Physiological and environmental factors affecting biofilm formation and activity in vapor phase bioreactors
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A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy m Chemical Engineering in
Montana State University


Abstract:
Vapor phase bioreactors (VPBRs) are being used increasingly to remediate vapor streams contaminated with volatile organic compounds (VOCs). VPBRs are packed columns of inert materials which support growth of biofilms. Physiological and environmental factors influence biofilm formation and activity significantly within VPBRs and these factors were investigated in the present dissertation. Throughout this investigation, monocultures of P. putida 54G were grown on toluene as the sole exogenous source of carbon and energy in planktonic and in biofilm cultures.

Kinetics of toluene degradation by suspended and biofilm cells were similar when specific activity (mg toluene degraded/unit biomass quantity-hr) was based on numbers of uninjured cells rather than total biomass. When based on total biomass, specific activity was lower for biofilm than for suspended cells, and may have been a result of prolonged toluene exposure.

Planktonic culture studies indicated that physiological stress and cell injury affected cell growth and bacterial respiration rates. Bacterial injury increased with increase in toluene concentration and duration of toluene exposure and coincided with formation of unconverted intermediates. Rate expressions for injury and irreversible loss of toluene degradation pathway were computed by fitting a theoretical injury model to experimental results.

In a flat plate VPBR, results from oxygen concentration profiles, microfluorimetry and cryosectioning combined with fluorescent cell staining indicated that stratified layers of respiratory activity formed in toluene-degrading biofilms with considerable activity observed at the base of the biofilm. This observation was in contrast to conventional biofilm models which indicate that the majority of the respiratory activity is located at the biofilm-liquid interface in thick biofilms.

Microscale parameters determined from planktonic cell studies and measured physical and chemical characteristics were used to calibrate a mathematical process model. The process model accurately predicted flat plate and column VPBR performance. A sensitivity analysis conducted on predicted results indicated that parameters adjusted to fit the model (biomass density and death and endogenous decay rates) showed a negligible effect on toluene degradation while Henry's law constant for toluene, biotransformation kinetics and reactor surface area affected toluene degradation significantly. Since these parameters were estimated accurately, confidence in predicting VPBR performance increased considerably.

# PHYSIOLOGICAL AND ENVIRONMENTAL FACTORS AFFECTING BIOFILM FORMATION AND ACTIVITY IN VAPOR PHASE BIOREACTORS

by

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APPROVAL

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This thesis has been read by each member of the thesis committee and has 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

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# ABSTRACT

Vapor phase bioreactors (VPBRs) are being used increasingly to remediate vapor streams contaminated with volatile organic compounds (VOCs). VPBRs are packed columns of inert materials which support growth of biofilms. Physiological and environmental factors influence biofilm formation and activity significantly within VPBRs and these factors were investigated in the present dissertation. Throughout this investigation, monocultures of *P. putida* 54G were grown on toluene as the sole exogenous source of carbon and energy in planktonic and in biofilm cultures.

Kinetics of toluene degradation by suspended and biofilm cells were similar when specific activity (mg toluene degraded/unit biomass quantity-hr) was based on numbers of uninjured cells rather than total biomass. When based on total biomass, specific activity was lower for biofilm than for suspended cells, and may have been a result of prolonged toluene exposure.

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# CHAPTER 1

# GOAL AND OBJECTIVES

Motivation: Vapor phase bioreactors (VPBRs), or biofilters as they are more commonly known, have been in use for more than twenty five years in Europe while their use as an alternate air pollution control technology is at an early phase of development in the U.S. Success in applying VPBR technology to controlling vapor phase volatile organic compounds (VOCs), such as TCE and toluene, depends on combining laboratory-scale studies with VPBR mathematical models which incorporate microscale and mesoscale phenomena for predictive purposes. At the microscale, it is important to study the effect of VOC degradation on the physiological and genetic response of bacteria and to investigate the kinetics of VOC degradation by biofilm-bound cells. Measurement of such processes can significantly increase confidence in the predictive capabilities of VPBR models, and can aid in identifying those parameters which most affect reactor performance.

Goal: Quantify microscale process dynamics of the progression of physiological state and activity during the biodegradation of toluene by *Pseudomonas putida* 54G in a vapor phase bioreactor.

Objectives: The objectives of the dissertation are detailed below:

- Determine the physiological response of suspended and biofilm cultures of *Pseudomonas putida* 54G during toluene degradation.
- Compare suspended and biofilm cell kinetics for toluene degradation by *P. putida* 54G to determine if suspended cell kinetics can be used to model bacterial activity in VPBRs.
- Determine stratification in respiratory activity (by using microscopy combined with cryosectioning) and oxygen concentration (by oxygen microsensors) in *P. putida* 54G biofilms during toluene degradation in a flat plate vapor phase bioreactor.
- Incorporate kinetic, stoichiometric, injury and genetic loss coefficients determined from suspended cell studies and measured values of Henry's law constant for toluene and gas-liquid and liquid-biofilm interfacial thicknesses into a predictive VPBR model.
- Assess the capabilities of the VPBR model for predicting performance of VPBRs under different loading rates during toluene degradation by *P. putida* 54G and determine which parameters affect reactor performance.

# CHAPTER 2

## INTRODUCTION

We live in a world in which the existence of carcinogens, mutagens and tetratogens is taken for granted. Waste materials released from industry and agriculture are responsible for considerable contamination of soil, water and air in the United States. Groundwater and soils in many regions of the U.S. contain various volatile organic compounds (VOCs), rendering the water unsuitable for human consumption. Stringent air emission standards in the U.S. have mandated the need to develop innovative and cost efficient technologies to remediate vapor streams contaminated with volatile organic compounds. Such streams are often produced as a result of remediation activities for treatment of gasoline and solvent spills in groundwater aquifers. Several treatment alternatives are available to remove VOCs from groundwater and soils, including soil excavation and subsequent disposal in a hazardous waste landfill and either *in situ* or above-ground volatilization/stripping followed by physico-chemical treatment.

There are many physico-chemical methods available for VOC elimination from the vapor phase. Adsorption on activated carbon, condensation, incineration, and liquid scrubbing are methods that have been used in the chemical industry for recovery or

destruction of contaminants (8). Activated carbon is frequently used as an appropriate adsorbent for VOCs. A major disadvantage of this process is the saturation of the adsorbent which necessitates carbon regeneration. Routine disposal of the spent activated carbon adds a significant expense to remediation costs. Organic compounds can be incinerated at sufficiently high temperatures which also entail high energy costs. The combustion temperature can be considerably reduced if a catalyst is used, the choice of which determines the cost (35). Typical operating conditions that have been used to ensure 95 - 99% removal of VOCs from waste gases require a retention time of 0.3 - 1.0 seconds and temperature of 1000°C - 1400 °C (18). Condensation and liquid scrubbing, as the names suggest, are methods which result in a phase transfer of the contaminants to the liquid phase rather than elimination.

Biological treatment of contaminated vapor streams is an attractive alternative because it offers the potential to: (1) permanently eliminate contaminants through biochemical transformation or mineralization, (2) provide an environmentally friendly solution by avoiding harsh chemical and physical treatments and intensive energy use, (3) be cost-effective and (4) provide an easily operable technique (45). In recent years, many bacteria have been isolated which show a high specificity, high activity and tolerance towards volatile organic compounds (34). Use of microorganisms to remove pollutants is well established in the area of wastewater treatment. However, not until recently have biological technologies been seriously considered in the U.S. for removal of pollutants from anything other than aqueous streams (28). The concept of using microorganisms for the removal of toxic compounds such as petroleum hydrocarbon and odorous compounds

in the vapor phase is well established in the Netherlands and Germany (9). Biofiltration is a standard air pollution control technology in Europe but is not well recognized in the U.S. because of limited governmental support for research and development, lack of regulatory programs, lack of descriptions written in the English language and a lack of full-scale demonstration projects in specific industries (28, 30).

Biological treatment can eliminate hazardous compounds by biotransforming them into innocuous forms, degrading them by mineralization, or anaerobically transforming them to carbon dioxide and methane (22). Biological processes have the additional advantage of being carried out at or near ambient temperature and pressure (36). Some volatile components sufficiently resemble biogenic structures to be eliminated biologically. VOCs are eliminated by microbial activity because they serve as energy and/or carbon source for microbial metabolism. Other xenobiotics, termed recalcitrant or persistent compounds, possess such unnatural chemical structures that their biological degradation is usually too slow to be of practical significance. Extensive studies on microorganisms that degrade recalcitrant and xenobiotic compounds have been carried out and literature in this field is ever expanding. New metabolic pathways continue to evolve by mutations and exchange of genetic properties. Such naturally transmitted changes may lead to the development of enzymes which are capable of degrading xenobiotics and other refractory compounds. Once a bacterial strain or a mixed microbial culture capable of degrading a specific compound has been isolated, its practical application may generally be extended to the field of biodegradation in biofilters, bioscrubbers and trickling filters. The microbial population can either be freely

dispersed in the water phase or immobilized on an inert support. The former is carried out in bioscrubbers, the latter in biofilters and biological trickling filters.

## Biofilters

A biofilter (Figure 2.1) can eliminate a broad range of inorganic and organic pollutants. In Europe, biofiltration has been successfully used to control odors and organic and inorganic air pollutants from a variety of industrial and public sources. A biofilter consists of a simply structured bed in which the flowing gas is contacted with an immobilized microbial flora which provides a high biological surface area. After sufficient time, a biofilm is formed on the filter material at the expense of the contaminant. Aerobic degradation of the contaminants in the filter reduces them to carbon dioxide, water and biomass. Continuous availability of water and transfer of oxygen from the vapor to the liquid phase are critical factors in the design of a biofilter. Maintaining the porosity of the compost by turning it over, and/or replacing it entirely, once spent, are the two major maintenance requirements for biofilters with compost-based filter materials (36).

Since inorganic nutrients are also required, natural materials such as compost, peat and bark are added. This type of a system provides a high biodegradation efficiency, up to 90% in some cases, with low operating costs. Carrier materials have been used for as long as five years before being discarded (8). Since the microorganisms cannot survive extreme temperatures commonly encountered in waste gas emissions, the gas temperature

is usually controlled before it is passed through the biofilter. Since the packing must be constantly wetted for continuous growth of biofilm, water quenching is used to achieve both objectives. Due to high porosity and low pressure drop of packing materials as well as an increased biological activity, high gas flow rates and high organic loads may be realized. Typical pressure drops in the region of 500 Pa/m are common for biofilters of mixtures of compost, peat and wood chips (8).

Until recently, most commercial biofilters have been built as single-bed systems that are open to the atmosphere. In open filters, the bed is exposed to fluctuations in weather conditions such as rain, sunshine and temperature which may directly influence the biological process resulting in higher pressure drop and channeling. However, there has been a shift towards closed systems because of their lower susceptibility to changing climatic conditions and the possibility of continuous off-gas monitoring. The increasing use of closed system biofilters has resulted in better control of humidity and temperature which are continuously monitored and recorded (8). The type of construction and installation of a biofilter for a given application usually depends on availability of space relative to the required filter volume. Biofilters can be operated in both a continuous and intermittent mode, based on the intended application. Pre-conditioning of the influent gas followed by transport to and distribution in the filter bed are other important components of a biofilter system. Table 2.1 lists some full scale applications of biofilters.

## Bioscrubbers

Bioscrubbers (Figure 2.2) are mainly suited for the treatment of waste air streams of medium concentration with components of a high or moderate water solubility. The gas is contacted with water in a spraying tower containing inert packing, resulting in absorption of waste gas components into the water phase. Microorganisms are suspended in an aqueous solution. In contrast to biofilters the liquid phase in bioscrubbers is mobile, which allows a better control of reaction conditions. Nutrients and buffers can be added and the liquid can be replenished and discharged in order to remove undesired products. Better control with reference to temperature, pH and ionic strength can be achieved. Other advantages compared to filters include a rapid adaptability to changing crude gas compositions, the possibility of discharging reactants and an easier heat dissipation. In comparison to biofilters, they are more expensive, complicated to use, inappropriate for low water-solubility contaminants and provide a lower specific gas/liquid surface area (5).

The bioscrubber process can be divided into two steps - adsorption and regeneration. In the absorption phase, water soluble components of waste gases are transferred to the liquid phase by continuous contact in a contacting apparatus like a spray tower. At steady state, the mass transfer rate is equal to the elimination rate of VOCs which can be estimated by the product of an overall mass transfer coefficient, total specific contact area between the liquid and the gas phase and the concentration gradient. The overall mass transfer coefficient depends on the transfer resistance in the continuous

(gas) phase as well as the dispersed (liquid) phase. For an efficient absorber performance, the concentration of the compound in the liquid phase must be as low as possible (11).

In the regeneration phase, microbial oxidation of the absorbed compounds leads to their elimination from the liquid phase in an activated sludge reactor. The sludge tank is usually aerated by diffused air or mechanical stirring which also helps in keeping the sludge in suspension and increases contact between the liquid phase and the sludge.

## Biological Trickling Filters

When biodegradability of contaminants is assured, the application of biofilters for treating contaminated vapor streams appears unlimited. However, problems may arise if acid metabolites are produced during the biological degradation of vapor phase contaminants. When the pH buffering of the packing material is effective for only a relatively short period of operation, the presence of a continuously flowing liquid phase is required for removal of the inhibiting metabolites or end products which may otherwise accumulate. This type of a situation is mainly encountered in the degradation of chlorinated hydrocarbons or reduced sulfur compounds.

In biological trickling filters, shown in Figure 2.3, the waste gas is forced to rise countercurrently through a column containing inert packing materials in order to obtain the greatest rate of absorption (21). Water soluble compounds are transferred to the liquid, from which they diffuse into the biofilm, growing on the packings, where they are eliminated by the constituent microorganisms. Inert materials used as a support media

include glass, plastics, ceramic and activated carbon. Their interfacial area/volume of reactor results in large void volume for gas flow which further leads to a reduced pressure drop. The flowing liquid allows for continuous control and adjustment of environmental physiological conditions which are necessary for optimal microbial activity (i.e. temperature, pH, and nutrients) as well as removal of acid metabolites.

A trickling filter is more adaptable for modeling purposes because inert manufactured packing material of distinct size and shape are used as support medium instead of natural materials that cannot be easily quantified. This is important for scale-up purposes especially when the results of a lab scale experiment are to be used in the design of a pilot plant. Trickling filters (packed column) also have the obvious advantage of maximizing bioavailability and limiting availability of waste products. The system can be modeled to quantify mass transfer characteristics independent of kinetics.

## Literature Review on Biofilters and Biological Trickling Filters

Biofilters and biological trickling filters have been used for many different applications including odor control problems at wastewater treatment plants, ammonia and sulfur odors and VOC control for methanol, phenol, formaldehyde, ethanol, toluene, and benzene. Kirchner et al. (20) used trickle-bed filters to biodegrade important solvents and substances such as aldehydes, methyl ethyl ketone and ethyl acetate at an influent concentration ranging from 5 - 40 ppm. Using suitable bacterial strains they obtained conversions of 90% at space velocities of 1500 $hr^{-1}$ and rate-determining parameters for

the biodegradation process were identified. Kirchner et al (21) obtained conversion rates of between 68 and 96% for propionaldehyde in trickle-bed biofilters. Ottengraf and van den Oever (33) and Ottengraf et al (35) investigated the removal of volatile organic compounds in biofilters and identified bacterial strains that could be used to convert many organic and inorganic compounds to mineral end-products. Kinetic models for the biodegradation process were developed to predict the elimination capacity of the biofilter bed. Diks and Ottengraf (7, 8) demonstrated the use of biological trickling filters for degrading dichloromethane from waste gases in concentration ranges of 0 - 10,000 ppm. They operated the filters in co- and counter-current mode and determined that flow direction did not significantly affect the elimination capacity of the bed. A simplified model, "Uniform-Concentration-Model", was developed and used to predict filter performance. Maximum dichloromethane elimination capacities of 157 g/($m^3$-hr) were obtained.

Apel et al. (2) applied gas phase bioreactors to treat vapor streams contaminated with methane, trichloroethylene (TCE) and p-xylene. Methanotrophic bacteria were used to process methane and TCE while a xylene resistant strain of *P. putida* was used to process p-xylene. For methane, the gas phase bioreactors demonstrated better efficiencies (almost twice) compared to sparged liquid phase bioreactors and conventional shaken cultures. At a feed rate of 140 μg of xylene $min^{-1}$, approximately 46 % of the xylene was mineralized to carbon dioxide in a single pass through a column scale gas phase bioreactor. Zilli et al (50) investigated the feasibility of biologically removing phenol from waste gases using a biofilter. During a one-year operation of a laboratory scale

biofilter, packed with peat and glass beads, degradation efficiencies of 93 - 99.6% were obtained for inlet phenol concentrations of 50 - 2000 mg $m^{-3}$ with a residence time of 54 s.

Shareefdeen et al (41) used laboratory columns containing bacteria supported on mixtures of peat and pearlite to biodegrade methanol vapors. Rates of up to 112.8 g-$hr^{-1}$ $m^{-3}$ were measured in the columns. Mathematical models were developed to validate the experimental results and based on experimental data and model predictions, the methanol biofiltration process was shown to be limited by oxygen diffusion and methanol degradation kinetics. Baltzis and Shareefdeen (4) developed a model for predictive and scale-up calculations of a methanol vapor degrading biofilter. The model indicates that oxygen availability can be the rate limiting factor for the biodegradation process and can be used to predict the performance of an existing biofilter or in preliminary design of a new unit.

Shareefdeen and Baltzis (42) used the same model to predict steady and unsteady state toluene degradation in a peat/pearlite biofilter. By incorporating kinetics for toluene degradation in suspended cell cultures and other experimentally determined parameters into a biofilter model, they satisfactorily predicted effluent toluene concentrations. Based on a sensitivity analysis conducted on predicted results, they concluded that an accurate estimate of biolayer surface area per unit volume was critical in modeling a biofilter.

A modular column vapor phase bioreactor has been in operation for three years at the Biotechnology Research Department at Orange County Water District (37). In the reactor a consortia of hydrocarbon degrading bacteria derived from a shallow gasoline-contaminated aquifer was established on the packing and grown on benzene, toluene, p-

xylene, hexane, cyclohexane, 2,2,4, trimethylpentane, octane and an inorganic minimal media. The maximum removal of contaminants occurred when conditions for unlimited microbial growth were present, and starvation for nitrogen (one of the components of the inorganic medium) significantly diminished contaminant removal. Groestijn and Hesselink (11) and Leson and Winer (28) have presented excellent reviews on applications of biofilters for industrial and domestic use.

## Scope of the Dissertation

Despite a rapidly expanding literature in the field of biofilters during the last five years, this technology is still far from being applied commercially as an alternate air pollution control technology. Relatively few studies address interactions among different scales of biofilter operation nor do they consider how phenomenological effects can be integrated to make predictions of field-scale process behavior. The relative absence of practitioner-oriented tools for decision making suggests that a process engineering approach is necessary to improve the state-of-the-art of biofilter practice. Process engineering, in the context of biofilters, involves the integration of chemical and microbiological characteristics and laboratory and field data, in order to make predictions and design decisions. To increase the applicability of biofilters in the field, a scale-up approach is essential to understanding and interpreting complicated biofilm processes within a biofilter. A possible biofilter scale-up scenario would appear in this fashion:

1) investigate microbiological and engineering phenomena at the microscale

using ideal laboratory reactors such as batch, continuous stirred tank and flat plate reactors or laboratory-scale biofilters,

2) calibrate a computer model that describes biofilter performance at the microscale, utilizing parameters determined from microscale studies and predict toluene degradation at this scale,

3) conduct mesoscale biofilter performance experiments using column laboratory reactors,

4) predict mesoscale reactor performance using the biofilter model and determine parameters which affect biofilter performance significantly and

5) utilize information from mesoscale reactors and the predictive biofilter model to design a pilot-scale system.

Figure 2.4 shows the focus of this dissertation designated as VPBR project, which attempts to address questions 1 through 4. Throughout this dissertation the term vapor phase bioreactors (VPBRs) is used to describe a biofilter containing inert manufactured packing material which support biomass growth and promote VOC degradation. The integration of different phenomena during scale-up are explained in the following.

Planktonic and Biofilm Cell Kinetics: Scale-up of VPBRs can best be achieved through use of predictive models, the success of which depends on accurate estimates of kinetic and stoichiometric coefficients. Most bioreactor models that are developed for predictive purposes incorporate planktonic cell kinetics to model substrate degradation and biofilm growth. No clear consensus is available

relating biofilm and suspended cell kinetics. Fletcher and Marshall (10), van Loosdrecht et al (47), Karel et al (17), Hamilton and Characklis (13) and Hamilton (12) have published extensive review articles that have shown both increases and decreases in activity for attached cells in comparison to freely suspended cells. Cellular processes involved during VOC removal by free and attached bacteria could be different from degradation of benign substrates such as glucose or acetate. LaMotta (25), Harremoës (14), Rittmann and McCarty (40), Skowlund and Kirmse (43) and Skowlund (44) are among many researchers that have developed mathematical models to evaluate steady-state biofilm kinetics but, in contrast very few studies on biofilm-mediated degradation kinetics of VOCs exist. Alvarez et al (1) and Arcangeli and Arvin (3) have presented information on toluene degradation kinetics without relating suspended and biofilm cell kinetics. With such conflicting information on kinetics and a need to understand degradation kinetics of VOCs such as toluene, the relation between kinetics of free and biofilm cells is important to understand. Chapter 3 titled "A Comparison of Toluene Degradation Kinetics by Planktonic and Biofilm Cells of *P. putida* 54G" details a comparative study for suspended and biofilm cells of *P. putida* 54G during toluene degradation.

Physiological Changes: Injury has been defined as the sub-lethal physiological structural consequence(s) resulting from exposure to injurious factors within aquatic environments (31, 32). This is reflected by the inability of

injured cells to reproduce under selective or restrictive conditions that are tolerated by uninjured cells. Although there is overwhelming evidence to indicate presence of stressed or injured bacteria in most environments, very few studies exist on the topic of hydrocarbon-related injury.

Tebbe et al (46) have determined that a majority of naphthalene- degrading microbes that they investigated only expressed their genotype after non-selective isolation while some organisms did not express their genotype at all. Love and Grady (29) determined that when continuous cultures of *P. putida* were grown on benzoate and m-toluate and transferred to plates containing the same medium, they lost culturability in comparison to continuous cultures grown on glucose. Ridgway (39) determined a similar phenomena wherein a stressed subpopulation of viable hydrocarbon-degrading bacteria from a gasoline-contaminated aquifer led to an underestimation of true viable-hydrocarbon degrading bacteria. Leddy et al (27) determined that *P. putida* 54G cells, when grown on vapor phase toluene for 10 - 15 days, formed Tol- mutants that showed a selective loss of catabolic functions. These mutants could not degrade toluene but continued to grow in the presence of toluene, metabolizing other carbon sources such as organic compounds leaking from wild-type cells.

In this context, it is important to determine how stress response can be related to the ability/efficiency of a microorganism to degrade hydrocarbon vapors. This information when obtained in a well defined system could be utilized to formulate rate expressions based on growth of stressed and injured

cells. Establishment of quantitative rate relationships would permit incorporation of stress response into process models, leading to a superior ability to design and control bioremediation systems. Chapter 4 titled "Physiological Stress and Injury in Batch Cultures of *P. putida* 54G during Toluene Degradation" covers the topic of stress and injury related to toluene degradation by *P. putida* 54G.

Stratification of Respiring Cells: Quantitative information on stratification of cell layers in biofilm reactors used in the degradation of volatile, potentially toxic, organic compounds such as toluene is non-existent. For over 25 years, researchers have presumed that the entire mass of attached microorganisms in fixed-film reactors was not uniformly active but different layers of activity developed within thick biofilms (15, 23). Many different techniques have been used to study the spatial and temporal variations of activity in biofilms (16, 19, 24, 26, 48, 49) but information on stratification of respiratory activity in toluene degrading biofilms has never been published. Toluene might cause injury to the layer of biofilm cells that are at the fluid/biofilm interface with active cells respiring in the depths of the biofilm. Clearly such a phenomena could exist in VPBRs degrading toluene and could effect the predictive capabilities of VPBR models if they are not accounted for. This study was carried out in a flat plate VPBR as detailed in Chapter 5 titled "Spatial Distribution of Respiratory Activity in *P. putida* 54G Biofilms during Toluene Degradation". In this study, oxygen profiles in the vapor, liquid and biofilm phases were measured using microsensors

and coupled with cryosectioning and fluorescent microscopy results to assess stratification of respiratory activity in *P. putida* 54G biofilms.

Process Model: Results obtained from the microscale experiments were used to calibrate a VPBR model which was developed by Peter Reichert at EAWAG, Switzerland (38). Measured values of kinetic and stoichiometric coefficients (Chapter 3), injury and irreversible loss coefficients (Chapter 4), were combined with Henry' law constant for toluene and boundary-layer thickness at the gas/liquid and liquid/biofilm interface. The model is used to predict toluene degradation along the length of the reactor and overall toluene degradation in a flat plate VPBR. Only three parameters are adjusted to fit the experimental results: biomass density, death and endogenous decay rates. A sensitivity analysis was conducted on predicted results to determine which parameters affected toluene degradation in the flat plate VPBR. This modeling approach provided a rational design for predicting an upper limit of toluene degradation in a VPBR. Chapter 6 titled "A Predictive Model for Toluene Degradation in a Flat Plate Vapor Phase Bioreactor", details the calibration of the process model from microscale experiments and the oxygen profiles from the flat plate VPBR experiment. Model results are compared with actual degradation measured in a flat plate VPBR operated under two different influent vapor phase toluene concentrations of 150 and 750 ppm. A section which includes the verification of column VPBR performance operated under the same conditions as the flat plate

VPBR except for a significantly higher flow rate and surface area has been added to Chapter 6. This chapter includes a detailed description on a sensitivity analysis conducted on parameters that affect column performance and a comparison of parameters that affect toluene degradation significantly at the micro- and mesoscale.

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Table 2.1. Biofilter applications for off-gas treatment in European industry (Adapted from van Groenestijn and Hesselink, 1993)

| **Compound** | **Industry type** | **Elimination efficiency(%)** | **Flow rate ($m^3 h^{-1}$)** | **Volumetric load ($h^{-1}$)** |
|---|---|---|---|---|
| Odor | Animal rendering | 94-99 | 214,000 | 66 |
| Odor | Vegetable oil | 97 | 39,000 | 120 |
| Odor | Cocoa roasting | >99 | 4,000 | 73 |
| Odor | Wastewater treatment | 80-90 | 5,000 | 7 |
| VOCs | Storage tanks | 90 | 2,000 | 8 |
| VOCs | Industrial wastewater treat. | 70-90 | 65,000 | 31 |
| VOCs | Fish processing | 95 | 6,300 | 105 |
| VOCs | Fish processing | 85 | 10,300 | 184 |
| $H_2S$ | Landfill gas | >99 | 300 | 17 |
| Alcohols | Foundry | >99 | 30,000 | 150 |
| Aromatics | Foundry | 80 | 40,000 | 120 |
| Styrene | Resins processing | 65 | pilot plant | 100 |
| Phenol | Phenol resins | 97 | pilot plant | 200 |

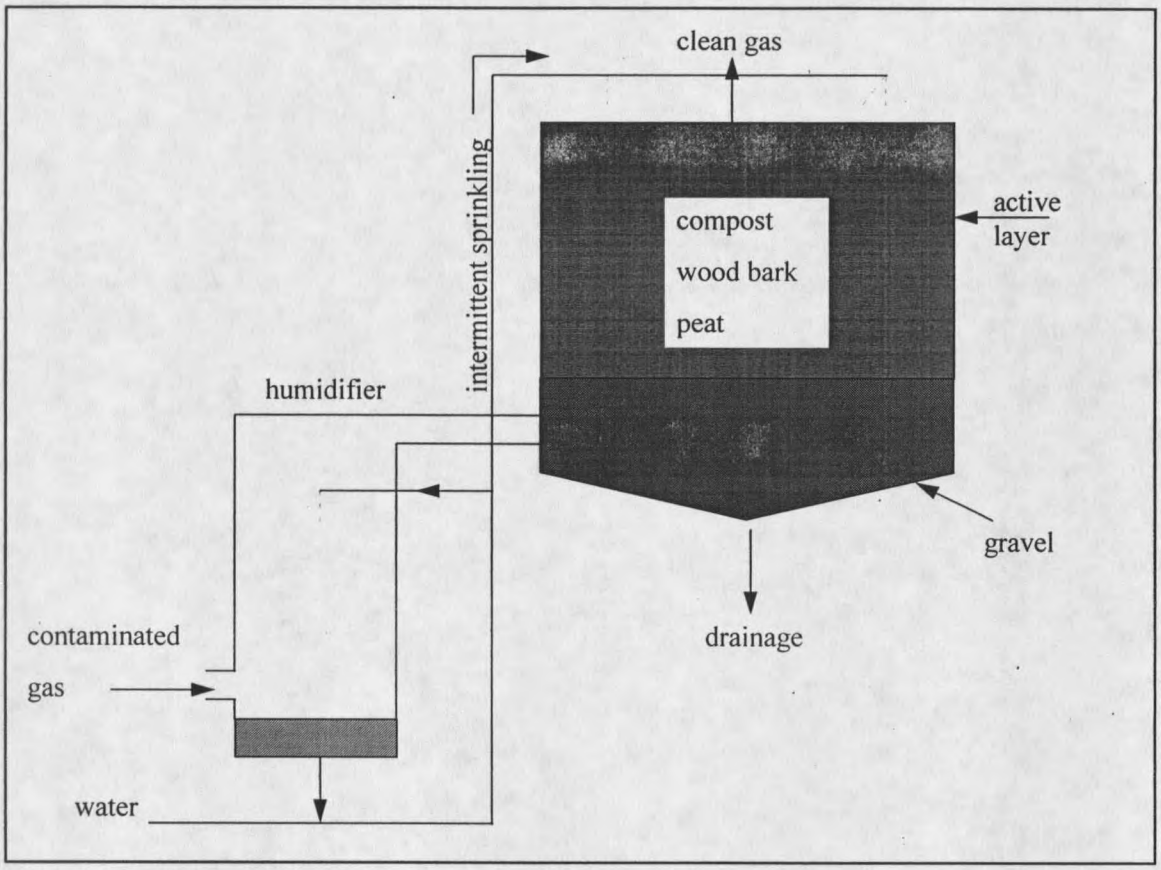


**Figure 2.1.** Schematic of a biofilter.