutions
 640 converging to it lie on the S -axis and these are either unbounded or leave X in backward time.
 641 Thus $\{M_0, M_1\}$ is an acyclic covering of Ω_2 . If M_1 were not a weak repeller for X_1 , there would
 642 exist an $x(0) \in X_1$ such that $x(t) \rightarrow E_1$ as $t \rightarrow \infty$. Let $V(t) = (u_+(t), \delta w_+(t))^t$ and define the matrix
 643 $P(f(S), u, w)$ ($f = (f_u, f_w)$) by

$$\begin{pmatrix} f_u(S)(1-c)(1-q) - D - \alpha_+ + \bar{\mu}u & \beta_+ \\ \alpha_+ & f_w(S)(1-c)(1-q) - \beta_+ + \mu w \end{pmatrix}. \quad (\text{A.3})$$

646 Then $A = P(f(\bar{S}), \bar{u}, \bar{w})$ and we may write the equation satisfied by $V(t)$ as

$$\dot{V} = P(f(\bar{S}), \bar{u}, \bar{w})V + [P(f(S), u, w) - P(f(\bar{S}), \bar{u}, \bar{w})]V.$$

649 If $P(f(\bar{S}), \bar{u}, \bar{w})^t W = qW$ where $q = s(P(f(\bar{S}), \bar{u}, \bar{w})) = s(C) > 0$ and $W = (m, n)^t$ with $m, n > 0$ is
 650 the Perron–Frobenius eigenvector, then on taking the scalar product of both sides of the differ-
 651 ential equation by W and using that $S(t) \rightarrow \bar{S}$ and $w(t) \rightarrow \bar{w}$, we have

$$\frac{d}{dt}(mu_+ + n\delta w_+) \geq q/2(mu_+ + n\delta w_+)$$

654 for all large t . But this leads to the contradiction to $x(t) \rightarrow E_1$, namely that $mu_+(t) + n\delta w_+(t) \rightarrow \infty$
 655 as $t \rightarrow \infty$. Thus M_1 is a weak repeller. The argument above together with the fact that E_1 is locally
 656 asymptotically stable relative to the subspace $(u_+, w_+) = (0, 0)$ implies that it is an isolated com-
 657 pact invariant set in X . Similar arguments show that M_0 is a weak repeller and an isolated com-
 658 pact invariant set in X . Therefore, Theorem 4.6 in [28], implies our result: there exists $\epsilon > 0$ such
 659 that $\liminf_{t \rightarrow \infty} d(x(t), X_2) > \epsilon$ for all $x(0) \in X_1$, where $d(x, X_2)$ is the distance from x to X_2 .

660 The existence of at least one coexistence steady state follows from Theorem 1.3.7 of [29]. \square

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