

<p style="text-align: center;">Biofilm Science and Engineering- University of Minho, Portugal Research Activities in 2002</p>

A. Team

Team (Permanent doctorate staff):

Rosário Oliveira (coordinator), Maria João Vieira, António Brito, Joana Azeredo, Olívia Pereira, Regina Nogueira (since December 2002),.

Other researchers:

Post-doc: Pilar Teixeira (since October 2002),

PhD students: Regina Nogueira (until December 2002), Pilar Teixeira (until September 2002), Filipa Lopes, Ana Cristina Rodrigues, Cláudia Alves, Mariana Henriques, Manuel Simões, Nuno Azevedo, Nuno Cerca.

MSc students: Isabel Lopes, Helena Carvalho, Lúcia Chaves, Manuela Caçador, Bárbara Cardoso.

Research students: Ana Paula Pacheco, Sanna Sillankorva, Salomé Pais.

Consultants: Ian Sutherland (Univ. of Edinburgh), Bill Keevil (Univ. of Southampton) and Gerald Pier (Univ. Harvard).

B. Research areas

The scientific activity of this group is divided in three main areas:

I – BIOFILM SCIENCE (*scientist in charge Rosário Oliveira*), focused on microbial initial adhesion, composition and structure of biofilms;

II – BIOFOULING (*scientist in charge Maria João Vieira*), focused on detrimental biofilm formation in industrial and health related surfaces.

III – BIOLOGICAL WASTEWATER TREATMENT (*scientist in charge António Brito*), comprising studies on the development and application of biofilm reactors.

It must be highlighted that, although these three areas are considered separately, a perfect entanglement is established among the research activities of the members of the group.

C. Research Activities in 2002

The research activities developed according to the scientific areas of the group are summarized as follows:

I – BIOFILM SCIENCE

This area is divided in the following sub-areas:

- Microbial adhesion and surface phenomena;
- Biofilm structure and composition
- Physiology of adhered microbial cells

a) Microbial adhesion and surface phenomena

The studies on the influence of physico-chemical properties of surfaces and cell wall on the adhesion process were mainly focused on the adhesion of: *Staphylococcus epidermidis* to different types of catheter surfaces (hydrophobic and hydrophilic ones); *Candida albicans* and *Candida dubliniensis* to buccal prosthetic materials and *Salmonella enteritidis* to materials used in kitchen ware.

Adhesion of *Staphylococcus epidermidis*

An intensive study of the methodology used to study adhesion of *Staphylococcus* strains under static conditions was performed. The objective was to set a more accurate assessment of adhesion extent by establishing correct adhesion periods, washing procedures and cell enumeration. The adhesion assays were performed with ten clinical isolates of *S. epidermidis*, gently ceded by Dr. Gerald Pier, to glass, acrylic and silicon. Those strains were characterised in terms of their surface physico-chemical properties and cell wall elemental composition. This work was carried out by a PhD student through a Project financed by FCT.

Adhesion of *Candida albicans* and *Candida dubliniensis*

Adhesion to oral devices (HAP and acrylic) of two strains of *Candida dubliniensis* and two strains of *Candida albicans* was studied in the presence and absence of chemically well-defined saliva. In order to interpret adhesion results, cell surface physico-chemical properties (hydrophobicity and zeta potential) and the surface elemental composition was assessed. This work was carried out by a PhD student. The results were selected for oral presentation in the next Biofilm Club Meeting in the U.K.

Adhesion of *Salmonella enteritidis*

Cross contamination by *Salmonella enteritidis* in kitchen environment was assessed by studying the adhesion to kitchen ware materials of strains of *Salmonella enteritidis* isolated from the muscles and packs of commercially available chickens. Both strains and materials were also characterised in terms of their physico-chemical properties and elemental composition. This work was carried out by a PhD student in collaboration with the University of Londrina, Brasil.

b) Biofilm structure and composition

The methods developed in previous years to study the composition of the biofilm matrix of bacteria were adapted to yeast biofilms. This work was carried out by a master student.

c) Physiology of adhered microbial cells

The effect of antifungal agents on adhered cells of *Candida albicans* and *Candida dubliniensis* was assed and compared to that of planktonic cells. Cell activity was evaluated by XTT or epifluorescence microscopy using specific life/dead and activity fluorochromes. This work was carried out by a PhD and a master student.

II - BIOFOULING

Biofouling (Scientist in charge: Maria João Vieira)

This area is divided in the following sub-areas:

- Biofilm monitoring;
- Biofilm formation in industrial processes;
- Biofilm formation in health related surfaces (especially indwelling devices);
- Biofilm formation in drinking water systems, mainly devoted to the survival of pathogens.

d) Biofilm monitoring

The studies concerned with the detection of biofilms formed on surfaces using cyclic voltammetry were continued with the collaboration of the Physical-Chemistry Group of the Chemistry Centre (Irene Montenegro) by a research student (through the project "On-line biofilm monitoring of biofilms" financed by FCT). The work carried out was focused on the application of cyclic voltammetry to detect the

biofilms formed on the surfaces (those biofilms were also observed by a complementary method- epifluorescence- to evaluate the death and the removal on the surface) the application of different conditions during cyclic voltammetry to evaluate the impact of those conditions on the biofilm that is attached to the surface and the effect of the formation of hydrogen bubbles on the removal of biofilms from the surfaces.

e) Biofilm formation in industrial processes

These studies, carried out at lab scale, were focused on the **Chemical and Biological Control of Biofilms**, in **Biofilm formation by sulphate reducing bacteria and in Biofilms in milk industries**. They were carried out by three Master students, a Research Student (through project “Biological and chemical control of biofilms” financed by FCT), two PhD students (one began his work in September 2001)

The studies performed included:

Chemical and Biological Control of Biofilms

- *Studies on the effects of surfactants on P. fluorescens cells adhered to the surfaces the surfaces.*

The efficacy of different concentration of the surfactant CTAB in detaching *P. fluorescens* from glass surface was evaluated in a parallel plate flow chamber. This device enables “in situ” determinations of cells detachment following the application of surfactants under well-controlled hydrodynamic conditions. The results showed that SDS was able to remove almost all adhering bacteria in a short period of time, whereas CTAB did not promote much cell desorption. On the contrary, this surfactant increased the adhesion strength between cells and glass. Both surfactants promoted different alterations of cell surface properties, which explain their dissimilar effectiveness as cleansing agents.

The effect of the surfactant DBSA in the detachment of *P.fluorescens* was also studied using the parallel flow chamber.

- *Effect of biocides to control biofilms and suspended bacteria*

The effect of different protocols of application of ortho-phthalaldehyde to control biofilms formed under laminar and under turbulent flow was evaluated, showing that this biocide is not effective in the inactivation and in the removal of the biofilm

from the surface. It was also proved (which is an important result from an industrial point of view) that this biocide increases the mechanical strength of the deposit, turning difficult its removal from the surface. The effect of the biocide on the cells adhered to the surface was also studied by using the parallel flow chamber.

A master student performed work with *P.fluorescens* in suspension in order to assess the effect of OPA concentration, pH of the medium and presence of different protein concentrations.

- ***The use of bacteriophages to control biofilms***

This work has been done with the collaboration of Prof. Ian Sutherland from the University of Edinburgh. Part of the work was devoted to the improvement of methods to study bacteriophage infection. An expedite method to quantify the burst size was developed based on the quantification of bacteriophages by spectrofluorimetry. The effect of temperature and physiological state of bacteria on phage infection was studied in planktonic cells and biofilms. It was also studied the phage infection in adhered bacteria in a parallel plate flow device.

- ***Biofilms Formed by Sulphate Reducing Bacteria***

These studies were mainly focused on the study of the population dynamics using FISH in biofilms formed in a heating district system. The PhD student began the writing of her thesis in the end of 2002.

f) Biofilm formation in health related surfaces

A master student carried out work on the effectiveness of chemical disinfectants used in hospital to clean medical devices. This work carried out was focused on the action of ortho-phthalaldehyde, OPA, to control *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *E. coli* and *Staphylococcus epidermidis* adhered to stainless steel surfaces. The action of the biocide was assessed, in situ, microscopically using LIVE/DEAD® BacLight™ Bacterial Viability Kit, developed by Molecular Probes, conversely to the traditional techniques used to assess the disinfection of the devices. The objective of this work was to assess the effect of the biocide concentration and the presence of proteins.

g) Biofilm formation in drinking water systems (pathogens survival)

A master student, a research student (through project "Behaviour of *Helicobacter pylori* in drinking water systems" financed by FCT) and a PhD Student (part of his work is

carried out at the University of Southampton with co-supervision of Prof. Bill Keevil) developed the following work:

- development of drinking water biofilms, formed under turbulent flow on removable stainless steel surfaces and PVC surfaces on a flow cell, in the presence and absence of added nutrients
- the identification of the bacteria present in the water and in the biofilms were continued and their hydrophobicities were determined
- Studies concerned with tests and development of solid media to recover *H. pylori* from water were carried out as well as the effect of some growth factors (such as the atmosphere and the presence of copper)
- The novel the novel technology peptide nucleic acid (PNA) molecular probes was used to detect and locate the pathogen within the biofilms.

III- BIOLOGICAL WASTE WATER TREATMENT

As in previous years, during 2002 the research work in this area continued directed to biofilm reactors focused on the biological removal of nitrogen and of polycyclic aromatic hydrocarbons (PAHs). However, a turning point was achieved: two PhD's were concluded and presented. Regina Nogueira concluded her studies on wastewater nitrification and Ana Cristina Rodrigues finished the work regarding bioremediation of oily sludges using a sequencing batch biofilm reactor.

On the other hand, the subject of modelling of nitrification/denitrification processes was still carried out using a SBBR. Thus, a substantial work on Poly- β -hydroxybutyrate accumulation/consumption in the suspended biomass and in the biofilm was performed. This work was necessary to clarify some points revealed by *Aquasim* tool when an improved IWA activated sludge model was used.

Using the available knowledge on biofilm wastewater treatment systems, a project on modelling biofilm kinetics of polluted aquatic systems was prepared in late 2002 and thus an experimental model-lake is already under construction. The experimental work will begin in 2003 and will be carried out by the Post -doc Ana Rodrigues.

D. Projects:

On Going

	Project	
2001-2004	On line monitoring of biofilms formed in industrial equipment	FCT- POCTI Chemical Engineering
2001-2004	<i>Helicobacter pylori</i> survival in drinking water systems	FCT- POCTI Health Sciences
2001-2004	Chemical and Biological Control of Biofilms	FCT- POCTI Biochemical Engineering Biochemical Engineering
2002-2005	Cleaning and disinfection in dairy industries	INIA- Agriculture Ministry
2002-2005	Physiology of Staphylococci attached to indwelling devices	FCT- POCTI Health Sciences
2002-2006	Surveillance and control of microbiological stability in drinking water networks (SAFER)	EC- 5 th framework

Approved to begin in 2003

2003-2006	Adhesion of <i>Candida albicans</i> to prosthetic devices surfaces and epithelial cells	FCT- POCTI Biotechnology
2003-2006	Biofouling in Contact lenses: Formation and Control	FCT- POCTI Health Sciences

E. Post-graduate studies

PhD Theses in 2001

Concluded

- Teixeira, P., "Denitrification in RBC reactors", supervisor: Rosario Oliveira.
- Rodrigues, A.C., "Biological degradation of hydrocarbons using Sequencing Batch Biofilm Reactors" supervisor: António Brito.
- Nogueira, R., "Nitrification and denitrification in moving bed biofilm reactors: population dynamics, mass transfer and biological kinetics" supervisor: Luis Melo; co-supervisor António Brito.

In Progress:

- Lopes, F.A., "Interactions between biofouling and biocorrosion in cooling water pipes", supervisor: Luis Melo; co-supervisor Rosário Oliveira.
- Alves, C., "Modelling and optimisation of Sequencing Batch Biofilm Reactors for nitrification/denitrification, supervisor: António Brito.
- Henriques, M., "Formation of biofilms by *Candida albicans* and *Candida dubliniensis* to oral prostheses and cells of buccal epithelium", supervisor Rosário Oliveira, co-supervisor Joana Azeredo.
- Simões, M.J., "Control of biofilms formed in industrial systems: use of biocides and surfactants", supervisor: Maria João Vieira
- Azevedo, N.; "Behaviour of *H. pylori* formed in drinking water systems", supervisor: Maria João Vieira, co-supervisor: Bill Keevil (University of Southampton)
- Cerca, N., "Physiology of adhered Staphylococci to indwelling devices", supervisor: Joana Azeredo

MSc Theses

Concluded

- Lopes, I.A. "Control of detrimental biofilms – use of surfactants" – supervisor: Maria João Vieira, co-supervisor: Joana Azeredo.

- - Costa, T.P. "SBBR for municipal wastewater treatment: design and operation of a pilot plant" supervisor: António Brito

In Progress:

- Carvalho, H.F. "Control of detrimental biofilms – alternative biocides" supervisor: Maria João Vieira. co-supervisor: Olivia Pereira.
- Chaves, L. "Study of the kinetics of biofilm formation on surfaces in contact with drinking water" supervisor: Maria João Vieira
- Caçador; M. "Assessment of the effectiveness of chemical disinfectants used in hospital disinfect medical devices" supervisor: Maria João Vieira
- Cachada, B. "Resistance to antifungal agents of adhered *Candida albicans* and *Candida dubliniensis*. Supervisor: Joana Azeredo

F. International Co-operation

Apart from other less regular collaborative activities, the group maintained strong cooperation with the following foreign entities:

Ondeo - France	Valentina Lazarova	nitrogen removal, biofilm reactors.
University of Santiago de Compostela - Spain	Ramon Mendez and Juan Lema	nitrogen removal, biofilm reactors.
Technical University of Munich - Germany	Peter Wilderer, Stefan Wuertz and Michael Wagner	use of genetic probes to study population dynamics in biofilms, biofilm reactors
University of Aalborg - Denmark	Per Nielsen	biofilms and biocorrosion by sulphate reducing bacteria (SRB).
University of Coruna - Spain	Maria Carmen Veiga	mass transfer in biofilms
Technical University of Delft - The Netherlands	M. van Loosdrecht	Biotobio project
Technical University of Denmark - Denmark	Poul Harremoës,	Biotobio project
University of Lublin - Poland	E. Chibowski	determination of surface properties of materials.
University of Liverpool - United Kingdom	David Schiffrin	Biofilm monitoring.
Catholic University of Leuven - Belgium	M. Quirynen Department of Periodontology	culture of epithelial cells and adhesion of microorganisms to epithelial cells
University of Edinburgh - United Kingdom	Ian Sutherland Department of Cell and Molecular Biology	isolation of bacteriophages and their utilization as biofilm controlling agents
University of Southampton - United Kingdom	Bill Keevil	pathogens in drinking water.
University of São Paulo - Brasil	Claudete Paula Institut of Biomedical Sciences	adhesion of yeasts to buccal prostheses.
University of Londrina- Brasil	Teresa Oliveira	adhesion of food born pathogens.
University of Harvard - USA	Gerald Pier	Adhesion of Staphylococcus
University of Badajoz- Spain	Maria Luisa Gonzales	Microbial adhesion
University of Davis - USA	Stefan Wuertz	Biofilm reactors

National Co-operation

PORTUCEL- cellulose company Viana do Castelo	new methodology to reduce biofouling
Universidade de Aveiro	ambiguity of genetic code of <i>Candida albicans</i>
ÁGUAS DO CÁVADO water distribution company	Utilization of the drinking water pilot plant
Direcção Regional de Agricultura entre Douro e Minho	Disinfection in dairy industries
Lacticínios das Marinhas	Disinfection in dairy industries
Direcção Regional do Ordenamento do Território e dos Recursos Hídricos	Modeling of biofilms in polluted aquatic systems

G. Publications in 2002

International Journals

Publications in 2002

1. Alves, C., Melo, L.F., Vieira, M.J., Influence of medium composition on the characteristics of a denitrifying biofilm formed by *Alcaligenes denitrificans* in a fluidised bed reactor. *Process Biochemistry*, 37:8, 837-845, 2002.
2. Guimarães, C., Matos, C., Azeredo, J., Mota, M., Oliveira, R., The Importance of the Morphology and Hydrophobicity of Different Carriers on the Immobilization and Sugar Refinery Effluent Degradation Activity of *Phanerochaete chrysosporium*. *Biotechnology Letters*, 24:10, 795-800, 2002.
3. Henriques M., Gasparetto K., Azeredo, J., Oliveira, R., Experimental Methodology to Quantify *Candida albicans* cell surface hydrophobicity. *Biotechnology Letters*, 24, 1111-1115, 2002.
4. Nakari-Setälä, T., Azeredo, J., Henriques, M., Oliveira, R., Teixeira, J., Linder, M., Penttilä, M. Expression of a fungal hydrophobin in *Saccharomyces* cell wall: Effect on cell surface Properties and immobilisation. *Applied and Environmental Microbiology*, 68:7, 3385-3391, 2002.
5. Nogueira, R., L.F. Melo, U. Purkhold, S. Wuertz, M. Wagner. Microbial population dynamics versus nitrification performance in biofilm reactors: Effects of hydraulic retention time and the presence of organic carbon. *Water Research*, 36:2, 469-481, 2002.
6. Pereira, M.O., Kuehn, M., Wuertz, S., Neu, T., Melo, L. Effect of flow regime on the architecture of a *Pseudomonas fluorescens* biofilm, *Biotechnology and Bioengineering*, 78:2, 164-171, 2002.

7. Pereira, M.O., Morin, P., Vieira, M.J.; Melo, L. F. A versatile reactor for continuous monitoring of biofilm properties in laboratory and industrial conditions, *Letters in Applied Microbiology*, 34:1, 22-26, 2002.
8. Pereira, M.O., Vieira, M.J., Melo L. F., The role of kaolin particles in the performance of a carbamate-based biocide for water bacterial control, *Water Environment Research*, 74:3, 235-241, 2002.
9. Teixeira P., Oliveira, R., Metabolism of *Alcaligenes denitrificans* in biofilm vs planktonic cells, *Journal of Applied Microbiology*, 92:2, 256-260, 2002
10. Wagner M., Loy A., Nogueira R., Purkhold U., Lee N. e Daims D. (2002) Microbial community composition and function in wastewater treatment plants. *Antonie van Leeuwenhoek* 81, 665-680.

In Press

1. Azeredo, J., Henriques M., Sillankorva S., Oliveira, R., Extraction of Exopolymers from Biofilms: the Protective Effect of Glutaraldehyde, *Water Science & Technology*, in press.
2. Azeredo, J., Pacheco, A.P., Lopes, I., Oliveira, R., Vieira, M.J., Monitoring Cell Detachment by Surfactants in a Parallel Plate Flow Chamber, *Water Science & Technology*, in press.
3. Azevedo, N.F., Vieira, M.J., Keevil, C.W., Establishment of a Continuous Model System to Study *Helicobacter pylori* Survival in Potable Water Biofilms, *Water Science & Technology*, in press
4. Garcia-López, L., Veiga, M. C., Nogueira, R., Aparicio, A., Melo L. F. A technique using a membrane flow cell to determine average mass transfer coefficients and tortuosity factors in biofilms, *Water Science & Technology*, in press.
5. Giao, M.S., Montenegro, M.I., Vieira, M.J., Monitoring biofilm formation by using cyclic voltammetry - effect of the experimental conditions on biofilm removal and activity, *Water Science & Technology*, in press.
6. Rodrigues A.C., Wuertz S., Brito A.G., Melo L.F. Three-dimensional distribution of GFP-tagged *Pseudomonas putida* during biofilm formation on solid PAHs assessed by confocal laser scanning microscopy, *Water Science & Technology*, in press.
7. Simões, M., Carvalho, H, Pereira, M.O., Vieira, M.J., Studies on the behaviour of *Pseudomonas fluorescens* biofilms after ortho-phthaldehyde treatment, *Biofouling*, in press.
8. Simões, M., Pereira, M.O., Vieira, M.J., Monitoring the Effects of Biocide Treatment of *Pseudomonas fluorescens* Biofilms Formed Under Different Flow Regimes, *Water Science & Technology*, in press.
9. Vieira, M.J., Pinho, I.A., Gião, M.S., Montenegro, M.I "Use of cyclic voltammetry to detect biofilms", *Biofouling*, accepted.

Chapters in Books

1. Azeredo, J., Oliveira, R., "The role of Hydrophobicity and Exopolymers in Initial Adhesion and Biofilm Formation", *Biofilms in Medicine, Industry and Environmental Biotechnology*, O'Flaherty, V., Moran, P., Lens P. and Stoodley P. (Eds.), IWA Publishing, London (in press).
2. Nogueira R., Melo L. F., Purkhold U., Wuertz S. e Wagner M. "Nitrifying and heterotrophic population dynamics in biofilm reactors: effects of hydraulic retention time and the presence of organic carbon" *Biofilms in Wastewater Treatment: an Interdisciplinary approach*, S. Wuertz, P L Bishop and P A Wilderer (Eds.), IWA Publishing, London (in press) ISBN: 1843390078
3. Oliveira, R., Azeredo J. and Teixeira, P. The importance of physicochemical properties in biofilm formation and activity "Biofilms in Wastewater Treatment: an Interdisciplinary approach", S. Wuertz, P L Bishop and P A Wilderer (Eds.), IWA Publishing, London (in press) ISBN: 1843390078.

International Conferences

1. Azeredo J., Pacheco, A.P., Lopes, I., Oliveira, R., Vieira, M.J., "Monitoring Cell Detachment by Surfactants in a Parallel Plate Flow Chamber", *Proc International Specialized Conference on Biofilm Monitoring*, Porto, 2002
2. Azeredo, J., Henriques M., Sillankorva S., Oliveira, R., "Extraction of Exopolymers from Biofilms: the Protective Effect of Glutaraldehyde", *Proc International Specialized Conference on Biofilm Monitoring*, Porto, 2002
3. Azevedo, N. F., Vieira, M. J., Keevil, C.W., "Establishment of a Continuous Model System to Study *Helicobacter Pylori* Survival in Potable Water Biofilms", *Proc International Specialized Conference on Biofilm Monitoring*, Porto, 2002
4. Caçador, M., Vieira, M.J., "Assessment of the efficiency of an ortho-phthaldehyde based biocide against *Pseudomonas fluorescens* adhered to stainless steel surfaces", *Proc International Specialized Conference on Biofilm Monitoring*, Porto, 2002
5. Marques, M. S.; Montenegro, M. I.; Vieira, M. J. "Monitoring Biofilm Formation by Using Cyclic Voltametry-Effect of the Experimental Conditions on Biofilm Removal and Activity", *Proc International Specialized Conference on Biofilm Monitoring*, Porto, 2002 (oral communication)
6. Nogueira R., Melo L. F., Purkhold U., Wuertz S. e Wagner M. "In situ study of the population dynamics of nitrifiers in biofilms and suspended cultures", *Proc International Specialized Conference on Biofilm Monitoring*, Porto, 2002.
7. Simões, M., Carvalho, H, Pereira, M.O., Vieira, M.J., "Studies on the behaviour of *Pseudomonas fluorescens* biofilms after ortho-phthaldehyde treatment" *Proc International Specialized Conference on Biofilm Monitoring*, Porto, 2002
8. Simões, M., Pereira, M. O., Vieira, M. J., "Monitoring the Effects of Biocide Treatment of *Pseudomonas Fluorescens* Biofilms Formed Under Different Flow Regimes", *Proc International Specialized Conference on Biofilm Monitoring*, Porto, 2002