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Inferring mixed-culture growth from total biomass data in a wavelet approach

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Abstract

It is shown that the presence of mixed-culture growth in batch fermentation processes can be very accurately inferred from total biomass data by means of the wavelet analysis for singularity detection. This is accomplished by considering simple phenomenological models for the mixed growth and the more complicated case of mixed growth on a mixture of substrates. The main quantity provided by the wavelet analysis is the Hölder exponent of the singularity that we determine for our illustrative examples. The numerical results point to the possibility that Hölder exponents can be used to characterize the nature of the mixed-culture growth in batch fermentation processes with potential industrial applications. Moreover, the analysis of the same data affected by the common additive Gaussian noise still lead to the wavelet detection of the singularities although the Hölder exponent is no longer a useful parameter.

Key words: bioreactor, mixed-cultures, total biomass, wavelets

1 Introduction

The growth of microbial species in media containing two or several growth-limiting substrates is of great importance in biotechnology and bioengineering.

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The mixed-culture growth occurs in many industrial processes. A first significant class of such processes is the traditional fermented foods and beverages in which either endemic microorganisms or an inoculum with selected microorganisms are used, see for instance (1). Some beverages get two or more different microorganisms in the inoculum with the purpose to provide a desired flavor. Evidence of this influence are presented in the recent paper of (2), in which the role of different yeast interactions on the wine flavor is discussed. However, the phenomenological details and the theory of the time evolution of the fermentation are as yet poorly understood. We can also mention the interesting case of the bioethanol production, in which the substrates used for fermentation typically consist of a mixture of glucose and fructose. Bioethanol is the product obtained from the metabolism of microbe mixtures feeding with this combination of hexoses and pentoses, see e.g., (3). The last relevant example we give is bioremediation, in which gasoline and chemical spills generally yield a complex mixture of water-soluble organic compounds. In gasoline spills, for instance, the four basic compounds are benzene, toluene, ethylbenzene, and xylene. The consumption of this mixture by microorganisms is what is defined as the bioremediation process.

In all the aforementioned cases, the presence of different populations of microorganisms and substrates is a key factor in the quality and quantity of the final product. Therefore, it is quite useful to detect the presence or lacking of process of mixed-culture growth type. Their presence could be used as an estimate of the right evolution of the process in its early stage. In addition, a rapid and reasonably accurate test is always useful for saving time and helping to take quick decisions. It is quite clear then that the biomass concentration is one of the most needed quantity that should be measured in fermentation monitoring. The most popular method to get the biomass concentration is by means of the measurement of the optical density of centrifugalized samples. However, this procedure has limited usefulness because it cannot distinguish neither the living cells from the dead ones, nor the different types of microorganisms involved in the process. In some cases it is also possible to correlate the total biomass concentration with the values of the redox potential of the fermentation.

Recently, new techniques have emerged to quantify the biomass and distinguish the different microorganisms present in a mixed-culture. Some of then based on sophisticated equipment ((5), (6) and (7)) and others resides on molecular biology techniques ((3) and (4)). All these techniques are very promising in the study of the dynamics of the mixed-culture growth, although, they require expensive or complicated procedures. In this paper, we show that it is possible to infer mixed-culture growth of microorganisms from their total biomass data, without using such complicated techniques. The alternative procedure that we put forth here is based on treating the total biomass data by means of the wavelet approach for detection of singularities in the growth

curves. The idea is to treat the mixed growth curves as more or less regular signals that can nevertheless display singularities due to their compound structure. In the wavelet literature there exist fundamental papers in which it has been shown that the wavelet techniques are very efficient in detecting any type of singularities.

The rest of the paper is organized as follows. In Section 2, we introduce a simple dynamics of the mixed-growth type and discuss its basic assumptions. Next, in Section 3, the method of the wavelet singularity analysis is briefly presented, whereas its application to the mixed type dynamical curves is enclosed in Section 4. A conclusion section ends up the paper. An appendix containing the standard definitions of Hölder exponents of singularities of functions is included as well.

2 A simple mixed-growth model

The technology of batch processes is well developed and numerous products are obtained in this way. Some products such as food, beverages, and pharmaceutical ones require precise tracking of the batch information for safety and regulatory purposes. The primary objective of monitoring batch processes is to ensure that significant and sustained changes in the quality of the product (caused by disturbances and/or faults) are detected as soon as possible. In that sense, the rapid detection of singularities in the output of the batch processes offers an interesting solution. The wavelet analysis for singularity detection is by now well established but there was no direct application to infer mixed-growth in the case of batch biochemical processes.

In order to achieve this task we will consider here a fermentation process consisting of a perfectly stirred tank, where no streams are fed into it. In the batch fermenter the substrate is converted by biomass into additional biomass and products. The general unstructured mass balances for the well-mixed bioreactor can be represented by the following equations for the concentrations of the cells and substrates:

$$\begin{aligned}\frac{\mathrm{d}x_{1,i}}{\mathrm{d}t} &= x_{1,i}\ \mu_i\left(x_{2,i}\right)\\ \frac{\mathrm{d}x_{2,i}}{\mathrm{d}t} &= -\frac{x_{1,i}}{Y_i}\ \mu_i\left(x_{2,i}\right)\end{aligned}$$

where $x_{1,i}$ represent the biomass concentrations, $x_{2,i}$ substrate concentrations and Y_i is the biomass yield, $\mu_i\left(x_{2,i}\right)$ is the specific growth rate and $i \in \mathbb{R}$ represent the i-th species, allowing for the possibility of multiple kinds of substrates and microorganisms. The growth rate relates the change in biomass

concentrations to the substrate concentrations. Two types of relationships for $\mu_i\left(x_{2,i}\right)$ are commonly used: the substrate saturation model (Monod Equation) and the substrate inhibition model (Haldane Equation). Both cases will be treated here. The substrate inhibited growth can be described by

$$\mu_i\left(x_{2,i}\right) \;=\; \frac{\mu_{max_i}\; x_{2,i}}{K_{1_i} + x_{2,i} + K_{2_i} x_{2,i}^2}$$

where K_{1_i} is the saturation (or Monod) constant, K_2 is the inhibition constant and μ_{max_i} is the maximum specific growth rate. The value of K_{1_i} expresses the affinity of biomass for substrate. The Monod growth kinetics can be considered as a special case of the substrate inhibition kinetics with $K_{2_i} = 0$ when the inhibition term vanishes. For the sake of simplicity, we will consider only two species and two substrates. Moreover, we consider that it is possible to measure only the total biomass concentration. That means that the output of the system (y) will be given by

$$y = \sum_{i=1}^{m} x_{1,i}$$

where m is the number of species of microorganisms growing in the bioreactor (in this work $m = 2$). We focus on the following four cases:

I The microorganism and substrate concentrations have the same initial conditions, but different growth rates, one with a Haldane type and one with a Monod type. In addition, quite different values of the Monod constant will be taken into account.

II The microorganism and substrate concentrations have different initial conditions, but the same growth rates.

III The microorganism and substrate concentrations have different initial conditions and different growth rates, one with a Haldane type and one with a Monod type.

IV The microorganism and substrate concentrations have the same initial conditions and the same growth rates, but with different values of the maximal growth rate.

Table 1 shows the variables and parameter values used to simulated the two species growing in the two different substrates, under the four cases under consideration.

Table 1
The initial conditions and the values of the employed parameters of the mixed-growth process model.

Symbol	Meaning	Values				Units
		Case I	Case II	Case III	Case IV	
μ_{max_1}	Maximal growth rate	1	1	1	0.9	$[l/h]$
K_{1_1}	Saturation parameter	0.03	0.03	0.03	0.03	$[g/l]$
K_{2_1}	Saturation parameter	0.5	0.5	0.02	0.5	$[g/l]$
Y_1	Yield coefficient	0.5	0.5	0.5	0.5	–
$x^0_{1_1}$	Initial biomass conc.	0.1	0.1	0.1	0.25	$[g/l]$
$x^0_{2_1}$	Initial substrate conc.	10	10	10	10	$[g/l]$
μ_{max_2}	Maximal growth rate	1	1	1	1	$[l/h]$
K_{1_2}	Saturation parameter	0.3	0.03	0.03	0.03	$[g/l]$
K_{2_2}	Inhibition parameter	0	0.5	0	0.5	$[l/g]$
Y_2	Yield coefficient	0.5	0.5	0.5	0.5	–
$x^0_{1_2}$	Initial biomass conc.	0.1	0.2	0.1	0.25	$[g/l]$
$x^0_{2_2}$	Initial substrate conc.	10	5	6	10	$[g/l]$

3 Mixed cultures on mixtures of substrates

When microbes are grown in a batch reactor containing a surplus of two substrates, one of the substrates is generally exhausted before the other, leading to the appearance of two successive exponential growth phases. This phenomenon could be noticeable at simple view in the biomass signal or unnoticed due to its nature or due to additive noise present in the signal. In general, such type of phenomenon is known as growth of mixed cultures on mixtures of substrates (MCMS).

The growth of MCMS is a phenomenon of practical and theoretical interest. The fundamental understanding of this problem has impact on many practical fields such as food processing, production of ethanol from renewable resources, bioremediation and microbial ecology, among many others.

To study the usage of the wavelet approach in the detection of MCMS growth, we consider the recent model proposed by Reeves 2004 (8), which takes into account such type of growth.

Within this section, the index i will denote the species number, and the index j will stand for the substrate number. Thus, c_i denotes the concentration of

the ith species, s_j denotes the concentration of the jth substrate, e_{ij} denotes the concentration of the lumped system of inducible enzymes catalyzing the uptake and peripheral catabolism of s_j by c_i. Here, c_i and s_j are based on the volume of the chemostat, and expressed in the units gdw/l and g/l, respectively. e_{ij} is based on the dry weight of the biomass, and expressed in the units g/gdw.

$$
\begin{aligned}
r_{ij}^{s} &= V_{ij}^{s}\, e_{ij}\, \frac{s_j}{K_{ij}^{s} + s_j} \\
r_{ij}^{x} &= k_{ij}^{x}\, x_{ij} \\
r_{ij}^{e} &= V_{ij}^{e}\, \frac{x_{ij}}{K_{ij}^{e} + x_{ij}} \\
r_{ij}^{ast} &= k_{ij}^{ast} \\
r_{ij}^{d} &= K_{ij}^{d}\, e_{ij}
\end{aligned}
$$

$$
\frac{d\, s_j}{d\, t} = D\left(s_j^{f} - s_j\right) - r_{1j}^{s}\, c_1 - r_{2j}^{s}\, c_2 \tag{1}
$$

$$
\frac{d\, e_{ij}}{d\, t} = V_{ij}^{e}\, \frac{e_{ij}\, \sigma_{ij}}{\bar{K}_{ij}^{e} + e_{ij}\, \sigma_{ij}} + k_{ij}^{ast} - k_{ij}^{d}\, e_{ij} - r_i^{g}\, e_{ij} \tag{2}
$$

$$
\frac{d\, c_i}{d\, t} = (r_i^{g} - D)\, c_i \tag{3}
$$

where

$$
\begin{aligned}
\bar{K}_{ij}^{e} &= \frac{K_{ij}^{e}\, k_{ij}^{x}}{V_{ij}^{s}}, \quad \sigma_{ij} = \frac{s_j}{K_{ij}^{s} + s_j} \\
r_i^{g} &= Y_{i1}\, r_{i1}^{s} + Y_{i2}\, r_{i2}^{s}
\end{aligned}
$$

Reeves et al (8) comment that a plausible experimental situation is the case of *Escherichia coli* and *Pseudomonas aeruginosa*, in which, *E. coli* prefers a sugar over an organic acid, and *P. aeruginosa* prefers the organic acid over the sugar.

In order to have the batch regime in the bioreactor we set parameter $D = 0$ and also employ Reeves' parameters $s_1^{f} = 1$ and $s_2^{f} = 2$. Table 2 shows the rest of the parameter values used to simulate the growth of the two species on the two different substrates in the MCMS conditions.

Table 2
Parameter values used in the MCMS growth model (8)

$V_{11}^s = 1000$	$V_{12}^s = 1000$	$V_{21}^s = 1000$	$V_{22}^s = 1000$	g/g h
$K_{11}^s = 0.01$	$K_{12}^s = 0.01$	$K_{21}^s = 0.01$	$K_{22}^s = 0.01$	g/l
$V_{11}^e = 0.0025$	$V_{12}^e = 0.0020$	$V_{21}^e = 0.0006$	$V_{22}^e = 0.0036$	g/gdw h
$\bar{K}_{11}^e = 0.0017$	$\bar{K}_{12}^e = 0.0032$	$\bar{K}_{21}^e = 0.0013$	$\bar{K}_{22}^e = 0.0030$	g/gdw
$k_{11}^d = 0.01$	$k_{12}^d = 0.01$	$k_{21}^d = 0.01$	$k_{22}^d = 0.01$	l/h
$k_{11}^* = 10^{-2}\, V_{11}$	$k_{12}^* = 10^{-2}\, V_{12}$	$k_{21}^* = 10^{-2}\, V_{21}$	$k_{22}^* = 10^{-2}\, V_{22}$	g/gdw h
$Y_{11} = 0.41$	$Y_{12} = 0.24$	$Y_{21} = 0.35$	$Y_{22} = 0.20$	g/g

4 Measuring regularity with the wavelet transform

Let us think of the total biomass of the mixed-growth curves as a signal. In general, performing the analysis of a signal means to find the regions of its regular and singular behavior. Usually the singularities are very specific features for signal characterization. As it has been pointed in the seminal paper of (14), the regularity of a signal treated as a function can be characterized by Hölder exponents. The wavelet transform has been demonstrated to be a tool exceptionally well suited for the estimation of Hölder exponents (for their definitions see the Appendix).

4.1 The wavelet transform

Let $L^2(\mathbb{R})$ denote the space of all square integrable functions on $\mathbb{R}$. In signal processing terminology, $L^2(\mathbb{R})$ is the space of functions with finite energy. Let $\psi(t) \in L^2(\mathbb{R})$ be a fixed function. The function $\psi(t)$ is said to be a wavelet if and only if its Fourier transform, $\hat{\psi}(\omega) = \int e^{i\omega t}\psi(t)dt$, satisfies

$$C_\psi = \int_0^\infty \frac{|\hat{\psi}(\omega)|^2}{|\omega|} d\omega < \infty. \tag{4}$$

The non-divergent relation given by Eq. (4) is called the *admissibility condition* in wavelet theory, see for instance (12) and (14). It implies that the wavelet must have a zero average on the real line

$$\int_{-\infty}^{\infty} \psi(t)dt = \hat{\psi}(0) = 0, \tag{5}$$

and therefore it must be oscillatory. In other words, ψ must be a sort of *wave* ((12; 14)). Based on $\psi(t)$, one defines the functions $\psi_{a,b}$ as follows

$$\psi_{a,b}(t) = \frac{1}{\sqrt{a}}\psi\left(\frac{t-b}{a}\right), \tag{6}$$

where $b \in \mathbb{R}$ is a translation parameter, while $a \in \mathbb{R}^+$ ($a \neq 0$) is a dilation or scale parameter. The factor $a^{-1/2}$ is a normalization constant such that $\psi_{a,b}$ has the same energy for all scales a. One notices that the scale parameter a in Eq. (6) is a measures of the dilations of the spatial variable $(t-b)$. In the same way the factor $a^{-1/2}$ measures the dilations of the values taken by ψ. Because of this, one can decompose a square integrable function $f(t)$ in terms of the dilated-translated wavelets $\psi_{a,b}(t)$. We define the wavelet transform (WT) of $f(t) \in L^2(\mathbb{R})$ by

$$\begin{aligned} W_f(a,b) &= \langle f, \psi_{a,b}\rangle = \int_{-\infty}^{\infty} f(t)\bar{\psi}_{a,b}(t)dt \\ &= \frac{1}{\sqrt{a}}\int_{-\infty}^{\infty} f(t)\bar{\psi}\left(\frac{t-b}{a}\right)dt, \end{aligned} \tag{7}$$

where $\langle\ ,\ \rangle$ is the scalar product in $L^2(\mathbb{R})$ defined as $\langle f, g\rangle := \int f(t)\bar{g}(t)dt$, and the bar symbol denotes complex conjugation. The WT given by Eq. (7) measures the variation of f in a neighborhood of size proportional to a centered on point b. In order, to reconstruct f from its wavelet transform (7), one needs a reconstruction formula, known as the resolution of the identity ((12; 14)).

$$f(t) = \frac{1}{C_\psi}\int_0^{\infty}\int_{-\infty}^{\infty} W_f(a,b)\psi_{a,b}(t)\frac{da\ db}{a^2}. \tag{8}$$

From the above equation we can see why the condition given by Eq. 4 should be imposed. One fundamental property that we require in order to analyze singular behavior is that $\psi(t)$ has enough vanishing moments as argued in the works of (9) and (13). A wavelet is said to have n vanishing moments if and only if it satisfies

$$\int_{-\infty}^{\infty} t^k\psi(t)dx\ =\ 0,\ \text{for } k = 0, 1, \ldots, n-1 \tag{9}$$

and

$$\int_{-\infty}^{\infty} t^k \psi(t) dt \neq 0, \text{ for } k \geq n. \tag{10}$$

This means that a wavelet with n vanishing moments is orthogonal to polynomials up to order $n-1$. In fact, the admissibility condition given by Eq. (4) requires at least one vanishing moment. So the wavelet transform of $f(t)$ with a wavelet $\psi(t)$ with n vanishing moments is nothing but a "smoothed version" of the n–th derivative of $f(t)$ on various scales. In fact, when someone is interested to measure the local regularity of a signal this concept is crucial (see for instance (12; 14)).

4.2 Wavelet singularity analysis

The local regularity of a function f at a point t_0 is often measured by its Hölder exponent. The Hölder exponent α measures the strength of a singularity at a particular point t_0, where t_0 belongs to the domain of f, see the Appendix. It is important to point out that if the singular part of a function f in the neighborhood of t_0 is of the type $|t-t_0|^\alpha$, then it corresponds to a *cusp* and in this case the singular behavior is fully characterized by its Hölder exponent. However, there exists functions that involve oscillating singularities which have to be described by an additional quantity: an oscillating exponent ((10; 11)). In such a case, the oscillation has to be analyzed carefully. Such functions can not be fully characterized only by the Hölder exponent. In this work, we will only consider functions whose singularities are not oscillating.

One classical tool to measure the regularity of a function $f(t)$ is to look at the asymptotic decay of its Fourier transform $\hat{f}(\omega)$ at infinity. However, the Fourier transform is not well adapted to measure the local regularity of functions, because it is global and provides a description of the overall regularity of functions ((13; 14)). Consequently, we need another way to characterize local signal regularity.

In the works (9; 12; 13; 14) it is shown that the WT provides a way of doing a precise analysis of the regularity properties of functions. This is made possible by the scale parameter. Due to its ability to focus on singularities in the signals, the WT is sometimes referred to as 'mathematical microscope' ((9; 12; 13; 14)), where the wavelet used determines the optics of the microscope and its magnification is given by the scale factor a.

The WT modulus maxima (WTMM) decomposition introduced by (13) provides a local analysis of the singular behavior of signals. In the works of Mallat (13; 14) it has been shown that for cusp singularities the location of the sin-

gularity can be detected and the related exponent can be recovered from the scaling of the WT along the so-called *maxima line* (WTMML for short), which is convergent towards the singularity. This is a line where the WT reaches local maximum with respect to the position coordinate. Connecting such local maxima within the continuous WT 'landscape' gives rise to the entire tree of maxima lines. Restricting oneself to the collection of such maxima lines provides a particularly useful representation of the entire WT. It incorporates the main characteristics of the WT: the ability to reveal the *hierarchy* of (singular) features, including the scaling behavior.

An other key concept, in addition to vanishing moments, used to characterize the regularity of a function in terms of WTMM is given next. Suppose that ψ has compact support $[-C, C]$. The *cone of influence* of ψ at point t_0 is the set of points (a, b) in the scale-space plane or domain, such that t_0 is in the support of $\psi_{a,b}(t)$. We will denote the scale–space plane or domain of the WT as the (a, b)-plane or the (a, b)-domain. Since the support of $\psi((t-b)/a)$ is $[b - Ca, b + Ca]$, the point (a, b) belongs to the cone of influence of t_0 if

$$|b - t_0| \leq Ca. \tag{11}$$

The function $f(t)$ has a Hölder exponent $\alpha \in (k, k+1)$ at t_0, if and only if there exists a constant $A > 0$ such that at each modulus maxima (a, b) in the cone defined by Eq. (11) one has

$$|W_f(a, b)| \leq Aa^{\alpha+1/2}, \quad a \to 0, \tag{12}$$

(see (13; 14)). Here it is assumed that the wavelet has at least $n > \alpha$ vanishing moments. If $f(t)$ is regular at t_0 or, if the number of vanishing moments is too small, i.e., $n < \alpha$, one obtains for $a \to 0$ a scaling behavior of the type

$$|W_f(a, b)| \leq Aa^{n+1/2}. \tag{13}$$

The scaling behavior of the WTMML is given in Eq. (12) and can be rewritten as follows

$$\log |W_f(a, b)| \leq \log A + \left(\alpha + \frac{1}{2}\right) \log a. \tag{14}$$

The global Hölder regularity at t_0 is thus the maximum slope $-\frac{1}{2}$ of $\log |W_f(a, b)|$ as a function of $\log a$ along the maxima line converging to t_0.

5 Results and discussion

In this section, we present the results we obtained using the singularity detection procedure described in the previous section. The signal to be analyzed, $f(t) = y$, represents the evolution in time of the total biomass concentration for the fermentation process described in Section 2 that includes four different cases as specified therein. In all the wavelet-related calculations we employed as mother wavelet the first derivative Gaussian $\psi'(t) = d/dt(e^{-t^2/2})$ having only one vanishing moment. The final goal is always to calculate the Hölder exponent of the singularities for such processes because it is a direct measure of the irregularity of a signal (function) at the singular point t_0, in the sense that higher values of it correspond to more regular functions than the lower values.

Figure 1 *a,b,c* shows the performance of the wavelet singularity analysis as applied to Case I (same initial conditions but different kinetic rates). We obtain a Hölder exponent of quite high value.

The following figure shows the performance of the scheme applied to Case II (same growth rates but different initial conditions). In this case, the Hölder exponent of the mixed growth singularity is lower than in Case I.

Similarly to the previous cases, Fig. (3) presents the graphical results for Case III (different initial conditions and different growth rates). Although, the singularity looks very mild in the time evolution of the total biomass concentration the wavelet analysis is able to detect it with high precision.

Finally, Case IV (same initial conditions, same growth rates but with different values of their maximal growth rates) is graphically analyzed in Fig. (4). For this case we obtained the lowest Hölder exponent.

Although the latter two cases seem to correspond to almost overlapping of the WTMML pointing to bifurcation phenomena we are still not at the threshold of a completely different behavior of the log plots generated by bifurcations. This could be explained by the fact that the strength of the first singularity is bigger with respect to the second one.

5.1 Wavelet analysis for the MCMS case

The MCMS case is the most interesting case that we discuss here because we will show that it is possible to infer in a very accurate manner by means of WT the moment in which the microorganisms switch their carbon source. In order to understand the detailed dynamics of this combined growth, we first

apply separately the WT approach to the two biomass signals $y = c_1$ (Fig. 5) and $y = c_2$ (Fig. 6) and then to the total signal $y = c_1 + c_2$ (Fig. 7).

It is worth noting that the Hölder exponent is bigger than one, a quite interesting feature which means that the singularity lies in the second derivative of the biomass signal. This result gives further opportunities to characterize the nature of the singularity because it suggests that the type of growth can be inferred from the order of the derivative in which the singularity occurs directly given by the value of the Hölder exponent. The latter fact is a great simplification with respect to the analytical search of the singularities which implies obtaining the analytical solution of the given dynamical growth model. Moreover, even in such fortunate cases, the analytical solutions could be subject to fixed parameter values of the model. On the other hand, the WT numerical approach allows the singularity analysis even in the case of time-varying parameters.

5.2 Wavelet analysis for the noisy data case

It is well known that in some cases the amplitude of the Gaussian noise affecting the on-line signals can be an important annoying factor. Therefore, a good analysis should be robust in such cases. Thus, we provide here the WT analysis for the MCMS biomass signals in the presence of white noise, that is, in the next figures (8-10) we consider signals of the form $y_i = c_i + \epsilon(t)$ and their sum, where $\epsilon(t)$ stands for the functional form of the noise.

We notice from the corresponding plots that the noisy data do not allow to obtain global Hölder exponents in a straightforward manner, which is a result already reported in the wavelet literature ((14) and (15)). On the other hand, the singularity detection is robust with respect to the noise for reasonable levels of its amplitude. In addition, Figure 9 gives us the hint that when the cones of influence produced by the Gaussian noise enter the scales of the singularity the cone of the latter becomes undistinguishable from those of the noise. This remark could be used as a sort of resolution criterium of the WT method in the presence of noise. Therefore, one can determine a critical amplitude of the noise for which the WT approach looses its applicability.

6 Concluding remarks

We showed here explicitly how the wavelet singularity analysis can be applied to infer mixed growth behavior of fermentation processes using only total biomass data. We prove that the wavelet analysis is very accurate for all the

cases we considered. A very interesting feature of our research is that the Hölder exponent is sensitive to the type of the mixed-growth phenomenon, more specifically depends on the parameters of the growth processes and on their initial conditions. The MCMS case points to the remarkable technological possibility of detecting the change of the substrate uptake since the singularity appears in the second derivatives of the biomass signal. This can lead one to think of the possibility to infer substrate contaminations based only in the analysis of the biomass data. In addition, our results for the noisy data clearly hint to the fact that the wavelet singularity analysis maintains its attractive features even in these more difficult but realistic case. We hope that in future works we could find out the mathematical relationships implied by this possible correlation. It might allow the usage of the Hölder exponent as an identification criterium of the more specific nature of mixed-growth processes.

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Appendix

A function $f : \mathbb{R} \to \mathbb{R}$ is said to be Hölder continuous of exponent α ($0 < \alpha < 1$) if, for each bounded interval $(c, d) \subset \mathbb{R}$, we can find a positive constant K such that

$$|f(t) - f(t_0)| \leq K|t - t_0|^\alpha \tag{15}$$

for all $t, t_0 \in (c, d)$.

The space of Hölder continuous functions is denoted C^α. A function is said to be $C^{n+\alpha}$ if it is in C^n and its nth derivative is Hölder continuous with exponent α. Thus, if we consider the Hölder exponent $n < \alpha < n + 1$, with $n \in \mathbb{N}$, the function can be differentiated n times, but the $(n+1)$th derivative does not exist. Therefore, a function with a Hölder exponent $n < \alpha < n + 1$ is said to be singular in the nth derivative. Keeping this in mind, let us give the following definition of the Hölder regularity of a function (12; 13; 14).

- Let $n \in \mathbb{N}$ and $n \leq \alpha < n + 1$. A function $f(t)$ has a *local* Hölder exponent α at t_0 if and only if there exist a constant $K > 0$, and a polynomial $P_n(t)$ of order n, such that

$$\forall t \in \mathbb{R}, \qquad |f(t) - P_n(t - t_0)| \leq K|t - t_0|^\alpha \tag{16}$$

- The function $f(t)$ has a *global* Hölder exponent α on the interval (c, d) if and only if there is a constant K and a polynomial of order n, $P_n(t)$, such that equation (16) is satisfied for all $t \in (c, d)$.
- The Hölder *regularity* of $f(t)$ at t_0 is the supremum of the α such that $f(t)$ is Hölder α at t_0.
- The nth derivative of a function $f(t)$ is *singular* at t_0 if $f(t)$ has a local Hölder exponent α at t_0 with $n < \alpha < n + 1$.

A function $f(t)$ that is continuously differentiable at a given point has a Hölder exponent not less than 1 at this point. If $\alpha \in (n, n + 1)$ in (16) then $f(t)$ is n times but not $(n + 1)$ times differentiable at the point t_0, and the polynomial $P_n(t)$ corresponds to the first $(n + 1)$ terms of the Taylor series of $f(t)$ around $t = t_0$. For example, if $n = 0$, we have $P_0(t - t_0) = f(t_0)$.

Figure Captions

Fig. 1

a) The time evolution of the total biomass concentration signal for Case I.
b) The wavelet cones of influence corresponding to this case showing a very accurate identification of the two singularity points presented in the signal, of which the first one allows to infer the presence of the mixed growth feature of the fermentation process whereas the second one is associated with the end of the fermentation batch cycle.
c) From the slope in the double logarithmic plot, the Hölder coefficient of the mixed growth singularity is calculated as $\alpha = 0.95$.

Fig. 2

a) The time evolution of the total biomass concentration signal for Case II.
b) The wavelet cones of influence corresponding to this case again showing the accurate identification of the two singularity points, of the same type, respectively, as in Fig. 1.
c) The Hölder coefficient of the mixed growth singularity is now $\alpha = 0.88$.

Fig. 3

Same caption comments as in the previous figures but for Case III. The Hölder coefficient of the mixed growth singularity is now $\alpha = 0.92$.

Fig. 4

Same caption comments as in the previous figures but for Case IV. The value of the he Hölder coefficient for the mixed growth singularity is $\alpha = 0.84$.

Fig. 5

Same caption comments as in the previous figures but for the MCMS biomass signal $y = c_1$. The Hölder coefficient of the mixed growth singularity is now $\alpha = 1.89$.

Fig. 6

Same caption as in the previous figures but for the MCMS biomass signal $y = c_2$. The Hölder coefficient of the mixed growth singularity is now $\alpha = 1.87$.

Fig. 7

Same caption comments as in the previous figures but for the total MCMS signal. The Hölder coefficient of the mixed growth singularity is now $\alpha = 1.88$.

Fig. 8

MCMS data corresponding to c_1 with a small amplitude Gaussian noise added. From the bottom plot *c*) one can see that because the curve is not a straight line one cannot get a global Hölder coefficient from its slope.

Fig. 9

MCMS data corresponding to c_2 with a small amplitude Gaussian noise added. The Hölder coefficient is not a useful concept in this case.

Fig. 10

MCMS data corresponding to the sum $c_1 + c_2$ with the same Gaussian noise added. The concept of global Hölder coefficient is again not useful.

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