Evaluation of Surface Colonization Kinetics in Continuous Culture

James A. Malone* and Douglas E. Caldwell*

Department of Biology, University of New Mexico, Albuquerque, New Mexico 87131, USA

Abstract. Two equations, describing surface colonization, were evaluated and compared using suspended glass slides in a continuous culture of Pseudomonas aeruginosa. These equations were used to determine surface growth rates from the number and distribution of cells present on the surface after incubation. One of these was the colonization equation which accounts for simultaneous attachment and growth of bacteria on surfaces:

\[ N = \frac{A}{\mu}e^{\mu t} - \frac{A}{\mu} \]

where \( N \) = number of cells on surface (cells field\(^{-1}\)); \( A \) = attachment rate (cells field\(^{-1}\)h\(^{-1}\)); \( \mu \) = specific growth rate (h\(^{-1}\)); \( t \) = incubation period (h).

The other was the surface growth rate equation which assumes that the number of colonies of a given size (\( C_i \)) will reach a constant value (\( C_{\text{max}} \)) which is equal to \( A \) divided by \( \mu t \):

\[ \mu = \frac{\ln\left(\frac{N}{C_i} + 1\right)}{t} \]

Both equations gave similar results and the time required to approximate \( C_{\text{max}} \) may not be as long as was previously thought. In all cases both \( A \) and \( \mu \) continuously decreased throughout the incubation period. These decreases may be due to various effects of microbial accumulation on the surface. Both equations accurately determined surface growth rates despite highly variable attachment rates. Growth rates were similar for both the liquid phase of the culture and the solid-liquid interface (0.4 h\(^{-1}\)). Use of the surface growth rate equation is favored over the use of the colonization equation since the former does not require a computer to solve for \( \mu \) and the counting procedure is simplified.

Introduction

Microbial accumulation on surfaces can be divided into 3 stages: adsorption, attachment, and colonization or growth [8]. Various methods have been used...
to quantitate this accumulation. Of particular interest is the determination of
the specific growth rate ($\mu$). Bott and Brock [1, 2, 3, 5] have used the exponential
growth equation to determine this value, and by their method the first 2 stages
are either ignored or accounted for by using an irradiated control slide. This
approach, however, fails to account for the progeny of cells that attached during
the incubation, and thus results in an inflated $\mu$ [6]. Subsequently, Caldwell et
al. [6] derived the colonization equation which accounts for simultaneous growth
and attachment:

$$ N = (A/\mu)e^{\mu t} - A/\mu \quad \text{(Eq. 1)} $$

where

$N =$ number of cells on surface (cells field$^{-1}$)
$A =$ attachment rate (cells field$^{-1}$h$^{-1}$)
$\mu =$ specific growth rate (h$^{-1}$)
$t =$ incubation time (h).

In this method, the first 2 stages are accounted for by $A$, the attachment rate.

Although the colonization equation better describes microbial surface growth,
its use requires some awkward counting procedures and a computer to solve
for $\mu$. As a result, Caldwell et al. [7] developed a new system of colonization
kinetics which simplifies the counting procedures and eliminates the need for
a computer. These kinetics yielded the surface growth rate equation:

$$ \mu = \frac{\ln\left(\frac{N}{C_i} + 1\right)}{t} \quad \text{(Eq. 2)} $$

where

$C_i =$ number of microcolonies with "i" cells (colonies field$^{-1}$)

This equation is based on the assumption that the number of microcolonies
of a particular size ($C_i$) will reach a steady-state number ($C_{\text{max}}$) as determined
by the following relationship involving the constants $A$ and $\mu$:

$$ C_{\text{max}} = \frac{A}{\mu} \quad \text{(Eq. 3)} $$

Using Equation 2, the only empirical data required to solve for $\mu$ are the total
number of cells and the number of colonies of a particular size. Furthermore,
the attachment rate can be calculated using Equation 3, once $\mu$ is known. One
possible drawback, however, is the time required for $C_i$ to approach $C_{\text{max}}$. A
computer simulation by Kieft and Caldwell [9] indicates that $C_i$ approaches
$C_{\text{max}}$ asymptotically and thus, long incubation times may be required.

Thus far, the colonization equation has only been evaluated under field
conditions [4]. In order to further evaluate it and examine the validity of the
newly proposed surface growth rate equation, glass surfaces were suspended in
a continuous culture. This method provided a controlled environment and