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RESEARCH NOTE

FLOCS VS GRANULES: DIFFERENTIATION BY FRACTAL DIMENSION

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Abstract—High rate anaerobic wastewater treatment systems usually give rise to biomass structured in different types of aggregates, depending on prevalent environmental conditions. Although highly dependent on wastewater characteristics, granules are generally formed and found in UASB reactors, whereas flocs are mainly found in fixed bed reactors. Different structures usually have different shapes and surface roughness. The aim of this work is to provide a contribution to the differentiation of those kinds of aggregates. A numerical parameter, the fractal dimension, was used to quantify the surface roughness. The fractal dimension, of two families of particles, was measured by two methods: (i) a box counting method; (ii) a method based on an area-size relationship. In both cases, the differences were highly statistically significant. Using the box counting method, for each of the 54 particles of each family, the average fractal dimension was 1.90 ± 0.02 for flocs and 1.95 ± 0.01 for granules ($\pm 99\%$ confidence interval). The log–log plot of area vs longest size was linear and the calculated fractal dimensions from this plot were 1.84 ± 0.13 and 2.14 ± 0.08 ($\pm 99\%$ confidence interval) for flocs and granules, respectively. Fractal dimension was proven to be a suitable parameter to quantify and differentiate surface roughness of different microbial aggregates present in high rate anaerobic digesters. © 1997 Elsevier Science Ltd

Key words—fractal dimension, anaerobic microbial aggregates, flocs, granules

INTRODUCTION

The study of microbial aggregates in anaerobic wasterwater treatment systems, especially after the development of the upflow anaerobic sludge blanket (UASB) concept (Lettinga *et al.*, 1980), has been the subject of several research works reported in literature (Hulshoff Pol *et al.*, 1986; Wu *et al.*, 1993). According to Dolfing (1987), three kinds of particles may be distinguished: (a) flocs, which are aggregates with a loose structure. After settling, they usually cannot be individualized; (b) pellets, which are aggregates with a more dense structure than flocs. After settling, they are still individualized entities; (c) granules, which are dense pellets with a granular shape. They are firm, withstand a certain amount of compression and are referred to as "well flocculated sludge" (Lettinga *et al.*, 1980); they have a regular shape and well defined surface (Alphenaar, 1994). Although morphological features can be qualitatively described based on visual inspection, they are rather difficult to characterize in a quantitative way.

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The importance of granulation has been conducted as an active research of this phenomena (Schmidt and Ahring, 1996), and granules have been defined by several parameters such as density, settling characteristics, strength and size (Hulshoff Pol *et al.*, 1986); flocs, probably due to their deformable nature and handling problems, are, however, difficult to characterize. Relationships between colour, activity and surface roughness of granules have been reported in literature (Kosaric and Blaszczyk, 1990). According to these authors black granules are, generally, more active and spherical than grey and white granules. This fact suggests that quantification of morphology can be useful to characterize and differentiate among several types of anaerobic microbial aggregates.

Surface roughness, as it affects the hydrodynamic environment of the surface–liquid interface, and the available area for exchange and reaction processes (Zahid and Ganczarckzyk, 1994) should be considered in the study of microbial aggregates and biofilms.

Surface morphology or roughness can be quantified by the value of fractal or Hausdorf dimension, a numerical parameter emerged from the fractal theory (Mandelbrot, 1982). This theory provides a new way to characterize irregular structures. Fractal dimension measures the space filling capacity of an object. Euclidean dimensions are integers varying from 0 for points to 3 for volumes. Fractal geometry considers that, as opposed to the classical Euclidean geometry, the dimension of an object may be a non-integer value. This new approach has been widely used to describe highly irregular and complex structures (Aon and Cortassa, 1994; Jones *et al*., 1993) and this concept has proven useful to explain some unexpected phenomena in aggregates formed in water and wastewater treatment processes (Li and Ganczarczyz, 1989). These authors reported that microbial and inorganic aggregates, found in water and wastewater treatment systems, have fractal properties and postulated that fractal dimension, as it reflects the environmental conditions in which aggregates were formed, can be used to study factors affecting the process of aggregation.

Hermanowicz *et al*. (1996) described the anisotropic morphology of a biofilm based on the fractal dimension concept, and Zahid and Ganczarczyk (1994) reported on the fractal characterization of a RBC biofilm surface. In both works the authors emphasized the potentialities of fractal concepts when applied to the biofilm characterization.

In anaerobic wastewater treatment systems, particularly in UASB reactor, the microbial aggregation (granulation) and maintenance of granules characteristics is, nowadays, of great importance. In fact, although the UASB concept, based on granular biomass development and sludge retention without packing media, is attractive for industrial purposes, examples of granule disintegration conducting to flotation and washout have been reported in the literature (Hawkes *et al*., 1995).

The aim of this work was to measure and compare the fractal dimension of two families of anaerobic microbial aggregates present in two different technological and operational systems. These two families of particles were very different in colour, shape and settling characteristics. The usefulness of this new approach as a contribution to differentiate and characterize surface roughness of anaerobic microbial aggregates is also discussed.

MATERIALS AND METHODS

Fractal dimension calculations

Particles were visualised in an image analysis system and all calculations were based on the projected image of each particle. Several algorithms and methods have been proposed to calculate the fractal dimension (Falconer, 1990; Dubuc *et al*., 1989).

In the present work, two different methods were used to measure the fractal dimension:

(i) a box counting method was applied to the projected area of each particle individually; the average fractal dimension was then calculated and statistically compared;

(ii) the average fractal dimension was determined via an area-size relationship.

Theoretical considerations

The box counting method. The box counting method is one of the most widely used (Soddell and Seviour, 1994) due to the relative ease of mathematical calculations and computations involved. It basically consists of drawing successively larger boxes and counting the number of boxes that touch the particle (any colour different from background colour). The slope of the log–log plot of the number of boxes vs their respective size is the fractal dimension. A commercial software package (Fractal Vision, Oliver, 1992), configured for a monochrome VGA display driver, was used for this purpose.

Area-size relationship. Fractal dimension relates the amount of some property in *n*th-dimension with the aggregate size. It can be seen as a generalization of the classical concept of Euclidean dimensions where the ordinary dimensions are used as exponents in area-size or volume-size power laws.

For example, fractal dimension of aggregates in two-dimensions is obtained from the following relationship (Logan and Wilkinson, 1991; Logan and Kilps, 1995):

$$A \propto l^{D_2} \tag{1}$$

where A is the projected aggregate area, l the longest size and D_2 is the fractal dimension in two dimensions. Since fractal objects are irregular, their projected area should be smaller than the circular area based on its longest size. Fractal dimensions calculated by the above expressions represent a statistical average of the whole sample of particles.

Microbial particles

The particles selected were considered as representative of two families of aggregates. Flocs were collected from a laboratory Upflow Anaerobic Filter fed with a synthetic dairy waste, working for more than 2 years. Flocs were light, whitish and apparently non-spherical. Granules were removed from an industrial UASB reactor treating paper mill effluents and were black, spherical and settled well.

Image analysis and measuring. After collection, each particle was picked up individually and randomly with a wide mouth pipette and put in a petri dish with distilled water. Care was taken to maintain its initial morphology. Image acquisition was started after the complete immobilization of liquid. In Fig. 1 a schematic flowsheet shows the different steps of image analysis and fractal dimension measurements.

A Zoom Stereo Microscope SZ4045TR (Olympus, Tokyo, Japan) connected to a Quantimet 500 Image Analysis System (Leica Cambridge, Cambridge, U.K.) was used to store the images in a digital form (TIFF format). Each image contained only one particle and magnification was manually adjusted in order to maximise the particle size on the screen. With this procedure, particles having similar, equivalent diameters were submitted to the same magnification. Several calibrated lenses were used for this purpose. The digitized images were converted from grey to black and white (binary) using a manual threshold technique. This method of threshold selection allowed the maximization of the contour definition. The aggregate properties measured were equivalent diameter, longest size and projected area.

Before calculations, the digitized images produced by the image analysis system were converted to binary code using the same threshold level previously selected in the image analysis system. Then, they were converted from TIFF to PCX format with a Corel Photopaint package version 5.0 (Corel Corp., Ottawa, Canada, 1995). The final binary image was used for fractal dimension measurements of each particle individually. Care was taken in order to have a good contrast during image acquisition. This condition was

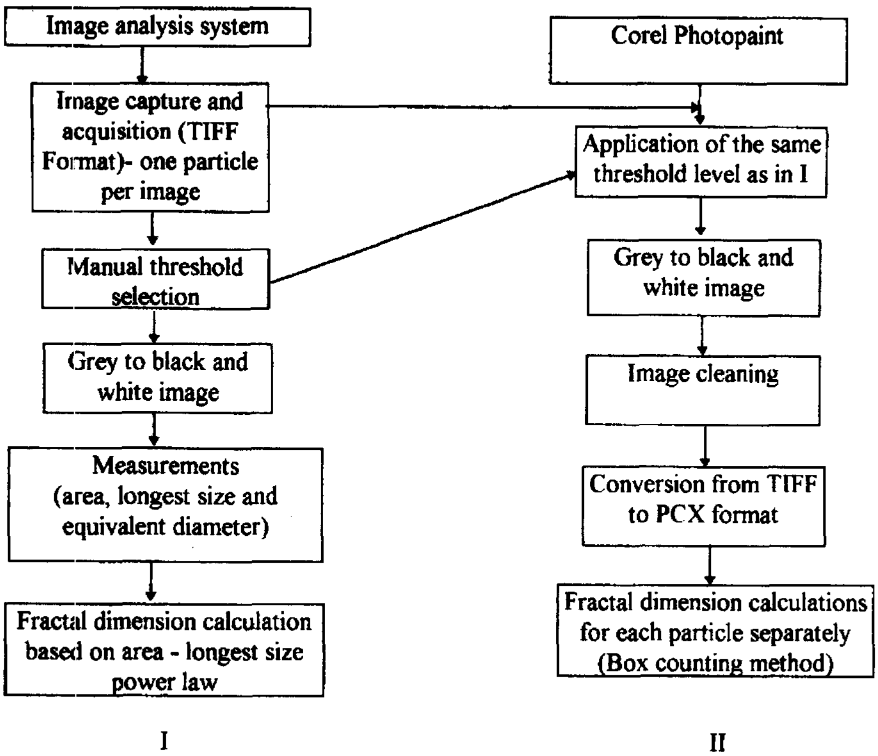

Fig. 1. Flowsheet of procedures of image acquisition and fractal dimension measures: (I) Image analysis system; (II) Corel Photopaint software.

important to eliminate the fractal dimension fluctuation with the threshold level selected.

RESULTS AND DISCUSSION

Figure 2 represents two digitized particles representative of each family studied. The different morphologies are evident from these pictures. Figure 3 represents the result of the log–log plot of area vs longest size for each family of particles. Fifty-four particles of each family were analysed. Table 1 displays the equivalent diameters and the fractal dimension calculated for each particle by the box counting method. The equivalent diameters ranged from 200 to 4000 μm for the two families.

For each calculation method, the average fractal dimension of each family of particles was compared.

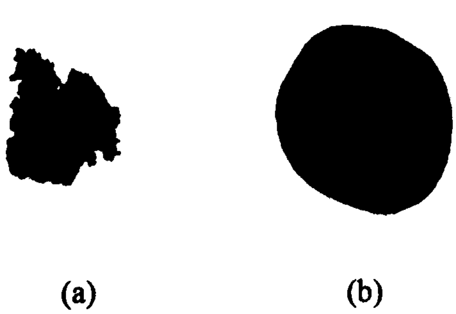

Fig. 2. Digitized images of a floc (a) and a granule (b).

Table 2 represents the obtained values as well as the respective confidence intervals.

The fractal dimension calculated by the slope of the log–log area-size plot showed to be significantly different for flocs and granules for a 99% of confidence level. On the other hand, a student's t-test applied to the difference between the two means of fractal dimension calculated by the box counting method, indicated that the fractal dimension for each group of particles was significantly different (p-value = 0.0001). Also with the same statistical high confidence level, the fractal dimension of granules, calculated by this method, was always higher than for flocs.

The results of fractal dimension indicate the value of D_2 obtained for granules is very close to the Euclidean dimension for circular shapes. The method based on the area-size power law was more sensitive to morphological differences between the two families of aggregates. The use of a log–log plot in this method may introduce some imprecision, which might explain the results above 2 obtained for the fractal dimensions of granules. However, from a practical point of view, this method is more suitable as it directly gives the average fractal dimension from a selected population.

Figure 4 represents the fractal dimension of flocs and granules, calculated for each particle individually by the box counting method, plotted against size (equivalent diameter).

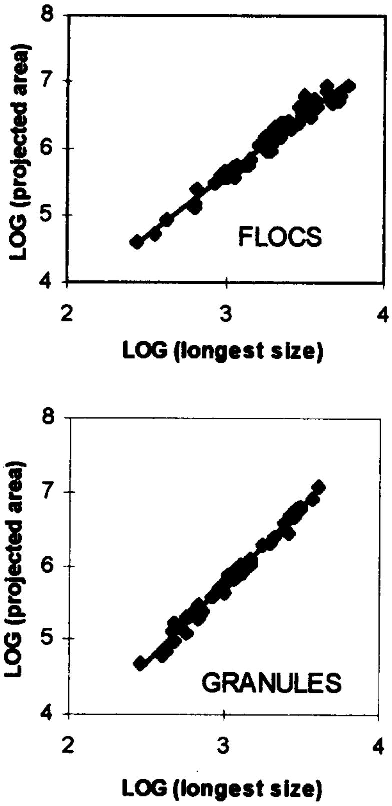

Fig. 3. Log–log plot of area-size power law for flocs and granules.

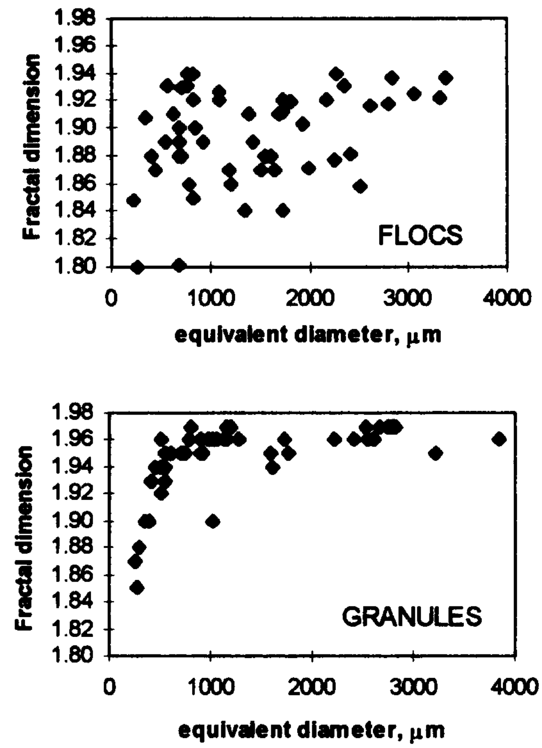

Fig. 4. Fractal dimension vs size for flocs and granules.

Comparing Fig. 4(a) with Fig. 4(b), it can be observed that for the flocs there is a random distribution of fractal dimension and size, whereas for granules, there are two situations: above an equivalent diameter of 700 μm, the fractal dimension is practically invariant. Below 700 μm there is a linear relationship between size and fractal dimension.

Small granules were more irregular than big granules and, over a range of sizes, a clear increasing

Table 1. Equivalent diameters and fractal dimensions for flocs and granules

Flocs				Granules			
Equivalent diameter (μm)	Fractal dimension	Equivalent diameter (μm)	Fractal dimension	Equivalent diameter (μm)	Fractal dimension	Equivalent diameter (μm)	Fractal dimension
227.7	1.85	1354.4	1.84	246.8	1.87	1024.1	1.90
256.2	1.80	1382.9	1.91	283.7	1.85	1057.6	1.96
332.5	1.91	1429.7	1.89	302.5	1.88	1059.6	1.96
408.6	1.88	1510.7	1.87	349.0	1.90	1142.2	1.96
437.5	1.87	1556.5	1.88	398.2	1.9	1157.5	1.97
551.5	1.89	1598.1	1.88	410.5	1.93	1159.1	1.96
553.9	1.93	1648.3	1.87	455.9	1.94	1165.4	1.97
613.2	1.91	1695.0	1.91	500.0	1.92	1204.7	1.97
673.7	1.90	1720.4	1.84	512.5	1.96	1271.6	1.96
674.3	1.80	1727.0	1.91	515.7	1.94	1589.4	1.95
682.8	1.89	1731.6	1.92	542.0	1.93	1609.7	1.94
685.7	1.88	1732.3	1.92	547.4	1.94	1721.7	1.96
695.3	1.88	1814.4	1.92	547.5	1.95	1755.1	1.95
696.2	1.93	1924.6	1.90	555.9	1.94	2208.0	1.96
758.4	1.94	1981.2	1.87	604.6	1.95	2403.0	1.96
764.6	1.93	2168.6	1.92	698.8	1.95	2535.7	1.97
789.2	1.86	2259.0	1.88	745.3	1.95	2538.7	1.97
822.9	1.94	2277.2	1.94	781.5	1.96	2545.0	1.96
825.9	1.92	2347.6	1.93	799.8	1.97	2600.8	1.96
829.4	1.92	2417.9	1.88	895.9	1.96	2667.3	1.97
830.6	1.85	2517.6	1.86	903.2	1.95	2736.4	1.97
849.8	1.90	2616.1	1.92	921.1	1.95	2776.7	1.97
926.9	1.89	2787.8	1.92	981.9	1.96	2776.8	1.97
1076.2	1.92	2839.6	1.94	984.6	1.96	2808.2	1.97
1088.9	1.93	3050.5	1.93	986.7	1.96	2819.3	1.97
1189.1	1.87	3315.2	1.92	1017.6	1.96	3208.6	1.95
1202.1	1.86	3378.9	1.94	1021.3	1.96	3842.5	1.96

Table 2. Average fractal dimensions for flocs and granules

Method	Average fractal dimension (± 99% confidence interval) Granules	Flocs
Power law	2.14 ± 0.08 (correl. coef = 0.98)	1.84 ± 0.13 (correl. coef = 0.96)
Box counting	1.95 ± 0.01	1.90 ± 0.02

in regularity of surface with size was observed. This fact can be helpful for following the granulation process.

CONCLUSIONS

As stated by other authors the fractal dimension can be used to differentiate aggregates generated in different environmental conditions in a more sophisticated way than the visual description (Li and Ganczarczyk, 1989). The method based on the area-size power law was not only more sensitive, but also more expeditious than the box counting method used in this work.

The quantification and differentiation of surface morphology of anaerobic microbial aggregates was possible using the fractal dimension concept. The observed linear trend between fractal dimension and size for small granules leads to the hypothesis that this method might also be applied to follow the granulation kinetics.

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