

## REPLY COMMENT

**Reply to Peters (2002) Overcoming linearisation errors in calculating bacterial growth rates**

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Peters (2002, this issue) revisits the topic covered by Kirchman (2002) and arrives at 'somewhat different conclusions' about how to calculate growth rates from data on production rates and biomass. In fact, Peters (2002) still implies that it is conceptually correct to use the equation I argued against, if one uses the proper production estimated over the right time interval. More problematic, his analysis leads to the counter-intuitive observation that errors in calculating growth rates increase as the incubation time decreases. Here I try to show why this analysis is incorrect, and I argue (again) that the correct way to calculate growth rates is to simply divide production rates by biomass.

In agreement with Kirchman (2002), Peters rightly identifies the problem in using:

$$\mu = \ln[(P_{\Delta t}/B_0) + 1]/\Delta t \quad (1)$$

to calculate growth rates ( $\mu$ ) from  $P_{\Delta t}$ , which is biomass production for the time interval  $\Delta t$ , and  $B_0$ , which is microbial biomass at an initial time point. The problem is one of trying to use a linear approximation for an inherently exponential process. He then goes on to suggest a solution: 'One just has to plug in the production obtained *during* [his italics] the incubation as  $P$  and the incubation time as  $\Delta t$ , without first calculating a daily production.' This seems reasonable, and in fact, the growth rate calculated using Eq. (1) and following Peters's suggestion is nearly the same as the growth rate calculated by:

$$\mu = P/B \quad (2)$$

where  $P$  is biomass production rate (units of biomass per volume or area per unit time) and  $B$  the microbial biomass (the same as  $B_0$ ) when  $P$  is measured. Although Peters's suggestion seems reasonable, I think his analysis of the errors is incorrect and leads to counter-intuitive conclusions.

The figures of Peters indicate that Eq. (1) seriously underestimates the true growth rate when the incubation time is shorter than the scale of the time units of

the growth rate (the reference time, as termed by Peters) and then it overestimates the growth rate when the incubation time exceeds the reference time. His analysis leads to the counter-intuitive argument that a short incubation time leads to bigger errors than a longer incubation time equal to the reference time, here 1 d. This paradox is created by how Peters analyzes the errors in these equations. Peters assumed a growth rate ( $1 \text{ d}^{-1}$  in Fig. 2) and biomass level at time zero, and then calculates biomass levels at successive time points using the exponential growth equation:

$$B_t = B_0 e^{\mu t} \quad (3)$$

Eq. (1) is then used to calculate a growth rate for that particular point in time. Peters estimates the error as being the difference between the calculated growth rate and the initial assumed growth rate. For time points far from 1 d, the error is great as illustrated in the figures of Peters. Why? Basically, it is because the calculated production rate is much lower (for time points  $< 1 \text{ d}$ ) or higher (time points  $> 1 \text{ d}$ ) than the production rate at  $t = 1 \text{ d}$ , because of the error in using a linear approximation for an exponential process. For example, in Peters's Fig. 2 the production rate at  $t = 1 \text{ d}$  is  $1.60 \times 10^9 \text{ cells l}^{-1} \text{ d}^{-1}$ , whereas it is calculated to be  $0.511 \times 10^9 \text{ cells l}^{-1} \text{ d}^{-1}$  at  $t = 1 \text{ h}$ . Consequently, the calculated growth rate is greatly underestimated at  $t = 1 \text{ h}$  relative to  $t = 1 \text{ d}$ .

I think this error analysis is at best misleading, mainly because it incorrectly allows biomass and production rates to vary during the incubation period (e.g. 1 h) and during the reference time. Allowing this variation leads to the appearance of errors for short time periods and to Peters's counter-intuitive and incorrect suggestion about trying to match incubation times and the reference time (here 1 d).

If the production data are to have any meaning, it is incorrect to allow the production rate to vary during the incubation and during the reference time. If biomass varied substantially during the incubation, incorporation of leucine, for example, would not be linear over time and the production rate estimate would be in error.

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Furthermore, when we wish production rates and growth rates to have a time unit longer than the incubation period ( $d^{-1}$ , for example, even though the incubation period was 1 h), it is necessary to assume or to have time course data showing that the biomass and production rate measured during a short incubation period hold for the longer time interval. There may be other problems with this assumption (no diel variation in rates and biomass?), but there are no calculation errors. Consequently, we cannot calculate errors in these equations for times shorter than 1 d (when growth rates are  $d^{-1}$ ), because biomass and the production rate cannot be allowed to vary during this time.

Peters recognizes that biomass must be constant, but the time frame he focuses on is incorrect. Peters argues that Eq. (2) — his Eq. (4) — ‘holds true if we assume the bacterial assemblage to be in steady state’, which he considers to be a ‘special case ... It occurs when all population loss factors ... match gross production so that the net assemblage production is zero.’ I agree that Eq. (2) requires a constant biomass, but not the steady state implied by Peters. Microbial growth is often balanced by mortality on short time scales, but certainly biomass levels do vary over some time scale, implying that Eq. (2) could never be used if we took Peters’s argument literally. If Eq. (2) cannot be used, then Eq. (1) could also never be used, because both depend on the same data (production rate and biomass) and the same assumption about exponential growth.

However, we only need biomass to be constant over the incubation and the reference time, rather than for all time. The necessity of having constant biomass for the incubation period should be obvious; if biomass is allowed to vary, the production rate will also vary, and it becomes meaningless to calculate a single growth rate. If biomass does vary, then the incubation period should be reduced until biomass and production rate are constant. Likewise, both biomass and production rate must be constant during the reference time; otherwise the time-dependent measures (production rate and growth rates) are rather meaningless. For example, we could extrapolate from an hourly production rate to an annual rate, assume constant biomass, and calculate a growth rate per year. But that growth rate would be rather meaningless, since production rates and biomass vary over a year, unless rates are extremely slow, such as in the deep ocean. The obvious solution is to reduce the reference time to some period during which production rate and biomass are constant and the calculated growth rate is meaningful.

Peters also examines errors for times longer than the reference time. This is necessary for Eq. (1) (see below), but it is unnecessary, and even misleading, for Eq. (2), which depends only on a measured production rate and biomass estimate, not on biomass levels

extrapolated into the future. Of course Eq. (2) would appear to be in ‘error’ when an initial growth rate is compared with new growth rates calculated with new biomass levels and production rates. Therefore, the error in using Eq. (2) suggested by Peters’s Fig. 2 is misleading; there is no error because biomass and production rates need not (and cannot) vary during the analysis.

But to use Eq. (1), it is necessary to carry out the calculations beyond the incubation time and to allow some change in biomass. Since that change is an exponential process, Eq. (1) is a linear approximation, as Peters rightly identifies. However, the approximation should be forward in time, not backward; a ‘backwards approximation’ leads to changing biomass and production rates during the reference time frame, invalidating the estimates, as argued above. If one must use Eq. (1) (although I see no reason to), a forward linear approximation avoids Peters’s counter-intuitive observation that short incubation times lead to large errors in growth rate calculations.

To conclude, I return to the primary argument made in Kirchman (2002). I began by pointing out that biomass production is simply the change in biomass over time ( $dB/dt$ ). If we assume exponential growth, then the production rate ( $P$ ) is:

$$P = dB/dt = \mu B \quad (4)$$

Peters also assumes exponential growth and uses the solution ( $B_0 e^{\mu t}$ ) to Eq. (4) in his derivation of Eq. (1). But if the solution to Eq. (4) is used, why not simply use Eq. (4) directly and derive an expression (Eq. 2) for the growth rate ( $\mu$ )? One cannot accept the validity of Eq. (1) and reject Eq. (4). If Eq. (4) is accepted, then Eq. (2) mathematically follows.

In conclusion, the easiest way to overcome linearization errors in calculating growth rates is to simply use Eq. (2). The difference in the growth rate value calculated by the 2 equations may not be large, but Eq. (2) is conceptually the correct one. It defines the proper relationships among the fundamental parameters of microbial assemblages: the production rate, biomass, and growth rate.

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#### LITERATURE CITED

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