M. da Motta*
M.N. Pons*
N. Roche**
A.L. Amaral***
E.C. Ferreira***
M. Alves*,**
M. Mota***
H. Vivier**
*Laboratoire des Sciences du Génie Chimique, CNRS-ENSIC-INPL, 1, rue Grandville, F-54001 Nancy cedex
** IUT de Marseille, Dept GCGP, UPRES 884, Université de Marseille, Traverse C. Susinni, F-13388, Marseille cedex, France
*** Centro de Engenharia Biologica IBQF, Universidade do Minho, P-4710-057 Braga, Portugal

AUTOMATED MONITORING OF ACTIVATED SLUDGE USING IMAGE ANALYSIS

Table of contents:

ABSTRACT
An automated procedure for the characterisation by image analysis of the morphology of activated sludge has been used to monitor in a systematic manner the biomass in wastewater treatment plants. Over a period of one year, variations in terms mainly of the fractal dimension of flocs and of the amount of filamentous bacteria could be related to rain events affecting the plant influent flow rate and composition.

KEYWORDS
Filamentous bacteria; flocs; image analysis; morphology; settleability;

INTRODUCTION
Activated sludge is a complex ecosystem constituted mainly by bacteria and protozoa. The bacteria, filamentous or not are agglomerated as flocs. A good balance between the different species is necessary for an efficient pollution removal, a good settleability of the sludge in the final clarifier and a low level of suspended solids in the effluent. If filamentous bacteria are essential to the floc structure, their over-production, due to substrate limitation for example, induces low settleability properties ("filamentous bulking"). There are different types of filamentous bacteria: *Microthrix parvicella* is frequently found in Europe (Eikelboom et al., 1998), when *Nocardia spp.* and Type 1701 are predominant in North America (Richard, 1991). Generally the amount of filamentous bacteria is estimated by manual counting under a microscope and that is a very tedious task for the plant operators. Automated image analysis can be used to make this task easier. It can be used also to characterise the flocs themselves, by their equivalent diameter and their fractal dimension, as proposed by various authors (Grijspeerdt and Verstraete, 1997).

METHODS
Mixed liquor samples were taken over a period of one year at the outlet of one of the aerated (gas diffusion) channels of Nancy-Maxéville (F) wastewater treatment plant (350 000 PE). The total treated flow rate was obtained from the plant manager. The daily average air temperature and rainfall were provided by the local Météo France station. Some samples were also obtained from the Braga (P) municipal wastewater treatment plant (45 000 PE), that is equipped with surface aerators.

The settleability characteristics were measured in a 10 l cylindrical column. The sludge concentration was measured by centrifugation of a sample aliquot for 15 min at 3000 rpm and drying the solid residue for 15 hours at 105°C. An optical microscope equipped with a video camera, connected to a PC via a grabbing board was used. A drop of mixed liquor was carefully deposited and covered with a

cover slip. A series of at least 70 images (magnification x100) is grabbed by a systematic examination of the slide. If necessary a second slide is used.

A procedure, called FlocMorph V.0, has been developed with the framework of Visilog™5 (Noésis, Les Ulis, France) (da Motta et al., 2000). The grey-levels images (768*576 pixels) are initially enhanced (delineation, halo suppression) and automatically segmented. On the resulting binary images the discrimination between flocs on one hand and small debris and filamentous bacteria on the other hand is based on their size: the objects still present after a 2-iterations opening are considered as flocs. The discrimination between small debris and filamentous is based on the size and the gyration diameter: objects with a projected area larger than 200 pixels and a gyration radius larger than 1 are filaments. The flocs that are not in contact with the image frame are characterised by their projected area (S) and fractal dimension (D_f). The fractal dimension is usually very sensitive to the definition of the contour of the particle. The method proposed by Russ (1995) has been used for its robustness. The floc diameter is defined as $D_{eq} = 2\sqrt{S/\pi}$. The number and the total length of filaments per image are calculated. The procedure is fully automated and the task of the operator is reduced to image grabbing. The result files are analysed using Excel (calculations of averaged values and standard deviations).

RESULTS

Figure 1 presents the results obtained by the monitoring of the Nancy-Maxéville plant for a one-year period. Large rainfalls were observed in winter 1999: the influent flow rate was kept almost constant and high (3500 m^3/d) between days 50 and 90. SVI decreases during that period. Simultaneously a decrease of the fractal dimension and of the amount of filamentous bacteria and an increase of the floc diameter are noticed.

For the rest of the year a diurnal variation pattern is observed for the influent flow rate, the average value of which is kept around 2200 m^3/d. Large rainfalls are again observed in early summer, between days 200 and 250: they seem to induce again a decrease of the fractal dimension, although it is not as large as in winter, and of the amount of filamentous bacteria. But this time the floc diameter decreases and SVI tends to increase. A similar event is observed in the fall, around day 300. No direct relationship was found between the different measured variables and more data should be collected to build a database containing enough weather events.

The activated sludge sampled in the Braga wastewater after an intense rain event present characteristics that are different from those observed in Nancy-Maxéville (Table 1).

TABLE 1: Comparison of Braga and Nancy-Maxéville activated sludge morphology at the same period of the year

Date	D_{eq} (µm)	D_f	Filament length (µm)
Braga		1.21	19
28/09/1999	31	1.22	70
29/09/1999	29	1.17	0
30/09/1999	29		
Nancy-Maxéville			
20/09/1999	46	1.30	1000
16/10/1999	46	1.33	2300

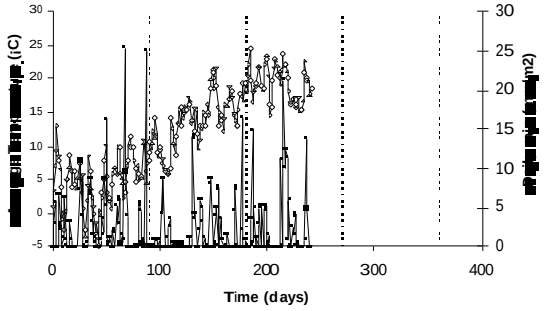
CONCLUSIONS

An automated image analysis procedure has been developed for the characterisation of flocs and filamentous bacteria. It has been used to monitor the activated sludge of a large municipal wastewater treatment plant, located in an area subject to a mild continental weather. The procedure has also been tested on an activated sludge sampled in a plant of different size and subject to very different weather conditions. Although the validation process is under continuation, the developed procedure produces

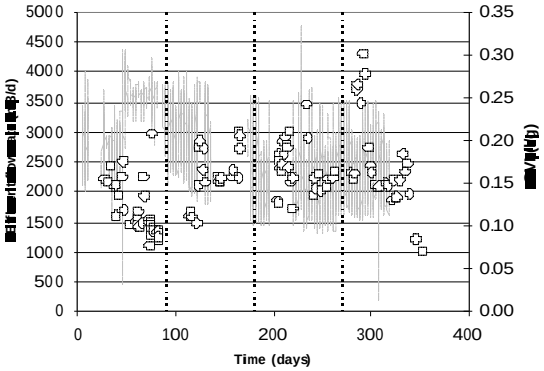
promising results for the characterisation of the flocs size and morphology and of the amount of filamentous bacteria.

Figure 1: Monitoring of Nancy-Maxéville activated sludge (a) average temperature and rainfall, (b) SVI, (c) floc diameter, (d) floc fractal dimension, (e) average filament length / image. Influent flow rate in grey in b, c, d and e.

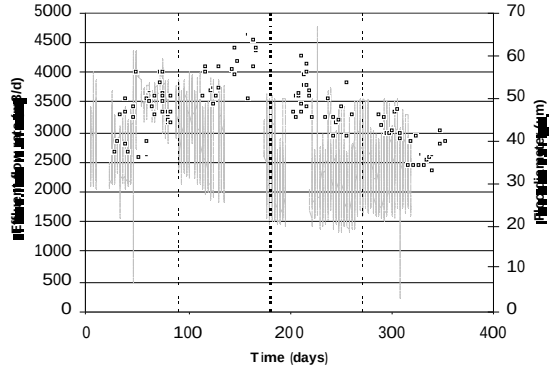
(a)



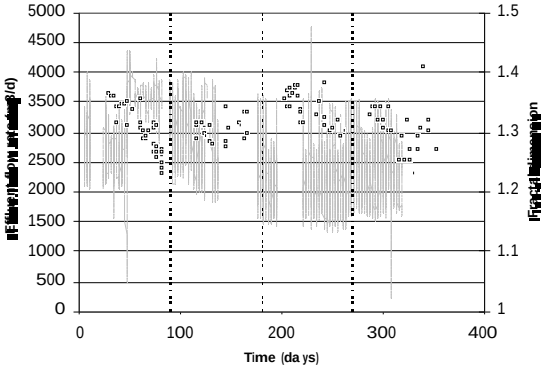
(b)



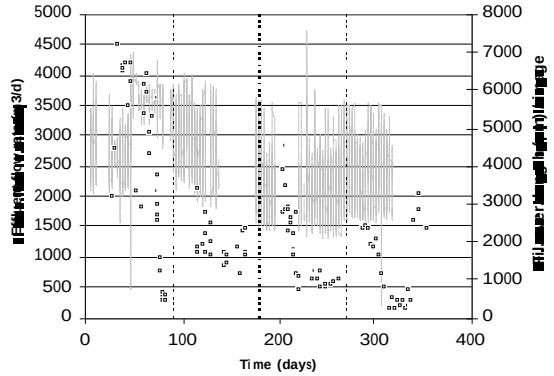
(c)



(d)



(e)



ACKNOWLEDGEMENTS

The authors are grateful to the Grand Nancy Council, the managers of the wastewater plants, Météo-France, CNPq (Brazil), the Embassy of France in Portugal and ICCTI.

REFERENCES

Eikelboom D.H., Andreadakis A. and Andreasen K. (1998) Survey of filamentous populations in nutrient removal plants in four European countries, *Wat. Sci. Technol.*, **37**, 281-289.

Grijspeerdt K. and Verstraete W. (1997) Image analysis to estimate the settleability and concentration of activated sludge, *Wat. Res.*, **31**, 1126-1134.

Da Motta M., Pons M.N., Roche N., Amaral A.L., Ferreira E.C. and Mota M. (2000) Analyse des flocs bactériens de la microfaune des boues activées par analyse d'image, submitted.

Richard M. (1991) *Activated sludge microbiology*, The Water Pollution Control Federation, 2nd ed., Virginia, USA.

Russ J.C. (1995) *The image processing handbook*, 2nd ed., CRC Press, Boca Raton.