

A SIMPLE AND LOW COST TECHNIQUE FOR DETERMINING THE GRANULOMETRY OF UASB SLUDGE

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ABSTRACT

Four techniques (microscope sizing, calculation from settling velocities, image and laser analysis) are available nowadays for determining the particle size distribution of UASB sludge. These techniques present however the disadvantage to be either tedious, poorly precise or expensive and hardly applicable in full scale treatment plants. There was then the need for a simple and low cost technique. In this study, a granulometry procedure based on manual humid sieving was evaluated. It was shown that no solid loss occurred during the screening and that the particle size profiles were reproducible when performed with sludge samples of 5, 10, 25 and 150 mL, but not 1 mL. Only the results between 10 and 25 mL were however fully identical. It was shown also that the sieving could be performed on sludge samples stored for as long as 50 days at refrigerator temperature and that tap water could be used for the wash and backwash operations without any impact on the particle size profile. The granulometry obtained by image analysis was not comparable to that given by sieving. Nevertheless, no evidence of granule erosion could be found. In any case, the technique allowed perfectly to follow the evolution of sludge granulometry over time. As a consequence, the manual humid sieving appears to be an adequate technique for determining the granule size distribution of UASB sludges.

KEYWORDS

Anaerobic reactor; granular sludge; image analysis; particle size distribution; sieving; UASB

INTRODUCTION

Among all the different types of anaerobic digesters applied at full scale, UASB (Upflow Anaerobic Sludge Blanket) reactors present the best commercial acceptance. The success of these reactors is related to their capacity of biomass accumulation by settling without the need of a carrier. Good settling properties are obtained through the flocculation of the biomass under the form of dense granules with diameters up to several

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millimeters. Actually, as individual cells and granules have similar densities, the greater settling velocity of the latter is only related to its larger particle size (Schmidt and Ahring, 1996). The study of this phenomenon has led to the development of several techniques for characterizing the resistance of the granules (Pereboom, 1997), their porosity (Alphenaar *et al.*, 1992), settling properties (Andras *et al.*, 1989), bacterial composition and organization (Dubourguier *et al.*, 1988), activity (Dolfing and Bloemen, 1985), nature and composition of exopolymers (Grotenhuis *et al.*, 1991a), as well as their size distribution. This last parameter is particularly useful for studying the physico-chemical factors promoting sludge granulation (i. e. trace metal and calcium concentrations, upflow velocity, organic load, addition of inorganic carriers or crushed granules, etc).

To date, four techniques are routinely used to determine the particle size distribution of UASB sludge. Two of them consist in the direct size measurement with a microscope of at least 100 sludge granules immobilized in a petri dish. This can be done either manually with a Porton graticule in the ocular or automatically using image analysis and a computerized data processing (Hulshoff Pol, 1989; Dudley *et al.*, 1993). The third method is indirect and consists in determining the settling velocities of a sludge sample and to extrapolate the corresponding diameters using equations such as the stoke's law (Hulshoff Pol, 1989; Grotenhuis *et al.*, 1991b). The last method involves particle size analysis by laser and has been introduced only recently due to the fact that few times ago the upper detection limit of the equipments was too low for this application and that some of them now can detect particles of up to 2.5 mm (Yan and Tay, 1997). Unfortunately all these procedures present several disadvantages. The graticule one is very tedious while the image and laser analysis are expensive (at least \$US 10 000 for image analysis and between 30 000 - 60 000 for the laser one) and the settling velocity procedure assumes that some correlations between size and velocity are correct which may not be always the case. This last procedure is also poorly sensible and presents a low degree of precision (Grotenhuis *et al.*, 1991b). Finally, none of the previous techniques, because of their complexity or cost can be easily set up in treatment plants and as a consequence are almost reserved to laboratory studies.

There is then the need for a low cost and simple granulometry technique which could be applied both at lab and full scale. A humid sieving could be such a method since it only requires a set of sieves which cost few hundred dollars, a furnace and a centrifuge or a filtering device always available in laboratories realizing water analysis. Such sieving has been used before for that purpose by several researchers (Noyola and Kano, 1994; Tur and Huang, 1997; Yoda and Nishimura, 1997), nevertheless its validity has never been assessed. For instance, based on some studies with digested sludge and automatic sieving (Leschber and Haacke, 1975), it is believed that this method causes granule erosion and yields erroneous measurements (Hulshoff Pol, 1989).

This paper presents the evaluation of a manual granular sludge sieving procedure. The points investigated are (1) the suspended solids recovery after sieving compared to the initial amount present in the sample, (2) the reproducibility of the technique for each sludge volume tested, (3) the range of sample volumes for which the technique gives comparable results, (4) the possibility of using tap water instead of phosphate buffer for granule washing, and (5) the effect of sludge storage at two temperatures on granulometry. The results are also compared to those obtained with image analysis in order to assess if erosion takes place during the sieving.

MATERIAL AND METHODS

Source of granular sludge

The sludge used for the study came from a 2400 m³ UASB reactor treating at a loading rate of 2.69 kg COD/m³.d. the low strength wastewater (1300-1700 mg COD/L) of "Central de Malta", a malt factory

belonging to the "Cerveceria Cuahtémoc Moctezuma" brewery group. The plant is located at Grajales, in the state of Puebla, south to Mexico City (Monroy *et al.*, 1997). This sludge was chosen because, with an activity of 1.2 g COD/g VSS.d, a SVI of 20 mL/g TSS, a settling velocity of 9 m/h and 83% of its TSS having a size higher than 0.6 mm (Noyola and Kano, 1996), it is a good example of granular biomass (TSS: Total Suspended Solids; VSS: Volatile Suspended Solids; SVI: Sludge Volume Index). The sludge was sampled at 2 different dates separated by 3 months. The effect of storage on granulometry was evaluated on the second sample and all the other factors on the first one as well as the image analysis.

Sieving procedure

The screening was performed with 5 stainless steel sieves of 8" diameter having respective mesh opening of 2, 0.925, 0.76, 0.59 and 0.23 mm which allow to cover the usual granule size range. The sieves were monted vertically one on the top of the other in increasing order of mesh opening (from bottom to top). A plate of the same diameter was located at the bottom to recover the particules smaller than 0.23 mm. Before analysis, the sludge was vigorously homogenized by shaking several time its storage container and then, transfered, in a beaker of 2 liters where it was quickly sampled. Depending of the sludge volume tested, sampling was made either with calibrated spoons (25, 150 mL) or pipettes (1, 5, 10 mL). In that last case, the pipette tip was broken in order to have a mouth of around 5 mm which is at least twice the size of the biggest granules. The spoons had a larger diameter (4 cm for the 25 mL one, 8.6 cm for the 150 mL one), so that the ratio of spoon to granule diameter was very high. The sample was afterwards deposited on the 2 mm mesh opening sieve and washed with phosphate buffer (KH_2PO_4 4 g/L, $\text{Na}_2\text{HPO}_4 \cdot 7 \text{H}_2\text{O}$ 5.09 g/L, K_2HPO_4 1.08 g/L, pH 7.3) or tap water to allow the particles less than 2 mm, to separate from the others and flow to the following sieve. The same washing operation was done successively for each sieve until reaching the last one. The granules retained on the different screens were recovered by a backwash using the same solvent. Each fractions were collected in different beakers and were later centrifuged at 5000 rpm during 15 min in order to obtain the solids and determine their TSS and VSS. Once, the amount of TSS and VSS retained on each sieve was determined, it was possible to calculate by simple addition of these data, the total amount of solids screened and then to determine for each class of size (< 0.23, [0.23-0.59[, [0.59-0.76[, [0.76-0.925[, [0.925-2[, > 2 mm) the percentage of the total weight that they represent.

Image Analysis

The first step in the image analysis consists of screening a sludge sample of 1 mL through a sieve of 0.149 mm in order to eliminate the small particles which cannot be detected by the system. The TSS of the sieved portion ($M_{0.149}$) are measured in parallel to the TSS of an unscreened 1 mL sample (M). Thereafter, an aliquot of the granules retained on the sieve is placed in a petri dish (with a diameter of 3.5 cm) followed by the addition of enough phosphate buffer (an equimolar solution of sodium and potassium phosphate, 0.1 M) to cover them. The petri dish is then observed with a stereomicroscope (WILD, Heerbrugg, Switzerland) equipped of a video camera coupled to a Quantimet Q520 Image Analysis System (Cambridge Instruments Ltd, Cambridge, UK) which automatically determines the equivalent diameter of each granules. This value corresponds to the diameter of a sphere with a surface identical to that of the particles. The image analysis gives therefore, a distribution of diameter (D) by number of granules. In order to compare it with the results obtained by sieving, this distribution must be converted to a distribution of diameter by weight. Assuming that all granules have equal densities, mass (M) and volume (V) distributions are equivalent since $M = V \cdot \text{density}$. The granules being assimilated to spheres, their volumes can be easily assessed ($V = 1/6 \pi D^3$). The procedures consists afterwards, (1) to classify the granules by increasing diameter, (2) to group them into classes of size corresponding to the sieving (< 0.23, [0.23-0.59[, [0.59-0.76[, [0.76-0.925[, [0.925-2[, > 2 mm), (3) to calculate for each class the volume that represent all the granules that they contain (= sum of granule

individual volumes) and finally (4) to determine in percentage the fraction that every class represents with respect to the total volume (V_t) of the granules found in the sample ($V_t = \text{sum of individual volumes (} v_i \text{) of all granules detected by image analysis + volume of particles inferior to } 0.149 \text{ mm} = \sum v_i / [M_{0.149} / M_t]$).

Statistical analysis

All the statistical analysis (Anova, Kolmogorov-Smirnov, Student t test, etc) of the data have been performed with the software SPSS version 7.5 for windows (SPSS Inc., Chicago, IL., USA).

Total and volatile suspended solids

The sludge TSS and VSS were determined respectively after drying at 105°C for 24 h and combustion during 1 h at 550°C of the dried solids following the procedure described in Standard Methods (1992). Before analysis, the suspended solids were obtained from the samples by centrifugation as indicated above.

RESULTS AND DISCUSSION

Excepted when mentioned differently, all screening experiments were performed using phosphate buffer as solvent.

Suspended solids recovery

For being valid, as first criteria, a granulometry technique must be able to take into account all the suspended particles present in a sample without any loss. It was therefore important to check if the total mass of solids recovered after screening was identical to that of the original unscreened sludge. For that purpose, the TSS and VSS of 10 sludge samples of 25 mL were determined directly, while 10 other 25 mL-samples were submitted to screening and the sum of the dry and volatile solids retained on the different sieves was calculated for each of them. As can be seen on Fig. 1, the direct measurements gave TSS and VSS values much more regular than those obtained after sieving. For TSS, the averages were however very similar, 73.7 (SD 7.12) and 74.08 g/L (SD = 1.08) with and without screening respectively. The difference was somewhat more important for VSS (mean of 57.84 g/L with a SD of 0.75 without sieving against 53.75 g/L and SD of 5.21 after sieving). A variance analysis which compared the means for the TSS and VSS with and without screening confirmed that no significant difference existed for the TSS values but that a significant difference (at a 2.5% level) existed for the VSS. No explanation could be found for this variation, except maybe, that the test should have been performed on a higher number of samples. Because of this result, for the rest of the evaluation only TSS were considered.

Reproducibility

Other important criteria that must cover a granulometry technique is reproducibility. In a first step, this one was tested again on 10 screening using a sludge sample of 25 mL. The results (Fig. 2) were analyzed in order to test that the %TSS obtained for each class of granule size followed a uniform distribution. The data were hence submitted to a Kolmogorov-Smirnov test which confirmed that for none of the classes the uniform distribution hypothesis could be rejected. Such uniform distribution indicated that the results obtained for the different sieving are similar and that as a consequence the method is reproducible. Later the same analysis was performed for sludge samples of 1, 5, 10 and 150 mL. For all of them, except 1 mL, the reproducibility was also confirmed (data not shown).

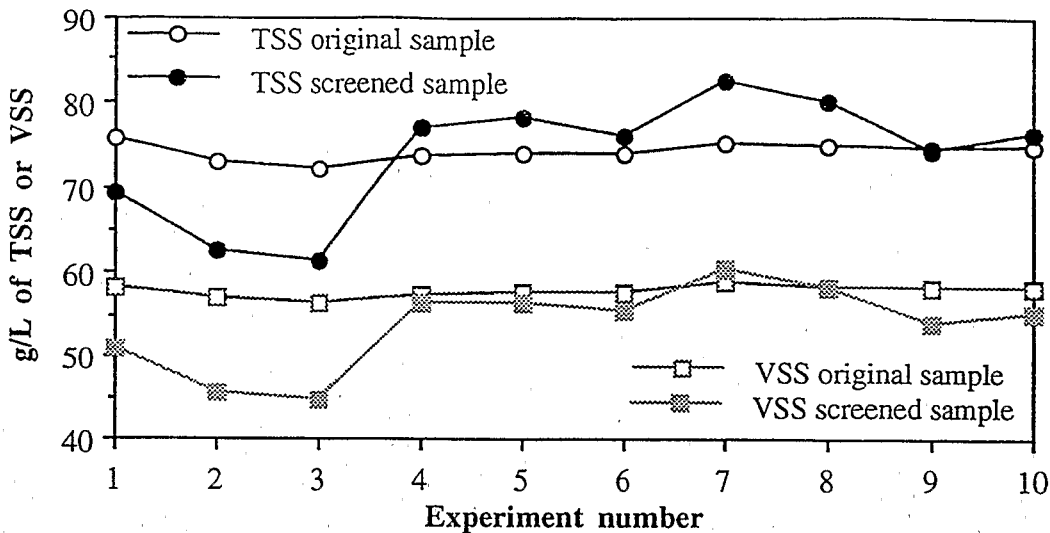


Figure 1. Values of the TSS and VSS obtained for ten sieved and unsieved sludge samples

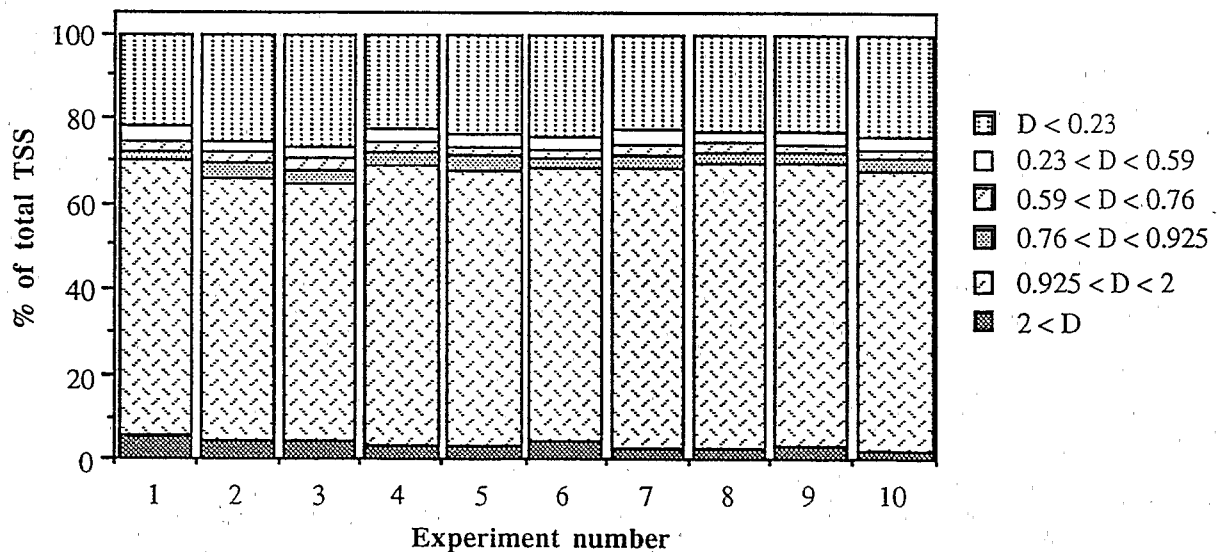


Figure 2. Reproducibility of sieving for each class of size and a sludge sample of 25 mL

Effect of sludge volume

Sludge sampling is not at all a problem for full scale reactors due to their huge volumes. Actually, various liters can be taken several times without any consequence on their performance. Unfortunately, this is not the case at lab scale where the total amount of sludge is usually limited to a few hundred milliliters. For instance, in the case of a reactor containing 500 mL of sludge, a sample of 25 mL represents already 5% of the total volume. Since the screening must be done at least in duplicate, this limit the frequency of sampling and can affect the experiments in progress causing overloading of the biomass. It was then important to know if the

volume of sample to be screened could be reduced to less than 25 mL without affecting the results of size distribution. As a consequence, a serie of screening in triplicate were performed with 1, 5, 10, 25 and 150 mL of sludge. This last value was included in order to see also the impact of using large volumes.

It appeared immediately from the results (Table 1, for clarity only mean values are given) that the screening of 1 mL of sludge gave a size distribution pattern completely different from the others. The fraction with a diameter higher than 2 mm disappeared while the fraction with a size between 0.925 and 2 mm was almost reduced by two. The rest of the fractions increased with respect to the other volumes. Such result is probably related to the fact that it is difficult for a 1 mL sample to be representative of the sludge. As shown by Pereboom (1994), usually in UASB sludge, the number of granules found in each class of size decreases with the increase of diameter. The number of small granules in the sludge, is then, always higher than the number of big ones. Since big granules have the weight of several small granules, their impact on mass is important even if their number is low. In case the granules bigger than 2 mm are only present at the density of 5 granules for each 25 mL of sludge, the probability to pick one in 1 mL is low. This will also affect the reproducibility, since the mass particle size distribution of such 1 mL sample will be highly different depending on the presence or absence of a big granule. The same argument can be used to explain the decrease of the [0.925-2[mm fraction and the increase of the small ones.

Table 1. Mean %TSS obtained for each class of granule size using different sludge sample volumes, phosphate buffer or water as solvent and image analysis instead of screening

Sample volume mL	Granule size (mm)					
	> 2	[0.925-2[[0.76-0.925[[0.59-0.76[[0.23-0.59[< 0.23
% TSS						
Sieving						
Phosphate buffer						
1	0	36.02	4.5	14.07	15.06	30.36
5	1.46	63.07	2.91	4.34	5.13	22.49
10	1.56	64.23	2.82	2.49	3.33	25.56
25	1.96	67.85	2.61	2.12	2.88	22.55
150	0.85	70.72	1.71	1.57	4.58	20.56
Tap water						
25	0.98	71.18	2.28	1.81	2.57	21.17
Image analysis						
1	15.48	52.5	2.24	0.9	0.4	28.48

For the other volume tested, on first approach, the size distribution looked like much more similar. For each class of granule size, the mean %TSS obtained at the distinct volumes were compared by a variance analysis. Later, with a multiple Tukey comparison, the means differing by more than 10% were identified (a 10% difference is tolerable for this type of analysis). No differences could be found between 10 and 25 mL, but some differences appeared between 5 and 150 mL and between them and the other volumes. For the 150 mL sample, one of the uncomparable values corresponded to the %TSS of the granules included in the [0.925-2[

and < 0.23 classes. In that case, the difference may be related to the fact that despite of using more phosphate buffer for washing the granules when screening 150 mL (1600 mL against 700 for 25 mL of sample, 300 for 10 and 150 for 5 mL), the ratio of buffer volume to sample volume was only of 10 compared to 28-30 mL/mL in the other cases. This implicates that the washing efficiency was probably lower and that as a consequence some small granules could not be separated from the big one in the $[0.925-2[$ class and stayed on the 0.925 mm sieve. Concomitantly the %TSS of the granules smaller than 0.23 mm decreased.

Phosphate buffer versus tap water as washing agent

Phosphate buffer was used at the beginning of the evaluation for the washing and backwash operations in order to provide to the granules an adequate environment in terms of pH and osmotic pressure. It was actually important to avoid any damage to the cells which could result in granule disruption. The preparation of phosphate buffer being time consuming and having a cost related to the chemicals, it was interesting to test the possibility of replacing it by tap water. Moreover, this would be particularly appreciated in full scale treatment plants where simple procedures are preferred. With this objective, 3 sieving with phosphate buffer and 3 other with tap water were performed on sludge samples of 25 mL (Tab.1). For each class of size, as previously, using a variance analysis and a Tuckey test for multiple comparison, it was tested if the differences between the mean %TSS resulting from the use of the two solutions were not higher than 10%. Actually, no difference was found. This result is in accordance with those of Leschber and Haacke (1975) who also observed no differences between the particle size distribution of sewage sludge screened in presence of distilled or tap water as well as the liquid recovered after sludge filtration. This absence of difference seems to indicate that the pH and ionic strength of tap water are adequate for that purpose or that the size of the granules is sufficient to make them not affected by osmotic stress.

Impact of sludge storage on granulometry

Analysis may not be always performed at the time of sampling, particularly when facilities are not available on site and the samples must be sent to other place. It is then important to know how long a sample can be stored without affecting the characteristics to be measured. Storage allows also to organize better the working schedule since several samples taken at different time can be processed at the same moment. In order to determine this parameter, two identical volumes of sludges sampled on the same day were stored for 50 days, one in a refrigerator and the other at ambient temperature. Every 2 weeks, the granulometry of both sludges was determined by duplicate using a sample volume of 25 mL.

At refrigerator temperature, the curves of %TSS versus time (Fig. 3) which could be drawn for each class of size were horizontal lines (slopes of zero). This means that at this temperature the %TSS did not change significantly with time, indicating therefore, that the sludge can be stored for 50 days in such conditions without any effect on its particle size distribution. At ambient temperature, the situation was different since for the $[0.76-0.925[$ and > 2 mm classes of granule size, the slope of the lines drawn were significantly different of zero. Statistical analysis of the data were performed using simple linear regression and the t-test for the time coefficient. It must be noted that by the time of performing the analysis, the ambient temperature increased above 30°C . As a consequence, the different comportment observed at the low temperatures could be the result of different metabolic activities. Cell lysis in starving conditions is actually more important at higher than lower temperature which could probably explain the reduction of size which was observed.

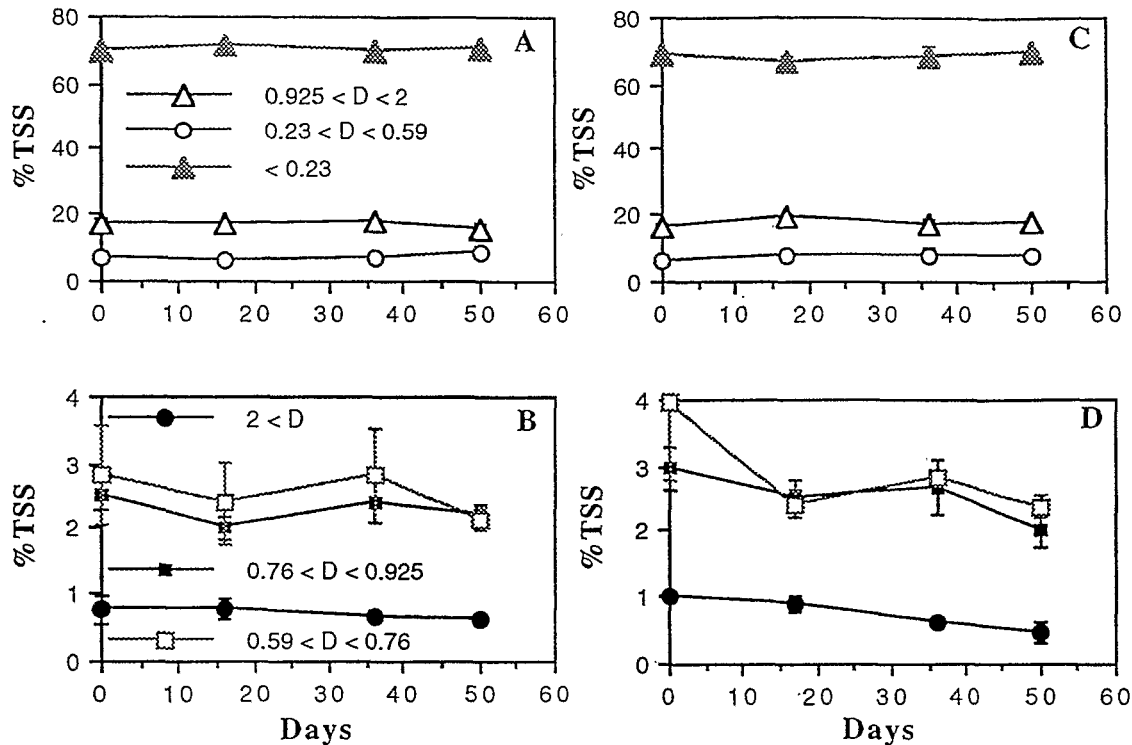


Figure 3. Evolution of sludge granulometry kept in a refrigerator (A, B) or ambient temperature (C, D) (bars represent standard deviation, when not visible they have the size of the symbols)

Image analysis

The image analysis involving only one screening in order to remove the undetectable small particles, the granules are supposed to be submitted to much less shear stress than during screening on multiple sieves and then to keep better their size integrity. As a consequence, image analysis was supposed to allow the detection of any damage caused to granule by erosion during the sieving procedure. Image analysis was therefore performed on 12 samples of the sludge used for the screening evaluation. The size distribution obtained (Tab. 1) appeared to be very different to those resulting from the sieving of the various sludge volumes. For instance, with image analysis, the granules bigger than 2 mm represented more than 15% of the total TSS against less than 2% for screening. The image analysis being performed with a sample of 1 mL, it was logical to compare it more in detail with the results obtained when sieving such volume. In their case, a t-test showed that for each classes of granule size, except 0.23 mm, the differences between the %TSS of the two techniques were significant at a level of 0.05 or less. The variations observed cannot be explained however by granule erosion since the 2 mm sieve is the first to be in contact with the sludge and its mesh opening is too high to cause an important shear stress during granules flowing. In fact the difference seems due to the point that the diameter given by image analysis are calculated assuming that granules are spherical. Central de malta's sludge contained unfortunately a certain amount of thin granules with a fusoid form. The surface of such particles may correspond to that of a sphere with a diameter higher than 2 mm but, their narrow width compared to their length could permit them to cross the 2 mm sieve in vertical position. This would result for image analysis in an artificial increase of the %TSS corresponding to the big granules and for the screening procedure to a decrease of this fraction without any correlation with erosion.

Practical application of the Technique

The sieving procedure with 25 mL-samples and phosphate buffer as washing agent was used to follow during 54 days the granulometry of a 2 L UASB reactor sludge fed with cheese wastewater and operated at an upflow velocity of 0.5 m/h. As can be seen on figure 4, the sieving technique permitted perfectly to visualize (1) a segregation of the sludge bed, the biggest granules being more abundant at the bottom and the smallest at the top, and (2) an increase of the fraction corresponding to the biggest granules along time, both at the bottom and top of the sludge bed. The techniques allows also to compare easily the granulometry of sludges from different sources (data not shown).

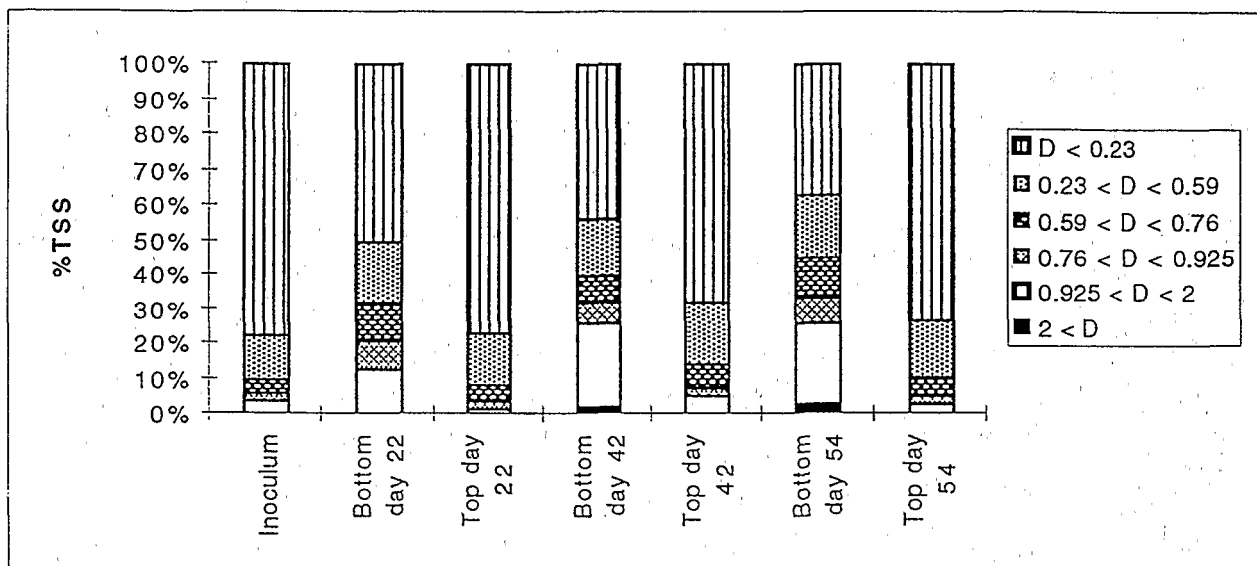


Figure 4. Evolution of the granulometry at the bottom and top of the sludge bed of a lab scale UASB reactor fed with cheese wastewater

CONCLUSIONS

The sieving technique can be used to determine the granulometry of UASB sludge. All screening performed with 5, 10, 25 and 150 mL will give reproducible results while only screening performed in the range of 10-25 mL will give identical values. In order to compare the screening done by different laboratories, this range of volume should be preferred. Tap water can be used for the washing and backwash operations since it yields identical results with phosphate buffer. The sludge can be stored up to 50 days in a refrigerator without any impact on its particle size distribution. Since screening and image analysis gave uncomparable distributions, the possibility of sludge erosion could not be discarded, but this is of little importance since the technique allows to observe the evolution of the sludge particle size profile over time. The main disadvantage of the technique is that it does not give any information on the number of granules and that as a consequence, a mean diameter cannot be determined unless similar intervals of mesh opening between sieves are used. However, an advantage of the technique is that it can be performed in series with SVI determinations. Three parameters (SVI, granulometry and TSS concentration) can, hence, be obtained with only one sludge sample. Finally, it should be mentioned that the sludge granulometry can be known with greater precision, simply by increasing the number of sieves. Sieving with automatic shakers is not advised unless no impact on granule

size integrity can be demonstrated. Such apparatus would require nevertheless probably higher sample volumes.

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