

Morphological Analysis from Images of Hyphal Growth using a Fractional Dynamic Model

J. M. Blackledge[†] and D. J. Barry[‡]

Dublin Institute of Technology, Ireland.

Abstract

*The development of methods capable of accurately characterising the morphology of filamentous microbes represents a significant challenge to biotechnologists. This is because the productivity of many industrial fermentation processes is heavily dependent on the morphological form adopted by an organism. It is therefore of significant value if a quantitative model and associated metric(s) for morphological forms determined by complex phenotypes can be determined non-invasively, e.g. through image analysis. Specific interest is in the quantification of the branching behaviour of an organism. This is due to the link between branching frequency, biomass and metabolite production. In this paper we present a model for three-dimensional microbial growth that is based on a fractional dynamic model involving separable coordinate geometry. This provides the focus for the approach reported in this paper where microbial growth can be quantified using a sample microscopic digital image. In particular, we study the fractal dimension of fungal mycelial structures by generating a 'fractal signal' based on the object boundary. In the analysis of a population of *Aspergillus oryzae* mycelia, both the fractal dimension and hyphal growth unit are found to increase together over time. Further, through an extensive analysis of different populations of *Penicillium chrysogenum* and *A. oryzae* mycelia, cultivated under a variety of different conditions, we show that there is a statistically significant logarithmic correlation between the boundary fractal dimension and hyphal growth unit.*

Categories and Subject Descriptors (according to ACM CCS): Computer Graphics [I.3.3]: L—ine and Curve Generation, Physically Based Modelling, Fractals, Feature Representation

1. Introduction

The optimisation of industrial fermentation processes involving filamentous microorganisms requires an in-depth knowledge of the relationship between biomass and metabolite production. The specific morphological form adopted by an organism is of critical importance to the clarification of this relationship which is dependent on a variety of factors [ZP01]. The accurate quantification of phenotypic variation in vegetative mycelia, as a means of process control, is therefore of the utmost importance. With the advent of image analysis systems, significant progress has been made in furthering the understanding of the relationship between morphology and productivity [Pap04]. However, the accurate quantification of complex morphologies still represents a major challenge in process optimisation.

At the microscopic level, filamentous microorganisms consist of Hyphae exhibiting strongly polarised growth that develop into a composite structure termed a mycelium, which is conventionally characterised based on the ratio of the total hyphal length to the number of branches formed. This measure, termed the hyphal growth unit and denoted by L_{HGU} , was first proposed by Plomley [Pl059] and is still a common means of morphological quantification [BCW09, PM06, Pap06, ESMH06]. The growth unit effectively provides an overview of the branching behaviour of an organism under a given set of environmental conditions; a low value indicates a high rate of branch formation, whereas a high value is indicative of a relatively non-branching structure. The extent to which an organism forms branches is often of interest in industrial processes, as metabolite excretion occurs primarily at hyphal tips [GAJ*00, MMHN02]. A knowledge of branching behaviour is therefore of significant interest in the design of a particular bioprocess.

At the macroscopic level, the dispersed mycelial morpho-

[†] SFI Stokes Professor

[‡] School of Biological Sciences

logical form may dominate, or an aggregation of biomass may result in mycelial ‘clumps’ being predominant. These clumps may develop into dense, approximately spherical structures termed ‘pellets’, which may be up to several millimetres in diameter as shown in Figure 1. In fermentations of certain microorganisms, such as *Aspergillus oryzae*, there is evidence that pellet-formation is driven by spore agglomeration [CSNV96] and, as such, the occurrence of ‘free’ mycelia may be rare. The characterisation of these complex macro-morphologies represents a far greater challenge to the fungal biotechnologist, as individual Hyphae cannot be isolated and enumerated. As such, the accurate determination of the extent of branching of the organism is often impossible. These large aggregates of biomass are conventionally characterised in terms of projected area (A_p), perimeter length (P), circularity ($C = 4\pi A_p P^{-2}$), or various other interpretations thereof [PM06, TKDT92, LSW*02]. As different morphological parameters are often utilised depending on the growth form present, a considerable amount of effort has been expended in designing imaging systems capable of discriminating between these different phenotypes [PM06, TKDT92].

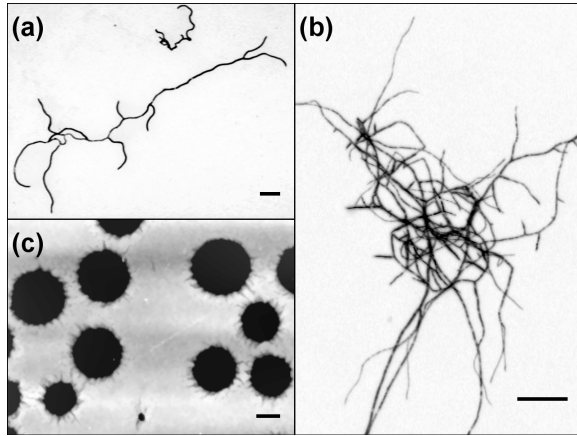


Figure 1: Typical morphologies found in submerged fermentations of filamentous microorganisms: (a) Freely dispersed mycelia (Bar = 50µm) (b) Mycelial clump (Bar = 100µm) (c) Pellets (Bar = 2.5mm)

An alternative approach to morphological quantification is to use fractal geometry [Man82] to characterise the spatial distribution of an organism (e.g. [Pap06], [PLL*07], [JL97], [KLK*05], [Ryo99], [HGR96], [GBD08], [MM92] and [JLM93]) although it has been suggested that the fractal dimension is often not sufficient for morphological characterisation, as microorganisms can sometimes appear to have different branching patterns, despite having similar values for the fractal dimension [BD08]. However, although numerous studies have been conducted in which fractal analysis is utilised to quantify morphology, few have attempted to link fractal dimension with conventional morphological pa-

rameters. Fractal analysis is of significant potential value in the study of filamentous microorganisms. This is because it lends itself to the quantification of all gross morphological forms that may be encountered and there remains the need to develop a quantitative relationship between the fractal dimension within a population of mycelia and the branching behaviour within that population. In this paper, we describe an alternative approach to fractal analysis, which directly relates the hyphal growth unit to the fractal dimension, based on an analysis of the mycelial boundary. The theoretical basis for this approach is considered in the following section.

2. Hyphal Growth based on Fractional Diffusion

Consider the three-dimensional fractional diffusion equation [Hi195], [Com96]

$$\left(\partial_x^2 + \partial_y^2 + \partial_z^2 - \sigma^q \partial_t^q\right) u(x, y, z, t) = -s(x, y, z, t)$$

where σ is the fractional coefficient of diffusion and where we consider a solution based on the separable case [Eis48]

$$s(x, y, z, t) = \delta(x)n_x(t) + \delta(y)n_y(t) + \delta(z)n_z(t)$$

and

$$u(x, y, z, t) = u_x(x, t) + u_y(y, t) + u_z(z, t)$$

The source function s is taken to model a system characterised by a separable spatial impulse with separable white noise function (n_x, n_y, n_z) . We are interested in a fractional dynamic model involving the time evolution of the system subject to changes in the ‘Fourier Dimension’ $q \in [1, 2]$ which is taken to decrease as a function of time. Hyphal growth in a three-dimensional space is taken to be described by the parametric curve $[u_x(t), u_y(t), u_z(t)]$ whose morphology is governed by the value of q . In this sense, we consider a fractional dynamic model based on the non-stationary operator [Bla10] $\partial_x^2 - \sigma^q \partial_t^q$.

2.1. Solution Method

The model described above allows us to consider Hyphal growth in terms of a solution to the following independent equations:

$$\left(\partial_x^2 - \sigma^q \partial_t^q\right) u_x(x, t) = -\delta(x)n_x(t) \quad (1)$$

$$\left(\partial_y^2 - \sigma^q \partial_t^q\right) u_y(y, t) = -\delta(y)n_y(t)$$

$$\left(\partial_z^2 - \sigma^q \partial_t^q\right) u_z(z, t) = -\delta(z)n_z(t)$$

The solution to equation (1) then applies to the solutions for u_y and u_z , i.e. we are required to find the general solution of the equation

$$\left(\partial_x^2 - \sigma^q \partial_t^q\right) u(x, t) = -\delta(x)n(t)$$

Let

$$u(x, t) \leftrightarrow U(x, \omega), \quad n(t) \leftrightarrow N(\omega)$$

and

$$\frac{\partial^q}{\partial t^q} u(x, t) \leftrightarrow U(x, \omega)(i\omega)^q$$

where \leftrightarrow denotes transformation from real to Fourier space. We can then transform our fractional diffusion equation to the form

$$\left(\frac{\partial^2}{\partial x^2} + \Omega_q^2 \right) U(x, \omega) = \delta(x)N(\omega)$$

where we take

$$\Omega_q = i(i\omega\sigma)^{\frac{q}{2}}$$

The Green's function solution to this equation is then given by

$$U(x, \omega) = N(\omega)g(|x|, \omega) \quad (2)$$

where [EBY99]

$$g(|x|, \omega) = \frac{i}{2\Omega_q} \exp(i\Omega_q |x|)$$

under the assumption that u and $\partial u / \partial x \rightarrow 0$ as $x \rightarrow \pm\infty$.

2.2. Asymptotic Solution

From equation (2), it is now clear that

$$\lim_{x \rightarrow 0} U(x, \omega) = \frac{iN(\omega)}{2\Omega_q}$$

or

$$U(\omega) = \frac{1}{2\sigma^{\frac{q}{2}}} \frac{N(\omega)}{(i\omega)^{\frac{q}{2}}}$$

The time series associated with this asymptotic solution is then obtained by Fourier inversion giving (ignoring scaling)

$$u(t) = \frac{1}{t^{1-q/2}} \otimes_t n(t) \quad (3)$$

where \otimes_t defines the convolution integral over time. This equation is the Riemann - Liouville transform (ignoring scaling) which is a fractional integral and defines a function $u(t)$ that is statistically self-affine, i.e. for a scaling parameter $\lambda > 0$,

$$\lambda^{q/2} Pr[u(\lambda t)] = Pr[u(t)]$$

where $Pr[u(t)]$ denotes the Probability Density Function of $u(t)$. Thus, equation (3) can be considered to be the temporal solution of equation (1) as $x \rightarrow 0$ and $u(t)$ is taken to be a random scaling fractal signal. Note that for $|x| > 0$ the phase $\Omega_q |x|$ does not affect the ω^{-q} scaling law of the power spectrum, i.e. $\forall x$,

$$|U(x, \omega)|^2 = \frac{|N(\omega)|^2}{4\sigma^q \omega^q}, \quad \omega > 0$$

Thus for a white noise function $n(t)$ with spectrum $N(\omega)$ the Power Spectrum Density Function of U is determined by $\omega^{-q}, \forall x$ and not just for the case when $x \rightarrow 0$. However, since we can write

$$U(x, \omega) = N(\omega) \frac{i}{2\Omega_q} \exp(i\Omega_q |x|) = N(\omega) \frac{1}{2(i\omega\sigma)^{q/2}}$$

$$\times \left(1 + i(i\omega\sigma)^{q/2} |x| - \frac{1}{2!} (i\omega\sigma)^q |x|^2 + \dots \right)$$

unconditionally, by inverse Fourier transforming, we obtain the following expression for $u(x, t)$ (ignoring scaling factors):

$$u(x, t) = n(t) \otimes_t \frac{1}{t^{1-q/2}} + i |x| n(t)$$

$$+ \sum_{k=1}^{\infty} \frac{i^{k+1}}{(k+1)!} |x|^{2k} \frac{d^{kq/2}}{dt^{kq/2}} n(t)$$

Here, the solution is composed of three terms composed of (i) a fractional integral, (ii) the source term $n(t)$; (iii) an infinite series of fractional differentials of order $kq/2$.

2.3. Fractional Differentials

Fractional differentials of any order need to be considered in terms of the definition for a fractional differential given by

$$\hat{D}^q f(t) = \frac{d^m}{dt^m} [\hat{I}^{m-q} f(t)], \quad m - q > 0$$

where m is an integer and \hat{I} is the fractional integral operator (the Riemann-Liouville transform) given by

$$\hat{I}^p f(t) = \frac{1}{\Gamma(p)} f(t) \otimes_t \frac{1}{t^{1-p}}, \quad p > 0$$

The reason for this is that direct fractional differentiation can yield divergences. However, there is a deeper interpretation of this result which relates to the 'memory' of a system and is based on observing that the evaluation of a fractional differential operator depends on the history of the function in question. Thus, unlike an integer differential operator of order m , a fractional differential operator of order q has 'memory' because the value of $\hat{I}^{m-q} f(t)$ at a time t depends on the behaviour of $f(t)$ from $-\infty$ to t via the convolution of $f(t)$ with $t^{(m-q)-1} / \Gamma(m-q)$. The convolution process is dependent on the history of a function $f(t)$ for a given kernel and thus, in this context, we can consider a fractional derivative defined by \hat{D}^q to have 'memory'. In this sense, the operator $\partial_x^2 - \sigma^q \partial_t^q$ describes a process, compounded in a field $u(x, t)$, that has memory association with regard to the temporal characteristics of the system it is attempting to model. This is not an intrinsic characteristic of systems that are purely diffusive $q = 1$ or propagative $q = 2$.

2.4. Relationship with the Hurst Exponent, Fractal Dimension and Lévy Index

Brownian motion is characterised by a Hurst exponent $H \in [0, 1]$ of 0.5 and random walk processes whose macroscopic behaviour is specified by the diffusion equation for a field u . By induction, the macroscopic behavior of a field generated by Hurst processes is determined by generalizing the diffusion operator to the fractional form $\partial_x^2 - \sigma^q \partial_t^q, q \in (0, 2]$. Fractional diffusive processes can therefore be interpreted as intermediate between classical diffusive (random phase walks with $H = 0.5$; diffusive processes with $q = 1$) and ‘propagative process’ (coherent phase walks for $H = 1$; propagative processes with $q = 2$). The relationship between the Hurst exponent H , the Fourier dimension q and the Fractal dimension D_F is given by [TBA] $D_F = D_T + 1 - H = 1 - (q/2) + (3/2)D_T$ where D_T is the topological dimension. Thus, a Brownian process, where $H = 0.5$, has a fractal dimension of 1.5. The relationship between q and the Lévy index γ (which specifies the characteristic function $\exp(-a |k|^\gamma), \gamma \in (0, 2]$ where a is a constant and k is the spatial frequency) is given by [Bla10] $q/2 = \gamma^{-1}$ so that diffusive processes with $q = 1$ are characterised by a Lévy index of 2 and Gaussian statistics.

2.5. Simulation

We consider the composite signal

$$\begin{bmatrix} u_x(t) \\ u_y(t) \\ u_z(t) \end{bmatrix} = \frac{1}{t^{1-q/2}} \otimes_t \begin{bmatrix} n_x(t) \\ n_y(t) \\ n_z(t) \end{bmatrix} \quad (5)$$

whose amplitude spectrum is given by (ignoring scaling constants)

$$\begin{bmatrix} U_x(\omega) \\ U_y(\omega) \\ U_z(\omega) \end{bmatrix} = \frac{1}{(i\omega)^{q/2}} \begin{bmatrix} N_x(\omega) \\ N_y(\omega) \\ N_z(\omega) \end{bmatrix} \quad (6)$$

The simulation involves the use of a uniform random number generator initiated with three different seeds to generate uncorrelated white noise fields $n_x(t)$, $n_y(t)$ and $n_z(t)$ in equation (5). A Discrete Fourier Transform is then used to transform these fields into Fourier space where upon each field is filtered by $(i\omega)^{-q/2}$ - equation (6). The real component of the inverse Fourier transform is then taken to compute the fields $u_x(t)$, $u_y(t)$ and $u_z(t)$ and a plot of the parametric curve $[u_x(t), u_y(t), u_z(t)]$ generated. Clearly, the output depends on the Fractal Dimension $D_F = 2.5 - q$ where $D_F \in [1, 2], q \in [0.5, 1.5]$. We consider the fractal dimension to increase linearly with time thereby simulating a Hyphae complex whose branching characteristics increase with time. This is taken to be due to the increased number of filamentations that develop as the Hyphae increase in length. Figure 2 provides an example of the three dimensional growth (a plot $[u_x(t), u_y(t), u_z(t)]$) using MATLAB function ‘plot3’ for different values of D_F . A qualitative comparison between ex-

ample simulations of the type given in Figure 2 and experimental images given in Figure 1, for example, points to the idea of using the fractal dimension as an indirect measure of the hyphal growth rate and thereby, biomass productivity. This is the basis for the study given in the following section. The rationale for this study is based on the following Hypothesis: If the replication (and thereby the growth) of bio-organisms is fractionally dynamic, then their structure should be interpreted using fractional or ‘fractal’ geometry.

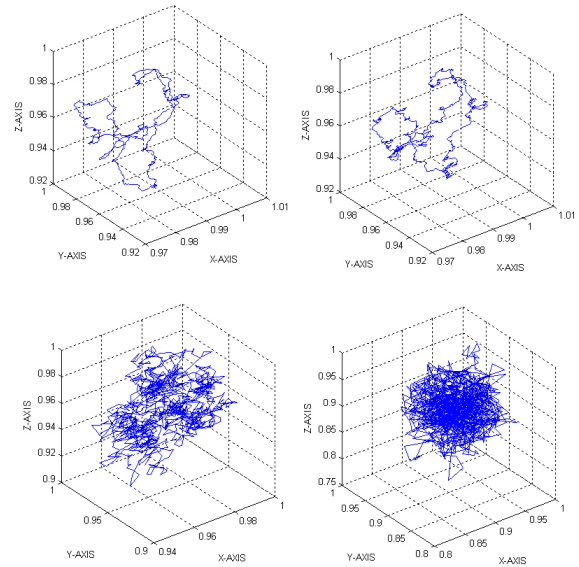


Figure 2: Evolution of three-dimensional Hyphal growth for values of D_F (from left to right and from top to bottom) given by 1.1, 1.3, 1.7 and 1.9.

3. Computation of the Fractal Dimension of Mycelial Structures

Penicillium chrysogenum (IMI 321325) spores were harvested with cultivation conditions and processing of cultures for image analysis as described in [BCW09]. Submerged fermentation of *A. oryzae* was also undertaken where images of submerged culture samples were captured with a Canon PowerShot S50 digital camera attached to a fluorescence microscope (Leitz Laborlux S) fitted with an epifluorescence illuminator (307-148.002 514687, Leitz Wetzlar). Images were captured at 100x magnification.

In all cases, only ‘free’ mycelial elements, exhibiting minimal overlapping Hyphae, were considered for image analysis, so that comparisons could be drawn between the fractal dimension and the hyphal growth unit. The generation of binary images and the enumeration of the hyphal growth unit were as undertaken as described in [BIM*]. The fractal dimension, D_F , of an object function, $f(x, y)$, was deter-

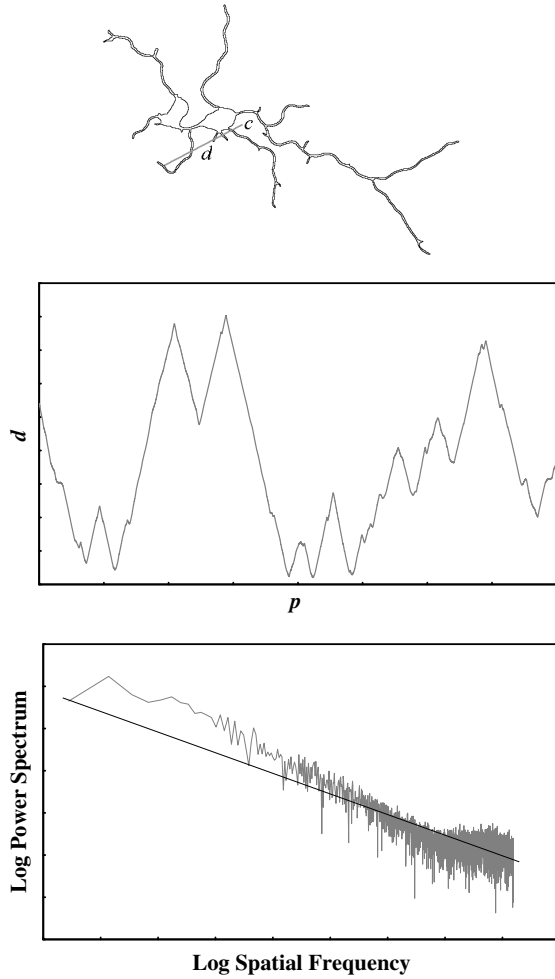


Figure 3: Illustration of the algorithm for determination of the fractal dimension of mycelial structures. Distance, d , between the centroid, c , and the boundary is plotted for each position on the boundary, p , and the fractal dimension derived from a log-log plot of the Fourier domain representation of the signal.

mined by first locating the object boundary in a binary image (all foreground pixels bordering background), which can be thought of as a ‘fractal curve’, consisting of a set of N coordinates, (x_i, y_i) . From this series of points, a digital ‘fractal signal’, u_i , can be constructed given by

$$u_i = \sqrt{(x_i - x_c)^2 + (y_i - y_c)^2} \forall 0 < i < N$$

where (x_c, y_c) is the average location of all $(x, y) \in f(x, y)$. If u_i is a digital fractal signal, then its Power Spectral Density Function will be of the form

$$P(\omega_i) = |U(\omega_i)|^2 = \frac{c}{\omega_i^q}$$

where $U(\omega_i)$ is the discrete Fourier transform of u_i and c is a constant. Thus,

$$\ln[P(\omega_i)] = \ln c - q \ln(\omega_i)$$

where the fractal dimension is given by $D_F = (5 - q)/2$. A value for q and thus D_F can therefore be determined by linear regression of a plot of $\ln(P(\omega_i))$ against $\ln(\omega_i)$ as illustrated in Figure 3. With regard to the results that follow, all numerical algorithms were implemented in Java using ImageJ v1.41o (US National Institutes of Health).

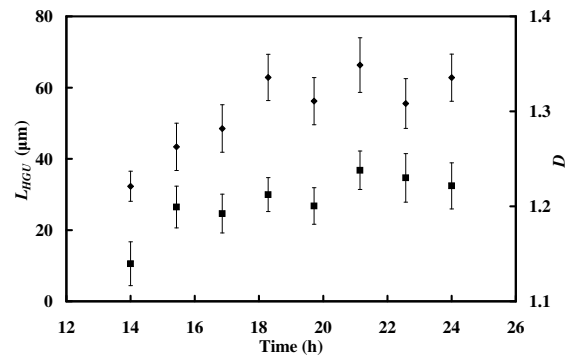


Figure 4: Temporal variation in mean hyphal growth unit (L_{HGU} ; \blacklozenge) and the mean fractal dimension (D_F ; \blacksquare) of populations of *A. oryzae* cultivated on malt agar. Error bars represent 95% confidence intervals.

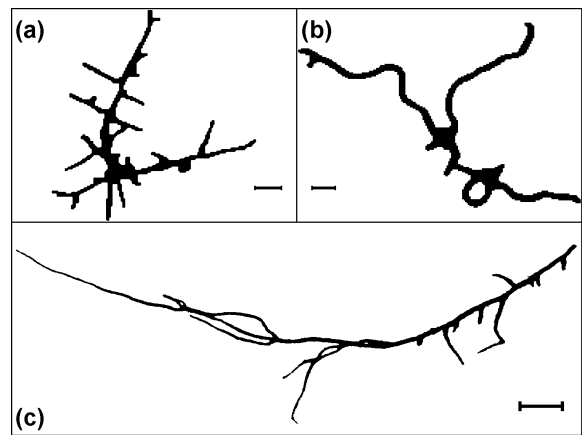


Figure 5: Different morphological forms of filamentous fungi. (a) *Penicillium chrysogenum* 27 hours after inoculation on malt agar; $D_F = 1.087$, $L_{HGU} = 25.8\mu\text{m}$ (bar = $20\mu\text{m}$). (b) *Aspergillus oryzae* 21 hours after inoculation on malt agar; $D_F = 1.243$, $L_{HGU} = 66.5\mu\text{m}$ (bar = $20\mu\text{m}$). (c) *Aspergillus oryzae* 46 hours after inoculation in submerged culture; $D_F = 1.271$, $L_{HGU} = 121.0\mu\text{m}$ (bar = $100\mu\text{m}$).

An analysis of the development of *A. oryzae* on malt agar shows that both D_F and L_{HGU} increased over time and both

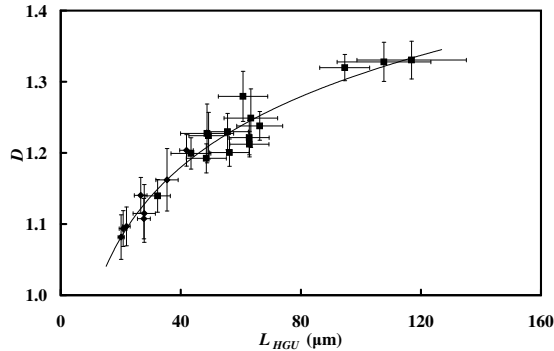


Figure 6: Relationship between the mean hyphal growth unit (L_{HGU}) and the mean fractal dimension (D_F) of populations of *Aspergillus oryzae* (■) and *Penicillium chrysogenum* (◆) mycelia, grown under a variety of different conditions. A logarithmic relationship of the form $D_F = a \ln(L_{HGU}) + b$ exists between the two parameters, where $a = 0.14$ and $b = 0.65$. Error bars represent 95% confidence intervals.

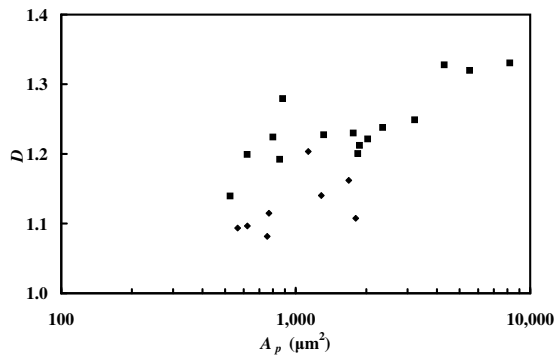


Figure 7: Relationship between mean fractal dimension (D_F) of populations of *Aspergillus oryzae* (■) and *Penicillium chrysogenum* (◆) mycelia and mean projected area (A_p).

tend toward approximately constant values (Fig. 4). This suggests that the value of L_{HGU} specific to *A. oryzae* under these growth conditions is reflected in the fractal dimension of the mycelia. The fractal dimension of *Ashbya gossypii* and *Streptomyces griseus* were also found to increase with time during the colonisation of solid substrates [OPS90]. *A. oryzae* and *P. chrysogenum* were grown under a variety of different conditions, producing mycelia of varying size and dimension (Fig. 5) that are quantified in the same manner. The resultant mean values of D_F obtained for each population were plotted against the mean values of L_{HGU} to yield an approximately logarithmic relationship (Fig. 6) of the form

$$D_F = a \ln(L_{HGU}) + b$$

where a and b are constants. This result demonstrates a strong correlation between the branching behaviour of mycelia and their space-filling properties. However, it has been shown in other studies that fractal dimension tends to increase as projected area of mycelial structures increases [Pap06]. This may also be the case here, as higher values of D_F tend to be biased toward high values of the projected area A_p (Fig. 7) although this result is inconclusive as the sizes of mycelia analysed fall within a relatively small range.

The results obtained demonstrate a clear relationship between the branching behaviour of filamentous organisms and the fractal dimension of the resultant mycelial structures, further emphasising the potential use of fractal analysis in morphological quantification. An ability to extract information on the branching behaviour of an organism by analysing the shape of the mycelial boundary would be highly advantageous in the study of more complex conformations where measures such as the hyphal growth unit are not readily obtainable. Furthermore, as has been demonstrated in other studies (e.g. [Pap06], [PLL*07], [KLK*05], [JLM93]) that fractal analysis can be applied regardless of the gross morphological form that results in a particular process, allowing a more thorough compilation of data. However, a more complete analysis, including more complex structures, is necessary to validate the universal application of fractal analysis.

It has been suggested that the box-counting method of fractal dimension enumeration may not be suitable for the analysis of small, relatively unbranched hyphal structures [Pap06], [OPS90]. This approach entails covering the object with a grid of side length ϵ and counting the number of boxes, $N(\epsilon)$ that are intersected by the object. If the object is fractal then

$$N(\epsilon) = c\epsilon^{-D_F}$$

The accuracy of the box-counting method relies on an object being sufficiently large in size so as to allow a reasonably large variation in ϵ (approximately one order of magnitude has been suggested [OPS90]). Given a value of approximately $4\mu\text{m}$ for ϵ_{min} (hyphal width is approximately $2-4\mu\text{m}$), this suggests a minimum value of approximately $40\mu\text{m}$ for ϵ_{max} in this study, equating to a minimum object ‘diameter’ of $160\mu\text{m}$. However, mycelia smaller than this dimension were often encountered, particularly in the case of *P. chrysogenum*. Further, the number of evaluations of $N(\epsilon)$ is restricted by the image resolution (approximately $1\mu\text{m}$ per pixel in this study).

By enumerating the fractal dimension based on the object boundary, considerations of resolution are obviated to some degree, as the boundary can be represented geometrically as a series of equations, or indeed as a single spline, to be sampled as often as is necessary to provide sufficient signal resolution. However, image resolution is still an important consideration, as low-resolution images may not contain an accurate representation of the object boundary. Consid-

eration must also be given to the means used to locate the boundary. In this study, Hyphae were uniformly stained and object segmentation from background was accurately performed by grey-scale thresholding. In cases where staining is non-uniform, thresholding may not be suitable and some form of edge-detection algorithm may be required.

While numerous studies have been conducted in which fractal analysis is utilised to quantify mycelial morphology, few have attempted to link fractal dimension with conventional morphological parameters. However, links have been established between fractal dimension and productivity in some processes. For example, in the optimisation of *Funalia trogii* fermentations, both fractal dimension and mean pellet area were monitored; while no link was established between the two parameters, it was suggested that a correlation may exist between fractal dimension and decolourisation of reactive black 5 [PLL*07]. A positive correlation was also found between fractal dimension and phenol-oxidase expression by *Pycnoporus cinnabarinus*, with both parameters being regulated by media composition [JL97].

Where links between fractal dimension and conventional Euclidean measures of morphology have been made, the relationship is often either ambiguous or qualitative in nature. An approximate correlation ($R^2 = 0.614$) was found between the convexity (defined as the ratio between convex perimeter and respective perimeter) of *Cupriavidus necator* DSM 545 flocs and the surface fractal dimension [FGL*07]. The fractal dimension was shown to be related to broth rheology in the submerged fermentation of *Cephalosporium acremonium* M25 and a relationship with other morphological measures, such as the number of arthrospores in the media, was also suggested, but not explicitly demonstrated [KLK*05]. A relationship between hyphal growth unit and fractal dimension of mycelia was previously noted in submerged fermentations of *Aspergillus niger*, but the differences in the recorded values of L_{HGU} were ambiguous [Ryo99]. Further studies of *A. niger* revealed that medium composition had a significant impact on the fractal dimension, the changes in morphology reflected in variations in the size and compactness of mycelial aggregates [Pap06]. The local fractal dimension (determined by the concentric circles method) within a colony of *Trichoderma viride* was found to increase with branching frequency (occurrence of 'loops' in the mycelium), although the result was rather qualitative in nature [HGR96]. However, successful attempts have been made in relating fractal dimension to growth kinetics. While colony expansion rates were found to differ between different strains of *Cryphonectria parasitica*, fractal dimension was found to correlate with the expansion rate, independent of strain [GBD08].

4. Conclusion

The optimisation of industrial fermentation processes involving filamentous microbes requires extensive knowledge

of morphological development, as productivity is heavily influenced by the specific phenotypic form adopted by an organism in a given process under specific operational conditions. The accurate quantification of morphological variation in vegetative mycelia is therefore of the utmost importance and the characterisation of complex morphologies represents a significant challenge. The utility of conventional measures employed in the analysis of these microbes (such as projected area, perimeter length and circularity) is limited, as they reveal little about the extent in the branching of the organism, which is known to be related to metabolite production.

The self-affine nature of mycelial structures has been demonstrated in numerous studies and there is clearly significant potential benefit in the application of fractal analysis to filamentous microorganisms. What has been lacking in these studies is a firm link between fractal dimension and conventional morphological parameters, such as the hyphal growth unit. We have considered a model for the morphology of a branching organism that is based on a coordinate separable fractional dynamic model in which increased branching behaviour is characterised by an increasing fractal dimension. This model provides the theoretical background to the work reported in this paper which indicates a strong correlation between the fractal dimension and hyphal growth unit in the analysis of 'free' mycelial elements. Further work is now required that focuses on elucidating a universal relationship between the fractal dimension, branching behaviour and productivity, independent of the gross morphological form encountered in a given process.

References

- [BCW09] BARRY D., CHAN C., WILLIAMS G.: Morphological quantification of filamentous fungal development using membrane immobilization and automatic image analysis. *Journal of Industrial Microbiology and Biotechnology* 36, 6 (2009), 787–800.
- [BD08] BODDY L., DONNELLY D. P.: *Fractal geometry and microorganisms in the environment*. John Wiley and Sons, 2008.
- [BIM*] BARRY D. J., IFEYINWA O. C., MCGEE S. R., RYAN R., WILLIAMS G. A., BLACKLEDGE J. M.: Relating fractal dimension to branching behaviour in filamentous microorganisms. *ISAST Trans. on Electronics and Signal Processing* 4, 1, 71–76.
- [Bla10] BLACKLEDGE J. M.: Systemic risk assessment using a non-stationary fractional dynamic stochastic model for the analysis of economic signals. *ISAST Trans. on Computers and Intelligent Systems* 2, 1 (2010), 76–94.
- [Com96] COMPTE A.: Stochastic foundations of fractional dynamics. *Phys. Rev. E* 53, 4 (1996), 4191–4193.
- [CSNV96] CARLSEN M., SPOHR A. B., NIELSEN J., VILLADSEN J.: Morphology and physiology of an alpha-amylase producing strain of *Aspergillus oryzae* during batch cultivations. *Biotechnol Bioeng* 49, 3 (1996), 266–76.
- [EBY99] EVANS G. A., BLACKLEDGE J. M., YARDLEY P.: *Analytical Solutions to Partial Differential Equations*. Springer, 1999.

- [Eis48] EISENHART L. P.: Enumeration of potentials for which one-particle schrodinger equations are separable. *Phys. Rev.* 74 (1948), 87–89.
- [ESMH06] EL-SABBAGH N., MCNEIL B., HARVEY L. M.: Dissolved carbon dioxide effects on growth, nutrient consumption, penicillin synthesis and morphology in batch cultures of *Penicillium chrysogenum*. *Enzyme and Microbial Technology* 39, 2 (2006), 185–190.
- [FGL*07] FINKLER L., GINORIS Y., LUNA C., ALVES T., PINTO J., COELHO M.: Morphological characterization of *Cupriavidus necator* DSM 545 flocs through image analysis. *World Journal of Microbiology and Biotechnology* 23, 6 (2007), 801–808.
- [GAJ*00] GORDON C. L., ARCHER D. B., JEENES D. J., DOONAN J. H., WELLS B., TRINCI A. P., ROBSON G. D.: A glucoamylase::gfp gene fusion to study protein secretion by individual hyphae of *Aspergillus niger*. *J Microbiol Methods* 42, 1 (2000), 39–48.
- [GBD08] GOLINSKI M. R., BOECKLEN W. J., DAWE A. L.: Two-dimensional fractal growth properties of the filamentous fungus *Cryphonectria parasitica*: the effects of hypovirus infection. *Journal of Basic Microbiology* 48, 5 (2008), 426–429.
- [HGR96] HITCHCOCK D., GLASBEY C. A., RITZ K.: Image analysis of space-filling by networks: Application to a fungal mycelium. *Biotechnology Techniques* 10, 3 (1996), 205–210.
- [Hil95] HILFER R. R.: Foundations of fractional dynamics. *Fractals* 3, 3 (1995), 549–556.
- [JL97] JONES C. L., LONERGAN G. T.: Prediction of phenoloxidase expression in a fungus using the fractal dimension. *Biotechnology Letters* 19, 1 (1997), 65–69.
- [JLM93] JONES C. L., LONERGAN G. T., MAINWARING D. E.: Mycelial fragment size distribution: an analysis based on fractal geometry. *Applied Microbiology and Biotechnology* 39, 2 (1993), 242–249.
- [KJK*05] KIM J. C., LIM J. S., KIM J. M., KIM C., KIM S. W.: Relationship between morphology and viscosity of the main culture broth of *Cephalosporium acremonium* M25. *Korea-Australia Rheology Journal* 17, 1 (2005), 15–20.
- [LSW*02] LI Z. J., SHUKLA V., WENGER K. S., FORDYCE A. P., PEDERSEN A. G., MARTEN M. R.: Effects of increased impeller power in a production-scale *Aspergillus oryzae* fermentation. *Biotechnol Prog* 18, 3 (2002), 437–44.
- [Man82] MANDELBROT B. B.: *The fractal geometry of nature*. W.H. Freeman, 1982.
- [MM92] MATSUYAMA T., MATSUSHITA M.: Self-similar colony morphogenesis by gram-negative rods as the experimental model of fractal growth by a cell population. *Appl. Environ. Microbiol.* 58, 4 (1992), 1227–1232.
- [MMHN02] MULLER C., MCINTYRE M., HANSEN K., NIELSEN J.: Metabolic engineering of the morphology of *Aspergillus oryzae* by altering chitin synthesis. *Appl Environ Microbiol* 68, 4 (2002), 1827–36.
- [OPS90] OBERT M., PFEIFER P., SERNETZ M.: Microbial growth patterns described by fractal geometry. *J Bacteriol* 172, 3 (1990), 1180–5.
- [Pap04] PAPAGIANNI M.: Fungal morphology and metabolite production in submerged mycelial processes. *Biotechnol Adv* 22, 3 (2004), 189–259.
- [Pap06] PAPAGIANNI M.: Quantification of the fractal nature of mycelial aggregation in *Aspergillus niger* submerged cultures. *Microb Cell Fact* 5, 1 (2006).
- [PLL*07] PARK C., LIM J.-S., LEE Y., LEE B., KIM S.-W., LEE J., KIM S.: Optimization and morphology for decolorization of reactive black 5 by *Funalia trogii*. *Enzyme and Microbial Technology* 40, 7 (2007), 1758–1764.
- [Plo59] PLOMLEY N. J. B.: Formation of the colony in the fungus *Chaetomium*. *Aust J Biol Sci* 12 (1959), 53–64.
- [PM] PAPAGIANNI M., MATTEY M.: Morphological development of a.
- [Ryo99] RYOO D.: Fungal fractal morphology of pellet formation in *Aspergillus niger*. *Biotechnology Techniques* 13, 1 (1999), 33–36.
- [TBA] TURNER M. A., BLACKLEDGE J. M., ANDREWS P.: *Fractal Geometry in Digital Imaging*. Academic Press.
- [TKDT92] TUCKER K. G., KELLY T., DELGRAZIA P., THOMAS C. R.: Fully-automatic measurement of mycelial morphology by image analysis. *Biotechnol. Prog.* 8, 4 (1992), 353–359.
- [ZP01] ZNIDARSIĆ P., PAVKO A.: The morphology of filamentous fungi in submerged cultivations as a bioprocess parameter. *Food technol. biotechnol.* 39, 3 (2001), 237–252.