# Reduction Of Diffuse Pesticide Contamination For A Sustainable Use Of Pesticides

Leticia Pizzul, Karin Önneby, John Stenström Department of Microbiology Swedish University of Agricultural Sciences (SLU) Uppsala, Sweden





## Bioprophylaxis - The Microbial Pro-Active Solution To Pesticide Pollution

Remediation Act of correcting an error or a fault or an evil





20 000 contaminated sites pose a large or a very large risk for humans and the environment in Sweden

> 4-5 billion € for remediation of the 1 500 most polluted sites

Prophylaxis

The prevention of disease





## Sources Of Pesticides In Surface- And Ground Waters



Parking

#### **Biobeds Bind And Degrade Pesticides** driving ramp **Biobed** mixture arass O50% Ο Straw 25% Peat Top-soil 25% Biobed 50 cm clay layer 10 cm Handling of concentrates Below the tank: -foaming - wash-off of residues 0 Dripping nozzles

Castillo, MdP, Torstensson, L. & Stenström, J. 2008. Biobeds for environmental protection from pesticide use - A review. J. Agric. Food Chem. 56(15), 6206-6219.

## Sources Of Pesticides In Surface- And Ground Waters

## Diffuse sources



### Spraying

Surface transportLeaching



## Microorganisms And Chemicals In Soil



# Chemicals "Age" In Soil





Young and available





Aged, low bioavailability

## How Reduce Contamination From Diffuse Sources?

Risk for surface transport and leaching, especially when the microbial activity is low New concept: Fast degradation after addition of active bacteria or enzymes that degrade pesticides before they "age"





## One Application - Herbicide Use On Railways



Soil Biology & Biochemistry 39 (2007) 473-484

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

#### Metabolic and cometabolic degradation of herbicides in the fine material of railway ballast

Harald Cederlund\*, Elisabet Börjesson, Karin Önneby, John Stenström

Department of Microbiology, Swedish University of Agricultural Sciences (SLU), Box 7025, SE-75007 Uppsala, Sweden

Received 15 March 2006; received in revised form 24 August 2006; accepted 29 August 2006 Available online 29 September 2006



#### S C I E N C E O F T H E T O T A L E N V I R O N M E N T 3 9 7 (2008) 205 – 214



available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/scitotenv





#### Functional microbial diversity of the railway track bed

#### Harald Cederlund<sup>a,\*</sup>, Tomas Thierfelder<sup>b</sup>, John Stenström<sup>a</sup>

<sup>a</sup> Department of Microbiology, Swedish University of Agricultural Sciences (SLU), Box 7025, SE-75007 Uppsala, Sweden
<sup>b</sup> Department of Energy and Technology, Swedish University of Agricultural Sciences (SLU), Box 7032, SE-75007, Uppsala, Sweden.

# - small microbial biomass, activity and diversity - heterogenous (sterile spots)





Microbial biomass		
Agricultural	Railway	
100-1500	<1-20	

No. of degraded carbon sources (of total 30)		
Agricultural	Railway	Railway
topsoil	0-10 cm	below 10 cm
28.5	19	10.5
(28-29)	(12-24)	(2-17)

## Model Substances





- Metabolic degradation (used as substrate)
- Sphingomonas sp. T51
  - Active at 2-30°C
  - Also degrades 2,4-D,
    2,4-DP and MCPP
- Cometabolic degradation (not used as substrate)
- Enzymatic (laccase, manganese peroxidase)

# Effect Of Adding An MCPA-Degrading Sphingomonas sp. T51 On MCPA-Degradation



Pesticide Properties DataBase: 50% degradation in 7-41 days in arable soil

## Realistic?

 $\blacktriangleright$  One can predict with 85% probability if it will rain within 24 hours. The degradation should thus be complete within 24 hours in order to eliminate the risk for tranport to surfaceand ground waters

> A normal initial concentration of MCPA is  $20\mu g$  MCPA per g of soil in the top cm. One bacterium degrades at least 0.25 pg MCPA per hour at  $10^{\circ}C$ 

> Degradation of all MCPA in 24 h then requires

3.3 x 10<sup>6</sup> bacteria per g of soil or
3.3 x 10<sup>14</sup> bacteria per ha or
33 liter fermentate with 10<sup>13</sup> bacteria per liter



### For A Useable Product It Is Also Necessary To ...



## Cultivate



### Formulate



### Risk assess



# Cultivation

- Development of new culture procedures
  - high biomass yield
  - stress-tolerant organisms
  - high viability
- Industrial cheap substrates



## Safety Profile Of Microbes

- Collection and evaluation of scientific literature and other information
- Determination of antibiotic resistance pattern
- Determination of temperature range for growth
- In vitro testing of production of toxic substances





The phagotrophic (particle feeding) unicellular ciliate *Tetrahymena pyriformis* is used as a biosensor for detection of toxic substances produced by microorganisms

# Formulation

- Facilitate packaging
- Extend shelf-life
- Render the preparation easy to handle
- Protect against compounds in pesticide product
- Protect against UV-light and desiccation
- Delay degradation until intended pesticide effect obtained



# Formulation

#### Freeze drying

- Well studied dehydration method
- Expensive



#### Fluidized bed drying

- Bed with fluid properties
- Carrier
- Harsh
- Less expensive





## Survival After Freeze-Drying And After Storage At Different Temperatures

- Freeze-drying of Sphingomonas sp. T51
- 15 % sucrose + 1.5 % PVP90 (polyvinylpyrrolidone)



## Ligninolytic Enzymes Degrade Many Different Pesticides ...



Biodegradation (2009) 20:751–759 DOI 10.1007/s10532-009-9263-1

ORIGINAL PAPER

#### Degradation of glyphosate and other pesticides by ligninolytic enzymes

Leticia Pizzul · María del Pilar Castillo · John Stenström



#### ... inclusive glyphosate





A bacterium encapsulated in CaptiGel-



Encapsulated bacteria and enzymes are protected against UV-light and chemicals

CaptiGel

Kessler, Seisenbaeva, Unell & Håkansson, 2008

## Formulation Of Laccase With CaptiGel

Almost no laccase activity after encapsulation of the enzyme in CaptiGel and 100% recovery of activity after dispersion of the gel in citrate buffer



### Degradation Of Glyphosate In Sand By CaptiGel-Encapsulated Laccase

> Addition of CaptiGel-encapsulated laccase and Roundup to sand

Incubated for 48h at 35°C



Pesticide Properties DataBase: 50% degradation in 4-180 days in arable soil