Wavelet analysis for morphological characterization of *Saccharomyces Cerivisiae*

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1 Introduction

Saccharomyces cerevisiae is used extensively in a large field of applications in biotechnology, e.g. biofuel, alcoholic beverages, baker yeast and recombinant protein. The intensification of bioreaction processes based on cell culture engineering can lead to changes of metabolic activities and biological functions (budding) because of high concentration of inhibitors, limitation of substrate. Exposed to increasing ethanol concentrations for instance, yeast cell morphology and activity are notably affected. The quantification of the budding and the cellular size of the yeast cells allow us to characterize the cell cycle and phenomena of stress encounter by the yeast. The analysis of microbial cells at the single-cell level may also lead to the quantification of subpopulations at different physiological states. Thereby, we have characterized the morphology of different population of yeasts during two discontinuous fed-batch fermentation by using image analysis. However the weakness of the traditional microscopy (with coloration or fluorescent probes) is the absence of the direct image analysis of cells and the time consuming of the image analysis.

One of the image processing is the detection of the budding cells. The idea is to find with a lot of precision the Hölder Coefficient corresponding to the curvature changing. The Hölder Coefficient is carry out by Wavelet Transform coupled to Genetic Algorithms.

2 Hölder's Coefficient, Wavelet Transform and Genetic Algorithms

A singularity in a point x_0 is characterized by the Hölder exponent. This exponent is defined like the most important exponent h allowing to verify the next inequality:

$$|s(x) - P_n(x - x_0)| \leq C|x - x_0|^h \tag{1}$$

We must remark that $P_n(x - x_0)$ is the Taylor Development and basically $n \leq h(x_0) < n + 1$. The Hölder exponent could be extended to the distribution. For example the Hölder exponent of a Dirac is -1. A fast computing leads to a very interesting result of the Wavelet Transform :

$$|W_{s,u} f(x)| \simeq a^{h(x_0)} \tag{2}$$

This relation is remarkable because it allows to measure the Hölder exponent using the behavior of the Wavelet Transform. Therefore, at a given scale $a = 2^N$ the $W_{a,b} f(x)$ will be maximum in the neighborhood of the signal singularities. The detection of the Hölder is linked to the vanishing moment of the wavelet: if n is the vanishing moment of the wavelet, then it can detect Hölder coefficient $\leq n$ (Mallat and Whang, 1992). The Hölder coefficient enables us to characterize the variation of the signals and to find the meaningful maxima.

For example, we had applied this method on analysis of bioreactor signals. We know that the sharp variations are due to operator's interventions and are sudden changes in the signal. Consequently, the maxima of these variations have Hölder coefficient equal to zero or negative (close to -1).

For the characterization of yeast morphology we have used different wavelets with a vanishing moment great less 1; consequently we can only detect Hölder coefficient smaller than 1. This is not a real problem because we are interesting by the singularities as step or dirac and the Hölder coefficient of these singularities are smaller than 1. Moreover for Hölder coefficient greater than 1 particularly for integer values, there are difficulties to interpret the Hölder coefficient (see Meyer, 1990; cited in Mallat, 1992). To evaluate the Hölder coefficient from the wavelet, there is two main ways:

1. the graphical method. It consists in finding the maximum line i.e. the maximum which propagates through the scales, and compute the slopes of this maximums (often with a log-log representation). The computed slope corresponds to the Hölder coefficient (Mallat and Whang, 1992).

2. the minimization method. It consists in minimizing a function whose one of the parameter is the Hölder coefficient(Mallat, 1992).

For the graphical method, the evaluation is generally made with a linear regression using a least squared method, but it is possible to also a more robust method called least median of squares regression (see Steele and Steiger, 1986; cited in Du and Hwang). This graphical method is the most fast and the most used method, but the evaluation of the Hölder coefficient is quite imprecise as observed in (Struzik, 1999) and (Nugraha and Langi, 2001). For the second method,*a priori*, all methods of minimisation can be used for the evaluation. In Mallat (1992), a gradient descent algorithm is proposed to resolved the minimisation, but this technique is very sensitive to local minima. Recently, a minimisation using Genetical Algorithms has been proposed (Manyri *et al.*, 2003). More precisely it uses differential evolutionary algorithms. Differential Evolution (DE) is one of Evolutionary Algorithms which are a class of stochastic search and optimization methods including Genetic Algorithms (GA), evolutionary programming, genetic programming and all methods based on genetic and evolution. The DE algorithms was introduced by Rainer Storn and Kenneth Price (Storn and Price, 1996) and is an implementation of Genetic Algorithms.

One of the lack of this method is the difficulty to analysis the oscillated singularities, but it is not our case. By using the evaluation of the Hölder coefficients by genetical algorithms, we have increase the accuracy of Hölder coefficient computing.

3 Conclusion

Using the Wavelet Transform we are able to detect the budding cells and also to construct a dictionary of singularities. We have worked on the correspondence between Hölder coefficient and cell age. The mathematical model for cell is ellipse allowing to obtain the next conclusions. During the growing phases, we show an increase of the ellipse form factor and a decrease of the volume variation of the cells (elongated growing cells). During the ethanol production phase when the ethanol concentration reached a concentration above 60 g/L, the ellipse form factor of the cells decreased (rod shape non growing cells).

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