INSTITUTE FOR BIOTECHNOLOGY AND BIOENGINEERING

Luísa Davies
Renata Ferreira
Carlos Antunes
Lília Alexandre
Luís Batista
Vera Ramalho
Adelaide Almeida
Gonçalo Cabrita
Filipe Freire
Júlio Novais
Susete Martins-Dias

PHYTOREMEDIATION MECHANISMS AND APPLICATIONS

enverg ENVIRONMENTAL AND ECO-PROCESS ENGINEERING RESEARCH GROUP
Phytoremediation tools and mechanisms

**Cost-effective** means of managing wastes using plants to enhance the degradation of polluted substrates (soil, water and air)

**Phytomechanisms:** Phytoextraction; Phytostabilization, Phytodegradation; Phytopumping; Phytovolatilization; Rhyzodegradation
Phytoremediation tools and mechanisms

**Cost-effective** means of managing wastes using plants to enhance the degradation of polluted substrates (soil, water and air)

**Phytomechanisms:** Phytoextraction; Phytostabilization, Phytodegradation; Phytopumping; Phytovolatilization; Rhyzodegradation

**Constructed Wetlands** are confined phytoremediation systems that mimic natural wetlands and enable effluent treatment under controlled operating conditions

**Applications:** Domestic, Municipal and Industrial wastewaters (sewage, wastewater from mining, swine, petrochemical, chemical, textile, aquaculture and food processing)
From Pilot to Full Scale (On going projects)

VFCW planted with *Phragmites sp.* treating azo dye contaminated wastewater

VFCW planted with *Vetiveria zizanioides* treating swine wastewater

Phytoremediation of soils contaminated by organic compounds

CW with light expanded clay aggregates matrix treating mine drainage and aquaculture wastewaters
From Pilot to Full Scale

**Vertical Flow Constructed Wetland**
Industrial effluent with nitroaromatic compounds (10000 m²), CUF-QI, 1994

**Horizontal Flow Constructed Wetland**
Denitrification in hydroponic reed bed (3000 m²), CUF-QI, 1999 (LECA®)

**Municipal Solid Wastes Leachates**

**Hybrid System**
Efluent from MSW Transfer Station (450 m²), Alcanadas, VALORLIS, 2000

**Hybrid System**
Landfill leachate treatment in Marinha Grande, (1200 m²) VALORLIS, 2003 (LECA®)
Research at ENVERG

Development of a mechanistic model able to understand the complex physical, enzymatic and hydraulic aspects.
(Design tool)

Role of plants in phytoremediation while integrated in constructed wetlands
(Enzymes activities, protein analysis and gene expression)

Applications of constructed wetlands at Full scale
Example of successful treatment of an azo dye (3 years)

\[ [\text{AO7}]^\text{IN} = 130 \text{ mg l}^{-1} \]

PILOT PLANT RESULTS \( (\text{C}_{16}\text{H}_{11}\text{N}_2\text{(OH)}\text{.SO}_3\text{Na}) \)

<table>
<thead>
<tr>
<th>HL (l m(^{-2}) d(^{-1}))</th>
<th>Inlet load ( (g_{\text{COD}} \text{ m}^{-2} \text{ d}^{-1}) )</th>
<th>COD Rem. (%)</th>
<th>TOC Rem. (%)</th>
<th>Colour Rem. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>5.82</td>
<td>91</td>
<td>89</td>
<td>98 - 99</td>
</tr>
<tr>
<td>40</td>
<td>10.3</td>
<td>93</td>
<td>90</td>
<td>98 - 99</td>
</tr>
<tr>
<td>53</td>
<td>11.2</td>
<td>86</td>
<td>86</td>
<td>82 - 97</td>
</tr>
<tr>
<td>108</td>
<td>26.1</td>
<td>89</td>
<td>85</td>
<td>74 - 84</td>
</tr>
</tbody>
</table>

Dilution of 1:20, no colour was detected, as required by EC Directives (76/464/EEC and 80/68/EEC)

- AO7 has 16 carbons
- TOC removal efficiencies (85-90%)
- Only linear aliphatic chains \( (C_2-C_5) \)

Davies et al., 2006. Water Research, 40, 2055-2063
Modelling the CW (Mechanistic analogy)

Davies et al., 2007. Water Science and Technology, 55, 127-134

This work was carried out in collaboration with CRERG-IBB
Mechanistic Model (MatLab 7.5)

Freire et al., 2009. Ecological Engineering, xx, xx-xxx
http://dx.doi.org/10.1016/j.ecoleng.2009.03.012

Non homogeneous bioreactors
• Rainfall and evapotranspiration are considered
• Richards’ equation describes water movement in variably saturated soils (large spatial heterogeneities)
• Nonlinear multivariate optimization of the model within the experimental data

\[ \frac{\partial \theta}{\partial t} = \frac{\partial}{\partial z} \left( k \frac{\partial h}{\partial z} \right) - \frac{\partial k}{\partial z} \]
Graphical User Interface

Ensaio
- data1.xlsx
- data2.xlsx
- data3.xlsx
- data4.xlsx
- data5.xlsx
- test.xlsx

Seleccionar Ensaio

Parametros

- kc: 0.1735
- ε: 0.1184
- α: 1.5763
- β: 65.234
- kd: 13.1728

Start

Modelo VasaFloWet

Caudal de Saida

Concentracao de Saida

Volume de agua Retida

Zoom

(c) FGF - IBB 2009
Research at ENVERG

- Development of a mechanistic model able to understand the complex physical, enzymatic and hydraulic aspects. (Design tool)

- Role of plants in phytoremediation while integrated in constructed wetlands (Enzymes activities, protein analysis and gene expression)

- Applications of constructed wetlands at Full scale
Plants cannot move away from stress!!!

Carias et al., 2008. Bioresource Technology, 99, 243-251

Plants are autotrophic but up-take water nutrients and pollutants from soil and groundwater

Recognition that plants can transform pollutants (metabolise Pesticides)

Plant enzymes from their natural defence system against a variety of Allelochemicals (peroxidases)

Plants can be seen as natural, solar-powered clean up vehicles for contaminated environments

Plants have an Enzymatic system to mineralize natural and exogenous xenobiotics
Plant Defence Mechanism Activation

**Pollutants**

- ROS – Reactive Oxygen Species
- GST (glutathione S-transferase)
- Metabolites (exudates)

**Unidirectional active transport**

**Conjugation reactions**

**Metabolic adjustments**

- ROS (reactive oxygen species)
- Metabolites (exudates)
- GST

**ROS generating reactions**

- ROS signal transduction pathway

**Metabolites**

- ROS scavenging mechanisms and gene overexpression of enzymes

The scavenging enzymes operate in order to eliminate and avoid protonation of $O_2^-$.

$H_2O_2$ is able to enter cellular membranes.

Prevent $OH\bullet$ formation that leads to cell death.
Wastewater treatment scale down

Glutathione Peroxidase and catalase gene expression in roots and leaves submitted to stress

Davies et al., 2009. Ecological Engineering, 35(6), 961-970

An indirect methodology has been established that enables the correlation between *Phragmites* Reactive Oxygen Species production induced by the AO7 through the measurement of mRNA accumulation
Genes sequenced so far...

- *Phragmites australis* putative catalase mRNA
  - Genbank accession number: DQ 786751

- *Phragmites australis* 23S ribosomal RNA, partial sequence, chloroplast; 301 bp rRNA linear
  - Genbank accession number: DQ 786752

- *Phragmites australis* glutathione peroxidase pseudogene mRNA, partial sequence, 383 bp mRNA linear
  - Genbank accession number: DQ 786753

- *Phragmites australis* 18S ribosomal RNA, partial sequence
  - Genbank accession number: DQ 786754
Towards *Phragmites’* Proteome (On going Work)

Differential analysis of the proteomes subjected to different oxidative stress induced by the pollutants

*Phragmites australis* leaves’ proteome (March 2009)

In collaboration with BSRG-IBB
Thank you for your attention!!!

luisadavies@ist.utl.pt