Bioremediation of Herbicide-Contaminated Soil and Water

Wisconsin Groundwater Research Project Summary

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Robin F. Harris (P.I.), Department of Soil Science, University of Wisconsin-Madison, Madison, WI 53706, Telephone: [608] 263-5691

ABSTRACT

The contamination of soils, groundwater and surface waters by herbicides poses major clean-up problems. At present, the most common approach is containment, involving costly removal of highly contaminated soils to landfill sites. Bioremediation methods for in situ treatment are needed as alternative and/or supplementary approaches for cost-effective and residue-free clean-up. This project focuses on biodegradation of atrazine, but involves development of a general bioremediation protocol applicable to other herbicides. Atrazine is the most commonly detected organic contaminant in groundwater in Wisconsin and all other high corn production areas of the Midwest. Laboratory studies have demonstrated that atrazine can be degraded by the concerted activity of consortia (mutually compatible groups) of microorganisms. Isolation of microbial consortia by traditional enrichment-culture techniques has been ineffective in the case of atrazine degraders. This report describes an improved method for obtaining these cultures utilizing a conceptual computer model to facilitate interpreting, predicting, and controlling the performance of the bioremediation system. Preliminary studies have focused on development of analytical procedures to determine the concentration of atrazine and its metabolites, microbial biomass, and ammonium. Values derived from these experiments have been used to determine initial input parameters for the model using *Pseudomonas sp*. strain D. Experimental values obtained for growth rate (biomass/hr) and specific growth yield (gr protein/mole nitrogen consumed) under nitrogen limited and carbon limited conditions agreed closely with the results from computer model simulations. These findings confirmed the conceptual adequacy of the model. The model was used to identify the basis of unusually low growth yields under carbon limited conditions. In this case, accumulation of intermediate metabolites was predicted. This application of the model provided an example of its utility for interpreting system performance. The model was also used to simulate atrazine degradation under a condition prevailing in groundwater, i.e., low levels of dissolved oxygen. However, *Pseudomonas sp.* strain D did not have the metabolic capabilities to survive in this environment.

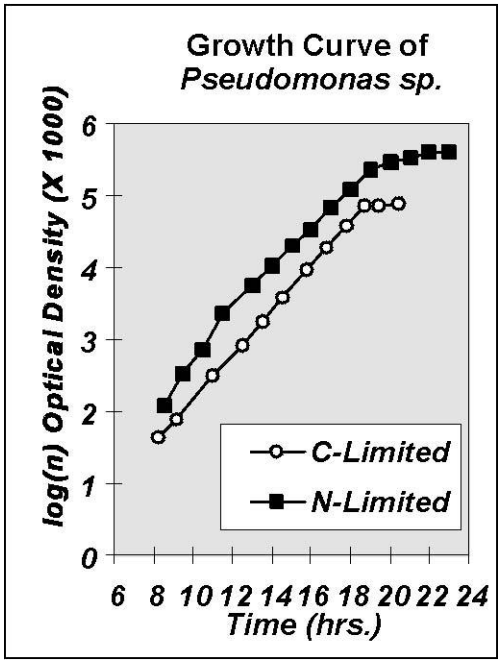
TECHNICAL SUMMARY

INTRODUCTION: Atrazine detection levels and frequencies in Wisconsin groundwater wells vary considerably. For example, in a 1990 survey of Grade A dairy farms, atrazine was detected in 12% of wells tested; 7% had concentrations exceeding the 1990 preventive action limit (PAL) of 0.35 ppb. It was detected in 71% of wells from high risk areas, with concentrations exceeding the PAL in 51% of cases. Atrazine enters groundwater after leaching through the soil at sites where it is mixed, improperly disposed of, or applied to fields for weed control. Atrazines*s leaching potential is increased by its relative persistence in the soil, slow chemical hydrolysis under normal soil pH conditions, and slow rate and extent of microbiological degradation. The bioenergetic potential for microbial degradation of atrazine is located in its two alkyl side chains since the ring carbons are at the same oxidation state as CO . 2 Degradation of desalkylated atrazine metabolites occurs only when they are utilized by microorganisms such as Pseudomonas sp. strain D as a source of nitrogen. This report describes an improved method for selective enrichment of such cultures by application of a model which incorporates several significant environmental variables that influence activity of the microorganism. The initial phase of the project focused on refining configuration of the model and developing methods to determine required input parameters, namely the concentration of atrazine and its metabolites, ammonium levels, and microbial biomass.

METHODS: Pseudomonas sp. strain D was obtained from CIBA-GEIGY and maintained on agar plates containing cyanuric acid-glucose media. An HP 5890A gas chromatograph (GC) fitted with a nitrogen-phosphorous detector and a fused silica capillary megabore column containing a 50% phenyl and 50% methylpolysiloxane matrix of intermediate polarity. Two high pressure liquid chromatography (HPLC) protocols were utilized on a Gilson

system: 1. an Alltech mixed mode cation exchange RP-C8 column with isocratic mobile phase consisting of 68% phosphate buffer (pH 6.7) and 32% methanol and 2. a 25 cm Supelco ODS column with a C-18 RP stationary phase and a mobile phase gradient from 100% 100 mM phosphate buffer at pH 6.7 to 70% methanol in 10 mM phosphate buffer. Recovery of atrazine and metabolites from sedimented incubation media was accomplished utilizing Superclean Envi-18 solid phase extraction tubes which were eluted with methanol. Growth of Pseudomonas sp. strain D was monitored by measuring turbidity, dry weight, and protein (Coomassie blue method) and ammonium was determined by a Kjeldahl steam distillation technique. The models consist of a set of spreadsheets defining ecophysiological properties, interlinked with spreadsheets defining system conditions and data output. These describe 1. cell physical composition, 2. cell and metabolite elemental composition and energy potential, 3. coefficients for the rate and efficiency of substrate uptake and cell growth, maintenance, death, and lysis, and, 4. stoichiometric mass balance coefficients for substrate uptake and product release. Data output parameters include media flow rate and output-input concentrations of substrates and products.

RESULTS: Measurement of atrazine and its metabolites by GC were found to be highly variable and further complicated by a low degree of precision associated with replicates. HPLC affords a better alternative because the quantitative data is of high quality and the tedious extraction procedure used for GC analysis is eliminated. Two separate experiments were conducted to determine growth characteristics of strain D and to test the accuracy of model simulations (Figure 1). The organism had a -1 specific growth rate of 0.27 ± 0.02 hr , 44.6 ± 1.5% protein content, andan average growth yield of 45.8 ± 1.5 g protein produced/mole cell nitrogen consumed when grown under limited nitrogen conditions. The model simulation predicted 44.3% protein and a growth yield of 46.4 g protein produced/mole cell nitrogen consumed which correlates closely with actual results. Accuracy of the model was also validated in experiments where strain D was grown under carbon limiting conditions, as the protein content and growth yield were within a few percentage points of the predicted values. The predicted and observed growth yields of 0.34 and 0.36 g cells produced/mole glucose consumed is much lower than the 0.5 g typically found. The low growth yields are likely to be the result of accumulating intermediate metabolites which are not utilizable as growth substrates. In addition, the computer model was applied to simulate chemical degradation of atrazine under conditions prevailing in groundwater. For this application, the models input parameters specify low levels of dissolved oxygen and the presence of a suitable alternate electron acceptor likely to be found in groundwater, such as nitrate. However, Pseudomonas sp. strain D did not have the metabolic capabilities necessary to survive in the simulated groundwater environment. Hence, application of strain D to groundwater remediation would require air or oxygen perfusion.



DISCUSSION: The high correlation of the computer model*s predictions with experimentally determined growth parameters enables the investigator to rapidly evaluate experimental methodologies. Thus it is evident that strain D will serve as a useful model organism to further validate the test system prior to beginning enrichment cultures.

PROJECT INFORMATION: This summary is based on the Remediation Project 91-REM-1 which was conducted between July 1, 1990 and June 30, 1992. The project was supported in part by General Purpose Revenue Funds of the State of Wisconsin through the University of Wisconsin System. Administrative management of the project was provided by the UW-Madison Water Resources Center (WRC). The final technical report for this project has been published and is available on loan from the WRC Library as document number WRC-GRR-95.03 (WRC Library call number 140702). To request a document call (608) 262-3069, email the library at askwater@macc.wisc.edu, or visit us on campus at 1975 Willow Drive, Madison, WI 53706.