$$\Delta\mu = \frac{m}{f_-^* - 1} \tag{14.38}$$

$$R = \frac{-m f_-^*}{1 - f^*} \tag{14.39}$$

This analysis provides a method to estimate experimentally the parameters of importance in predicting genetic instability. Once these parameters are known, eq. 14.21 can be used to predict chemostat performance. However, the reader should recall the large number of assumptions that went into these expressions. Case 1 is the one relevant to most commercial systems, where high expression levels are coupled with high-copy-number plasmids. Case 2 will occur only for low-copy-number plasmids that do not have a par locus or other stabilizing features. The results for case 3 must be applied very cautiously. For μ_- to be less than μ_+, selective pressure usually must be applied (e.g., antibiotic resistance or auxotrophic hosts). Equations 14.7 and 14.8 do not recognize the possibility of leakage of nutrients or enzymes, which would degrade the antibiotics. If there were no leakage and the antibiotic-to-cell ratio were high enough to leave an effective residual level of antibiotic, then the analysis for case 3 could be applied if μ_- were interpreted correctly. For a newly born plasmid-free cell, there will be a carry-over of the complementing factor, which will be gradually reduced by turnover and dilution (recall Fig. 14.5). Thus, in the first generation after plasmid loss, μ_- may even be greater than μ_+, but μ_- will decrease from generation to generation until it becomes zero. The analysis from case 3 will give an effective average value of μ_-.

Removal of many of these assumptions to yield a more realistic analysis increases complexity and makes the development of simple analytical expressions difficult. Let us consider how this simple analysis may be used and how it can be extended.

Example 14.2.

The data in Table 14.4 were obtained for *E. coli* B/r-pDW17 at two different dilution rates in glucose-limited chemostats. The average plasmid copy number for pDW17 is about 40 to 50 copies per cell. About 12% of the total protein synthesized is due to the plasmid. The proteins are retained intracellularly in soluble form. Use these data to estimate $\Delta\mu$, R, and P.

Solution Note that data on plasmid stability are usually given in terms of the number of generations, which is usually calculated as $(\ln 2)/D = 1$ generation. Clearly, the trend of the data in Table 14.4 is a sigmoidal dependence of f_- on time, which corresponds to growth-rate-dependent instability or a case 1 situation (see Fig. 14.6). Thus, we need to plot the data as $\ln f_-$ versus time. Such a plot is given as Fig. 14.7. Recall that eq. 14.32, which gives the bounds on t, suggests that only data at intermediate times can be used to estimate $\Delta\mu$ and R. In this case, data for $0.01 < f_- < 0.40$ were used as a basis for the initial estimates of values of $\Delta\mu$ and R. Equation 14.32 can be used later to check the appropriate data range.

For $D = 0.3\ h^{-1}$ the slope, m, is 0.15 gen^{-1} or 0.066 h^{-1}, and the intercept is −5.3, implying $f_{-1} = 0.005$. For $D = 0.67\ h^{-1}$, $m = 0.12\ gen^{-1}$ or 0.11 h^{-1}, an intercept of −5.6 gives $f_{-1} = 0.0037$. Recall that, for case 1, $m = \Delta\mu$ and $R = m(f_{-1} - f_{-0})$. The value of f_{-0} presents a problem, as an accurate value is difficult to determine experimentally when it is low (many colonies must be plated to obtain any statistical accuracy). R will be maximal if f_{-0} is assumed to be zero. If a culture is developed under strong selective pressure, with plasmid-