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A reappraisal of the plasmid stability problem in chemostat cultures

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Segregational instability models of recombinant bacteria conventionally assume that —(a) the probability of loss of a unit of a plasmid is constant and (b) upon cell division, the progeny either inherit the mother's plasmid content entirely or not at all. However, two previous studies have shown that neither assumption is correct. So, in this work, both assumptions have been relaxed to formulate a more general model of plasmid dynamics in a chemostat. Unlike earlier work, this model predicts a time-dependent dilution rate to maximize segregational stability. Some biological implications are discussed.

Keywords: chemostat, decay probability variation, *Escherichia coli*, plasmid stability

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Introduction

In a recent publication, Baheri *et al*[1] drew our attention to a weakness in models of chemostat cultures based on the classic mechanism for recombinant cell propagation proposed by Imanaka and Aiba[2]. That mechanism considers that when a recombinant cell divides, not always do both the daughter cells inherit copies of the plasmid of interest that the mother cell contained. Over a population of cells and across successive generations of cell division, this phenomenon (called segregational stability) results in a gradual depletion in the proportion of plasmid-bearing cells even under supposedly 'steady state' conditions.

While Baheri *et al*[1] accepted the observations of segregational instability, they questioned Imanka and Aiba's[2] premise that each daughter cell may have either all copies of a plasmid or none at all. They argued that when a recombinant mother cell contains a multi-copy plasmid, as is often the case, it is possible for the progeny to have fewer copies, i.e. less of plasmid mass per unit cell mass, rather than be totally without the plasmid. This concept of partial loss of plasmid is an important departure from the all or none demarcation of plasmid inheritance. On a macroscopic scale, the Imanaka-Aiba formalism translates to just two kinds of cells in a population, those with and those without the entire plasmid content, whereas Baheri *et al*'s model allows a distribution of plasmid copy numbers. Apart from being more realistic, a distribution of copy numbers also enables attainment of a greater activity of the recombinant protein[3].

In support of their model, Baheri *et al*[1] showed that, for their *Escherichia coli* culture, it expressed the observed rates of decline of the fraction of plasmid-bearing cells at different dilution rates more accurately than Imanaka and Aiba's[2] model did. Nevertheless, the improved model also deviated from experimental data at large dilution rates and over long durations of time. One reason for this weakness may lie in an assumption implicit in Baheri *et al*'s model.

To quantify the extent of plasmid loss, they considered two probabilities: one for the loss at each stage of cell division, p, and the other for complete loss, P. The latter process creates plasmid-free cells from plasmid-containing cells. In relating P to p, Baheri *et al*[1] allowed P to vary but assumed p constant. However, Mosrati *et al*[4] have shown earlier that p may vary with the specific growth rate of the plasmid-containing cells, which in turn depends on the dilution rate. So it is relevant to ask how the predicted dynamics of a chemostat will change upon including the two non-ideal features proposed by Baheri *et al* and Mosrati *et al*. The present communication addresses this question.

Model Development

Baheri *et al*[1] expressed the rate of loss of plasmid material from an ensemble of plasmid-harbouring cells as a fermentation progresses by Eqn (1).

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$$\frac{dN}{dt} = -k_p N \quad \ldots (1)$$

Since N is a macroscopic average concentration, the extent of decrease in N over an interval of time may not produce an equivalent decrease in the number of plasmid-harbouring cells. Only the complete absence of plasmid transfer, i.e. N=0, generates plasmid-free cells. If p denotes the probability of loss of an individual copy of plasmid and P the probability of complete loss, then[1]:

$$P = p^N \quad \ldots (2)$$

Baheri *et al*[1] pointed out that many models of chemostat dynamics incorrectly use p in place of P. Obviously P = p only in the elementary case of N=1 at all times. With Eqn (1), the standard mass balances for a chemostat become slightly modified.

$$\frac{dX^+}{dt} = (1-p^N)\mu^+ X^+ - DX^+ \quad \ldots (3)$$

$$\frac{dX^-}{dt} = p^N \mu^+ X^+ + \mu^- X^- - DX^- \quad \ldots (4)$$

At this point, we relax Baheri *et al*'s assumption of a constant p and use the equation proposed by Mosrati *et al*[4] for the dependence of p on μ^+:

$$p = \alpha\left[\mu^+ - \frac{\beta(\mu^+/K_h)^n}{1+(\mu^+/K_h)^n}\right] \quad \ldots (5)$$

where K_h is the saturation constant for the host component of the plasmid replication rate[5].

Mosrati *et al*[4] did not, however, employ Eqns (1) and (2), preferring instead to assume a constant value of N=1. This assumption, as we know now, is not correct, and neither is Baheri *et al*'s[1] use of a constant p. Therefore we incorporate Eqn (5) into Eqns (3) and (4) and also replace another simplifying assumption of Baheri *et al*, namely that $\mu^+ = \mu^- = D$. Generally $\mu^+ < \mu^-$ because of the extra metabolic load on plasmid-bearing cells[6], and Patnaik[7] showed that for recombinant *E. coli* cultures the linear relations presented below are good approximations for many different strains.

$$\mu^+ = 0.8856D \quad \ldots (6)$$

$$\mu^- = 1.1148D \quad \ldots (7)$$

Eqns (1)-(7) constitute a more general formulation of the plasmid stability problem in a chemostat than those of Imanaka and Aiba[2], Baheri *et al*[1] and Mosrati *et al*[4]. These equations were solved with the same values for the parameters as in Baheri *et al* but allowing the presence of a small concentration of plasmid-free cells at the start (Table 1). This allowance is a practical consideration because some segregational instability is unavoidable during the growth period of a seed culture before it is transferred to the main reactor.

Application and Discussion

Figs 1-3 portray the results of this study. The shaded surfaces in Figs 1 and 2 are for a variable plasmid loss probability. This variation is seen to be an approximately linear function of the dilution rate (Fig. 3); such a variation has also been reported for a recombinant *Pseudomonas* sp.[8], thus underlining both the importance and the diversity of the variation of p with μ^+ or D. For comparison, a second set of surfaces have also been plotted in Figs 1 and 2. These transparent surfaces correspond to a constant probability of $p = 1.659 \times 10^{-3}$, which is the mean value for the range of variation in Fig. 3.

The differences between the two pairs of surfaces emphasize the importance of including in a chemostat model the variability both in the single-cell plasmid loss probability, p, and in the probability, P, for the population as a whole. A constant probability, p, predicts a higher segregational instability, resulting in less of plasmid-containing cells at all times. The separation between each pair of surfaces in Figs 1 and 2 increases with time, indicating that the simplified model predicts that the fermentation ceases to be viable much sooner than predicted by the more accurate model used here. Another significant difference is in the shapes of the surfaces. With a

Table 1—Values of the parameters and initial conditions

Parameter	Value	Variable	Initial value
K_h (h^{-1})	0.132	N (g g^{-1})	1.0
k_p (h^{-1})	0.044	X^+ (g L^{-1})	0.2
n	1.78	X^- (g L^{-1})	0.02
α	0.015		
β	2.15×10^{-4}		

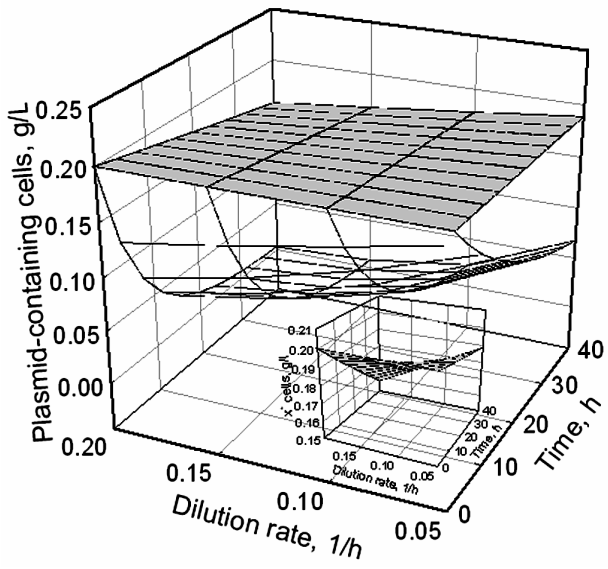

Fig. 1—Variation of the concentration of plasmid-containing cells with time and dilution rate. The shaded surface is for variable plasmid loss probability and the clear surface for constant probability.

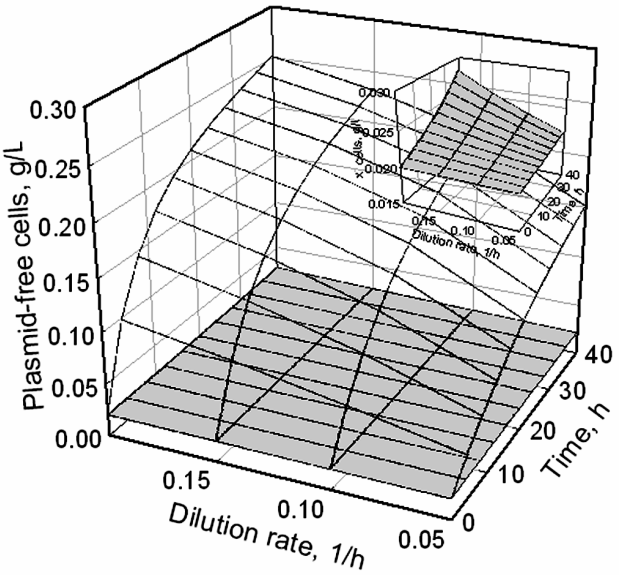

Fig. 2—Variation of the concentration of plasmid-free cells with time and dilution rate. The shaded surface is for variable plasmid loss probability and the clear surface for constant probability.

constant p, the surfaces are concave upward (for plasmid-harboring cells, Fig. 1) or downward (Fig. 2) and segregational instability increases with the dilution rate.

Accommodation of variability in p [Eqn (5)] and in the extent of plasmid loss across a population of cells [Eqn (2)] generates quite different concentration profiles. To highlight the differences, the shaded

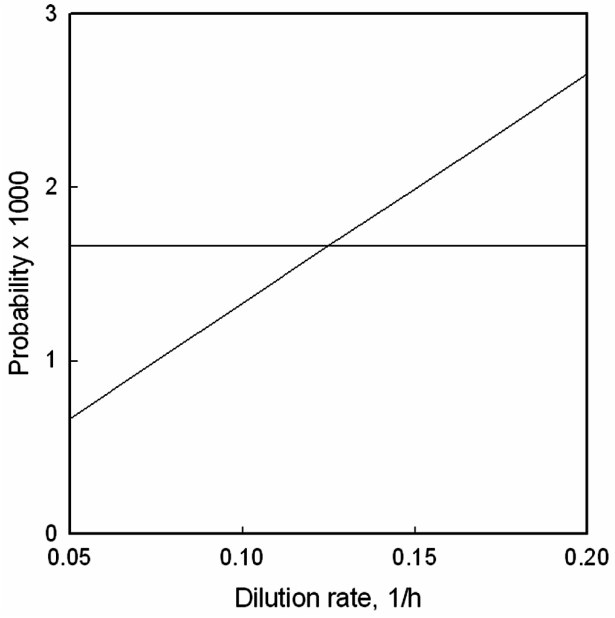

Fig. 3—Variation in the single unit plasmid loss probability with time. The horizontal line is the constant mean value.

surfaces in Figs 1 and 2 have been projected on magnified sets of axes as insets of these figures. Now, it is seen that, whereas the plasmid-free surface (Fig. 2, inset) slants upward and is weakly convex, for recombinant cells (Fig. 1, inset) it is skewed in the three-dimensional space. These two shapes together imply that the relative growth of plasmid-containing cells with respect to plasmid-free cells is favoured by a time-dependent optimal dilution rate. This inference generalizes previous studies with simpler models[9,10], which have shown that discrete variations in the dilution rate promote plasmid stability. The physiological basis of the beneficial effects of dilution rate variation is yet unclear, but its possible role in the growth rates of the two kinds of cells and in the plasmid copy number distribution have been discussed recently[11]. An optimal distribution is meaningful especially for multi-copy plasmids, where too large a concentration becomes metabolically unsustainable and too few copies result in premature quenching of the fermentation[3,6]. Since partial segregational loss of plasmid affects this distribution, the present analysis suggests that controlled feeding may be utilized to maintain a near-optimal distribution throughout the cultivation period.

Nomenclature

D dilution rate (h^{-1})

K_h saturation constant for host component of plasmid replication rate (h^{-1})

k_p plasmid decay constant (h^{-1})

n empirical constant in Eqn (5)
N plasmid concentration inside the cells (g g^{-1})
p probability of loss of a unit of plasmid
P probability of loss of entire plasmid content
t time (h)
X^+ concentration of plasmid-containing cells (g L^{-1})
X^- concentration of plasmid-containing cells (g L^{-1})

Greek letters

α empirical constant in Eqn (5) (h)
β maximum plasmid replication rate (h^{-1})
μ^+ specific growth rate of plasmid-containing cells (h^{-1})
μ^- specific growth rate of plasmid-free cells (h^{-1})

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