FINAL REPORT
Plant beds and constructed wetland for phytoremediation of storm water runoff
Period of performance: October 16-December 31, 2003
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Overall goal: Selection and development of elite phytoremediator plants for a storm water recycling/remediation system based on a constructed wetlands populated with suitable plants to serve the Green Dorm (West Quadrangle – Residential Center for Sustainable Futures). The residential center and the grounds were designed in such a way so that storm water, which would otherwise run off into the sewer or the nearby stream would be directed to go through terraces with plants and would collect at the bottom of the property in an artificial wetland that overflows into the neighboring stream. The plant beds and wetlands would serve two purposes (i) reduction and recycling of surface runoff water and (ii) filtration and phytoremediation, i.e. biological cleaning. The runoff water potentially is contaminated with refined oil derivatives such as gas and gasoline leaks.

Objectives:

(i) selection of wetland plant species
(ii) selection of growth substrate and determination of hydraulic parameters of root colonized substrate
(iii) establishment of plant-microbe associations with known oil-eating bacteria
(iv) determination of areal rate constants for oil contamination and establishing criteria for effluent water quality
(v) cost/savings analysis

Results:

(i) Wetland species were selected by the following criteria:

- Ornamental value, also evidenced by availability from ornamental plant nurseries
- Perennial life cycle
- Availability of aseptic in vitro cultures so that artificial colonization by oil-eating bacterial is facilitated and verified
- Salt tolerance - optional

The following species met these criteria:

1. Giant reed – *Arundo donax*
2. Carex acuta – Slender pond sedge
3. Carex nudata – Black flower sedge
4. Scirpus californicus – Bulrush
5. Juncus roemerianus – Black needle rush (salt tolerant)
6. Juncus effuses – Soft rush
7. Typha latifolia – Common cattail
8. Spartina alterniflora – Salt marsh cordgrass (salt tolerant)

Not all species from this selection were used for the subsequent experiments.

(ii) Selection of substrate and determination of hydraulic parameters of root colonized substrate. Water retaining capacity of root colonized sand, fine gravel (West Columbia), and potting soil (Baccto), was determined for giant reed. Knowing these parameters will allow the arrangement of plants in the proper sequence for optimal water retention.

Root colonization	Substrate	Density [g cm-3]	Water holding capacity % of dry weight
none	Sand	1.49	47%
none	Potting soil	0.21	100%
Arundo	Sand	1.04	45%
Arundo	Potting soil	0.78	105%

(iii) Establishment of plant-microbe associations with known oil-eating bacteria. First we colonized dissected Arundo roots with oil eating bacterial strains, C/22 (#381), B/106 (#382), and A/3 (#384), from our collection to screen for nonpathogenic associations. Excised fresh roots of giant reed were dipped into liquid cultures of individual strains of our existing collection of oil-eating bacteria. No discoloration or necrosis was observed on excised roots or intact plants.

Bacterium-plant associations were synthesized by co-cultivation of individual microbial strains with aseptically grown Arundo plants, a technology that exists in the PI's laboratory. Loose bacteria were be rinsed off after three days and the persistence of the bacterium is monitored on the long term. Presence of the bacteria was ascertained by growth assays on rich (YEP) medium. Molecular markers such as strain specific PCR probes were not possible to find within the project period.

Disappearance of asphaltum (quick drying, Matheson, Coleman & Bell, L-944) is an indication that refined petroleum products can be metabolized in contact with roots, and the disappearance/clearing can be visualized by a microfilm degradation assay. A thin film of asphaltum was dried on the surface of minimal nutrient medium without carbon source by evaporation from a hexane solution. The region cleared up by the bacteria appears opaque.

(iv) Determination of areal rate constants for oil contamination and establishing criteria for effluent water quality was proposed but not completed because the plants went to dormancy in October to December period.

(v) Cost/savings analysis is very limited in this case to estimating the cost of nursery stock that is does not need to be purchased from outside sources as plants are propagated in vitro at USC. The plant species that we have selected cost $3-12/plant in local and mail-order nurseries. The number of plants required for populating the GreenDorm structures cannot be determined at this point because the available space is not certain. Determination of the production cost of in vitro plants was not within the scope of this project.