IMAGES ACQUISITION OF MULTIPHASE DISPERSIONS IN FERMENTATION PROCESSES

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ABSTRACT

Multiphase mixing is a common operation in fermentation process. However, one of the main problems for on-line automatic monitoring of dispersions occurring in microbial cultures in a mechanically stirred bioreactor, is the difficulty in acquiring images (in motion) clear enough to characterize its elements (mainly air, water, oil and biomass) and their interactions during cultivation. Once the images to be analyzed have been acquired, other problems arise related to the complexity and diversity of objects/artifacts captured in the visual field. The heterogeneous transparency of some objects, low contrast and similarity between different classes of objects are, among others, major problems for the automation of image analysis procedures. The purpose of this work is to present a system that allows the on-line acquisition of images inside a mechanically stirred tank. The images are digitally obtained by connecting a TV camera to a stereomicroscope. The scanning of the camera is synchronized to the flashing of a stroboscope, which acts as the light source and is equipped with a submergible probe. These illumination conditions allow obtaining high quality images that can be further analyzed to quantify size distributions of air bubbles and oil drops in multiphase dispersion, and to observe the dynamics of phase interactions (solid, liquid and gaseous) in a model culture.

RESUMEN

El mezclado de multifases es una operación común en el proceso de fermentación. Sin embargo, uno de los principales problemas que presenta la automatización del monitoreo en línea de las dispersiones que ocurren dentro de un cultivo microbiano en un fermentador bajo condiciones de agitación mecánica, es la dificultad en la adquisición de imágenes (en movimiento) lo suficientemente claras que permitan caracterizar sus elementos (principalmente aire, agua, aceite y biomasa) y su interacción durante su cultivo. Una vez que las imágenes a ser analizadas han sido adquiridas, otro problema que se presenta está relacionado con la complejidad y diversidad de objetos/artefactos capturados en el campo visual. La transparencia heterogénea de algunos objetos, el bajo contraste y la similitud entre las diferentes clases de objetos son, entre otros, problemas mayores para la automatización de los procedimientos de análisis de imágenes. El objetivo de este trabajo es presentar un sistema que permite la adquisición de imágenes en línea de lo que ocurre dentro de un tanque agitado mecánicamente. Las imágenes son digitalmente obtenidas mediante una cámara de TV conectada a un estereomicroscopio. El barrido de la cámara es sincronizado con la luz de un estroboscopio, el cual actúa como fuente de luz y está equipado con una sonda sumergible. Estas condiciones de luz permiten obtener imágenes de alta calidad que pueden posteriormente ser analizadas para cuantificar el tamaño de las distribuciones de burbujas de aire y gotas de aceite en una dispersión multifásica y observar las dinámicas de las interacciones de las fases (sólida, liquida y gaseosa) en un cultivo.

KEYWORDS: Four-phase system, bubble sizes, drop sizes, image acquisition.

1. INTRODUCTION

Fermentation industry currently produces a wide range of products. Many industrial processes involve filamentous fungi, witch are cultivated for the production of important molecules such as enzymes, organic acids, antibiotics, and aroma compounds amongst others. Usually, this fermentation process entails the mixing of up to four phases [1]. Therefore, it is important to determine the influence of bioreactor operational parameters (stirring speed, impeller type, power drawn, etc) over the efficiency of the phases dispersions and ultimately on cultures performance. However, few works have been published about the monitoring of the phases dispersion in these types of fermentation with the use of image processing. Some of the early studies were conducted by photographing the tank wall [2]. However, the analysis of photographs is a tedious and costly activity, involving a relatively long processing time period. Other approach has been the study of fungal growth during the early growth stages [3] by using a small growth chamber, which is mounted on a microscope and periodically fed with medium. Although providing interesting results, growth in this system occurs under conditions differing to those found inside a mechanically stirred bioreactor. Moreover, in situ microscopy has been used for the characterization of microbial cultures [4,5] and in model for phases fermentation systems [6], although no details have been reported about the difficulties in image acquisition. One of the main problems arising from automatic monitoring of multiphase fungal cultures in a bioreactor, is the difficulty of acquiring images in motion clear enough to characterize the elements involved in the culture (air, oil and biomass, all immersed in an aqueous solution of salts). On the other hand, the diversity and complexity of these objects, as well as the presence of artifacts, the heterogeneous transparency of some objects, their low contrast and similarity of classes complicate the automation of the image analysis process [7].

The purpose of this work is to present a system that allows the on-line acquisition of images inside a mechanically stirred tank. The generated images can be further analyzed to quantify size distributions of air bubbles and oil drops in a multiphase dispersion, and to observe the dynamics of phase (solid, liquid and gaseous) interactions in a model culture.

2. PROBLEMS WITH THE ACQUISITION OF IMAGES IN MOTION

The acquisition of images in motion presents several difficulties. The most evident comes from the velocity of the particles being analyzed in contrast to the limitations of the sensors used to capture such images. A standard video camera generates an interlaced image by scanning out 480 horizontal lines (American standard) over 1/30 sec. (two interlaced 1/60 sec fields of 240 even and odd lines) [8]. This means that if the objects to be captured are moving faster than the scanning speed, the resulting image will be a blurred one (Fig. 1). Moreover, single objects may appear twice or overlapped due to the interlacing effect of the two 1/60 sec fields. This phenomenon is typically observed for images taken inside of a stirred tank, even at low stirring speeds.

One solution to this problem could have been to utilize a high-speed video camera. However, such equipment could cost several thousands of dollars. Hence, the alternative was to use a conventional video camera synchronized to the flashing of a stroboscope. This type of illumination provided by the immersion of a narrow probe in the stirred tank, had allowed to decrease the frame rate required for obtaining sharp and non-overlapped interlaced images. Moreover, heterogeneous transparency, diversity and low contrast between different classes of objects are, amongst others, serious problems for the automation of the image analysis process, specially when high oil and mycelia density are involved. Therefore, the high luminescence provided by the stroboscopic lighting also helped substantially to avoid this problem by diminishing field darkening.

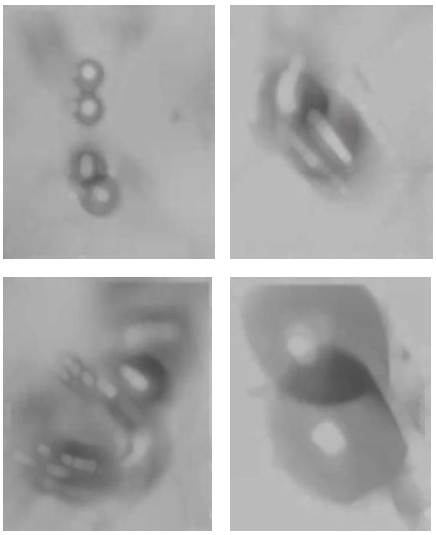

Figure 1. Examples of images of air bubbles captured without a stroboscopic light. Each one of these images show one bubble in motion, appearing as two overlapped or separated objects.

3. SYSTEM DESCRIPTION

3.1 Optics

A revolving nose-piece of an Olympus SZ-STU1 (SZ1145) stereomicroscope was used to vary lens focal length. A magnification range from 1.8 to 11X was covered from a relatively long distance of 110 mm. This nose-piece was mounted on an Olympus SZ-STB1ESD stand. Image acquisition was achieved using a Hitachi KP-D50 Color NTSC digital video camera. The camera was attached to the video tube of the stereomicroscope revolving nosepiece by means of an Olympus SZ-CTV coupling piece. A stroboscope (MVS-2600, EG&G Optoelectronics) was used as the light source, connected with a one meter long submergible fiber optic probe with a 0.4 mm diameter window light on its tip (Figure 2).

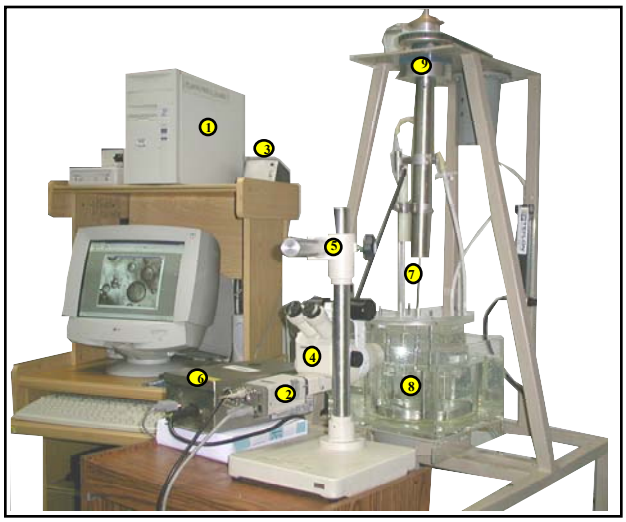

Figure 2. Photograph of the image acquisition system for the on-line capture of events occurring inside the stirred tank. 1) Computer with frame-grabber card and image analysis software. 2) Video camera. 3) Video camera-stroboscope synchronization device. 4) Stereomicroscope revolving nosepiece. 5) Stand. 6) Stroboscopic light source. 7) Light robe. 8) Cylindrical stirred glass tank and square glass tank. 9) Agitation stand.

Concerning the bioreactor, due to its cylindrical geometry, the objects (bubbles and drops) appear distorted (Figure 3) by the refraction on its curved wall when they are observed from outside (9). This was overcome by placing the cylindrical tank inside of other transparent square tank that served as a curvature-correcting lens when clear water fills the space between the two tank.

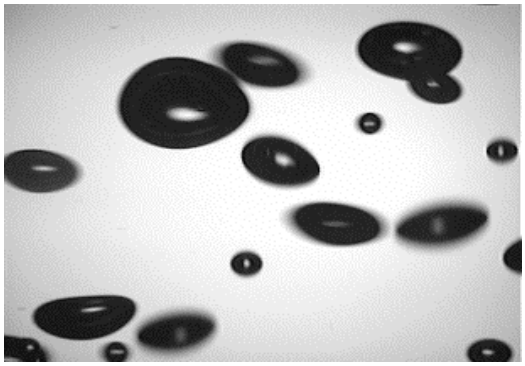

Figure 3. Example of bubbles image distorted by the refraction on the cylindrical tank. They were acquired using the synchronised camera-stroboscope system developed, but without placing the ellipsoidal stirred tank inside a square tank.

3.2 Digital System

A 733 MHz Intel III processor (Intel Technologies) personal computer was used. A Flash Point® 128 (Integral Technologies) video capture card was installed on the computer, providing the RBG/YC/SYNC input for the image acquisition and synchronization for the strobe triggering.

3.3 Synchronizing System

In order to obtain good quality images, it was necessary to synchronise the stroboscope triggering with the vertical RBG/YC/SYNC signal of the video camera [10,11], which was externally coupled by a TTL inverting buffer. Moreover, in order to keep the flash lamp life, the power source of the stroboscope was controlled with a computer command, enabling flashing only in acquisition mode. For this purpose, it was constructed an optical coupled interface that activates via the serial port, a mechanical relay providing AC source to the stroboscope. The light of the flashing pulses is sent via a submergible fibre optic probe inside the stirred tank.

3.4 Software

A C++ program was written to enable stroboscope switching. The commercial software Image-Pro Plus v. 4.1 (Media Cybernetics, USA) was used to create the subroutines (macros) needed to digitize and store images on the computer.

4. RESULTS

The synchronization of a conventional video camera with the flashing of a stroboscope decreased the frame rate required to minimize the problem of overlapping objects and artifacts in a moving image. Besides, the high luminescence provided by the stroboscopic lighting helped to obtain sharp images as well as to diminish darkening of the visual field. By introducing the cylindrical stirred tank into a square tank filled with water, the ellipsoidal distortion of bubbles and drops was eliminated. The necessary programs and subroutines were developed to acquire and store images as well as to switch the stroboscope. Figure 4 shows some examples of the types of images that can be obtained with the developed system.

a) b) c)

Figure 4. Examples of images obtained using the fully implemented system. a) Air bubbles, b) Oil drops c) Oil drops and air bubbles.

5. CONCLUSIONS

An on-line image acquisition system was successfully coupled to a mechanically stirred tank. The quality of the images generated by the system allows the quantification of the size distribution of oil drops and air bubbles in multiphase dispersions, as well as to make inferences about phase (solid, liquid and gaseous) interaction mechanisms in model and actual cultures. For the second stage, we are currently developing algorithms for the automatic quantification of drop and bubble sizes, thereby saving time and effort to the researchers.

6. REFERENCES

[1] Cordova A.M.S., Sanchez A., Serrano C.L., Galindo E., 2001 Oil and Fungal Biomass Dispersion in Stirred Tank Containing a Simulated Fermentation Broth, J.Chem.Technal. Biotechnal, 76, 1101-1106.

[2] Chen H.T., Middleman S., 1967 "Drop Size Distributions in Stirred Liquid-liquid Systems", AIChE J, 13, (5), 989-998.

[3] Spohr A., Dam M.C., Carlsen M., Nielsen J., Villadsen J., 1998 On-line Study of Fungal Morphology During Submerged Growth in a Small Flow-through Cell. Biotechnol, Bioeng. 58, (5), 541-553.

[4] Suhr H., Wehnert G., Schneider K., Bittner C., Scholz T., Geissler P., Jahne B., Scheper T., 1995 In Situ Microscopy for On-line Characterization of Cell-Populations in Bioreactors, Including Cell-Concentration Measurements by Depth from Focus" Biotechnol. Bioeng. 47, (1), 106-116.

[5] Dalmau E., Sánchez A., Montesinos J.L., Valero F., Lafuente F.J., Casas C., 1998 Study of Drop Size Distributions Frequencies in a Microbial Growth System with an Aqueous-Organic Culture Medium: Lipase Production from Candida Rugosa, J. Biotechnol, 59,183-192.

[6] Galindo E., Pacek A.W., Nienow A.W., 2000 Study of Drop and Bubble Sizes in a Simulated Mycelia Fermentation Broth of up to Four Phases, Biotechnal. Bioing., 69, 213-121.

[7] Russ J.C, The Image Processing Handbook, 1992, CRC Press.

[8] Kenneth R.S., Fundamentals of Video Imaging, National High Magnetic Field Laboratory, Microscopy Premier.

[9] Hecht E., 1989 Optics, 2nd. Ed., Addison-Wesley, New York, N.Y.

[10] Manual of Hitachi KP-D50 Color NTSC Digital Video Camera.

[11] Manual of Stroboscope MVS-2600, EG&G Optoelectronics.

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