Characterisation by image analysis of anaerobic microbial aggregates under shock conditions

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KEYWORDS

Image analysis; anaerobic biomass; shock conditions

ABSTRACT

In the present work the characterisation by image analysis of anaerobic biomass under organic and hydraulic shocks was performed. The digester was fed with a synthetic substrate, containing 50% of oleic acid (as COD). Organic and hydraulic shocks were performed by increasing the substrate concentration or by lowering the hydraulic retention time. In both cases the organic loading rate changed from 6 to 30 kgCOD/m^3.d. Hydraulic shock induced fast and persistent changes both in the number and length of filaments, but in the organic shock significant changes were detected later and initial values were recovered 840 hours after the shock.

INTRODUCTION

In the last two decades, anaerobic digestion technology was significantly improved by the development of the sludge bed digesters, based on granular biomass. Usually the processes of granulation and granules-disintegration are coupled with macroscopic transformation of morphology. Several works refer to the characterisation of these morphological changes, using image analysis, although most of them are mainly focused on size determinations (Bellouti *et al*., 1997, Jeison and Chamy, 1998). It is accepted that filamentous organisms play a key role in the process of granulation, being responsible for the first nuclei of aggregated biomass. Systematic microscopic examinations have not been used to follow the granulation/disintegration process, because they are tedious and difficult to implement in a quantitative way. However, the use of automatic image analysis coupled to microscopic observations can overcome this problem. Due to inherent slow growth rate of the methane producing bacteria, a better knowledge of the dynamic growth of microbial populations during transient conditions can contribute to the development of appropriate modifications in design and operation, leading to improvements in the stability of the system (DeLorme and Kapuscinski, 1990). In the present work image analysis was applied to the characterisation of anaerobic biomass under shock load conditions.

METHODS

Experimental set-up and operation mode

The anaerobic reactor configuration was described in detail elsewhere (Alves *et al*., 1998) and allowed the regular withdrawal of biomass with minimum operation disturbances. The reactor was a cylindrical tank constructed in PVC, with a diameter

of 48 cm and a total volume of 86.8 L. In the central section 27 mini-bioreactors, with a total volume of 989 cm^3 each, were arranged in parallel constituting the support matrix. The support medium consisted of PVC Raschig rings of 21 mm in size (86 in each mini-bioreactor), and had a specific surface area of 230 m^2/m^3 and a porosity of 92.5 %. The effluent leaving the reactor entered a Plexiglas settler, and the settled biomass was recycled. The substrate was based on oleic acid (50% COD) with skim milk as co-substrate (50% COD). Macro and micronutrients were supplemented. During the steady state conditions the total COD was 4 g/L and the hydraulic residence time (HRT) was 16 hours. The first load shock - organic shock, was accomplished by increasing the substrate concentration to 20 gCOD/L, during 4 days, keeping the HRT and the relative proportion of skim milk and oleic acid. The second load shock – hydraulic shock, was accomplished by decreasing the HRT to 3.2 hours during 4 days, keeping the substrate concentration. The reactor operated 86 days under the referred steady state conditions between the two shocks.
The inoculum was taken from a local municipal sludge anaerobic digester. 15L with 10 g volatile suspended solids /L were inoculated. After collection, the biomass samples were homogenised, and concentrated in order to have similar concentration of VSS.

Image Analysis

The morphological parameters were determined by a programme developed in Cool language and run in the commercial software package Visilog 5.1 (les Ulis, France). This program consists of four major parts:

- Image improvement and binarisation

A series of morphological openings followed by morphological closings are applied to obtain the background image. An histogram equalisation and a delineation are then applied to improve the image. The background image is afterwards subtracted from the later one. In order to binarise the image, a method based on the histogram variance is applied to determine the threshold level.

- Filament / debris and flocs identification

A morphological opening (2 pixels size) and a logical subtraction to the binary image are applied. The resulting image contains only flocs. The filaments / debris image is obtained by subtracting the flocs image to the total binary image.

- Filament identification

A labelling the filament / debris image is then performed: the 2nd order moments and the area of the objects are determined. With these parameters, the program is able to determine of the Gyration radius (G_r):

$$G_r = \frac{\sqrt{M_{2X} + M_{2Y}}}{D_{eq}/2} \qquad (1)$$

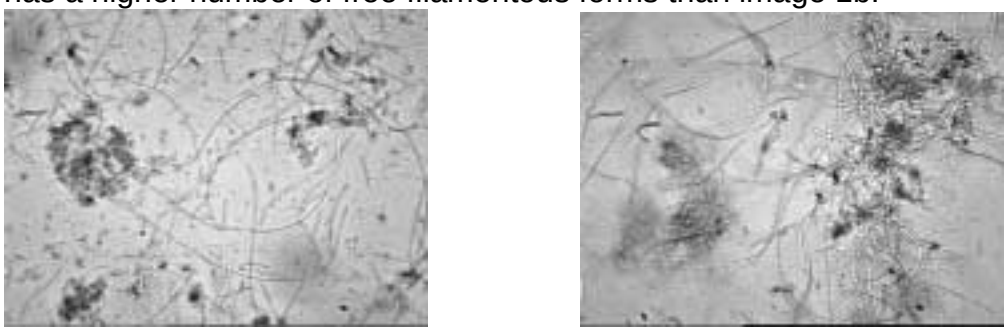
where D_{eq} is the equivalent diameter ($D_{eq} = 2\sqrt{\frac{Area}{\pi}}$) and, M_{2x} and M_{2y} are the 2nd order moments X and Y.
The objects that do not meet the Gyration radius and Area criteria (Gr >1, Area >200) are subsequently considered as debris and removed. The remaining objects are filaments. A morphological skeletonisation is then performed and the number and length of the objects (filaments) are determined.

- Floc characterisation

A dilation, a hole-fill function and an erosion are then applied in order to fill possible holes in the flocs. Next, the objects lying in the image borders are discarded by a border-kill function. A morphological erosion and subsequent reconstruction followed by image labelling and area determination of the remaining objects (flocs), are the final steps of this stage.

- Total biomass

The total biomass is given by the sum of the areas of the filaments and the flocs. Figure 1 represents examples of images to be processed by the software. Image 1a has a higher number of free filamentous forms than image 1b.



(a) (b)

Figure 1 – Images from two samples collected before (a) and 51 hours after (b) the beginning of hydraulic shock

RESULTS AND DISCUSSION

Figures 2 a and b represent the number of filaments per total detected surface during the organic and the hydraulic shocks, respectively. During both shocks a decreasing oscillating behaviour was observed. In the organic shock a minimum value of 72% of the initial value was achieved 200 hours after the beginning, and after 840 hours, the initial value had been recovered. However, in the hydraulic shock a minimum of 82% of the initial value was achieved 51 hours after the beginning, but there was no evidence of recovery 340 hours after. This suggests that washout or aggregation of filaments occurred by suddenly increasing the concentration or the flow rate. However this effect was more persistent after the hydraulic shock. The decreasing in the number of filaments was expected under load shock conditions. It is known that microbial filamentous forms prevail under low food:micro-organisms ratio. On the other hand, the selective pressure imposed by the hydraulic shock could have induced the aggregation of biomass as suggested by figure 1b.

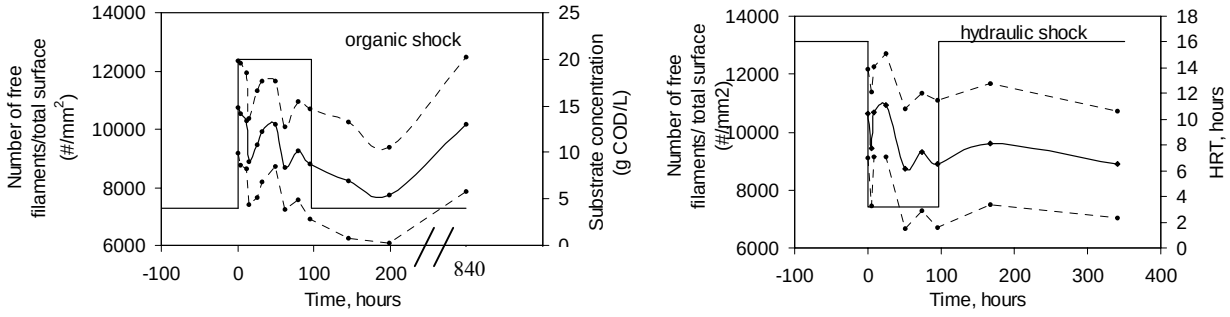


Figure 2 – Number of free filaments per total detected surface. (a) organic shock. (b) hydraulic shock. The dashed lines represent the boundaries of standard deviation.

The average total filament length detected per image processed is represented in Figure 3. As obtained for the number of filaments, a minimum value was detected, 200 hours after the beginning, representing 60% of the initial filament length. After 840 hours 90% of the initial value had been recovered.

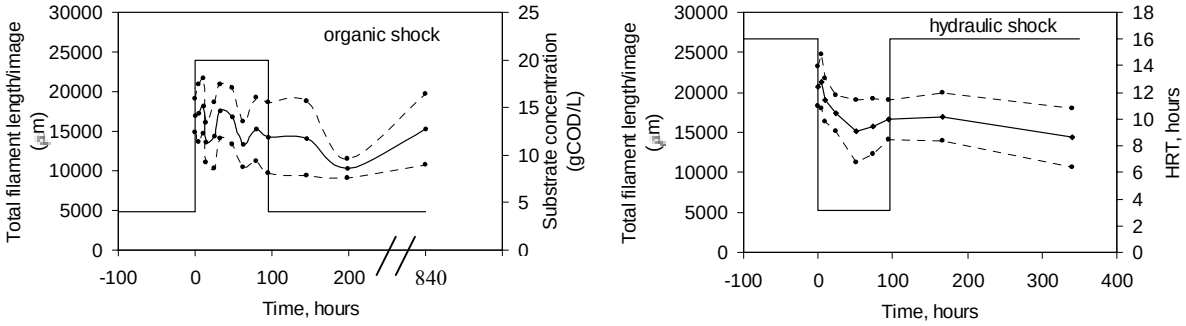


Figure 3 – Average total filament length detected per image. (a) organic shock. (b) hydraulic shock. The dashed lines represent the boundaries of standard deviation.

As detected for the number of filaments, in the hydraulic shock a minimum of 73% of the initial filament length was achieved after 51 hours. No significant recovery was detected, and after 340 hours the filament length was only 70% of the initial value. The filament length can be related to the filament number, but also to the filament morphology. One of the more known filamentous methanogenic bacteria is the acetoclastic *Methanosaeta*. This bacterium can have different morphologies from very long filaments (> 1000 units) to short filaments of 5-10 units (Wiegant, 1988).

CONCLUSIONS

The number and length of filaments detected by image analysis were more affected by an hydraulic load shock than by an organic load shock. Hydraulic shock induced fast and persistent changes both in the number and length of filaments, but in the organic shock significant changes were detected latter and initial values were recovered 840 hours after the shock.

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REFERENCES

Alves, M.M., Bellouti, M., Pereira, M.A., Álvares Pereira , M.R., Mota Vieira, J.A., Novais, J.M., Mota, M. (1998) A new method to study interactions between biomass and packing material in anaerobic fixed bed reactors. *Biotechnol. Tech.*, 12, 4, 277-283.

Bellouti,M.; Alves,M.; Novais,J.M.; Mota,M. (1997) Flocs versus Granules: Differentiation by Fractal Dimension. Water Res. 31:5, 1227-1231

DeLorme, A.J., Kapuscinski, R.B. (1990) On performing experimental studies on transient states of continuous-flow methanogenic reactors. *Biotechnol. Bioeng.*, 35, 746-750.

Jeison, D., Chamy, R. (1998) Novel Technique for Measuring the Size Distribution of Granules from Anaerobic Reactors for Waste-Water Treatment. Biotechnology Techniques 12:9, 659-662.

Wiegant, W.M. (1988) The Spaghetti theory on anaerobic granular sludge formation, or the inevitability of granulation, In Proc Granular Anaerobic Sludge; Microbiology and Technology (GASMAT) Workshop, G. Lettinga, A.J.B. Zehnder, J.T.C. Grotenhuis, L.W. Hulshoff Pol (Eds.), Puduc Wagningen, Wageningen, 170-178.