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Novel technique for measuring the size distribution of granules from anaerobic reactors for wastewater treatment

D. Jeison and R. Chamy*

School of Biochemical Engineering, Universidad Católica de Valparaíso, Av. Brasil 2147, Valparaíso, Chile
Email: rchamy@ucv.cl

A new technique for measuring the size distribution of anaerobic granular sludge, which involves the use of digital image analysis, is presented. Sludge samples are embedded in gelatin, spread over glass dishes, which are then placed over a flat-bed scanner where an image is captured. The images are processed with an image analysis software. The technique is simple, reliable and does not need any special equipment.

Introduction

During the last twenty years, anaerobic digestion processes for wastewater treatment have experienced an important development. They have become one of the preferred alternatives for the biological treatment of wastes. The key to that success has been the second and third generation reactors, like UASB (upflow anaerobic sludge blanket) and EGSB (expanded granular sludge bed), due to the high organic loading that they are able to treat. A common factor of UASB and EGSB reactors is the immobilization of the biomass inside the reactor, by the formation of spherical, mechanically stables particles, known as granules.

Naturally, the quality and stability of such granules directly dictates the behavior of the entire treatment system. One of the most important characteristics of anaerobic granules is their size distribution. This size, with their density, defines the settling properties of the sludge, attribute that is fundamental from the operational point of view. Besides, various researchers have shown the influence of several operational conditions (organic loading rates, chemical oxygen demand, superficial velocity) on the granule size, and therefore on the complete system performance (Arcand *et al.*, 1994; Guiot *et al.*, 1992).

It is therefore of great importance to have a technique that allows the evaluation, in a reliable and reproducible way, of the size distribution of anaerobic granular sludge. Such procedure should keep the original structure of granules intact and avoid mechanical erosion. With this in mind, techniques based on image analysis represent an interesting alternative to traditional methods, since the sludge damage is minimal. The techniques based on image analysis for the measurement of biological particles usually involve sophisticated equipment, such as digital cameras (Dudley *et al.*, 1993; Thaveesri *et al.*, 1995; Grijspeerdt and Verstraete, 1997), or intermediate steps that include the digitalization of traditional photographs (Rebac *et al.*, 1995). The first of these methods involve costs that may be too high for certain applications, and the second requires additional steps that can be time consuming.

This article presents a new approach to the problem, introducing a simple and economical alternative to the measurement of size distribution of anaerobic granules or other biological particles.

Materials and methods

Scanner

An Epson scanner was used to acquire the images, using 140 pixels per mm^2.

Software

The images were analyzed with the free UTHSCSA Image Tool program, a software developed at the University of Texas Health Science Center at San Antonio, Texas. The program is available through the Internet by anonymous FTP from ftp://maxrad6.uthscsa.edu. The images were acquired and processed using a desktop computer with Windows 95.

Standards

Several metallic spheres were used as standards for the construction of calibration curves. Each sphere was measured using a micrometer, allowing a margin of error of 10 μm.

Methodology

The proposed method consists of the direct digitalization of samples of sludge spread over a glass plate and fixed

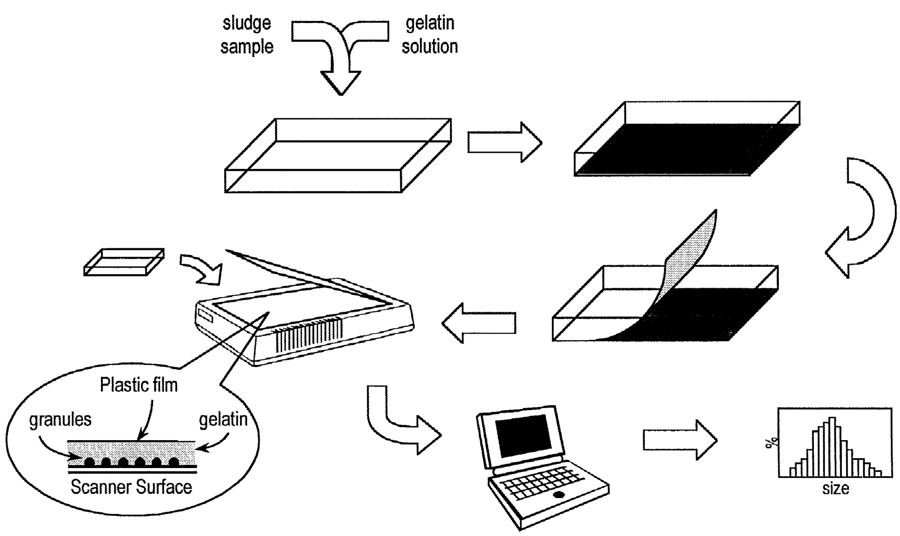

Figure 1 Steps involve in the presented technique.

within a transparent gelatin solution. The technique has three main steps (Figure 1):

1. Fixing of the sample using gelatin.
2. Digital Image acquisition with a scanner
3. Analysis of the images through a software.

Sample fixing
To facilitate the manipulation of the sample and its subsequent digitalization, the sludge is suspended in 25 g gelatin/l solution and then spread over a glass dish. In choosing the amount of sludge to be used in the measurement, it is recommended to prepare samples with two to three granules per cm^2 and to use samples free of fine or flocculent sludge to increase the contrast between the granules and the background. Once the gelatin solidifies, a white plastic film should be placed over its surface, to increase the contrast between the granules and the surrounding media (see Figure 1).

Image acquisition
After the sample dishes have been prepared, they are placed over the scanner surface. An eight-bit greyscale image is acquired, operating the scanner in the same manner as for a photograph or a printed page. The result is a digital image like the one shown in Figure 2. In order to obtain a clear and sharp picture, it might be necessary to adjust certain parameters of the scanner software.

Analysis
Once the images are obtained, they are then analyzed. The software gives information about the area, perimeter and other characteristic of the particles in the image. It should be noted that this method is not limited to the study of size and number of granules. Shape factors, like roundness, can also be obtained and traced during a study. Overlapped particles will yield erroneous information, and should therefore be excluded from the analysis (see Figure 2).

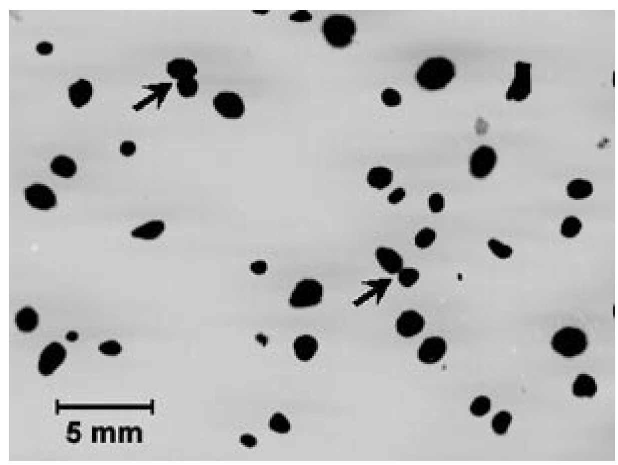

Figure 2 Images of granular sludge. Arrows indicate overlapped granules that must be excluded from the analysis.

Results and discussion

Digital image analysis

Techniques based on digital image analysis have some interesting characteristics that are improvements over traditional approaches. Some of them are:

- The samples do not suffer physical stress, thus ensuring their integrity during the measurement process.

- Each granule is analyzed individually, thereby yielding high quality information. In addition, the size distributions are obtained as a function of the number of particles (number of granules in a class), not as a function of another property related to the number of granules (such as mass in the case of sieve analysis).
- The images obtained during the process can be stored for further analysis or comparison.
- Small samples of sludge are required

This last point is particularity important for laboratory studies where it is generally impossible to obtain large samples without affecting the original conditions of the reactors. Nevertheless, working with small samples increases the importance of the sampling process and care should be taken to ensure that the sample is representative of the granules inside the anaerobic reactor.

Absolute size achievement

Following the steps described in Material and Methods it is possible to obtain reproducible measurements, as long as the scanner exploration parameters are maintained constant. If all that is needed are comparisons between different samples, it is sufficient to consider the area, in pixels, projected by the granules. However, it is possible to convert these results to absolute quantities by the use of calibration curves. The glass dishes used to hold the samples increase the distance between the surface of the scanner and the granules. Therefore, the relationship between pixels and mm^2 is different from that given by the scanning resolution. Considering that the distance between the granules and the scanner surface is constant, only a scaling factor is needed to correct the data. The expression that relates the projected area of a particle in pixels and its area in square millimeters is:

$$\text{Area [mm}^2\text{]} = \frac{1}{R\left[\frac{\text{pixels}}{\text{mm}^2}\right]} \times f \times \text{Area[pixels]}$$

$$\text{Area [mm}^2\text{]} = F \times \text{Area[pixels]}$$

where R is the scanning resolution (in pixels per mm^2) and f is the correction factor. Figure 3 presents a calibration curve which shows clearly the proportional relationship between pixels and mm^2.

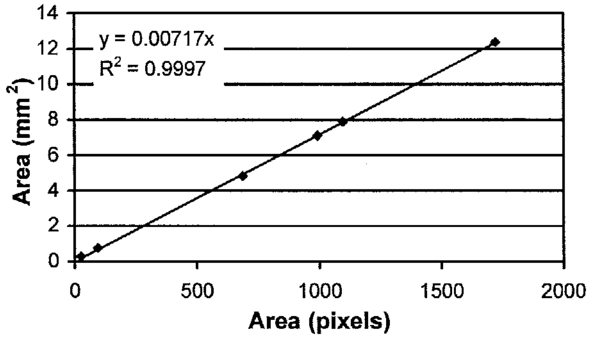

Figure 3 Calibration curve to convert pixels to mm^2.

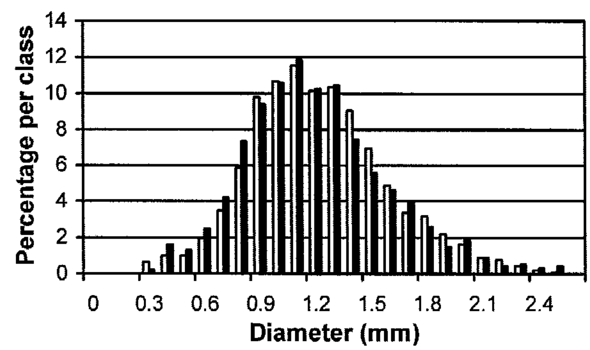

Figure 4 Size distribution of a sample of anaerobic sludge from a laboratory EGSB reactor. Two replicas, approximately 1500 granules each. Class width: 0.1 mm.

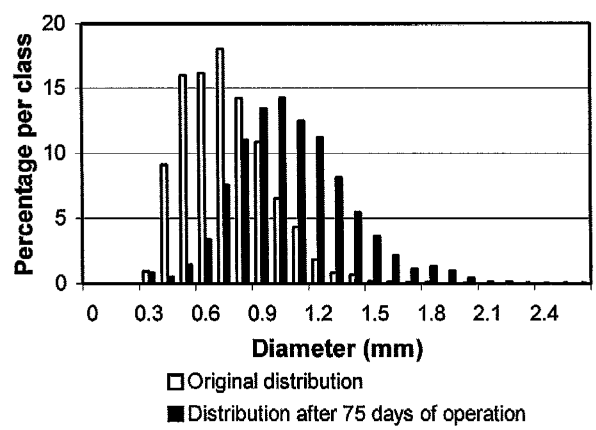

Figure 5 Granular size increase after 75 days of operation in a laboratory EGSB reactor. Class width: 0.1 mm.

Real samples analysis

The following figures show the application of the technique to real samples obtained from a laboratory EGSB reactor. Figure 4 shows the size distribution of two samples obtained at the same time. As expected, both distributions are almost coincident. The distributions where measured from samples of approximately 1500 granules (approximately 2–3 ml of settled sludge). This sample size has proven to be large enough to obtain a reliable distribution, of course if the sampling process is carried out properly.

Figure 5 shows the growth of granules in the EGSB reactor after 75 days of operation. Differences between samples are notorious, showing that the technique is suitable for used in studies of granule growth and development.

Conclusions

A technique presented that is a simple and inexpensive for the measurement of anaerobic granular sludge. No sophisticate equipment is needed and results can be obtained within a couple of hours. The analyses are completely reproducible, with good precision and exactitude. Small samples of sludge are needed (2–3 ml of settled sludge), which is especially important in the case of laboratory scale reactors, where large samples are not available.

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