Automated characterization of protozoa in activated sludge

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Protozoa play an important part in the wastewater treatment by activated sludge. They live using smaller microorganisms such as bacteria as feed by grazing. Information on their specific growth rates and response to operation conditions (availability of food) [1] is not yet well documented. However they have been found to be a good indicator of the good operation of a plant [2].

Protozoa are very diverse and their study is not easy. An automated species recognition would be useful for a systematic study. For that purpose the possibilities of characterization by image analysis have been investigated.

Activated sludge has been sampled in the aeration tank of treating municipal and brewery wastewater in separate lines. After sampling the protozoa have been maintained in an aerated tank. They have been examined by optical microscopy and monochrome images have been captured by a video camera. The commercial software Visilog™ (Noesis, France) has been used for the image treatment and analysis. The characterization is based on the silhouette of the microorganism. The determination of the silhouette is semi-automated as manual editing is generally necessary to separate the grazing protozoa from the sludge flocs. Five shape descriptors of the silhouette are calculated such as elongation factors and indices related to concavity [3]. These descriptors are size-invariant. A Principal Component Analysis [4] is then performed to reduce the dimensionnality of the problem and enable a graphical representation of the different types of protozoa.

About 20 different protozoa have been first manually recognized [5, 6] and their shape descriptors calculated. The main species are *Bodo edax*, *Vorticella convallaria* and *Vorticella microstoma*. Their abundance have been recorded over a 8-week period. The difficulties come from the different shapes which can be taken by an individual. The size range would probably be helpful to improve the performance of the protocol and obtain an automated characterization procedure.

[1] Nicolau, A., Lima, N., Mota, M., Madoni, P., *Boletim de Biotecnologia*, 56, 14-19, 1997.
[2] Drakides, C., *T.S.M. L'eau*, 2, 85-98, 1978.
[3] Pons, M.N., Vivier, H., Dodds, J., *Part. Part. Syst. Charact.*, 14, 272-277, 1997.
[4] Einax, J.W., Zwanziger, H.W., Geiβ, S., Chemometrics in Environmental Analysis, Wiley, 1997.
[5] Madoni, P., *Atlante Fotografico - Guida all'analisi microscopica del fango attivo*, Università degli Studi de Parma, AGAC, Reggio Emilia, 1996.
[6] Jahn, T.J., Bovee, E.C., Jahn, F.F., *How to know the protozoa*, The Pictured Key Nature Series, Wm.C. Brown Company Publishers, Dubuque (Iowa), 1979.