Image Analysis Applications in Biological Wastewater Treatment Processes

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Opportunities for Image Analysis Applications

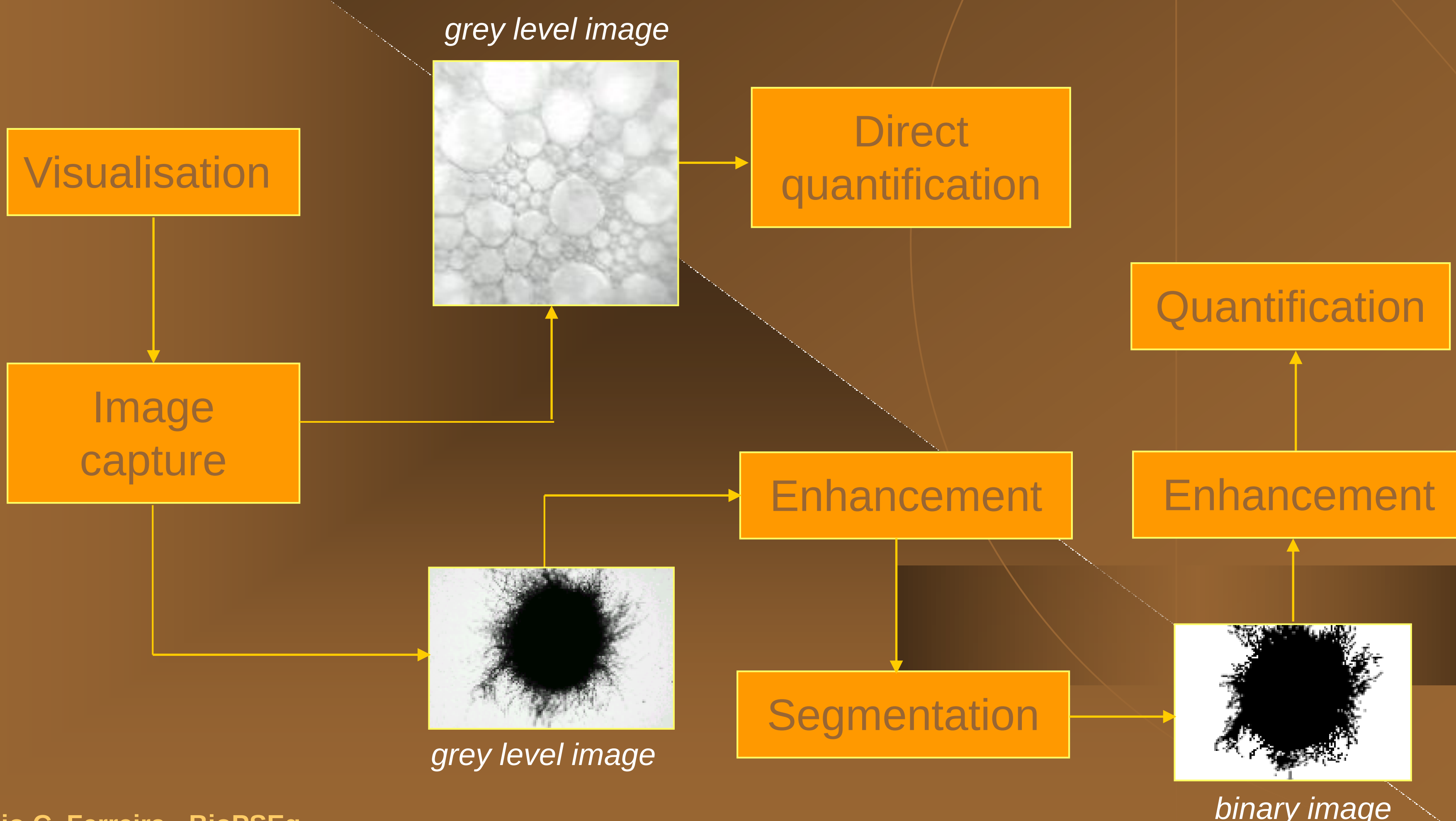
- ☞ Development of faster computers
- ☞ Advanced frame grabbers
- ☞ Sophisticated software

→ $/quality ↓

Image Analysis allows for:

- Enhancement of pictures
- Automatic identification and isolation of particles
- Fast means of getting morphologic information, thus saving tremendous effort and time

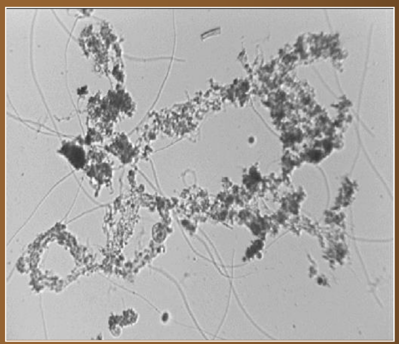
Principles of Image Processing

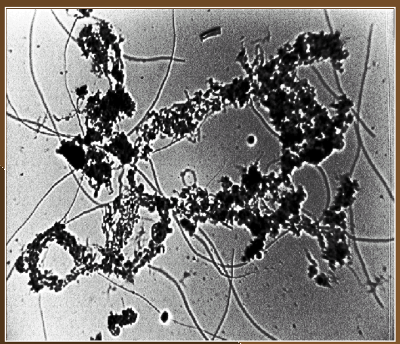


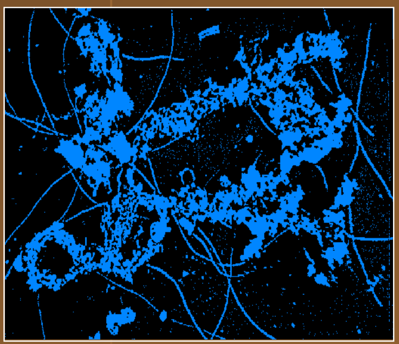
Application of Image Analysis Techniques in Wastewater Treatment

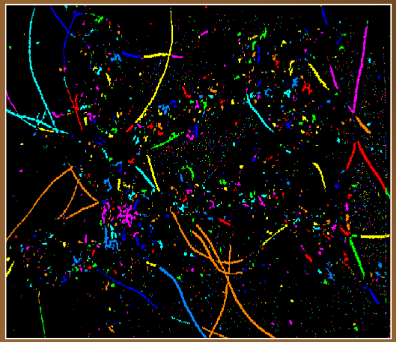
- Recognition and identification of several protozoa species present in Wastewater Treatment Processes (WWTP)
- Morphological Characterization of microbial aggregates in anaerobic digesters
- Morphological Characterization of microbial aggregates in aerobic reactors and abundance of filamentous bacteria in WWTP

IA in Wastewater Treatment – Activated Sludge

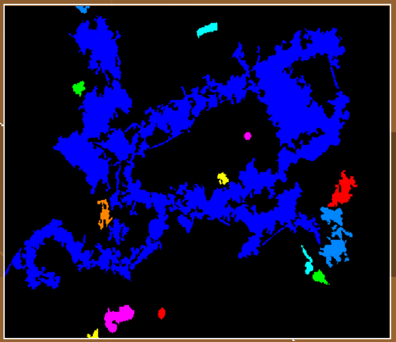

Initial Image


Enhancing


Binary Image


Labelling


Filaments

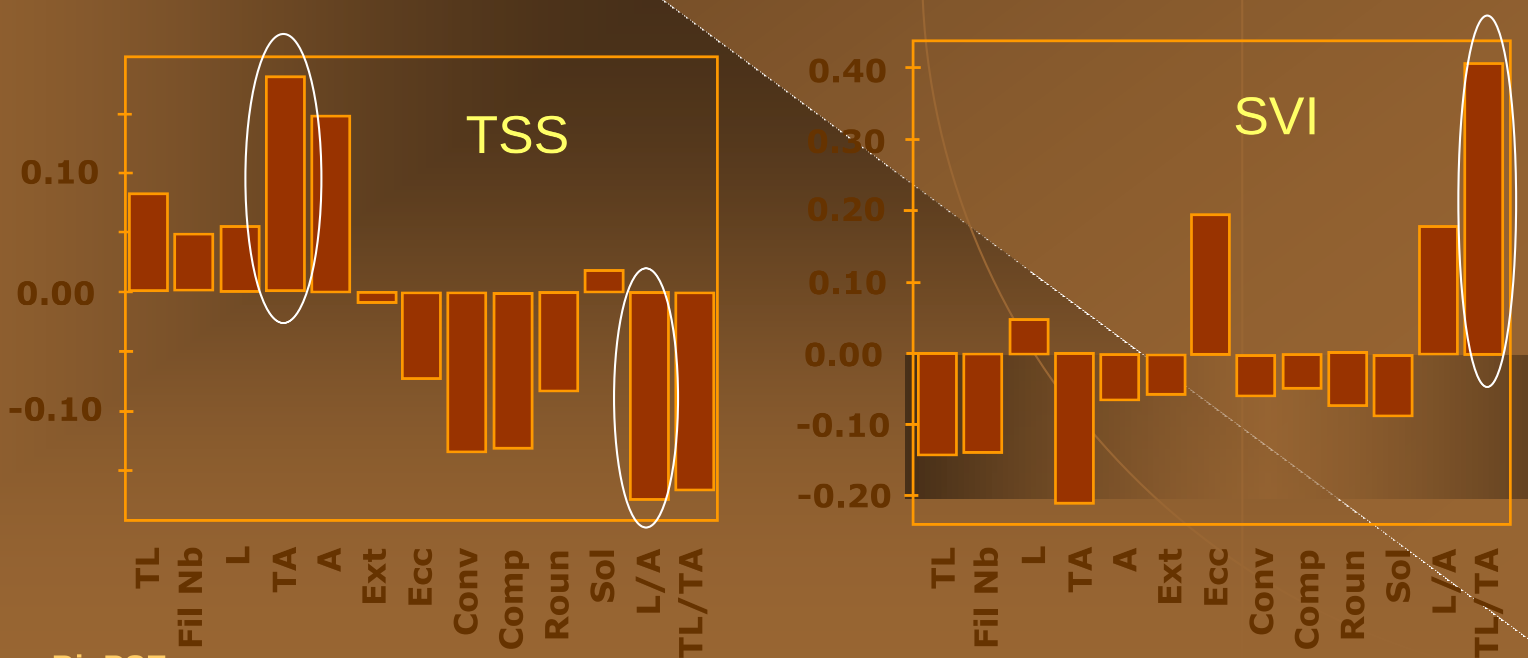

Flocs

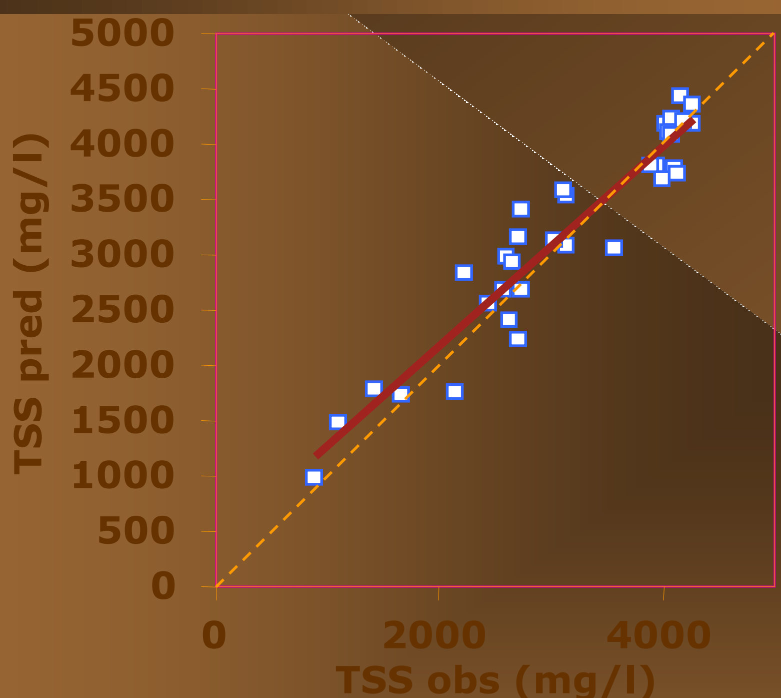
Morphological sludge characterization in a WWTP using Partial Least Squares

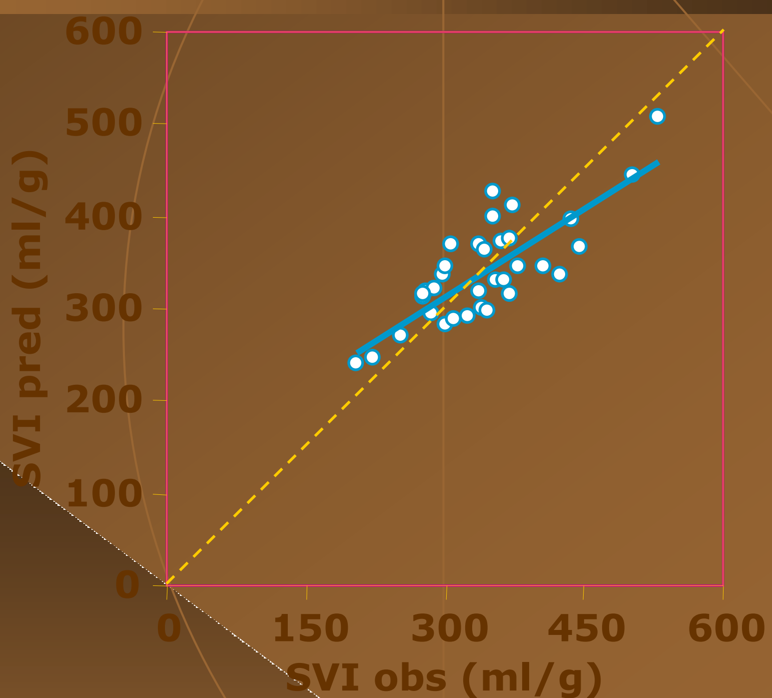
- The biomass present in a wastewater treatment plant was surveyed and their morphological properties related with operating parameters such as the Total Suspended Solids (TSS) and Sludge Volume Index (SVI).
- Image analysis was used to provide the morphological data subsequently treated by Partial Least Squares regression (PLS) multivariable statistical technique:
  - extracts linear combinations of the essential features of the original data while modelling the data dependence on the work set being therefore well suited for multivariate calibration.

Correlation with settleability, SVI

- PLS regression was performed to this dataset with the TSS and SVI as Y variables and the morphological parameters as X variables.
- 5 major aspects were studied: free filamentous bacteria contents (total filamentous bacteria length and number and filaments contents vs. aggregates contents); free filamentous bacteria characterization (filamentous bacteria mean length and filaments mean length vs. aggregates mean area); aggregates contents (total aggregates area and number); aggregates size (aggregates mean area) and aggregates morphology (convexity, compactness, extent, solidity, roundness and eccentricity)
- With respect to the PLS analysis for the TSS only 3 key aspects were studied: free filamentous bacteria contents; aggregates contents and aggregates morphology. The parameters most suited for representing each of these major aspects were found to be respectively TL/TA, TA and Convexity)



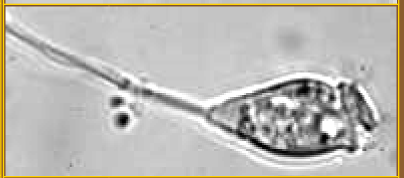
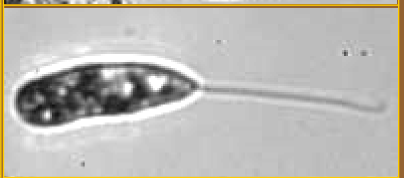
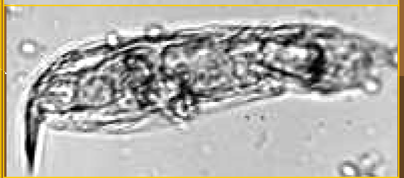




Amaral, A.L. Ferreira, E.C. Activated Sludge Monitoring of a Wastewater Treatment Plant using Image Analysis and Partial Least Squares Regression. **Analytica Chimica Acta**, in press

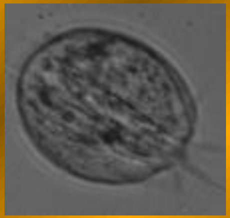
Automatic Recognition of Protozoa by Image Analysis

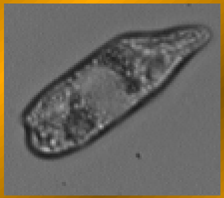
- Protozoa are commonly used as biological indicators of the performance of wastewater treatment. Their identification is not only time consuming but also demands high expertise.
- Programs were created to automatically analyse protozoa digitised images.
- A PCA and Discriminant Analyses techniques were explored for the species identification. Several protozoa species could be completely separated from the others.

Ciliates	Carnivorous	
	Crawling	
	Stalked	
	Free Swimming	
Flagelates		
Metazoan		
Testate Amoebae		

Ciliate Protozoa in Wastewater Treatment Plant


Colpidium


Glaucoma


Litonotus


Tetrahymena


Trachelophyllom

Free swimming


Epistylis


Zoothamnium


Opercularia

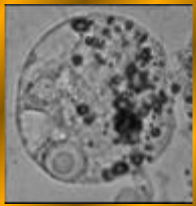

V. convallaria


V. microstoma

Sessiles


Euplotes

Crawling


Prorodon

Carnivorous

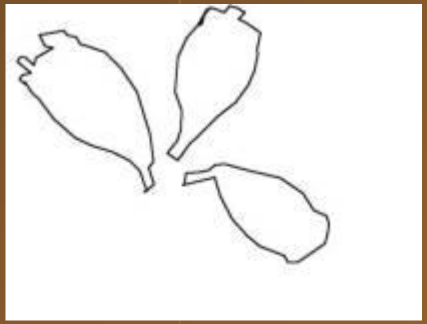
Some steps of the image processing programme (v. 1)

1. Initial image with a x400 magnification
2. Contour enhancement by histogram local equalization
3. Background suppression by opening and closing to remove the halo.
4. Semi-automated segmentation based on the Euclidian Distance Map.
5. When the protozoan is not in contact with the frame, part of the flocs are eliminated by a border-killing routine. The protozoan contour is closed by openings.
6. Hole-filling of the silhouette and semi-automated segmentation based on the Euclidian Distance Map.
7. Elimination of flocs by a series of erosion and reconstruction of the protozoa silhouette. If flocs are larger than protozoa, they are isolated and discarded by a logical subtraction.

Some steps of the image processing programme (v. 2)

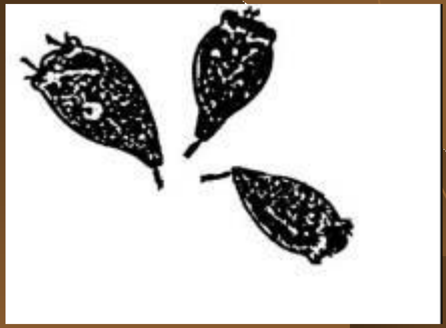
Acquired image

Pre-Treated image

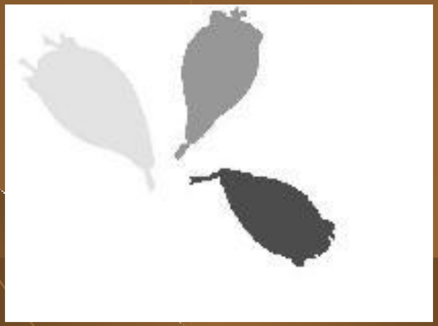


Regions of interest

Recovered protozoan



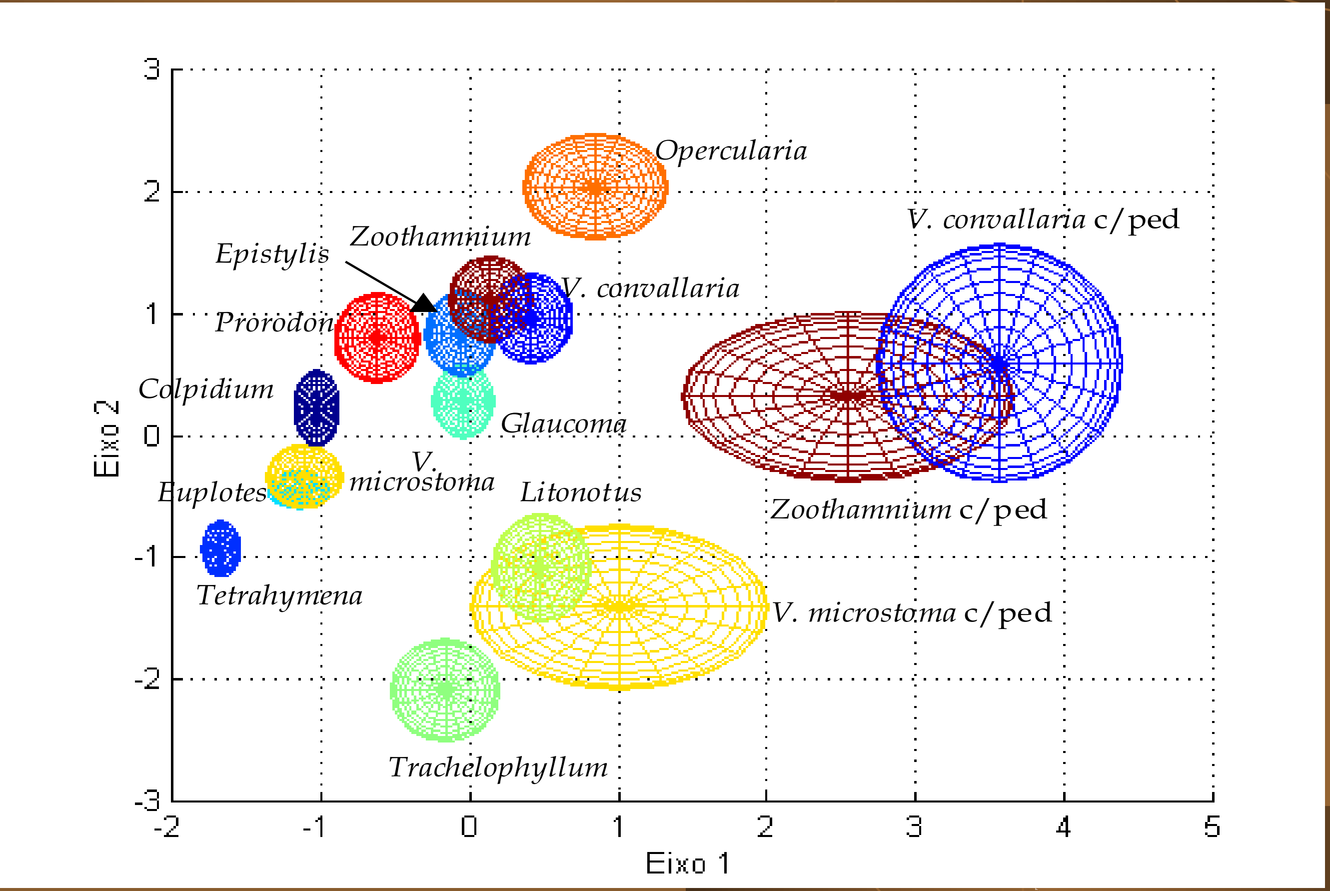
Binary image



Final labeled image

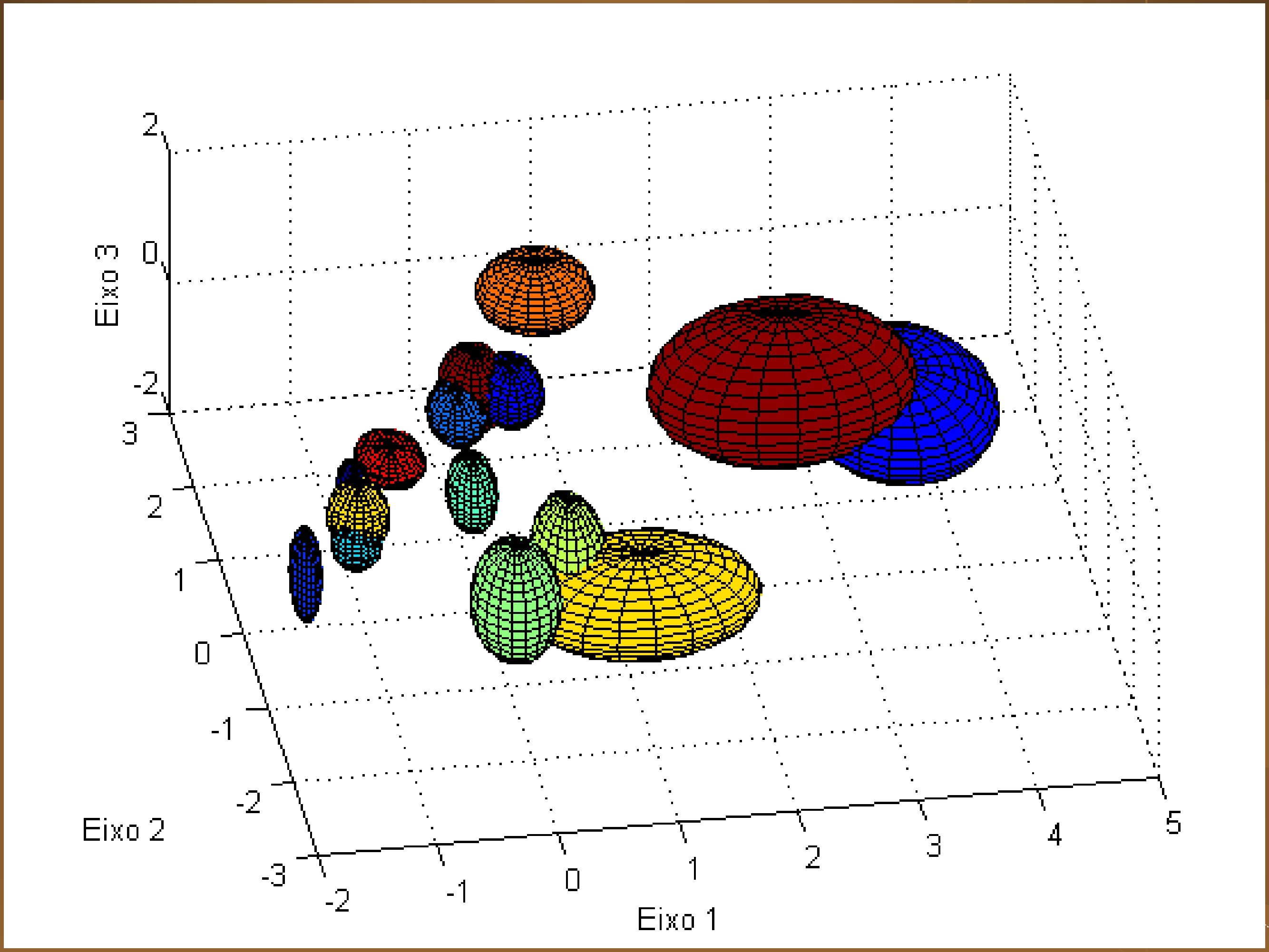
Principal Component Analysis

Axis:
Linear
combination
of
A/P Shape,
Feret shape,
Eccentricity,
Area,
Length



V. microstoma and *Opercularia* sp., indicators of a poor efficiency of a wastewater treatment, are quite well isolated, thus allowing the determination of possible anomalies in the performance of the plant.

3D



Species	% Rec.	
Nematoda	100 %	Very Good
Suctoria	98 %	
Trochilia	95 %	
Litonotus	90 %	
Peranema	88 %	Good
Arcella	86 %	
Trachellophyllum	86 %	
Euplotes	84 %	
Aelosoma	81 %	
Euglypha	80 %	
Aspidisca cicada	78 %	
Vorticella aquadulcis	78 %	
Monogononta	76 %	
Trithigmostoma	74 %	Reasonable
Digononta	71 %	
Vorticella microstoma	70 %	
Vorticella convallaria	66 %	
Epistylis	56 %	
Zoothamnium	52 %	
Carchesium	43 %	Poor
Opercularia	42 %	

	% Rec
Ciliates	94 %
Flagelates	88 %
Metazoan	86 %
Testate Amoebae	83 %

Ciliates	% Rec
Carnivorous	94 %
Crawling	92 %
Stalked	90 %
Free Swimming	86 %

IA in Wastewater Treatment – Protozoa

- Amaral, A.L., da Motta, M., Pons, M.N., Vivier, H., Mota, M., Ferreira, E.C. Survey of Protozoa and Metazoa Populations in Wastewater Treatment Plants by Image Analysis and Discriminant Analysis. **Environmetrics** 15:4, 381-390, 2004.

- Dias, N., Amaral, A., Ferreira, E.C., Lima, N. Automated image analysis to improved bead ingestion toxicity test counts in the protozoan Tetrahymena pyriformis. **Letters in Applied Microbiology** 37:3, 230-233, 2003.

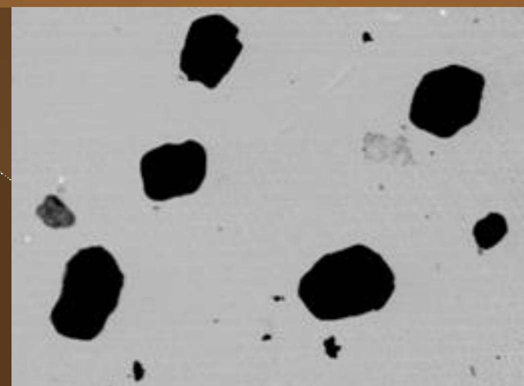
- da Motta, M., Pons, M.N., Vivier, H., Amaral, A.L., Ferreira, E.C., Mota, M. Study of Protozoa Population in Wastewater Treatment Plants by Image Analysis. **Brazilian Journal of Chemical Engineering**, 18:1, 103-111, 2001.

- Amaral, A.L., Baptiste, C., Pons, M.-N., Nicolau, A., Lima, N., Ferreira, E.C., Mota, M., Vivier, H. Semi-Automated Recognition of Protozoa by Image Analysis. **Biotechnology Techniques**, 13:2, 111-118, 1999.

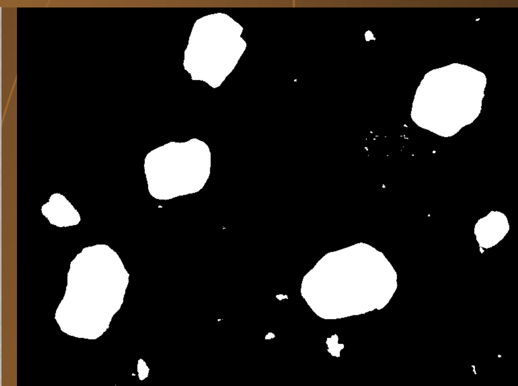
Granulation in Anaerobic Digestion

Some steps of the *Flocs* image processing

Acquired image



After background subtraction



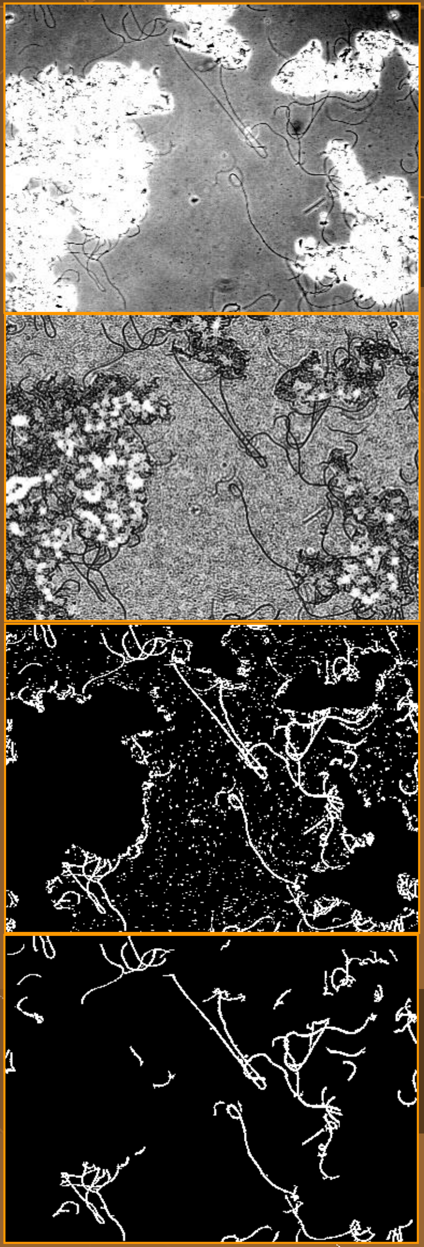
Final image

The *Flocs* program consists of three major parts:

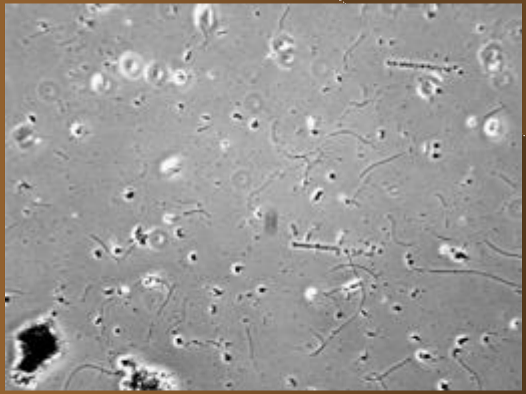
- **Image improvement and thresholding**: subtraction of background image and thresholding by a defined threshold.
- **Floc identification**: elimination of the objects (debris) smaller than 5x5 pixels; border-kill and labelling of the remaining flocs.
- **Floc characterisation**: determination of the morphological parameters area, equivalent diameter, breadth (minimum Feret diameter), and roundness.

The *Filaments* program consists of 3 major parts:

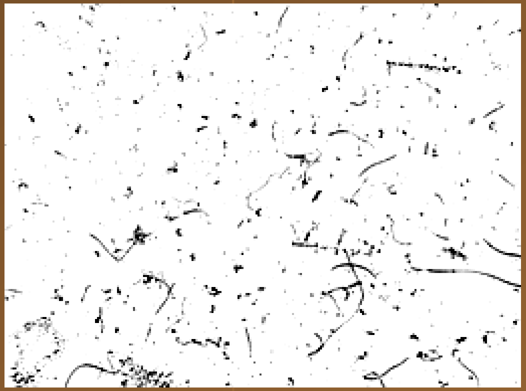
- Image improvement and thresholding: Mexican-hat filter; background homogenisation, Wiener filtering and histogram equalization. Subsequently, the image is thresholded by a defined threshold.
- Filament identification: skeletonisation; end-points removal (10 pixels length); reconstruct and labelling of the remaining filaments.
- Filament characterisation: determination of the parameters number of filaments and average filament length.

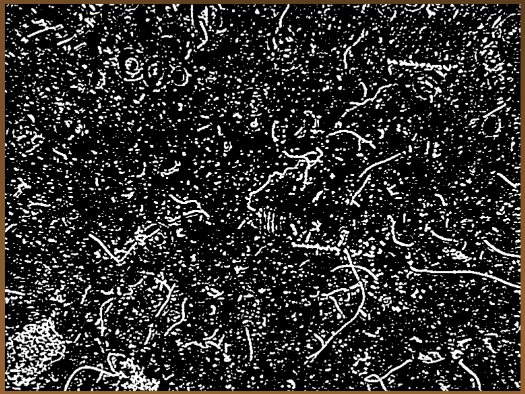


Some steps of the *Filaments* image processing


Acquired image


Mexican hat image


Homogenisation image

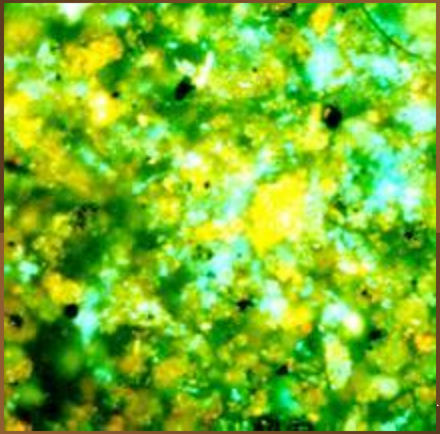

First binary image


Filaments image

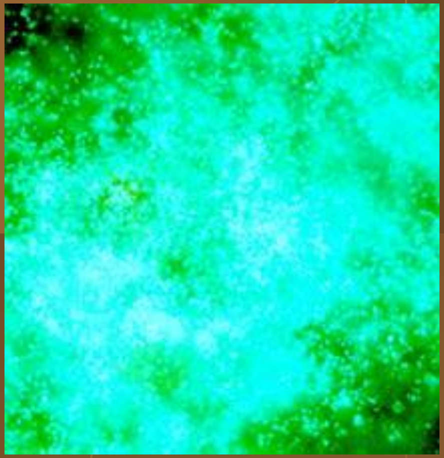
Monitoring Methanogenic Fluorescence by Image Analysis

- The co-factor F_{420} gives to the methanogenic bacteria the specific ability of auto-fluorescence when excited at a wavelenght of 420 nm. The Blue-Green (B-G) autofluorescence allows to differentiate between methanogenic and non-methanogenic bacteria.
- IA was used to quantify the B-G light intensity developed during the start-up of a CSTR fed with a VFA based synthetic substrate and during the S.S. operation of an anaerobic filter fed with a synthetic dairy waste
- A program was written to calculate the number of bacterial cells and its fluorescence intensity.

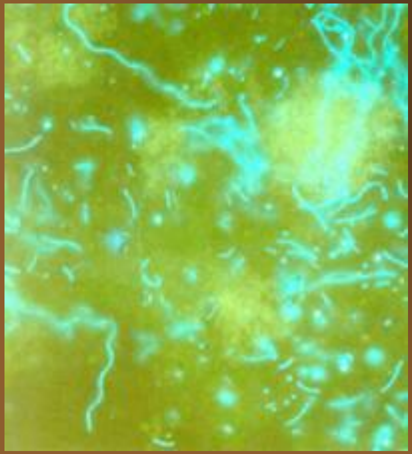
Examples of fluorescent anaerobic sludges



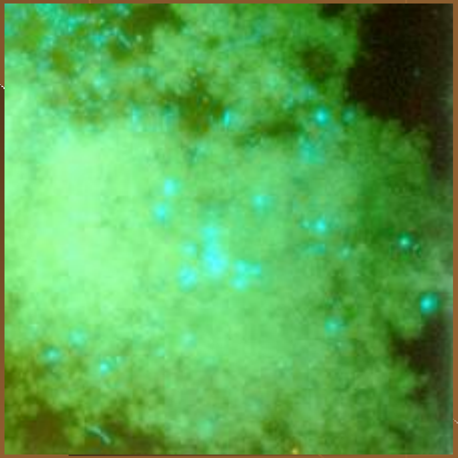
Low intensity



High intensity



Different morphologies



floc

Image Analysis in Anaerobic Digestion

- Characterisation by Image Analysis of Anaerobic Sludge from Two EGSB Reactors Treating Oleic Acid: Automatic Detection of Granules Disintegration
- Image analysis as a tool to recognize anaerobic granulation time
- Image analysis, methanogenic activity measurements and molecular biological techniques to monitor granular sludge from an EGSB reactor fed with oleic acid
- Characterization by Image Analysis of Anaerobic Microbial Sludge under Shock Conditions

Image Analysis in Anaerobic Digestion

- Amaral, A.L., Pereira, M.A., da Motta, M., Pons M.-N., Mota, M., Ferreira, E.C., Alves, M.M. Development of Image Analysis Techniques as a Tool to Detect and Quantify Morphological Changes in Anaerobic Sludge: II. Application to a Granule Deterioration Process Triggered by Contact With Oleic Acid. Biotechnology and Bioengineering 87:2, 194-199, 2004.
- Araya-Kroff, P., Amaral, A.L., Neves, L., Ferreira, E.C., Pons, M.-N., Mota, M., Alves, M.M. Development of Image Analysis Techniques as a Tool to Detect and Quantify Morphological Changes in Anaerobic Sludge: I. Application to a Granulation Process. Biotechnology and Bioengineering 87:2, 184-193, 2004.
- Pereira, M.A., Roest, K., Stams, A.J.M., Akkermans, A.D.L., Amaral, A.L., Pons, M.-N., Ferreira, E.C., Mota, M., Alves, M.M. Image analysis, methanogenic activity measurements and molecular biological techniques to monitor granular sludge from an EGSB reactor fed with oleic acid. Water Science & Technology 47:5, 181-188, 2003.
- Alves, M.M., Cavaleiro, A.J., Ferreira, E.C., Amaral, A.L., Mota, M., da Motta, M., Vivier, H., Pons, M.-N. Characterization by Image Analysis of Anaerobic Microbial Aggregates under Shock Conditions. Water Science & Technology, 41:12, 207-214, 2000.

Other Applications (Biotechnology and Food Technology)

- Classification of *Saccharomyces cerevisiae* morphology using image analysis
- Morphological Analysis of *Yarrowia lipolytica* under Stress Conditions through Image Processing
- Automatic counting of viable/non-viable yeasts by epifluorescence microscopy with acridine orange as dying agent
- Characterization of bubbles in a bubble column by image analysis
- Simultaneous monitoring of lactic acid bacteria and yeast during Vinho Verde fermentation using phase contrast microscopy coupled to image analysis

More Information about Projects and Resources may be browsed throughout the BioPSE group's web page

http://BioPSEg.deb.uminho.pt