Reactor optimization of volatilized p-xylene metabolism
by Barbara Christine Vaughn

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering
Montana State University

Abstract:
VOC emissions are subject to increasingly strict regulations dictating a need for innovative, cost-effective control technologies. The degradation of volatile, higher molecular weight organic compounds such as those in the BTEX group are of special interest due to their association with hydrocarbon fuel spills. Certain bacteria are capable of metabolizing these contaminants as their sole carbon and energy source and the one used in this study, Pseudomonas putida Idaho, has been shown to be resistant to high concentrations of p-xylene.

Packed tower bioreactors offer several advantages over suspended cell systems for contaminant removal including continuous degradation, high volumetric reaction rates, reduced VOC stripping losses, and simplified downstream processing. Because they can operate as a semi-closed system, bacterial strains can be introduced that target degradation of specific pollutants without competition from indigenous soil or water microorganisms.

The goal of this research was to evaluate the performance of vapor phase bioreactors in response to various process parameters, gas and liquid flow rates and xylene inlet concentration. The columns were packed with two different elements, diatomaceous earth (D.E.) pellets (porous) and glass spheres (non-porous).

Results indicated that when normalized to exterior packing surface area, the D.E. pellet reactor exhibited significantly higher degradation rates than the glass sphere reactor at comparable loading rates. Degradation rates increased with increased mass loading except at the highest gas flow rate. Maximum elimination capacity for the D.E. pellet reactor was 66 mg xylene/m^2 packing per hour with less than 2 millimeters pressure drop. Protein levels were significantly higher in the D.E. pellet reactor while total organic carbon levels were significantly higher in the glass sphere reactor. Both systems maintained maximum degradative capacity after five months of operation under changing organic loads and loss of pure culture.

REACTOR OPTIMIZATION OF VOLATILIZED *p*-XYLENE METABOLISM

by

Barbara Christine Vaughn

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Environmental Engineering

MONTANA STATE UNIVERSITY
Bozeman, Montana

March 1993

APPROVAL

of a thesis submitted by

Barbara Christine Vaughn

This thesis has been read by each member of the thesis committee and been found to be satisfactory regarding content, English usage, format, citations, bibliographic style and consistency and is ready for submission to the College of Graduate Studies.

April 19, 1993
Date

Chairperson, Graduate Committee

Approved for Major Department

20 April 1993
Date

Head, Major Department

Approved for the College of Graduate Studies

4/28/93
Date

Graduate Dean

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library.

If I have indicated my intention to copyright this thesis by including a copyright notice page, copying is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for permission for extended quotation from or reproduction of this thesis in whole or in parts may be granted only by the copyright holder.

Signature Barbara C Caughlin

Date April 20, 1983

ACKNOWLEDGEMENTS

The completion of this thesis was possible only with the encouragement and help of a host of folks. In particular, I owe my sanity to my advisor, Warren Jones, who was ever patient, understanding, and full of insight. My thanks to him and to the rest of the crew at the Engineering Research Center for their time and knowledge, especially Jim Wolfram, Anne Camper, Paul Sturman, Rick Veeh, and Robert Mueller. Most of all, a toast to my son, Cade, "To life after graduate school."

TABLE OF CONTENTS

TABLE OF CONTENTS-Continued

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES-Continued

ABSTRACT

VOC emissions are subject to increasingly strict regulations dictating a need for innovative, cost-effective control technologies. The degradation of volatile, higher molecular weight organic compounds such as those in the BTEX group are of special interest due to their association with hydrocarbon fuel spills. Certain bacteria are capable of metabolizing these contaminants as their sole carbon and energy source and the one used in this study, *Pseudomonas putida* Idaho, has been shown to be resistant to high concentrations of *p*-xylene.

Packed tower bioreactors offer several advantages over suspended cell systems for contaminant removal including continuous degradation, high volumetric reaction rates, reduced VOC stripping losses, and simplified downstream processing. Because they can operate as a semi-closed system, bacterial strains can be introduced that target degradation of specific pollutants without competition from indigenous soil or water microorganisms.

The goal of this research was to evaluate the performance of vapor phase bioreactors in response to various process parameters, gas and liquid flow rates and xylene inlet concentration. The columns were packed with two different elements, diatomaceous earth (D.E.) pellets (porous) and glass spheres (non-porous).

Results indicated that when normalized to exterior packing surface area, the D.E. pellet reactor exhibited significantly higher degradation rates than the glass sphere reactor at comparable loading rates. Degradation rates increased with increased mass loading except at the highest gas flow rate. Maximum elimination capacity for the D.E. pellet reactor was 66 mg xylene/m^2 packing per hour with less than 2 millimeters pressure drop. Protein levels were significantly higher in the D.E. pellet reactor while total organic carbon levels were significantly higher in the glass sphere reactor. Both systems maintained maximum degradative capacity after five months of operation under changing organic loads and loss of pure culture.

INTRODUCTION

The Superfund Amendments and Reauthorization Act (SARA) emission summary for the petroleum and chemical industries reveals that the largest environmental releases of chemicals are volatile organic compounds (VOC's) into air. VOC's are emitted primarily from industrial operations involving organic solvents or hydrocarbon fuels. Because they can lead to a variety of health and environmental problems, VOC emissions are subject to increasingly strict regulations dictating a need for innovative, cost-effective control technologies.

Removal of volatile organic compounds from air streams is generally approached in two ways; sorption or reaction. Sorption is accomplished either by absorbing compounds into a solvent liquid (gas absorption) or adsorbing them onto the surface of a solid material, usually granulated activated carbon. Reaction processes include combustion, photochemical oxidation, and microbial degradation (Schroeder, 1990). Although the abiotic processes can achieve high elimination efficiencies, the disadvantages can be characterized by a relatively high cost (Know and Canter, 1988; Chang et al., 1989; Guensler, 1989; Kosky and Neff, 1988), a phase transfer of the pollution problem, and the formation of toxic products (Diks,1992).

Microbial degradation is an effective alternative particularly when the pollutant concentration is low and the volumetric flow rate is high which is often the case in odorous or toxic waste gas emissions (Leson and Winer,

1991). Biological processes operate at low pressures and temperatures and require low maintenance making them cost-effective. The pollutants are not just shifted into another phase but are ultimately eliminated by metabolizing the contaminants to carbon dioxide, water, and cellular constituents.

Although biological treatment of easily metabolized organic compounds has been used for decades to treat municipal and industrial waste, its use for recalcitrant compounds has been developed only in the past ten years. A number of microorganisms, primarily bacteria, have been isolated that show a high activity and stability towards several xenobiotic compounds. The biodegradation of volatile, higher molecular weight organic compounds such as those in the BTEX group (benzene, toluene, ethylbenzene and xylene) are of particular interest due to their association with hydrocarbon fuel spills (Apel et al.,1991). These aromatic compounds are readily biotransformed in aerobic environments (Leahy and Colwell, 1990). Certain Gram negative heterotrophic bacteria are capable of metabolizing these contaminants as their sole carbon and energy source and one in particular, *Pseudomonas putida* Idaho, has been shown to be resistant to high concentrations of xylene (Rogers et al., 1992).

When substrate biodegradation is desired, the formation of biofilms can be an advantage over traditional batch fermentation because it applies continuously operated processes (Characklis, 1990). In suspended cultures the cell retention time and the hydraulic retention time are coupled. The

immobilization of cells in a packed tower allows the accumulation of high biomass concentrations and thus high volumetric reaction rates, while the hydraulic residence time can be reduced below the washout condition for the suspended system (Diks, 1992). Because there is less liquid effluent in packed towers, downstream processing is generally simplified. Conventional liquid phase bioreactors use mechanical aeration which can result in significant VOC stripping losses to the air stream rather than biodegradation. Gas-in-water solubility limitations in chemostats often produce unacceptably low conversions (Rogers et al.,1992). Fixed film, vapor phase bioreactors provide a solution to these problems.

Industrial manufacturing practices produce dilute gaseous effluents that cannot be recovered or disposed of economically particularly in the case of mixed wastes such as spent scintillation cocktail. On-line processing employing vapor phase bioreactors would lower treatment costs, reduce user liability, and allow continuance of existing manufacturing processes (Rogers, et al., 1992).

Contaminated air streams are produced during soil venting of unsaturated subsurface material and air stripping of ground water pumped to the surface. Degradation of VOC's by vapor phase bioreactors at the wellhead provides a promising alternative for remediation on site. Diverse geochemistries of groundwaters present problems to biological-based systems for treating water; treating air would circumvent this complication (Canter et al., 1989). Pumping air instead of water also lowers processing costs. Another potential advantage

is the dilution of high concentrations of toxic organic compounds in water by the 10 to 1 or 20 to 1 air-to-water ratios of packed towers (Canter et al., 1989). Recent findings indicate many hydrocarbon-degrading bacteria in the environment are genetically unstable or physiologically impaired which can interfere with remediation effectiveness (Ridgway and Phipps, 1991; Ridgway, 1990). Because vapor phase bioreactors can operate as a semi-closed system, it may be possible to introduce recombinant or genetically improved bacterial strains into such a system to target degradation of specific hydrocarbon pollutants without competition from indigenous groundwater or soil microorganisms.

Before vapor phase bioreactors can be developed, fundamental studies are needed to optimize reactor design and operation. Practical applications of the process are limited, primarily due to a lack of empirical data. An enormous amount of literature has been published on physical aspects of packed bed reactors and on intrinsic microbial degradation kinetics but few studies are available that evaluate the mass transfer and reaction processes occurring in a single bioreactor system.

Goal and Objectives

The goal of this project is to evaluate the performance of a vapor phase bioreactor in response to various process parameters in order to produce a maximum feed rate to reactor volume ratio and a minimum contaminant breakthrough.

Specific objectives relevant to this goal follow: 1) Identify nutrient requirements and/or limitations of a xylene-degrading organism, *Pseudomonas putida* Idaho. 2) Compare mass transfer rates of xylene for two packings (one porous and one non-porous) under abiotic conditions. 3) Determine the response of inoculated vapor phase bioreactors to variations in gas flow rate, liquid flow rate, and influent vapor phase xylene concentrations. 4) Compare the relative importance of mass transfer and biodegradation kinetics in a vapor phase bioreactor.

BACKGROUND

Biofilters

The use of biological systems for elimination of volatile compounds can be found in literature as early as 1923 when Bach discussed control of H_2S emissions from sewage treatment plants. The systems originally built in the US were mostly "soil beds" where mineral soils were used as filter materials (Leson and Winer, 1991). Pomeroy (1963) received a US patent in 1957 for a soil bed concept and described a successful biofilter installation in California for the treatment of sewage-related odor including organic sulfides. The first systematic research on the biofiltration of H_2S was conducted by Carlson and Leiser in the early 1960's. They demonstrated that biodegradation rather than sorption accounted for odor removal in several soil filters (Carlson and Leiser, 1966). In 1988, a manufacturing facility of SC Johnson & Son Inc. was able to satisfy regulatory requirements for the reduction of VOC emissions from an aerosol can filling operation by using a prototype biofilter. The soil bioreactor reduced concentrations of propane, isobutane, and n-butane in a waste air stream by at least 90% (Kampbell et al.,1987).

Large-scale use of biofilters in the US has been sporadic due to low biodegradative capacity of the soils, correspondingly large space requirements of the beds, lack of regulatory programs, and little governmental support for research and development. In contrast, increasingly stringent regulatory

requirements and funding from the federal government in The Netherlands and West Germany have established biofiltration as a well used air pollution control (APC) technology. It is considered the best available control technology (BACT) in a variety of VOC and odor control operations such as chemical plants, foundries, print shops, and coating operations. These sources typically emit large volumes of waste gases that contain low concentrations (less than 1000 ppm as methane) of the target pollutant (Leson and Winer, 1991). Ottengraf and Van den Oever (1983), Hartenstein (1987), Kosky and Neff (1988), and Bohn (1989) have all reported excellent success in the removal of VOC's from various sources employing packed bed microbial processes.

A packed bed biofilter consists of one or more beds of a biologically active material, primarily mixtures based on compost, peat, or soil. Contaminated waste gas is vented through the filter which, given sufficient residence time, diffuses into biofilm immobilized on the filter particles. Aerobic degradation of the target pollutants will occur if the microorganisms are present that can metabolize them (Figure 1).

In order for a biofilter to operate efficiently the filter material must provide optimum environmental conditions for the microbial population, large reactive surfaces, and low pressure drops. Problems arise with the development of microbial growth which can result in plugging, high head losses, and compaction, requiring replacement of the filter material. The biodegradation of air pollutants often generates acidic by-products and end-

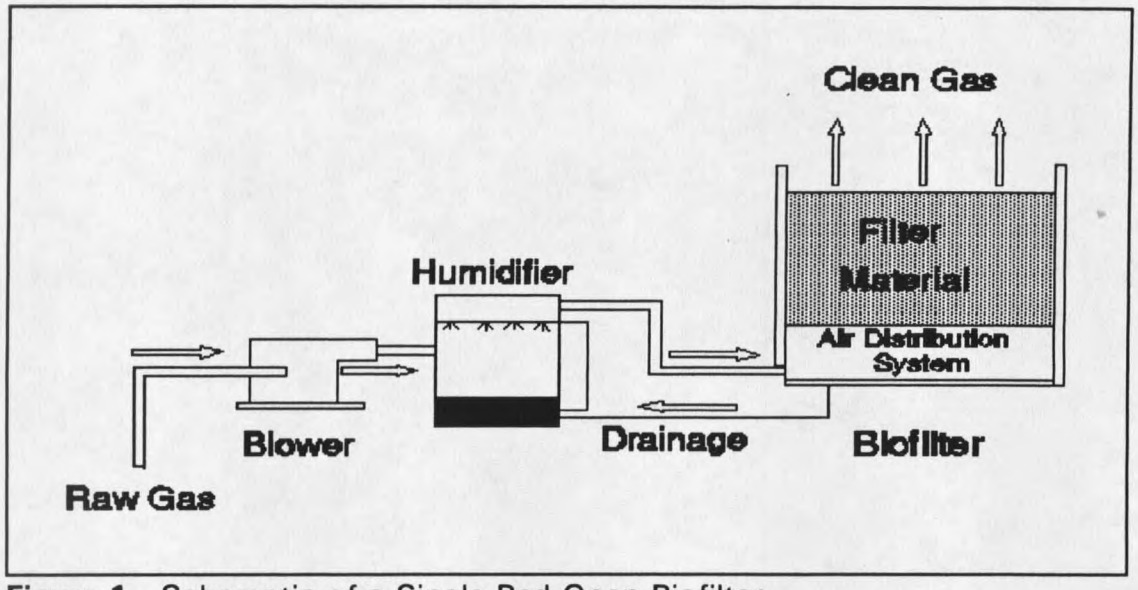

Figure 1. Schematic of a Single Bed Open Biofilter

products. Most bacteria and actinomycetes proliferate in a pH range between 7 and 8 (Atlas, 1981) so a resultant drop in pH can severely inhibit the resident population and reduce or eliminate the filter's degradative capacity. Because they lack a continuous liquid flow, biofilters cannot remove metabolic waste products or replace depleted nutrients. Removal of toxic compounds by adsorption to the filter material transfers rather than alleviates the environmental problem.

The approach to designing biofilter systems is usually empirical. Mobile pilot units are often used for treatability studies and the sizing of full scale systems. Predictive modelling of these systems is complicated for a variety of reasons. Because the surface area of the soil matrix cannot be determined, substrate depletion as a result of mass transfer from the gas to liquid phase, mass flux from the liquid to biofilm surface, and reaction within the biofilm

cannot be quantified. Soil may contain as many as 10^7-10^9 cells/g soil of naturally occurring microorganisms making enumeration of existing species and mass on a spatial and temporal basis impossible. This precludes development of genuine kinetic models of the biofilm. The metabolic activity of the biofilm may change in response to buildup of acid metabolites and reduction of nutrients resulting in an inherently "unsteady state" system. In a biofilter, it is difficult to differentiate between, and subsequently model, contaminant removal due to adsorption or to biodegradation.

Biological Trickling Filters

Biological trickling filters address some of the problems encountered in biofilter operation and design. They consist of columns with manufactured packing elements onto which a suitable microbial culture is immobilized. The specific surface area of the packing is high for maximum biofilm development with a large void fraction between elements for gas flow which minimizes the pressure drop. This also prevents obstruction of the bed due to biofilm growth and sloughing. The waste gas is forced through the packed bed, in co- or countercurrent flow with a continuously circulated water phase. Inert packing materials maximize bioavailability, minimize compaction, and prevent accumulation of pollutants in the filter material. A flowing liquid phase allows for continuous control of the physiological conditions, such as nutrient requirements, pH, temperature, and concentration of inhibiting compounds.

The increase in the specific wetted area optimizes gas-liquid mass transfer. An initially aseptic bioreactor system can be inoculated with selected organisms without competition from existing soil microbes.

Quantification of an overall net substrate flux from transport and reaction phenomena occuring at the microscale is facilitated by using packing materials of known geometry and surface area, aiding modelling efforts. Mass transfer characteristics can be measured independently by running the system abiotically. Intrinsic kinetic parameters (i.e. maximum growth and Monod constant) can be evaluated for a pure culture or known consortia. When continuous liquid flow and substrate availability are insured, steady state performance can be assumed implying the presence of a biofilm with constant activity, thickness, and contact area, independent of time.

One of the earliest uses of immobilized biofilms to degrade undesirable compounds was in a biological trickling filter. The technique was known in wastewater treatment by 1898 (Diks, 1992). The method was applied commercially in a plant constructed in Palm Springs, California, in 1960. At Avila Beach, California, a primary plant has operated a small trickling filter since 1968 solely for the deodorization of waste gas. Brown and Caldwell have designed "odor reduction towers" at six locations in California. Hydrogen sulfide removal efficiencies of 98 to 99% are attained (Pomeroy, 1982).

Unlike biofilters, commercial use of biological trickling filters for VOC elimination is limited outside the wastewater industry. The recent increase in

the availability and the stability of isolates capable of degrading xenobiotic compounds and the disadvantages of biofiltration systems have refocused attention on this process. The majority of studies employing microbial trickling filters and specifically vapor phase bioreactors for VOC removal have been conducted in the last five years. Most fundamental research has been done at the laboratory scale with little data available at the pilot or industrial scale.

Vapor Phase Bioreactors

To demonstrate the advantage of immobilized over suspended cell systems, Hill et al. (1991) compared the performance of batch, CSTR, and packed bed reactors for their ability to biodegrade liquid phase phenol with *Pseudomonas putida*. The processing rates for the packed bed bioreactor were an order of magnitude above those achieved by either batch or continuous, well-mixed bioreactors. Air stripping in the batch reactors resulted in losses of phenol of up to 25% while no loss of phenol was detected in the air stream of the packed bed reactor. Mass transfer and fluid mechanic parameters were measured with and without biomass and compared to traditional chemical engineering correlations. Comparisons differed by up to an order of magnitude. Hill concluded that actual column performance should be measured to prevent the use of incorrect functions in a model resulting in erroneous design calculations.

Kirchner et al. (1989) used a combination of bacterial monocultures fixed

on various carriers for degradation of a mixture of pollutants. By using different compounds (eg. acetone, propionaldehyde, napththalene and toluene, crude gas concentrations:5-35 ppm) the effect of the water solubility of the gaseous substances on removal efficiency was studied. They showed that the elimination capacity of the systems treating low solubility compounds was still high but less so than that of the more hydrophilic compounds. When the fixed cells were present in high densities, the reaction rate was independent of the cell count. The conversion of the pollutant did not depend to a significant extent on the carrier as long as it supported a high cell density. These and some other results indicated to Kirchner that the catalysed biological oxidation was limited by mass transfer, i.e., the biological reaction takes place comparatively rapidly and the mass transfer of the reactants through the liquid film is rate-determining (mass transfer regime) (Kirchner et al. 1987).

A later study by Kirchner, et al. (1991) focused on one of the systems described above; *Pseudomonas fluorescens* as the bacterial strain and propionaldehyde as the pollutant in a model bioreactor. Propionaldehyde is a relatively water-soluble substance. The carrier materials used were open-pore sintered glass, smooth glass, and plastic. The absorption and degradation of propionaldehyde was measured by systematic variation of the gas and liquid flow rates. The reactor was run in co- and countercurrent mode. Co-current mode has a potential disadvantage of limited mass transfer at the column outlet due to the concentration difference tending to zero. Results showed, however,

that the driving concentration gradient was practically independent of the mode of operation because the pollutant concentration in the liquid throughout the reactor remained very low. Tubes of sintered glass were compared with Raschig rings of a similar specific surface area. Although the tubes had advantages with regard to pressure loss and operational stability, the conversions were on average 20% lower, owing to poorer mass transfer (less turbulence in the oriented packing). At high pollutant loads, increasing inhibition (termed "retardation") of the biological degradation reaction was indicated. The cell density with the open-pore sintered glass was at least an order of magnitude higher than that for the plastic and smooth glass packings which resulted in improved degradation efficiencies. Intermittent operation of the bioreactor caused no significant decrease in conversion.

A microbial consortium that utilized methanol, butanol, acetonitrile, hexane, benzene, and toluene was selected by Oh and Bartha (1991) and fixed on a highly porous peat-perlite matrix packed into glass columns. Uninoculated columns served as a control for abiotic vapor removal by absorption alone. The experimental setup allowed the independent variation and measurement of solvent vapor concentrations and air flow rates. At very high solvent concentrations performance declined, presumably due to solvent toxicity. Performance also declined at very high flow rates, presumably because of insufficient retention time. Performance was steady around the maximum removal rate. In general, hydrophobic solvents were more difficult to remove

than water-miscible solvents.

Oh, et al. (1991) did a follow up study on methanol vapor removal using a similar experimental design. Steady state conditions for most experiments were attained after 7-10 days of operation. The data indicated that the removal rate increased with the inlet concentration with a tendency to reach a maximum value at an intermediate inlet methanol concentration (6-7 g/m^3). The data also indicated that at constant inlet concentrations removal rates remained essentially unchanged as the superficial air velocity increased except for a drop at the highest flow rate possibly due to a toxicity effect. This was indicative of reaction rather than mass transfer limitation for the process. Oh's (1991) study used the value of the kinetic constants derived from batch experiments in a model for the biofilter. The data from the suspensed system showed a drop in specific growth rate at high concentrations of methanol indicating inhibition kinetics. Oh assumed that the biomass in the biofilter remained essentially constant over considerable time periods which he justified by the low yield coefficient value (0.28) and the low methanol concentrations used.

One of the most extensive studies on vapor phase bioreactors was conducted in the Netherlands by Diks (1992). He achieved stable dichloromethane (DCM) elimination over two years of operation with feed concentrations ranging from 0-10000 ppm using the strain Hyphomicrobium sp GJ21. Even though DCM is poorly water-soluble, Diks showed that high

degrees of conversion could be reached. The maximum elimination capacity remained constant with a constant average organic load which Diks explained by the existence of a balance between biomass accumulation by growth and biomass removal by decay and sloughing, resulting in a constant holdup of active biomass. Biofilm was unaffected by short-term fluctuations in the organic load, which was important in view of the dynamic behavior of the filter system at briefly changing process conditions. He concluded that the assumption of steady-state was valid in experiments which required a changing organic load, if the total period of time consumed only amounted to several days. On a longer term, steady-state existed if a constant average organic load was maintained.

Experimental and theoretical results found that the gas-liquid mass-transfer resistance in the trickling filter bed was negligible and that the gas and liquid phases were close to equilibrium. The biological process inside the biofilm was the rate limiting step. The specific activity of the biomass was determined to be $R_s = 0.08$ g DCM/g TSS/h. This value was one eighth the specific activity of the suspended pure culture. This indicated that only about 12% of the biomass present in the bioreactor actually degraded dichloromethane which led Diks to question the reliability of applying intrinsic kinetic growth parameters calculated from suspended systems to fixed-cell system.

Diks evaluated the effects of both oxygen limitation and temperature

fluctuations on system performance. By comparing the substrate availability and the rate of reaction for oxygen and dichloromethane theoretically, Diks found that no oxygen limitation occurred throughout the substrate concentration range indicated. To investigate the influence of temperature, experiments were performed at 20°C. and 30°C. It is well known that temperature can affect a biological reaction rate by increasing the growth rate of the organism and the diffusion rate of the substrate. At the same time Henry's constant increases which reduces the concentration, and therefore availability, of the substrate in the liquid phase. Diks found that the elimination capacity of the system in the diffusion limited range was not affected by a temperature rise. He concluded that the increase of the reaction rate in the biofilm was offset by the decrease in the mass transfer rate from the gas to the liquid phase. The trickling filter system was operated co-currently and counter-currently. Diks expected co-current flow to give better results because of the lack of stripping effects but only small differences existed throughout the parameter ranges indicated. The phenomenon was explained by the smoothing effect the recirculation of the liquid phase had on the axial concentration profile. Although the overall filter efficiency depended on the local liquid concentration it was unaffected by the relative flow direction of the mobile phases.

Gas velocity was measured because of its effect on the system's mean residence time. The elimination capacity increased at higher superficial gas

velocities for inlet concentrations smaller than 11 g/m^3 (diffusion limited range) because the average gas phase concentration in the system increased. At higher inlet concentrations, the biofilm was fully penetrated with substrate and filter performance was limited only by reaction rate with no effect from the increased average gas phase concentration. Diks concluded that the degree of conversion was determined by the superficial gas contact time rather than superficial gas velocity or reactor height.

Canter et al.(1989) studied cometabolic removal of TCE, TCA, and butane with two glass bioreactors packed with either celite biocatalyst carrier R635 (.27 m^2/g) or R630 (1.3 m^2/g) by Mannville. Up to 94% degradation of TCE was achieved with the R635 reactor. Removal efficiency of all three compounds was greater in the R635 reactor than in the R630 reactor. Four pairs of butane sampling ports were located along each column. Butane removal was evident at each port indicating that the celite biocatalyst carriers allowed microbial growth throughout the entire length of the bioreactor.

The Idaho National Engineering Laboratory conducted a limited study assessing vapor phase bioreactors for their potential in bioprocessing methane, TCE, and *p*-xylene. Rates of methane removal were 2.1 and 1.6 fold greater than those exhibited by batch and chemostat reactors respectively. Cometabolism of TCE using methanotrophs removed 9 micrograms TCE/day per bioreactor. At a feed rate of 139.9 micrograms xylene carbon/min, 46% of the xylene was mineralized to carbon dioxide in a bench scale gas phase reactor

inoculated with *Pseudomonas putida* Idaho (Apel,1991).

Aromatic Hydrocarbons

The above vapor phase reactor study and this project evolved from work done at INEL involving isolation of microorganisms with the ability to use methylated aromatics, such as xylene and toluene, as their sole carbon source. The ability to utilize naturally occurring hydrocarbons is widely distributed among diverse microbial populations. When natural populations are contaminated with petroleum hydrocarbons, the indigenous microbial communities are likely to contain populations of differing taxonomic relationships which are capable of degrading the contaminating hydrocarbon (Atlas, 1981). Adaptation can occur by three interrelated mechanisms, namely; induction and or depression of specific enzymes, genetic changes which result in new metabolic capabilities, and selective enrichment of organisms able to transform the compound or compounds of interest. Selective enrichment has been widely observed in studies of hydrocarbon and petroleum degradation in the environment (Leahy and Colwell, 1990). Several reports by Colwell and Walker (1977), Atlas (1981), Floodgate (1984), and Cooney (1984) have shown that the numbers of hydrocarbon-degrading microorganisms and their proportion in the heterotrophic community increase upon exposure to petroleum or other hydrocarbon pollutants and that the levels of hydrocarbon-utilizing microorganisms generally reflect the degree of contamination of the ecosystem.

Petroleum is a complex mixture of hydrocarbons. Several classes, based on related structures, are recognized from the hundreds of individual components. The petroleum mixture can be fractioned by silica gel chromatography into a saturate or aliphatic fraction, an aromatic fraction, and an asphaltic or polar fraction (Atlas, 1981). Aromatic hydrocarbons constitute a major fraction of gasoline. These compounds are more water soluble and less volatile than many aliphatic constituents which favors the prevalence of aromatic hydrocarbons in groundwater contaminated by gasoline (Ridgway, 1990). Ridgway et al. (1990) isolated 297 gasoline-degrading bacteria from well water and core material from a shallow coastal aquifer contaminated with unleaded gasoline. Their responses on 15 gasoline hydrocarbons were evaluated on the basis of aerobic growth. Toluene, *p*-xylene, ethylbenzene, and 1,2,4-trimethylbenzene were most frequently utilized as growth substrates, whereas cyclic and branched alkanes were least utilized.

INEL Studies

Pseudomonas putida Idaho Isolation

Identification of 244 of the isolates in Ridgway's study revealed four genera: *Pseudomonas, Alcaligenes, Nocardia,* and *Micrococcus,* with pseudomonads making up 86.9% of bacteria identified. Jensen studied the bacterial flora of soil after application of oily waste and found that the predominant species of oil degraders belonged to the genera *Arthrobacter* and

Pseudomonas (Atlas, 1981).

INEL collected and screened several petroleum-contaminated soil and water samples for organisms that could degrade toluene, xylenes, and pseudocumene. After several transfers were done on a basal agar medium and incubated in a dessicator with an open beaker of *p*-xylene or toluene, the pure colonies that grew on these plates were introduced into a liquid broth with 1-10 ppm of the aromatic compound. Growth was determined by turbidity. A chemostat was set up and inoculated to control the essential parameters of aeration and pH. Initially toluene or *p*-xylene was introduced via vaporization. Cells grew to a density of 10^8-10^9 cells/ml and survived under continuous feed conditions for three years. One isolate was not only tolerant to high concentrations of toluene and *p*-xylene but utilized these compounds as its sole carbon source and grew under a layer of liquid *p*-xylene on basal salts agar media. The isolate was determined to be a strain of *Pseudomonas putida* (Rogers et al.,1990).

Cruden et al. (1992) analyzed some physiological properties of the INEL isolate named *Pseudomonas putida* Idaho. Aromatic compounds that can serve as growth substrates for *P. putida* Idaho include toluene, *m*-xylene, *p*-xylene, 1,2,4-trimethylbenzene, 3-ethyltoluene, benzylalcohol, benzoic acid, *m*-toluic acid, *p*-toluic acid, *p*-hydroxybenzyl alcohol, *m*-cresol, and *p*-cresol. Growth was also observed on solid media when the surface of the agar was overlaid with 5.0 ml of toluene, *m*-xylene, *p*-xylene, 1,2,4-trimethylbenzene, or 3-

Table 1. Growth of selected pseudomonas strains in the presence of organic solvents[a] (Cruden, 1992).

Solvent	Log P_{oct}[c]	Growth[b] *P.putida* Idaho	*P.mendocina* KR1	*P.putida* mt-2	*P.putida* F1
Decane	5.6	+ + +	+ + +	+ + +	+ + +
Hexane	3.5	+ + +	+ + +	+ + +	+ + +
Cyclohexane	3.2	+ + +	+ + +	+ + +	+ + +
Pentane	3.0	+ + +	+ + +	+ + +	+ + +
p-Xylene	3.1	+ + +	-	-	+ + +
Cyclopentane	2.5	+ + +	-	-	+ + +
Toluene	2.5	+ + +	-	-	-
1-Heptanol	2.4	+ + +	-	-	-
Benzene	2.0	-	-	-	-

[a]Cells were grown in LB medium in the presence of 25%(vol/vol) solvent.
[b]Symbols indicating turbidity values at 600 nm: + + +, >1 after 24 hr; + + 0.7 to 1.0 after 48 hr; -, <0.2 after 48 hrs.
[c]Logarithm of the octanol-water partition coefficient.

ethyltoluene. Three other species of *Pseudomonas* which can metabolize toluene were examined for their tolerance of selected organic solvents(Table 1). None of the strains tested was able to tolerate organic solvents to the same extent as *P. putida* Idaho. Results suggested that this isolate utilizes the same metabolic pathway as *P. putida* mt-2 which contains the TOL plasmid pWWO for the degradation of alkyl-substituted aromatic hydrocarbons. Attempts to detect the presence of a catabolic plasmid in *P. putida* Idaho were not successful. Southern hybridization experiments indicate significant homology

between fragments of *P. putida* Idaho DNA and a fragment of *P. putida* mt-2 DNA. The ability of this isolate to degrade toluene and *p*-xylene is stable through numerous transfers on nonselective media, as is the resistance to solvents. It is possible that the chromosomal location of the genes may make its ability to degrade aromatic hydrocarbons more stable.

Chemostat Studies

Commercial use of *Pseudomonas putida* Idaho required development of a bioprocess system capable of continuously degrading aromatic compounds. INEL modified their chemostat to allow mass balances to be taken. They concentrated on observing the response of the system to very high xylene loading rates and to changes in the dilution rate within the system. The studies were useful in determining the operating parameters for a system under extreme loadings but they could not be used for estimation of the fundamental kinetic parameters of the organism due to the extreme heterogeneity of the reactor. Experiments are in progress at Montana State University to determine the kinetic parameters for the organism's growth at significantly lower loading rates. The resulting data will be analyzed to evaluate μ_m, K_s, and the stoichiometry of xylene utilization (growth yield) for *Pseudomonas putida* Idaho.

VPBR Design Criteria

Because aromatics are highly volatile, traditional aeration procedures used in INEL's chemostat studies produced a significant xylene-saturated gas

stream leaving the system. This project was initiated to concentrate on biodegradation of this vapor phase xylene. Two gas phase reactors were designed and constructed to be operated concurrently. Inert, manufactured packings were used because of the modelling advantages. One column was packed with glass spheres and the other with diatomaceous earth pellets of a comparable external surface area with both resembling natural surfaces (in contrast to plastics) that appear to be readily colonized (Diks, 1991). A potential benefit of the porous pellets is a degree of protection for interior colonized organisms from both surface shear conditions, microbial competition, and shock loadings. The nominal pore size of 20 micrometers provides a high surface area but does not seriously impede movement and colonization of all pellet interior surfaces (Sturman, 1991). Gas and liquid loading rates are important process parameters due to their effect on mass transfer and interfacial area (Gossett, 1985). The system was designed so that the gas and liquid flow rates could be varied independently to measure their effect on column performance. No chemical engineering correlations describing mass transfer for the pellets were available. This and the fact that actual performance can differ significantly from general correlations prompted separate abiotic studies to determine mass transfer characteristics independent of kinetics. A countercurrent flow regime offered the advantage of a consistent concentration gradient for mass transfer between the gas and liquid phases for the entire column even in the absence of biodegradative activity. Once

inoculated, however, Diks and Kirchner both concluded that the overall reactor efficiency was not dependent on the flow regime. A flow rate sufficient to merely wet the biofilm surface was chosen to minimize the liquid waste effluent. Oxygen was delivered to the system via the air stream carrying the xylene vapor. Although oxygen is required for biological reaction, the Monod constants are generally extremely low (10^{-3}-10^{-1} mg/l) and so reaction kinetics are regarded as zero order in oxygen concentration (Kampbell et al., 1987). *Pseudomonas putida* is a mesophylic bacteria which grows at a temperature of about 20-45°C.(Kampbell et al., 1987). The reactor temperature was maintained between 20°C. and 30°C.

METHODS AND MATERIALS

The experimental approach can be divided into the following four parts: 1) Growth and preliminary chemostat studies on *Pseudomonas putida* Idaho. 2) Abiotic reactor studies. 3) Biotic reactor studies. 4) Biomass determination studies. All experiments were conducted with Baker Analyzed Reagent (J.T. Baker Chemical Co.) *p*-xylene and *Pseudomonas putida* Idaho.

Growth Studies

Batch Studies

Batch growth studies were conducted at MSU to determine whether

Table 2. Basal salts media.

Compound	Concentration (mg/l)
KH_2PO_4	700
K_2HPO_4	700
MgCl2	300
$(NH_4)_2SO_4$	500
Trace Compounds*	
$FeSO_4*7H_2O$	1.0
$MnSO_4*H_2O$	1.0
$NaMoO_4*7H_2O$	1.0
$ZnSO_4*7H_2O$	1.0

*Trace compounds added to M.S. media for chemostat and reactor studies.

Note: 10 liters autoclaved 90 minutes at 121°C.
20 " " 4 hours at 121°C.

certain trace minerals and metals enhance or inhibit *Pseudomonas putida* growth on *p*-xylene. All studies were carried out in closed flasks containing 100 ml aliquots of sterile basal salts media (Table 2), 1 ml of xylene, and 1 ml of cells from a batch culture in log phase of growth. One ppm of Fe, Mo, Co, Mn, Zn and 10 ppm of Ca were added to the first culture (G-1). The second culture included 0.1 ppm of Cu (G-2). The third culture contained 10 ppm of Fe and Mo, 1 ppm Co,Mn, and Zn, and 100 ppm Ca (G-3). The fourth culture contained 1ppm of Fe, Mo, Co, Zn, Mn, and Cu, and 10 ppm Ca (G-4). Incubations were performed at 22°C. on a gyratory shaker. Cell growth was demonstrated by measuring increases in optical density on a Varian DMS 90 UV Visible Spectrophotometer set at a wavelength of 600 nm. Growth rates were determined by cell enumeration done at 2-3 hour intervals for 25-30 hours. Each culture was diluted and spread in duplicate on Difco (Detroit, MI) nutrient agar plates. The plates were incubated in a xylene and water saturated atmosphere at room temperature for three days. Colonies were counted after incubation. The arithmetic mean of the observations was used as a colony forming unit (cfu) count. When possible, the dilution counted contained between 30 and 300 cfu per plate.

Chemostat Studies

A New Brunswick Bioflo III computer controlled reactor was used to conduct preliminary kinetic studies. Chemostat experiments were carried out