The use of biobeds for the depuration of wastewaters from the fruit packaging industry –
Turning from on-farm to post-farm applications

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Establishing the problem...

- **Fungicides** and **antioxidants** are applied for the control of postharvest fungal decays by *Penicillium* and physiological disorders (apple scald) in apples, pomes, citrus, oranges.

- Production of **large amounts of wastewaters** (10-100 m³) containing high pesticide loads (10-200 mg/L).
Fungicides used at EU level in fruit packaging plants

<table>
<thead>
<tr>
<th>Fungicides</th>
<th>Registration Status</th>
<th>Registration in member states</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imazalil</td>
<td>Yes until 31/7/2021</td>
<td>Cyprus, Greece, France, Spain, Italy, Portugal</td>
</tr>
<tr>
<td>Thiabendazole</td>
<td>Yes until 31/12/2015</td>
<td>Cyprus, France, Spain, Italy, Portugal</td>
</tr>
<tr>
<td>Ortho-phenylphenol</td>
<td>Yes until 31/12/2019</td>
<td>Cyprus, Greece, Spain</td>
</tr>
</tbody>
</table>
Antioxidants used at EU level in fruit packaging plants

<table>
<thead>
<tr>
<th>Fungicides</th>
<th>Registration Status</th>
<th>Registration in member states</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethoxyquin</td>
<td>Not inclusion in Annex I since 2009</td>
<td>-</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>Not inclusion in Annex I since 2009</td>
<td>120 days authorization was granted in Greece, Spain, Portugal</td>
</tr>
</tbody>
</table>
All those pesticides share a common paragraph in their registration documents.....

Member States should pay particular attention to ensure that appropriate waste management practices to handle the waste solution remaining after application, including for instance the cleaning water of the drenching system and the discharge of the processing waste are put in place. Prevention of any accidental spillage of treatment solution. Member States permitting the release of waste water into the sewage system shall ensure that a local risk assessment is carried out
How do we handle these wastewaters today?

- Direct Disposal into creeks, sewage treatment plants, evaporation ponds
- Land spreading on adjacent field sites
- Physicochemical treatment with CONTROL TEC ECO® based on filtration through activated carbon (Unacceptably High Cost)
So we aim….

• To provide a **viable, effective and economic solution** for the local fruit packaging plants to decontaminate the wastewaters produced by their phytosanitary activities
First problem: wastewater volumes..

- Large fruit-packaging plants producing 50 - 100 m$^3$ or more wastewaters
  
  *More engineering based bioreactor system is needed*

- Small to medium size fruit packaging plants producing 10-50 m$^3$ of wastewaters
  
  *Biobeds*
Bioreactor treatment of wastewaters

- Development of microbial inocula for rapid degradation of the pesticides contained in wastewaters
  - Two thiabendazole-degrading consortia composed of *Pseudomonas*, *Sphingomonas*, other *a*- and *b*-proteobacteria
  - *Pseudomonas monteilli* strain degrading diphenylamine
  - *Pseudomonas stutzeri* and *Sphingomonas haloaromaticans* degrading ortho-phenylphenol
Biobeds for treatment of wastewaters from post-harvest activities

Optimization Steps

- Selection of biomixture showing high degradation potential for the given pesticides
- Water management (frequency/rate of wastewater discharge on the biobed)
- Evaluation of pilot biobed systems
- Handling of spent biobed substrate
- Handling of biobed effluent
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  - Handling of biobed effluent

Optimization Steps
Biomixture Composition

- Soil
- Straw
- Spent Mushroom Substrate: Pasteurized straw colonized by *Pleurotus ostreatus* edible basidiomycetes (good pesticide degrader) which is considered a waste for mushroom growers after 2-4 harvests and they would like to find a way to get rid off it
Ortho-phenylphenol and diphenylamine are not persistent chemicals

Karas P., PhD
Imazalil and thiabendazole more persistent

Karas P., PhD
# DT50 (days) of the given pesticides

<table>
<thead>
<tr>
<th>Substrates</th>
<th>OPP</th>
<th>DPA</th>
<th>IMZ</th>
<th>TBZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>1,5</td>
<td>1,9</td>
<td>79,3</td>
<td>53,4</td>
</tr>
<tr>
<td>SMS/Soil (50:50)</td>
<td>1,1</td>
<td>1,7</td>
<td>24,2</td>
<td>33,6</td>
</tr>
<tr>
<td>SMS/Straw/Soil (50:25:25)</td>
<td>1,0</td>
<td>1,9</td>
<td>26</td>
<td>28,3</td>
</tr>
<tr>
<td>Straw/Soil (50:50)</td>
<td>1,5</td>
<td>1,6</td>
<td>55,4</td>
<td>236,5</td>
</tr>
<tr>
<td>Straw/SMS/Soil (50:25:25)</td>
<td>1,6</td>
<td>1,9</td>
<td>46</td>
<td>60,4</td>
</tr>
</tbody>
</table>

**OPP**: ortho-phenylphenol, **DPA**: diphenylamine, **IMZ**: Imazalil, **TBZ**: Thiabendazole

Karas P., PhD
Degradation of fungicides in alternative biomixtures used in Cyprus

<table>
<thead>
<tr>
<th>Substrates</th>
<th><strong>R^2</strong></th>
<th><strong>DT50</strong></th>
<th><strong>X^2</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ortho-phenylphenol – Na (SOPP)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OTP</td>
<td>0.93</td>
<td>33.0</td>
<td>2.1</td>
</tr>
<tr>
<td>GVP</td>
<td>0.93</td>
<td>21.1</td>
<td>1.8</td>
</tr>
<tr>
<td>GM</td>
<td>0.94</td>
<td>19.5</td>
<td>4.6</td>
</tr>
<tr>
<td>GSS-1</td>
<td>0.96</td>
<td>4.9</td>
<td>7.2</td>
</tr>
<tr>
<td>GSS-2</td>
<td>0.94</td>
<td>13.1</td>
<td>9.7</td>
</tr>
<tr>
<td>SS</td>
<td>0.98</td>
<td>31.1</td>
<td>4.1</td>
</tr>
<tr>
<td>S</td>
<td>0.92</td>
<td>43.3</td>
<td>3.6</td>
</tr>
<tr>
<td><strong>Thiabendazole (TBZ)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OTP</td>
<td>0.82</td>
<td>57.4</td>
<td>2.6</td>
</tr>
<tr>
<td>GVP</td>
<td>0.90</td>
<td>28.8</td>
<td>3.4</td>
</tr>
<tr>
<td>GM</td>
<td>0.91</td>
<td>40.8</td>
<td>1.4</td>
</tr>
<tr>
<td>GSS-1</td>
<td>0.93</td>
<td>26.7</td>
<td>5.9</td>
</tr>
<tr>
<td>GSS-2</td>
<td>0.98</td>
<td>26.2</td>
<td>10.1</td>
</tr>
<tr>
<td>SS</td>
<td>0.98</td>
<td>89.5</td>
<td>3.2</td>
</tr>
<tr>
<td>S</td>
<td>0.87</td>
<td>77.8</td>
<td>9.1</td>
</tr>
<tr>
<td><strong>Imazalil (IMZ)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OTP</td>
<td>0.71</td>
<td>48.8</td>
<td>8.3</td>
</tr>
<tr>
<td>GVP</td>
<td>0.69</td>
<td>15.5</td>
<td>10.4</td>
</tr>
<tr>
<td>GM</td>
<td>0.90</td>
<td>36.8</td>
<td>9.6</td>
</tr>
<tr>
<td>GSS-1</td>
<td>0.87</td>
<td>34.4</td>
<td>8.2</td>
</tr>
<tr>
<td>GSS-2</td>
<td>0.88</td>
<td>31.7</td>
<td>6.4</td>
</tr>
<tr>
<td>SS</td>
<td>0.91</td>
<td>19.2</td>
<td>7.2</td>
</tr>
<tr>
<td>S</td>
<td>0.87</td>
<td>28.6</td>
<td>11.4</td>
</tr>
</tbody>
</table>

**GSS1**
- Soil (25%)
- Compost from grape marc and grape stalks (25%)
- Straw (50%)

**S**: Soil
Biobeds for treatment of wastewaters from post-harvest activities

Optimization Steps

Selection of biomixture showing high degradation potential for the given pesticides

Water management (frequency/rate of wastewater discharge on the biobed)

Evaluation of pilot biobed systems

Handling of spent biobed substrate

Handling of biobed effluent
The % of the pesticides leached from columns which received different water loads of wastewaters

<table>
<thead>
<tr>
<th>Water loading (L m(^{-3}))</th>
<th>biobed substrate</th>
<th>Soil: Perlite (1:3 v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SOPP</td>
<td>IMZ</td>
</tr>
<tr>
<td>463 (H)</td>
<td>8.1</td>
<td>0.1</td>
</tr>
<tr>
<td>242 (M)</td>
<td>6.1</td>
<td>0.1</td>
</tr>
<tr>
<td>161 (L)</td>
<td>5.6</td>
<td>0.09</td>
</tr>
</tbody>
</table>
Biobeds for treatment of wastewaters from post-harvest activities

Optimization Steps

Selection of biomixture showing high degradation potential for the given pesticides

Water management (frequency/rate of wastewater discharge on the biobed)

Evaluation of pilot biobed systems

Handling of spent biobed substrate
Handling of biobed effluent
Three 1.1 m$^3$ Pilot Biobeds
Two 0.25 m$^3$ Pilot Biobeds bioaugmented with bacteria ($10^6$ cells/g)

Application of 1.1 m$^3$ wastewater (3 x 10 min/d) containing 100 mg/L of each pesticide in a period of 5 months (Oct 2012 – Feb 2013)
Biomixture

- Soil (25%)
- Straw (25%)
- Spend Mushroom Substrate (50%)
Evaluation of Depuration Efficiency

- Daily measurements of DO & temperature
- Regular measurement of pesticide concentration in the inflowing wastewater and in the efflux of the biobed
- Distribution of pesticides in biobed profile at the end of the treatment
- Microbial measurements before and after treatment in the biobed material (PLFAs, qPCR for functional genes)
Offset type BIOBED in Cyprus used for the treatment of both on-farm and post-farm wastewaters from citrus production

Omirou et al. Environmental Pollution (2012)
Dissipation of ortho-phenylphenol in biobed Cyprus

0% of OPP in the leachate
Dissipation of thiabendazole in biobed Cyprus

0% of thiabendazole in leachate
Dissipation of **imazalil** in Biobed Cyprus

0% of **imazalil** in the leachate
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Handling of spent biobed substrate
Handling of biobed effluent
How do we treat this waste?

- **Composting** with fresh organic matter (straw + manure)
- **Storage** at standard temperature and moisture
- **Bioaugmentation** with pesticide-degrading microbes
- **Bioaugmentation** (10 days) and **composting**

In progress…….
Cost of a Biobed for use in Fruit Packaging Plants

- **Biobeds**: 6000-7500 €
  - Storage Tank (10-25 m³): 1400-3300 €
  - Plastic membrane for water proofing: 300-900 €
  - Cost of dig up and preparation of the site: 1000 €
  - Pumps (2): 500 €
  - Tubing: 500 €
  - Small storage tank (max 5 m³): 800 €
  - Plastic for covering the biobed during heavy rainfall events: 100 €
  - Various extra costs: 200 €

- **Cost of treatment by subcontractor:** 17500 € annually

0.70 – 3 € (depending on the volume)

For a medium size plant producing 25 m³ wastewaters = 17500 €/year
Biobeds use for on-farm activities......
Many thanks to....

Chiara Perruchon, PhD student
Scholar State Scholarship Foundation of Greece

Panagiotis Karas, PhD student

Dr Michalis Omirou
Agricultural Research Institute of Cyprus

Dr Constantinos Ehaliotis
Agricultural University of Athens

Prof. Nikolaos Tsiropoulos
University of Thessaly

Dr Evangelos Karanasios
Benaki Phytopathological Institute
Publications related to biobeds work by our group


