INSTITUTO SUPERIOR TÉCNICO

INSTITUTE FOR BIOTECHNOLOGY AND BIOENGINEERING

ENVERG | Environmental and Eco-Process Engineering Research Group

2008

Annual Report

Contents



Executive Summary

I am proud to present to you the 2008 report of the activities of ENVERG, the Environmental and Ecoprocess Engineering Research Group of IBB, Institute for Biotechnology and Bioengineering, the recently created Associate Laboratory of the Ministry of Science, Technology and Higher Education in Portugal.

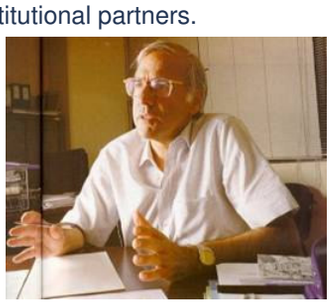
Thought initially as an Environmental Engineering Laboratory, it now also includes Ecoprocess Engineering in the title, due to the specific interests and expertise of some of its researchers. The group includes now thirteen Ph.D., eight of them being Faculty members. They teach Environmental Engineering in the Biological and Chemical Engineering degree courses as well as other matters such as Mass Transfer, Biotechnology Processes and Plant Design. They also participate in several units of the Environmental Engineering degree course.

The objectives of research are described in detail in the next chapters of this report, but will be outlined here. The area of constructed wetlands is one of the oldest in the laboratory and is now benefitting from studies at the plant molecular level and using new analytical equipment such as a LC-MS and a GC-MS. Biodegradation of industrial wastewater components (textile dyes, surfactants and their metabolites) in activated sludge sequencing-batch reactors has also been studied for several years. More recently, spectrometric monitoring (UV-vis, NIR and MIR) has been applied to municipal and industrial wastewaters and to waste sludge quality. In the field of solid waste management, characterization and optimization of solid recovered fuels (SRF) have been further developed and are now the target of industrial contracts. In a completely different field, benthic invertebrates are being studied in estuarine and river environments and an invasive species was already found in the Tagus Estuary. As to the line of the ecoprocess engineering research, an application for waste glycerol (a by-product of biodiesel production) was developed, namely the bacterial production of 3-hydroxybutyrate co-polymers in high cell density cultures. In a more basic level, mass transfer studies in gas-liquid-liquid systems are under way with application to biodegradation of gaseous effluents and/or biosynthesis of aromatic and biosurfactant compounds. The activities of ENVERG also contemplated work in the area of food (involving supercritical fluid extraction of substrates of plant origin to produce extracts with phytotherapeutic properties, namely antioxidants, or minimal processing of fruit compatible with extended high quality life) and in the area of health (studies on the adaptation of several strains to drugs contributed to the understanding of the responses of bacteria to antibiotics and antineoplastic agents and to the deterrence of established infection-related biofilms).

Research at ENVERG will proceed with the major concern of maintaining scientific quality at international standards while meeting the interests of the industry and of the institutional partners.

Júlio M. Novais

ENVERG Head






Environment and Eco-Process Engineering Research Group | ENVERG

ENVERG

ENVIRON

ECOPROCESS

The Environmental and Eco-Process Engineering Research Group (ENVERG) is a research unit in the development of innovative technologies for industrial application and industrial pollution control at the Centre for Biological and Chemical Engineering (CEBQ). CEBQ is the leading Centre of the Associate Laboratory Institute for Biotechnology and Bioengineering (IBB), a network of research centres across Portugal. IBB has been identified by the Portuguese Ministry of Science, Technology and Higher Education as a strategic infrastructure for the development of the Portuguese R&D and innovation policies in the areas of Biotechnology, Bioengineering, Biomaterials and Life, Biomedical and Agricultural Sciences.

Research activities at ENVERG are either financed by Fundação para a Ciência e a Tecnologia or through contracts with private industry and state departments. This fact hinders the number of possible publications, as some of the results cannot be released during a pre-fixed time and contributes to increase the number of grant holders that, for some time, are not working to get a degree. However, “public” research work is guaranteeing a high and international scientific level and is usually the basis for the specific industrial contracts.

ENVERG research priorities have special emphasis on Phytoremediation, Solid Waste Management, Monitoring of Waters and Wastewaters, Separation Processes, Novel Food Technologies and Products, Biotransformation and Transfer Phenomena in multiphase systems, featuring an integrated cross-disciplinary approach through two thematic lines:

- **Environmental Engineering (ENVIRON)**
- **Eco-Process Engineering (ECOPROCESS)**

Environmental Engineering

Objectives

The Environmental Science and Technology Laboratory of ENVERG pursues research and application activities in liquid effluent and solid waste monitoring, treatment and valorisation.

Research Topics

1. *Constructed Wetlands (CW) Modelling and Design* -Sewage has been treated for a long time in CW built according to a *rule of thumb*. Vertical flow (VF) CW using new support matrices for plants and microbes are being designed based on the knowledge of system pollutant removal kinetics and clogging phenomena hydrodynamics.

2. *Phytoremediation of Polluted Soils* - Self–regeneration of planted polluted areas has been observed albeit the mechanisms involved are frequently linked to *black-box* systems. The study of the role of plants in the detoxification of chemicals aims at correlating pollutants physical-chemical properties to bioavailability and phytodegradation mechanisms. Research at molecular scale is being pursued. *Phragmites sp.* and organic compounds are being used as model systems.

3. *Constructed Wetlands for swine effluent nitrification and denitrification* -Intensive piggeries farming are of major environmental concern due to the large amount of effluents produced with high solids and nutrient content. A nitrification/denitrification VFCW pilot system using light expanded clay aggregates and *Vetiveria zizanioides* is under study in order to enable full application. Mechanisms behind nitrogen high removals kinetics by plant enzymatic array are being established.

4. *Surface waters quality monitoring by macro -invertebrates communities* - Zêzere river is the main affluent of Castelo do Bode dam which is the supplier water body for 2.5 million inhabitants in the Lisbon region. Our main objective is to assess the ecological impact of several wastewater treatment plants discharges found in Zêzere river basin. Macro-invertebrates communities characterization will be also correlated with diffuse pollution by several endocrine disruptors.

Our interest is also to study the accompanying fauna and how it behaves in stressed or modified situations in Tagus estuary and even when its marine and freshwater limits are considered.

5. *Solid recovered fuels design and production* -Solid wastes could be considered as raw materials for different purposes and even highly contaminated fractions may suffer further processing and valorization. The production of solid recovered fuels with high calorific fractions enriched in biogenic content is understudy. Research focus on hygienization and stabilization of fractions with high biogenic content prior to the development of technologies enabling a safe storage and transportation are main goals to be achieved.

6. *Sequencing-batch reactor (SBR) technology* -The treatment of textile wastewater pollutants in activated sludge SBR is being investigated. Establishment of tests to assess the reductive biodecolourisation of dyes and the auto-oxidation sensitivity of the resulting amines, both involving UV-visible and infrared (NIR-MIR) spectral monitoring with chemometric data processing are underway. Online spectra acquisition is being implemented in laboratory SBR units, to monitor studies into the effects of SBR cycle and wastewater composition manipulation on dye decolourisation capacity. Aromatic amine desulfonation, as a first step in bio-mineralisation, is being studied with mixed and pure (*Rhodococcus*) cultures, with promising results.

7. *Spectral monitoring of wastewater and sludges* -The use of UV-visible spectra for the qualification (i.e., detection of change trends or abnormal situations) of industrial and municipal wastewater samples is being studied. The valid estimation of quality parameter values (suspended solids, COD, BOD) has been demonstrated. UV-visible and NIR-MIR spectroscopy with chemometric analysis is being also applied to wastewater sludges destined for agricultural use and to their receiving soils, aiming at the estimation of quality parameters of sludges (water and organic fractions) and the qualification of soils in the context of long-term monitoring.

Major Achievements

- Clarification of *Phragmites sp.* chemical stress answer to pollutants detoxification through survey of enzymatic array activities and genomic studies
- Development of a vertical flow constructed wetland model able to correlate systems hydraulic performance with clogging phenomena and overall system pollutant removal kinetics.
- Development of field sampling methodologies and laboratory sample preparation for heterogeneous materials from solid wastes.
- Establishment and publication of the Portuguese Standardization for Solid Recovered Fuels production and use (NP 4486:2008)
- Discovery of a not previously reported invasive species in the Tagus estuary (Mya arenaria)
- Continued development of the anaerobic-aerobic SBR bioreactor configuration applied to azo dye biodegradation, with insights into the fate of intermediate metabolites and the role of specific populations.
- Demonstration of the potential of spectral analysis (UV-visible, NIR-MIR) with chemometric processing for the qualification and quantitative monitoring of wastewaters and sludges.

Eco-Process Engineering

Objectives

The group aims at applying an interdisciplinary approach to tackle basic and applied topics in the development of sustainable bioprocesses, namely for gas cleaning and bioremediation, as well as for the production of bio-polyesters from renewable/waste sources and of value-added food and cosmetics and/or their ingredients.

Research Topics

The current projects cover (i) fundamental research on *G-L* mass transfer in multiphase systems, (ii) studies on the bacterial adaptation to organic solvents and anti-microbial agents, (iii) the upgrade of residual glycerol in the production of valuable bioplastics and (iv) the production of extracts with phytotherapeutic activity, namely antioxidants.

1. *Study of Multiphase Systems* -The development of *L-L-G* systems for VOC cleaning of off-gases by bacteria has been attempted, as well as the study of mass transfer limitations in such systems, namely mass transfer between: (i) gas and organic liquid; (ii) gas and liquid-liquid emulsions and also the effect of additives. The absorption of a gaseous solute into a two liquid system was studied in stirred tanks. The solute may be either oxygen or a VOC, or both. Applications include: (i) absorption of a VOC for biodegradation in the liquid phase(s); (ii) absorption of a volatile organic substrate for biosynthesis; (iii) a combination of (i) and (ii); (iv) oxygen supply for bioreactions in *L-L* systems.

2. *Bacterial Adaptation Studies* - Recently, we have focused on understanding the effect of organic solvents and of drugs on the cell membrane of gram-positive cells, with applications in Biocatalysis and Medicine and also on the adaptation of bacteria to high concentrations of xenobiotics for bioremediation purposes. The group has acquired, in recent years, a solid expertise on the monitoring of fermentation and biotransformation processes by image analysis. This has become an invaluable tool for the quantification of morphological and physiological aspects of single bacterial cells and the measurement of the extent and thickness of biofilms. Plus, strategies to prevent and destroy biofilms could be developed.

3. *Preparation of value-added food and cosmetics* -The main focus has been the implementation of environmentally clean processing technologies, namely supercritical fluid extraction, in the preparation of value-added food and cosmetics and/or their ingredients, from vegetable origin, as well as the evaluation and modelling of the methodologies and products obtained.

The group has also developed, in cooperation with Instituto Superior de Agronomia, packed and fluidized-bed enzyme reactors for the production of fragrance and flavor compounds and for the transesterification of fat bases for margarine. The operational stability of the immobilized preparations was evaluated and the lipase microenvironment was modelled in terms of substrate/product concentration and water activity.

4. Production of Bacterial Polyesters - Residual glycerol (GRP) from the biodiesel industry utilised as primary C source for cell growth and product accumulation can, to a considerable extent, contribute to lower large-scale production costs of polyhydroxyalkanoates obtained by bacterial cultivation. The process to obtain poly(3-hydroxybutyrate) (P(3HB)) from GRP has been optimised, in terms of production and productivity. Another production scheme on GRP as major C source is currently being developed for the polymer P(3-HB-co-4HB). By varying the % incorporation of 4HB, it was possible to obtain co-polymers with an array of different thermal and mechanical properties.

Major Achievements

- A bacterial population could be adapted to concentrations as high as 52% (v/v) toluene, which is much above the usually concentration required to kill most bacteria, 0.1% (v/v).
- The ability of bacterial cells to modulate their membrane in response to toxic compounds was used to influence the hydrophobicity of the cells. By influencing the hydrophobic or hydrophilic character of the membrane, biofilm formation or destruction could be promoted.
- Productivities up to 1.1 $g.L^{-1}.h^{-1}$ of P(3HB) were attained on the glycerol rich-phase from a biodiesel plant using high cell density cultures. To our knowledge, higher values were achieved only when noble substrates or surplus materials combined with noble substrates were used.

- The group has been successful in the area of minimal processing of fruit, an ever more demanding activity due to consumer acceptance and demand.
- Extracts from *Lavandula luisieri* and *Myrthus communis* with antioxidant activity similar to BHT were obtained.

Selected Publications

C.C.C.R. de Carvalho and M.J. Caramujo (2008) The Open Biotechnol J, 2, 235-246

N. M. Osório, M.H. Ribeiro, M.M.R. da Fonseca, S. Ferreira-Dias (2008) J Mol Cat B: Enzymatic 52-53, 58-66.

A. S. Pires, A. C. Nascimento, F. van Keulen, M.M.R. da Fonseca, S. Ferreira-Dias (2008), Eur. J. Lipid Sci. Technol. 110, 893-904.

R. Maceiras, S.S. Alves, M.A. Cancela, E. Álvarez, (2008), Chem. Eng. J., 137 (2) 422-427.

ECOPROC

Bacterial Adaptation Studies

- Application to Biofilms, Biocatalysis, Bioremediation and Medicine

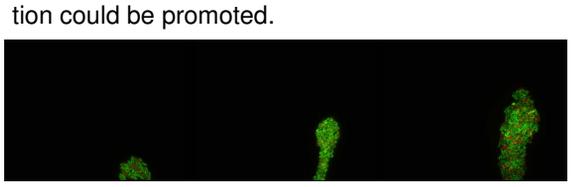
Carla C.C.R. de Carvalho, M. Manuela R. da Fonseca

1. Adaptation of bacterial cells to toxic substrates for application in

1.1 Biofilms

Bacterial cells have the ability to adhere to each other and to surfaces through an exo-polymeric matrix, creating cell communities called biofilms. These can be found in nearly all earth environments and, although beneficial in waste treatment processes, they are disadvantageous in the vast majority of cases, as they can decrease the efficiency of heat and mass transfer processes and increase corrosion. Biofilms are also a threat to public health since they are a source of contamination in water supply systems and in hospital environments.

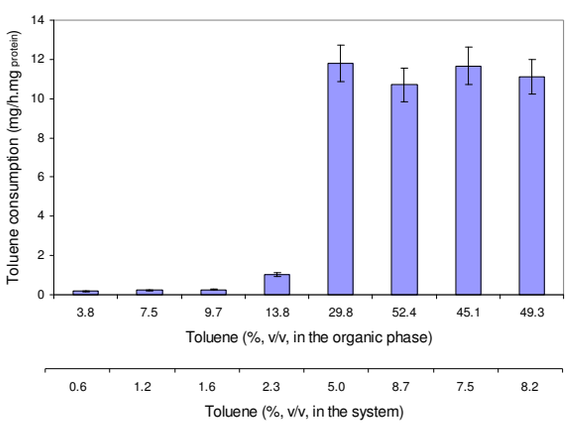
In the studies carried out in our laboratory, the ability of bacterial cells to modulate their membrane in response to toxic compounds was used to influence the hydrophobicity of the cells. By influencing the hydrophobic or hydrophilic character of the membrane, biofilm formation or destruction could be promoted.



1.2 Bioremediation

Leakage of petroleum, oils and combustibles occur throughout the world on a daily basis. To biodegrade their constituents (e.g. benzene, toluene, xylene, n-alkanes), the cells must be able to synthesise the appropriate enzyme(s) and be resistant to high concentrations of toxic compounds.

One of the objectives of our studies was the cleaning of gaseous and liquid effluents containing toluene. By adding slowly increasing concentrations of toluene in a mechanically stirred reactor, the bacterial population could be adapted to concentrations as high as 52% (v/v) toluene, which is much above the usually concentration required to kill most bacteria, 0.1% (v/v). Cross-resistance of the adapted cells to antibiotics and disinfectants was also evaluated.



1.3 Biocatalysis

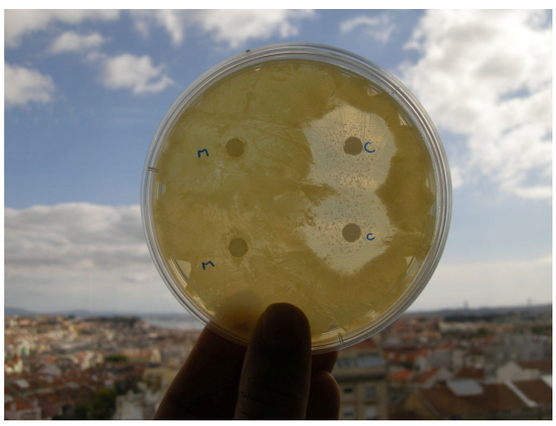
Most of the potential substrates used in the production of commercially interesting compounds (e.g. building blocks for the pharmaceutical industry, aroma compounds and food additives) present low water solubility. This can be overcome by the introduction of an organic phase to the biocatalytic system, which will act as a reservoir of substrates and/or products. The behaviour of the bacterial cells in the presence of this organic phase will determine the success of the biocatalysis or biotransformation process. The effect upon the cellular membrane will be crucial since cell viability, membrane potential and the transfer of substrates and products between the inside and outside of

the cell will depend on its integrity. By favouring the adaptation of bacteria to increasing concentrations of solvent, substrate(s) and product(s), we found a way to significantly increase the productivity and yield of these cells, when compared to their non-adapted counterparts.

The composition of exo-polymeric substances produced during the adaptation periods and its importance to the overall performance of the cells was also studied.

1.4 Medicine

The mechanisms involved in the adaptation of bacteria (e.g. *Staphylococcus aureus*, *Rhodococcus* and *Mycobacterium* strains, *Escherichia coli*) to antineoplastic agents were studied. Cross-resistance between these compounds and antibiotics was also evaluated.



2. Relevant techniques used

2.1 Analysis of FAMES from bacterial membranes

Since the major target of organic compounds toxicity is the bacterial membrane, the lipid composition of membranes was studied in detail before and after exposure to a toxic compound. The phospholipids were analysed after extraction and methylation as FAMEs by gas chromatography. In certain strains, the composition in glycolipids and neutral lipids was also evaluated after extraction, fractioning and methylation.

2.2 Fluorescence microscopy and image analysis

In biological processes, the physiological state of the cells is of paramount importance. In our laboratory, we've been using several fluorescent dyes to monitor the integrity of the cell membrane and the membrane potential, the intra-cellular pH and the adhesion properties of the cells. The positioning of the cells in organic-aqueous biocatalytic systems and the several stages of bacterial adhesion during biofilm formation was also monitored. Image analysis programs were successfully developed to transform the visual information from the acquired images into useful data to determine the values of the studied parameters.

Polyhydroxyalkanoates (PHAs) from waste glycerol

M. Manuela R. da Fonseca, Catarina Almeida

Biopolyesters are increasingly being considered as an alternative to conventional plastics. Being biodegradable, produced from renewable sources and (when adequately processed) biocompatible, they can advantageously replace many petroleum -derived plastics if obtained at competitive prices from improved bioprocesses.

Some microbial strains are able to synthesize and accumulate biopolymers under specific environmental conditions. Plus, 3-hydroxybutyrate (3HB) copolymers, *e.g*, poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P(3HB-co-4HB)) were found to have interesting properties in terms of polymer processing, as well as in the medical and pharmaceutical fields.

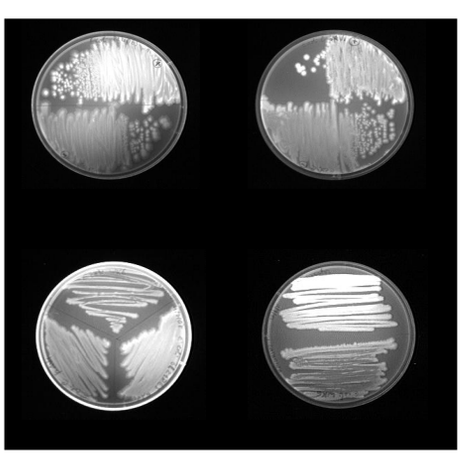


Fig. 1: Bacterial colonies stained with Nile blue; increased fluorescence results from higher intracellular PHA accumulation.

Fig. 2: Lyophilized cells and a film of bacterial P(3HB) obtained from waste glycerol.

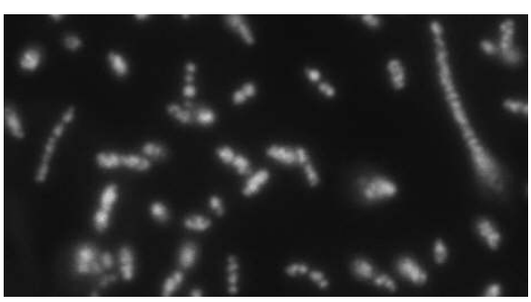


Fig. 3: *Cupriavidus necator* cells containing P(3HB-co-4HB) after Nile-blue staining of the polymer granules.

Economical evaluation studies indicated that about 48 % of the total PHA production costs is ascribed to the raw materials, in which the C source for growth and polymer accumulation can account to up to 80 % of the total cost [1]. Since glycerol is a by-product of the biodiesel industry, there is nowadays an excess of crude glycerol (GRP) in the market.

At ENVERG, non diluted waste glycerol from a biodiesel plant, originated during the transesterification of vegetable oils, is currently being tested as the main C source for PHA production by a wild type bacterial strain. Processes are being developed to reach high volumetric productivities and adequate end-product properties. Bench scale assays were developed and monitorized for polymer production both in a stirred tank reactor (STR) and in an air-lift reactor (ALR) on GRP and on commercial glycerol as control substrate.

P(3HB) was produced at high productivities with the wild type strain grown on GRP [2]. The same strain could accumulate P(3HB-co-4HB) at different 4HB monomer percent incorporation, again on GRP, using gamma-butyrolactone (GBLact) as precursor of the 4HB units.

Fig. 4: High cell density cultivation of *Cupriavidus necator* in the ALR

The highest molar monomer percentage of 4HB (12.4 %) was obtained in the STR at a volumetric productivity of 0.38 g $L^{-1}h^{-1}$ with a polymer accumulation of 40 %. In the ALR the PHA percentage accumulation was always higher than in the STR (attaining 82 %), but only in one cultivation 4HB monomers were found in the chain by H-NMR analysis at Université de Liège.

Extraction of PHB from lyophilized cells with chloroform followed by polymer precipitation in ethanol allowed for the recovery of a highly pure polymer, with a high average molecular weight (Mw) in the range of 3.2 to 6.7 x 10^5 Da and a PDI between 2.4 and 3.9.

Table 1–Results of some of the cultivations for the production of P(3HB-co-4HB) in the STR (rows 1-3) and in the ALR (row 4)

DW ($g.L^{-1}$)	Prod ($g_{pol}.L^{-1}.h^{-1}$)	4HB (%)	$Y_{P/S}$ ($g.g^{-1}$)
67.4	0.64	1.84	0.30
36.3	0.30	1.51	0.24
47.6	0.38	12.5	0.51
24.7	0.31	0.56	0.34

It was observed that, even at low % of 4HB monomers in the polyester chain, the properties of the copolymers differ from those of the homopolymer P(3HB), namely regarding changes in the crystallinity and melting temperatures. Differential Scanning Calorimetry performed at Université de Liège has suggested that the copolymers adopt polymorph arrangements. A significant and monotonous decrease of the glass transition temperature with the 4-HB % was observed.

Partners are searched to exploit the potential of the co-polymers in the production of composite materials for medical applications.

References

[1] Suriyamongkol, P., Weselake, R., Narine, S., Maloney, M. and Shah, S. (2007), Biotechnological approaches for the production of polyhydroxyalkanoates in microorganisms and plants – A review, Biotechnol. Adv., 25: 148 - 175.

[2] Cavalheiro, J.M.B.T. , de Almeida, M.C.M.D. , Grandfils, C. , da Fonseca, M.M.R. (2009) Poly(3-Hydroxybutyrate) Production by *Cupriavidus necator* Using Waste Glycerol, Process Biochem. 44, 509-515.

Phytoremediation Studies

- From Pilot to Molecular Scale

Susete Martins Dias, Júlio Novais, Filipe Freire, Luísa Davies

Phytoremediation systems, such as Constructed Wetlands (CW), have emerged as a cost effective, non-invasive and publicly acceptable way to address the removal of environmental contaminants. ENVERG has a pilot scale vertical flow CW (VFCW) planted with *Phragmites australis* (*P. australis*) used for trials, Fig. 1, which have led to successful industrial applications, and scientific studies, aimed at obtaining an in depth understanding of phytoremediation. These studies range from the development of a mechanistic model of the overall treatment process down to studies at molecular scale, including the identification of plant genes involved in phytoremediation. In 2008 our studies centred on the treatment of an azo dye wastewater. Dye wastewaters represent a challenge for conventional treatments, since dyes must have a high degree of chemical, photolytic and microbiological stability, due to the need to resist fading on exposure to sweat, soap, water, UV light and oxidizing agents in order to fulfil consumer requirements. Small amounts of dyes are highly visible in water courses and even when degradation occurs colourless aromatic amines with carcinogenic and mutagenic potential are released. The treatment of a synthetic wastewater containing the azo dye acid orange 7 (AO7) and of the resulting colourless aromatic amines from the azo-bond cleavage has been achieved using the pilot scale VFCW.

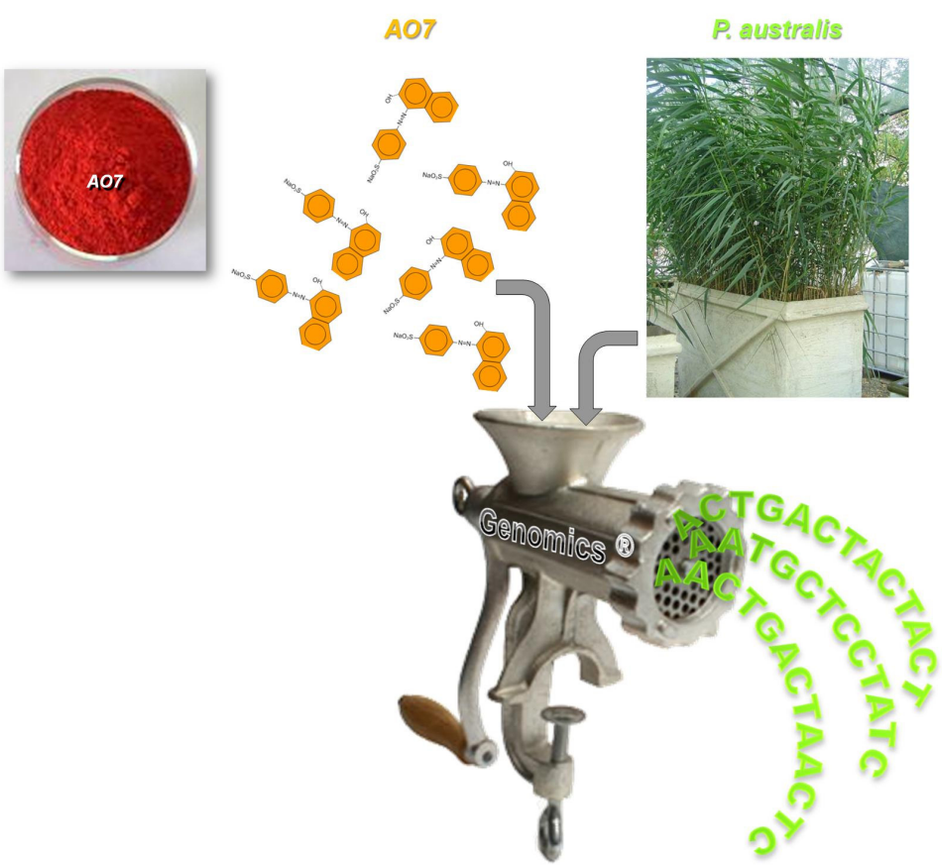


Fig. 1: *P. australis* oxidative stress response to Acid Orange 7

Modelling Constructed Wetlands

By regarding the VFCW as bioreactors working as CSTRs, Fig.2, we have developed a model that is able to predict the treated wastewater quality, the hydraulic profile of the system and an overall kinetic constant, taking into account all of the contributions from the chemical and biological processes, in function of the AO7 inlet concentration and hydraulic load applied. Rainfall and evapotranspiration are both accounted for. It enables us to predict the number of VFCW units operating in series and hydraulic retention times needed in order to obtain a treated wastewater fulfilling European discharge legislation. This is an important feature, enabling the reuse of the treated wastewater in the textile industry, irrigation of surrounding areas, toilet flushes, etc., making the CW a truly sustainable treatment solution. The modelling work was carried out as collaboration between ENVERG and CRERG.

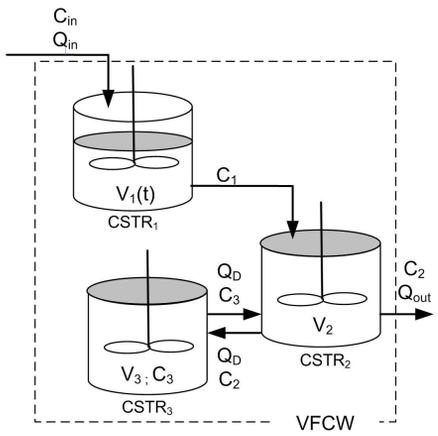


Fig. 2: Mechanistic analogy between the VFCW with CSTRs.

Role of Plants in Constructed Wetlands

Plant survival in CWs that are in use for a long time in the treatment of wastewaters containing xenobiotic compounds is proof that plants are able to biochemically self-engineer. At ENVERG, and as pioneers in this area, an in-depth study of the genes and enzymes involved in phytoremediation and how they can be induced is underway. Despite the fact that *P. australis* is one of the most common plants in CWs, the exact number of its enzymes involved in the metabolism of pollutants remains unknown, which is an awkward situation as there are many reed beds that seem to be very successful in the removal of pollutants from soil and water.

When AO7 is applied to the VFCW, reactive oxygen species (ROS) are generated by the photosynthetic system, followed by the activation or inhibition of the downstream signalling enzymes. Some genes that code for the enzymes involved in phytoremediation have already been identified by the isolation of mRNA transcripts (Catalase, Superoxide Dismutase and Glutathione Peroxidase) as a response to AO7 uptake. Therefore, it was possible to measure indirectly the level of ROS produced after AO7 was in contact with plant root system, Fig 3.

The *P. australis* gene expression and enzymatic

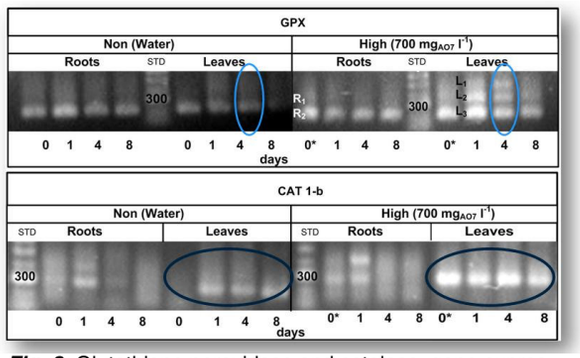


Fig. 3: Glutathione peroxidase and catalase gene expression in roots and leaves from 15 min to 8 days in the absence and presence of AO7

activities reached a maximum one day after feeding with AO7, Fig.4, thus indicating that plant response to the stress posed by the xenobiotic is a rapid process.

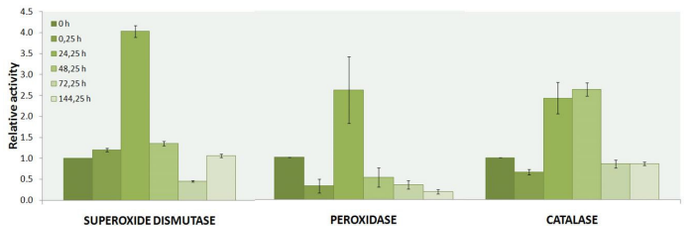


Fig. 4: *P. australis* foliar enzymatic activities

Currently, a parallel approach based on the identification of the *P. australis* proteome is underway, in collaboration with BSRG.

Production and design of Solid Recovered Fuels

Susete Martins-Dias, Júlio Novais

At ENVERG, a recent active research area on solid waste management, towards solid recovered fuels (SRF) is being carried out. In recent years an increasing demand for alternative fuels and its safe use led to the development of new reliable assessment of these fuels quality.

Main research focus on physical-chemical characterization of non hazardous wastes high calorific fractions (HCF) aims at the establishment of a neuronal network of different well characterized HFC as a tool for an optimized production scheme for tailor made and eco-friendly SRFs.

Since heterogeneous materials with different properties and contaminants content compose HFC, a quality assurance management of SRF production will be only possible if materials selection is based on reliable analytical characterization. Moreover, SRF biogenic content should be increased and biological stability guaranteed for safe storage and transport.

Sampling and major and minor elements characterization

HFC sampling procedures are scarce and since 2007 our research is focused on the development of successful plans for sampling and sample preparation based on material characteristics like, for instance, particle size and hardness.

Several shredding and milling stages were introduced in order to assure representativity at field and lab scale and cryogenic milling was also applied, avoiding volatile losses and ductile materials segregation.

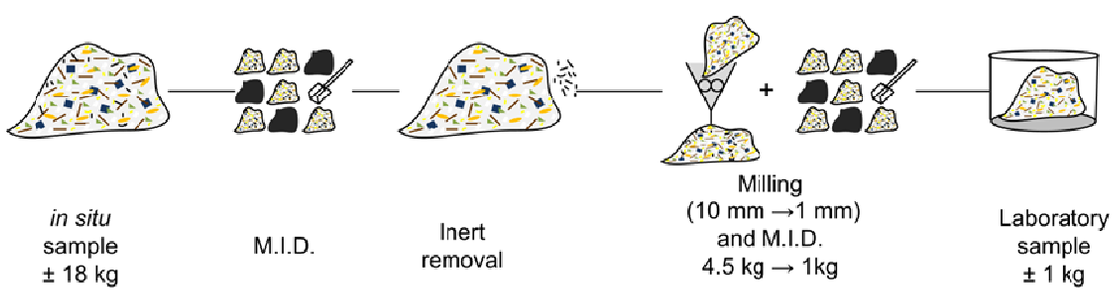


Fig. 1: *Schematic representation of the different stages of comminution and mass reduction steps involved in laboratory sample preparation (M.I.D.- manual increment division).*

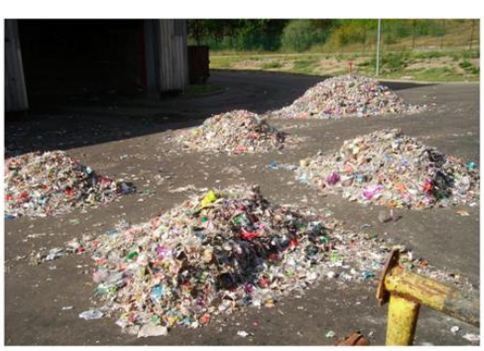


Table 1–*Influence of sample particle size on metals quantification*

d_{95} (mm)	10	4	0.5
Pb (mg/kg)	43	43	136
	73	38	128
	74	68	107
	89	47	166
	96	66	213
Cd (mg/kg)	**n.d.**	n.d.	2.5
			2.1
			4.8
			3.8
			3.5

SRF environmental impact is influenced for example by its metal content and respective volatility. In Table 1 some results are shown denoting the importance of the size of the sampling material for metals analysis, taking also into account that Cd is easily transferred to raw combustion gas and Pb distributes between bottom ash and raw gas.

Running projects, four in 2008, consist of public and private funded research tasks towards waste characterizing, development and optimization of industrial processes.

Main focuses of research are waste biogenic content, biological stability and the relation between calorific value and contaminants presence. Major goals are the development of new processes aiming at increasing biogenic fraction in SRF without compromising biological stability.

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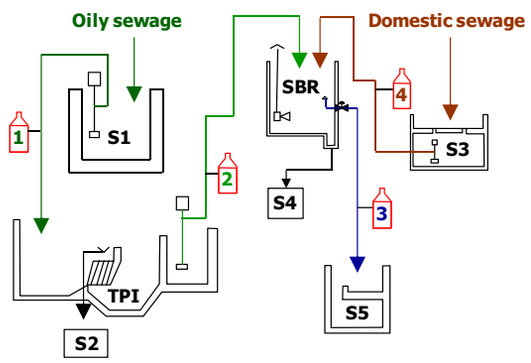
Wastewater Treatment Monitoring with UV -VIS Spectra and Chemometrics

Helena M. Pinheiro and Nídia D. Lourenço

Wastewater treatment monitoring traditionally consists of the quantification of incoming and treated wastewater quality parameters in order to assess the compliance of the legal requirements. However, the standard analytical methods currently used are most often applied off-line, are retrospective and time-consuming, not allowing a real-time knowledge of the parameters influencing the process performance. Moreover, the use of high-cost and/or toxic reagents and the production of wastes that require further treatment also contribute to a low frequency of analyses, preventing a real knowledge of the time-dependent wastewater characteristics. Thus, online sensors are fast becoming compulsory tools for wastewater quality monitoring. Ultraviolet-visible (UV-Vis) spectrophotometry is a fast and simple technique that has been increasingly considered in this context due to the availability of high-quality optical fibers and to the development of robust spectrometers. The potential of UV-Vis spectrophotometry coupled to chemometrics for wastewater quality monitoring was assessed in a fuel park wastewater treatment plant (WWTP), in several municipal WWTP's and in a laboratorial sequencing batch reactor (SBR).

Fuel park WWTP

UV spectra were coupled to Principal Component Analysis (PCA) to characterize samples taken from a fuel park WWTP (Fig.1) without using further analytical information. The score plot (Fig. 2) resulting from PCA identified two different groups of spectra, one (group 1) including the influents to the biological reactor (SBR) and the other (group 2) the treated wastewater samples. Among the latter, weekend (2a) and weekday (2b) samples



S1 - oily sewage sump
S2 - oil sump
S3 - domestic sewage sump
S4 - sludge sump
S5 - treated wastewater sump
TPI - Tilted Plate Interceptor
SBR - Sequencing Batch Reactor
- selected sampling points

Fig. 1: *S*implified scheme of the fuel park WWTP (CLC – Companhia Logística de Combustíveis, Aveiras, Portugal).

could be further distinguished. The score plot also allowed the tentative identification of employed process chemicals (lubricant and detergents) as residual contaminants in the treated effluent. This information can support the selection of monitoring investments in quality assessment and provide data for process and WWTP optimization.

Successful Partial Least Squares (PLS) calibration models were developed and validated (Fig. 3) using UV-Vis spectra of treated wastewater samples (sampling point 3 of Fig.1) and the corresponding analytical data obtained by reference standard methods (IST Laboratory of Analyses - LAIST) for three parameters legally required at discharge: Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD_5) and Total Suspended Solids (TSS). The use of these models for the fast, simple and cost-effective (no sample preparation, no reagents consumption) estimation of COD, BOD_5 and TSS can constitute a highly advantageous alternative to the Total

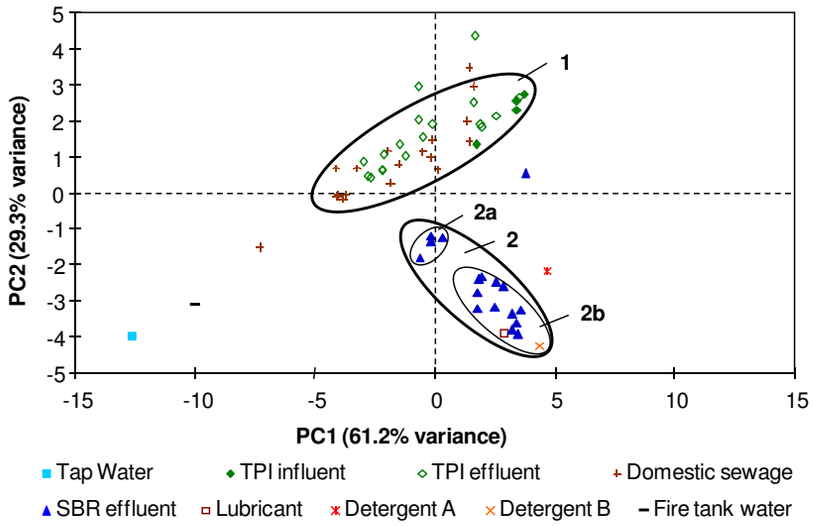


Fig. 2: Score-plot of the first two principal components from the PCA model of the UV spectra from the fuel park sample set. Group 1 includes the influents to the SBR and group 2 to the treated wastewater (2a - weekend and 2b - weekday).

Organic Carbon (TOC) analyzer installed at the fuel park for the on-line control of the discharged wastewater quality.

Municipal WWTP's

PCA was used to analyze spectral data from samples collected along three municipal WWTP process lines with different primary and secondary treatment units. The clustering observed in PCA score plots was mainly attributed to the suspended solids fraction present in the wastewater and highlighted differences in solids quality between plants and along the treatment lines. Thus, satisfactory Partial Least Squares (PLS) calibration models to estimate Total Suspended Solids (TSS) from the acquired spectra could only be established per plant. The results revealed the potential of the near-ultraviolet and visible wavelength ranges for suspended solids qualification and quantification, the latter in relation to the use of the standard gravimetric TSS method.

Textile dye decolorization in an anaerobic/aerobic SBR

A laboratory-scale SBR system was implemented and operated for the biodecolorization of textile dyes in the context of the research project PDCT/AMB/59388/2004 (FCT, Portugal). PCA of UV-Vis spectra of wastewater samples collected along successive SBR 24-h cycles allowed the detection of abnormal cycles with respect to the fed carbon source load, aeration conditions and the presence of an electron transporting additive. The collected UV-Vis spectra together with further on-line analyses will be used for Multivariate Statistical Process Control (MSPC) model development. These models allow the identification of deviations from normal process operation and the diagnosis of possible causes, enabling the application of early corrective actions for the prevention of non-compliant wastewater discharge.

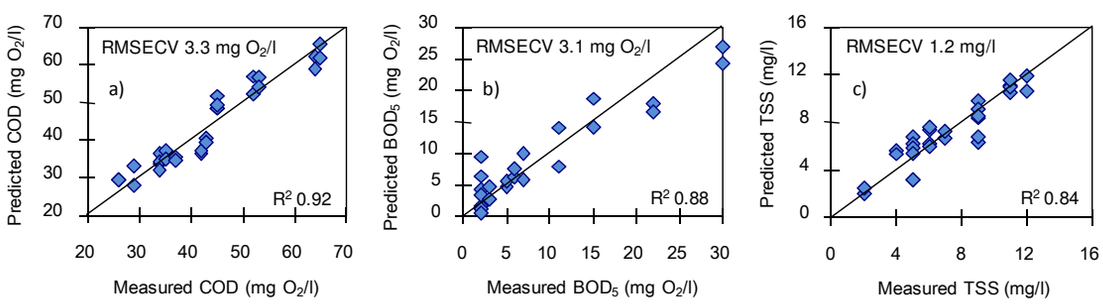


Fig. 3: Measured against predicted values for the PLS calibration models developed for COD (a), BOD_5 (b) and TSS (c) using UV-Vis spectra of samples from the fuel park treated wastewater. RMSECV - Root Mean Squared Error of Cross Validation.

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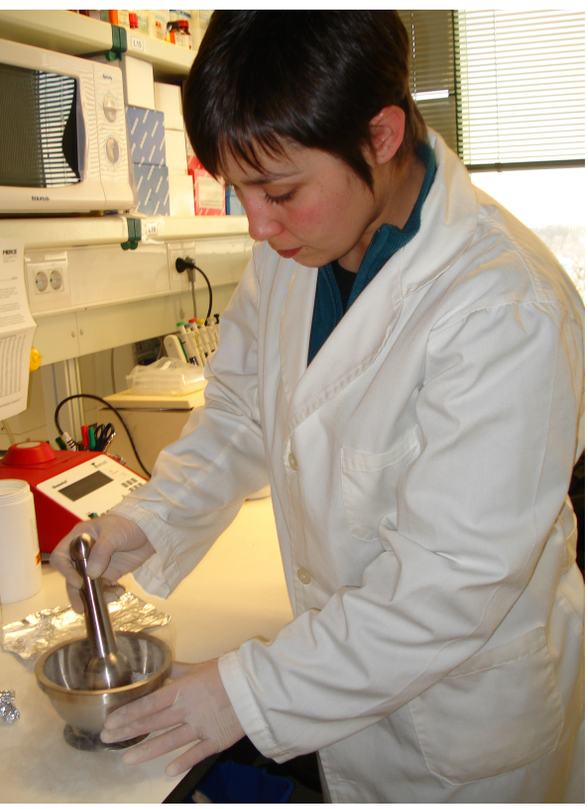

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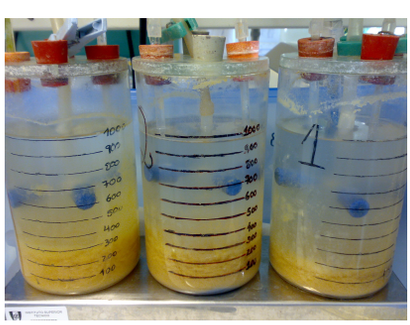

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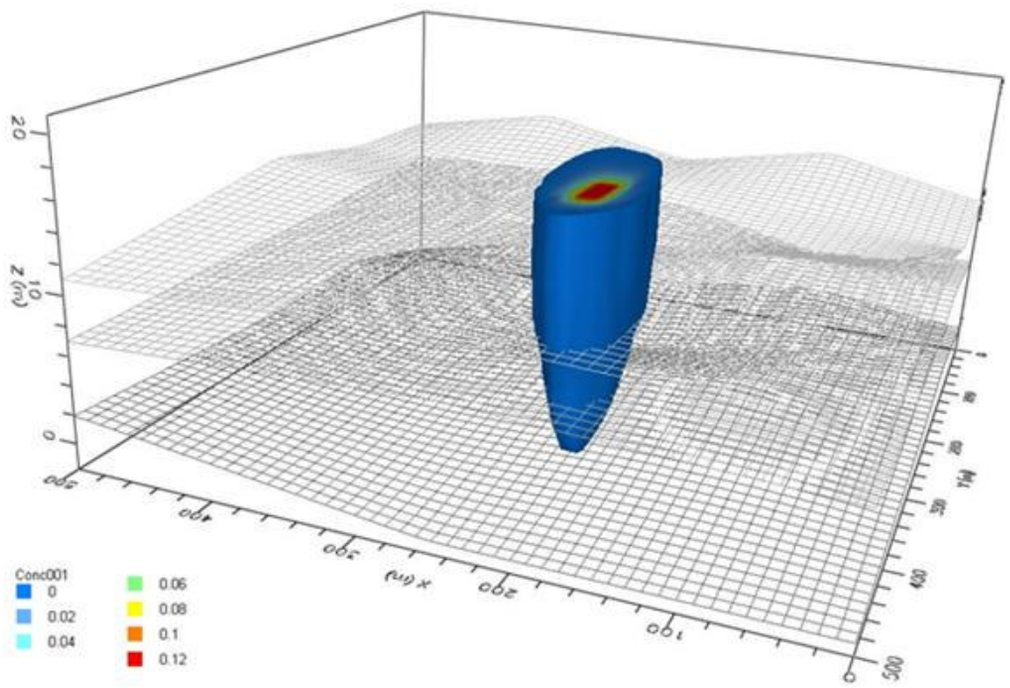

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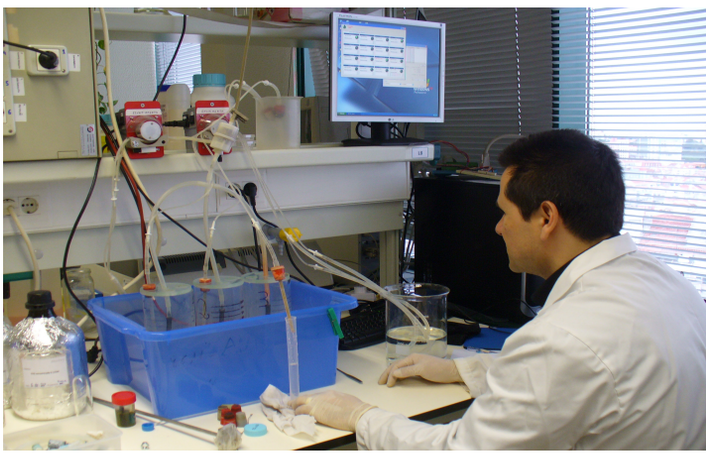

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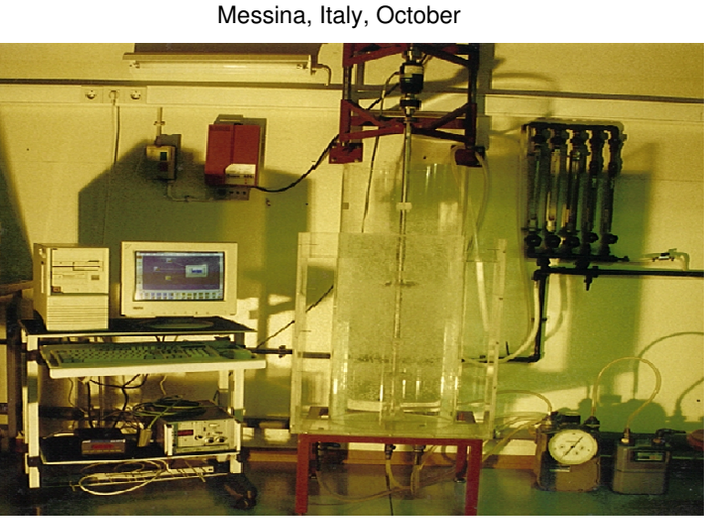

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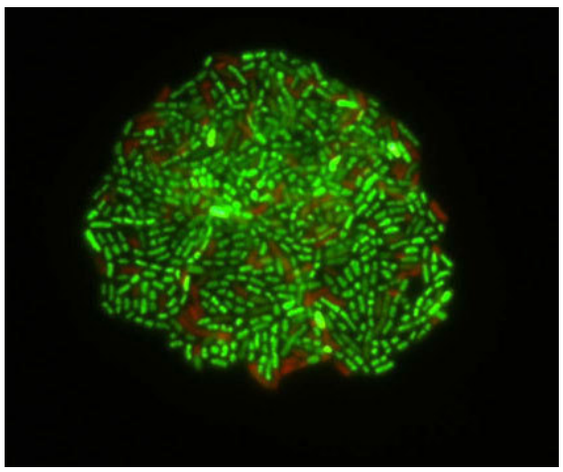

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Project Funding

Administração do Porto de Lisboa (IST Coordinator: *Júlio Maggiolly Novais*) R&D contract " Study of invasive species in the Tagus estuary"

CUF-QI (IST Coordinator: *Susete Martins Dias*) R&D contract "Phytoremediation of soils and waters contaminated with aromatic compounds"; "Development of tools towards Industrial site environmental management sustainability".

Secretaria Regional do Ambiente e Mar do GR dos Açores (IST Coordinator: *Susete Martins Dias*) "Reference and strategic documents to support decision makers on Clinical and Hospital wastes management"; Municipal Solid Waste Management and Construction and Demolition wastes. In cooperation with private experts and FCT-UNL.

MAXIT (IST Coordinator: *Susete Martins Dias*) R&D contract "Development of environmental applications for expanded clay aggregates". Support to co-combustion of alternative fuels IPPC licensing.

EDP (IST Coordinator: *Júlio Maggiolly Novais*) "Reference and strategic document on co-combustion of solid recovered fuels on Large Combustion Plants. In cooperation with IST Mechanical Enginnering Department and ISEG from the Technical University of Lisbon.

Empresa Geral de Fomento / Secretaria de Estado do Ambiente (IST Coordinator: *Susete Martins Dias*). "Reference and strategic documents to support decision makers on refuse derive fuels production and co- combustion".

Tratolixo (IST Coordinator: *Susete Martins Dias)* "Characterization of municipal solid waste high calorific fractions for solid recovered fuels production" .

Serurb/Lipor (IST Coordinator: *Susete Martins Dias*) " Reference and strategic document to decision makers on Refuse Derived Fuel production and its energetic valorization at the Municipal Incineration Plant, Lipor II"

PRIME/IDEIA/70/00326 (AdI) (IST Coordinator: *Gabriela Bernardo Gil*), "VALORALFA, Carob pulp valorisation for functional food ingredients"

PTDC/AGR-ALI/67194/2006 (IST Coordinator: *Gabriela Bernardo Gil*), "Nano-entrapment and controlled release of bioactive compounds to improve food quality and human health".

PTDC/AMB/67641/2006 (IST Coordinator: *Susete Martins Dias*; Principal Investigator: *Luísa C. Davies*) "Phytoremediation of soils contaminated by organic compounds".

PTDC/AMB/59388/2004 (IST Coordinator: *Helena Pinheiro*) "Wastewater treatment bioreactor operation for synthetic dye decolourisation".

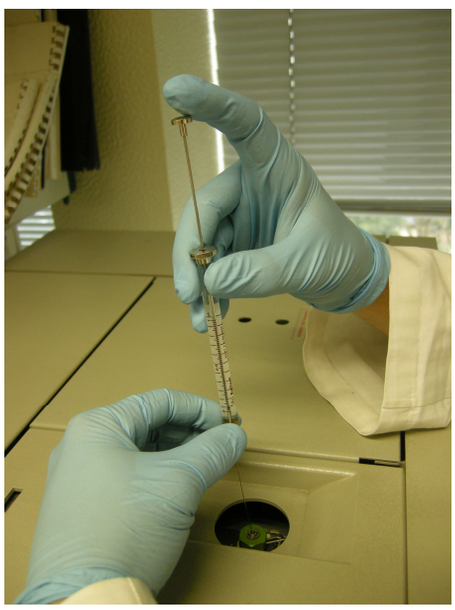
PDCT/AMB/59392/2004 (IST Coordinator: *Helena Pinheiro*) "Aromatic amine monitoring and degradation in the treatment of industrial wastewaters".

PPCDT/EQU/5972/2004 (IST Coordinator: *Sebastião Alves*) "Gas liquid mass transfer mediated by a second immiscible liquid"

EU Integrated Project NMP2-CT-2007-026515 BIOPRODUCTION - "Sustainable Microbial and Biocatalytic Production of Advanced Functional Materials"
(collaboration of *Manuela Fonseca* with ICTPOL).

Prizes

"Jovens Investigadores UTL/Deloitte 2008" award given to Carla C.C.R. de Carvalho by the Technical University of Lisbon and Deloitte in the area "Biotechnology"



Best Poster Award given to Carla C.C.R. de Carvalho for the poster *"Simultaneous degradation of linear and aromatic hydrocarbons by Rhodococcus erythropolis cells"*, C.C.C.R. de Carvalho, P.S. Ramos-Silva, S.S. Alves, M.M.R. da Fonseca, ESBES 7 - 7th European Symposium on Biochemical Engineering Science, Faro, Portugal, September

Best Oral Presentation given to Carla C.C.R. de Carvalho for the communication *"The importance of the bacterial membrane to biofilm formation*", IWA Biofilm Technologies Conference, Singapore, January

Thesis Award given to Sandra C. S. Calisto, Honorable Mention in the area of Quality for the thesis *"Interlaboratory Comparisons Contribution for Testing Laboratories"* (advisors: Mercedes Esquível (IST) and M.A. da Silva Trancoso (LAACQ – INETI)), 33º Colóquio da Qualidade, Évora, Portugal, November

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