

A bioreaction–diffusion model for growth of marine sponge explants in bioreactors

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Abstract Marine sponges are sources of high-value bioactives. Engineering aspects of in vitro culture of sponges from cuttings (explants) are poorly understood. This work develops a diffusion-controlled growth model for sponge explants. The model assumes that the explant growth is controlled by diffusive transport of at least some nutrients from the surrounding medium into the explant that generally has a poorly developed aquiferous system for internal irrigation during early stages of growth. Growth is assumed to obey Monod-type kinetics. The model is shown to satisfactorily explain the measured growth behavior of the marine sponge *Crambe crambe* in two different growth media. In addition, the model is generally consistent with published data for growth of explants of the sponges *Disidea avara* and *Hemimycale columella*. The model predicted that nutrient concentration profiles for nutrients, such as dissolved oxygen within the explant, are consistent with data published by independent researchers. In view of the proposed model's ability to explain available data for growth of several species of sponge explants, diffusive transport does play a controlling role in explant growth at least until a fully developed aquiferous system has become established. According to the model and experimental

observations, the instantaneous growth rate depends on the size of the explant and all those factors that influence the diffusion of critical nutrients within the explant. Growth follows a hyperbolic profile that is consistent with the Monod kinetics.

Introduction

Of the marine invertebrate species, sponges have attracted significant attention as potential sources of bioactive compounds (Osinga et al. 1999; Proksch et al. 2002; Belarbi et al. 2003a; Mayer and Gustafson 2003; Donia and Hamman 2003; Jha and Zi-rong 2004; Thakur and Müller 2004; Sipkema et al. 2005). Natural sponge populations are relatively inaccessible, or insufficient, for use in extraction of bioactives. Consequently, substantial interest exists in artificial culture of sponges and sponge cells. Obtaining cell lines that can be cultured as freely suspended cells without reverting to whole sponge has not proved possible. Instead, sponge culture technology is focusing mostly on culturing sponge explants or cuttings and primmorphs (cellular aggregates) (Müller et al. 1999) derived from the mother sponge and primary sponge cells, respectively. Engineering aspects of growth of sponge explants and primmorphs are poorly understood (García Camacho et al. 2005). No models have been published to satisfactorily describe the observed growth behavior.

Whole sponge and explants have an aquiferous system of pores and channels that distributes feeds and nutrients to the entire sponge biomass (Nickel and Brümmer 2003). Primmorphs lack a developed aquiferous system. Unlike the whole sponge, aquiferous systems of recently healed explant cuttings appear to be poorly developed. Flow

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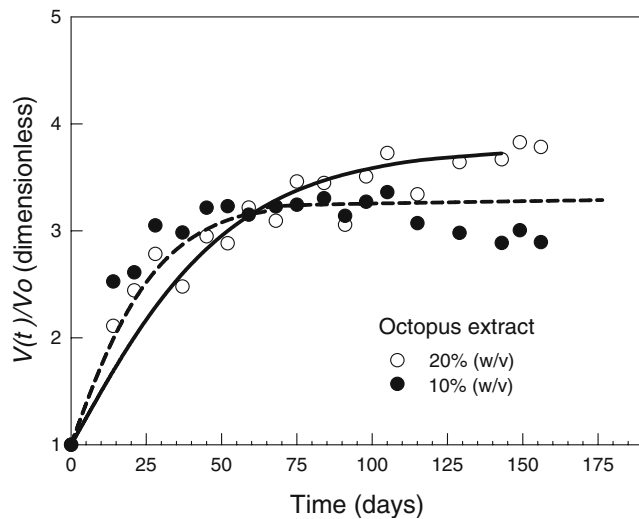


Fig. 1 Time course of the explants' dimensionless volumes ($V(t)/V_0$). Comparison of measured data (symbols) and the model predicted curves

through the aquiferous system is produced by movement of cilia on choanocytes, the cells that line the expanded chambers within aquiferous channels. The channels of the aquiferous system have diameters of the order of micrometers and, therefore, the flow through these channels is mostly laminar. Dissolved nutrients such as oxygen must diffuse from the channels of the aquiferous system into the body of the sponge. In contrast with convective transport, diffusive transport of at least some nutrients likely plays a role in growth of primmorphs and explants with poorly formed aquiferous systems. Similarly, diffusive transport of dissolved oxygen is important in satisfactory growth of the fully developed sponge. For example, dissolved oxygen concentrations that are significantly lower than in the surrounding water have been demonstrated within sponges (Gatti et al. 2002; Hoffmann et al. 2005). Limitations in oxygen diffusion have indeed been cited as a cause of explant death once the explant size reached a certain value (Gatti et al. 2002; Hoffmann et al. 2005). In view of diffusion limitations, zones of widely differing metabolic activity may exist within explants and relative dimensions of these zones could affect growth.

This work examines the implications of the increasing explant size on the rate of nutrient transport in the explant and its growth. A growth model that is potentially applicable to in vitro growth of sponge cell aggregates and explants is used to explain our data and other published information. The quasimechanistic model discussed in this study is useful in explaining the differences in growth rates that are observed at different growth stages of explants of the same adult sponge and different individuals of the same species.

Materials and methods

The sponge used was the pocilosclerid *Crambe crambe* (Schmidt). This sponge is known to possess an array of potentially active metabolites such as crambines and crambescidins (Rinehart and Jares-Erijman 1999). Specimens were collected by SCUBA diving off the coast of Almería (SE Spain, Western Mediterranean).

Adult individuals of the sponge were cut into small pieces of uniform size such that each piece had a volume of approximately 0.045 cm^3 . Explants were sanitized by immersion (5 min) in a solution of sodium hypochlorite (5% w/v) in seawater (Muldford and Villena 2000). Subsequently they were held for 1 h in seawater that had been supplemented with antibiotics (gentamycin 50 mg l^{-1} , nistatin 1.25 mg l^{-1} , and penicillin 50 mg l^{-1}). Afterwards, the explants were transferred to sterile seawater where they remained for 2–3 days before further experiments. Explant preparation was carried out at 17°C .

The culture medium and procedure used are described in detail in García Camacho et al. (2006). Briefly, the culture medium consisted of commercial RPMI 1640 (Sigma, St Louis, MO, USA) supplemented with (1) various inorganic salts at concentrations that are typically used for making artificial seawater, (2) various amino acids and ascorbic acid, and (3) an aqueous extract of octopus (*Octopus vulgaris*) added at 10 or 20% (v/v) in different experiments. The explants were placed in the culture medium (1.5–4.0 ml) held in glass vials (4–15 ml). The amount of the medium used and the size of the vials depended on the elapsed time since initial inoculation. The medium in the vials was changed from once to four times per week, depending on the time elapsed from inception. The vials were incubated ($17 \pm 2^\circ \text{C}$) under an atmosphere of carbon dioxide ($4.0 \pm 0.5\%$ v/v) at pH 7.2 ± 0.2 . The vials were held in an orbital shaker to ensure good mixing. The growth of the explants was monitored by photographic measurements as described in García Camacho et al. (2006).

Results

Data on growth of *C. crambe* explants in two media are shown in Fig. 1, in terms of increases in dimensionless apparent volumes of explants ($V(t)/V_0$). Each data point shown is an average of measurements from four explants. In both media, a typically Monod-type of growth pattern was observed (Fig. 1). Explants increased in volume by up to fourfold within 120-days from inoculation, suggesting a relatively good growth environment. As the explants were not provided with particulate feed and the media were free of bacteria that could serve as feed, all observed growth

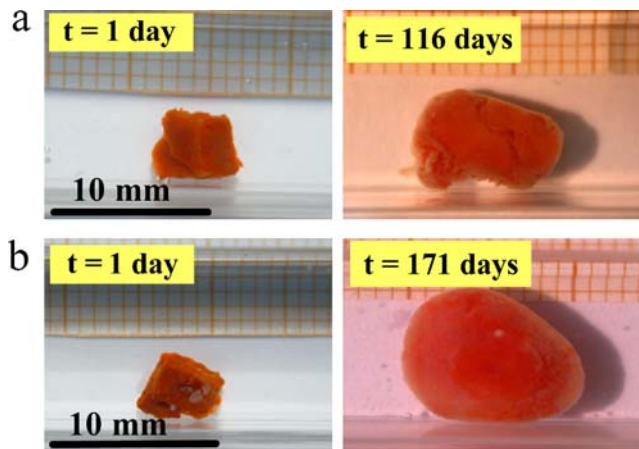


Fig. 2 Increase in size of two explants grown in media with octopus extract concentrations of **a** 10% (w/v) and **b** 20% (w/v)

was attributed to dissolved organic carbon sources present in the media (García Camacho et al. 2006).

The growth pattern shown in Fig. 1 has been seen for other sponge explants. For example, for explants of the Mediterranean sponge *Chondrosia reniformis* (Nickel and Brümmer 2003) grown mostly on particulate feeds. Initial rapid growth followed by a stationary phase, as seen in Fig. 1, has been reported also for *C. crambe* explants that were fed on microalgal cells but not exclusively on organic nutrients (Belarbi et al. 2003b).

During the first 15 days after inoculation, the explants all underwent similar morphological changes. Thus, the cut surfaces became smooth and healed, the straight edges become rounded, and the rounding continued until the explants became fully ovoid. These changes are clearly seen in Fig. 2a,b; for two explants grown in media that were supplemented with 10% (v/v) and 20% (v/v) extracts of octopus, respectively. Histological observations of explants in advanced stages of growth revealed a lack of well-developed openings of the aquiferous system and, therefore, a likely poor capacity to filter feed in comparison with *C. crambe* adults in the wild (García Camacho et al. 2006).

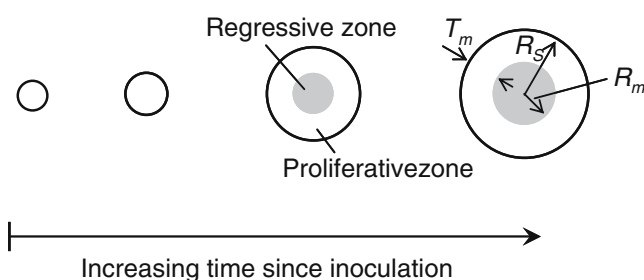


Fig. 3 Idealized growth pattern of sponge aggregates and explants. The explant initially has a diameter that is smaller than the thickness (T_m) of the outer proliferative layer. A zone of low activity, or regressive zone, develops in the interior when the explant diameter exceeds T_m . The diameter of this zone increases until eventually the explant dies

A similarly poorly developed aquiferous system has been reported by Nickel and Brümmer (2003) in explants of *C. reniformis*.

In view of the ability of explants to grow in the absence of well-developed aquiferous systems and on media that contain only dissolved nutrients, diffusive transport was postulated as a significant mechanism of nutrient uptake by explants. Consequently, a reaction-diffusion model was used in interpreting the growth observed. This model is discussed next.

Bioreaction–diffusion model of explant growth

The irregular shape of sponge explants and cell aggregates can be approximated as spherical (García Camacho et al. 2006). Furthermore, for short durations, if the external levels of nutrients are held constant, the nutrient concentration profiles within an explant can be assumed to be in steady state because explants grow extremely slowly. Because nutrients diffuse from the outside to the interior of the explant, the volume of the explant can be divided into at least two zones (Fig. 3). These are a proliferative external zone in which the biomass grows at its maximum possible rate (i.e., the growth is not limited by any nutrient) and an inner regressive zone in which the concentration of a growth limiting nutrient is below the level necessary for maintaining metabolically proliferative biomass. These zones are demarcated by the radial position R_m (Fig. 3). The total volume of the spherical explant with an equivalent radius $R_s(t)$ is given by the equation:

$$V(t) = \frac{4}{3}\pi R_s(t)^3 \quad (1)$$

The rate of change of mass $M(t)$ of the explant is given as follows:

$$\frac{dM(t)}{dt} = r_{\max}M_m(t) - bM_D(t) \quad (2)$$

where M_m is the mass of explant in the proliferative layer, M_D is the mass in the regressive core, r_{\max} is the maximum specific growth rate of the proliferative layer (i.e., the specific growth rate equivalent to that of a unlimited population of freely suspended cells), and b is the specific rate of biomass decay in the regressive core. Regression of tissue and apoptosis in marine sponges when environmental conditions are unfavorable for growth has been widely described (Elvin 1976; Barthel 1989; Turón et al. 1998; Kuhns et al. 1997).

The characteristic density ρ_B of the explants is assumed to be constant, i.e., tissue growth and regression are assumed to occur without affecting the ratio mass/volume within the explant. This assumption is commonly accepted in microbial flocs (Martins et al. 2004). Then dividing Eq. 2

by ρ_B , it can be rewritten in terms of explant volumes in the various zones, as follows:

$$\frac{dV(t)}{dt} = r_{max}V_m(t) - bV_D(t) \quad (3)$$

where V_m and V_D are the volumes of the proliferative and regressive zones, respectively, and $V(t)$ is the total volume of the explant. Equation 3 simply expresses any increase in volume of the explant in terms of growth and decay processes. The various volumes in Eq. 3 relate to the radial dimensions of the corresponding zones (Fig. 3), as follows:

$$\begin{aligned} V_m(t) &= \frac{4}{3}\pi(R_S(t)^3 - R_m(t)^3) \\ &= \frac{4}{3}\pi(R_S(t)^3 - [R_S(t) - T_m]^3) \end{aligned} \quad (4)$$

$$V_D(t) = \frac{4}{3}\pi R_m(t)^3 = \frac{4}{3}\pi(R_S(t) - T_m)^3 \quad (5)$$

where T_m is the thickness of the proliferative layer given as follows:

$$T_m = R_S(t) - R_m(t) \quad (6)$$

For a given set of culture conditions and sponge species, we assume T_m to be a constant irrespective of the size of the explant. This assumption is reasonable because a nutrient diffusing from the outside of the explant to the interior will penetrate to the same fixed depth before being fully consumed by a metabolically proliferative population of cells that are growing at their maximal rate.

Using the definitions in Eqs. 4 and 5, Eq. 3 can be written in terms of the radial distance R_S , as follows:

$$\begin{aligned} \frac{dR_S(t)}{dt} &= \frac{r_{max}(R_S(t)^3 - [R_S(t) - T_m]^3)}{3R_S(t)^2} \\ &\quad - \frac{b(R_S(t) - T_m)^3}{3R_S(t)^2} \end{aligned} \quad (7)$$

when the explant size is such that $R_S \leq T_m$, the entire volume of the explant will be in exponential growth and the explant will not have a regressive core zone (i.e., $V_D=0$). For such a fully proliferative explant, Eqs. 3 and 7 become Eqs. 8 and 9, respectively:

$$\frac{dV(t)}{dt} = r_{max}V(t) \quad (8)$$

$$\frac{dR_S(t)}{dt} = \frac{r_{max}R_S(t)}{3} \quad (9)$$

Integrated forms of Eqs. 8 and 9 can be used to estimate the explant volume $V(t)$ and the radius $R(t)$, respectively.

Once the explant radius exceeds T_m , its growth rate will decline continuously because of the presence of a regressive core of increasing size; i.e., the net growth rate will decline with time. Under these conditions, the integration of Eq. 7 leads to an implicit equation that enables the calculation of the radius of the explant as a function of time; thus,

$$\int_{R_0}^{R_S} \frac{dR_S(t)}{\frac{r_{max}}{3} \left(R_S(t) - \frac{[R_S(t) - T_m]^3}{R_S(t)^2} \right) - \frac{b[R_S(t) - T_m]^3}{3R_S(t)^2}} = t \quad (10)$$

In Eq. 10, R_0 is the initial radius of the explant. In these equations, r_{max} , b , and T_m are model parameters that depend on the physiological state of the explant, the culture medium used, and the growth environment.

Estimation of T_m

If $C_S(R)$ is a factor that relates to the concentrations of possible multiple growth limiting substrates at the radial position R within the explant and explant responds to changes in $C_S(R)$ in conformance with the commonly observed Monod growth kinetics, then an explant response function $g(R)$ can be defined at position R as follows:

$$g(R) = \frac{C_S(R)}{K_S + C_S(R)} \quad (11)$$

The response function $g(R)$ is dimensionless. In Eq. 11 K_S is a saturation constant that is comparable to that found in Monod kinetics. Equation 11 uses a grouped response factor $g(R)$ and a grouped concentration factor $C_S(R)$ because growth is determined by a combination of feed-related factors, including the concentrations of dissolved oxygen, dissolved organic nutrients, and particulate organic matter that sponges feed on. This concept of grouped response is commonly used in the literature on filter feeders like marine sponges (Jeshcke et al. 2004) and on microbial flocs (Martins et al. 2004). Equation 11 can be written as follows:

$$g(R) = \frac{c_s(R)}{1 + c_s(R)} \quad (12)$$

where $c_s(R)$ is the dimensionless substrate concentration at radial position R ;

$$c_s(R) = \frac{C_S(R)}{K_S} \quad (13)$$

The response function $g(R)$ decreases from its maximum value at the surface ($g(R_S) \approx 1$) of the explant to a lower threshold value at the interface between the proliferative and regressive zones. The functional response at the interface is $g_m (=g(R_m))$. At the interface of the proliferative

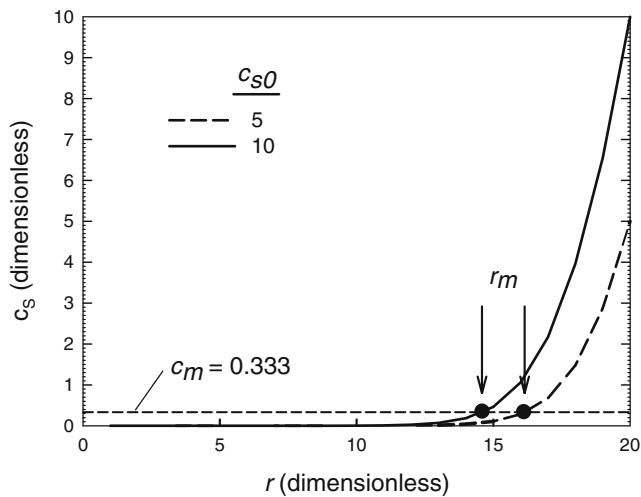


Fig. 4 Idealized profiles of dimensionless substrate concentrations (c_s) in an explant of dimensionless size $r_s=20$ for dimensionless external substrate concentrations of $c_{s0}=10$ and $c_{s0}=5$. Graphical estimation of r_m is shown for an arbitrary substrate concentration $c_m=0.333$

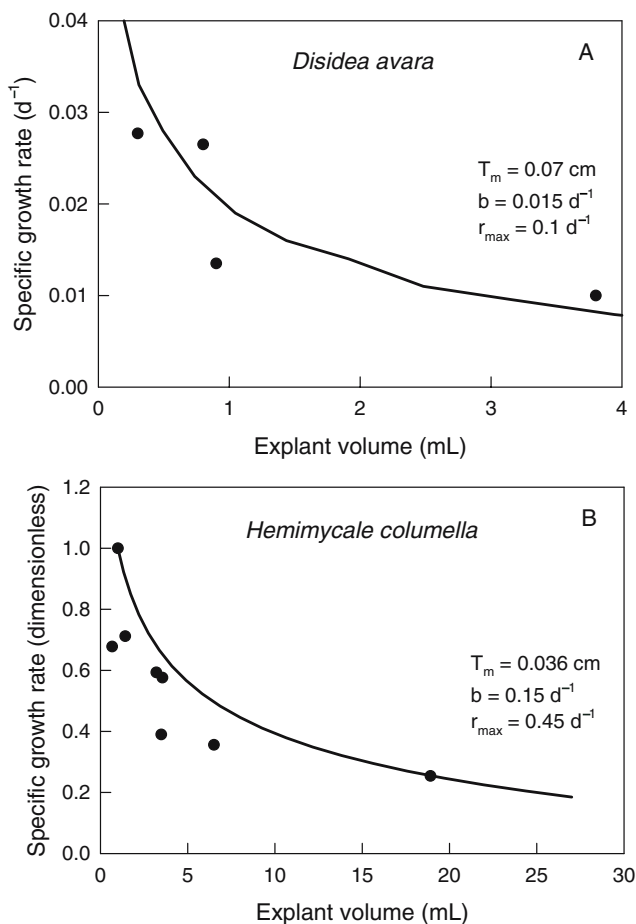


Fig. 5 Variation of specific growth rate with explant volume for sponges *D. avara* (a) and *H. columella* (b). The data points shown are as reported by Sipkema (2004). The solid lines are the model predicted trends

and regressive zones, the substrate uptake rate is that associated with a decaying population; thus,

$$g_m r_{max} - b = 0, \text{ or } g_m = \frac{b}{r_{max}} \tag{14}$$

At radial position R_m , the substrate concentration C_m equals $C_S(R_m)$. The dimensionless substrate concentration c_m at position R_m is as follows:

$$c_m = \frac{C_S(R_m)}{K_S} = \frac{g_m}{1 - g_m} \tag{15}$$

The radial position R_m (or T_m) where the substrate concentration declines to C_m , is calculated from the substrate concentration profile within the explant. Assuming diffusive transport of the limiting substrates in the explant, well-mixed culture medium of constant composition around the explant, and Eq. 11 for the local growth response of the explant, the substrate profile at quasisteady state in the explant can be written as follows:

$$\frac{D_e}{R^2} \frac{\partial}{\partial R} \left\{ R^2 \frac{\partial C_S(R, t)}{\partial R} \right\} = \frac{C_S(R) q_{sm} \rho_B}{C_S(R) + K_S} \tag{16}$$

In Eq. 16, q_{sm} is the maximum specific consumption rate of the limiting substrate, ρ_B is the apparent density of the explant, and D_e is the effective diffusion coefficient for the limiting substrate.

If we define a modified diffusion coefficient (equivalent to the well-known Thiele modulus) as follows,

$$\phi_{D_e} = \sqrt{\frac{D_e K_S}{q_{sm} \rho_B}} \tag{17}$$

Equation 16 modifies to the following dimensionless form:

$$\frac{\partial^2 c_s(r)}{\partial r^2} + \frac{2}{r} \frac{\partial c_s(r)}{\partial r} = \frac{c_s(r)}{c_s(r) + 1} \tag{18}$$

where

$$r = \frac{R}{\phi_{D_e}} \tag{19}$$

The boundary conditions of Eq. 18 are as follows:

$$c_s(r_s) = c_{s0}; \frac{\partial c_s(0)}{\partial R} = 0, \text{ and } r_s = \frac{R_s}{\phi_{D_e}} \tag{20}$$

Equation 18 can be integrated numerically to obtain the substrate profile and from this profile, r_m can be read as the value of r at which c_s equals c_m . Thus, T_m can be estimated as the difference between r_s and $r(c_m)$.

Figure 4 shows the hypothetical dimensionless concentration profiles for two initial substrate concentrations and the corresponding values of r_m for a fixed value of c_m . Oxygen concentration gradients similar to those shown in

Fig. 4, have indeed been measured by Gatti et al. (2002) and Hoffmann et al. (2005) in sponge explants and cell aggregates.

Using this value of r_m in Eqs. 17 and 19, we can calculate R_m ; thus,

$$R_m = r_m \phi_{D_e} = r_m \sqrt{\frac{D_e K_S}{q_{sm} \rho_B}} \quad (21)$$

In view of Eq. 21, the R_m value is influenced by factors such as q_{sm} , K_S , and D_e that can vary with time and culture conditions. Variations in these factors can explain at least partly the observed variations in growth rates of explants of a given species under different conditions of culture. Furthermore, as discussed next, the model propounded in this study does explain the frequently observed hyperbolic growth kinetics of laboratory cultured sponges where the growth ceases without explanation once the explant has attained a certain size.

Discussion

The proposed reaction-diffusion model closely fitted the growth data of Fig. 1 as shown by the model-generated curves shown in the figure. The modeled growth curves that best fitted the experimental data were produced using the following values of the model parameters: $T_m=0.039$ cm, $b=0.1$ d⁻¹, and $r_{max}=0.4$ d⁻¹ for the medium that was supplemented with 10% of octopus extract; and $T_m=0.050$ cm, $b=0.175$ d⁻¹, and $r_{max}=0.85$ d⁻¹ for the medium that was supplemented with octopus extract to 20% level. The best fit T_m values (i.e., the thickness of the fully proliferative layer that did not experience any substrate limitation) were quite reasonable in being approximately 10% of the maximum radii attained by the explants. These T_m values are consistent with other independent evidence. For example, measurements of dissolved oxygen profiles near the surfaces and within tissue of 10-day-old explants of the marine sponge *Geodia barrette* have revealed a complete depletion of oxygen at a depth of only 1 mm below the surface of the explant (Hoffmann et al. 2005). The volume of explants used by Hoffmann et al. (2005) ranged from 2 to 4 cm³ with corresponding equivalent explant radii of between 7.8 and 9.8 mm. In view of the reported T_m value of 1 mm and the aforementioned values of explant radii (i.e., R_S), the T_m/R_S ratios were 13 and 10%, respectively. This concurs with our own data for *C. crambe*. In view of such steep gradients in concentration of dissolved oxygen, the aquiferous system in explants is clearly less effective than in adult sponges.

There is a paucity of information on the maximum specific growth rates (i.e., r_{max}) of freely suspended sponge

cells. Nonetheless, our best fit model values of r_{max} noted above are consistent with the few available independently published data. For example, cultivated sponge cells of *Acanthella cavernosa* and *Ircinia muscarum* using enriched organic media grew, respectively, at maximum specific growth rates of 0.46 and 0.638 d⁻¹ (McMahon 2000; De Rosa et al. 2003).

The proposed growth model further explains the commonly observed decrease of the specific growth rate of explants with their increasing size. Declining specific growth rates with increasing volume of explants are shown in Fig. 5 for the sponges *Disidea avara* and *Hemimycale columella*, as measured by Sipkema (2004). Figure 5 also shows the trends in growth rates as predicted by the proposed model. The values of the model parameters that best fitted the two sets of experimental data are given in Fig. 5. For both sponges, the model reasonably described the experimentally observed behavior for parameter values (Fig. 5) that were quite consistent with expectations.

The modeled curves in Figs. 1 and 5 were generated using different fixed values of T_m for the different curves (i.e., T_m was assumed to be constant for a given set of growth conditions). In principle, model curves can be generated using varying values of T_m for a given curve. In this case, if T_m increases with time, the specific growth rate will also increase. Conversely, if T_m decreases with time, the growth rate would also do so. This behavior potentially explains observed fluctuations in specific growth rate of explants from the same sponge under slightly differing conditions. The value of T_m is influenced by R_m and, therefore, by all those factors that affect R_m as established in Eq. 21. Thus, changes in the apparent density (ρ_B) of explant will affect T_m . For example, Nickel and Brümmer (2003) did report a decrease in biomass density of *C. reniformis* with increasing age. This was a consequence of extensive accumulation of collagen in the mesohyl (mesenchyme).

Any change in the density of the sponge biomass will inevitably affect the effective diffusivity D_e of the biomass and, therefore, its T_m value (Eq. 21). For example, increases in biomass density are known to reduce the effective diffusion coefficient in biofilms and bacterial flocs (Stewart 1998). Sponge explants shown in Fig. 2 had a largely undifferentiated internal structure with few channels. In contrast, wild type *C. crambe* has a well-organized aquiferous system (Turón et al. 1997).

Although convective transport through the aquiferous system is the primary mechanism of nutrient transport in sponges (Osinga et al. 1999) in the wild, this does not automatically apply to sponge explants certainly in the early stages of growth. Except for oxygen, in the wild sponges rely mainly on particulate feeds as opposed to dissolved nutrients. Furthermore, convective transport of

nutrients in explants cannot explain the hyperbolic growth curves that have been consistently observed for them even though their volumes have remained small in comparison with parent sponge in the wild.

Plausibly, the poorly developed aquiferous systems in young explants do allow some convective transport. For example, Stoodley et al. (1994) have demonstrated the occurrence of flow of water in apparently homogeneous bacterial biofilms, by microscopically measuring the movement fluorescent latex balls (0.28 μm in diameter). When the velocity of water surrounding the biofilm was 6.6 cm/s, particles within the biofilm at a depth of 70 μm were moving at velocities between 10 and 20 $\mu\text{m/s}$ in a biofilm that was 175 μm thick. Clearly, this convective flow in a biofilm matrix with a poorly developed porous structure is miniscule compared with the maximum estimated velocities in pores of adult sponges. For the latter, Osinga et al. (1999) estimated a pore velocity of 0.48 $\text{cm}\cdot\text{s}^{-1}$ based on data for pumping reported by Reiswig (1973, 1974).

Concluding remarks

A model is developed to explain the observed growth behavior of sponge explants, using diffusive transport of growth-limiting nutrients as the principal factor that influences growth in explants with poorly developed aquiferous systems. The explant growth is characterized by Monod-type kinetics with the limiting nutrients supplied via diffusion into the explant from the surrounding medium. The model fits the observed growth data for explants of the marine sponge *Crambe crambe* grown in two different media. Furthermore, the model satisfactorily explains the independently published data on variation of the specific growth rates of explants with the explant volume for the sponges *D.* and *H. columella*. In view of the evidence presented, diffusive transport of nutrients in explants is a controlling influence on their growth at least during the stages when the explant has not developed a fully functional aquiferous system. Based on the model and experimental evidence, growth is influenced by explant size and other factors that influence the effective diffusivity of nutrients into explants.

Nomenclature

b	Biomass decay rate in the regressive layer (s^{-1})
C_m	Substrate concentration at the interface between the proliferative and regressive zones ($\text{kg}\cdot\text{m}^{-3}$)
$C_S(R_m)$	Substrate concentration at radial location R_m ($\text{kg}\cdot\text{m}^{-3}$)
$C_S(R_S)$	Substrate concentration at surface of explant ($\text{kg}\cdot\text{m}^{-3}$)

c_m	Dimensionless substrate concentration at R_m , defined by Eq. 15
c_s	Dimensionless substrate concentration
c_{s0}	Dimensionless substrate concentration at the surface of explant
$c_s(R)$	Dimensionless substrate concentration at radius R , defined by Eq. 13
D_e	Effective diffusion coefficient for substrate in explant ($\text{m}^2\cdot\text{s}^{-1}$)
g_m	Functional growth response at the interface between the proliferative and regressive zones
$g(R)$	Functional growth response of explant at radius R
$g(R_m)$	Functional growth response at the radial location R_m
K_S	Saturation constant ($\text{kg}\cdot\text{m}^{-3}$)
M_D	Sponge mass in the regressive core of the explant (kg)
$M(t)$	Total mass of explant at time t (kg)
M_m	Sponge mass in the proliferative layer (kg)
q_{sm}	Maximum specific consumption rate of the limiting substrate (s^{-1})
$R(t)$	Radial position at time t (m)
$R_m(t)$	Radius of the regressive zone at time t (m)
R_o	Initial radius of the explant (m)
$R_S(t)$	Radius of explant at time t (m)
r	Dimensionless radius defined by Eq. 19
$r(c_m)$	Dimensionless radial position at which the dimensionless concentration is c_m
r_m	Dimensionless radius of the regressive zone
r_{max}	Maximum specific growth rate of the proliferative layer without substrate limitation (s^{-1})
r_S	Dimensionless radius of the explant
T_m	Thickness of the proliferative layer of the explant (m)
t	Time (s)
$V(t)$	Volume of spherical explant at time t (m^3)
$V_D(t)$	Volume of the regressive zone of explant (m^3)
$V_m(t)$	Volume of proliferative zone of explant (m^3)
V_0	Initial volume of explant (m^3)
Greek symbols	
ρ_B	Density of sponge biomass ($\text{kg}\cdot\text{s}^{-1}$)
ϕ_{De}	Parameter defined by Eq. 17 (m)

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References

- Barthel D (1989) Growth of the sponge *Halichondria panicea* in the North Sea habitat. In: Klekowski RZ, Styczynska-Jurewicz E, Falkowski L (eds) Proceedings of the 21st European Marine Biology Symposium, Gdansk, 14–19 September 1986 pp 23–30
- Belarbi EH, Contreras Gómez A, Chisti Y, García Camacho F, Molina Grima E (2003a) Producing drugs from marine sponges. *Biotechnol Adv* 21:585–598
- Belarbi EH, Ramírez Domínguez M, Cerón García M, Contreras Gómez F, García Camacho F, Molina Grima E (2003b) Cultivation of explants of the marine sponge *Crambe crambe* in closed systems. *Biomol Eng* 20:333–337
- De Rosa S, De Caro S, Iodice C, Tommonaro G, Stefanov K, Popov S (2003) Development in primary cell culture of demosponges. *J Biotechnol* 100:119–125
- Donia M, Hamman M (2003) Marine natural products and their potential applications as anti-infective agents. *Lancet Infect Dis* 3:338–348
- Elvin DW (1976) Seasonal growth and reproduction of an intertidal sponge, *Haliclona permollis* (Bowerbank). *Biol Bull* 151:108–125
- García Camacho F, Belarbi EH, Cerón García MC, Sánchez Mirón A, Chile T, Chisti Y, Molina Grima E (2005) Shear effects on suspended marine sponge cells. *Biochem Eng J* 26:115–121
- García Camacho F, Chileh T, Cerón García MC, Sánchez Mirón A, Belarbi EH, Contreras Gómez A, Molina Grima E (2006) Sustained growth of explants from Mediterranean sponge *Crambe crambe* cultured in vitro with enriched RPMI 1640. *Biotechnol Prog* (in press). DOI 10.1021/bp050341m
- Gatti S, Brey T, Müller WEG, Heilmayer O, Holst G (2002) Oxygen microoptodes: a new tool for oxygen measurements in aquatic animal ecology. *Mar Biol* 140:1075–1085
- Hoffmann F, Larsen O, Rapp HT, Osinga R (2005) Oxygen dynamics in choanosomal sponge explants. *Mar Biol Res* 1:160–163
- Jeshcke JM, Kopp M, Tollrian R (2004) Consumer-food systems: why type I functional responses are exclusive to filter feeders. *Biol Rev* 79:337–349
- Jha RK, Zi-rong X (2004) Biochemical compounds from marine organisms. *Mar Drugs* 2:123–146
- Kuhns WJ, Ho M, Burger MM, Smolowitz R (1997) Apoptosis and tissue regression in the marine sponge *Microciona prolifera*. *Biol Bull* 193:239–241
- Martins AMP, Piciorenu C, Heijnen JJ, Van Loosdrecht MCM (2004) Three-dimensional dual-morphotype species modeling of activated sludge flocs. *Environ Sci Technol* 38:5632–5641
- Mayer AM, Gustafson KR (2003) Marine pharmacology in 2000: antitumor and cytotoxic compounds. *Int J Cancer* 105:291–299
- McMahon P (2000) System for the cell culture and cryopreservation of marine invertebrates. US Patent 6,054,317, 2000
- Muldford AL, Villena AJ (2000) Cell cultures from crustaceans: shrimps, crabs and crayfish. In: Mothersill C, Austin B (eds) Aquatic invertebrate cell culture. Praxis Publishing, Chichester, pp 63–134
- Müller WEG, Wiens M, Batel R, Steffen R, Borojevic R, Custodio MR (1999) Establishment of a primary cell culture from a sponge: primmorphs from *Suberites domuncula*. *Mar Ecol Progr Ser* 178:205–219
- Nickel M, Brümmer F (2003) In vitro sponge fragment culture of *Chondrosia reniformis* (Nardo, 1847). *J Biotechnol* 100:147–159
- Osinga R, Tramper J, Wijffels RH (1999) Cultivation of marine sponges. *Mar Biotechnol* 1:509–532
- Proksch P, Edrada RA, Ebel R (2002) Drugs from the seas—current status and microbiological implications. *Appl Microbiol Biotechnol* 59:125–134
- Reiswig HM (1973) Population dynamics of three Jamaican demospongiae. *Bull Mar Sci* 23:191–226
- Reiswig HM (1974) Water transport, respiration and energetics of three tropical marine sponges. *J Exp Mar Biol Ecol* 14:231–249
- Rinehart KL, Jares-Erijman EA (1999) Antiviral and cytotoxic compounds from the sponge *Crambe crambe*. US Patent 5,952,332, 1999
- Sipkema D (2004) Cultivation of marine sponges: from sea to cell. Ph. D. thesis, Wageningen University, Wageningen
- Sipkema D, Osinga R, Schihatton W, Mendola D, Tramper J, Wijffels RH (2005) Large-scale production of pharmaceuticals by marine sponges: sea, cell, or synthesis? *Biotechnol Bioeng* 90:201–222
- Stewart PS (1998) A review of experimental measurements of effective diffusive permeabilities and effective diffusion coefficients in biofilm. *Biotechnol Bioeng* 50(3):261–272
- Stoodley P, de Beer D, Lewandowski Z (1994) Liquid flow in biofilm systems. *Appl Environ Microbiol* 60:2711–2716
- Thakur NL, Müller WEG (2004) Biotechnological potential of marine sponges. *Curr Sci* 86:1506–1512
- Turón X, Galera J, Uriz MJ (1997) Clearance rates and aquiferous systems in two sponges with contrasting life-history strategies. *J Exp Zool* 278:22–36
- Turón X, Tarjuelo I, Uriz MJ (1998) Growth dynamics and mortality of the encrusting sponge *Crambe crambe* (Poecilosclerida) in contrasting habitats: correlation with population structure and investment in defence. *Funct Ecol* 12:631–639