Design and operating strategies for bioreactors treating dynamically varying concentrations of gas-phase volatile organic compounds (VOCs)

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DESIGN AND OPERATING STRATEGIES FOR BIOREACTORS TREATING DYNAMICALLY VARYING CONCENTRATIONS OF GAS-PHASE VOLATILE ORGANIC COMPOUNDS (VOCs)

A Dissertation

Submitted to the Graduate Faculty of the
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Doctor of Philosophy

in

The Department of Civil and Environmental Engineering

by

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ABSTRACT

The dynamically varying concentrations of volatile organic compounds (VOCs) in most waste gas streams present a challenge in design and operation of biofilters treating such off-gases. Studies described in this dissertation were directed toward development and experimental testing of two design modifications/operating strategies that have the potential to improve biofilter performance during unsteady-state loadings.

In the first design modification studied, activated carbon was incorporated into biofilter packing media and a novel periodic operating strategy, Sequencing Batch Biofilter (SBB) operation, was tested under “normal” and various “shock” loading conditions treating methyl ethyl ketone contaminated air. Results demonstrate how the operational flexibility of the SBB system can lead to higher overall removal efficiency and higher minimum instantaneous removal efficiency than are achieved in a conventional continuous flow biofilter (CFB) during transient loading. Denaturing gradient gel electrophoresis of PCR-amplified genes coding for 16S rRNA was used to assess differences in bacterial community structure as a function of height in the SBB and CFB columns. Results suggest that SBB operating strategy can impart a sufficiently large selective pressure to influence microbial community structures.

In the second design modification studied, an activated carbon column was placed in series before a biofilter to assess the potential of the combined system to effectively treat discontinuously loaded VOC mixtures. Abiotic fixed-bed sorption experiments and numerical modeling were conducted to assess the degree of load equalization achieved by GAC columns for gas streams containing intermittent concentrations of acetone and toluene present as single-component contaminants and as a mixture. Performance of the GAC-biofilter integrated system was experimentally evaluated in comparison to a conventional biofilter in treating a mixture of acetone and toluene at various discontinuous loadings. Results demonstrate that load dampening
achieved by a passively-operated GAC buffering system can lead to more complete contaminant removal during biofilter treatment. Further, this can minimize diminished performance caused by starvation encountered in conventional biofilters under discontinuous loadings. Because of competitive adsorption, however, the degree of load equalization achieved by a passively operated GAC system for different constituents in multi-contaminant gas streams can vary markedly.
CHAPTER 1  INTRODUCTION

1.1. Rationale

Manufacturing facilities, waste treatment operations, and environmental remediation activities generate vast quantities of waste gases containing a wide variety of volatile organic compounds (VOCs), many of which are classified by the US EPA as hazardous air pollutants (HAPs). Regulatory pressure to control these emissions has intensified in response to the 1990 Clean Air Act Amendments and new EPA regulations lowering the National Ambient Air Quality Standard for ozone (Kamarthi and Willingham, 1994). Biofilters have attracted increasing popularity for removal of VOCs from contaminated air streams because of their low costs, operational simplicity, and lack of secondary pollutant generation (Devinny et al., 1999; van Groenestijn and Hesselink, 1993). In biofiltration, contaminants are transferred from an air stream into a biofilm immobilized on solid support media. Once in the biofilm, contaminants are metabolized by microorganisms into environmentally acceptable end products such as carbon dioxide, water, and additional biomass. This treatment technology is particularly attractive for treating high volumetric flow airstreams containing low concentrations of biodegradable pollutants.

Although biofilters have been used successfully for many applications, several design and operational challenges remain. One of these challenges is understanding the appropriate basis of design and selection of appropriate operating strategies for treatment of dynamically-varying waste gas streams typical of industrial operations. The contaminant concentration in most waste gas streams varies with time due to the unsteady-state nature of the industrial processes that generate them (Tang and Hwang, 1997; Webster et al., 1999; Dirk-Faitakis and Allen, 2003). The sometimes severe variation in VOC concentration can result in dynamic “shock” loads that exceed biological reaction capacities. Such transient periods of elevated contaminant loading
can result in contaminant emission from biofilter systems (Martin and Loehr, 1996; Deshusses et al., 1999; Martin et al., 2002; Moe and Li, 2004; Atoche and Moe, 2004). Excessive contaminant emission during transient loading conditions is particularly problematic in cases where air pollution control regulations require a specified removal efficiency (e.g., 95%) on a continuous basis.

A similar phenomenon can exist with respect to design and operation of biofilters for processes that generate VOC-contaminated gas on a discontinuous basis (e.g., because of 8-hr work days or process shutdown). Intervals of low or no contaminant loading in biofilter systems are problematic because of starvation conditions imposed on the microbial populations. Although short-term interruptions in contaminant loading (on the order of minutes) are unlikely to cause problems (Dirk-Faitakis and Allen, 2003), diminished contaminant removal for a period lasting several hours or even days following resumption of contaminant loading has been reported for cases where the duration of no loading was longer (on the order of several hours or days) because microbial populations can undergo sufficient endogenous decay or shifts in metabolism to result in diminished performance (Martin and Loehr, 1996; Mohseni et al., 1998; Webster et al., 1999; Park and Kinney, 2001; Cox and Deshusses, 2002; Moe and Qi, 2004). In general, longer duration shutdown periods require longer time for performance recovery.

In spite of the inherently unsteady-state nature of most waste gas streams, conventional biofilters are generally designed and operated to receive a continuous stream of contaminated air without appreciable variation in organic load. In typical biofilter installations, control is quite passive and is often restricted to adjustment of the medium’s moisture content or nutrient supply. Although inexpensive, such designs limit implementation of engineering decisions which could improve performance during steady and unsteady-state loading conditions.
To address the limitations described above, during the course of research described in this dissertation, two design modifications were investigated in this study as potential solutions to mitigating contaminant emissions from biofilters under unsteady-state loading conditions. In the first, sorptive material (i.e., activated carbon) was incorporated into the biofilter packing media and a novel periodic operating strategy, Sequencing Batch Biofilter (SBB) operation, was experimentally tested under “normal” and “shock” loading conditions. In the second, an activated carbon column was placed in series before a biofilter to assess the potential of the combined system to effectively treat dynamically varying VOC concentrations. Experiments were conducted to assess the effectiveness of each system for removing contaminants, and the microbial population was analyzed to determine the effects of SBB operating strategy on selection and enrichment in microbial consortia.

1.2. Objectives

The overall objective of this dissertation was development and testing of design modifications/operating strategies with the potential for improving biofilter performance in handling unsteady-state VOC loadings via use of sorptive material and/or periodic operation. The research also involved characterization of microbial populations under different biofilter operating strategies using DNA-based techniques. Model simulation and experimental testing of adsorption processes under discontinuous loading conditions were also conducted as a basis for preliminary design or analysis of granular activated carbon (GAC) buffering systems. The specific objectives were as follows:

(1) To assess the feasibility of using a controlled SBB system to treat VOC contaminated air and determine if SBB operation can be used effectively to remove contaminants during transient periods of elevated contaminant load.
(2) To assess differences in performance during both steady state loading conditions and uncontrolled transient loading conditions in a sequencing batch biofilter and a continuous flow biofilter.

(3) To determine whether applying Sequencing Batch Biofilter (SBB) operating strategies can affect selection and enrichment in the mixed microbial consortia in biofilters.

(4) To evaluate the range of load dampening achieved by GAC columns subjected to various inlet concentrations of intermittently loaded single or multi-component contaminants.

(5) To evaluate the impact of a GAC buffering system on biofilter performance for treatment of a model gas stream containing a two-component VOC mixture under intermittent loading conditions.

1.3. Dissertation Organization

The organization of this dissertation is as follows. Chapter 2 of the dissertation contains a brief literature review summarizing the background of biofiltration and periodically operated biological reactors for waste treatment. Chapters 3 through 7 elaborate on the experimental methods and results from studies that were aimed at meeting the aforementioned objectives. Specifically, Chapter 3 describes initial experiments conducted to determine the proof-of-concept of using a SBB operating strategy to treat methyl ethyl ketone (MEK) contaminated air under both “normal” and “shock” loading conditions at a fixed empty bed residence time. Laboratory manufactured polyurethane foams impregnated with powdered activated carbon were used as the biofilter packing media. To determine the broader applicability of the SBB operating strategy and to determine the effect of such operating strategy on biofilter performance during both normal loading and unsteady-state conditions, in the research conducted as described in Chapter 4, commercially available activated carbon coated polyurethane foam media with much higher sorption capacity was selected as biofilter packing media. Differences in performances
were assessed between biofilters operated under a continuous flow mode (CFB) and a sequencing batch mode (SBB). Both biofilters were operated under progressively higher MEK loading rates during normal loading and transient elevated MEK shock loading greater in magnitude than that described in Chapter 3. Chapter 5 describes how differences in bacterial community structure as a function of spatial location along the height of the CFB and SBB previously tested in Chapter 4 were assessed using Denaturing Gradient Gel Electrophoresis (DGGE) analysis of polymerase chain reaction (PCR)-amplified genes coding for 16S rRNA. Chapter 6 describes abiotic fixed-bed sorption experiments and numerical modeling conducted to assess the degree of load-equalization achieved by GAC columns for gas flows containing intermittent concentrations of acetone and toluene present as single-component contaminants and as a mixture. Chapter 7 describes an experimental evaluation of the impact of a carbon buffering system on biofilter performance for treatment of a model gas stream containing a two-component mixture of acetone and toluene under intermittent loading conditions. Lastly, Chapter 8 contains overall conclusions as well as recommendations for future research. Chapters 3 to 7 are prepared as self-contained units intended for publication as separate journal papers.
CHAPTER 2 BACKGROUND AND LITERATURE REVIEW

2.1. Biofilter VOC Removal Mechanisms

As air pollution regulations increase the need for cost-effective control technologies, biofilters have attracted increasing popularity for removal of volatile organic compounds (VOCs) from contaminated air streams because of their low costs, operational simplicity, and lack of secondary pollutant generation (Devinny et al., 1999). In biofiltration, contaminants are transferred from an air stream into a biofilm immobilized on solid support media where they are biodegraded by microorganisms into environmentally acceptable end products including CO₂, water, and additional biomass. This treatment technology is particularly attractive for treating high volumetric flow airstreams containing low concentrations of biodegradable pollutants (Devinny et al., 1999).

Figure 2.1 illustrates the schematic diagram of a typical biofilter system. The basic components of a biofilter include a packed bed reactor, a system for maintaining moisture in the reactor, and a blower to push or pull contaminated air through the porous medium. Several process configurations may be used. For example, air flow may be either upflow or downflow and water may be added to maintain moisture content by humidifying influent air or to the filter bed via a sprinkler or soaker hose (Groenestijn and Hesselink, 1993).

The principles governing biofiltration are similar to those of other biofilm processes. Conceptually, a three-step process occurs within the packed bed of a biofilter (Swanson and Loehr, 1997). Figure 2.2 depicts the basic transfer of the pollutant and oxygen from the air phase into the biofilm where it is taken up by the microbes. First, contaminants in the gas phase cross the interface between gas flowing in the pore space and the aqueous biofilm surrounding the solid medium. Then, contaminants diffuse through the biofilm to a consortium of acclimated microorganisms. Finally, the microorganisms obtain energy from oxidation of the contaminants.
as a primary substrate, or they cometabolize the contaminants via nonspecific enzymes. Simultaneously, there is diffusion and uptake of nutrients, such as nitrogen and phosphorous, and oxygen within the biofilm. Concentration gradients created by utilization of the contaminant, \( \text{O}_2 \), and nutrients serve as driving force for diffusive transport in the biofilm. A properly designed and operated biofilter converts target waste contaminants to end products such as \( \text{CO}_2 \), \( \text{H}_2\text{O} \), and biomass.

**Figure 2.1**: Schematic diagram of a conventional biofilter system.

**Figure 2.2**: Biological degradation in a biofilter at the microscale level (redrawn from Kazenski, 2000).
2.2. Biofilter Performance Criteria Parameters

There are several variables by which biofilter performance can be measured and evaluated. Listed in Table 2.1 are the definitions and typical ranges of reported values of such variables.

Table 2.1: List of biofilter performance criteria parameters.

<table>
<thead>
<tr>
<th>Parameter name</th>
<th>Calculated as</th>
<th>Typical units</th>
<th>Typical or desired range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empty bed residence time</td>
<td>V/Q</td>
<td>seconds</td>
<td>15-60</td>
<td>Devinny <em>et al.</em> (1999)</td>
</tr>
<tr>
<td>Surface loading</td>
<td>Q/A</td>
<td>m³/m²/h</td>
<td>50-200</td>
<td>Swanson and Loehr (1997)</td>
</tr>
<tr>
<td>Mass loading</td>
<td>QC_i/V</td>
<td>g/m³/h</td>
<td>10-160</td>
<td></td>
</tr>
<tr>
<td>Removal Efficiency</td>
<td>(C_i-C_e)/C_i × 100%</td>
<td>%</td>
<td>95-99</td>
<td></td>
</tr>
<tr>
<td>Elimination capacity</td>
<td>Q(C_i-C_e)/V</td>
<td>g/m³/h</td>
<td>10-160</td>
<td></td>
</tr>
</tbody>
</table>

Note: V=biofilter packed bed volume, Q=gas flow rate; A=Cross-sectional area; C_i=influent concentration; and C_e=effluent concentration.

- **Empty Bed Residence Time (EBRT)**. EBRT is a relative measure of gas residence time within the biofilter packed bed. The actual gas residence time in the reactor can be calculated as the EBRT divided by the air-filled porosity available to gas flow, but such porosity is rarely known. EBRT is a simplified, relative measure of chemical residence time in a biofilter. Sufficient EBRT is necessary to allow transport and degradation of the pollutant to occur, which makes EBRT a critical design and operating parameter (Swanson and Loehr, 1997).

- **Surface Loading Rate**. Commonly expressed in units of cubic meters of gas supplied per square meter of packed bed cross-sectional area per hour (m³/m²/h), surface loading is a measure of the volumetric gas loading applied to a biofilter. Upper limits on surface loading exist due to bed-drying concerns and EBRT requirements. Thus, maximum surface loads with
efficient moisture control systems are generally less than 200 m$^3$/m$^2$/h (Swanson and Loehr, 1997).

- **Mass Loading Rate.** Biofilter mass loading rate is defined as the VOC mass applied to a biofilter per unit medium volume per unit time. As mass loading includes the effects of both flow and concentration, a single biofilter can perform differently under identical mass loadings. Because there is an intrinsic limitation on maximum growth rates that microorganisms can exhibit, removal efficiency eventually decreases with higher mass loading and there is potential limit on mass loading rates at which good performance will be achieved. High loading rates can result in biomass clogging of biofilter media and accumulation and/or emission of partially transformed intermediates (Devinny and Hodge, 1995).

- **Removal Efficiency (RE).** Expressed as a percentage, removal efficiency is the fraction of inlet contaminant removed by the biofilter. It is the operating parameter most often used to assess the success of a biofilter, and likely to be of paramount interest from a regulatory perspective. Removal efficiency varies with inlet contaminant concentration, airflow, and biofilter size and only reflects the specific conditions under which it is measured (Devinny et al., 1999).

- **Elimination Capacity (EC).** Defined as the mass of contaminant degraded per unit volume of packed bed per unit time, EC is a normalized measure of VOC removal capacity at a given mass loading. EC is a function of mass load, EBRT, packing medium type, VOC type, microbial population, and environmental conditions (Swanson and Loehr, 1997).

### 2.3. Biofilter Design and Operation Considerations

Many factors influence performance, treatment costs, and long term-stability of biofilters for air pollution control. The following sections briefly discuss the important operating
parameters that are of particular interest to the design and operation of biofilters for VOC treatment.

- **Packing Media.** Selecting or engineering the proper biofilter medium is an important step toward developing a successful biofiltration operation. Desirable media properties include the following: optimal microbial environment, large specific surface area, structural integrity, high moisture retention, high porosity, and low bulk density (Swanson and Loehr, 1997). A variety of materials have been used as biofilter support media including compost, peat, soil, wood chips, bark, sawdust, activated carbon, ceramic pellets, sintered glass, polystyrene beads, polyurethane foam, polyurethane hydrogel, and some combinations of them (Medina et al., 1995; Defilippi et al., 1996; Hodge, 1997; Kinney, 1998; and Moe and Irvine, 2000a).

- **Moisture Content.** Control of the packing medium moisture content is important for biofilter operation (Swanson and Loehr, 1997). Media that is too wet or too dry is prone to failure. Excess water can cause high backpressure and low gas retention times due to filling of the pore space with water. It may also cause oxygen transfer problems due to reduced air/water interface per unit biofilm volume and creation of anaerobic zones that promotes odor formation and slow degradation rates. Excess drying of a biofilter medium can cause cracks and channeling in the biofilter and deactivation of VOC-degrading microorganisms. For most “natural” materials (e.g., compost), the optimal biofilter medium moisture content generally ranges from 40 to 60% (wet weight) (Leson and Winner, 1991).

- **Microorganisms.** For media lacking indigenous microorganisms, seeding of biofilters with microorganisms such as activated sludge cultures which contain microorganisms already adapted to certain contaminants has been used in attempts to reduce acclimation times (Swanson and Loehr, 1997). The predominant microorganisms in biofilters treating VOCs are
heterotrophs. Bacteria and fungi are especially common (Leson and Winer, 1991). Although active biomass is necessary for VOC degradation, biofilters treating moderate to high carbon loadings eventually accumulate excess biomass within the packing material. Excess biomass reduces the effective porosity, residence time and surface area per unit volume within the packed bed. Therefore, excessive microorganism growth should be controlled to avoid plugging in the biofilter (Kinney et al., 2003).

- **Nutrient, pH, and Temperature.** In order for a biofilter to operate efficiently, a sufficient supply of nutrients such as nitrogen, phosphorus, potassium, sulfur, and trace metals, and proper pH, temperature ranges are also desired (Swanson and Loehr, 1997).

2.4. **Challenges to Conventional Biofilter Operation and Design**

Although biofilters have been used successfully for many applications, several operational problems have been frequently reported. These operational problems include: 1) biofilters’ sensitivity to unsteady-state contaminant loading (e.g., contaminant emission during transient periods of elevated loading; diminished performance following discontinuous loading), 2) complex kinetics caused by inhibition and/or catabolic repression which often exist in systems where multiple contaminants are present and thus achieving consistent removal of all compounds can be problematic even when the compounds are easily removed when present as single contaminants. Each of these difficulties is further described below.

2.4.1. **Unsteady-State Contaminant Loading Conditions**

Contaminant concentrations in most waste gas streams vary with time due to the inherent nature of processes that generate them (Webster et al., 1999; Dirk-Faitakis and Allen, 2003). The sometimes severe variations in VOC concentration can result in dynamic “shock” loads that exceed biological reaction capacities. These transient periods of elevated contaminant loading often result in contaminant emission from biofilter systems. Contaminant emission during short-
term unsteady-state loading conditions have been reported for a number of applications including wastewater treatment off-gases, wood processing, soil vapor extraction, sponge manufacturing, and treatment of BTEX compounds (Ergas, 1992; Boyette et al., 1995; Chang and Yoon, 1995; Yang and Alibekoff, 1995; Martin and Loehr, 1996, Kinney, 1996b; Mohseni et al., 1998; Irvine and Moe, 2001). Excessive contaminant emission during transient loading conditions is particularly problematic because air pollution control regulations sometimes require a specified removal efficiency (e.g., 95%) on a continuous basis. Furthermore, temporary high concentrations can be toxic for the microorganisms in the reactor, resulting in inactivation of the system (Devinney et al., 1999).

Intervals of low or no contaminant loading in biofilter systems are also problematic because of starvation conditions imposed on the microbial populations. Although short-term interruptions in contaminant loading (on the order of minutes) are unlikely to cause problems (Dirk-Faitakis and Allen, 2003), diminished contaminant removal for a period lasting several hours or even days following resumption of contaminant loading has been reported for cases where the duration of no loading was longer (on the order of several hours or days) because microbial populations can undergo sufficient endogenous decay or shifts in metabolism to result in diminished performance (Martin and Loehr, 1996; Mohseni et al., 1998; Webster et al., 1999; Park and Kinney, 2001; Cox and Deshusses, 2002; Moe and Qi, 2004). In general, longer duration shutdown periods require longer time for performance recovery.

Conventional biofilters are designed and operated to receive a continuous stream of contaminated air without appreciable variation in organic load. If a biofilter is designed to meet “average” loading conditions for a waste stream with transient elevated concentrations, then dynamic mass loadings may exceed biological reaction capacities and result in unacceptably high
contaminant emissions. Conversely, designing a biofilter based on infrequently-occurring peak concentration in the waste gas to ensure reliable operation would pose an economically unfavorable situation. A similar phenomenon can exist with respect to design and operation of biofilters for processes that generate VOC-contaminated gas on a discontinuous basis. In a conventional biofilter, contaminants must be treated essentially at the same rate that they are generated. A biofilter sized to treat a waste gas generated during only a portion of the day can sit idle with available (but unutilized) capacity during portions of the day when no contaminants are loaded. From this perspective, the system is over-designed in the sense that it has excess capacity.

In addition, in typical biofilter installations, control is quite passive and is often restricted to adjustment of the medium’s moisture content or nutrient supply (Swanson and Loehr, 1997; Irvine and Moe, 2001). Although inexpensive, such designs limit implementation of engineering decisions which could improve performance during steady and unsteady-state loading conditions.

Various methods for mitigating the effects of dynamic loading on biofilters have been proposed; however, none have been extensively tested experimentally. For example, Ottengraff (1986) suggested that addition of granular activated carbon (GAC) or other sorptive media to biofilter media would be advantageous for biofilters subjected to variable inlet loading. The author proposed the possibility of contaminant sorption to the GAC during periods of high VOC loading and then contaminant desorption followed by utilization in the biofilm during periods of low VOC loading period (i.e., bioregeneration). Although this approach has been recommended by additional authors (e.g., Tang et al., 1995; Tang and Hwang, 1997; Abumaizar et al., 1998), experimental results testing systems with activated carbon added to the packing medium have
produced somewhat conflicting results. For example, in a comparison of compost-based biofilters augmented with either GAC or an equal volume of perlite (a material with similar surface area but without appreciable sorption capacity) treating an α-pinene contaminated waste gas stream, it was reported that addition of activated carbon produced little benefit in performance during transient loading experiments conducted after long term operation (i.e., five months) (Mohsini and Allen, 1997; Mohsini et al., 1998). Use of granular activated carbon as sole biofilter support medium in an attempt to better transient performance has also been proposed and tested experimentally (Amanullah et al., 2000), however, there has also been report on its loss of adsorption capacity during long-term operation (Medina et al., 1995). Further, such sole GAC based biofilter medium is generally not considered for full-scale applications as it is apparently economically unfavorable.

A similar approach of performing load equalization is by applying an activated carbon column prior to the biofilter to adsorb and then desorb transient spikes in contaminant concentration so that a more steady-state inlet biofilter load could be created (Weber and Hartmans, 1995). In their studies, three biofilter configurations were compared for treating a gas stream containing 900 mg/m$^3$ of toluene for a period of 8 hours per day and uncontaminated air for 16 hours per day. In the first configuration a biofilter was used without activated carbon (i.e., a conventional biofilter). In the second configuration, activated carbon was mixed with compost in the biofilter. In the third system, a separate activated carbon column installed in series prior to the biofilter was used. The biofilter without activated carbon had a removal efficiency of about 50%. Similar removal efficiency was observed for the reactor in which activated carbon was mixed with the compost. When a separate column with activated carbon was placed before the biofilter the fluctuating toluene concentrations were buffered to a concentration of about 300 mg
m³, which was completely degraded in the biofilter. It should be noted, however, that the systems tested may have been kinetically limited by an insufficient nutrient supply (Weber and Hartmans, 1995), so drawing more general conclusions about the performance of the various reactor configurations is somewhat tenuous. Furthermore, such a system has been experimentally tested only for a single-component waste gas stream containing toluene. Little or no research has been conducted to assess performance of passively-operated activated carbon load-dampening systems for treating multi-contaminant waste gas streams during unsteady-state loading conditions. When multiple contaminants are present, competitive sorption can occur (Ruthven, 1986; Yang, 1987). As a result, activated carbon may exhibit differing buffering capacities for different contaminants. Furthermore, an appropriate basis of design for buffering systems is currently lacking.

Another method of damping transient loading to biofilters was described by Webster et al. (2000) who conducted preliminary experiments associated with passing a contaminated gas through a moving-bed GAC adsorber and then using a microwave regeneration system to desorb contaminants. Solvent vapors desorbed from the GAC were transferred to pressurized gas storage tanks using nitrogen purge gas before being supplied to a biofilter along with dilution air. Although these techniques are promising, they require substantial equipment in the case of microwave regeneration, have not been demonstrated for many applications, and have not been adopted for widespread use.

Until recently, periodic processes used to select, enrich, and manipulate the physiology of microbes were limited to applications in wastewater treatment and soil remediation. Recent studies have shown that biofilters could be designed and operated as controlled, unsteady-state, periodic processes for the destruction of gas-phase contaminants (Irvine and Moe, 2001;
Norman, 2002). Irvine and Moe (2001) conducted experiments using one continuous biofilter and two periodically operated biofilters to treat air contaminated with toluene. The two periodically operated biofilters received contaminated air during one-third and one-sixth, respectively, of their operating cycle. Results demonstrated that the removal efficiency of contaminants for both periodically operated biofilters during shock-loading conditions was superior to that of the continuously operated biofilter. Superior performance for the periodically operated biofilters in comparison to the continuously operated biofilter was also observed in treatment of air contaminated with MEK during transient conditions of high loading (Norman, 2002). Periodic operating strategies can provide operators with an effective alternative for controlling biofilters during transient conditions of high loading and enhancing contaminant removal (Moe and Irvine, 2000b).

2.4.2. Kinetics Inhibition and/or Catabolic Repression for Multiple Contaminants Treatment

Although many gas-phase contaminants have been successfully treated using biological methods when they are present as individual compounds or simple mixtures, complex mixtures can be problematic when biological treatment processes are applied (Ottegraaff et al., 1987). Complex kinetic inhibition and/or catabolic repression often exist in systems where multiple contaminants are present (Ottegraaff et al., 1986; Deshusses et al., 1995; Webster et al., 1998; Deshusses et al., 1999; Kazenski and Kinney, 2000). In such cases, achieving consistent removal of all compounds can be problematic even when the compounds are easily removed when present as single contaminants (Deshusses et al., 1995). In treatment of mixtures of VOC contaminants, numerous researchers have reported that one or more compounds is not degraded until after other compounds have been degraded to very low concentrations. This frequently results in a spatial separation of zones for degradation of different compounds as a function of
height in a biofilter bed. For example, during the operation of several laboratory-scale biofilters and biotrickling filters for treatment of a simulated paint spray booth waste stream, Kazenski and Kinney (2000) observed that degradation of toluene and $p$-xylene did not occur until after methyl $n$-propyl ketone, $n$-butyl acetate, and ethyl 3-othoxypropionate reached very low concentrations.

Similar results were reported by Webster et al. (1998) for contaminants commonly found in paint spray booth applications when treated in bench-scale and pilot-scale biotrickling filters. For a mixture of toluene, xylene, methyl ethyl ketone, and $n$-butyl acetate, although overall removal efficiencies were relatively high (greater than 90%), removal of toluene was lower (approximately 70%) at an EBRT of 39 seconds. Deshusses et al. (1999) reported that in treating a mixture of toluene and ethyl acetate, toluene removal was inhibited by presence of ethyl acetate. Deshusses et al. (1995) observed that methyl isobutyl ketone degradation rates were adversely affected by presence of methyl ethyl ketone. Such effects, particularly for mixtures of ketones, have been known for quite some time. For instance, Mahmoud and Davis (1970) reported that in batch experiments with suspended growth cultures, acetone was not removed until concentrations of methyl ethyl ketone and methyl propyl ketone reached low levels even though acetone removal was immediate for samples where it was the sole compound. Irvine et al. (1973) reported inhibition of acetone degradation in the presence of methyl ethyl ketone and diethyl ketone in batch activated sludge cultures.

Although problems associated with inhibition or other complex kinetics can sometimes be overcome by designing biofilters with sufficiently deep beds or sufficiently long residence times to allow for complete degradation of different compounds at different spatial locations in the bed, such solutions can be problematic for the unsteady-state conditions frequently encountered in many industrial operations. It has been reported that during transient periods of
elevated contaminant loading (i.e., a “shock load”) when the contaminant loading rate exceeds the biological reaction capacity for the most readily degraded compounds, essentially no degradation of less readily degraded compounds occurs (Deshusses et al., 1999). Such results are especially problematic when the less readily degraded compounds (e.g., toluene or benzene) pose larger health risks or are of a higher priority from a regulatory perspective than the more readily degraded compounds.
CHAPTER 3 SEQUENCING BATCH BIOFILTER OPERATION FOR TREATMENT OF METHYL ETHYL KETONE (MEK) CONTAMINATED AIR

3.1. Introduction

In recent years, biofiltration has gained increasing acceptance worldwide as an economical air pollution control technology for treatment of gases contaminated by low concentrations of biodegradable volatile organic compounds (VOCs) (Devinney et al., 1999). In biofiltration, a fan or blower forces gases containing VOCs through a packed bed that contains an unsaturated solid medium that supports a biofilm. As contaminated air flows through the support medium and past the biofilm, contaminants partition to the aqueous or solid phases where they are transformed by microorganisms into innocuous products such as carbon dioxide, water, and biomass.

Contaminant concentrations in most waste gas streams vary with time due to the inherent nature of processes that generate them. The sometimes severe variations in VOC concentration can result in dynamic “shock” loads that exceed biological reaction capacities. These transient periods of elevated contaminant loading often result in contaminant emission from biofilter systems. Contaminant emission during short-term unsteady-state loading conditions have been reported for a number of biofilter applications including wastewater treatment off-gases, wood processing, soil vapor extraction, sponge manufacturing, and treatment of BTEX compounds (Ergas, 1992; Boyette et al., 1995; Chang and Yoon, 1995; Yang and Alibeckoff, 1995; Martin and Loehr, 1996; Kinney, 1996b; Mohseni et al., 1998; Irvine and Moe, 2001). Excessive contaminant emission during transient loading conditions is particularly problematic because air pollution control regulations sometimes require a specified removal efficiency (e.g., 95%) on a continuous basis. Thus, if a biofilter is not able to maintain a high removal efficiency during
short term periods of shock loading, it may not meet regulatory compliance even if it is able to achieve the required removal efficiency during “normal” operations over long time periods.

In spite of the inherently unsteady-state nature of most waste gas streams, conventional biofilters are designed and operated to receive a continuous stream of contaminated air without appreciable variation in organic load. In typical biofilter installations, control is quite passive and is often restricted to adjustment of the medium’s moisture content or nutrient supply. Although inexpensive, such designs limit implementation of engineering decisions which could improve performance during normal operation or allow effective handling of short-term variations in the waste stream (i.e., shock loads).

A potential solution to the operational difficulties described above is use of operating strategies based on sequencing batch treatment. Periodically operated bioreactors designed to treat hazardous and non-hazardous contaminants present in wastewaters are used to select and enrich for microbial consortia that readily handle appreciable variation in organic load (Irvine and Ketchum, 1989; Irvine et al., 1997; Wilderer et al., 2001). The most widely used controlled, unsteady-state periodic process for wastewater treatment is the Sequencing Batch Reactor (SBR). In SBR operation, each reactor in a system has five basic operating modes or periods, each of which is named according to its primary function: FILL, REACT, SETTLE, DRAW and IDLE. Operating strategies that periodically change oxygen tension and electron donor availability force microbes to compete over wide ranges of feast and famine, which, in turn, has a dramatic impact on the selection of robust microbial consortia in SBRs (Chiesa et al., 1985; Irvine et al., 1997; Wilderer et al., 2001). Microorganisms that experience controlled load variations on a regular basis (i.e., once each cycle) are better able to handle unexpected uncontrolled shock or
transient loads than consortia enriched under conventional continuous flow conditions (Irvine et al., 1997).

A variation of the SBR sometimes used for treatment of wastewaters containing volatile or inhibitory compounds is the Granular Activated Carbon – Sequencing Batch Biofilm Reactor (GAC-SBBR). In this process, granular activated carbon (GAC) placed in the SBR adsorbs a fraction of the influent contaminants during the FILL period. During the REACT period, microorganisms growing attached to the GAC or other support surfaces biologically regenerate the activated carbon to allow reuse in the process. For wastewaters containing toxic or inhibitory compounds, the GAC serves an important role as a temporary sink for contaminants. GAC-SBBR systems, some of which utilized membrane aeration, have been used successfully for treatment of industrial wastewaters containing volatile and/or inhibitory components including toluene and mixtures of phenolic compounds (Chozick and Irvine, 1991; Kolb and Wilderer, 1995; Kolb and Wilderer, 1997; Ha et al., 2000; Buitron et al., 2001).

The studies described herein demonstrate how the basic principles employed by SBRs treating wastewater can be extended successfully to biofilters treating contaminated gases. Terminology proposed for this operating strategy is Sequencing Batch Biofilter (SBB) operation. Implementation of the SBB operating strategy consists of one or more biofilters packed with medium containing powdered activated carbon (PAC) or other sorptive material and operated with three distinct periods that comprise one complete cycle: FEED, REACT, and IDLE.

During the FEED period, contaminated air enters the biofilter, and treated air exits. The mechanism for contaminant removal is expected to be a combination of biodegradation, bioaccumulation, and contaminant sorption to the PAC-containing medium. The sorption capacity of the PAC is “filled” during this portion of the cycle and microbes degrade a portion of
the incoming organics as they do during the FILL period of an SBR. For toxic and inhibitory constituents, the PAC can provide an important role as a buffer that reduces the aqueous-phase concentration of contaminants. During transient loading periods, the system’s sorption capacity can provide the important role of temporarily storing contaminants when their loading rate exceeds the biological reaction capacity. Because biodegradable contaminants can be accumulated in the biofilter, it is hypothesized that there can be an appreciable reduction in the empty bed residence time (EBRT) without contaminant breakthrough. This can facilitate a separation in time between when the contaminants enter the biofilter and when they are degraded, a clear advantage in treating unsteady-state waste streams, and it can result in a system comparable in size or smaller than a conventional continuous-flow biofilter system.

The SBB operating sequence is graphically depicted in Figure 3.1. The black filter bed shown in the FEED period represents a biofilter with contaminants entering the system and accumulating within the packing medium. The gray biofilter bed shown in the REACT period represents a biofilter with some stored substrate but in a quantity less than the peak amount accumulated during FEED. The white biofilter bed shown in the IDLE period represents a biofilter with no stored substrate.

At some point before an unacceptably large contaminant concentration occurs in the biofilter effluent, the influent contaminated air is diverted to a second biofilter and the first biofilter will operate in a batch REACT mode with air or pure oxygen addition and/or recirculation as necessary to maintain aerobic conditions. The task of biotransformation initiated during the FEED period will take place during REACT. As the contaminant concentration in the gas, liquid, and solid phases is lowered due to microbial degradation, the contaminant mass initially sorbed in the system (e.g., adsorbed on the PAC) will desorb, providing additional
Recirculating air through the filter medium during REACT allows the electron donors and electron acceptors to be more uniformly distributed throughout the filter medium and thus allows for more spatially homogenous growth of microorganisms than is expected in continuous-flow systems where excess microbial growth near the biofilter inlet can cause clogging. Famine conditions will occur after the biodegradable contaminants are fully transformed. During IDLE, the period between FEED and REACT, the biofilter awaits the beginning of a new cycle. The biofilter’s excess capacity is measured by the total time in IDLE because the time could be easily re-allocated to REACT (or FEED) if necessary, thus providing appreciable operational flexibility in full-scale operations.

Figure 3.1: Cycle for one biofilter in a periodically operated multiple-reactor biofilter system.

In applications where there is an intermittent discharge of contaminated gases (e.g., during an eight hour work day), it may be possible to use a single biofilter, while in cases where a continuous contaminated gas flow is generated, multiple units installed in parallel and operated in sequence will be necessary. In multiple biofilter systems, the length of time for one biofilter
to complete REACT and IDLE will be set equal to the total FEED time of all other biofilters in the system. For example, if two hours are needed to bring the PAC to the desired sorption capacity during FEED, but four hours are needed for REACT and IDLE, then the system will require three biofilters operated in sequence as represented in Figure 3.2. As shown, the three-biofilter system can be installed in parallel and operated in sequence according to the solid arrows that connect the time periods I-III. The EBRT for this system is equal to that of one “properly” designed conventional biofilter. In this case, biofilter A is undergoing FEED in time period I, while the other biofilters are undergoing REACT. Biofilter A is undergoing REACT during time periods II and III. In the case of a transient shock load, an operator could simultaneously direct gas flow to all of the biofilters as illustrated in period IV, resulting in an EBRT that is three times longer than when the biofilters are loaded one at a time. Such an active control strategy has the potential to result in more complete contaminant removal during a transient period of elevated contaminant loading than would otherwise occur. The number of biofilters required to be operated in sequence to treat a continuous gas flow can be generalized as shown in Equation 3.1 below.

\[(n-1) t_{\text{Feed}} = t_{\text{React}} + t_{\text{Idle}} \]  

(3.1)

where: \( n \) = number of biofilters in the system, and  
\( t \) = time devoted to a specific period of operation

Studies described herein addressed two issues: first, to evaluate whether or not a controlled SBB system is feasible; second, to determine if SBB operation could effectively remove contaminants during uncontrolled transient periods of elevated contaminant load. Experiments utilized a laboratory-scale biofilter that was subjected to a variety of steady and unsteady-state loading conditions to treat a model waste gas stream containing methyl ethyl
ketone (MEK) over a period lasting more than 130 days. MEK is one of the 188 compounds regulated as Hazardous Air Pollutants (HAPs) under the 1990 Clean Air Act Amendments in the US (Brownell, 1998). Additionally, the US EPA lists MEK as one of the top 20 chemicals in terms of largest total on-site and off-site releases with 35,547,685 pounds of total releases in 2000 (US. EPA, 2002). Although previous studies indicate that MEK can be effectively removed from contaminated gas streams using a variety of bioreactor configurations including biofilters, biotrickling filters, and tubular biofilm reactor (Agathos et al., 1997; Deshusses, 1997; Chou and Huang, 1997; Amanullah et al., 2000), systematic evaluation of system performance during short-term transient loading conditions is currently lacking.

![Figure 3.2: Schematic diagram of three biofilters loaded periodically with FEED for one-third of the operating cycle during normal loading conditions (periods I, II, and III) and active control strategy during transient shock loading (period IV).](image)

### 3.2. Materials and Methods

#### 3.2.1. Experimental Apparatus

Laboratory studies employed a biofilter consisting of a glass column reactor with an inner diameter of 9.9 cm and an overall height of approximately 1.5 m. As depicted in Figure 3.3, the biofilter consisted of five sections, each of which was filled to 20 cm depth with biofilter medium to provide a total packed bed depth of 1 m and a total bed volume of approximately 7.7
L. Compressed air from a laboratory air tap flowed through an activated carbon filter to remove any contaminants. A pressure regulator then reduced the pressure to 15 psi. The air was split into two streams with 95% of the air flow passing through an aeration stone submerged in water in a 20 L glass carboy heated by electrical heating tape, and the remaining 5% of air flow was used to volatilize contaminant into the air stream. A KD Scientific model 1000 syringe pump (Boston, MA) delivered MEK (ACS Reagent, Sigma Chemical Co.) from a glass gas-tight syringe (Hamilton Co., Reno, NV) through a needle that pierced a septum-filled (Thermogreen™ LB-1 half-hole type septa, Supelco, Bellefonte, PA) glass injection port and into the air stream. Glass marbles were placed in the bottom of the column to evenly distribute air flow. All surfaces that contacted the contaminated air were made of glass, stainless steel, Teflon™, or Viton™.

Figure 3.3: Schematic diagram of laboratory-scale Sequencing Batch Biofilter (SBB). Components used for fixed bed sorption test were identical except that the components inside the dashed line were omitted.

Solenoid valves with stainless steel bodies and flow tubes (Automatic Switch Company, New Jersey) were used to turn airflows on and off as needed. A diaphragm pump with stainless
steel heads and Teflon diaphragm (Air Dimensions Incorporated, FL) was used to recirculate air through the biofilter during REACT periods. A gas scrubbing bottle (Fisher Scientific) was placed between the bottom recirculation outlet of the biofilter and the diaphragm pump to drain condensation from the biofilter system. A microprocessor controller (Model XT, Chron-Trol Corp., San Diego, CA) was used to control operation of the syringe pump, diaphragm pump, and air flow valves. During FEED periods, only the syringe pump and air flow valves 1, 2 and 5 (see Figure 3.3) were switched on. During REACT/IDLE periods, only the diaphragm pump and valves 3 and 4 were switched on to recirculate air in the closed system.

Packing media for the biofilter consisted of polyurethane foam/powdered activated carbon cylinders described previously (Martinez, 2001). The foam cylinders were each manufactured using 45 g PAC per cylinder and had Freundlich isotherm constants for adsorption of MEK of $K_f$ equal to $5.1 \times 10^{-2}$ and $1/n$ equal to 1.19 (with equilibrium concentration expressed as mg L$^{-1}$ and $x/m$ in mg g$^{-1}$). Each of the five column sections contained approximately 102 g of foam (dry mass basis).

3.2.2. Fixed-bed Abiotic Sorption and Desorption Tests

Abiotic sorption and desorption of MEK into the polyurethane foam was tested prior to biofilter inoculation and start-up. The experimental apparatus was identical to that described above in Figure 3.3 except that the gas recirculation components inside the dashed line were omitted. The foam cylinders were adjusted to 65% moisture content before each sorption test. Sorption studies were conducted at a target influent MEK concentration of 106 ppm, under flow rates of 23.1 and 46.2 L/min, respectively, which corresponded to empty bed residence times in the column of 20 and 10 sec, respectively. Effluent MEK was measured until complete breakthrough was observed (i.e., the effluent concentration was equal to the influent). Fixed bed desorption experiments were conducted immediately after each adsorption experiment. In the
desorption tests, the syringe was removed from the syringe pump to stop contaminant loading, only the MEK-free air flowed through the column. Effluent MEK was measured over time until below detection limit. Triplicate runs were conducted under each test condition.

3.2.3. Biofilter Inoculation and Start-up

Laboratory studies employed a 4.0 L glass kettle reactor (Pyrex, Acton, MA) with a working liquid volume of 3.0 L as depicted in Figure 3.4 to enrich for MEK-degrading microorganisms. The sparged-gas reactor was inoculated by adding 1.0 mL of liquid drained from the bottom of a lab-scale biofilter that was fed MEK as sole carbon and energy source to 3.0 L of a nutrient solution consisting of the compounds listed in Table 3.1. The reactor was operated for a period of 180 days prior to inoculation of the biofilter. At the time of biofilter start-up, the mixed liquor suspended solids (MLSS) concentration of the sparged-gas reactor was 2800 mg l⁻¹ and the specific oxygen uptake rate (SOUR) was 11.4 mg g⁻¹ hr⁻¹, mean cell residence time was 10 days, and the influent gas-phase MEK concentration was 106 parts per million by volume (ppm).”

For biofilter inoculation, 1.5 L of MLSS settled from the sparged-gas reactor was combined with 3.5 L of nutrient solution. Each foam cylinder was immersed in this solution and compressed to dislodge entrapped gas bubbles. Cylinders were adjusted to approximately 65% moisture content by compressing them to remove excess liquid and then placed in the biofilter column. No special attempt was made to retain biomass on the foam. The foam swelled when wet, and the swelling held foam cylinders in place inside the column.

Following inoculation, during a 130 day start-up period, the biofilter was operated using sequencing batch operation with a FEED period lasting from 25 min to 360 min, a REACT/IDLE period from 60 min to 335 min, and a total cycle length varying from 4 hr to 12 hr. The EBRT was 20 seconds, and the target influent MEK concentration was 106 ppm. After 49, 73, and 102
days of operation, the biofilter was dismantled and each foam cylinder was removed and placed in a plastic container with 1.0 L of the previously described nutrient solution. Each foam cylinders was compressed several times to simultaneously add nutrients and remove biomass following the procedure of Moe and Irvine (2000). Each time, the cylinders were adjusted to approximately 65% moisture content before being returned to the biofilter column. The glass column and its associated components were periodically washed with hot tap water to prevent biomass accumulation at these locations.

**Figure 3.4: Schematic diagram of sparged gas reactor used to culture inoculum.**

**Table 3.1: Nutrient solution composition for culture enrichment and biofilter operation.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃</td>
<td>29,300</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>2380</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>1000</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>1290</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>630</td>
</tr>
<tr>
<td>(NH₄)₆Mo₇O₂₄·4H₂O</td>
<td>0.04</td>
</tr>
<tr>
<td>FeSO₄·7H₂O</td>
<td>480</td>
</tr>
<tr>
<td>ZnSO₄·H₂O</td>
<td>2</td>
</tr>
<tr>
<td>MnSO₄·H₂O</td>
<td>0.4</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>0.04</td>
</tr>
<tr>
<td>Co(NO₃)₂·6H₂O</td>
<td>0.04</td>
</tr>
<tr>
<td>CoCl₂·6H₂O</td>
<td>0.033</td>
</tr>
<tr>
<td>Na₂B₄O₇·10H₂O</td>
<td>0.04</td>
</tr>
<tr>
<td>EDTA</td>
<td>1.0</td>
</tr>
</tbody>
</table>
3.2.4. Biofilter Operation

After the startup period during which the cycle length varied, the previously described nutrient addition procedure was conducted, the biofilter was restarted, and time was recorded in days with Day 0 set as the end of the startup period. Subsequently, the SBB operational cycle was fixed with a FEED time of 4 hr and a REACT time of 4 hr (total cycle length of 8 hr). The empty bed residence time (EBRT) was set at 20 seconds, and the target influent MEK concentration was 106 ppm, for “normal” operation. This simulates the loading condition experienced by one biofilter in a set of two biofilters constructed in parallel and operated in sequence to treat a continuous gas flow.

To assess biofilter response to uncontrolled variation in influent contaminant concentration, the biofilter was periodically subjected to an influent MEK concentration of 530 ppm, (5 times that of the normal loading) for a period lasting one hour. Studies gave consideration to the fact that a shock loading could occur at any point during the FEED period and that the biofilter response may vary depending on the initial condition (e.g., essentially contaminant-free condition with microbes experiencing famine-like conditions at the start of the FEED period or accumulated contaminant and actively growing microorganisms toward the end of the FEED period). Each of the four shock loading conditions, conducted at least three times, is further described below and summarized in Table 3.2.

One shock loading condition (arbitrarily named Shock Loading I) was conducted with the period of elevated contaminant concentration during the last hour of the FEED period. A second shock loading condition (arbitrarily named Shock Loading II) was conducted with the period of elevated contaminant concentration during the first hour of the FEED period. A third shock loading study (arbitrarily named Shock Loading III) was conducted with the period of elevated contaminant concentration during the first hour of the FEED period, but in this case the EBRT
was adjusted to 40 seconds during both the shock loading condition and the following three hours FEED at normal (106 ppm\textsubscript{v}) MEK concentration. A fourth shock loading study (arbitrarily named Shock Loading IV) was conducted with the period of elevated contaminant concentration during the first hour of the FEED period with an EBRT of 40 seconds, but the EBRT was adjusted to 20 seconds during the following three hours of the FEED period. Shock Loading III and IV simulate conditions where an operator simultaneously diverts contaminated air to both biofilters in a SBB system containing two biofilters constructed in parallel and operated in sequence. A minimum of three cycles of “normal” loading occurred between each shock loading experiment.

Table 3.2: Summary of biofilter operation during shock loading studies.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Shock Loading I</th>
<th>Shock Loading II</th>
<th>Shock Loading III</th>
<th>Shock Loading IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period prior to transient</td>
<td>FEED</td>
<td>REACT</td>
<td>REACT</td>
<td>REACT</td>
</tr>
<tr>
<td>EBRT prior to transient (sec)</td>
<td>20</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Mass flow rate of MEK before transient (mg hr\textsuperscript{-1})</td>
<td>434</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MEK loading rate before transient (g hr\textsuperscript{-1} m\textsuperscript{-3})</td>
<td>56.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EBRT during transient (sec)</td>
<td>20</td>
<td>20</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Time for transient (hr)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>MEK concentration during transient (ppm\textsubscript{v})</td>
<td>530</td>
<td>530</td>
<td>530</td>
<td>530</td>
</tr>
<tr>
<td>Mass flow rate of MEK during transient (mg hr\textsuperscript{-1})</td>
<td>2170</td>
<td>2170</td>
<td>1085</td>
<td>1085</td>
</tr>
<tr>
<td>MEK loading rate during transient (g hr\textsuperscript{-1} m\textsuperscript{-3})</td>
<td>282</td>
<td>282</td>
<td>141</td>
<td>141</td>
</tr>
<tr>
<td>Period after transient</td>
<td>REACT</td>
<td>FEED</td>
<td>FEED</td>
<td>FEED</td>
</tr>
<tr>
<td>EBRT after transient (sec)</td>
<td>N.A.</td>
<td>20</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>Mass flow rate of MEK after transient (mg hr\textsuperscript{-1})</td>
<td>0</td>
<td>434</td>
<td>217</td>
<td>434</td>
</tr>
<tr>
<td>MEK loading rate after transient (g hr\textsuperscript{-1} m\textsuperscript{-3})</td>
<td>0</td>
<td>56.4</td>
<td>28.2</td>
<td>56.4</td>
</tr>
</tbody>
</table>

Approximately once per month (on days 0, 29, 63, 86, and 118), nutrients were added to the biofilter using the same procedure as during the start-up period. The glass column and its associated components were periodically washed with hot water to minimize unintended biomass accumulation on reactor components.
3.2.5. **Analytical Techniques**

Gas-phase MEK concentrations were measured using a MiniRAE 2000 programmable photoionization detector (PID) (RAE Systems, Sunnyvale, CA). The instrument was operated using a 10.6 eV discharge lamp and a 0.40 L min⁻¹ intake flow rate. Calibration was performed using nitrogen gas and 198 ppmv certified MEK calibration standard (BOC Gases, Port Allen, LA). Calibration was verified using a 1090 ppmv MEK calibration standard (BOC Gases, Port Allen, LA). The MEK concentration was measured at one-second intervals, and the data reported are the average of these measurements over one-minute intervals. Gas-phase CO₂ and O₂ concentrations were measured using an Oxygen and Carbon Dioxide Meter (Servomex, UK). The instrument intake flow rate was approximately 0.20 L min⁻¹. Calibration was performed using nitrogen gas and 953, 5,080, or 50,000 ppmv CO₂ and 20% O₂ certified calibration standards (BOC, Port Allen, LA). Data reported are the average over 0.5 or 1.0 second intervals. Because the concentration of CO₂ in the biofilter influent fluctuated over time, results are reported indicating the concentration of CO₂ above the inlet concentration.

Prior to the nutrient addition procedure described above, a pre-cut and pre-weighed foam wedge was removed from each biofilter section. Each foam wedge was placed in an Erlenmeyer flask containing 250 mL of deionized water and mixed vigorously. The NO₃⁻-N concentration of the resulting solution was then measured using a HACH Nitrate Test Kit (HACH Company, Loveland, CO). Absorption was measured using a spectrophotometer (UV-1201, Shimadzu, Japan) at a wavelength of 500 nm.

For aqueous-phase measurements associated with the sparged-gas reactor used to grow the inoculum, OUR was calculated after measuring DO using a YSI model 5300 Biological Oxygen Monitor. Total suspended solids and volatile suspended solids were measured using standard method 2540-D and 2540-E, respectively (APHA, 1998).
3.3. Results and Discussion

3.3.1. Abiotic Sorption and Desorption of MEK on Packing Media

Figure 3.5 presents the sorption (top) and desorption (bottom) of MEK by the wet foam medium. Data reported are the average of triplicate runs. At an EBRT of 20 sec, 10% breakthrough occurred approximately 0.5 hour after the start of the sorption test, and the column reached complete breakthrough after a total of 7 hours. In contrast, at an EBRT of 10 sec, 10% and 100% breakthrough occurred approximately 5 min and 3.5 hours after the start of the sorption test, respectively. Due to the water present in the foam medium, MEK sorption was accounted for by a combination of absorption into the water and adsorption onto the foam medium itself. The total mass of MEK sorbed to either the foam medium and water was 838 and 765 mg at EBRTs of 20 and 10 sec, respectively. Assuming the aqueous phase MEK was in equilibrium with the influent gas-phase MEK concentration of 106 ppmv (i.e., 0.314 mg/L) at the end of each adsorption experiment when the influent and effluent gas-phase MEK concentrations were equal, and based on dimensionless Henry’s law constant of $2.33 \times 10^{-3}$ for MEK (Perry and Green, 1997), the theoretical equilibrium aqueous MEK concentration was expected to be 134.8 mg/L for each sorption test. Assuming a constant total water volume of 0.947 L in the column based on a moisture content of 65% of the foam medium, the theoretical mass of MEK absorbed in the water after equilibrium has reached is 135.7 mg for each sorption test. This accounted for only 16 and 17.7% of the total mass sorbed in the wet foam medium at EBRTs of 20 and 10 sec, respectively. Therefore, it was concluded that sorption of MEK into the wet foam medium was dominated by adsorption to the foam medium itself rather than absorption into the water.

During desorption tests, effluent MEK concentration decreased rapidly during the initial period following removal of MEK from the influent gas stream. At an EBRT of 20 sec, it took approximately 0.6, 4.5, and 9.5 hours for the dimensionless effluent concentration to decrease
from 1.0 to 0.5, 0.1, and 0.05, respectively. Desorption occurred more quickly at an EBRT of 10 sec (i.e., higher flow rate) where the dimensionless effluent concentration decreased from 1.0 to 0.5, 0.1, and 0.05 after approximately 0.25, 2.2, and 4.6 hours of desorption, respectively. The mass of MEK desorbed from the column was 823 and 778 mg at EBRTs of 20 and 10 sec, respectively. In comparison to the mass originally sorbed, such data demonstrate a good closure on contaminant mass balance in the column, indicating that MEK sorption to the wet foam medium is reversible. Such sorptive characteristics of the packing medium make possible both accumulation of MEK during FEED period and subsequent desorb of MEK during REACT period essential for successful SBB operating strategy.

![Graph](image1)

![Graph](image2)

**Figure 3.5: Abiotic sorption and desorption of MEK on the foam medium (with a moisture content of 65%) at influent MEK concentration of 106 ppm.**
3.3.2. Biofilter Performance during Normal Loading

Effluent MEK concentrations during normal loading FEED periods were measured for more than 120 days after nutrient addition on day 0. The overall removal efficiencies are depicted in Figure 3.6. On day 0, approximately 10% of the influent MEK concentration was detected in the biofilter effluent within one hour after the FEED began, and the effluent MEK concentration remained near 10 ppm, during the remainder of the FEED period during that cycle. As shown in Figure 3.6, MEK removal efficiency increased to 98.3% and 99.4% in the third and fourth cycles, respectively, indicating rapid recovery of the MEK degrading microbes in the foam. The lower removal efficiency during the first cycle was likely due to biomass loss during the nutrient addition process in which each foam cylinder was submerged in 1.0 L of nutrient solution and then compressed to remove excess moisture. The rapid recovery is consistent with that associated with growth of new biomass during the subsequent time period.

On days 49 and 50, there was a slight decrease in MEK removal efficiency. A visual inspection of the column revealed a slight drying of the biofilter packing media. Following adjustment of the packing medium’s moisture content to approximately 65% on day 51, MEK removal efficiency rapidly increased and remained greater than 99% during normal loading for the remainder of the study period, even in cycles immediately following nutrient addition.

During each normal loading cycle, at the start of the FEED period, the effluent CO\textsubscript{2} concentration increased rapidly to a maximum value within the first 10 seconds after the FEED period began and air flow through the biofilter resumed. Likewise, the O\textsubscript{2} concentration reached a minimum value within the first 10 seconds after the FEED period began. Typical effluent CO\textsubscript{2} and O\textsubscript{2} concentrations for the first 15 minutes of the FEED period (from day 44) are depicted in Figure 3.7 (top). As shown in the figure, the CO\textsubscript{2} concentration reached a maximum value of 5400 ppm\textsubscript{v} (0.54%) and the O\textsubscript{2} concentration reached a minimum value of 16.5% within the first
10 seconds after the FEED period began. The pattern of elevated CO$_2$ concentration and depleted O$_2$ concentration during the first few minutes of the FEED period is consistent with that expected from degradation during the REACT period of MEK sorbed into the column during previous cycle’s FEED and endogenous respiration. Approximately one minute after the start of the FEED period, the effluent O$_2$ concentration increased to approximately 21% and remained relatively constant for the remainder of the FEED cycle. The effluent CO$_2$ concentration rapidly decreased to below 1000 ppm within two minutes after FEED began, and then the CO$_2$ concentration decreased gradually during the next 100 minutes of FEED before becoming relatively constant for the remainder of the FEED cycle (see Figure 3.7, bottom).

![Figure 3.6: Removal efficiency and average loading rate of MEK during normal operation.](image)

An estimate of the mass of MEK sorbed by the system during FEED can be made from the mass of CO$_2$ produced during REACT period if complete degradation of MEK was achieved during REACT. Assuming that the majority of the CO$_2$ produced during REACT was subsequently released during the first 15 minutes of the next cycle’s FEED period and neglecting the amount of CO$_2$ produced by endogenous respiration, the corresponding MEK-equivalent degraded during REACT was 214 mg. This covered only a small percentage of the total MEK (1737 mg) entering the system during FEED (i.e., 12%). This indicates that during normal
loading, most of the MEK entering the biofilter was biodegraded at approximately the same rate that it entered the system rather than accumulating via sorption to the packing medium. Because MEK, CO₂, and O₂ concentrations were not measured at time intervals during the REACT period, it is not possible to determine from the data collected how much time was needed to degrade the accumulated MEK and what fraction of the time could have been allocated to IDLE. However, because the MEK was not detected in the effluent during the initial stages of the FEED period, it is clear that four hours was sufficient for REACT for the loading conditions of normal operation.

![Graph showing CO₂ and O₂ concentrations](image)

**Figure 3.7:** Typical effluent CO₂ and O₂ concentration during the first 15 minutes of FEED for normal loading (top), and effluent CO₂ concentration during the remainder of the FEED period on day 44 (bottom).
Figure 3.8 depicts the maximum CO$_2$ concentration and the minimum O$_2$ concentration at the end of the REACT period (start of the FEED period) during normal operation. As clearly shown in the figure, a higher CO$_2$ concentration was observed for a period of several days following each nutrient addition. During the nutrient addition process, an appreciable amount of biomass was removed from the packing medium. The higher CO$_2$ concentration during cycles immediately following nutrient addition was likely caused by a greater mass of MEK accumulating in the column (i.e., sorbed to the packing medium) rather than degrading at a rate approximately equal to the influent loading rate. The average maximum CO$_2$ concentration at the end of REACT (start of FEED) during normal loading was approximately 7800 ppm$_{v}$. The average minimum oxygen concentration at the end of REACT during normal loading was approximately 16.5%. Consequently, it is not expected that oxygen limited contaminant biotransformation in this system. However, in other applications or at higher loading rates, it may be necessary to supply air or pure oxygen to the system to avoid stoichiometric and kinetic limitations.

Because undegraded contaminants can accumulate in the SBB system, the period during which contaminants enter the system can be separated in time from the period in which they are biotransformed. Consequently, the commonly used term “elimination capacity” (mass of contaminant removed per unit volume of packing medium per unit time) is not directly comparable to continuous flow systems because the elimination capacity clearly changes as a function of time during the operation cycle. Nevertheless, the average elimination capacity was calculated to provide a general comparison. During the FEED period of normal loading, the mean elimination capacity was 56.4 g m$^{-3}$ h$^{-1}$, which is comparable to the maximum MEK elimination capacity reported by previous researchers studying MEK degradation in biofilters,
biotrickling filters, and tubular biofilm reactors (Deshusses et al., 1995; Agathos et al., 1997; Chou and Huang, 1997; Amanullah et al., 2000; Deshusses 1997). The mean elimination capacity averaged over each complete cycle of normal loading (4 hr FEED plus 4 hr REACT) was 28.2 g m⁻³ h⁻¹.

![Image](image.png)

**Figure 3.8**: Maximum effluent CO₂ and minimum O₂ concentration at the end of REACT (start of FEED) during normal loading. Arrows denote dates of nutrient addition.

### 3.3.3. Shock Loading I

Shock Loading I tests (elevated MEK concentration applied during the last hour of the FEED period at a loading rate of 282 g m⁻³ h⁻¹), were conducted on days 13, 21, 31, 40, 43, 46, 51, 58, 61, 65, and 68. Figure 3.9 depicts typical effluent MEK and CO₂ concentration during Shock Loading I as measured on day 46. During the first three hours (normal loading), there was no measurable MEK in the effluent. This is consistent with the performance observed during “normal” loading. After the influent MEK concentration increased from 106 to 530 ppmv during the last hour of the FEED period, the effluent MEK concentration reached approximately 10% breakthrough (50 ppmv) after the shock loading had been applied for 18 minutes. During the same time period, the effluent CO₂ concentration increased rapidly from approximately 400 ppmv to 800 ppmv, where it remained relatively constant during the remainder of the FEED
period. The effluent MEK concentration increased to more than 140 ppm, after an additional 42 minutes (the end of the shock loading). Other Shock Loading I tests (data not shown) showed a similar pattern of MEK breakthrough. No MEK was detected in the biofilter effluent at the beginning of the FEED period immediately following the REACT period of the shock-loading event. This indicates that the REACT period of four hours was sufficiently long for MEK sorbed and/or accumulated by organisms during the Shock Loading I FEED period to be biotransformed.

The MEK removal efficiency for Shock Loading I experiments conducted throughout the study period are depicted in Figure 3.10. The mass of MEK entering the biofilter during the one-hour shock load (assuming a constant influent concentration of 530 ppm) was 2160 mg (with a loading rate of 282 g m⁻³ h⁻¹). The mass of MEK exiting the biofilter during the one-hour shock period ranged from 281 mg to 950 mg and averaged 465 mg (average of all Shock Load I replicates). Considering only the one-hour shock-loading period, the MEK removal efficiency ranged from 56% to 87% with a mean of 78.5% removal. The removal efficiency averaged over the entire four-hour FEED period that included the shock loading (3 hours normal FEED and 1 hour shock load) was 86.5%.

The average maximum CO₂ concentration at the end of the REACT period following Shock Loading I (start of FEED period of subsequent normal loading cycle) was approximately 20,400 ppm. The average minimum oxygen concentration for the same time period was 11.6%. The markedly higher CO₂ concentration and lower oxygen concentration (in comparison to normal loading) provide clear evidence that MEK accumulated in the system by sorption or production of intracellular storage compounds was biodegraded during the subsequent REACT period.
As shown in Figure 3.10, MEK removal efficiency was higher during experiments conducted during the three weeks following each nutrient addition than during later time periods.
The decrease in performance during the transient loading conditions at later time periods is consistent with that expected from a kinetic limitation caused by lack of nutrient availability as previously reported by Moe and Irvine (2001). This is further supported by nitrate concentrations measured in samples removed from the biofilter just prior to nutrient addition (data not shown). Results indicate that nitrate-nitrogen was nearly depleted in the packing media near the biofilter inlet four weeks after nutrient addition. However, an appreciable amount of nitrate-nitrogen remained in the medium in sections of the column furthest from the inlet.

3.3.4. **Shock Loading II**

Shock Loading II experiments (elevated MEK concentration applied during the first hour of FEED) were conducted on days 72, 74, and 75. Typical effluent MEK concentrations during Shock Loading II are depicted in Figure 3.11 (from day 74). As shown in the figure, the effluent MEK concentration remained 0 ppm during the first 10 minutes of the shock loading. The effluent MEK concentration reached 10% breakthrough (50 ppm) approximately 20 minutes after the start of the shock-loading, and the effluent MEK concentration had stabilized at approximately 200 ppm when shock loading condition was terminated after a total of 60 minutes. Approximately ten minutes after the end of the shock loading condition when the influent MEK concentration decreased to 106 ppm, the effluent MEK concentration began to rapidly decrease. During the next 20 minutes, the effluent MEK concentration decreased to near zero.

As was observed with Shock Loading I, the effluent CO₂ concentration during the one-hour shock loading period was almost twice the concentration observed during normal loading (see Figure 3.11 bottom graph). This indicates that there was an increase in the MEK biodegradation rate during the transient loading period; however, the increased biodegradation rate was not sufficient to prevent MEK breakthrough. After MEK was no longer observed in the
biofilter effluent (approximately 90 minutes into the FEED period and approximately 30 minutes after the influent concentration returned to 106 ppm), the effluent CO\textsubscript{2} concentration began to decrease and reached a stable concentration of approximately 330 ppm within an additional 30 minutes (120 minutes into the FEED period). The lag period between the time that MEK was no longer present in the effluent (90 min) and the time that the CO\textsubscript{2} concentration reached a stable, lower value (120 min) was likely caused by degradation of bioaccumulated MEK during this time period or by MEK desorption from the support medium at a rate equal to the biodegradation rate.

For the three replicates, an average of 393 mg MEK exited the biofilter column during the first hour of the Shock Loading II experiment, and an additional 215 mg of MEK exited the system during the 30 minutes following return to normal loading. Thus, an average of 608 mg MEK exited the system during the FEED period. The corresponding overall MEK removal efficiency during the Shock Loading II cycle was 82.4%, lower than the 86.5% overall removal efficiency during the Shock Loading I cycle FEED period. When the shock load was applied during the last hour of the FEED period (Shock Load I), MEK sorbed in the column during the FEED period was degraded during the following REACT period. However, when the shock loading condition occurred during the first hour of the FEED period (Shock Load II), a portion of the MEK initially sorbed to the packing medium subsequently desorbed and exited the system without being degraded. Thus, the difference in overall performance was caused by sorbed MEK degrading in the case of Shock Loading I while being stripped from the system in Shock Loading II. This is further supported by the fact that the average maximum CO\textsubscript{2} concentration at the end of the REACT period following Shock Loading II (start of FEED period of subsequent normal loading cycle) was approximately 14,800 ppm. The average minimum oxygen concentration for
the same time period was 14.7%. The lower maximum CO\textsubscript{2} concentration and higher minimum oxygen concentration (in comparison to Shock Load I) provide clear evidence that less MEK accumulated in the system during Shock Load II than during Shock Load I.

![Figure 3.11: MEK concentration (top) and effluent CO\textsubscript{2} concentration (bottom) during Shock Loading II on day 74.](image)

**3.3.5. Shock Loading III**

Shock Loading III experiments were conducted on days 84, 86, and 89. When this shock loading condition was applied, the EBRT increased from an initial value of 20 seconds to 40 seconds (i.e., the flow rate decreased to half the original value) during the entire four hour FEED period. Because the influent MEK concentration increased by a factor of 5.0 and the gas flow rate decreased by a factor of 2.0, the MEK loading rate increased by a factor of 2.5 (to 141 g m\textsuperscript{-3} h\textsuperscript{-1}) compared to normal loading conditions. This simulates the loading condition imposed on one biofilter in a set of two SBBs under conditions where an operator simultaneously loads both
biofilters. It is anticipated that an operator could make such adjustments to the SBB loading strategy when on-line instrumentation or process knowledge indicates that higher than normal VOC concentrations are present in the influent to the gas treatment system.

Typical results (from day 89) are depicted in Figure 3.12. As shown in the top graph of the figure, there was no measurable breakthrough of MEK in the biofilter effluent during the FEED period in any of the three replicates of this loading condition. As also shown in bottom graph of Figure 8, the effluent CO\textsubscript{2} concentration during the shock-loading period (first hour of FEED) was almost twice that of Shock Loading II. This indicates that a larger fraction of the MEK was biodegraded as it entered the system. Thus, it is expected that the mass of MEK accumulated in the system was appreciably smaller during Shock Load III than in Shock Load II.

Following the return to 106 ppm\textsubscript{v} MEK in the influent, the higher EBRT (lower gas flow rate) provided conditions for the adsorbed MEK to be degraded before the MEK was stripped from the system. During Shock Loading II (EBRT of 20 seconds), the desorption rate was so rapid (compared to the biodegradation rate) that within 30 minutes after shock loading was terminated, much of the originally sorbed MEK was stripped from the system. During Shock Loading III (when the air flow rate was lower, 40 seconds), the MEK sorbed during shock loading desorbed during the remaining three hours of normal loading FEED at a sufficiently slow rate that it could be biodegraded before it exited the system. This is supported by the effluent CO\textsubscript{2} concentration data. During Shock Loading III, the effluent CO\textsubscript{2} concentration began to decrease almost immediately after the end of the shock-loading period (60 min) and gradually decreased to a stable value only after an additional 90 min (150 min into the FEED period). In contrast, the effluent CO\textsubscript{2} concentration reached a stable value approximately 30 minutes earlier during Shock Loading II. This is further supported by the fact that the average maximum CO\textsubscript{2}
concentration at the end of the REACT period following Shock Loading III (start of FEED period of subsequent normal loading cycle) was approximately 9,300 ppmv. The lower maximum CO$_2$ concentration (in comparison to Shock Load II) provides clear evidence that less MEK accumulated in the system during Shock Load III than during Shock Load II.

![Graph of MEK and CO$_2$ concentrations](image)

**Figure 3.12**: MEK concentration (top) and effluent CO$_2$ concentration (bottom) during Shock Loading III on day 89.

Application of an active control strategy (e.g., simultaneously loading more than one biofilter in a multiple-biofilter SBB system) during Shock Loading III clearly resulted in more complete contaminant removal during the transient period of elevated contaminant loading than would have otherwise occurred. It is expected that the operational flexibility of the SBB system containing packing medium with sorptive capacity can be adapted to other operating conditions and waste gas streams to result in high removal efficiency during transient periods of elevated contaminant loading.
3.3.6. Shock Loading IV

Shock Loading IV experiments were conducted on days 83 and 91. This loading condition represent the conditions expected in a biofilter where an operator controls the system by simultaneously loading both biofilters in a SBB system containing two biofilters during only conditions when higher than normal VOC concentrations are observed in the influent (rather than continuing to simultaneously load both biofilters for a period of time following the transient condition as occurred with Shock Loading III).

Typical results (from day 91) are depicted in Figure 3.13. As shown in the figure, no breakthrough of MEK occurred during the first hour of the shock loading when the EBRT was 40 seconds. However, almost immediately after the normal loading FEED resumed and EBRT returned to 20 seconds, there was immediate release of MEK which peaked at around 50 ppm, 15 minutes after normal loading began. Subsequently, the effluent concentration dropped to near zero after an additional 15 minutes (30 minutes after the shock loading period ended). This indicates that at a higher flow rate, a portion of the contaminant originally sorbed to the foam during the first hour (shock loading) was stripped from the column. Shock Loading IV verified that the lack of MEK breakthrough during Shock Loading III was due to combination of sorption and biological activity.

An estimate of the mass of MEK sorbed by the system during shock loading FEED cycles can be made based on the same assumptions described previously for the normal loading. On the average, the mass of CO₂ released during the first 15 minutes of FEED in shock loading I, II, and III was 1082 mg, 712 mg, and 660 mg, respectively. The corresponding MEK-equivalent accumulated during FEED and subsequently degraded in REACT was 443, 291, and 270 mg, respectively, which accounted for 12.8, 8.3, and 15.5% of the total MEK entering the system during Shock Loading I, II, and III cycles, respectively. Sorption capacity of the lab-
manufactured foam impregnated with powdered activated carbon was lower than the theoretical adsorption capacity of the PAC alone. Possible explanations for this have been made previously (Moe and Irvine, 2000a; Martinez 2001). First, during the manufacture process many of the adsorptive sites of the activated carbon may become occupied by any of the components of the foam matrix (e.g. surfactant, polymer, or cross linking agents). Second, during manufacture, PAC becomes part of the polyurethane matrix, and PAC completely surrounded by the polyurethane may be physically blocked from contact by the MEK.

![Figure 3.13: MEK concentration during Shock Loading IV on day 91.](image)

If packing media with contaminant sorption capacity higher than the polyurethane/PAC foam tested in the study reported herein were used, it is expected that appropriate operating strategies could be developed to further decrease or eliminate contaminant emission during these and other shock loading conditions. Furthermore, it is expected that such an approach can result in a robust air pollution control technology adaptable to many contaminated gas streams with unsteady-state VOC concentrations.
3.4. Conclusions

Data presented herein establish that sequencing batch biofilter (SBB) operation is a feasible technology for treating air streams contaminated by low concentrations of MEK. The laboratory-scale SBB was able to achieve greater than 99% contaminant removal when subjected to a loading of 106 ppm, MEK with an EBRT of 20 seconds. The system exhibited stable long-term performance (more than 130 days) when nutrients were added on a monthly basis. During normal loading periods (106 ppm, MEK influent), the majority of the MEK was biodegraded during the FEED period, and the fraction of MEK sorbed or bioaccumulated in the system was degraded during the subsequent REACT period.

SBB operation of a system containing packing medium with sorptive capacity can result in high removal efficiency during transient periods of elevated contaminant loading. Furthermore, such a system allows application of active control strategies (e.g., simultaneously loading more than one biofilter in a multiple-biofilter SBB system) that can provide a practical approach for effectively removing contaminants during transient periods of elevated contaminant loading. In cases where active control strategies are not applied during shock loading conditions, overall treatment efficiency was limited by the sorption capacity of the biofilter packing medium. If packing media with contaminant sorption capacity higher than the polyurethane/PAC foam tested in the study reported herein were used, it is expected that appropriate operating strategies could be developed to further decrease or eliminate contaminant emission during shock loading conditions even without implementation of active control strategies.
CHAPTER 4  COMPARISON OF CONTINUOUS AND SEQUENCING BATCH OPERATED BIOFILTERS FOR TREATMENT OF GAS-PHASE MEK

4.1. Introduction

Although biofilters have been used successfully for many applications, several design and operational challenges remain. One of these challenges is selection of the appropriate basis of design for treatment of dynamically-varying waste gas streams. Contaminant concentrations in most waste gas streams vary with time due to the unsteady-state nature of the industrial processes that generate them. In spite of the inherently unsteady-state nature of most waste gas streams, conventional biofilters are designed and operated to receive a continuous stream of contaminated air without appreciable variation in organic load. Short-term increases in VOC concentration can result in dynamic “shock” loads that exceed biological reaction capacities and result in contaminant emission from biofilter systems (Martin and Loehr, 1996; Deshusses et al., 1999; Martin et al., 2002).

A promising alternative for improving biofilter performance during transient loading conditions while minimizing the disadvantages of conventional treatment technologies is a combination of conventional biofilters with adsorption processes in a hybrid system that utilizes batch operating strategies. Preliminary results reported previously in Chapter 3 indicate that combining use of biofilter packing medium that contains appreciable contaminant sorption capacity with implementation of sequencing batch operating strategies can greatly enhance treatment performance and mitigate many of the disadvantages encountered with conventional biofilter operation. Terminology proposed for this new operating strategy is Sequencing Batch Biofilter (SBB) operation. Implementation of the SBB operating strategy utilizes one or more biofilters packed with medium containing powdered activated carbon (PAC) or other sorptive

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material and is operated with three distinct periods that comprise one complete cycle: FEED, REACT, and IDLE (see Chapter 3 for detailed definition).

Although results reported in Chapter 3 demonstrated that SBB operation is feasible and can be used in conjunction with active control strategies to better control biofilter performance during transient loading conditions, further research is required to determine the broader applicability of this previous work. For example, the packing medium used in the previously reported research consisted of polyurethane foam monoliths that are likely impractical for full scale applications. Additionally, the sorption capacity of the previously tested medium was comparatively low, and therefore, the magnitude of transient loading condition that could be effectively handled by the biofilter without excessive breakthrough was limited. Finally, the previously tested system did not include a direct comparison to a conventional, continuous flow biofilter.

The investigation described herein determined the effect of sequencing batch operation on biofilter performance during both “normal loading” and unsteady-state conditions. Experiments utilized two laboratory biofilters, one operated as a continuous flow biofilter (CFB) and the other operated as a sequencing batch biofilter (SBB) to assess differences in performance based on the operating strategy imposed on the system. The biofilters, packed with cubed activated carbon coated polyurethane foam media with much higher sorption capacity than those used in previous SBB experiments, were subjected to a variety of steady and unsteady-state loading conditions to treat a model waste gas stream containing methyl ethyl ketone (MEK) over a period lasting more than 300 days.
4.2. Materials and Methods

4.2.1. Experimental Apparatus

Laboratory studies employed two biofilters consisting of glass column reactors with an inner diameter of 9.9 cm. As depicted in Figure 4.1, each biofilter consisted of six sections each of which was filled to 25 cm depth with biofilter medium to provide a total packed bed depth of 1.5 meters and a total bed volume of 11.5 L. Perforated stainless-steel support plates placed at the bottom of each section supported the packing medium.

Figure 4.1: Schematic diagram of the laboratory-scale sequencing batch biofilter (SBB) apparatus. The continuous-flow biofilter (CFB) was identical except for that the components inside the dashed line were omitted.

For each biofilter, compressed air from a laboratory air tap flowed through an activated carbon filter to remove contaminants. A pressure regulator then reduced the pressure to
approximately 15 psi. The air was split into two streams with 95% of the air flow passing through an aeration stone submerged in water in a 20 L glass carboy heated by electrical heating tape. The remaining 5% of airflow was used to volatilize MEK into the air stream. A KD Scientific model 1000 syringe pump (Boston, MA) delivered MEK (ACS Reagent, Sigma Chemical Co.) from a glass gas-tight syringe (Hamilton Co., Reno, NV) through a needle that pierced a septum-filled (Thermogreen™ LB-1 half-hole type septa, Supelco, Bellefonte, PA) glass injection port and into the air stream. Glass marbles were placed in the bottom of the column to evenly distribute airflow. All surfaces that contacted the contaminated air were made of glass, stainless steel, Teflon™ or Viton™.

One of the biofilters contained additional components (shown inside the dashed line in Figure 4.1) to allow it to be operated as an SBB. Solenoid valves with stainless steel bodies and flow tubes (Automatic Switch Company, Florham Park, NJ) were used to turn airflows on and off as needed. A diaphragm pump with stainless steel heads and Teflon diaphragm (Air Dimensions Incorporated, Deerfield Beach, FL) was used to recirculate air through the biofilter during REACT periods (with gas flow exiting the bottom of the biofilter and then being reintroduced at the top). A gas-scrubbing bottle (Fisher Scientific) was placed between the bottom recirculation outlet of the biofilter and the diaphragm pump to drain condensation. A microprocessor controller (Model XT, Chron-Trol Corp., San Diego, CA) was used to control operation of the syringe pump, diaphragm pump, and air flow valves. During FEED periods, only the syringe pump and air flow valves 1, 2 and 5 (see Figure 4.1) were turned on. During REACT/IDLE periods, only the diaphragm pump and valves 3 and 4 were turned on to allow gas recirculation in the closed system. The second biofilter, operated as a conventional CFB, was
identical to the SBB except that valves and other equipment associated with gas recirculation were omitted.

The packing medium for both biofilters consisted of type M-2CC activated carbon coated polyurethane foam cubes (Honeywell-PAI, Lakewood, CO). The medium, obtained as cubes approximately 5.0 cm per side, was cut into cubes approximately 1.25 cm per side prior to use in biofilter experiments. Previous experiments conducted with this packing medium determined Freundlich isotherm constants for adsorption of MEK of $K_f$ equal to $1.4 \times 10^{-3}$ and $1/n$ equal to 0.60 (with equilibrium concentration expressed as mg·L$^{-1}$ and $x/m$ in mg·mg$^{-1}$). MEK sorption to the medium was determined to be almost completely reversible (Martinez, 2001). Each of the six column sections in each biofilter contained approximately 75 g of foam medium (dry mass basis), and the resulting porosity of material in the columns was approximately 0.96. For the biofilter operated in SBB mode, the volume of gas recirculated in the system during the REACT period immediately following startup was approximately 12.3 L.

4.2.2. Biofilter Inoculation and Start-up

To prepare the inoculum for each biofilter, 1.0 L of rinsate from a previously completed biofilter experiment conducted in Chapter 3 was added to 5.0 L of nutrient solution as previously described in Table 3.1. The packing medium from each biofilter section was submerged separately in the resulting 6.0 L of activated sludge, and the medium was then placed in the biofilter column. After this inoculation, excess liquid was allowed to drain by gravity from the foam cubes for 30 minutes before each of the two biofilters began to receive MEK-contaminated air. No special attempt was made to retain biomass on the foam packing media. Time was measured in days from the time of inoculation.
4.2.3. Biofilter Operation

Following inoculation, one of the biofilters was operated as an SBB and the other was operated as a CFB (i.e., conventional biofilter). A summary of the operating conditions and loading rates for each of the biofilters during each phase of the study is presented in Table 4.1.

Table 4.1: Summary of biofilter operation during normal loading operation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Continuous Flow Biofilter (CFB)</th>
<th>Sequencing Batch Biofilter (SBB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period of operation (days)</td>
<td>Phase 1: 0–141</td>
<td>Phase 2: 141–187</td>
</tr>
<tr>
<td>EBRT (sec)</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>Influent MEK conc. (ppm$_v$)</td>
<td>106</td>
<td>106</td>
</tr>
<tr>
<td>MEK loading rate (g·hr$^{-1}$·m$^{-3}$)</td>
<td>19.0</td>
<td>12.7</td>
</tr>
<tr>
<td>Mass MEK / day (g)</td>
<td>5.25</td>
<td>3.50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Continuous Flow Biofilter (CFB)</th>
<th>Sequencing Batch Biofilter (SBB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period of operation (days)</td>
<td>0–141</td>
<td>141–187</td>
</tr>
<tr>
<td>EBRT (sec)</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Length of FEED (min)</td>
<td>240</td>
<td>45</td>
</tr>
<tr>
<td>Length of REACT (min)</td>
<td>240</td>
<td>90</td>
</tr>
<tr>
<td>Total cycle length (min)</td>
<td>480</td>
<td>135</td>
</tr>
<tr>
<td>Ratio of FEED:REACT</td>
<td>1:1</td>
<td>1:2</td>
</tr>
<tr>
<td>Number of cycles / day</td>
<td>3</td>
<td>10.7</td>
</tr>
<tr>
<td>Influent MEK conc. (ppm$_v$)</td>
<td>106</td>
<td>106</td>
</tr>
<tr>
<td>Loading rate during FEED (g·hr$^{-1}$·m$^{-3}$)</td>
<td>38.0</td>
<td>38.0</td>
</tr>
<tr>
<td>Mass MEK / day (g)</td>
<td>5.25</td>
<td>3.50</td>
</tr>
</tbody>
</table>

During the initial stage of biofilter operation (arbitrarily named Phase 1), which lasted from days 0 to 141, the SBB operational cycle consisted of a FEED period of 240 min (4 hr) and a REACT period of 240 min (total cycle length of 8 hr). The empty bed residence time (EBRT) was set at 30 seconds, and the target influent MEK concentration was 106 parts per million by volume (ppm$_v$). This corresponds to an MEK loading rate of 38.0 g·m$^{-3}$·h$^{-1}$ (grams of MEK supplied per cubic meter of packed bed volume per hour) during the FEED period and simulates the loading condition experienced by one biofilter in a set of two biofilters constructed in parallel and operated in sequence to treat a continuous gas flow. Because the SBB received contaminated air during only half of the cycle length, the average MEK loading rate considering
the entire cycle length was 19.0 g·m⁻³·h⁻¹. During Phase 1, the CFB had an EBRT of 60 seconds and target influent MEK concentration of 106 ppm, for “normal” operation. This corresponds to an MEK loading rate of 19.0 g·m⁻³·h⁻¹ and simulates the loading condition experienced by a conventionally operated (continuous-flow) biofilter identical in size to the SBB. The influent MEK concentration (106 ppm,) and influent mass of MEK treated per day were identical in both the SBB and CFB.

During Phase 2 of the study (days 141 to 187), the FEED period in the SBB was adjusted to 45 min and the REACT period was adjusted to 90 min. The EBRT remained 30 sec and the influent MEK concentration remained 106 ppm. This corresponds to an MEK loading rate of 38.0 g·m⁻³·h⁻¹ during the FEED period (average MEK loading rate of 12.7 g·m⁻³·h⁻¹ considering entire cycle length) and simulates the loading condition experienced by one biofilter in a set of three biofilters constructed in parallel and operated in sequence to treat a continuous gas flow. During Phase 2, the continuous-flow biofilter had an EBRT of 90 seconds, and target influent MEK concentration of 106 ppm,. This corresponds to an MEK loading rate of 12.7 g·m⁻³·h⁻¹ and simulates the loading condition experienced by a conventionally operated (continuous-flow) biofilter identical in size to the SBB. The influent MEK concentration (106 ppm,) and influent mass of MEK treated per day were identical in both the SBB and continuous-flow biofilters.

During Phase 3 of the study (days 187 to 300, the MEK loading rate was adjusted to be twice that of Phase 2. To accomplish this, the EBRT in the SBB was adjusted to 15 sec and the EBRT of the continuous-flow biofilter was adjusted to 45 sec. The influent MEK concentration remained 106 ppm, in both biofilters, and the length of the FEED and REACT periods of the SBB remained 45 min and 90 min, respectively. This corresponds to an MEK loading rate of 76.0 g·m⁻³·h⁻¹ during the FEED period of the SBB (average of 25.4 g·m⁻³·h⁻¹ considering entire
cycle length) and simulates the loading condition experienced by one biofilter in a set of three biofilters constructed in parallel and operated in sequence to treat a continuous gas flow. The MEK loading rate was 25.4 $\text{g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ in the continuous-flow biofilter. The influent MEK concentration (106 ppm,) and influent mass of MEK treated per day were identical in both the SBB and continuous-flow biofilters.

Axial concentration profiles of MEK along the depth of each biofilter were measured during each phase of the CFB and Phase 2 of the SBB during normal loading. Profile studies for the SBB were conducted during the last 30 min of FEED period.

To assess biofilter response to uncontrolled variation in influent contaminant concentration, each biofilter was periodically subjected to an influent MEK concentration of 1060 ppm, (10 times that of the normal loading) for a period lasting one hour (during Phase 1) or 45 min (during Phases 2 and 3). Loading conditions for each of the shock loading studies are summarized in Table 4.2.

**Table 4.2: Summary of biofilter operation during shock loading studies.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Continuous Flow Biofilter (CFB)</th>
<th>Sequencing Batch Biofilter (SBB)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phase 1</td>
<td>Phase 2</td>
</tr>
<tr>
<td>EBRT (sec)</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>Length of shock load (min)</td>
<td>60</td>
<td>45</td>
</tr>
<tr>
<td>Influent MEK conc. (ppm,)</td>
<td>1060</td>
<td>1060</td>
</tr>
<tr>
<td>MEK loading rate (g·hr$^{-1}$·m$^{-3}$)</td>
<td>190</td>
<td>127</td>
</tr>
<tr>
<td>Mass MEK influent during shock load (g)</td>
<td>2.19</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBRT (sec)</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Length of shock load (min)</td>
<td>60</td>
<td>45</td>
</tr>
<tr>
<td>Influent MEK conc. (ppm,)</td>
<td>1060</td>
<td>1060</td>
</tr>
<tr>
<td>MEK loading rate (g·hr$^{-1}$·m$^{-3}$)</td>
<td>380</td>
<td>380</td>
</tr>
<tr>
<td>Mass MEK influent during shock load (g)</td>
<td>4.37</td>
<td>3.28</td>
</tr>
</tbody>
</table>
Studies conducted during Phase 1 gave consideration to the fact that a shock loading could occur at any point during the FEED period and that the biofilter response may vary depending on the initial condition (e.g., essentially contaminant-free condition with microbes experiencing famine-like conditions at the start of the FEED period or accumulated contaminant and actively growing microorganisms toward the end of the FEED period). During Phases 2 and 3, the period of elevated contaminant loading in each of the biofilters lasted for 45 min, the entire length of one FEED period in the SBB. During Phase 3 shock loading conditions, consideration was given to the fact that SBB operation allows the system’s operator to implement active control strategies. During Phase 3A, the SBB was operated using a passive control strategy (i.e., the predefined operating strategy in place during normal loading was utilized, and no change was made to the operation strategy when the influent concentration increased ten-fold). During Phase 3B shock loading conditions, the SBB was operated using an active control strategy. In this case, the EBRT of the SBB was adjusted to 45 sec to simulate conditions where an operator simultaneously diverts contaminated air to all biofilters in a SBB system containing three biofilters constructed in parallel and operated in sequence. Because an active control strategy is not possible in a continuous-flow biofilter, there was no separate 3-A and 3-B designation for the shock loading condition in the continuous-flow system during Phase 3. A minimum of three cycles of “normal” loading occurred between each shock loading experiment.

To quantify endogenous respiration, the biofilter was occasionally subjected to a non-loading condition (0 ppm, MEK influent). For these experiments, the syringe containing MEK was temporarily removed from the system for one cycle in the SBB and an equal amount of time in the continuous-flow biofilter.
At approximately two-week intervals, nutrients were added to the biofilter by filling each column with freshly-prepared nutrient solution described previously. The columns were then allowed to drain by gravity for a period of approximately 15 minutes before being re-started. Portions of the glass column and its associated components were periodically dismantled and washed with hot water to minimize unintended biomass accumulation on reactor components. During the washing procedure, care was taken to avoid disturbing the packing medium, and the packing medium itself was not washed.

At the end of the biofilter operation, the spatial distribution of biomass within each biofilter was estimated using a water displacement procedure as described in Atoche (2003). In this procedure, each of the biofilter columns was filled starting at the bottom with freshly prepared nutrient solution by means of a peristaltic pump. The volume of nutrient solution required to fill the void space of each section was recorded. The volume of wet biomass was calculated by Equation 4.1 as shown below.

\[ V_{wb} = V_s - V_f - V_v \]  

(4.1)

where:

- \( V_{wb} \) = volume of wet biomass,
- \( V_s \) = total volume of each empty section,
- \( V_f \) = volume of packing material originally placed in each section, and
- \( V_v \) = measured void volume of each section

The calculated volume of wet biomass of each section was then divided by the total volume of each empty section in order to obtain the volume of wet biomass per unit volume of packed bed (cm\(^3\) L\(^{-1}\) packed bed).
4.2.4. **Analytical Techniques**

Gas-phase MEK concentrations were measured using three different methods, gas-phase CO\textsubscript{2} concentrations were measured using three different methods, and gas-phase oxygen concentrations were measured using one method. The various methods were used depending on the frequency of data collection required, the range of constituent concentrations, and the presence or absence of gas flow to allow on-line measurement. Each of the methods is described below.

The first method for measuring gas-phase MEK concentrations used an on-line MiniRAE 2000 programmable photoionization detector (PID) (RAE Systems, Sunnyvale, CA). The instrument was operated using a 10.6 eV discharge lamp and a 400 mL/min intake flow rate. Calibration was performed using nitrogen gas, as well as 198 ppm \textsubscript{v} and 1090 ppm \textsubscript{v} certified MEK calibration standards (BOC Gases, Port Allen, LA). The MEK concentration was measured at one-second intervals, and the data reported are the average of these measurements over one-minute intervals.

The second technique for measuring gas-phase MEK concentrations used an Agilent 6890 gas chromatograph equipped with a flame ionization detector (FID). 100 µL samples collected from the biofilters were directly injected using a glass gas-tight syringe (Hamilton Co., Reno, NV). A 60 m model DB-624 capillary column (Hewlett Packard, Palo Alto, CA) was used. The injector and detector temperatures were set at 225°C, and oven temperature was 50°C for 4 min followed by an increase at a rate of 50°C per min to 150°C where it held for 4 min before increasing at a rate of 50°C per min to 200°C where it held for 2 min. The helium carrier gas flow rate was 45 mL/min, hydrogen flow rate was 40 mL/min, and air flow rate was 200 mL/min. MEK eluted with a retention time of approximately 7.5 min. Calibration curves were
prepared using nitrogen gas as well as 198 and 1090 ppm\textsubscript{v} MEK standards (BOC, Port Allen, LA).

The third technique for measuring gas-phase MEK concentrations allowed simultaneous on-line measurement of MEK, CO\textsubscript{2}, and H\textsubscript{2}O concentrations. Gas streams were passed through a model 130 Genie membrane filter (A+ Corporation, Prairieville, LA) to remove excess moisture prior to analysis by a California Analytical Instruments (Orange, CA) model 1312 photoacoustic multigas monitor equipped with four optical filters (UA# 0971, 0974, 0983, and SB0527). The instrument was factory calibrated using concentrations of 0, 5.7, 52.1, and 143 ppm\textsubscript{v} MEK, and 0, 101, 505, and 1010 ppm\textsubscript{v} CO\textsubscript{2}. An additional external calibration using 198 and 1090 ppm\textsubscript{v} MEK and 953 and 5,080 ppm\textsubscript{v} CO\textsubscript{2} was also performed. Because the concentration of CO\textsubscript{2} in the biofilter influent fluctuated over time, results are reported indicating the concentration of CO\textsubscript{2} above the inlet concentration (i.e., CO\textsubscript{2} production in the biofilters).

During some experiments, CO\textsubscript{2} and O\textsubscript{2} concentrations were measured using an Oxygen and Carbon Dioxide Meter (Servomex, UK). The instrument intake flow rate was approximately 200 mL/min. Calibration was performed using nitrogen gas and 953, 5,080, or 50,000 ppm\textsubscript{v} CO\textsubscript{2} and 20\% O\textsubscript{2} certified calibration standards (BOC, Port Allen, LA). Data reported are the average over 0.5 or 1.0 second intervals.

Gas-phase CO\textsubscript{2} concentrations were also measured using an Agilent 6890 gas chromatograph equipped with a thermal conductivity detector (TCD). 100 µL samples collected from the biofilters were directly injected using a glass gas-tight syringe (Hamilton Co., Reno, NV). A 6.0 ft model 80/100 Chromosorb 102 packed column (Supelco, Bellefonte, PA) was used with the oven temperature isothermal at 40°C. The injector and detector temperatures were 225°C. A calibration curve for CO\textsubscript{2} was developed using nitrogen gas as well as 953 ppm\textsubscript{v} CO\textsubscript{2},

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5080 ppm, CO₂, and 50,000 ppm, CO₂ certified calibration standards (BOC Gases, Port Allen, LA).

Gas flow meters were calibrated using an Aalborg Instruments (Orangeburg, NY) model GFM-37 electronic gas mass flow meter. A water manometer was used to measure pressure drop across each of the biofilter’s packing medium. Head loss was recorded to the nearest 1 mm.

4.3. Results and Discussion

4.3.1. Biofilter Performance during Phase 1

On the first day of biofilter operation, immediately after startup, no MEK was detected in the effluent of the SBB during the FEED period of the first cycle of operation (4 hours), and no MEK was detected in the effluent of the CFB 4 hours after startup (the first time that samples were analyzed). As depicted in Figure 4.2, both the CFB and SBB systems continued to exhibit stable long-term performance with essentially complete MEK removal (greater than 99% removal efficiency) throughout all of the Phase 1 normal loading periods (in the absence of shock loading). Initial studies on a column packed with identical packing media but without biomass inoculation indicated that complete breakthrough would have occurred within a period of several hours at the loading conditions tested if biodegradation did not occur (data not shown). The rapid start-up provides a clear demonstration that an inoculation procedure using an enrichment culture acclimated to the contaminant and nutrient medium can provide rapid start-up of polyurethane foam based biofilters operated with either sequencing batch or continuous flow operation. In contrast, start-up periods of considerable length (several weeks) have been reported for some biofilters using alternate media and inoculation procedures (Devinny et al., 1999).

For the CFB, MEK loading rate during Phase 1 normal loading was 19 g·m⁻³·h⁻¹, and all of the MEK was removed to below detection limit. The SBB, which received the same influent
MEK concentration and MEK mass per day, also exhibited complete MEK removal. Direct comparison of the two systems on the basis of the commonly used term “elimination capacity” (mass of contaminant removed per unit volume of packing medium per unit time), however, is not entirely straightforward. During the 4 hour FEED period of the SBB, the normal loading rate was 38 g·m$^{-3}$·h$^{-1}$ (double that of the CFB); however, during the REACT period (which comprised one half of the SBB cycle time during Phase 1) the loading rate was 0 g·m$^{-3}$·h$^{-1}$. Because undegraded contaminants can accumulate in the SBB system (in fact they may be purposely added at a rate greater than they can be biotransformed), the period during which contaminants enter the system can be separated in time from the period in which they are biotransformed. Consequently, the elimination capacity is not directly comparable to continuous flow systems because the elimination capacity clearly changes as a function of time during the operation cycle. As further discussed below, a similar difficulty in using the term elimination capacity also arises in describing the performance of continuous-flow systems containing sorptive packing media when they are subjected to unsteady-state loading conditions. Removal efficiency is a more useful measure of performance and basis for comparison.

On the basis of overall performance during normal loading, both the CFB and the SBB were essentially identical in terms of removal efficiency. On a daily basis, both biofilters received the same influent MEK mass and concentration, and both removed essentially all of the influent MEK. The CFB was essentially at steady-state. The MEK and CO$_2$ concentration profiles measured along the height of the CFB were essentially constant as a function of time throughout the Phase 1 normal loading. In contrast, the SBB was clearly operated as an unsteady-state process.
Figure 4.2: Average loading rate and MEK removal efficiency of SBB (top) and CFB (bottom) during normal operation; for the SBB, the loading rate is the average over each complete cycle (FEED and REACT).

At the start of the FEED period during each normal loading cycle in the SBB, the effluent CO$_2$ concentration increased rapidly to a maximum value within the first 30 seconds after the FEED period began and air flow through the biofilter resumed. Likewise, the O$_2$ concentration reached a minimum value within the first 30 seconds after the FEED period began. The pattern of elevated CO$_2$ concentration and depleted O$_2$ concentration during the first few minutes of the FEED period is consistent with that expected from degradation during the REACT period of MEK sorbed into the column during previous cycle’s FEED and endogenous respiration. Approximately one minute after the start of the FEED period, the effluent O$_2$ concentration increased to approximately 21% and remained relatively constant for the remainder of the FEED cycle. The effluent CO$_2$ concentration rapidly decreased and was then relatively constant for the remainder of the FEED cycle.
During the REACT period of the SBB, gas was recirculated within the closed system. Assuming that the gas flow through the biofilter occurred with minimal back-mixing, the minimum effluent O₂ concentration and maximum CO₂ concentration measured in the biofilter outlet at the start of FEED are equal to the concentration of the recirculating gas at the end of REACT. As summarized in Table 4.4, the CO₂ concentration at the end of the REACT period during Phase 1 normal loading ranged from 0.77 to 1.70% (percent by volume, where 1% by volume equals 10,000 ppm_v) with a mean of 1.21% (12,100 ppm_v). The O₂ concentration at the end of REACT (measured at the start of the subsequent FEED period) ranged from 14.6% to 15.5% (percent by volume) and with a mean of 15.0%. Direct GC measurement of the CO₂ concentration during the REACT period confirmed that the maximum CO₂ concentration measured by online analyzers during the start of the FEED period was equivalent to that measured at the end of REACT.

During Phase 1 experiments, the model transient loading condition applied to each biofilter consisted of an influent MEK concentration of 1,060 ppm_v (ten times that of normal loading) for a duration of 60 minutes. Typical results for the CFB (data from day 60) are shown in Figure 4.3 (a), and results from the various shock loading studies are summarized in Table 4.3. Because it was not possible to simultaneously measure the inlet and outlet MEK concentrations using the on-line analyzers, the inlet concentration depicted in the figure is the target concentration based on the gas flow and syringe pump flow rates. Direct measurement of the influent MEK concentration during other shock loading time periods confirmed that the measured concentrations were approximately the same as the target values.

As shown in Figure 4.3 (a), the CFB effluent concentration during the 60 minutes of the shock-loading period remained below the instrument detection limit indicating essentially 100%
contaminant removal. Following the return to “normal loading” (106 ppm\textsubscript{v} MEK influent); however, MEK was detected in the effluent starting 9 min after the end of the shock load. The MEK concentration increased to a maximum concentration of 49.6 ppm\textsubscript{v} after an additional 109 minutes and then subsequently decreased to zero after approximately 160 additional minutes. Because of the packing medium’s sorptive capacity and consequent peak attenuation, the maximum effluent concentration was not reached until after the transient loading condition ended and the influent concentration was returned to 106 ppm\textsubscript{v} (the “normal” concentration). The effluent concentration of 49.6 ppm\textsubscript{v} at the time when the influent was 106 ppm\textsubscript{v} resulted in a minimum instantaneous removal efficiency of 53% (calculated as the influent MEK concentration minus the effluent MEK concentration divided by the influent MEK concentration times 100%, on an instantaneous basis). The “overall removal efficiency” (calculated as the total mass of MEK input to the system during the 60-min. transient loading period minus the total mass of MEK escaping the system during and after the transient loading period divided by the mass of MEK input to the system during the 60-min. shock loading period times 100%) for the CFB during Phase 1 ranged from 74.9% to 90.3% with a mean of 83.0%.

Phase 1 shock loading studies on the SBB gave consideration to the fact that a shock loading could occur at any point during the FEED period and that the biofilter response may vary depending on the initial condition (e.g., essentially contaminant-free condition with microbes experiencing famine-like conditions at the start of the FEED period or accumulated contaminant and actively growing microorganisms toward the end of the FEED period). Typical results (data from day 61) for the SBB are shown in Figure 4.3 (b) for the case in which the transient period of elevated contaminant loading occurred during the first hour of the FEED period. As shown in the figure, the effluent MEK concentration began to increase approximately 45 minutes after the
inlet concentration was increased to 1,060 ppm\textsubscript{v}. The effluent MEK concentration increased to a maximum concentration of 244 ppm\textsubscript{v} at 87 minutes after the start of the shock loading, well after the influent concentration returned to the normal concentration of 106 ppm\textsubscript{v}. This resulted in a minimum instantaneous removal efficiency of -130%. The negative instantaneous removal efficiency reflects the fact that the effluent concentration was higher than the influent concentration. The more rapid on-set of contaminant breakthrough in the SBB (in comparison to the CFB) was due to a combination of the higher gas flow rate and higher contaminant loading rate during the FEED period. The overall MEK removal efficiency (again, accounting for the total mass of MEK escaping the system during and after the transient loading period) associated with this shock-loading scenario ranged from 73.5 to 81.1% and averaged 77.3%.

Typical results (data from day 68) for the SBB are shown in Figure 4.3 (c) for the case in which the transient period of elevated contaminant loading occurred during the last hour of the FEED period. As shown in the figure, effluent MEK concentration reached an appreciable amount approximately 45 minutes into the shock load period, and it reached a maximum concentration of 65.5 ppm\textsubscript{v} at the end of the shock load period (when the subsequent REACT period began). This resulted in a minimum instantaneous removal efficiency of 93.8%. The much higher instantaneous removal efficiency (in comparison to the case where the shock load occurred during the first hour of FEED) results from the fact that the peak effluent concentration was reached at a time when the influent concentration was 1,060 ppm\textsubscript{v} (rather than 106 ppm\textsubscript{v}, as was the case with the previously described shock loading conditions) and that the maximum effluent MEK concentration was lower. As summarized in Table 4.3, the overall removal efficiency associated with this shock loading scenario ranged from 99.0 to 99.7% and averaged 99.3%. 
Figure 4.3: Typical results of shock loading experiments conducted during Phase 1: (a) Continuous-flow biofilter (day 60), (b) SBB shock loading during the first hour of FEED (day 61), and (c) SBB shock loading during the last hour of FEED (day 68).

At the end of the FEED period, the REACT period started, and gas was recirculated within the closed SBB system. Gas samples removed from the sampling port noted in Figure 4.1 were analyzed for MEK and CO₂ concentration during the REACT period. Average results from two such track studies (conducted for the case where the shock loading was applied during the
last hour of FEED) are depicted in Figure 4.4. Error bars denote the range of concentrations measured.

### Table 4.3: Summary of biofilter performance during shock loading studies.

<table>
<thead>
<tr>
<th>Period</th>
<th>Continuous Biofilter (CFB)</th>
<th>Sequencing Batch Biofilter (SBB)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range of Removal Efficiency (%)</td>
<td>Average Removal Efficiency (%)</td>
</tr>
<tr>
<td>Phase 1</td>
<td>74.9 - 90.3</td>
<td>83.0</td>
</tr>
<tr>
<td>Phase 2</td>
<td>87.5 - 98.1</td>
<td>95.2</td>
</tr>
<tr>
<td>Phase 3</td>
<td>75.0 - 93.8</td>
<td>83.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Figure 4.4: CO₂ and MEK concentrations in the SBB during the REACT period during Phase 1 shock loading experiments (1060 ppmv MEK influent applied at the last hour of FEED).

As shown in the figure, during the REACT period, the average maximum MEK concentration measured in the recycle line was 472 ppmv at a time of 15 minutes into the REACT period. The MEK concentration in the recirculating gas decreased to a concentration of 10.5 ppmv after 90 minutes in the REACT period, and it was below detection after 120 minutes.
The CO2 concentration increased to an average level of 7.5% (75,000 ppmv) (average of the two track studies) at the end of REACT. During the same time interval, the oxygen concentration decreased from an initial concentration of approximately 21% to a final concentration of approximately 8%. The markedly higher CO2 concentration and lower oxygen concentration during the REACT period following this shock loading (in comparison to normal loading in which the CO2 and O2 concentration were 1.21% and 15.0%, respectively, at the end of the REACT period – see Table 4.4) provide clear evidence that MEK accumulated in the system by sorption or production of intracellular storage compounds during the FEED period was biodegraded during the subsequent REACT period. In contrast, when the shock load was applied during the first hour of FEED in the SBB, a portion of the MEK initially accumulated in the column during the shock loading event subsequently desorbed and was emitted from the column following the return to normal loading even though the peak height was attenuated somewhat by the packing medium’s sorption capacity. Conversely, in the case that the shock load was applied during the last hour of FEED, accumulated contaminants were degraded in the closed system and were therefore not emitted.

No MEK was detected in the biofilter effluent at the beginning of the FEED period immediately following the REACT period of the shock-loading event. This, combined with data depicted in Figure 4.4, indicates that the REACT period was sufficiently long for MEK sorbed and/or accumulated by organisms during the shock loading FEED period to be biotransformed, and the shock loading did not have any apparent negative impact on subsequent performance.

The Phase 1 shock loading studies indicated that for the SBB, appreciable MEK breakthrough was not encountered until after approximately 45 minutes of shock loading during the FEED period (see Figures 4.3 [b] and 4.3 [c]). Likewise, MEK concentration was quite low
after 90 minutes and was not detected in the gas phase after 120 minutes in the REACT period (see Figure 4.4). This suggested that an operating strategy with a maximum FEED period length \( t_{\text{FEED}} \) of 45 minutes and a REACT period length \( t_{\text{REACT}} \) of between 90 and 120 minutes would be sufficient to minimize MEK discharge in the effluent. This operating strategy was adopted for use in Phase 2 experiments.

**Table 4.4: CO\(_2\) and O\(_2\) concentrations in the SBB at the end of REACT under different loading conditions.**

<table>
<thead>
<tr>
<th>Period</th>
<th>Loading Strategy</th>
<th>Range of CO(_2) conc. (%)</th>
<th>Average CO(_2) conc. (%)</th>
<th>Range of O(_2) conc. (%)</th>
<th>Average O(_2) conc. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1</td>
<td>Normal loading</td>
<td>0.77-1.7</td>
<td>1.21</td>
<td>14.6-15.5</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>Shock loading (last hour)</td>
<td>4.91-9.95</td>
<td>6.28</td>
<td>5.89-9.53</td>
<td>7.8</td>
</tr>
<tr>
<td>Phase 2</td>
<td>Endogenous</td>
<td>0.40-0.64</td>
<td>0.50</td>
<td>18.3-18.5</td>
<td>18.4</td>
</tr>
<tr>
<td></td>
<td>Normal loading</td>
<td>0.53-0.82</td>
<td>0.67</td>
<td>17.2-18.4</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>Shock loading</td>
<td>3.40-4.98</td>
<td>3.97</td>
<td>8.23-12.3</td>
<td>11.0</td>
</tr>
<tr>
<td>Phase 3</td>
<td>Endogenous</td>
<td>0.48-0.62</td>
<td>0.55</td>
<td>17.0-17.3</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td>Normal loading</td>
<td>0.64-0.83</td>
<td>0.77</td>
<td>16.4-17.0</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>Shock loading 3A</td>
<td>4.14-6.24</td>
<td>5.45</td>
<td>2.69-6.75</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>Shock loading 3B</td>
<td>2.22-3.26</td>
<td>2.74</td>
<td>10.1-12.7</td>
<td>11.4</td>
</tr>
</tbody>
</table>

**4.3.2. Biofilter Performance during Phase 2**

At the start of Phase 2 experiments on day 141, the FEED period was adjusted to 45 minutes and the REACT period was adjusted to 90 minutes in the SBB. The EBRT of the SBB remained 30 seconds and the influent concentration remained 106 ppm. To maintain the mass of MEK loaded to each biofilter per day the same in both biofilters, the EBRT of the CFB was changed to 90 seconds.

As shown in Figure 4.2, following the change in operating strategy, both biofilters continued to exhibit greater than 99% removal efficiency during the normal loading condition. This was not surprising considering that the mass of MEK loaded to each biofilter per day was lower than that in Phase 1 when complete removal was observed.
Figure 4.5 (top) depicts typical MEK and CO$_2$ concentrations as a function of time in the CFB (data from day 158) for the shock loading condition during Phase 2 (loading rate increased to 127 g·m$^{-3}$·h$^{-1}$ for a duration of 45 min). As shown in the figure, the effluent concentration during the 45 minutes of the shock-loading period remained below the instrument detection limit indicating essentially 100% contaminant removal. Following the return to “normal loading” (106 ppm$_v$ MEK influent); however, MEK was detected in the effluent starting 30 minutes after the end of the shock load. The MEK concentration increased to a maximum concentration of 6.4 ppm$_v$ after an additional 125 minutes and then subsequently decreased to below the detection limit after approximately 150 additional minutes. As also shown in the figure, the effluent CO$_2$ concentration increased rapidly from a concentration of approximately 400 ppm$_v$ at the beginning of the shock load to 730 ppm$_v$ after 15 minutes. The effluent CO$_2$ concentration in the system continued to increase at a slower rate during the next 78 minutes, reaching a maximum of 995 ppm$_v$ at 93 minutes after the start of the shock load (48 minutes after the end of the shock load). The effluent CO$_2$ concentration then decreased, reaching a concentration of 562 ppm$_v$ after an additional 367 minutes, at which point monitoring was discontinued.

As with the shock loading during Phase 1, the packing medium’s sorptive capacity resulted in peak attenuation, and the maximum effluent concentration was not reached until after the transient loading condition ended and the influent concentration was returned to 106 ppm$_v$ (the “normal” concentration). The sorptive mechanism is also evident from a comparison of the time difference between the peak CO$_2$ production (peak effluent CO$_2$ concentration 48 minutes after the end of shock loading) and the peak effluent MEK concentration (155 minutes after the end of shock loading). The effluent concentration of 6.4 ppm$_v$ at the time when the influent was 106 ppm$_v$ resulted in a minimum instantaneous removal efficiency of 94%. The overall removal
efficiency associated with the shock-loading event for the CFB during Phase 2 ranged from 87.5 to 98.1% with an average of 95.2%, higher than that observed in Phase 1 (83.0%). The higher removal efficiency during Phase 2 was expected because the EBRT was longer (90 sec vs. 60 sec), the duration of the shock load was shorter (45 min vs. 60 min), and the overall loading rate during the shock load was lower (127 g·m⁻³·h⁻¹ vs. 190 g·m⁻³·h⁻¹).

Figure 4.5: Effluent MEK and CO₂ concentrations exiting the CFB (top) and SBB during the FEED period (bottom) during Phase 2 shock loading (data from day 158).

Figure 4.5 (bottom) depicts typical MEK and CO₂ concentrations as a function of time during the FEED period in the SBB (data from day 158) for shock loading during Phase 2 (loading rate increased to 380 g·m⁻³·h⁻¹ for a duration of 45 min). For clarity of presentation, the CO₂ concentrations during the first 2 minutes of the FEED period (during which the effluent CO₂ concentration rapidly increased and then subsequently decreased due to accumulated CO₂ from the REACT period being purged from the system following resumption of gas flow at the start of
FEED) are not shown. As shown in the figure, the effluent MEK concentration reached a maximum of only 2.2 ppm when the FEED period terminated after 45 minutes. This corresponds to a minimum instantaneous removal efficiency of 99.8%. The overall removal efficiency for the SBB during the Phase 2 shock loading study ranged from 99.8 to 99.9% with a mean of 99.9% (average of all replicates), higher than that observed in the CFB even though the loading to the SBB was three times higher (380 g·m⁻³·h⁻¹ vs. 127 g·m⁻³·h⁻¹). No MEK was detected in the effluent of the SBB during the FEED period following REACT.

Figure 4.6 depicts the MEK and CO₂ concentrations in the recirculating gas during the REACT cycle of the SBB for periods immediately following FEED periods of 0 ppm, influent MEK (i.e., endogenous) (top), 106 ppm, influent MEK (i.e., normal loading) (middle), and 1,060 ppm, influent MEK (i.e., shock loading) (bottom). Error bars indicate the range of measurements conducted in at least triplicate track studies. In the case of endogenous loading, the CO₂ concentration increased throughout the 90 min REACT period, reaching an average maximum concentration of 5,550 ppm at the end of REACT during the track studies. As expected, no MEK was detected because there was no MEK in the influent gas stream during the immediately preceding FEED period. In the case of normal loading, MEK was initially present in the recirculating gas when the REACT period began, but it decreased to below detection limit within 15 minutes. CO₂ increased to an average maximum of 7,790 ppm at the end of REACT during the track studies. In the case of shock loading, the average maximum MEK concentration measured in the recirculating gas was 365 ppm at a time of 15 minutes in the REACT period. The MEK concentration rapidly decreased and was below detection after 60 minutes. The CO₂ concentration increased to an average level of 40,200 ppm (4.02%) at the end of REACT during the track studies.
Figure 4.6: CO₂ and MEK concentrations in the SBB during the REACT period (gas recirculated in the closed system) during Phase 2 experiments (EBRT 30 sec, FEED 45 min, REACT 90 min). (a) Endogenous loading condition (0 ppmv MEK influent). (b) Normal loading condition (106 ppmv MEK influent). (c) Shock loading condition (1060 ppmv MEK influent).

As summarized in Table 4.4, the O₂ concentration at the end of the REACT cycle immediately following the shock-loading event averaged 11.0%. In contrast, the O₂ concentration at the end of the REACT cycle following a normal FEED period averaged 18.0% and following a FEED period with no MEK in the influent (endogenous condition) averaged 18.5%. The higher CO₂ concentration and lower O₂ concentration during normal loading compared to that of the endogenous loading condition, further supported by the fact that MEK was measured in the recycle line at the start of the REACT period, indicates that a portion of the
MEK entering the system during the normal loading FEED period accumulated in the system without being fully transformed.

As also depicted in Table 4.4, the markedly higher CO\textsubscript{2} concentration and lower O\textsubscript{2} concentration during the REACT period following the shock loading conditions, further supported by the appreciable MEK concentration measured in the gas recycle line, clearly demonstrate that a considerable fraction of the contaminants accumulated in the system during the shock loading FEED conditions and then were subsequently degraded during the REACT period. The lower maximum MEK concentration observed in Phase 2 shock loading (in comparison to Phase 1) is likely caused by the fact that the length of the shock loading period was shorter (45 min vs. 60 min) leading to a smaller mass of MEK being accumulated. The lower maximum CO\textsubscript{2} and higher minimum O\textsubscript{2} concentrations observed in Phase 2 studies for the endogenous loading condition in comparison to those observed during Phase 1 is consistent with the fact that the length of the REACT period was considerably shortened (90 min vs. 240 min) and therefore less CO\textsubscript{2} was accumulated and less O\textsubscript{2} was consumed due to endogenous respiration of the microbial population. The lower maximum CO\textsubscript{2} and higher minimum O\textsubscript{2} concentrations observed in Phase 2 studies for the normal and shock loading conditions in comparison to those observed during Phase 1 is consistent with that expected from the shortened FEED period and the lower mass of MEK accumulated.

4.3.3. Biofilter Performance during Phase 3

At the start of Phase 3 experiments on day 187, the EBRT of both biofilters was decreased by a factor of two (i.e., the gas flow rate doubled). The influent concentration remained the same (106 ppm\textsubscript{v}), and thus, the MEK loading rate to each biofilter increased by a factor of two to 25.4 g·m\textsuperscript{-3}·h\textsuperscript{-1} in the CFB and a loading rate of 76.0 g·m\textsuperscript{-3}·h\textsuperscript{-1} in the SBB during the FEED period (average loading rate of 25.3 g·m\textsuperscript{-3}·h\textsuperscript{-1} accounting for both FEED and REACT
periods). As shown in Figure 4.2, both biofilters continued to maintain greater than 99% removal efficiency during normal loading period throughout Phase 3, even immediately following the increase in loading on day 187.

Typical effluent MEK concentrations for the CFB during a shock-loading event during Phase 3 (data from day 220) during which the loading rate was temporarily increased to a rate of 254 g·m⁻³·h⁻¹ for a duration of 45 minutes are depicted in Figure 4.7 (top). As shown in Figure 4.7 (top), the MEK concentration increased to a maximum concentration of 91 ppmv approximately 80 minutes after the start of shock loading and then subsequently decreased to zero after approximately 120 additional minutes. Because the peak effluent MEK concentration occurred well after the return to normal loading (106 ppmv influent), the average minimum instantaneous removal efficiency was 14%. The overall pattern of CO₂ production in the biofilter was similar to that observed in Phase 2. The overall MEK removal efficiency associated with the shock-loading events for the CFB during Phase 3 ranged from 75.0 to 93.8% with a mean of 83.8%. The lower overall removal efficiency observed during Phase 3 shock loadings (in comparison to those from Phase 2) likely resulted from the finite sorption capacity available to dampen the transient elevated loading at the higher rate experienced during Phase 3.

Typical effluent MEK concentrations in the SBB during the FEED period during Phase 3A shock loading (1,060 ppmv MEK influent) conditions (data from day 229) are depicted in Figure 4.7 (bottom). During the shock loading FEED period, MEK was detected in the effluent after only 6 minutes, and after 45 minutes (the end of the FEED period), the effluent MEK concentration had increased to 438 ppmv. Clearly, the loading rate exceeded the biodegradation rate and sorption capacity of the system. The more rapid on-set of contaminant breakthrough in the SBB (in comparison to the CFB) was due to the fact that the gas flow rate and MEK loading
rate to the SBB was three times that of the CFB (see Table 4.3). As shown in the figure, no MEK was emitted from the column during REACT periods. During the REACT periods in the SBB, air was recirculated in the closed system with no inflow or outflow of gas. As further discussed below, MEK that accumulated in the system during the shock loading FEED period was not completely degraded during the subsequent REACT period. Consequently, MEK breakthrough was observed during the first 27 minutes of the next FEED period. Although the maximum effluent MEK concentration observed in the SBB was higher than for the CFB, the minimum instantaneous removal efficiency observed in the SBB, 38%, observed at the start of the FEED period after the shock loading, was appreciably higher than the 14% observed in the CFB.

Figure 4.7: MEK concentrations exiting the CFB during Phase 3 shock loading conditions (top) (day 220) and MEK concentrations exiting the SBB during the FEED period of Phase 3A shock loading conditions (bottom) (day 229). In both graphs, time 0 indicated the start of the 45-min shock load. There was no airflow into or out of the SBB during the REACT time periods.
Figure 4.8 depicts the MEK and CO\textsubscript{2} concentrations in the SBB during the REACT period when subjected to conditions of no MEK loading (endogenous condition – top graph), normal loading (middle), and shock loading (bottom). As shown in the top graph of Figure 4.8, CO\textsubscript{2} production as a function of time during the REACT period following a condition of endogenous loading during Phase 3 was essentially identical to that observed during Phase 2. As summarized in Table 4.4, the CO\textsubscript{2} concentration at the end of the REACT cycle following FEED periods with no MEK in the influent (endogenous condition) averaged 0.55% and the O\textsubscript{2} concentration averaged 17.2%. In the case of normal loading, the pattern of MEK and CO\textsubscript{2} concentration as a function of time was also very similar to that observed during Phase 2 even though the load to the system during the FEED period was doubled. The CO\textsubscript{2} concentration at the end of the REACT cycle following a normal FEED period averaged 0.77% and the O\textsubscript{2} concentration averaged 16.6%.

During the REACT period following the shock loading condition (see Figure 4.8, bottom graph), the average maximum MEK concentration in the recirculating gas was 650 ppm\textsubscript{v} near the start of the REACT period. Unlike previous phases in which all of the accumulated MEK was transformed by the end of the REACT period, the MEK concentration in the recirculating gas reached a minimum concentration of 36 ppm\textsubscript{v} at the end of REACT. Consequently, during the subsequent FEED cycle, MEK was present in the effluent (see Figure 4.7, bottom). During the next REACT period, although the MEK concentration as a function of time returned to the same as during normal loading (none detected after 15 min), the final CO\textsubscript{2} concentration at the end of the REACT period was approximately three times that observed following normal FEED cycles (data not shown). No MEK was detected during the next FEED period (second cycle after shock loading) and the CO\textsubscript{2} and MEK concentrations were the same as for normal loading (data not
shown). This indicates that the system recovered within one cycle following the shock-loading event.

Figure 4.8: CO$_2$ and MEK concentrations in the SBB during the REACT period (gas recirculated in the closed system) during Phase 3A experiments (EBRT 15 sec, FEED 45 min, REACT 90 min). (a) Endogenous loading condition (0 ppm, MEK influent). (b) Normal loading condition (106 ppm, MEK influent). (c) Shock loading condition (1060 ppm, MEK influent).

The overall removal efficiency in the SBB for this shock loading condition (accounting for all MEK emitted from the system during the shock loading FEED period as well as the subsequent FEED period) ranged from 82.6 to 83.3% with an average of 82.9%, quite similar to the 83.8% removal observed in the CFB. The CO$_2$ concentration at the end of the REACT cycle
immediately following the shock-loading event in the SBB averaged 5.45% and the O₂ concentration averaged 5.2%. The low oxygen concentration may have led to a kinetic limitation on MEK degradation in the system. Addition of air or pure oxygen during the REACT period or use of a longer REACT period, t_{REACT}, may have allowed more complete MEK removal. In any case, the relatively high overall removal efficiency (82.9%) for the SBB at such a high loading rate (760 g·m⁻³·h⁻¹) reflects a much higher MEK removal rate than previously reported for MEK in biofilters (Deshusses et al., 1995; Agathos et al., 1997; Chou and Huang, 1997; Amanullah et al., 2000; Li and Moe, 2003).

For the SBB, the difference between Phase 3A and Phase 3B shock loading conditions is that in the latter, an active control strategy was applied during the FEED period. In Phase 3B, when the shock loading condition was applied (the inlet MEK concentration increased from 106 to 1,060 ppm v.), the EBRT was simultaneously increased from an initial value of 15 sec to 45 sec (i.e., the flow rate decreased to one-third of the original value) during the entire 45-min FEED period. Because the influent MEK concentration increased to 10 times that of normal operation and the gas flow rate decreased to 1/3 that of normal operation, the MEK loading rate increased to 3.33 times that of normal loading conditions. This simulates the loading condition imposed on each biofilter in a set of three SBBs under conditions where an operator simultaneously loads all biofilters. It is anticipated that an operator could make such adjustments to the SBB loading strategy when on-line instrumentation or process knowledge indicates that higher than normal VOC concentrations are present in the influent to the gas treatment system. For the Phase 3B shock loading conditions, the SBB had an MEK loading rate, EBRT, and influent concentration during the 45-min shock-loading period identical to that used for the CFB during all Phase 3 shock loading experiments.
Figure 4.9 (top) depicts typical effluent MEK and CO\textsubscript{2} from the SBB during the FEED period in Phase 3B shock loading (data from day 242). As shown in the graph, there was no measurable breakthrough of MEK in the biofilter effluent during the FEED period in any of the replicates of this loading condition. During the subsequent REACT period (see Figure 4.9, bottom graph), the average maximum MEK concentration in the gas recirculation line was approximately 580 ppm\textsubscript{v} near the start of REACT, and the MEK concentration decreased to below the detection limit within 75 minutes. The maximum CO\textsubscript{2} concentration increased to an average of 2.74\% by the end of the REACT period and the O\textsubscript{2} concentration decreased to an average of 11.4\%. Clearly, the MEK accumulated in the SBB during shock loading FEED period was desorbed and biodegraded during the subsequent REACT period. The lower maximum CO\textsubscript{2} concentration and higher minimum O\textsubscript{2} concentrations associated with the REACT period in this shock-loading scenario (in comparison to Phases 1, 2, and 3A) provide clear evidence that less MEK accumulated in the system during Phase 3B shock loading than occurred during previous phases. This was expected because the MEK loading rate to the system was lower. No MEK was detected in the SBB effluent during the next FEED period. In this case, unlike the Phase 3A shock loading condition, the mass of oxygen supplied and the time allocated for REACT were sufficient to allow degradation of the accumulated MEK.

Application of the active control strategy (e.g., simultaneously loading more than one biofilter in a multiple-biofilter SBB system) during Phase 3B shock loading clearly resulted in more complete contaminant removal during the transient period of elevated contaminant loading than would have otherwise occurred. It should be noted that with the operating strategy utilized for the SBB in Phase 3B, simultaneously loading all biofilters in a set of SBBs normally operated in sequence, an operator would need to transition to a return to “normal” loading. If all of the
biofilters in a multi-biofilter system were immediately put into a REACT period after their transient elevated FEED period was completed simultaneously, then none would be immediately available to treat the continuously incoming air. Thus, one or more of the biofilters would need to undergo a FEED period of extended duration.

![Graph showing MEK and CO2 concentrations](image)

**Figure 4.9**: CO\textsubscript{2} and MEK concentrations exiting the SBB during the FEED period during Phase 3B shock loading experiments (top) (data from day 242), and MEK and CO\textsubscript{2} concentrations in the SBB during REACT following the Phase 3B shock loading (bottom).

An alternate active-control loading strategy would be to simultaneously load all but one of the biofilters in a system with multiple biofilters installed in parallel and operated in sequence. In the latter case, an operator would be able to return immediately to “normal” operation following the shock loading because the remaining biofilter not loaded during the transient loading condition would have completed its REACT (or IDLE) period and would be immediately available to receive contaminated air at the beginning of its regularly scheduled FEED period. For the experimental system described in this paper, such a loading condition would be simulated by increasing the EBRT of the SBB to twice that of normal loading for the
FEED period during shock loading (to simulate simultaneously loading two biofilters in a set of three SBBs). That scenario is identical to the shock loading condition tested in the SBB during Phase 2 experiments (EBRT of 30 sec for 45-min period with influent MEK concentration of 1060 ppm) that resulted in greater than 99% contaminant removal. It is expected that the operational flexibility of the SBB system containing packing medium with sorptive capacity can be adapted to other operating conditions and waste gas streams to result in high removal efficiency during transient periods of elevated contaminant loading.

This novel biofilter operating strategy periodically exposes microorganisms to periods of high (e.g., feast) and low (e.g., famine) substrate concentrations. This “feast-famine” operating strategy has been used successfully in the SBR and other biological waste treatment processes to increase the biological reactor’s ability to handle shock and intermittent loads. Periodic operation of biofilters has the potential to overcome the limitations conventional biofilters have when treating industrial waste streams (e.g., inability to handle shock and intermittent loads). Likewise, because biodegradation would occur in a batch-mode during REACT, in treating waste streams containing multi-component mixtures, it may be possible to overcome kinetic limitations caused by inhibition and repression encountered in continuous flow systems provided that REACT is sufficiently long to allow for degradation of the most slowly degraded constituent.

4.3.4. MEK Axial Concentration Profile Study and Biomass Distribution

MEK concentration profiles measured along the height of the CFB and SBB during “normal” loading are depicted in Figure 4.10. As shown, the majority of MEK was degraded in the first two sections of column near the inlet during each phase of CFB and Phase 2 of SBB. As the EBRT decreased from Phases 1 to 2 to 3 for the CFB, the MEK profile shifted and MEK traveled a longer length of column before complete removal during Phase 3 compared to the previous phases.
Figure 4.10: MEK concentration profile along the height of the column during each phase for the CFB column and Phase 2 for SBB column (FEED period) during normal loading.

The wet biomass spatial distribution within each column near the end of the experiment (day 286) is illustrated in Figure 4.11. For each column, the amount of biomass was much greater near the inlet of the column than further away from the inlet, consistent with the above observation that the majority of MEK degradation occurred in the first two columns near the inlet. At the same height of section, biomass from the SBB was slightly greater than the CFB, probably due to the reason that the SBB removed greater amount of MEK during shock loading than the CFB, thus resulting in a higher net yield in biomass. A more uniform distribution of biomass was also observed in the SBB.

Figure 4.11: Spatial distribution of biomass within the SBB and CFB column on day 286.
4.4. Conclusions

Following inoculation of the biofilters on day 0, both the CFB and SBB exhibited essentially complete MEK removal. The rapid attainment of high removal efficiency following system start-up indicates that the inoculation procedure used in this study (using an enrichment culture acclimated to the contaminant and nutrient medium) can provide rapid start-up of activated carbon coated polyurethane foam based biofilters.

The laboratory-scale CFB and SBB both exhibited stable long-term performance (more than 300 days) with greater than 99% contaminant removal when subjected to “normal” loading conditions consisting of influent MEK concentration of 106 ppm\(_v\) at average loading rates ranging from 12.7 to 25.4 g·m\(^{-3}\)·h\(^{-1}\) when nutrients were added on a biweekly basis.

Results are presented which demonstrate how SBB systems can be utilized to minimize or eliminate contaminant emissions from biofilters during unsteady-state loading conditions. During transient periods of elevated contaminant loading, the SBB system was able to accumulate MEK during the FEED period and subsequently degrade the accumulated organics during the following REACT period. The ability of the system to accumulate MEK during the FEED period was demonstrated even after long-term operation. Once an appropriate operating strategy was selected, the SBB was able to remove more than 99% of the influent MEK at a transient loading rate of 380 g·m\(^{-3}\)·h\(^{-1}\) and 83% of the influent MEK at a transient loading rate of 760 g·m\(^{-3}\)·h\(^{-1}\). The operational flexibility of the SBB system facilitated selection of operational conditions that led to higher overall removal efficiency and higher minimum instantaneous removal efficiency than were achieved in the CFB.

Application of an active control strategy (e.g., simultaneously loading more than one biofilter in a multiple-biofilter system), made possible by SBB operation, can result in more complete contaminant removal during the transient period of elevated contaminant loading than
would have otherwise occurred. This provides an effective alternative for removing contaminants during transient periods of elevated contaminant loading in cases where on-line instrumentation or sufficient process knowledge allow implementation of process control decisions.
CHAPTER 5  ASSESSMENT OF MICROBIAL POPULATIONS IN MEK DEGRADING BIOFILTERS BY DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE)*

5.1. Introduction

Biofiltration is increasingly used as an air pollution control technology for treatment of gases contaminated by low concentrations of biodegradable volatile organic compounds (VOCs). In conventionally designed biofilters, contaminated gases are humidified and then vented through beds packed with solid media that support a biologically active layer. As gases flow through the support medium and past the biofilm, contaminants partition from the gas phase into the aqueous and solid phases (e.g., support medium and biofilm) where a mixed consortia of microorganisms biodegrade the contaminants into innocuous products such as carbon dioxide, water, and cell mass (Song and Kinney, 2000).

The diversity and relative abundance of microbial species present in a biofilter may be influenced by the inoculum source, composition of the influent waste gas, environmental conditions (e.g., pH, temperature), empty bed residence time, oxygen and pollutant concentrations, and the overall operating strategy employed. Several researchers have characterized microbial populations present in different biofilter systems using conventional culturing techniques, analysis of biomarkers such as phospholipid fatty acids (Webster et al., 1997), molecular techniques such as fluorescent in-situ hybridization (Møller et al., 1996), and cloning and sequencing of ribosomal RNA genes (Friedrich et al., 2002; Roy et al., 2003). Although these studies have provided insights into the diversity of microbial species present in biofilters, most of these studies have involved characterization of microorganisms at a particular location within a biofilter and do not allow quantitative assessment of differences in microbial population structure as a function of spatial location within the biofilters.

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Previous studies described in Chapter 4 demonstrated that a novel biofilter loading strategy that utilizes adsorptive packing media and implementation of sequencing batch biofilter (SBB) operation can be used to remove a larger portion of contaminants than continuously loaded biofilters of equivalent size during transient periods of elevated contaminants loading (i.e., shock loads). A large body of research in the field of biological wastewater treatment has demonstrated that periodic operating strategies that force microbes to compete over wide ranges of “feast” (high growth rate conditions imposed by high electron donor concentrations) and “famine” (starvation conditions imposed by very low electron donor concentrations) can be used to preferentially increase the relative abundance of microorganisms with desirable characteristics like high specific substrate uptake rates (Irvine et al., 1997). In light of this, it is of interest to know whether unsteady-state operating strategies can serve as a sufficiently large selective pressure to impact microbial population structure in biofilters treating gas phase contaminants.

The research described herein was conducted to: 1) assess whether the microbial population structure changes as a function of height in a continuous-flow biofilter (CFB); 2) assess whether the microbial population structure changes as a function of height in a sequencing batch biofilter (SBB); and 3) to compare the microbial populations in a CFB and SBB in an attempt to better understand whether the operating strategies imposed on biofilters treating gas phase contaminants can affect the relative abundance of microbial species in the mixed microbial consortia. The two biofilters were inoculated with an identical mixed culture and then operated for more than 300 days using different operating strategies (i.e., CFB or SBB). A detailed comparison of the biofilters’ performance in terms of contaminant removal was described in Chapter 4. In the research described herein, bacterial population diversity at various spatial
locations in the biofilters was compared on the basis of DNA banding patterns of PCR amplified genes coding for 16S rRNA subjected to denaturing gradient gel electrophoresis (DGGE).

DGGE allows separation of DNA segments that are identical or nearly identical in length but different in nucleotide sequences on the basis of differences in denaturing properties, and hence migration distance, in a gel system containing a linearly increasing gradient of denaturants (Muyzer et al., 1993). Because different bacterial species have different 16S rRNA sequences, DGGE of PCR amplified 16S rRNA gene segments from mixed microbial populations produce unique DNA banding patterns, often referred to as DNA “fingerprints.” The number, position, and intensity of the bands reflect the number and relative abundance of dominant rRNA types in the sample and thus allow a comparison of different microbial communities. Using DGGE analysis, species present in the community that comprise more than 1% of the target population can generally be detected (Muyzer et al., 1993, 1998). In contrast, culture-dependent techniques typically include less than 15% of the cells observed by direct counts (Amann et al., 1995). DGGE has been used successfully to monitor spatial or temporal differences in bacterial communities in a variety of environmental settings as well as bioreactors used for waste treatment (Muyzer et al., 1998; Kaewpipat and Grady 2002; LaPara et al., 2002; Tresse et al., 2002; Lacey et al., 2002).

The organization of this chapter is as follows. Section 5.2 provides background information regarding various approaches for characterizing selection and enrichment in mixed microbial populations with an emphasis on DGGE, the technique employed in the research described herein. Section 5.3 describes the materials and methods used to conduct the experimental procedures and analyze resulting data. Section 5.4 contains results and discussion, and Section 5.5 summarizes conclusions.
5.2. Background

5.2.1. Methods for Analysis of Microbial Diversity

5.2.1.1. Culture-dependent Approach

Microbial diversity in an environmental sample can be characterized using a variety of methods that can be broadly classified in two groups: 1) culture-dependent approaches and 2) culture-independent approaches. In the culturing approach, different cells are separated from each other by isolation of colonies on solid culture media, and these colonies propagate to result in a pure culture. The microorganisms in a sample can be recovered by direct plating of the sample, or plating after an initial enrichment phase that selects for an organism or group of organisms of interest. The main advantage of the culturing approach is that strains are maintained and are available for further characterization, which may provide insight into their ecological role (Perry and Staley, 1997).

The culturing approach cannot, however, be considered to furnish a complete picture of prokaryotic diversity in a given system because of biases inherent to the methods and materials employed. Limitations of the culture-dependent methods include the following.

(1) Culture-dependent methods do not accurately reflect the actual bacterial community structure, but rather the selectivity of growth media for certain bacteria. Every facet of the enrichment and isolation process imposes a selective pressure on the natural prokaryotic populations present in the sample. Some of these pressures include the nutrient composition of the culture medium, the temperature and gaseous conditions of incubation, exposure to ambient light, competition between organisms, and the human bias associated with the selection of colonies for further study. The cumulative effect of these selective pressures is to reduce the spectrum of prokaryotic diversity that can be recovered from a sample by the culturing approach (Head et al., 1998).
(2) It is generally accepted that only a minor fraction (0.1 to 10%) of all bacteria can be cultivated using standard culture techniques (Theron and Cloete, 2000). Reasons for this include the fact that the precise microhabitats in which many microbes grow cannot be easily mimicked. For example, microbes may require the presence of other organisms for symbiotic relationships. The “unculturables” may only be sustainable in a niche in which some exact combinations of resources (nutrients, light, etc.) are present (Muyzer et al., 1993).

(3) Techniques that rely on cultivation are time consuming and expensive as are the physiological and biochemical differentiation tests necessary to identify isolates (Temmerman et al., 2003).

(4) After many generations necessary to form plate colonies, an organism may deviate from its physiology, and possibly even from genotypic mix, of the population in nature (Theron and Cloete, 2000).

(5) Culturing techniques offer a limited insight into the spatial distribution of the microorganisms in the system from which they were isolated (Theron and Cloete, 2000).

5.2.1.2. Culture-independent Approach

Recently developed molecular techniques such as denaturing gradient gel electrophoresis (DGGE) allow assessment of microbial diversity and population dynamics based on 16S rDNA sequences without the need for isolation or culture. These strategies circumvent the cultivation step and its associated biases by extracting DNA or RNA sequences directly from the environmental sample (Ward et al., 1990; Muyzer et al., 1993; Ludwig et al., 1994; Amann et al., 1995). For several reasons, 16S rRNA (or 16S rDNA) sequence comparison is currently considered the most powerful tool for the classification of microorganisms (Woese, 1987). The 16S rRNA genes are found in all bacteria, and their gene sequences differ between species. The 16S rRNA contains both highly variable and conserved regions that allow one to distinguish
between organisms on all phylogenetic levels. In addition, a substantial body of data exists in publicly available databases (Maidak et al., 1999), which can be used to compare the DNA-sequence of unknown microorganisms and allow a phylogenetic identification.

The DGGE technique allows separation of partial 16S rDNA amplified fragments of identical length but different sequences due to their different melting behavior in a gel system containing a gradient of denaturants. As a result, a band pattern is obtained, which reflects the complexity of the microbial community. The reliability of the technique is very high; all species present in the community that comprise more than 1% of the target population can generally be detected by DGGE analysis (Muyzer et al., 1993; Murray et al., 1996). This percent representation is much higher than can be achieved using cultivation-based techniques in complex microbial communities (Muyzer et al., 1993). The amount of sample required to study microbial communities using molecular techniques is normally smaller than that needed for cultivation efforts. DGGE is relatively rapid to perform, and many samples can be run simultaneously. The method is, therefore, particularly useful when examining time series and population dynamics. By excising individual DGGE bands from the gel and reamplifying the DNA, it is possible to get sequence information of single community members (Muyzer et al., 1993; Muyzer and Smalla, 1998). Once the identity of an organism associated with any particular band has been determined, fluctuations in individual components of a microbial population, for example those caused by environmental perturbations, can be rapidly assessed. Overall, DGGE represents a powerful tool for monitoring microbial communities.

The following sections provide necessary background on DNA isolation from environmental samples followed by a summary of the principles of DGGE, its applications,
advantages, limitations, and finally, guidelines and methods for interpretation of DGGE fingerprinting patterns.

5.2.2. Isolation of DNA from Environmental Samples

Before any of the molecular techniques may be applied, nucleic acids of sufficient quality and quantity must be recovered from the sample of interest. In the field of environmental engineering, nucleic acids isolation are basically either from aquatic environments or from soil and sediment (i.e., from a solid matrix).

In general, water samples are the easiest environmental matrix from which to isolate nucleic acids. In many cases, water samples need only be filtered to collect microorganisms that may then be lysed to isolate their nucleic acids (Steffan and Atlas, 1991).

Two general strategies have been used to recover DNA from soils and sediments. The first approach involves separation of cells from the environmental matrix by successive washing and differential centrifugations (cell fractionation), followed by cell lysis and purification of DNA by standard methods (Faegri et al., 1977). The second approach is based on the lysis of cells in the presence of particulates or soil particles (direct lysis), followed by extraction of the released nucleic acid from the particulates and separation of the nucleic acid from organic carbon present in the sample (Ogram et al., 1987).

In order to obtain purified DNA from cells, DNA must first be separated from the rest of the cellular material. This involves destruction of the cell membrane (and/or cell wall), elimination of structural materials, and separation of proteins and RNA from the DNA. Current protocols for DNA extraction from microorganisms begin with cell lysis followed by purification and concentration of the DNA.
5.2.2.1. **Cell Lysis Methods**

The efficiency of lysis of microorganisms varies considerably among species and may vary among cell ages within the same species. In general, gram-negative species are more easily lysed than gram-positive ones, older cells may be more easily lysed than younger cells, and larger cells may be lysed more easily than small cells. Some fungal species and gram-positive endospores may be extremely difficult to lyse compared with most bacterial cells. Complete lysis of all microorganisms in a target sample is required if the recovered nucleic acids are to be truly representative of the sample, although this may be an unattainable ideal for many applications or may not be desirable for other applications. If the organisms of interest are lysed easily, a gentle lysis procedure that will enrich the target sequences in the recovered nucleic acid may be used. If all of the nucleic acids present in the sample are of interest, however, a more harsh lysis procedure should be used. A harsh lysis procedure may have the unintended consequence of damaging DNA from easily lysed cells. Therefore, the appropriate lysis procedure for the desired application should be selected carefully and the limitations of the selected procedure should be understood (Burlage *et al.*, 1998).

Most lysis procedures may be divided into two very general categories: chemical lysis and mechanical disruption. Chemical lysis involves the use of detergents (such as SDS), or lysozymes. Mechanical procedures include bead beating, sonication, French press, freeze/thaw cycles, and heating. Chemical lysis procedures are generally thought to be less efficient than mechanical procedures, but have the advantage that the recovered DNA has a higher molecular weight distribution. Procedures that fragment DNA to smaller sizes may not be desirable for cloning and may also increase the formation of chimeric amplification products during polymerase chain reaction (PCR) that would confound analysis of rDNA sequences. Most lysis
procedures combine elements of both chemical and mechanical procedures (Burlage et al., 1998).

Lysis procedures involving combination of lysozyme, SDS treatment, and freeze/thaw cycles are considered to be relatively gentle, and the DNA recovered is typically of high molecular weight (greater than 30 kbp). This method does not lyse endospores, however, and may not be efficient at lysing fungi and some difficult-to-lyse bacteria (Holben, 1994; Burlage et al., 1998).

Aside from freeze-thaw cycles, the most commonly used mechanical disruption procedure is bead-mill homogenization. In this process, the environmental sample or cells are mixed with either glass or zirconium/silica beads in a homogenizer, such as the Bead-Beater. Shear force generated in the homogenizer disrupt cell walls and lyse most cells and endospores. Homogenization in a bead-mill has been described as the most rigorous lysis procedure yet described and is likely to yield the least biased recovery of nucleic acids from a sample, but it has certain disadvantages (Burlage et al., 1998). DNA isolated by most bead-mill methods may be sheared to fragments smaller than 10 kbp, possibly increasing the potential for artifact formation during PCR. Very rigorous lysis methods should be used with caution in some cases with direct lysis procedures since they may yield nucleic acid from a variety of organisms, including non-microbial species (Burlage et al., 1998).

5.2.2.2. Purification of DNA

After cells are lysed and cell debris is removed through centrifugation, the DNA in solution can be extracted with phenol/chloroform. Phenol and chloroform are commonly used as denaturants of protein and for the solubilization of lipopolysacharides. After protein and lipopolysacharides are removed from the DNA solution, DNA may be concentrated through precipitation by addition of monovalent cations and alcohol (usually ethanol, polyethylene...
glycol, or isopropanol), followed by incubation at 0 to -70°C. The DNA may then be pelleted by centrifugation. A subsequent wash with ethanol, followed by brief centrifugation, removes residual salt and small organic molecules. If the extracted DNA is still high in humic content, it can be subjected to further purification by chromatographic separations or agarose gel electrophoresis, or CsCl-ethidium bromide density-gradient ultracentrifugation before any downstream application (Burlage et al., 1998).

5.2.3. Denaturing Gradient Gel Electrophoresis (DGGE)

5.2.3.1. Theoretical Principles of DGGE

The overall process for performing microbial community analysis using DGGE is depicted graphically in Figure 5.1. As shown in the figure, prior to DGGE, cells are lysed and the DNA is subsequently extracted. For environmental samples, cells may be lysed and DNA extracted without first separating the cells from their native matrix (e.g., soil). Following extraction and purification, DNA is amplified by polymerase chain reaction (PCR) using oligonucleotide primers complimentary to DNA segments flanking the region of the gene segment of interest. PCR, with target primers, serves three functions. First, it increases the quantity of DNA to detectable levels. Second, the primers target a gene sequence of particular interest. In microbial ecology, the target sequence is usually on the 16S rDNA gene, which is unique to prokaryotes; however, other genes can also be examined. Third, the primers are selected to produce amplicon lengths in the range of 200-500 base pairs. 500 base pairs is the approximate maximum fragment size limit for separation by DGGE (Muyzer and Smalla, 1998). Once PCR is complete, samples are ready for DGGE.

In the DGGE process, amplified 16S rDNA gene fragments of the same length but with different sequences can be separated (Muyzer et al., 1993). The process for this separation involves a polyacrylamide gel containing a linear gradient of DNA denaturants. The DNA
fragments are placed at the end of the gel containing the lowest concentration of denaturant. A voltage is applied across the gel so the DNA fragments move electrophoretically. For each of these DNA molecules (ds DNA), a transition from a double-stranded, helical structure to a partially single-stranded secondary structure will occur at a certain position in the gel. This will stop, or at least strongly slow down, migration of the respective gene fragment. To aid in the separation, so that nearly 100% of the sequence variations can be detected, a 30-40 nucleotide GC-clamp is attached to the 5’-end of one of the primers, coamplified and thus introduced into the amplified DNA fragments. The GC-rich sequence acts as a high melting domain preventing the two DNA strands from complete dissociation into single strands (ss DNA). As sequence variations cause a difference in the melting behavior of ds DNA, sequence variants of particular fragments will stop at different positions in the denaturing gradient gel and hence can be separated effectively as depicted in Figure 5.1 (Muyzer et al., 1993; Muyzer et al., 1998). To obtain the best separation of different DNA fragments, it is necessary to optimize the gradient and the duration of electrophoresis prior to DGGE analysis of DNA fragments (Muyer et al., 1993).

Figure 5.1: Flow chart of a community analysis by DGGE.
The most common method for viewing the resulting pattern of bands in the DGGE gel is staining the bands with ethidium bromide, SYBR Green or Silver, followed by UV transillumination. The resulting fragment band pattern is called a DGGE “fingerprint”. In theory, a band will form for each unique DNA sequence, and the number of bands corresponds to the number of genetically distinct members in the microbial community. If the GC clamp is not used, the bands can be subsequently excised, eluted and sequenced to identify the community members (Muyer et al., 1993). If the GC clamp is used, the DNA can be amplified by PCR using primers lacking the GC clamp, and then sequencing can be performed.

Manual scoring based on direct visual inspection is arduous and may suffer from error and bias from the investigator. However, image analysis can now be automated with appropriate hardware and software tools. The image can be recorded via a high-resolution camera, scanning device, or phosphorimage analyzer, edited to reduce background and allow band alignment, and then analyzed for band matching. The analysis usually takes into account the mobility and intensity of the bands permitting to set thresholds of tolerance for each of these parameters.

5.2.3.2. Applications of DGGE in Microbial Ecology

In microbial ecology, DGGE is primarily used to study microbial complexity, community stability, microbial spatial distribution, to observe population shifts in a system (due to time or a change in operating condition), to monitor the enrichment and isolation of bacteria, and to determine the phylogeny of bacterial species in a system (Muyzer et al., 1993; Amann et al., 1998; Muyzer, et al., 1998; Casamayor et al., 2000; Theron et al., 2000). DGGE of PCR-amplified 16S rDNA fragments has also been used to compare the efficacy and reproducibility of different DNA extraction protocols and to determine the PCR and cloning biases in the study of microbial ecology (Muyzer et al., 1993).
DGGE has been applied in the field of biological wastewater treatment, and to a limited extent in biological waste gas treatment. For example, Lapara et al. (2002) used PCR-DGGE to study the stability of bacterial communities in a seven-stage biological process treating pharmaceutical wastewater. Yoshie et al. (2001) used DGGE to compare the relative complexity of microbial communities and the relative efficiencies of two different bioreactor types for the remediation of metallurgic wastewater. Tresse et al. (2002) analyzed the bacterial population dynamics of free-floating and attached cells in a styrene-degrading biotrickling filter using the DGGE technique and found that the complexity of the bacterial community growing attached to the solid packing medium as a biofilm was different from the bacterial population suspended in the recirculating liquid. Lacey et al. (2002) used PCR-DGGE to monitor microbial populations in nitric oxide biofilters to ascertain the effects of oxygen exposure on the microbial populations.

It should be noted that a band at a given site of a DGGE gel has no intrinsic biological meaning. The overall “DNA fingerprints” (i.e., banding patterns) generated from two or more environmental samples; however, can be used to discern differences between the populations present in each. To learn more about the population represented by a certain band, this band needs to be further characterized, which can be achieved, in principle, by two techniques. Individual bands (fragments) may be excised from the gel, subjected to a second round of PCR amplification and sequenced. Alternatively, the DNA can be transferred to nylon membranes and then challenged with group- and species-specific oligonucleotide probes to identify specific populations within the microbial community (Muyzer et al., 1993).

5.2.3.1 DGGE Limitations

Whereas DGGE analysis of rDNA PCR products is a powerful tool to analyze diversity and dynamics of microbial communities, it has inherent limitations due to the experimental protocols employed. These include:
(1) Biases can result from the protocols employed for sample handling, extraction, and PCR amplification. Rochell et al. (1994) found that different sample handling procedures, such as aerobic or anaerobic storage or direct freezing of the samples, can result in preferential replication of certain types of bacteria. The recovery efficiency of nucleic acid isolation is subject to reliable, reproducible lyses. Some cells break more easily than others. Cells that do not break during lysis procedures are not represented in the DGGE data. Several substances may interfere with the PCR reaction and should be avoided in DNA preparation. These substances include heavy metals and humic acids commonly found in soil samples, as well as detergent, phenol, EDTA (frequently used in DNA extraction), and bacterial exopolysaccharides (Muyzer et al., 1993). It is possible that PCR preferentially amplifies certain DNA templates. Although all DNA should be amplified in principle, the microbial types could be disproportionately amplified with respect to their original numbers (Suzuki and Giovannoni, 1996). Additionally, heteroduplex molecules, formed when strands of two different PCR products anneal during PCR amplification, may be a problem for analysis of mixed bacterial populations because their presence will lead to an overestimation of the real number of community constituents (Muyzer et al., 1998).

(2) Although DGGE is able to separate sequences that differ by only one base pair, it cannot always separate fragments with a certain amount of sequence variation (Buchholz-Cleven et al., 1997). This can result in co-migration of nonrelated DNA sequences to an identical gel position, in which case a single band will represent more than one member of the bacterial community. This effect is especially problematic when treating complex community patterns (Kowalchuk et al., 1997).
(3) Separation of DNA fragments with high resolution is restricted to a maximum size of approximately 500 bp. Consequently, the phylogenetic information that can be retrieved by sequencing is limited (Muyzer et al., 1993).

(4) Individual organisms could potentially contribute multiple bands on a DGGE gel because of the presence in some bacteria of multiple rDNA operons with sequence heterogeneity (Rainey et al., 1996). This may lead to overestimation of the number of bacteria within natural communities (Nübel et al., 1996).

(5) Because of the limited quantity of DNA loaded in each gel lane, DGGE will only display the rDNA fragments obtained from the predominant species present in the community. DGGE is generally not capable of detecting populations that make up less than 0.1-1% of the total populations (Muyzer et al., 1993).

In spite of the limitations described above, DGGE is a useful and widely accepted technique for characterizing microbial populations. Differences in DGGE profiles are expected to reflect major differences in the microbial composition, and many of the limitations described above can be eliminated or minimized by properly treating all samples in a given set in an identical manner so that any biases introduced are applied to all samples (Fromin et al., 2002).

5.2.3.3. Guidelines for Interpretation of DGGE Fingerprinting Patterns

Classically, the variations between DGGE profiles have been described based on visual observation of single DGGE gels on the basis of disappearance, appearance, or changes in intensity of selected bands (Murray et al., 1996). An increasing number of studies, however, have proposed statistical investigations of DGGE banding patterns that can lead to refined interpretation of the spatial and temporal functioning of microbial communities (McCaig et al., 2001; Müller et al., 2002; Schauer et al., 2003). In these more sophisticated analyses, based on a computer-assisted characterization of the banding patterns and the subsequent treatment of the
data using a statistical approach, each band is described by its position (Y, in pixels on the image file) and its relative intensity in the lane ($P_i$), which can be calculated by the relative quantity of the band in the profile ($P_i=n_i/N$, where $n_i$ is the intensity of the band, $N$ is the total intensity of all the bands in the lane) (Fromin et al., 2002). Using these data, various statistical methods can be applied based either on analysis of a single band or on a complete DGGE profile (Fromin et al., 2002).

The following features of the fingerprinting techniques are considered before applying statistics for analysis of DGGE profiles. In DGGE analysis, the generated banding pattern is considered as an “image” of the whole bacterial community. To facilitate interpretation of the fingerprints, assumptions are made that no heterduplex molecules are formed during PCR amplification, no multiple bands are produced by a single species, and species with any sequence difference are well resolved as different bands. Therefore, each individual discrete band is treated as a discrete bacterial species (Muyzer et al., 1995; Van Hannen et al., 1999). PCR fragments generated from a single species are expected to display identical electrophoretic mobility in the analysis.

Another assumption for DGGE fingerprinting interpretation is that the band intensity is directly related to the density of corresponding bacterial phylotypes within the sample (Murray et al., 1996). Band intensities are also influenced by 16S rDNA gene copy numbers (Farrelly et al., 1995), by differential DNA extraction (Rochelle et al., 1994), by PCR biases (Reysenbach et al., 1992; Suzuki et al., 1996), by comigration of two or more sequence types (Murray et al., 1996), or by a combination of these events. Therefore, such an assumption implies that no bias was obtained during the whole extraction-amplification procedure of the bacterial genomes. Thus, DGGE analysis should probably be restricted to samples treated using identical methods, in
which DNA extraction and amplification biases are assumed to occur homogenously (Fromin et al., 2002).

Moreover, it is commonly accepted that only the main populations (those representing more than 0.1-1% of the target organisms in terms of relative proportion) can be visually observed in the DGGE profiles (Muyzer et al., 1993; Murray et al., 1996). As a result, all microbial species present within an environmental sample do not appear in the DGGE banding patterns. The DGGE fingerprinting pattern, therefore, provides a general indication of the microbial population structure (i.e., major species in the population) rather than an indication of its total richness (i.e., minor species present at low concentrations).

5.2.3.4. Interpretation of DGGE Fingerprinting Patterns

Various methods of analysis of denaturing gel electrophoresis fingerprinting patterns have been summarized by Fromin et al. (2002). The following is a brief summary of the most common ways to interpret the fingerprinting patterns.

- **Analysis of DGGE Profiles Based on Single Bands.** One way to analyze DGGE fingerprinting patterns is to observe the possible changes in the presence/absence or in the variation of intensity of a single band (Murray et al., 1996). Bands of interest can be excised from the gel and their sequences analyzed using a cloning-sequencing procedure (Kowalchuk et al., 1997). The variation in band presence or intensity can be correlated to biological, physicochemical, or environmental parameters (Fromin et al., 2002).

- **Comparison of DGGE Profiles Based on Similarity Matrix.** Quantification of pairwise similarities or differences between fingerprints can be done using different algorithms. Similarity between banding patterns can be expressed as a percentage value of a similarity coefficient such as Jaccard or Dice coefficient, or a distance coefficient such as Euclidean
distance or City-block distance (Pielou, 1984; Fromin et al., 2002). The Dice coefficient is the most widely used of the similarity indices available to ecologists (Pielou, 1984). A similarity matrix can be calculated based on either presence/absence of bands (non-weighted data) or on band relative intensity (weighted data) (Simpson et al., 2000; Lapara et al., 2002). For the non-weighted approach, the Dice coefficient (Hereafter referred to as $C_s$) is calculated using Equation 5.1 (Pielou, 1984; Lapara et al., 2002).

$$C_s = 100 \times \frac{2j}{(a+b)} \quad (5.1)$$

Where:

- $j$ is the number of bands common to samples A and B, and
- $a$ and $b$ are the number of bands in samples A and B, respectively.

This $C_s$ index (also known as Sorensen’s similarity index [Pielou, 1984]) ranges from 0 (no common bands) to 100 (identical band type patterns).

For the intensity-weighted approach, the weighted Dice’s similarity coefficient (hereafter referred to as $C_{sw}$) was calculated based on band relative quantities using Equation 5.2 (Simpson et al., 2000).

$$C_{sw} = 100 \times \frac{\sum_{i=1}^{B} \text{Min}(s_i, t_i)}{\sum_{i=1}^{B} (s_i + t_i)} \quad (5.2)$$

Where:

- $s_i$ and $t_i$ equal the relative quantity (expressed as a percentage of the total band trace quantities detected in the lane) of the band assigned to the $i$th band type in samples $s$ and $t$, respectively. $s_i$ or $t_i$ equals 0 if the lane does not have a band assigned to the $i$th type.

- $B$ is the number of band types in the two lanes’ band set. Min represents minimum.
This $C_{sw}$ index (also known as Czekanowski’s index of similarity [Pielou, 1984]) ranges from 0 (no common bands) to 100 (identical band types and relative quantities).

Similarity or distance matrices can be displayed graphically by means of clustering techniques as a dendrogram (most frequently referred to by ecologists as a phylogenetic tree) (Pielou, 1984). There are numerous phylogenetic reconstruction methods, including single linkage, complete linkage, UPGAMA (Unweighted pair group method using arithmetic averages), WPGAMA (Weighted pair group method using arithmetic averages), Centroid, Ward’s method, or Neighbor joining algorithms (Sneath and Sokal, 1973; Saltou and Nei, 1987; Pielou, 1984). Among these, UPGAMA is one of the most widely used and simplest methods for phylogenetic tree construction (Sneath and Sokal, 1973).

- **Analysis of DGGE Whole Profile Based on Diversity Indices.** Diversity indices, such as Shannon-Weaver and Evenness indices (Magurran, 1988; Martin, 2002; Müller, 2002), can be calculated to describe possible changes in the dominance among phylotypes based on the DGGE fingerprinting pattern (Fromin et al., 2002). The Shannon diversity index is a mathematical measure of species diversity in a community and is the most common diversity index used by ecologists (Washington, 1984). This diversity index provides more information about community composition than simply species richness (i.e., the number of species present); it also takes the relative abundance of different species into account. The Shannon diversity index ($H'$) is calculated using Equation 5.3 (Müller, 2002).

$$H' = -\sum_{i=1}^{n} x_i \ln x_i$$  \hspace{1cm} (5.3)

Where:

$n$ is the number of bands in the sample, and

$x_i$ is the fraction of relative quantity of the $i$th band.
Shannon indices between samples can be statistically compared, or the index can be correlated with environmental parameters. For instance, Nübel et al. (1999) found a positive linear correlation between Shannon-Weaver indices calculated from DGGE patterns and carotenoid types in oxygenic-phototrophic microbial communities.

- **Analyzing DGGE Profiles Using Ordination Methods.** Another way of analyzing DGGE profiles is to bring out major tendencies of the variance of the samples for the whole set of descriptors using multivariate ordination methods (Fromin et al., 2002). Common ordination methods include non-metric multidimensional scaling (NMDS), principal component analysis (PCA), correspondence analysis (CA), canonical variate analysis (CVA) and canonical correspondence analysis (CCA) (McSpadden Gardener and Lilley, 1997; Legendre and Legendre, 1998). The major advantage of these methods is to display the whole set of samples on a simple scheme, and to highlight the possible descriptors which are governing their dispersion (ter Braak et al., 1995).

5.3. **Materials and Methods**

5.3.1. **DNA Extraction**

For both of the biofilters described in Chapter 4 (SBB and CFB), biomass samples were collected for DNA extraction on day 290 of biofilter operation. The biofilter columns were temporarily disassembled, and three foam cubes were removed from the upper portion of each section of each biofilter using sterile tweezers. Each cube, processed separately, was immediately placed in a 15 mL sterile centrifuge tube containing 5 mL of cold (4°C) sterile TE buffer (10 mM Tris, 1 mM EDTA, pH=8). Each sample was vortexed for 5 min to remove biomass from the foam packing to the liquid suspension. The polyurethane foam cube was then removed from each centrifuge tube and discarded. Samples of the remaining bacterial suspension were then vortexed for one minute prior to DNA extraction using Mo Bio Ultraclean™
Microbial DNA isolation kits (Mo Bio Laboratories, Inc., Solana Beach, CA) with the following modification of the manufacturer’s protocol. A Biospec Mini-Beadbeater 3110BX (Biospec Products) was used for cell disruption instead of utilizing a Mo Bio Vortex Adapter tube holder. Bead beating was performed for 180 sec at 4,800 rpm. Extracted DNA was frozen at -20°C until further analysis.

To verify that the method used to remove biomass from foam cubes prior to DNA extraction did not bias results, a supplemental experiment was conducted in which biomass on foam cube samples was subjected to direct lysis (i.e., without first removing biomass via vortexing) by treatment with lysozyme, proteinase K, SDS, and freeze-thaw in conjunction with a chloroform-phenol extraction. PCR and DGGE were then performed as described in the following sections. A detailed description of the experimental protocol and results for this supplemental experiment appears in Appendix B. Comparison of results from the two cell lysis and DNA extraction methods indicated that biomass removal by vortexing followed by the Mo Bio DNA extraction method captured microbial population richness in a manner essentially indistinguishable from the direct lysis extraction method.

### 5.3.2. PCR Amplification

The extracted DNA was diluted 1:50 with distilled water prior to PCR amplification. Oligonucleotide primers 341f (Casamayor et al., 2000) and 907r (Lane, 1991) were used to amplify partial 16S rRNA genes of bacteria. The sequence of the 341f primer, which included a 40 base pair GC-clamp attached to the 5' end, was 5'-CGCCCGCCGCCGCCCCGCCGCCGCCCCGCCCCGCCCCCTACGGGAGGCAGCA-3', and the sequence of the 907r primer was 5'-CCGTCAATTCMRTTARAGTTT-3' (where M=C:A, R=A:G).
PCR amplification was performed using iQ PCR reagents with a hot-start iTaq™ DNA polymerase (BioRad Laboratories, Hercules, CA). Each of the 100 μL PCR reactions contained 1 μL of template DNA (after 1:50 dilution), 0.5 μM of each primer, 2.5 units of iTaq™ DNA Polymerase, 200 μM of each dNTP, 1× iTaq buffer, 1.5 mM MgCl₂, and sterile water added to make a final volume of 100 μL. PCR took place in a Mastercycler (Eppendorf Scientific) with denaturation at 95ºC for 3 min to activate the DNA polymerase, followed by 30 cycles of denaturation at 94ºC for 1 min, annealing at 53ºC for 1 min and extension at 72ºC for 1 min, then a single final extension at 72ºC for 7 min. Samples were then refrigerated at 0-4ºC until further analysis. Sizing and quantification of DNA amplicons from PCR was performed using DNA 1000 Assays in conjunction with an Agilent 2100 Bioanalyzer (Agilent Technologies) following the manufacturer’s recommended protocol.

5.3.3. DGGE

Three individual DGGE gels (arbitrarily designated Replicates A, B, and C) were made. Each consisted of a 24 mL polyacrylamide gel (6% w/v acrylamide) containing a denaturing gradient ranging from 40% to 70% denaturant (where 100% denaturant contains 7 M urea and 40% v/v formamide) cast using a BioRad Model 475 Gradient Delivery System and then covered by a 5 mL acrylamide stacking gel without denaturant. Between 20 and 50 μL of solution containing 250 ng DNA mixed with an equal volume of 2× gel loading dye (0.05% bromophenol blue, 0.05% xylene cyanol, 70% glycerol, BioRad) was loaded into individual wells of the gel. Each gel contained 12 lanes loaded with PCR products derived from different sections of both the periodic and continuous flow biofilters. PCR products from foam cubes removed from the same biofilter sampling location were loaded in separate gels. DGGE was performed using a D-Code™ Universal Mutation Detection System (BioRad Laboratories, Hercules, CA) essentially
as described by Myers et al. (1987) with electrophoresis performed in 1× TAE buffer at 60°C and 65 V for 15 hours.

Following electrophoresis, gels were stained for 10 min with ethidium bromide (1 µg/mL, BioRad), destained for 12 min, then visualized and photographed on a UV transilluminator (ChemiDoc XRS gel documentation system, BioRad). A “flat-fielding” technique (which involved capturing an image of a fluorescent reference plate on the ChemiDoc XRS sample stage) was used to ensure uniform background intensity.

5.3.4. Quantitative Analysis of DGGE Fingerprints

High-resolution digital DGGE images were analyzed using Quantity One software (BioRad). Lanes were framed manually. A pixel intensity profile was generated through each lane, and lane-based background subtraction was made by applying a rolling disk size ranging from 6 to 10. Bands in each lane were automatically detected and quantified at a sensitivity of 10, shadow bands as common gel artifacts were rejected, and normalization was used to compensate for differences in intensity between lanes for band detection. Faint bands with a relative quantity (expressed as percentage of the trace quantity of a particular band relative to the total of all bands in the lane) less than 0.2% were omitted in further analysis. Bands from different lanes in a gel occupying the same position were matched at a tolerance of 1%. No attempt was made to correlate bands between different gels.

Similarity coefficients for pairwise comparisons of DGGE gel lanes were calculated using two different methods. First, in the non-weighted method, Dice’s similarity coefficient (Cs) based on the presence or absence of individual bands was calculated using Equation 5.1. Second, in the intensity-weighted method, the weighted Dice’s similarity coefficient (Csw) was
calculated based on band relative quantities using Equation 5.2. Both Equations 5.1 and 5.2 were defined previously in Section 5.2.3.5.

Dendrograms based on both non-weighted and weighted similarity matrices were constructed by UPGAMA algorithm in cluster analysis using SAS 8.0 (SAS Institute Inc., Cary, NC).

Finally, the number of bands and the relative quantity of each band were used to calculate the Shannon-Weaver diversity index \( (H') \) as defined in Equation 5.3 (see Section 5.2.3.5). Statistical analysis of the Shannon diversity indices for the triplicate DNA samples was performed by analysis of variance (One way ANOVA) or two sample t-test using SAS 8.0 (SAS Institute Inc., Cary, NC).

5.4. Results and Discussion

5.4.1. DGGE Band Identification and Quantification

The bacterial community DNA fingerprints for each section of the two biofilters were generated by DGGE of PCR-amplified 16S rDNA gene fragments. Three individual DGGE gels (arbitrarily designated Replicates A, B, and C) were made. Each gel contained PCR products corresponding to one foam cube removed from each section of each biofilter (i.e., PCR products from foam cubes removed from the same location were loaded in separate gels). Gel images from DGGE Replicates A (top), B (middle), and C (bottom) are shown in Fig. 5.2. Lanes are labeled with a letter (either “S” or “C”) followed by a number. “S” denotes the Sequencing Batch Biofilter (SBB) and “C” denotes the Continuous Flow Biofilter (CFB). The number denotes the spatial location in the biofilter column corresponding to where the DNA originated, with “1” designating the section closest to the biofilter inlet and “6” designating the section
Figure 5.2: Negative images of DGGE fingerprints from samples along the height of SBB and CFB columns in replicates A, B, and C. Lanes are labeled with a letter (either “S” or “C”) followed by a number. “S” denotes SBB and “C” denotes CFB. The number denotes the spatial location in the biofilter column corresponding to where the DNA originated, with “1” designating the section closest to the biofilter inlet and “6” designating the section closest to the biofilter outlet. Identified bands were numbered sequentially on each gel.
closest to the biofilter outlet. Individual bands identified for Replicate A are shown in Table 5.1, and individual bands identified for Replicates B and C are shown in Tables A.1 and A.2 in Appendix A. In the tables, grey shading denotes the presence of a band, and white indicates absence of a band. The number listed in grey-shaded boxes indicates the relative quantity of that band (expressed as a percentage) relative to the total detected in the lane (100%).

**Table 5.1: Relative quantity of bands in DGGE fingerprints from replicate A (gray color denotes presence of band).**

<table>
<thead>
<tr>
<th>Band Number</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
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<tbody>
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<td>2.2</td>
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<td>3.6</td>
<td>1.5</td>
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<td>0.6</td>
<td>1.0</td>
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<tr>
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<td>0.0</td>
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<td>19.6</td>
<td>7.6</td>
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</tr>
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</tr>
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<td>14</td>
<td>16</td>
<td>16</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

A total of 23, 22, and 24 unique bands were identified in Replicates A, B, and C, respectively. Most bands appeared in at least one section of both biofilters. For example, of the
23 unique bands detected in Replicate A, 20 of them (all except for bands A6, A10, and A20) appeared in at least one of the sequencing batch biofilter sections, and 22 (all except band A16) were identified in at least one of the continuous flow biofilter sections. The similar total number of bands detected for the two biofilters (taking all sections into account) indicates that the microbial populations in the SBB and CFB columns had essentially the same magnitude of species richness.

The banding pattern observed in samples removed from different biofilter sections clearly differ, with many bands appearing in certain lanes but not others. Furthermore, common bands (i.e., those identified at the same relative position in different lanes) were observed to have varying relative intensities, which reflects a difference in relative abundance of certain common bacterial species along the height of each biofilter and between the two biofilters.

5.4.2. **Cluster Analysis Based on Non-weighted Similarity Matrices**

Non-weighted Dice’s similarity coefficients ($C_s$) were calculated in a section-by-section comparison based on presence/absence of bands for Replicates A, B, and C as shown in Tables A.3, A.4, and A.5, respectively (see Appendix A). Data were then averaged for the same section-by-section comparisons among the triplicate matrices so that an averaged non-weighted similarity matrix was obtained (see Table 5.2). Averaged $C_s$ values ranged from a low of 49.6 (S2 vs. C1) to a high of 90.8 (S4 vs. S5).

Cluster analyses were performed to graphically display the similarity data in the form of dendrograms based on these non-weighted similarity matrices. The dendrograms revealing the relatedness of PCR-DGGE fingerprints for each biofilter based on presence/absence of band for Replicates A, B, and C (with each replicate considered separately) were shown in Figure A.1 (see Appendix A). Only the dendrograms generated based on the averaged similarity matrices are discussed (see below).
Table 5.2: Non-weighted Dice’s similarity coefficient ($C_s$) matrix based on presence/absence of bands (expressed as mean ± standard deviation).

<table>
<thead>
<tr>
<th></th>
<th>AVE</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2</td>
<td>69.4±</td>
<td>6.9</td>
<td>74.5±</td>
<td>72.0±</td>
<td>10.7</td>
<td>5.4</td>
<td>56.0±</td>
<td>57.9±</td>
<td>76.5±</td>
<td>6.9</td>
<td>8.4</td>
<td>6.2</td>
</tr>
<tr>
<td>S3</td>
<td>6.9</td>
<td>10.7</td>
<td>5.4</td>
<td>56.0±</td>
<td>57.9±</td>
<td>76.5±</td>
<td>61.7±</td>
<td>66.5±</td>
<td>76.7±</td>
<td>90.8±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S4</td>
<td>6.9</td>
<td>8.4</td>
<td>6.2</td>
<td>61.7±</td>
<td>66.5±</td>
<td>76.7±</td>
<td>70.9±</td>
<td>64.5±</td>
<td>78.7±</td>
<td>63.7±</td>
<td>66.6±</td>
<td></td>
</tr>
<tr>
<td>S5</td>
<td>5.4</td>
<td>8.1</td>
<td></td>
<td>6.9</td>
<td>10.7</td>
<td>5.4</td>
<td>5.4</td>
<td>8.1</td>
<td></td>
<td>2.9</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>S6</td>
<td>3.8</td>
<td>4.4</td>
<td>2.7</td>
<td>3.8</td>
<td>4.4</td>
<td>2.7</td>
<td>13.9</td>
<td>18.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>10.9</td>
<td>5.0</td>
<td>9.0</td>
<td>10.9</td>
<td>5.0</td>
<td>9.0</td>
<td>10.9</td>
<td>5.0</td>
<td>9.0</td>
<td>11.1</td>
<td>6.6</td>
<td>4.7</td>
</tr>
<tr>
<td>C2</td>
<td>10.8</td>
<td>4.5</td>
<td>7.3</td>
<td>10.8</td>
<td>4.5</td>
<td>7.3</td>
<td>10.8</td>
<td>4.5</td>
<td>7.3</td>
<td>11.1</td>
<td>6.6</td>
<td>4.7</td>
</tr>
<tr>
<td>C3</td>
<td>5.2</td>
<td>4.0</td>
<td>5.5</td>
<td>5.2</td>
<td>4.0</td>
<td>5.5</td>
<td>5.2</td>
<td>4.0</td>
<td>5.5</td>
<td>12.1</td>
<td>16.0</td>
<td>9.1</td>
</tr>
<tr>
<td>C4</td>
<td>5.6</td>
<td>2.0</td>
<td>11.0</td>
<td>5.6</td>
<td>2.0</td>
<td>11.0</td>
<td>5.6</td>
<td>2.0</td>
<td>11.0</td>
<td>11.6</td>
<td>8.2</td>
<td>10.5</td>
</tr>
<tr>
<td>C5</td>
<td>6.3</td>
<td>10.3</td>
<td>2.1</td>
<td>6.3</td>
<td>10.3</td>
<td>2.1</td>
<td>6.3</td>
<td>10.3</td>
<td>2.1</td>
<td>9.3</td>
<td>6.1</td>
<td>20.7</td>
</tr>
<tr>
<td>C6</td>
<td>6.0</td>
<td>12.2</td>
<td>4.0</td>
<td>6.0</td>
<td>12.2</td>
<td>4.0</td>
<td>6.0</td>
<td>12.2</td>
<td>4.0</td>
<td>10.8</td>
<td>6.6</td>
<td>12.3</td>
</tr>
</tbody>
</table>

Cluster analysis of DNA banding patterns in samples from the various sections of the SBB based on the averaged $C_s$ matrix is shown in Figure 5.3 (top). The six sections of the column could be grouped into two main clusters with regard to bacterial populations. Sections S4 and S5 clustered at the highest band type similarity of 90.8%, and the other four sections (S1, S2, S3 and S6) formed the other main cluster. These two main clusters had a similarity of 65.7%.

Cluster analysis of DNA banding patterns in samples from the various sections of the CFB based on the averaged $C_s$ matrix is shown in Figure 5.3 (bottom). The upper four sections of the column (C3, C4, C5 and C6) shared higher similarities in band types than did the bottommost two sections (C1 and C2).
Figure 5.3: Dendrograms revealing the relatedness of PCR-DGGE fingerprints from the SBB (top) and CFB (bottom) generated from averaged $C_s$ matrix (based on presence/absence of bands).

5.4.3. Cluster Analysis Based on Intensity-weighted Similarity Matrices

Intensity-weighted Dice’s similarity coefficients ($C_{sw}$) were calculated in a section-by-section comparison based on band relative quantities for Replicates A, B, and C as shown in Tables A.6, A.7, and A.8, respectively (see Appendix A). Data were then averaged for the same section-by-section comparisons so that an averaged weighted similarity matrix was obtained (see Table 5.3). $C_{sw}$ values ranged from a low of 17.6 (C2 vs. S1) to a high of 83.6 (S4 vs. S5).

Cluster analyses were performed to graphically display the similarity data based on these intensity-weighted similarity matrices. Figure A.2 shows the dendrograms revealing the relatedness of PCR-DGGE fingerprints for each biofilter based on relative quantities of band for Replicates A, B, and C (with each replicate considered separately). Figure A.3 shows the corresponding dendrograms for sections of both the CFB and the SBB sections for Replicates A, B, and C (again, with each replicate considered separately). As shown in the figures, the
similarity matrices (from Replicates A, B, and C) produced identical or nearly identical dendrograms in each of the three cases considered (SBB sections only, CFB sections only, and SBB and CFB combined). Therefore, these dendrograms are not discussed here on an individual basis. Instead, only the dendrograms generated based on the averaged similarity matrices as shown in Figure 5.4 are discussed (see below).

**Table 5.3: Intensity-weighted Dice’s similarity coefficient ($C_{sw}$) matrix based on band relative quantities (expressed as mean ± standard deviation).**

<table>
<thead>
<tr>
<th></th>
<th>AVE</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2</td>
<td>49.6±</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td>36.5±</td>
<td>37.4±</td>
<td>14.4</td>
<td>19.7</td>
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<td></td>
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</tr>
<tr>
<td>S4</td>
<td>20.7±</td>
<td>21.5±</td>
<td>52.9±</td>
<td>2.4</td>
<td>7.4</td>
<td>16.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S5</td>
<td>22.1±</td>
<td>21.7±</td>
<td>53.6±</td>
<td>83.6±</td>
<td>7.3</td>
<td>4.1</td>
<td>20.3</td>
<td>8.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S6</td>
<td>27.7±</td>
<td>23.5±</td>
<td>65.9±</td>
<td>53.3±</td>
<td>56.4±</td>
<td>1.2</td>
<td>8.6</td>
<td>4.5</td>
<td>5.2</td>
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<td></td>
</tr>
<tr>
<td>C1</td>
<td>42.5±</td>
<td>32.6±</td>
<td>23.0±</td>
<td>22.2±</td>
<td>21.8±</td>
<td>22.2±</td>
<td>15.2</td>
<td>7.0</td>
<td>5.7</td>
<td>7.5</td>
<td>8.9</td>
<td>5.1</td>
</tr>
<tr>
<td>C2</td>
<td>34.5±</td>
<td>21.6±</td>
<td>23.2±</td>
<td>17.6±</td>
<td>21.2±</td>
<td>23.3±</td>
<td>46.8±</td>
<td>1.9</td>
<td>7.1</td>
<td>14.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>28.6±</td>
<td>25.0±</td>
<td>43.1±</td>
<td>53.5±</td>
<td>59.0±</td>
<td>43.4±</td>
<td>59.7±</td>
<td>4.9</td>
<td>1.9</td>
<td>7.0</td>
<td>6.5</td>
<td>2.1</td>
</tr>
<tr>
<td>C4</td>
<td>26.3±</td>
<td>23.8±</td>
<td>46.6±</td>
<td>59.7±</td>
<td>63.9±</td>
<td>47.2±</td>
<td>23.6±</td>
<td>31.6±</td>
<td>28.5±</td>
<td>82.4±</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>28.4±</td>
<td>25.6±</td>
<td>49.2±</td>
<td>71.5±</td>
<td>73.7±</td>
<td>50.8±</td>
<td>26.4±</td>
<td>27.3±</td>
<td>68.7±</td>
<td>75.8±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td>35.5±</td>
<td>31.8±</td>
<td>49.5±</td>
<td>56.7±</td>
<td>60.6±</td>
<td>53.3±</td>
<td>39.1±</td>
<td>42.0±</td>
<td>53.5±</td>
<td>54.8±</td>
<td>69.6±</td>
<td></td>
</tr>
</tbody>
</table>

Cluster analysis of DNA banding patterns in samples from the various sections of the SBB based on the averaged $C_{sw}$ matrix is shown in Figure 5.4 (top). The microbial communities present in the SBB were grouped into two main clusters. The bottommost two sections (those closest to the biofilter inlet) formed one main cluster at a similarity value of 49.6%, the upper four sections formed the other main cluster. These two main clusters had a similarity value of only 26.4%. Among the upper four sections, sections S4 and S5 clustered at the highest similarity value, 83.6%, and sections S3 and S6 clustered at the second highest similarity value,
65.9%. The cluster formed by section S3 and S6 had a similarity value of 54% with the cluster formed by section S4 and S5.

Cluster analysis of DNA banding patterns in samples from the various sections of the CFB based on the averaged $C_{sw}$ matrix is shown in Figure 5.4 (bottom). The microbial communities present in the CFB were also grouped into two main clusters. The bottommost two sections (those closest to the inlet) formed one main cluster at a similarity value of 46.8%, the upper four sections were each most closely related to their adjacent sections and formed the other main cluster. These two main clusters had a similarity of only 30.4%.

Results of cluster analysis including data from both the SBB and CFB based on the averaged $C_{sw}$ matrix are depicted in Figure 5.5. As shown, based on the DNA banding patterns, the microbial communities present in the biofilters were grouped into two main clusters. Each cluster was formed by several subsets. In the first main cluster, sections S4, S5, C5, C3, C4, and
C6 formed one complex subset, joined with subset formed by sections S3 and S6. In the second main cluster, the bottommost two sections of SBB clustered with the counterpart of CFB at a similarity of 32.8%. These two main clusters (i.e., bottommost two sections of both biofilters vs. upper four sections of both biofilters) had a similarity value of only 26.7%.

![Dendrogram revealing the relatedness of PCR-DGGE fingerprints from both the SBB and CFB sections generated from averaged Csw matrix (based on band relative quantities).](image)

**Figure 5.5:** Dendrogram revealing the relatedness of PCR-DGGE fingerprints from both the SBB and CFB sections generated from averaged $C_{sw}$ matrix (based on band relative quantities).

### 5.4.4. Assessment of Degree of Microbial Community Diversity Based on Shannon Diversity Index

As a mathematical measure of species diversity in a community that takes both species richness and relative abundance into consideration, Shannon diversity index was calculated for each section of both biofilters based on the triplicate DGGE image band types and relative band intensities as shown in Table 5.4. The Shannon diversity indices were further subjected to statistical comparisons for testing the hypotheses of whether there was significant difference in the degree of microbial community diversity along the height of each biofilter column as well as between the two biofilters operated under different strategies. The statistical comparisons are shown in Table 5.5.
Table 5.4: Shannon’s diversity indices based on band type and band relative quantity in the different sections of both SBB and CFB.

<table>
<thead>
<tr>
<th>Section</th>
<th>Replicate A</th>
<th>Replicate B</th>
<th>Replicate C</th>
<th>Mean±Std</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>2.04</td>
<td>2.14</td>
<td>2.48</td>
<td>2.22 ± 0.23</td>
</tr>
<tr>
<td>S2</td>
<td>1.88</td>
<td>1.78</td>
<td>1.85</td>
<td>1.84 ± 0.05</td>
</tr>
<tr>
<td>S3</td>
<td>1.56</td>
<td>2.15</td>
<td>2.21</td>
<td>1.97 ± 0.36</td>
</tr>
<tr>
<td>S4</td>
<td>1.91</td>
<td>1.76</td>
<td>1.93</td>
<td>1.87 ± 0.09</td>
</tr>
<tr>
<td>S5</td>
<td>2.02</td>
<td>1.90</td>
<td>2.18</td>
<td>2.03 ± 0.14</td>
</tr>
<tr>
<td>S6</td>
<td>1.97</td>
<td>2.11</td>
<td>2.08</td>
<td>2.05 ± 0.07</td>
</tr>
<tr>
<td>C1</td>
<td>1.97</td>
<td>2.00</td>
<td>1.99</td>
<td>1.99 ± 0.02</td>
</tr>
<tr>
<td>C2</td>
<td>2.17</td>
<td>2.30</td>
<td>1.86</td>
<td>2.11 ± 0.22</td>
</tr>
<tr>
<td>C3</td>
<td>2.29</td>
<td>2.02</td>
<td>2.51</td>
<td>2.27 ± 0.25</td>
</tr>
<tr>
<td>C4</td>
<td>2.10</td>
<td>2.19</td>
<td>2.45</td>
<td>2.25 ± 0.18</td>
</tr>
<tr>
<td>C5</td>
<td>2.22</td>
<td>2.08</td>
<td>2.38</td>
<td>2.22 ± 0.15</td>
</tr>
<tr>
<td>C6</td>
<td>2.38</td>
<td>2.45</td>
<td>2.49</td>
<td>2.44 ± 0.06</td>
</tr>
</tbody>
</table>

As shown in Table 5.5, for the CFB, based on a p-value of 0.089, there was moderately sufficient evidence to indicate that at least one of the six sections of Shannon diversity indices is different from the others. The null hypothesis that the CFB column sections had the same Shannon diversity index is therefore rejected at a significance level of α=0.1. Moreover, the combination of sections C1 and C2 differed significantly in Shannon indices from combination...
of sections C3, C4, C5, and C6 ($p=0.01$). Consequently, there was statistically significant difference in the degree of microbial community diversity between the bottommost two sections and the upper four sections of the CFB column.

For the SBB, there was insufficient evidence to indicate that at least one of the six sections of Shannon diversity indices is different from the others based on a $p$-value of 0.232. The null hypothesis that the SBB column sections had the same Shannon diversity index is therefore not rejected at a significance level of $\alpha=0.1$. This indicates that there was no statistically significant difference in the degree of microbial population diversity along the height of the SBB column.

When the two biofilters were compared, there was sufficient evidence to indicate that the overall Shannon diversity index of the SBB is different from that of the CFB based on a $p$-value of 0.0032. The null hypothesis that SBB and CFB had the same overall Shannon diversity index is therefore rejected at $\alpha=0.1$ level of significance. Therefore, the overall degree of microbial population diversity of the SBB was significantly different from that of the CFB. Furthermore, the significantly higher value of average Shannon index for the continuous flow biofilter ($H'=2.21\pm0.202$) than that of the periodic biofilter ($H'=2.0\pm0.207$) indicates that the continuous flow biofilter probably had a relatively more diverse microbial population than the periodic one.

Shannon diversity indices comparison in the above analysis aided in the interpretation of the community structure differences between samples. It should be noted however, that the Shannon diversity test tends to be conservative in differentiating the “real diversities” among samples, as the calculation of Shannon index only involves species richness and relative abundance, without accounting for difference in species types. Therefore, while Shannon indice comparisons of the SBB sections indicated no significant difference in the degree of microbial
diversity along the height of the column, it provides no indication whether or not the microbial community structure differences were significant along the SBB column height by taking into account the species richness, relative abundance, and species types as well. Nevertheless, relatedness between the SBB biofilter sections could still be well interpreted based on the magnitude of similarities in the dendrogram in Figure 5.4.

5.4.5. Discussion

As shown in Figure 5.2, the DNA banding patterns in the three gel images were similar but not identical. This is reflected in the standard deviation presented in the averaged Dice’s similarity coefficient matrices (see Tables 5.2 and 5.3). Differences in samples removed from the same spatial location in each biofilter, however, were generally much smaller than differences between samples removed from different spatial locations. Differences between the DNA banding pattern for samples removed from the same biofilter section indicates either some local spatial heterogeneity in terms of microbial community structure (i.e., the populations growing on adjacent foam cubes were not identical) or some variation associated with sample collection, DNA extraction, PCR amplification, or the DGGE process. From the supplemental experiment in Appendix B it is learned that variation introduced by sample collection, DNA extraction, PCR amplification, or the DGGE process was minimal, indicating that the former rather than latter explanation is more likely.

Although all sections of both biofilters were inoculated with the same mixed culture, DGGE results clearly indicate that microbial community structures differed as a function of height in each of the biofilters after long-term operation (290 days). For the CFB, the most readily observed difference was between the predominant phylotypes in the bottommost two sections and those in the upper four sections. For conventional, continuous-flow biofilters, it has been reported previously that cell densities vary as a function of biofilter height with highest cell
concentrations observed near the inlet where the greatest contaminant concentration and highest biodegradation rates are observed (Song and Kinney, 2000). Both the cluster analysis and Shannon diversity index comparison data presented herein suggest that microbial community structure also differs as a function of height in CFB. When band relative quantity was taken into account in Dice similarity coefficient comparisons, there was a larger difference than when only presence/absence of bands was considered. This suggests that microbial community structures differed more in terms of relative abundance of each species rather than in terms of species richness (i.e., number of species present) along the height of the CFB.

A reasonable explanation for the differences in microbial community structure along the height of the CFB is that a difference in pollutant concentrations along the column applied a sufficiently large selective pressure to influence the microbial community composition. Measurements of MEK concentrations along the height of the CFB revealed that contaminants were rapidly degraded in the bottommost two sections of the biofilter (those closest to the inlet), and hence, microorganisms in the upper four sections (those closest to the outlet) were exposed to markedly and successively lower MEK concentrations (see Figure 4.10 in Chapter 4). Differences in specific growth rates at the prevailing contaminant concentrations at each location likely served as a selective pressure to increase the relative abundance of certain microorganisms with respect to others.

For the SBB, the MEK concentration profiles measured during the FEED portions of the operating cycle revealed that the bottommost two sections (those closest to the inlet) were exposed to higher MEK concentrations than were the upper four sections (those closest to the outlet) (see Figure 4.10 in Chapter 4), similar to what was observed for the CFB. During REACT portions of the operating cycle, however, air inside the closed system was recirculated from the
bottom of the biofilter to the top. This allowed a portion of the MEK initially accumulated in the system during the FEED period to be redistributed from the bottom section of the column to the top section (Moe and Li 2003). Therefore, the upper part of the SBB column was exposed to a higher contaminant concentration during a portion of the REACT period than was the upper section of the CFB. The length of the REACT period employed in the SBB was sufficiently long for the accumulated MEK to be fully transformed, and after depletion of the contaminant, the microbes located throughout the height of the biofilter were subjected to famine conditions. Such SBB operating strategy likely resulted in a relatively more homogenous distribution of the microbial population than the CFB, which could possibly account for the non-significantly different Shannon diversity indices along the SBB column as discussed earlier.

Cluster analysis of the DGGE banding patterns for samples removed from the various sections of the SBB revealed that the microbial population associated with the outlet section (S6) was more similar to that of one of the middle sections (S3), than it was to the spatially more proximate S5 and S4 sections. This likely results from the fact that the S6 section was exposed to higher contaminant concentrations for at least a portion of the operating cycle due to gas recirculation during the REACT period. Similar to what was observed for the CFB, the microbial community structure differed more in terms of relative abundance of each species rather than in terms of the species richness along the height of SBB column, and the predominant phylotypes in the bottommost two sections of SBB were relatively distinct from those in the upper four sections. In an SBB operated with long cycle times, the microbial population may change as a function of time within each cycle. In the system described herein, the cycle length was short (45 min FEED and 90 min REACT) so changes in population structure as a function of time within each cycle were likely minimal.
Because microbial populations differed as a function of height in each of the biofilters, an overall comparison of the populations between the two biofilters is somewhat complicated. The dendrogram showing relatedness of PCR-DGGE fingerprints from both the CFB and SBB (based on the intensity-weighted Dice’s similarity coefficient matrix, see Figure 5.5) in combination with the Shannon diversity indices comparison suggests that microbial populations differed between the two biofilters. Although the bottommost two sections of the CFB clustered with the bottommost two sections of the SBB, similarity between the two subsets was only 32.8%. As shown in Fig. 5.5, similarity of the upper four sections of CFB with the counterpart of SBB was somewhat higher, but sections from the CFB generally clustered more closely to other sections of the CFB than they did to sections of the SBB. This is indicative of differences in microbial community structure in terms of both predominant species types and relative abundance between the SBB and CFB.

Use of molecular techniques to analyze functionally-stable populations in anaerobic bioreactors treating glucose (Fernández et al., 1999) and aerobic bioreactors treating synthetic wastewater (Kaewpipat and Grady, 2002) has revealed that population structure can vary over time even in bioreactors inoculated with the same starting culture and operated under identical conditions. Innate temporal and spatio-temporal variability in the microbial community obviously complicates interpretation of microbial community differences in systems subjected to different operating conditions (Kaewpipat and Grady, 2002; Curtis et al., 2003). Samples collected and analyzed in the research described herein provide only a snapshot of the composition of the microbial communities populating the biofilters. The populations may have been dynamically varying, and further research is needed to unequivocally establish a link
between biofilter operating strategies, microbial community structure, and treatment performance.

Nevertheless, the results reported herein support the notion that biofilter operating strategies can impose a selective pressure on the microbial population large enough to influence relative abundance of microbial species that predominate over time. Differences in selection and enrichment may be partially responsible for superior performance of the SBB during shock loading conditions (in comparison to the CFB), and imposing operating strategies that purposely manipulate microbial populations in biofilters treating gas-phase contaminants may open new possibilities for achieving stable and favorable treatment performance.

5.5. Conclusions

A DGGE “DNA fingerprinting” technique was successfully applied to evaluate differences in microbial diversity of biofilters subjected to continuous flow and sequencing batch loading strategies. Microbial populations differed more in terms of relative abundance of each species rather than in terms of the species richness (i.e., number of species present) along the height of each column as well as between these two biofilter columns.

Both the SBB and CFB biofilter had stratified microbial community structures along the height of each column. There was a significant difference in the degree of microbial diversity between the bottommost two sections and the upper four sections of the column subjected to continuous loading (i.e., the CFB). On the other hand, there was no significant difference in the degree of microbial diversity along the height of the SBB column.

The microbial diversity in the biofilter subjected to sequencing batch operation (i.e., the SBB) was significantly different from that of the conventional continuous flow biofilter (CFB). Although it does not provide conclusive proof, this supports the notion that periodic operating
strategies can be utilized in biofilters treating gas phase contaminants to impose a sufficiently large selective pressure to influence the microbial population composition in the column.
6.1. Introduction

Biofiltration has proven successful at removing biodegradable VOCs from a wide variety of industrial and waste treatment operations under conditions of relatively steady loading; however, unsteady-state loading conditions pose challenges in biofilter design and operation. For example, contaminant concentrations in most waste gas streams vary with time due to the unsteady-state nature of industrial processes that generate them (Dirk-Faitakis and Allen, 2003). If a biofilter is designed to meet “average” loading conditions for a waste stream with dynamically varying contaminant concentrations, then dynamic mass loadings may exceed biological reaction capacities and result in unacceptably high contaminant emissions (Martin et al., 2002). Conversely, designing a biofilter based on peak concentration in the waste gas, a loading condition that may occur infrequently, in order to ensure reliable operation poses an economically unfavorable situation.

A similar phenomenon can exist with respect to design and operation of biofilters for processes that generate VOC-contaminated gas on a discontinuous basis (e.g., 8 hour work days, or weekend shutdown). In a conventional biofilter, contaminants must be treated essentially at the same rate that they are generated. A biofilter sized to treat a waste gas generated during only a portion of the day can sit idle with available (but unutilized) capacity during portions of the day when no contaminants are loaded. From this perspective, the system is over-designed in the sense that it has excess capacity (i.e., it could degrade contaminants during the no-loading period if they were loaded to the system). Intervals of low or no contaminant loading in biofilter systems are also problematic because of starvation conditions imposed on the microbial populations. Although short-term interruptions in contaminant loading (on the order of minutes
or a few hours) are unlikely to cause problems (Dirk-Faitakis and Allen, 2003), diminished contaminant removal for a period lasting several hours or even days following resumption of contaminant loading has been reported for cases where the duration of no loading was longer (on the order of several hours or days) because microbial populations can undergo sufficient endogenous decay or shifts in metabolism to result in diminished performance (Martin and Loehr, 1996; Mohseni et al., 1998; Webster et al., 1999; Park and Kinney, 2001; Cox and Deshusses, 2002; Moe and Qi, 2004). In general, longer duration shutdown periods require longer time for performance recovery.

In the field of industrial wastewater treatment, equalization basins are widely employed as a pre-treatment process. This can serve several purposes including 1) dampening fluctuations in organic loading to prevent shock loading of biological systems, 2) providing continuous feed to biological systems over periods when wastes are not being generated, 3) preventing high concentrations of toxic materials from entering the biological treatment plant, and 4) providing capacity for controlled discharge of wastes in a more even manner (Eckenfelder, 2000). In the field of biological treatment of contaminated gases, however, similar processes have not been widely studied or implemented.

In biofilters treating gas-phase contaminants, there is generally a greater need for organic loading dampening than for flow dampening because the flow rate is generally relatively constant (because of fixed-speed blowers). A potential method for achieving the goal of “buffering” dynamic loading (i.e., more evenly distributing contaminant loading over time) is to install an activated carbon column in series prior to the biofilter. The rationale for such a system is that during periods of high contaminant loading, the adsorbent could temporarily accumulate contaminants and then subsequently desorb contaminant during periods when concentrations in
the waste gas are low. In this manner, it could dampen fluctuations in organic loading to prevent shock loading of the biofilter and provide continuous feed to the biological system over periods when wastes are not generated. It may also allow smaller (and therefore less expensive) biofilters for treating discontinuously generated waste gases because the biofilter bed could be used more efficiently.

Although this strategy, first proposed by Ottengraff (1986), has been recommended by multiple authors (Weber and Hartmans, 1995; Swanson and Loehr, 1997; Devinny et al., 1999), such a system has been experimentally tested only for a single-component waste gas stream containing toluene (Weber and Hartmans, 1995). Little or no research has been conducted to assess performance of passively-operated activated carbon load-dampening systems for treating multi-contaminant waste gas streams during unsteady-state loading conditions. When multiple contaminants are present, competitive sorption can occur (Ruthven, 1986; Yang, 1987). As a result, activated carbon may exhibit differing buffering capacities for different contaminants. Furthermore, an appropriate basis for design or analysis of buffering systems is currently lacking.

In research described in Chapters 6 and 7, studies were conducted to evaluate use of granular activated carbon (GAC) as a passively-operated load-equalization mechanism for biofilters treating gas streams with dynamically varying (intermittent) pollutant loading. The first stage of this part of research is described here in Chapter 6 where abiotic fixed-bed adsorption/desorption experiments and numerical simulation were conducted to evaluate the range of load dampening expected for GAC columns subjected to various inlet concentrations of acetone and toluene as intermittently loaded single-component contaminants and as a two-component mixture. Both acetone and toluene are frequently encountered in industrial
operations (Marg, 1991; Ayer and Hyde, 1992; Chang and Lu, 2003), and toluene is regulated as a Hazardous Air Pollutant (HAP) in the US (USEPA, 2002). Intermittent loading conditions were intended to simulate a process where contaminants are generated 8 hr/day. The pore and surface diffusion model (PSDM) described by Crittenden et al. (1986) was used for the model simulation of contaminant breakthrough behavior through the GAC columns. Simulations using the PSDM were performed using AdDesignSTM software (Michigan Technological University). The model was calibrated and validated for predicting dynamic behavior of the sorption systems. The second stage of this part of research is presented in Chapter 7 where the impact of a carbon buffering system on biofilter performance was tested for treatment of a model gas stream containing a two-component mixture of acetone and toluene under intermittent loading conditions.

6.2. Background

6.2.1. Pore and Surface Diffusion Model (PSDM)

The pore and surface diffusion model (PSDM) described by Crittenden et al. (1986) and Hand et al. (1997) was used for predicting dynamic behavior of contaminant adsorption onto the granular activated carbon (GAC) column under discontinuous loading conditions. The PSDM is a dynamic fixed bed model that incorporates the following assumptions: (1) constant flow rate; (2) plug-flow conditions exist in the bed; (3) linear driving force describes the local bulk phase mass flux at the exterior surface of the adsorbent particle; (4) local adsorption equilibrium exists between the solute adsorbed onto the GAC particle and the solute in the intraaggregate stagnant fluid; (5) intraparticle mass flux is described by surface and pore diffusion; (6) adsorption equilibrium of individual compounds can be represented by the Freundlich isotherm equation and Ideal Adsorbed Solution Theory (IAST) describes the competition between the compounds; (7) there are no interactions between adsorbing compounds during the diffusion process (Mertz et
A diagram illustrating the mechanisms and assumptions incorporated into the PSDM is shown in Figure 6.1. In this figure, $K_f$ is film diffusion coefficient (m/s); $C_b$ is the adsorbate concentration in the bulk gas phase (mol/m$^3$); $C_s$ is the adsorbate concentration on the external surface of the adsorbent (mol/m$^3$); $q$ is the solid-phase concentration in the pores (mol/kg); $\rho_a$ is apparent adsorbent density (kg/m$^3$); $D_s$ is the surface diffusion coefficient (m$^2$/s); $\varepsilon$ is particle void fraction (porosity); $D_p$ is the pore diffusion coefficient (m$^2$/s); $C_p$ is the adsorbate concentration in the pores (mol/m$^3$); and $R$ is average adsorbent particle radius.

**Figure 6.1: Mechanisms and assumptions that are incorporated in Pore and Surface Diffusion Model (Crittenden et al., 1987).**

Dynamic adsorption calculations using this model require equilibrium parameters, kinetic parameters, physical properties of adsorbing compound(s) and adsorbent, fluid properties,
influent concentrations, and column dimensions. From the mass balances on the bulk phase and adsorbent phase, two partial differential equations are obtained for each adsorbing component. A coupling equation between the bulk phase and the adsorbent phase is obtained from assuming local equilibrium at the exterior of the adsorbent particle. In cases where pore and surface diffusion are present, local equilibrium is assumed along the pore walls.

The partial differential equations describing the adsorber dynamics in a fixed-bed adsorber are given below. The detailed derivation of these equations is given by Friedman (1984). The gas-phase mass balance for component $i$ is given by:

$$
\frac{\partial C_i(z,t)}{\partial t} + V \frac{\partial C_i(z,t)}{\partial z} + 3 \frac{k_f(i)}{\varepsilon R} \left[ C_i(z,t) - C_{p,i}(r = R, z,t) \right] = 0 \quad (6.1)
$$

Where $C_i$ is gas-phase concentration (mmol/L) of adsorbate $i$ at time $t$ (sec) and axial position along the bed $z$ (cm); $C_{p,i}$ is adsorbate concentration in adsorbent pores (mmol/L); $V$ is loading rate (cm/sec); $k_f$ is film transfer coefficient for adsorbate $i$ (cm/sec); $R$ is average adsorbent particle radius (cm); and $\varepsilon$ is particle void fraction (porosity). The initial condition for Equation 6.1 is:

$$
C_i(z,t) = 0 \quad at \quad 0 \leq z \leq L , t = 0 \quad (6.2)
$$

Where $L$ is bed length (cm). The boundary conditions for Equation 6.1 is:

$$
C_i(z,t) = C_{0,i} \quad at \quad z = 0 , t > 0 \quad (6.3)
$$

Where $C_{0,i}$ is initial concentration of adsorbate (mmol/L). The intraparticle phase mass balance for component $i$ is given by:

$$
\frac{1}{r^2} \frac{\partial}{\partial r} \left[ r^2 D_{p,i} \frac{\partial q_i(r,z,t)}{\partial r} + \frac{r^2 D_{p,i}}{\rho_a} \frac{\partial}{\partial r} C_{p,i}(r,z,t) \right] = \frac{\partial}{\partial t} \left[ q_i(r,z,t) + \frac{\varepsilon}{\rho_a} C_{p,i}(r,z,t) \right] \quad (6.4)
$$
Where \( q_i(r, z, t) \) is solid-phase concentration of adsorbate \( i \) (mmol/g); \( D_{p,i} \) is pore diffusion coefficient for component \( i \); \( D_{s,i} \) is surface diffusion coefficient for component \( i \); and \( \varepsilon \) is particle void fraction (porosity). The initial condition for Equation 6.4 is:

\[
q_i(r, z, t) + \frac{\varepsilon}{\rho_a} C_{p,i}(r, z, t) = 0 \quad \text{at} \quad 0 \leq r \leq R, \quad t = 0 \quad (6.5)
\]

Where \( \rho_a \) is apparent adsorbent density (g/cm\(^3\)). The first boundary condition for Equation 6.4 is:

\[
\frac{\partial}{\partial r} \left[ q_i(r, z, t) + \frac{\varepsilon}{\rho_a} C_{p,i}(r, z, t) \right] = 0 \quad \text{at} \quad r = 0, \quad t \geq 0 \quad (6.6)
\]

The second boundary condition for Equation 6.4 is:

\[
D_{s,i} \rho_a \frac{\partial q_i}{\partial r}(r = R, z, t) + D_{p,i} \varepsilon \frac{\partial C_{p,i}}{\partial r}(r = R, z, t) = k_{f,i} \left[ C_i(t) - C_{p,i}(r = R, z, t) \right] \quad (6.7)
\]

The equation coupling the gas phase concentration of component \( i \) within the adsorbent pores to the adsorbent phase concentration of component \( i \) is given by:

\[
C_{p,i}(r, z, t) = \frac{q_i(r, z, t)}{\sum_{k=1}^{m} n_k q_k(r, z, t) \left( \frac{n_i K_i}{n_j K_j} \right)^{m_j}} \quad (6.8)
\]

Where \( m \) is the number of components. This equation is derived assuming that the adsorption reaction rate is much faster than the mass transfer rate (assumption of local equilibrium).

In AdDesignS\(^{TM}\) software, the above partial differential equations were converted to a dimensionless form. An orthogonal collocation method is used to convert the dimensionless partial differential equations into a set of ordinary differential equations that are solved using a
backward differential method (Crittenden et al., 1980; Crittenden et al., 1986; Mertz et al., 1999; Ramakrishnan et al., 2004).

6.2.2. Ideal Adsorbed Solution Theory (IAST)

In the PSDM model, the competitive interactions between multicomponent VOCs were estimated using Ideal adsorbed solution theory (IAST). IAST, proposed by Myers and Prausnitz (1965), requires the use of single solute isotherm parameters for each adsorbing compound. In IAST, the fundamental thermodynamic equations for liquids or gases are applied to the adsorbed phase. The validity of using thermodynamic equations relies on three assumptions (Cal, 1995):

(1). The adsorbent is assumed to be thermodynamically inert, meaning that a change in a thermodynamic property, such as internal energy, during an adsorption process at constant temperature is assumed to be negligible compared with the change in the same property for the adsorbing gas.

(2). The adsorbent possesses a temperature-invariant area which is the same for all adsorbates. This assumption is not valid for a molecular sieve adsorbent, because the area available for adsorption depends upon the size of the adsorbate molecule.

(3). The Gibbs definition of adsorption applies. In most cases, this definition corresponds to the usual methods in which volumetric or gravimetric adsorption experimental results are obtained.

In IAST, the following basic equations are used to predict multicomponent behavior from single-component adsorption isotherms (Crittenden et al., 1985):

\[ q_T = \sum_{i=1}^{N} q_i \]  \hspace{1cm} (6.9)

\[ z_i = q_i / q_T \]  \hspace{1cm} (6.10)
\[ C_i = z_i C_i^0 \quad (6.11) \]

\[ \frac{1}{q_T} = \sum_{i=1}^{N} \frac{z_i}{q_i^0} \quad (6.12) \]

\[ \frac{\pi_m A}{RT} = \int_0^{q_i^0} \frac{d \ln C_i^0}{d \ln q_i} dq_i^0 = \frac{\pi_i^0 A}{RT} = \int_0^{q_j^0} \frac{d \ln C_j^0}{d \ln q_j} dq_j^0 = \frac{\pi_j^0 A}{RT} = \ldots \text{ for } j=2 \text{ to } N \quad (6.13) \]

Where \( N \) is the total number of adsorbates in the mixture; \( q_T \) is the total solid-phase adsorbates concentration (M/M); \( q_i \) is the solid-phase concentration for component \( i \) (M/M); \( z_i \) is the mole fraction on the surface of the carbon for component \( i \); \( C_i \) is the gas-phase concentration for component \( i \) (M/L\(^3\)); \( q_i^0 \) is the single-solute solid-phase concentration for component \( i \) that causes the same spreading pressure, or reduction in surface tension, as the mixture (M/M); \( C_i^0 \) is single-solute gas-phase concentration in equilibrium with \( q_i^0 \) for component \( i \) (M/L\(^3\)). Equations 6.9 and 6.10 define \( q_T \) and \( z_i \), respectively. Equation 6.11 is analogous to Raoult’s law. Equation 6.12 is the expression for no area change per mole upon mixing in the mixture from the single-solute isotherms at the spreading pressure of the mixture. Equation 6.13 equates the spreading pressures of the pure component systems to the spreading pressure of the mixture. The spreading pressure, \( \pi \), corresponds to the difference in surface tension between a clean surface and an adsorbate covered surface, and can be expressed as:

\[ \pi = -\left( \frac{\partial U}{\partial A} \right)_{S,V} \quad (6.14) \]

Where \( U \) is the internal energy of the adsorbate, \( A \) is the surface area occupied by the adsorbate, \( S \) is entropy, and \( V \) is volume.
If the Freundlich isotherm equation as described in Equation 6.15 is used to represent single-solute behavior in Equation 6.13, then Equation 6.13 will simplify to the expression described in Equation 6.16:

\[ q_i = K_i C_i^{1/n_i} \]  \hspace{1cm} (6.15)

\[ n_i q_i^0 = n_j q_j^0 \quad j=2 \text{ to } N \]  \hspace{1cm} (6.16)

After a great deal of algebraic manipulation, the following equation for each adsorbate was derived (Crittenden et al., 1985):

\[ C_i = \frac{q_i}{\sum_{j=1}^{N} q_j \left( n_j K_i \right)^{n_j}} \quad \text{for } i=1 \text{ to } N \]  \hspace{1cm} (6.17)

In general, the Freundlich equation does not fit pure-component isotherms well at low adsorption coverages. This leads to one serious flaw when incorporating the Freundlich equation into IAST: it does not reduce to Henry’s law at low adsorbate coverage. Therefore Crittenden’s modification to IAST will not work well when trying to predict an entire adsorption isotherm. Nevertheless, the simplifications introduced to the IAST equations by Crittenden’s modifications have proved useful in modeling multicomponent adsorption over regions where the Freundlich equation is valid for the adsorbates modeled (Cal, 1995).

6.3. Materials and Methods

6.3.1. Fixed-bed Granular Activated Carbon (GAC) Adsorption/Desorption Experiments

6.3.1.1. Apparatus and Materials

A diagram of the experimental apparatus for the adsorber column for each individual fixed-bed adsorption/desorption test is shown in Figure 6.2. Each column was constructed of PVC (ID 7.62 cm) and contained a perforated plate that supported 6 cm depth glass beads (5 mm
diameter), a thin layer of glass wool, 84.4 cm depth of GAC, another thin layer of glass wool, and another 6 cm of glass beads. The GAC, with a total mass of 1.69 kg, consisted of Calgon BPL 4×6 mesh carbon (Calgon Carbon Corp., Pittsburgh, PA) which has characteristics summarized in Table 6.1. The carbon was rinsed with distilled water to remove particles of carbon dust and dried at 105 °C for 7 days prior to use. Initial tests conducted prior to the placement of activated carbon demonstrated that the column components (other than GAC) had little or no adsorption capacity for acetone or toluene. Channeling and wall effects should be avoided because the ratio of column diameter to GAC particle diameter was greater than 20.

![Figure 6.2: Schematic diagram of apparatus used for fixed-bed adsorption/desorption experiments.](image)

**Table 6.1: Properties of Calgon BPL 4×6 mesh activated carbon (data provided by Calgon Carbon Corp., Pittsburgh, PA).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle apparent density (g/cm³)</td>
<td>0.85</td>
</tr>
<tr>
<td>Average particle diameter (cm)</td>
<td>0.3715</td>
</tr>
<tr>
<td>Particle void fraction</td>
<td>0.595</td>
</tr>
<tr>
<td>Particle shape factor</td>
<td>0.72</td>
</tr>
</tbody>
</table>
Contaminant-free compressed air (with an average relative humidity of 20%) flowed through a pressure regulator to reduce pressure to approximately 15 psi and a rotameter (Cole-Parmer Instrument Co., Veron Hill, IL) was used to control the flow rate. Individual contaminant compound were delivered by a syringe pump (KD Scientific model 1000, Boston, MA) through a needle into a glass injection port where they evaporated into the air stream. A microprocessor-based controller (Chron-Trol Corp., San Diego, CA) was used to turn the syringe pumps on and off during each loading cycle. The contaminated air stream then flowed downward through the GAC column before being discharged to a fume hood.

Solenoid valves with stainless steel flow tubes (Asco Valve Inc., NJ) were installed immediately prior to the GAC column inlet and immediately after the GAC column outlet to allow automated sampling of inlet and outlet contaminant concentrations. When a solenoid valve was turned on using a microprocessor controller (Chron-Trol Corp., San Diego, CA), a portion of the gas flow was diverted to the analytical instrument used to measure contaminant concentrations. The gas sampling lines were constructed using Teflon tubing. Details regarding the analytical instrument and its operation can be found in section 6.3.1.3 of this chapter.

6.3.1.2. GAC Column Operation

The dynamic loading scenario investigated in this study consisted of 8-hr contaminant loading followed by 16-hr non-loading each day. During non-loading intervals, uncontaminated air flowed through the columns at the same rate as during contaminant loading intervals. Gas flow rates were 42.1, 38.4, and 38.4 L/min for GAC columns used to test adsorption of toluene, acetone, and their binary mixture, respectively. These correspond to empty bed contact times (EBCTs) in the GAC columns of 5.5, 6.1, and 6.1 sec, respectively. For toluene adsorption tests, target influent contaminant concentrations during the daily 8-hr loading cycle were 868 ppm\textsubscript{v} during the first 12 days, 488 ppm\textsubscript{v} for 7 days, and then 217 ppm\textsubscript{v} for 6 days (25 days total). For
acetone adsorption tests, target influent concentrations during the daily 8-hr loading cycle were 960 ppm\textsubscript{v} for the first 8 days, 545 ppm\textsubscript{v} for 7 days, and then 243 ppm\textsubscript{v} for 6 days (21 days total). During the mixture adsorption test, target influent concentrations for acetone and toluene during the 8-hr loading cycle were 550 ppm\textsubscript{v} each (21 days total). All experiments were conducted at ambient laboratory temperature of 23±2\degree C.

6.3.1.3. Analytical Techniques

Acetone and toluene concentrations were measured on-line using a model 1312 photoacoustic multigas monitor (California Analytical Instruments, Orange, CA) equipped with four optical filters (UA# 0971, 0974, 0983, and SB0527). Gas streams were passed through a model 130 Genie membrane filter (A+ Corporation, Prairieville, LA) to remove excess moisture prior to analysis. The instrument was calibrated using concentrations of 0, 800 ppm\textsubscript{v} acetone and 0, 100 ppm\textsubscript{v} toluene (BOC, Port Allen, LA).

Relative humidity was measured using a Traceable\textsuperscript{®} Digital Hydrometer-Thermometer (Fisher Scientific, Pittsburgh, PA).

6.3.2. PSDM Modeling of Adsorption/Desorption in the GAC Column

The pore and surface diffusion model (PSDM) described by Crittenden \textit{et al.} (1986) and Hand \textit{et al.} (1997) was used to predict the degree of load dampening achieved by GAC columns under discontinuous loading conditions. The PSDM is a dynamic fixed-bed model that incorporates assumptions and governing equations described previously (Crittenden \textit{et al.}, 1986; Mertz \textit{et al.}, 1999). Simulations using the PSDM were performed using AdDesignS\textsuperscript{TM} software (Michigan Technological University). Influent contaminant concentrations and flow rates input to the model were identical to those experimentally tested. Measurements in the experimental system revealed that influent contaminant concentrations increased to near the target level within 3 min after the start of loading and decreased to below detection within 3 min after syringe
pumps turned off (data not shown). Accordingly, in model simulations, contaminant concentrations entering the GAC column as a function of time were assumed to linearly increase from zero to the target level over a period of 3 min at the start of each 8 hr loading period and linearly decrease to zero 3 min after the end of the 8 hr loading interval.

To obtain a qualitative and quantitative appreciation of the influence of various kinetic and isotherm parameters on the overall GAC column dynamic adsorption, sensitivity analysis was performed for model simulation of single-component adsorption for toluene and acetone. A detailed description of the method and results for the sensitivity analysis appears in Appendix C. The model was calibrated by optimizing the film mass-transfer coefficients for toluene or acetone single-component adsorption to achieve the best fit with experimental data obtained for the first loading condition of single-component adsorption experiments (i.e., the first 12 and 8 days of experimentally determined sorption data for toluene and acetone, respectively). Data from the last two loading conditions of single-compound sorption experiments (lasting 7 and 6 days, respectively) as well as the entire duration of the binary mixture adsorption test were used for model validation.

6.4. Results and Discussion

6.4.1. Experimental Testing of Fixed-bed GAC Adsorption/Desorption of Single Components at Discontinuous Loadings

Experimentally measured GAC breakthrough curves for intermittent loading of toluene and acetone as single-component adsorption are depicted in Figures 6.3a and 6.4a, respectively. As shown, under the discontinuous loading conditions, initial toluene and acetone breakthrough occurred after 6.3 and 2.4 days, respectively. Quasi-steady state conditions were attained rather quickly with both contaminants exhibiting a consistent pattern of attenuated effluent concentrations reoccurring on a daily basis. Daily peak effluent concentrations differed only
slightly (within 5%) and mass balance calculations verified that contaminant mass entering and exiting GAC columns on a daily basis were essentially the same (within 3%) after quasi-steady state was reached at each loading condition.

**Figure 6.3: Experimental measurement and modeling of toluene adsorption under three discontinuous loading conditions (EBCT = 5.5 sec).**

The experimental data demonstrate that a GAC column with low EBCT (5.5 and 6.1 sec for toluene and acetone, respectively) can markedly dampen dynamic (intermittent) loading for both compounds. Peak contaminant concentrations exiting the GAC column were less than half of the influent concentration during each loading condition for both compounds. Furthermore, during the third loading conditions (influent concentration 217 ppm<sub>v</sub> toluene or 234 ppm<sub>v</sub> acetone), “ideal” buffering was achieved. Contaminants loaded to the GAC column during only one third of the cycle duration (i.e., 8 hr per 24 hr period), exited the system evenly distributed in time at a level one third of the influent concentration. This nicely demonstrates that contaminant
Mass temporarily accumulated in the GAC column during intervals when influent contaminant concentrations are high can desorb within a sufficiently short time interval (i.e., during each loading cycle when influent contaminant concentrations are low) to be of practical benefit as a passively-operated load equalization mechanism prior to biofilter treatment. Under the discontinuous contaminant loading conditions, the only driving force necessary for contaminant desorption was the naturally occurring decrease in influent contaminant concentration. Regeneration of the GAC column through other means (e.g., heating) was not necessary for successful load dampening.

**Figure 6.4:** Experimental measurement and modeling of acetone adsorption under three discontinuous loading conditions (EBCT=6.1 sec).

For single-contaminant waste gas streams, load equalization such as that depicted in Figures 6.3a and 6.4a could provide an obvious practical benefit in design and operation of biofilters. Peak loading to the biofilter could be greatly reduced and starvation during periods of low or no contaminant loading could be minimized or entirely avoided. If a biofilter’s design is
to be based on peak loading as advocated by some authors (Martin et al., 2002; Martin et al., 2004), then the biofilter could be markedly reduced in size compared to a system without buffering. A decrease in the peak contaminant concentration entering the biofilter as a result of such load attenuation could also be used to avoid toxicity (Devinney and Hodge, 1995) or O$_2$ limitation (Zhu et al., 2004) in cases where they would otherwise occur.

**6.4.2. Modeling of Fixed-bed GAC Adsorption/Desorption of Single Components at Discontinuous Loadings**

Results from the sensitivity analysis described in Appendix C revealed that the predicted breakthrough curves were highly sensitive to the film transfer coefficient $K_f$, and Freundlich isotherm parameters $K$ and $n$. Therefore, the model could be calibrated by adjusting one or more of these parameters to fit the experimental data. The film transfer coefficient, $K_f$, was selected as the single parameter adjusted and optimized for the purpose of model calibration in the study described herein. Alternative methods for model calibration and the corresponding model validation using different parameter adjustments are described in Appendix C.

For modeling of breakthrough curves using the PSDM, physical properties of adsorbent and adsorbate required for model input were obtained using the *AdDesignSTM* database and Software to Estimate Physical Properties (StEPP). The Freundlich parameters were generated using Calgon Carbon’s proprietary model. For the kinetic parameters, the surface diffusion ($D_s$) coefficient was determined using the Sontheimer correlation equation (Mertz et al., 1999):

$$D_s = \frac{D_G \varepsilon C_0}{\tau_p \rho_q q_o} \times SPDFR$$

(6.18)

where $C_0$ is the initial inlet concentration (mg/L); $D_G$ is the adsorbate gas phase diffusivity ($cm^2/s$); $D_s$ is the surface diffusion coefficient ($cm^2/s$); $q_o$ is the solid phase concentration in equilibrium with $C_0$ for a single-solute equilibrium (mg/g); $\varepsilon$ is the void
fraction (porosity) of the carbon (-); \( \rho_a \) is the apparent adsorbent density (which includes pore volume) (g/L); \( \tau_p \) is the adsorbent tortuosity (-); and \( \text{SPDFR} \) is the surface to pore diffusion flux ratio. The gas diffusivity, \( D_G \), is calculated using the Wilke-Lee modification of the Hirschfelder-Bird-Spotz method.

The pore diffusion coefficient \( D_p \) was determined using the Wilke-Lee modification of the Hirschfelder-Bird-Spotz equation with user-defined tortuosity as shown in the following equation (Mertz et al., 1999):

\[
D_p = \frac{D_G}{\tau_p}
\]

(6.19)

where: \( D_G \) is the adsorbate gas or liquid phase diffusivity (cm\(^2\)/s); \( \tau_p \) is the adsorbent tortuosity (-); and \( D_p \) is the pore diffusion coefficient (cm\(^2\)/s).

Initial model simulations, conducted for each single-component adsorption by inputting the above parameters and the base values of film diffusion coefficients \( K_f \) (i.e., 2.55 and 2.99 cm/s for toluene and acetone, respectively) calculated using Gnielinski correlation (Mertz et al., 1999), predicted the same general pattern of breakthrough curves as were experimentally measured (see Figures C.1 and C.2 in Appendix C). Peak effluent concentrations, however, were over-predicted and minimum effluent concentrations were under-predicted, in the initial uncalibrated model simulations employing the Gnielinski correlation for \( K_f \).

In calibrating the model by optimizing the \( K_f \) value, the most important criterion was to match the predicted minimum and maximum concentrations and shape of the daily breakthrough curves with those of the measured data. Best agreement between experimental and modeled data for the first loading condition was achieved with a \( K_f \) value of 1.02 and 0.45 cm/sec for toluene and acetone adsorption, respectively. Such \( K_f \) values correspond to 0.4 and 0.15 times the values calculated using Gnielinski correlation for toluene and acetone adsorption, respectively. The
model was validated by comparing the fit of the model with the experimental data at the other two loading conditions for each single-component adsorption. A summary of parameter values and sources of data input to the calibrated model for single-component adsorption is presented in Table 6.2.

**Table 6.2: Parameter values used in modeling of single-component adsorption.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fixed Bed Properties</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bed length</td>
<td>84 (^a)</td>
<td>cm</td>
</tr>
<tr>
<td>Bed diameter</td>
<td>7.62 (^a)</td>
<td>cm</td>
</tr>
<tr>
<td>Bed mass</td>
<td>1.69 (^a)</td>
<td>kg</td>
</tr>
<tr>
<td>Bed Porosity</td>
<td>0.483 (^a)</td>
<td>dimensionless</td>
</tr>
<tr>
<td><strong>Adsorbent Properties</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Particle apparent density ((p_a))</td>
<td>0.85 (^b)</td>
<td>g/cm(^3)</td>
</tr>
<tr>
<td>Average particle diameter ((d_p))</td>
<td>0.3715 (^b)</td>
<td>cm</td>
</tr>
<tr>
<td>Particle void fraction ((\epsilon))</td>
<td>0.595 (^b)</td>
<td>dimensionless</td>
</tr>
<tr>
<td>Particle shape factor</td>
<td>0.72 (^b)</td>
<td>dimensionless</td>
</tr>
<tr>
<td><strong>Freundlich Isotherm Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K (Freundlich constant for acetone)</td>
<td>82 (^b)</td>
<td>(mg/g) (L/mg)(^{1/n})</td>
</tr>
<tr>
<td>(1/n) (Freundlich constant for acetone)</td>
<td>0.291 (^b)</td>
<td>dimensionless</td>
</tr>
<tr>
<td>K (Freundlich constant for toluene)</td>
<td>305 (^b)</td>
<td>(mg/g) (L/mg)(^{1/n})</td>
</tr>
<tr>
<td>(1/n) (Freundlich constant for toluene)</td>
<td>0.107 (^b)</td>
<td>dimensionless</td>
</tr>
<tr>
<td><strong>Air Properties</strong></td>
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<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>23 (^a)</td>
<td>°C</td>
</tr>
<tr>
<td>Density</td>
<td>1.18×10(^{-3}) (^c)</td>
<td>g/cm(^3)</td>
</tr>
<tr>
<td>Viscosity</td>
<td>1.80×10(^{-4}) (^c)</td>
<td>g/(cm sec)</td>
</tr>
<tr>
<td><strong>Kinetic Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(K_f) (acetone film mass-transfer coefficient)</td>
<td>0.45 (^d)</td>
<td>(cm/s)</td>
</tr>
<tr>
<td>(K_f) (toluene film mass-transfer coefficient)</td>
<td>1.02 (^d)</td>
<td>(cm/s)</td>
</tr>
<tr>
<td>(D_s) (acetone surface diffusion coefficient)</td>
<td>2.64×10(^{-5}) (^e)</td>
<td>(cm/s)</td>
</tr>
<tr>
<td>(D_s) (toluene surface diffusion coefficient)</td>
<td>8.75×10(^{-6}) (^e)</td>
<td>(cm/s)</td>
</tr>
<tr>
<td>(D_p) (acetone pore diffusion coefficient)</td>
<td>1.07×10(^{-1}) (^f)</td>
<td>(cm/s)</td>
</tr>
<tr>
<td>(D_p) (toluene pore diffusion coefficient)</td>
<td>8.24×10(^{-7}) (^f)</td>
<td>(cm/s)</td>
</tr>
<tr>
<td>Surface to pore diffusion flux ratio</td>
<td>16 (^g)</td>
<td>dimensionless</td>
</tr>
<tr>
<td>Tortuosity</td>
<td>1 (^f)</td>
<td>dimensionless</td>
</tr>
<tr>
<td><strong>Collocation Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total simulation time (days)</td>
<td>25</td>
<td>21</td>
</tr>
<tr>
<td>Time step (hr)</td>
<td>0.8</td>
<td>1.3</td>
</tr>
<tr>
<td>Number of axial elements</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Number of axial collocation points</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Number of radial collocation points</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

\(^a\)Experimentally measured.

\(^b\)Data provided by Calgon Carbon.

\(^c\)Calculated by correlation using the StEPP\(^\text{TM}\) database (Mertz et al., 1999).
The calibrated and validated model simulations of toluene and acetone as intermittently-loaded single-component contaminants are shown as solid lines in Figures 6.3b and 6.4b, respectively. As shown, under the dynamic loading conditions tested, by optimizing the $K_f$ value to get the best fit of the experimental data under one loading condition, the PSDM model was successful in qualitatively and quantitatively predicting the pattern of VOC concentrations exiting the GAC column for single-component adsorption under other loading conditions (i.e., at other inlet concentration levels). Before applying the model to conditions outside the range of those experimentally evaluated, however, it is recommended that further experimental testing and a more rigorous validation procedure be established.

6.4.3. Experimental Testing of Binary Mixture Adsorption/Desorption of Acetone and Toluene at Discontinuous Loadings

Target inlet concentrations and experimentally measured concentrations exiting the GAC column for the mixture of toluene and acetone under discontinuous (8 hr/day) loading are depicted in Figure 6.5a and b, respectively.

As illustrated in Figure 6.5, under an EBCT of 6.1 sec and at an influent concentration of 550 ppm, for each compound loaded 8 hr/day, toluene was effectively buffered, and its breakthrough was similar to that observed for single-component adsorption (though with longer interval before initial breakthrough, consistent with that expected for lower influent concentration in comparison to data depicted in Figure 6.3). Breakthrough for acetone, however, was markedly different from that observed in the single-component adsorption process. Although initially well-buffered (e.g., day 5), peak daily acetone concentrations exiting the GAC column progressively increased over time until both toluene and acetone breakthrough reached...
quasi-steady state. On day 12, peak effluent acetone concentration was actually 16% higher than
the influent. Mass balance calculations revealed that during the interval when acetone
breakthrough was observed while toluene was not (days 5 to 12), mass of acetone exiting the
GAC column was greater than mass entering on a daily basis. After both acetone and toluene
reached quasi-steady state (day 14), only a small fraction of acetone remained in the GAC bed.

![Figure 6.5: Experimental measurement and model simulation of binary mixture adsorption of toluene and acetone under discontinuous loading conditions (EBCT = 6.1 sec).](image)

This phenomenon can be readily explained by competitive sorption effects caused by
differences in physical properties of the adsorbates (Ruthven, 1986; Yang, 1987). VOC
adsorption to activated carbon is governed by both activated carbon surface characteristics (e.g.,
pore volume, specific surface area, and polarity) and VOC properties (e.g., dipole moment, boiling point, and molecular weight) (Chiang et al., 2001). Because the surface of activated carbon is non-polar or slightly polar, it generally has higher affinity and higher adsorption capacity for non-polar organics (e.g., toluene, dipole moment of 0.45 debyes) than it does for polar organics (e.g., acetone, dipole moment of 2.87 debyes) (Yang, 2003). Furthermore, toluene has a higher boiling point than acetone (111 vs 56.3 °C), and it also has a higher molecular weight (92 vs 58 g/mol), characteristics favoring adsorption. Thus, as the more strongly adsorbed toluene accumulated in the carbon bed, it displaced the more weakly adsorbed acetone. The combination of initially adsorbed acetone being displaced and emitted at the same time as additional acetone entering the system passed through unadsorbed resulted in effluent acetone concentration exceeding the influent for a period of time before quasi-steady state was reached. This effect (displacement of more weakly adsorbed component resulting in effluent concentrations for one or more compounds temporarily exceeding influent) is consistent with patterns observed for competitive adsorption in processes treating multi-component waste streams subjected to steady (rather than intermittent) loading conditions (Ruthven, 1986; Yang, 1987). For multi-component contaminant mixtures with similar physicochemical properties (e.g., toluene vs. p-xylene, or ethylbenzene), however, the competitive interactions between adorption of such compounds would become less pronounced, and attenuation of all compounds with the same or similar magnitude might be expected.

Because numerous reports suggest that aromatic compounds (e.g., toluene) are more slowly degraded than ketones (e.g., acetone) in biofilters (Webster et al., 1998; Song et al., 2003; Qi et al., 2004), and in view of the fact that toluene poses larger health risks and is of a higher priority from a regulatory perspective than acetone, decreasing the peak loading of toluene may
be necessary and beneficial for improving biofilter performance even if acetone is less attenuated in such passively operated activated carbon load dampening systems treating multi-component contaminants. However, such competitive adsorption has to be accounted for when designing GAC buffering systems for biofilters treating waste streams containing multiple contaminants.

6.4.4. Modeling of Binary Mixture Adsorption/Desorption of Acetone and Toluene at Discontinuous Loadings

For the parameters used in the binary mixture adsorption modeling, values of the fixed bed properties, adsorbent properties, Freundlich isotherm parameters, air properties, film mass-transfer coefficient for each compound, surface to pore diffusion flux ratio, and tortuosity were the same as described in Table 6.2. The surface diffusion coefficients were $5.77 \times 10^{-6}$ and $1.78 \times 10^{-5}$ cm$^2$/s for toluene and acetone, respectively; and the pore diffusion coefficients were $8.24 \times 10^{-2}$ and $1.01 \times 10^{-1}$ cm$^2$/s for toluene and acetone, respectively, both following corresponding correlations as were described in Table 6.2. The total simulation time was 21 days, with a time step of 1.3 hours. The number of axial elements, axial collocation points, and radial collocation points were 6, 12, and 6, respectively. Results of the model simulation of binary mixture adsorption/desorption are shown in Figure 6.5c.

As shown, the PSDM was successful in qualitatively and quantitatively predicting the pattern of VOC concentrations exiting the GAC column for the multi-component gas stream using the optimized $K_f$ values determined in single-contaminant experiments. Both modeled and measured data demonstrate that for multi-component waste gas streams containing constituents with different physicochemical properties (e.g., acetone and toluene), competitive adsorption can result in buffering of more weakly adsorbed components markedly different from that observed when contaminants are present as single-components.
6.5. Conclusions

For single-component adsorption, a GAC column was able to effectively attenuate both dynamic loadings of acetone and toluene under a wide range of influent concentrations. Because of competitive sorption, the degree of load equalization achieved for different constituents in multi-contaminant gas streams can vary markedly. Acetone was less attenuated in the presence of toluene.

Under the discontinuous contaminant loading conditions, the only driving force necessary for contaminant desorption was the naturally occurring decrease in influent contaminant concentration. The contaminant mass temporarily accumulated in the GAC column can be desorbed within a sufficiently short time interval (i.e., during each loading cycle) to be of practical benefit as a load dampening mechanism for biofilter pretreatment. Regeneration of the GAC column through other means (e.g., heating) was not necessary for successful load dampening.

A PSDM model was calibrated successfully using experimental data by optimizing only a single parameter, the film transfer coefficient $K_f$. The calibrated PSDM was successful in qualitatively and quantitatively predicting the pattern of VOC concentrations exiting the GAC column for both single and multi-component gas streams under discontinuous loading conditions. This model may be useful in developing a design basis for activated carbon load dampening systems for biofilters treating dynamically varying VOC concentrations.
CHAPTER 7    EFFECTS OF ACTIVATED CARBON LOAD EQUALIZATION ON BIOFILTER TREATMENT OF DISCONTINUOUSLY GENERATED WASTE GAS MIXTURES

7.1. Introduction

Use of an integrated system consisting of a column packed with granular activated carbon (as a passively-controlled load dampening mechanism) in series before a biofilter has great potential for mitigating the effects of unsteady-state loading and minimizing the uncertainty that accompanies design and operation of biofilter systems. However, such a GAC-biofilter integrated system has been experimentally tested only for a single-component waste gas stream containing toluene (Weber and Hartmans, 1995). Little or no research has been conducted to assess performance of passively-operated activated carbon load-dampening systems for treating multi-contaminant waste gas streams during unsteady-state loading conditions. As described in Chapter 6, abiotic fixed-bed sorption experiments and numerical modeling revealed that for waste gas streams containing multi-component contaminants with different physicochemical properties, the degree of load equalization achieved for different constituents can vary markedly. Acetone was much less attenuated in the presence of toluene because of competitive sorption.

To determine whether passively-operated activated carbon load dampening systems can improve performance of biofilters treating multi-component mixtures, the treatment performance of an integrated system (i.e., carbon buffering system placed in series before a biofilter) was experimentally evaluated in comparison to a conventionally designed biofilter (i.e., one lacking an activated carbon column). The model waste gas stream contained a two-component mixture of acetone and toluene. The systems were operated with 8 hr/day contaminant loading to simulate conditions expected in industrial operations.
7.2. Materials and Methods

7.2.1. Experimental Apparatus

The experiments described herein utilized two laboratory-scale biofilter systems. One system (Figure 7.1) consisted of an activated carbon column (to achieve load dampening), a packed column (to achieve humidification), and a biofilter (to achieve contaminant biodegradation) installed in series. A second system, identical to the first except for the fact that the activated carbon buffering column was omitted, was used for comparison purposes. These were designated as “buffered” and “unbuffered” biofilters, respectively. Gas supply and equipment for regulating air flow and introducing VOCs were identical to those used in abiotic sorption experiments as described in Chapter 6. In the buffered system, the activated carbon column, constructed of PVC, had an inner diameter of 7.62 cm. A perforated aluminum plate supported a 6 cm depth of glass beads (5 mm diameter), a thin layer of glass wool, 42.2 cm depth of GAC, another thin layer of glass wool, and another 6 cm depth of glass beads. The GAC, with a total mass of 0.85 kg, consisted of Calgon BPL 4×6 mesh carbon (Calgon Carbon Corp., Pittsburgh, PA) which has characteristics previously summarized in Table 6.1 (see Chapter 6).

The humidification column, constructed of PVC, had an inner diameter of 7.62 cm and a total height of 153 cm. The column was divided into two sections by a perforated stainless steel support plate with 9 mm openings, held in position by a PVC coupling. The bottom section had a height of 40 cm and served as a reservoir for liquid recirculation (with 1.3 L of water filled to a depth of 20 cm). The top section had a height of 110 cm packed with 100 cm of stainless steel 10 mm Interpack® packing media (Jaeger Company, Houston, TX). A peristaltic pump (Cole Parmer, Barrington, IL) withdrew water from the bottom reservoir and pumped it to the top of the column at a flow rate of 200 mL/min. There, water was distributed over the packing medium by a spray nozzle (1/8G-316SS3, Spray System Co., Wheaton, IL) mounted 10 cm above the
packing media. Water draining through the packing media was collected in the bottom reservoir for recirculation. Contaminated air (with an average original relative humidity of 20%) entered the humidification column at a point between the lower water reservoir and the upper packed bed and exited from the top of the column. Make-up water was added on an hourly basis by a peristaltic pump to compensate for water lost to evaporation into the air stream. Measurements of the gas exiting the humidification column demonstrated that this system achieved greater than 95% relative humidity in the gas stream entering the biofilter.

![Figure 7.1: Schematic diagram of experimental apparatus for the buffered biofilter system. Components used for the unbffered biofilter system were identical except that the activated carbon column was omitted.](image)

Each biofilter, operated in an up-flow mode, consisted of a glass column with a bottom, top, and six sections (9.9 cm ID). A perforated stainless steel plate supported packing medium in each section. The column had total packed bed depth of 1.45 m and packed bed volume of 11.2 L. Glass marbles were placed in the bottom of the columns to evenly distribute airflow. The biofilter packing medium consisted of polyurethane foam cubes (Honeywell-PAI, Lakewood,
CO). The medium, supplied by the vendor in the form of cubes approximately 5.0 cm per side, was cut into cubes approximately 1.25 cm per side prior to use. This packing medium contained no activated carbon coating, different from the type of packing medium previously used in SBB operation as described in Chapter 4. Preliminary abiotic fixed-bed sorption experiments with this packing medium (with moisture content adjusted at 30%, and experimental setup similar to that described in Section 3.2.2, Chapter 3), conducted at a stable influent toluene and acetone concentrations of 80 and 400 ppmv, respectively under an EBRT of 30 sec, revealed that this packing medium had relatively low sorption capacity for the VOCs used in these experiments, with complete toluene and acetone breakthrough occurring within 30 and 60 minutes, respectively after start of contaminant loading.

Solenoid valves with stainless steel flow tubes (Asco Valve Inc., NJ) installed prior to the GAC column inlet and after the GAC column, humidification column, and biofilter allowed automated sampling of contaminant concentrations at different spatial locations. When a solenoid valve was turned on, a portion of the gas flow was diverted to the analytical instrument used to measure contaminant concentrations. Gas sampling lines were constructed using Teflon tubing.

7.2.2. Biofilter Inoculation and Start-up

Laboratory studies employed two 4.0 L glass kettle reactor (Pyrex, Acton, MA), each with a working liquid volume of 3.0 L, to enrich for acetone and toluene-degrading microorganisms, respectively. Initially, each of the sparged-gas reactors was filled with 100 mL of activated sludge from a municipal wastewater treatment facility (Port Allen, LA) and 2.9 L of a previously described nutrient solution (See Table 3.1). Contaminated air passed through an aeration stone submerged in the liquid. Additional mixing was provided by a Teflon-coated magnetic stir bar. The reactor for enriching toluene-degrading microorganisms was operated
with an influent toluene concentration of 100 parts per million by volume (ppm) and gas flow rate of 5 L min\(^{-1}\). The reactor for enriching acetone-degrading microorganisms was operated with an influent acetone concentration of 450 ppm, and gas flow rate of 5 L min\(^{-1}\). Both reactors had a solid and hydraulic residence time of 6 days and were operated for a period of 86 days prior to biofilter inoculation. Biomass wasting was achieved by removing 1/6 of the reactor’s liquid volume (0.5 L) without settling and then replacing with an equal volume of freshly-prepared nutrient solution. At the time of biofilter inoculation, the total suspended solids (TSS) concentration was 2100 and 2600 mg L\(^{-1}\) for reactors supplied with toluene and acetone, respectively.

For inoculation of each biofilter, 1.5 L of suspended biomass from each of the two sparged-gas reactors was mixed with 3.0 L of freshly prepared nutrient medium (6.0 L total per biofilter). The packing medium for each biofilter section was submerged in a separate 1.0 L portion of the resulting culture. The packing medium was then placed in the biofilter columns and allowed to drain by gravity. No special attempt was made to retain biomass on the packing medium. Time was measured in days from the time of inoculation.

7.2.3. Biofilter Operation

7.2.3.1. Loading Conditions

Following inoculation, both biofilters underwent three distinct periods of operation (arbitrarily named Phase 1 (days 0-40), Phase 2 (days 41-60), and Phase 3 (days 61-82)) that involved progressively higher contaminant loading rates. For the buffered system, target influent concentrations during the 8 hr/day loading interval were 430 ppm, acetone and 100 ppm, toluene during all three phases. Gas flow rates to the buffered system were 11.5, 23, and 46 L/min for Phases 1, 2, and 3, respectively. These correspond to empty bed contact times (EBCTs) in the GAC column of 10, 5.0, and 2.5 sec, and EBCTs in the biofilter of 58, 29, and 14.5 sec,
respectively. During Phases 1 and 2, the unbuffered biofilter system had the same gas flow rate and intermittent contaminant concentrations as the buffered system. For comparison purposes, during Phase 3, gas flow rate to the unbuffered biofilter was 39.4 L/min so that the unbuffered biofilter had the same EBCT (17 sec) as the total EBCT of the activated carbon column (2.5 sec) plus biofilter (14.5 sec) in the buffered system. Target influent concentrations for the unbuffered biofilter system were lowered to 418 ppm \( v \) acetone and 96.6 ppm \( v \) toluene during Phase 3 due to limited flow increments available using the syringe pumps. Loading conditions for each biofilter system during the various phases are summarized in Table 7.1. All experiments were carried out at ambient laboratory temperature of 23±2°C.

**Table 7.1: Summary of biofilter loading conditions.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Buffered biofilter</th>
<th>Unbuffered biofilter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phase 1</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Period of operation (days)</td>
<td>0-40</td>
<td>41-60</td>
</tr>
<tr>
<td>EBRT of biofilter (sec)</td>
<td>58</td>
<td>29</td>
</tr>
<tr>
<td>EBRT of carbon column (sec)</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>(^1) Influent acetone concentration (ppm,(v))</td>
<td>430</td>
<td>430</td>
</tr>
<tr>
<td>(^1) Influent toluene concentration (ppm,(v))</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>(^2) Target daily biofilter acetone loading (g⋅m(^{-3})⋅d(^{-1}))</td>
<td>506</td>
<td>1012</td>
</tr>
<tr>
<td>(^2) Target daily biofilter toluene loading (g⋅m(^{-3})⋅d(^{-1}))</td>
<td>185</td>
<td>370</td>
</tr>
<tr>
<td>(^2) Target total daily VOC loading (g⋅m(^{-3})⋅d(^{-1}))</td>
<td>691</td>
<td>1382</td>
</tr>
<tr>
<td>(^3) Contaminant loading interval (hr/day)</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

\(^1\) The influent concentration is the target value entering the activated carbon column in the case of the buffered system and the target value entering the humidification column in the case of the unbuffered biofilter.

\(^2\) Target daily biofilter contaminant loading is calculated as the mass of contaminants delivered by the syringe pumps per day divided by the packed bed volume of the biofilter. Because contaminants were supplied during only one third of the cycle length (i.e., 8 hr/day), the instantaneous loading rate during the 8 hr loading period was equal to three times the daily loading rate.

\(^3\) During all loading conditions, contaminants were supplied in the influent gas stream for 8 hr/day. During the remaining 16 hr/day, contaminants were not supplied; however, uncontaminated air continued to flow through the system at the same rate as during the loading period.

To avoid a lengthy start-up interval during which abiotic adsorption to GAC would account for a large percentage of contaminant removal in the buffered system, the activated carbon column was pre-loaded with contaminants prior to biofilter startup. In the pre-loading
interval, the activated carbon column was operated with the same daily loading conditions as in Phase 1 described above. Biofilter start-up did not occur until after the patterns of effluent acetone and toluene concentrations exiting the GAC column were similar in a day-to-day comparison.

7.2.3.2. **Analysis of Biofilter Performances**

Due to the dynamic changes of both the biofilter inlet and outlet contaminant concentrations, removal efficiency of each contaminant in the biofilter for each 24-hour loading cycle was determined by calculating the percent difference between the mass of contaminant entering and exiting the biofilter column during the entire 24-hour loading cycle.

The mass of contaminant entering or exiting each biofilter column per day was calculated by integrating the humidification or biofilter outlet contaminant mass flow rates (g/h) (converted from humidification or biofilter outlet contaminant concentrations measured on either a 0.5 or 1 hour interval basis) over the 24-hour loading cycle using the trapezoidal rule.

For each biofilter, the biofilter daily contaminant mass loading rate (volumetric basis) was defined as the measured mass of contaminants exiting the humidification column per cubic meter of biofilter packed bed volume per day. For the buffered biofilter, this is equivalent to the measured mass of contaminants exiting the GAC column per cubic meter of biofilter packed bed volume per day.

7.2.3.3. **Axial Concentration Profile Studies**

Concentration profiles of toluene, acetone and CO\textsubscript{2} along the depth of each biofilter were measured on various days during each of the three phases of operation. Due to the dynamic changes of inlet concentrations for each biofilter, axial profile studies for the unbuffered biofilter were made during the last hour of the loading period when the inlet concentration had reached its
peak concentration. Axial profile studies for the buffered biofilter were made when the inlet acetone concentration was either at its minimum or maximum concentration.

### 7.2.3.4. Toluene Elimination Capacity Tests

At the end of Phase 3, a series of elimination capacity tests on toluene were performed on the buffered biofilter under an EBRT of 17 seconds, and results were compared to the unbuffered biofilter tested under the same conditions. To further investigate whether substrate interactions exist, toluene elimination capacities in both biofilters were measured under two scenarios: in the presence of high concentrations of acetone (430 ppm) and in the absence of acetone.

During the EC tests on the buffered system, air flow bypassed the activated carbon and entered directly into the humidification column so that toluene was not buffered prior to biofilter treatment. During the tests in the presence of acetone, acetone was preloaded for 3 hours prior to step increase of toluene concentration to ensure that absorption of acetone in the humidification column had reached equilibrium and the biofilter inlet acetone concentration remained near the target level of 430 ppm, throughout the elimination capacity test. At a fixed EBRT of 17 seconds, the toluene concentration was increased stepwise from 8.7 to 600 ppm. The biofilter was operated at each contaminant concentration for approximately 40 minutes with on-line measurement of VOCs and CO₂ sampled every minute. Effluent toluene concentrations averaged over the last 5 minutes were used for calculating the elimination capacity. Continuous measurement of the effluent indicated that the biofilter has reached a steady-state removal of toluene approximately 15 minutes after each loading was applied.

The mass loading rate (volumetric basis), \( L \), and elimination capacity, \( EC \), were calculated using Equations 7.1 and 7.2 as follows:

\[
L = \frac{Q \times C_{in}}{V} \tag{7.1}
\]
\[ EC = \frac{Q \times (C_{in} - C_{out})}{V} \] (7.2)

Where: \( Q \) is the air flow rate (m³ h⁻¹), \( C_{in} \) and \( C_{out} \) are the inlet and outlet concentrations (g m⁻³), \( V \) is the biofilter packed bed volume (m³), and \( L \) and \( EC \) are expressed as grams of pollutant per cubic meter of packing medium per hour (g m⁻³ h⁻¹).

Two sample student-t tests were performed on the maximum elimination capacities between different loading scenarios measured during the last 6 loading rates under each loading scenario using SAS 8.0 (SAS Institute Inc., Cary, NC).

7.2.3.5. Nutrient Addition, Biomass Distribution Studies, and Head Loss

To supply nutrients, on day 8 of biofilter operation, 2.0 L of freshly-prepared nutrient solution identical in composition to that used to grow the initial inoculum was added to the column from the top of each column. Then, the columns were drained by gravity for a period of approximately 15 minutes before being re-started. On day 9, nutrients were added by filling each of the biofilter columns with freshly-prepared nutrient solution. Then, the columns were drained by gravity for approximately 15 minutes before they were restored to normal operation. This latter nutrient addition procedure was repeated at approximately three-day intervals for the remainder of biofilter operation, except that on day 72 for the buffered biofilter and day 73 for the unbuffered biofilter, nutrient solution with a different nitrogen source (use of ammonium nitrate (27.6 g/L) in place of sodium nitrate; with other components remaining identical) was added and filled each biofilter column before drained by gravity, which was followed by nutrient addition with original nitrogen source 12 hours later.

During each phase of operation, the spatial distribution of biomass within each biofilter was estimated using a water displacement procedure as described previously in Chapter 4 (see Section 4.2.3). The volume of wet biomass was calculated by Equation 4.1. The calculated
volume of wet biomass of each section was then divided by the total volume of each empty section in order to obtain the volume of wet biomass per unit volume of packed bed (cm$^3$ L$^{-1}$ packed bed). At the end of each of the three phases of biofilter operation, pressure drop across the biofilter’s packed bed depth was measured using a water manometer with head loss recorded to the nearest 1 mm.

7.2.4. Analytical Techniques

Acetone, toluene, and CO$_2$ concentrations in the air entering and leaving the activated-carbon column and the biofilter were measured on-line using a model 1312 photoacoustic multigas monitor (California Analytical Instruments, Orange, CA) equipped with four optical filters (UA# 0971, 0974, 0983, and SB0527). Gas streams were passed through a model 130 Genie membrane filter (A+ Corporation, Prairieville, LA) to remove excess moisture prior to analysis. The instrument was calibrated using concentrations of 0, 800 ppm$_v$ acetone; 100, 46.6, and 11 ppm$_v$ toluene; and 0, 101, 505, and 1010 ppm$_v$ CO$_2$. Because the concentration of CO$_2$ in the biofilter influent fluctuated over time, results are reported indicating the concentration of CO$_2$ above the inlet concentration (i.e., CO$_2$ production in the biofilters).

Relative humidity was measured using a Traceable® Digital Hydrometer/Thermometer (Fisher Scientific, Pittsburgh, PA). TSS concentrations were measured using Standard Methods (APHA, 1998).

7.3. Results and Discussion

7.3.1. Buffering Effect of Humidification Systems and GAC Column

Typical contaminant concentrations measured over 48-hr periods (i.e., two sequential loading cycles) at spatial locations prior to the biofilter inlets during the three different phases of biofilter operation are illustrated in Figures 7.2 and 7.3 for the unbuffered and buffered systems, respectively. For the buffered biofilter, data depicted in Figure 7.3 represented the contaminant
concentrations measured after adsorption of both acetone and toluene reached quasi-steady state at each phase. Time zero in both figures corresponds to start of an 8-hr loading period. As shown, measured influent acetone and toluene concentrations closely matched target concentrations (within 5%).

The humidification column located in series prior to the unbuffered biofilter somewhat dampened loading for acetone but not toluene (see Figure 7.2). This is consistent with a previous report that scrubbing water in a packed bed humidification system can dampen concentrations of highly water soluble compounds with low Henry’s Law constants (e.g., acetone) but is ineffective for less water soluble compounds with higher Henry’s Law constants (e.g., toluene) (Al-Reyes et al., 2003). In this case, the volume of water recirculated in the humidification system was rather small (approximately 1.3 L); therefore, the load dampening effect was exhausted rather quickly and contaminant concentrations entering the biofilter were essentially the same as those entering the system for a portion of the loading interval each day.

As air flow rates and contaminant mass flow rates increased from Phases 1 to 2 and to 3, the time for acetone to reach equilibrium in absorption and desorption in the water was consequently shortened. Humidification outlet acetone concentrations equaled the influent approximately 5, 3, and 2 hours after the onset of the daily 8 hr loading interval; and subsequently decreased to below detection approximately 6, 4, and 2.5 hours after the loading was terminated during Phases 1, 2, and 3, respectively. On a daily basis, approximately 21, 10.5, and 7.7% of the corresponding inlet acetone loadings were absorbed to the water in the humidification column before reaching equilibrium and were subsequently completely desorbed after the loadings were finished during Phases 1, 2, and 3, respectively. The daily mass of
contaminants exiting the humidification column was essentially the same as that entering the column (within 1%) indicating excellent mass balance closure.

As depicted in Figure 7.3, for the buffered biofilter system, the GAC column markedly buffered the dynamic loadings for toluene during all the three phases. The dynamic loading of acetone was also buffered by the GAC column but to a lesser extent than toluene, and the dampening decreased as EBCT decreased. The buffered toluene concentration exiting the GAC column remained relatively stable at around one third of the inlet concentration (i.e., 33 ppm\textsubscript{v}) during both loading and non-loading periods of Phases 1 and 2. A slight fluctuation in the buffered toluene concentration per loading cycle was observed during Phase 3, which ranged from a low of 30.8 ppm\textsubscript{v} to a high of 41.9 ppm\textsubscript{v}. For acetone, there was substantial fluctuation in

Figure 7.2: Contaminant concentrations measured at spatial locations prior to the inlet of the unbuffered biofilter during Phase 1 (top, data from days 37 and 38), Phase 2 (middle, data from days 55 and 56), and Phase 3 (bottom, data from days 68 and 69).
the GAC column outlet concentration per loading cycle during each phase, yet the pattern of such fluctuation per cycle showed very consistent reproducibility on a day-to-day comparison once quasi steady-state was reached. During each 24-hour loading cycle, minimum GAC outlet acetone concentration reached approximately 48, 15.7, and 1.2 ppm, during Phases 1, 2, and 3, respectively. Maximum GAC outlet acetone concentration reached approximately 275, 403, and 443 ppm during Phases 1, 2, and 3, respectively. At each quasi-steady state condition, the difference between the daily mass of each contaminant entering and exiting the GAC column was within 3% determined by integration.

Figure 7.3: Toluene (left) and acetone (right) concentrations measured at spatial locations prior to the inlet of the buffered biofilter during Phase 1 (top, data from days 28 and 29); Phase 2 (middle, data from days 56 and 57); and Phase 3 (bottom, data from days 69 and 70).
Interestingly, acetone concentrations exiting the GAC column were out of phase with the inlet concentrations during Phase 1. This was caused by a unique combination of column dimensions, gas flow rate, contaminant loading rate, and interval of contaminant loading, and would not be expected under all circumstances. Acetone concentrations exiting the GAC column were further attenuated after passing through the humidification column, however, because the volume of recirculated water was small, the dampening effect was rather small. The daily mass of contaminants exiting each humidification outlet was within 1% of that entering the humidification column, indicating excellent mass balance closure.

The difference in the degree of dampening observed for acetone and toluene can be attributed to several different phenomena. First, the influent concentration and loading rate were several times higher for acetone than for toluene, the degree of load dampening is expected to decrease at higher influent concentrations as described in Chapter 6. Second, as previously discussed in Chapter 6, toluene has characteristics (e.g., non-polar, higher molecular weight and higher boiling point) favoring gas-phase adsorption onto activated carbon in comparison to acetone (Chiang et al., 2001). Therefore, in competitive sorption, toluene would be expected to displace acetone until steady state is reached, as has been experimentally tested in the previously described abiotic binary mixture adsorption tests (see Figure 6.15, Chapter 6). This also explains the fact that the mass of acetone exiting the GAC column exceeded that entering the column immediately after there was an increase in the loading rates when the phase changed. Acetone was less attenuated at higher gas flow rates, consistent with the observation that sharper breakthrough curves occur at lower EBCTs in continuously loaded systems (Mansour et al., 1985).
As depicted in Figure 7.3, acetone was dampened to a greater extent in comparison to the attenuation of acetone shown in the abiotic binary mixture adsorption test depicted in Figure 6.15 (see Chapter 6). This difference can be partially attributed to the lower feed concentration of toluene than that used in the previously described binary mixture adsorption test (i.e., 100 vs. 550 ppm\(_v\)). At higher concentrations (see Figure 6.15), the more strongly adsorbed component (toluene) has a greater competitive edge and consequently displaces more of the weakly adsorbed component (acetone) (Mansour et al., 1984). Model simulations demonstrated that the magnitude of the initial “rollup” for acetone (before quasi-steady state of acetone adsorption is reached) increases in the presence of higher inlet toluene concentration (data not shown).

Because of load equalization by the GAC column, peak contaminant loading to the buffered biofilter, especially for toluene, was substantially lower than peak loading to the unbuffered biofilter. Because numerous reports suggest that aromatic compounds (e.g., toluene) are more slowly degraded than ketones (e.g., acetone) in biofilters (Webster et al., 1998; Song et al., 2003; Qi et al., 2004), decreasing the peak loading of toluene may be beneficial for biofilter treatment even if acetone is less attenuated. Over the 82 days of operation, the difference between the total mass of each contaminant entering and exiting the GAC column was 0.5% and 4% for acetone and toluene, respectively. Considering analytical error associated with gas measurements and the integration method employed in calculations, these data indicate a good closure on contaminant mass balance for the GAC column. These data, supported by fixed-bed sorption tests and model results described in Chapter 6, demonstrate the success of the GAC buffering system in redistributing contaminant concentrations more evenly as a function of time. Eventual exhaustion of the GAC bed is not expected under these intermittent loading conditions because the contaminant mass temporarily accumulated in the GAC column can be desorbed.
within a sufficiently short time interval (i.e., during each loading cycle). Thus, application of
this technique is amenable to passive operation and can be implemented without the need for
active operator control or more aggressive means of carbon regeneration (e.g., heating).

7.3.2. Biofilter Performance Comparison

7.3.2.1. Buffered Biofilter

Figure 7.4 (top) and Figure 7.4 (bottom) depict daily contaminant loading rates and
contaminant removal efficiencies for the buffered biofilter during three phases of operation,
respectively. Data points depicted in Figure 7.4 (top) represent the measured biofilter daily
contaminant loading rate defined as the measured daily mass of contaminants exiting the GAC
column divided by biofilter packed bed volume (see Section 7.2.3.2 for detailed definition).
Lines depicted in Figure 7.4 (top) represent target contaminant loading rates as previously
defined in Table 7.1.

Figure 7.4: Loading rate (top) and removal efficiency (bottom) in the carbon buffered
biofilter.
At the start-up of the buffered biofilter (day 1), the measured daily biofilter acetone loading rate was 4% lower than the target loading. Considering analytical errors associated with gas measurements and the integration method employed in calculations, this small difference suggests that when the buffered biofilter was started, the preloaded GAC column had essentially reached quasi steady-state for acetone adsorption (i.e., mass of acetone adsorbed by the GAC column per loading cycle is equal to the mass of acetone desorbed per loading cycle). On the other hand, the measured daily biofilter toluene loading rate was approximately 17.8% lower than the target level on day 1. However, the measured daily biofilter toluene loading rate gradually increased and equaled the target level after day 24. This indicates that toluene adsorption and desorption approached quasi-steady state on a daily basis within 25 days after the biofilter was started.

At the start of Phases 2 (day 41) and Phase 3 (day 61) when both acetone and toluene loading rates increased to twice those of the previous phase, the measured daily biofilter acetone loading rate increased slightly above the target level (<10%), indicating more mass of acetone exited the GAC column than that entered the GAC column when the phase changed; meanwhile, the measured daily biofilter toluene loading rate decreased slightly below the target level (<12.5%) indicating less mass of toluene exiting the GAC column than that entering the GAC column when the phase changed. However, both acetone and toluene regained quasi-steady state in adsorption and desorption within 5 days after a change in phase.

The procedure used to calculate the removal efficiencies depicted in Figure 7.4 (bottom) explicitly accounts for contaminant removal only by the biofilter. Because a portion of the influent toluene mass was still accumulated daily by the GAC column within 25 days after the buffered biofilter was started up during Phase 1, toluene contaminant removal considering the
entire integrated system was somewhat higher than the removal efficiency for just the biofilter portion of the system depicted in Figure 7.4 (bottom).

As shown in Figure 7.4 (bottom), when the biofilter was operated under an EBRT of 58 seconds during Phase 1, during the first day following biofilter start-up, 32% of the acetone and 16% of the toluene entering the biofilter were removed by the biofilter. During this start-up cycle, acetone was first detected in the biofilter outlet 30 minutes after loading began. The zero breakthrough of acetone during the first 30 minutes was primarily due to absorption to water in the humidification column, absorption to water associated with nutrient solution retained by the packing medium, and adsorption to the biofilter foam packing medium. This attenuation mechanism was depleted quickly because the volume of water either recirculated in the humidification column or retained in the packing medium was small, and the polyurethane packing medium itself provided minimal adsorption capacity. For the remainder of day 1, acetone concentration measured at the biofilter outlet was consistently 15 to 25 ppm\textsubscript{v} below the inlet concentration, and the effluent CO\textsubscript{2} concentration was higher than the inlet. Both of these factors indicate that acetone biodegradation was occurring even in the period immediately following startup. The effluent toluene concentration reached a value near that of the biofilter inlet shortly after start-up and remained relatively constant until a slight decrease in toluene concentration was observed starting 12 hours after start-up. Outlet CO\textsubscript{2} concentration increased gradually from 25 to 65 ppm\textsubscript{v} above the inlet CO\textsubscript{2} concentration.

As shown, during the week following biofilter start-up, performance of both biofilters decreased over time. On day 9, a nutrient addition procedure was conducted, and performance of both biofilters began to improve. Thereafter, nutrients were added at approximately 3-day
intervals. It is evident that a sufficient nutrient supply is vital for rapid biofilter start-up as reported previously (Moe and Irvine, 2001).

By day 20, the buffered biofilter removed >99% of both contaminants and remained at that level until the end of Phase 1. There was a small but noticeable decrease in performance of the buffered biofilter immediately after contaminant loading rate was doubled (EBRT decreased to 29 sec) at the start of Phase 2. The system quickly acclimated to the new loading condition, and high removal efficiency (>98% for acetone and >99% for toluene) was observed until the end of Phase 2. At the start of Phase 3, when the contaminant loading rate was doubled again (EBRT decreased to 14.5 sec), there was no decrease in removal efficiency for toluene (>99%). However, appreciable breakthrough of acetone was observed during a portion of the loading cycle when the biofilter inlet acetone concentration was high. Acetone removal efficiency declined to 66.4% on the first day of Phase 3 (day 61) and increased gradually to 78.5% by day 71. A substantial decrease in acetone and toluene removal was observed on days 72 and 73. Such deterioration, however, was due to inhibitory effects of high concentration of ammonium on the degradation of VOCs in a failed attempt to minimize possible nutrient limitation, if any, by adding nutrient with ammonium nitrate as alternate nitrogen source on day 72. 12 hours after such inhibitory effect was noticed, the biofilter column was filled with nutrient of original formula that used sodium nitrate as nitrogen source and was drained after 15 minutes to abate such inhibitory effects of ammonium. Afterwards, performance began to recover immediately and reached 83 and 98% removal efficiencies for acetone and toluene, respectively by day 75. Toluene remained completely degraded since and the system leveled with an average of 87% removal efficiency for acetone throughout the remainder of Phase 3.
Typical VOCs and CO$_2$ concentrations exiting the buffered biofilter for each phase of operation are depicted in Figure 7.5. In particular, VOCs and CO$_2$ concentrations both during and after biofilter start-up period were investigated in Phase 1. CO$_2$ concentrations depicted in the figure are concentrations above the inlet (i.e., CO$_2$ production within the biofilter).

During the startup period before complete removals of both compounds were achieved, a consistent pattern of VOC removal was observed from days 9 to 19. Figure 7.5a depicts biofilter inlet and outlet concentrations of both contaminants on days 18 and 19. During intervals when the acetone concentration entering the biofilter was low, complete acetone and toluene removal were observed. However, as the acetone concentration entering the biofilter increased, the acetone loading rate exceeded the biological reaction capacity of the system and acetone breakthrough occurred until after the inlet acetone concentration decreased again. Although the buffered inlet toluene concentration remained essentially constant as a function of time as previously described in Figure 7.3, toluene breakthrough occurred concurrently with breakthrough in acetone when acetone loading was high. The magnitude of breakthrough of each compound, however, decreased gradually over the days until no breakthrough of either compound was observed after day 20. This suggests that during the start-up period of the buffered biofilter, there was a relationship between acetone and toluene degradation, and toluene degradation was adversely affected when acetone was not completely removed from the biofilter.

As depicted in Figure 7.5 (b, c, and d), after the start-up period, during each phase, toluene removal was essentially complete under the reasonably steady inlet concentrations achieved by GAC buffering. CO$_2$ production increased as influent acetone concentration increased. During Phases 1 and 2, although there was variation in acetone loading as a function of time each loading cycle, the buffered biofilter was able to exhibit a sufficiently high
Figure 7.5: Typical effluent VOCs and CO\textsubscript{2} concentrations for the buffered biofilter during the start-up period of Phase 1 (a); after the start-up period of Phase 1 (b); Phase 2 (c); and Phase 3 (d).
During Phase 3, however, acetone was transiently detected for approximately 6 hr/day during intervals of high acetone loading, apparently, acetone loading rate during such a portion of the loading cycle exceeded the system’s maximum biodegradation capacity (see Figure 7.5d). The system reached a maximum instantaneous acetone elimination capacity of around 185 g m\(^{-3}\) h\(^{-1}\) during peak loadings at the end of Phase 3, which is comparable to the maximum acetone elimination capacity reported elsewhere (Devinny et al., 1999). This difference in acetone removal between different phases is consistent with the degree of buffering observed in the system. During Phases 1 and 2, peak acetone concentration entering the biofilter was appreciably lower than that entering the GAC column (i.e., activated carbon attenuated peak acetone loading). With a further decrease in EBRT in Phase 3, peak acetone concentration entering the biofilter was essentially the same as that entering the GAC column (see Figure 7.3 c). In cases where greater than 95% removal efficiency of both compounds on a continuous basis is required, it is suggested that a larger water reservoir in humidification column be used to increase further dampening of acetone by absorption prior to biofilter treatment.

### 7.3.2.2. Unbuffered Biofilter

Figure 7.6 (top) and Figure 7.6 (bottom) depict the loadings and removal efficiencies, respectively for the unbuffered biofilter during the three phases. As shown in Figure 7.6 (top), contaminant loading to the unbuffered system on a daily basis was essentially the same with the buffered system during Phases 1 and 2, and the loading of the unbuffered biofilter was purposefully adjusted to be 16.7% less than the buffered biofilter during Phase 3 so that the unbuffered biofilter had the same total EBRT (17 seconds) as the activated carbon column plus the buffered biofilter in the buffered system. The two systems differed greatly, however, in terms of the fraction of time during which contaminants entered the biofilter.
Figure 7.6: Loading rate (top) and removal efficiency (bottom) in the unbuffered biofilter.

In contrast to relatively rapid startup and high contaminant removal efficiency observed in the buffered biofilter, the unbuffered biofilter exhibited lower treatment performance of both compounds over the entire interval of study. As shown in Figure 7.6 (bottom), during Phase 1, while acetone removal efficiency increased gradually from 7.4% on day 1 to 93% on day 40 (at the end of Phase 1), the unbuffered biofilter system exhibited markedly lower removal efficiency for toluene than did the buffered system. The system removed only 10.5% of the influent toluene on day 1 and 40.5% of the toluene averaged over the last three days of Phase 1. At the start of Phase 2 (day 41), when the EBRT was decreased to 29 seconds, acetone and toluene removal efficiency declined to 46.8% and 28.8%, respectively. The system removed 67.6% of the acetone and 58.1% of the toluene averaged over days 55, 56 and 59, at the end of Phase 2. At the start of Phase 3 (day 61), when the EBRT was decreased to 17 seconds, acetone and toluene removal efficiency declined to 44.7% and 48.0%, respectively. The system removed
80.4% of the acetone and 54% of the toluene averaged over days 79, 80, and 82, at the end of Phase 3. Performance worsened on days 73, 74, and 75, due to the same inhibitory effects of ammonium nitrate as described previously.

Toluene, acetone, and CO$_2$ concentrations exiting the unbuffered biofilter measured on different days during each phase of operation are shown in Figure 7.7. During all three phases, toluene breakthrough was observed shortly after start of contaminant loading each day. Acetone breakthrough, somewhat delayed by attenuation in the humidification system (see Figure 7.2), was consistently observed. An increase in CO$_2$ production rate observed as acetone and toluene concentrations entering the biofilter increased indicates that the microbial population was able to rapidly increase its contaminant degradation rate; however, effluent contaminant concentration data demonstrate that the biofilter’s increased degradation rate was insufficient to remove all of the contaminants at the rate they entered the system. During all three phases of operation, there was a small but noticeable decrease in effluent VOC concentrations over time during the loading period after inlet VOC concentrations stabilized at their peak influent concentrations. This suggests that the 16-hr/day non-loading interval was a sufficiently long starvation period to result in decreased activity of toluene and acetone degrading microorganisms. Diminished performance following starvation conditions is consistent with previous reports on biofilter performance following non-loading periods (Martin and Loehr, 1996; Cox and Deshusses, 2002; Dirk-Faitakis and Allen, 2003). Such effects were not observed in the buffered biofilter where contaminants entered the biofilter more uniformly as a function of time due to the attenuation achieved by the GAC column.

At the end of experiments described above, an additional short-term test was conducted to further evaluate the effect of the GAC buffering system. The GAC column initially in series
with the buffered biofilter was moved to a location in series prior to the humidification system in the previously unbuffered biofilter. Performance was evaluated over a two-day period. Overall performance improved dramatically, with average toluene and acetone removal efficiency reaching 98% and 84%, respectively. This performance is comparable to that observed in the originally buffered system, and it provides further confirmation that load equalization provided by the GAC column accounted for the superior performance observed in the buffered system.

Figure 7.7: Typical effluent VOCs and CO$_2$ concentrations for the unbuffered biofilter during Phase 1 (a), Phase 2 (b), and Phase 3 (c).

7.3.3. Carbon Balance in the Biofilter Systems

A mass balance on carbon was conducted for each biofilter accounting for CO$_2$, acetone, and toluene entering and exiting the systems in the gas phase during the entire period of operation (82 days), and biomass present at the end of the experiments.
The total mass of carbon entering each biofilter as acetone during the entire period of operation (hereafter referred to as acetone-C) was obtained by integrating the area under the curve of the daily mass loading rate of acetone entering the biofilter system (g m$^{-3}$ d$^{-1}$) (shown in Figures 7.4a and 7.6a for the buffered and unbuffered biofilters, respectively) over the entire period of operation (days), then multiplied by the biofilter bed volume (m$^3$), and finally, multiplied by the grams of carbon per gram of acetone (36 g acetone-C per 58 g acetone). Likewise, the total mass of carbon entering each biofilter as toluene during the entire period of operation (hereafter referred to as toluene-C) was obtained by integrating the area under the curve of the daily mass loading rate of toluene entering the biofilter system (g m$^{-3}$ d$^{-1}$) (shown in Figures 7.4a and 7.6a for the buffered and unbuffered biofilters, respectively) over the entire period of operation (days), then multiplied by the bed volume (m$^3$), and finally, multiplied by the grams of carbon per gram of toluene (84 g toluene-C per 92 g toluene). The total mass of carbon entering each biofilter as CO$_2$ (hereafter referred to as CO$_2$-C) was calculated based on an averaged inlet CO$_2$ concentration of 440 ppm$_v$. The total mass of acetone-C, toluene-C, and CO$_2$-C entering the buffered biofilter was 594.1, 305.3, and 604.6 g, respectively. The mass of acetone-C, toluene-C, and CO$_2$-C entering the unbuffered biofilter was 539.2, 290.6, and 559.1 g, respectively. The less mass of constituents entering the unbuffered biofilter was due to the fact that the mass loading rates in the unbuffered biofilter were purposefully adjusted to be lower (i.e., the gas flow rate was lower) than the buffered biofilter during Phase 3 operation.

The total mass of acetone-C escaping each column during the entire period of operation was approximated by calculating the mass of acetone-C escaping the column for each day data were collected, and then integrating them over the entire period of operation using the trapezoidal rule. The total mass of toluene-C, and CO$_2$-C escaping each column during the entire
period of operation were calculated likewise. The total mass of acetone-C, toluene-C, and CO$_2$-C escaping the buffered biofilter was 107.8, 18.0, and 1260.2 g, respectively during the entire period of operation. The total mass of acetone-C, toluene-C, and CO$_2$-C escaping the unbuffered biofilter was 243.1, 174.4, and 981.3 g, respectively during the entire period of operation.

To calculate an estimate of the mass of carbon present in biomass at the end of the experiments, it was assumed that: 1) the mass of biomass-C present at the start of the experiment (from the inoculum) and the quantity of biomass-C removed from the system during nutrient addition procedures were assumed to be negligible; 2) the wet biomass had a density of 1100 g/L (consistent with the range of biomass densities reported by Tchobanoglous and Schroeder, 1987); 3) the wet biomass was 92% water on a mass basis (based on previous measurements of biofilter samples, Atoche, 2003); and 4) biomass was 53% carbon on a dry mass basis (consistent with the range of previous measurements of the elemental composition of biomass produced in biofilters containing polyurethane foam packing media reported by Moe and Irvine (2001). Using these assumptions and the volume of wet biomass present at the end of the experiments as determined from water displacement studies (3001.1 and 2060.8 mL for buffered and unbuffered biofilters, respectively, see Figure 7.18), the mass of biomass-C produced in the buffered and unbuffered biofilter was calculated to be 140.0 and 96.1 g, respectively.

Figure 7.8 depicts the total mass of acetone-C, toluene-C, CO$_2$-C entering each biofilter (denoted as C-in), and total mass of acetone-C, toluene-C, CO$_2$-C escaping each biofilter plus the mass of biomass-C present in each biofilter at the end of the experiments (denoted as C-out) for the 82 days of operation.

For the buffered biofilter, the total C-in and C-out during the entire period of operation was 1504 and 1526 g, respectively. For the unbuffered biofilter, total C-in and C-out during the
entire period of operation was 1389 and 1495 g, respectively. The total C-out was 1.5 and 7.6% greater than the total C-in for the buffered and unbuffered biofilter, respectively. Taking into account the assumptions used in making these calculations, the percentage difference between the mass of carbon entering and exiting the biofilter systems represents reasonable closure on the carbon balance.

![Carbon Mass Balance](image)

**Figure 7.8: Carbon Mass Balance.** C-in includes total mass of CO\(_2\)-C, acetone-C, and toluene-C entering the biofilter column. C-out includes total mass of CO\(_2\)-C, acetone-C, and toluene-C exiting the biofilter column plus the biomass-C present in the column at the end of the experiments.

During the entire period of operation, the total mass of acetone degraded by the buffered and unbuffered biofilter (calculated as the total mass of acetone influent minus total mass of acetone effluent) was 486.3 and 296.1 g, respectively. The total mass of toluene degraded by the buffered and unbuffered biofilter was 287.3 and 116.2 g, respectively. Using balanced stoichiometric equations for mineralization of acetone and toluene (see equations 7.4 and 7.5), the COD equivalent of the degraded contaminants was calculated. The total mass of COD degraded by the system was 1974.0 and 1018.1 g COD, respectively for the buffered and unbuffered biofilter.
\[
\begin{align*}
(-1 \text{ g}) \text{C}_3\text{H}_6\text{O} + (-2.21 \text{ g}) \text{O}_2 & \rightarrow (2.28 \text{ g}) \text{CO}_2 + (0.93 \text{ g}) \text{H}_2\text{O} \\
(-1 \text{ g}) \text{C}_7\text{H}_8 + (-3.13 \text{ g}) \text{O}_2 & \rightarrow (3.347 \text{ g}) \text{CO}_2 + (0.782 \text{ g}) \text{H}_2\text{O}
\end{align*}
\] (7.4) (7.5)

The estimated yield of biomass in the two systems was then calculated by dividing the total mass of dry biomass present at the end of the experiment by the total mass of COD degraded by each biofilter system. Therefore, the calculated yield of biomass for the buffered biofilter was approximately 0.133 g biomass (dry basis) produced per 1.0 g COD consumed. For the unbuffered biofilter, the calculated yield of biomass was approximately 0.178 g biomass (dry basis) produced per 1.0 g COD consumed. More than 80% of the carbon associated with acetone or toluene removed by the biofilters was transformed to CO$_2$.

### 7.3.4. Axial VOC and CO$_2$ Concentration Profile Study

Concentration profiles of toluene, acetone and CO$_2$ along the depth of each biofilter were measured on different days during each phase in order to better understand the degradation rate of each compound and whether there is VOC interactions in each column. Axial profile studies for the unbuffered biofilter measured during the last hour of the loading period when the inlet concentration had reached its peak are depicted in Figures 7.9, 7.10, and 7.11 for Phases 1, 2, and 3, respectively. Axial profile studies for the buffered biofilter measured when the inlet acetone concentration was either at its minimum or maximum are depicted in Figures 7.12, 7.13, and 7.14 for Phases 1, 2, and 3, respectively.

As depicted in Figures 7.9 to 7.11, for the unbuffered biofilter, during each phase, the concentration profile for both acetone and toluene shifted gradually, with an increasing degradation rate over time. Contaminants were removed as a relatively linear function of bed depth in the column. Correspondingly, the CO$_2$ concentration increased relatively linearly along the bed depth.
Figure 7.9: Axial VOC and CO$_2$ concentration profiles in the unbuffered biofilter during Phase 1.

Figure 7.10: Axial VOC and CO$_2$ concentration profiles in the unbuffered biofilter during Phase 2.
Figure 7.11: Axial VOC and CO$_2$ concentration profiles in the unbuffered biofilter during Phase 3.

As depicted in Figures 7.12 to 7.14, for the buffered system, when the acetone concentration entering the biofilter was at its minimum daily concentration (i.e., approximately 50, 15, 2 ppm, for Phases 1, 2, and 3, respectively), acetone was readily removed within the first section of the column. In the presence of low concentrations of acetone, complete removal of toluene was achieved before exiting the last column section during each phase of operation.

When the acetone concentration was at its maximum (i.e., approximately 275 ppm$_v$) in the buffered biofilter during Phase 1, both acetone and toluene was quickly removed within the first three sections of the column at the end of Phase 1, with an increasing degradation rate of both compounds over time. During Phase 2, when the acetone concentration was at its maximum value of approximately 400 ppm$_v$, toluene was removed rapidly and relatively linearly within the first three sections of the column, while there was a stepwise removal of acetone throughout the column, with 80% of inlet acetone removed within the first three sections near the inlet and complete elimination by the end of the last section. During Phase 3, toluene was still
steadily and completely removed by the end of the column. There was only partial removal of acetone at its peak concentration of approximately 440 ppm. The system was able to remove approximately 64% of the peak acetone loading by the end of the column.

Finally, in the buffered biofilter, under both minimum and maximum acetone loading conditions, CO₂ concentration during Phases 1 and 2 increased rapidly in the first three sections and leveled off afterwards, indicating that the majority of VOC degradation was achieved in the first three sections of column near the inlet. CO₂ concentration increased along the bed in Phase 3, as contaminant removal occurred throughout the bed height.

Interestingly, the above profile data suggested that toluene removal seemed not adversely affected by the presence of high concentrations of acetone in both buffered and unbuffered biofilters during each phase, which was surprising in the context of several previous reports on the interactions of ketones on the degradation of aromatics in biofilter operations. In treatment of mixtures of VOC contaminants, many researchers have reported that one or more compounds is not degraded until after other compounds have been degraded to very low concentrations. This frequently results in a spatial separation of zones for degradation of different compounds as a function of height in a biofilter bed. For example, during the operation of several laboratory-scale biofilters and biotrickling filters for treatment of a simulated paint spray booth waste stream, Kazenski and Kinney (2000) observed that degradation of toluene and p-xylene did not occur until after methyl n-propyl ketone, n-butyl acetate, and ethyl 3-othoxypropionate reached very low concentrations.

Similar results were reported by Webster et al. (1998) for contaminants commonly found in paint spray booth applications when treated in bench-scale and pilot-scale biotrickling filters. For a mixture of toluene, xylene, methyl ethyl ketone, and n-butyl acetate, although overall
removal efficiencies were satisfactory (greater than 90%), removal of toluene was lower (approximately 70%) at an EBRT of 39 seconds. For treatment of binary mixtures of acetone and toluene as the specific VOCs investigated in the research described in this chapter, there has been only one other study, to date, by Chang and Lu (2003) who reported that in a trickle-bed air biofilter treating toluene and acetone mixtures, a significant reduction of acetone was found only after the depletion of toluene. Further, their research indicated that the inhibition which toluene exerts on the removal of acetone is much stronger than the inhibition exerted by acetone on the removal of toluene. However, it should be noted that their study was investigated under a condition where influent toluene and acetone concentration were 3:1 by volume, with toluene concentrations ranging from 150 to 300 ppm, and EBRTs ranging from 60 to 20 seconds.

Figure 7.12: Axial VOC and CO₂ concentration profiles in the buffered biofilter during Phase 1.
Figure 7.13: Axial VOC and CO\textsubscript{2} concentration profiles in the buffered biofilter during Phase 2.

Figure 7.14: Axial VOC and CO\textsubscript{2} concentration profiles in the buffered biofilter during Phase 3.
7.3.5. **Toluene Elimination Capacity Tests**

In order to define the maximum range of operating conditions for the treatment of toluene in the biofilters, and to further investigate whether substrate interactions exist, toluene elimination capacity tests were conducted for each column under two loading scenarios: in the presence of high concentrations of acetone (at a stable biofilter inlet acetone concentration of 430 ppm\textsubscript{v}) and in the absence of acetone under an EBRT of 17 seconds. Elimination capacities were plotted against the loading rates as shown in Figure 7.15.

![Figure 7.15: Toluene elimination capacity tests in the presence and absence of acetone.](image)

As depicted in Figure 7.15, for the buffered biofilter tested in the presence of a constant acetone concentration of 430 ppm\textsubscript{v}, there was a very high toluene removal rate (greater than 95%) at loading rates as high as 61.8 g m\textsuperscript{-3} h\textsuperscript{-1} (corresponding to 75 ppm\textsubscript{v} inlet toluene). The elimination capacity essentially equaled the loading rate within this range. As the loading increased between 77 and 154 g m\textsuperscript{-3} h\textsuperscript{-1} (corresponding to a range of 95 to 190 ppm\textsubscript{v} inlet toluene), toluene was no longer completely removed, still, the elimination capacity increased to 98.3 g m\textsuperscript{-3} h\textsuperscript{-1} at a loading of 154 g m\textsuperscript{-3} h\textsuperscript{-1}. When the inlet loading increased further from 154 to 463 g m\textsuperscript{-3} h\textsuperscript{-1} (corresponding to an increase of inlet toluene concentration from 190 to 598 ppm\textsubscript{v}),
the EC leveled off at an average of 101 g m\(^{-3}\) h\(^{-1}\) (with a standard deviation of 4.1) measured during the last six loading rates.

When the buffered biofilter was tested in the absence of acetone, at loadings less than 77 g m\(^{-3}\) h\(^{-1}\), elimination capacity was approximately 8.7% lower than compared to the corresponding buffered biofilter EC test in the presence of acetone, still, it increased linearly within this range of loading, approximately 91% of toluene was removed at a loading of 77 g m\(^{-3}\) h\(^{-1}\). The elimination capacity increased to 96 g m\(^{-3}\) h\(^{-1}\) when the loading was increased to 193 g m\(^{-3}\) h\(^{-1}\). When the inlet loading increased further from 193 to 463 g m\(^{-3}\) h\(^{-1}\), the EC leveled off at an average of 102.9 g m\(^{-3}\) h\(^{-1}\) (with a standard deviation of 3.8) measured during the last six loading rates.

For the unbuffered biofilter, there was a lower range of loading conditions than the buffered biofilter in terms of linear complete removal of toluene, both in the presence and absence of acetone, as breakthrough occurred shortly after the loading rate was increased to 23 g m\(^{-3}\) h\(^{-1}\). Elimination capacities under both loading conditions continued to increase before the loading was increased to 270 g m\(^{-3}\) h\(^{-1}\). Afterwards it stabilized at an average of 86.2 g m\(^{-3}\) h\(^{-1}\) (with a standard deviation of 3.5) during the remainder of the increasing loadings when acetone was present. When acetone was not present, the EC reached and remained at an average of 91.9 g m\(^{-3}\) h\(^{-1}\) (with a standard deviation of 3.1) measured during the last six loading rates.

Such response of biofilter elimination capacity to increasing loading conditions has been known to follow the Michaelis-Menten Kinetics (Ottengraf and Van den Oever 1983; Devinny et al., 1999). At low inlet concentrations, especially when the contaminant has low water solubility, as is the case here for toluene, removal of a component is controlled by the rate at which it can reach and penetrate the gas-liquid interfaces, and the rate is proportional to
concentration. As the concentration of the contaminant increases, the rate of degradation increases as well. Therefore, below the critical air phase concentrations, diffusion in the biolayer is the rate limiting factor and the degradation kinetics are first order. In the case of high inlet contaminant concentrations, the pollutant could more fully penetrate the active biofilm, there is no longer diffusion limitation to the wet biolayer, and because removal is limited by the biodegradation rate, the degradation rate became independent of the contaminant concentration. Therefore, degradation kinetics are zero order at high contaminant concentrations. Thus, the elimination capacity of the reactor is constant at a high inlet concentration (Devinny et al., 1999).

The corresponding maximum elimination rate in the biofilm may be limited by the limited transfer of oxygen into the biofilm, availability of nutrients, or any other event in the biodegradation scheme (Deshusses and Johnson, 1999; Song et al., 2003). Furthermore, in some cases, substrate inhibition has been found in bioreactors where the enzyme-catalyzed reaction is diminished by extremely excess substrate (Devinny et al., 1999). However, from Figure 7.15, no substrate inhibition was noticed at inlet concentrations as high as 598 ppm, as the elimination capacities remained relatively stable.

As is easily observed from Figure 7.15, under both the “diffusion limited” and “reaction limited” loading ranges as defined above, the EC of the unbuffered biofilter in the presence of acetone was lower than that at the condition without acetone, whereas there is not much difference in the EC of the buffered biofilter tested with or without acetone. When tested both in the presence or absence of acetone, the buffered biofilter outperformed the unbuffered biofilter. To determine whether the difference was statistically significant, two sample student-t tests were performed on the maximum elimination capacities between different loading scenarios, by taking
into account the mean, standard deviation, or variance of the elimination capacities measured during the last six loading rates under each loading scenario. The results are shown in Table 7.2.

Table 7.2: Statistical analysis of the maximum toluene elimination capacities under different operating scenarios using two sample t-test.

<table>
<thead>
<tr>
<th>Toluene loading scenario</th>
<th>EC with acetone (g m⁻³ h⁻¹)</th>
<th>EC with no acetone (g m⁻³ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>buffered biofilter</td>
<td>101.1±4.1</td>
<td>102.9±3.8</td>
</tr>
<tr>
<td>unbuffered biofilter</td>
<td>86.2±3.5</td>
<td>91.9±3.1</td>
</tr>
</tbody>
</table>

**Two-sample t test**

<table>
<thead>
<tr>
<th>Two-sample t test</th>
<th>p value</th>
<th>Significance (α=0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>buffered biofilter, presence vs. absence of acetone</td>
<td>0.4373</td>
<td>No</td>
</tr>
<tr>
<td>unbuffered biofilter, presence vs. absence of acetone</td>
<td>0.0146</td>
<td>Yes</td>
</tr>
<tr>
<td>buffered vs. unbuffered biofilter both in the presence of acetone</td>
<td>&lt;.0001</td>
<td>Yes</td>
</tr>
<tr>
<td>buffered vs. unbuffered biofilter both in the absence of acetone</td>
<td>0.0002</td>
<td>Yes</td>
</tr>
<tr>
<td>buffered vs. unbuffered biofilter, overall</td>
<td>&lt;.0001</td>
<td>Yes</td>
</tr>
</tbody>
</table>

From the t tests, at a significant level of α=0.05, several conclusions can be drawn. First, for the buffered biofilter, there was no significant difference in the maximum toluene elimination capacity between the conditions when acetone was present and when not present. Second, for the unbuffered biofilter, maximum toluene elimination capacity in the absence of acetone was greater than that in the presence of acetone. Third, the buffered biofilter had a maximum toluene elimination capacity significantly greater than that of the unbuffered biofilter when tested both in the presence and absence of acetone.

From the above analysis, it appears that high concentrations of acetone adversely affected toluene degradation in the unbuffered biofilter, but not in the buffered biofilter. To further investigate contaminant interactions, effluent acetone concentrations versus toluene loading rates during the EC tests were plotted for both biofilters as shown in Figure 7.16. Somewhat surprisingly, for both biofilters, acetone removal was found to be adversely impacted by
increasing toluene loading over a wide range of influent toluene concentrations. This seems to support similar findings by Chang and Lu (2003) as described previously, thus complicating understanding of the interactions between the binary mixture. Further study is required to better understand the relationship between the mixtures and to optimize the system performance.

![Graph showing effluent acetone concentration during toluene elimination capacity tests in the presence of 430 ppm influent acetone.](image)

**Figure 7.16:** Effluent acetone concentration during toluene elimination capacity tests in the presence of 430 ppm, influent acetone.

### 7.3.6. Headloss and Biomass Distribution

Figure 7.17 depicts the spatial distribution of biomass along each section within the two biofilters over time. Figure 7.18 depicts the total volume of biomass accumulated within each column over time. As shown in Figure 7.17, in both biofilters, the biomass within each section increased over time. In the buffered biofilter, biomass was always highest in the first section and decreased gradually along the column. This is consistent with the contaminant removal and CO₂ concentrations measured as a function of height in Figures 7.12, 7.13, and 7.14 which indicate that the majority of contaminant was degraded in the first three sections closest to the inlet. The biomass in the unbuffered biofilter was more uniformly distributed as a function of column height, especially during Phase 1, and 2, compared to that of the buffered biofilter. This is also
consistent with the unbuffered biofilter profile data which indicated that the contaminants were removed more linearly along the entire column (see Figures 7.9, 7.10, and 7.11).

Figure 7.18 shows that the total wet biomass in the buffered biofilter was 27, 43, and 31% higher than that of unbuffered biofilter during Phases 1, 2, and 3, respectively. A plausible explanation for this is that because the buffered biofilter removed an appreciably higher contaminant mass, a higher net yield in biomass would be expected. It should be noted, however, the yield coefficient of the buffered biofilter (0.133 g Biomass/g COD) was still lower than that of the unbuffered biofilter (0.178 g Biomass/g COD), as described previously. The wet biomass occupied 27.5 and 18.8% of the total void space of the foam media, respectively, in the buffered and unbuffered biofilter at the end of the experiments. More biomass accumulation in the buffered biofilter led to a higher pressure drop in the buffered biofilter, as illustrated in Figure 7.19. As the pressure drop through a biofilter bed typically ranges from 20 to 100 Pa/m and can even go up to 980 Pa/m (Devinny et al., 1999), the head loss in the two biofilters both fell in the lower range of typical biofilter operations. In general, the increase in pressure drop as a result of biomass development in biofilters can be explained by a decrease in the bed interparticle void space or the effective porosity or both and by the microbial degradation of the support matrix, as in the case of natural media, which results in decreased specific permeability (Morgan et al., 2001).

Finally, it should to be noted that excess biomass accumulation in the biofilter might cause a major problem for stable long-term performance. Biofilm accumulation decreases the reactor’s porosity, and therefore, causes a gradual decrease in the interfacial surface area of the biofilm exposed to the gas phase, which further results in reductions of mass transfer rates of contaminants across the air-water/biofilm interface (Alonso et al., 1997). Therefore, the positive
effects of biofilm accumulations on biofilter elimination capacities (e.g., the presence of higher cell mass concentrations to degrade contaminants as biofilm accumulates) will be much likely offset by the detrimental effects of mass transfer limitations associated with biofilm accumulation during the long-term operation. Additionally, biomass accumulation might cause channeling and increased pressure drops, which translates into higher operating and maintenance costs that become significant for long-term biofilter operation (Morgan et al., 2001). Ideally, the biofilter system must be designed and operated such that it promotes the degradation of the contaminants, while limiting biomass clogging (Song et al., 2003).

Figure 7.17: Biomass distribution in each biofilter on days 39 (Phase 1), 59 (Phase 2), and 82 (Phase 3).
7.4. Conclusions

Under the discontinuous contaminant loading conditions imposed on the system, a GAC column applied prior to biofilter treatment effectively dampened the dynamic toluene loading to reasonably stable concentration throughout the entire operation under GAC column empty bed residence times (EBCTs) ranging from 2.5 to 10 seconds. The dynamic loading of acetone was also buffered by the GAC column but to a lower degree than toluene, and the dampening decreased as EBCT decreased. Eventual exhaustion of the GAC bed is not expected under these intermittent loading conditions because the contaminant mass temporarily accumulated in the
GAC column can be desorbed within a sufficiently short time interval (i.e., during each loading cycle).

The hybrid buffered system could remove 100%, 99%, and 100% of toluene and 100%, 98%, and 87% of acetone when subjected to progressively increasing discontinuous VOC loading rates of 185, 370, and 740 g m$^{-3}$ d$^{-1}$ for toluene and 506, 1012, and 2024 g m$^{-3}$ d$^{-1}$ for acetone, respectively. In contrast, the unbuffered biofilter removed only 40.5%, 58.1%, and 54% of toluene and 93%, 67.6%, and 80.4% of acetone when subjected to progressively increasing discontinuous VOC loading rates of 185, 370, and 618 g m$^{-3}$ d$^{-1}$ for toluene and 506, 1012, and 1686 g m$^{-3}$ d$^{-1}$ for acetone, respectively.

The buffered system exhibited more stable and higher removal efficiencies of contaminants than the unbuffered biofilter throughout the entire phases of operation, which clearly demonstrates that a passively-operated GAC load-dampening system installed in series before a biofilter is a feasible and superior technology for effectively treating dynamically varying concentrations of acetone and toluene mixtures in contaminated air.

A passively-operated activated carbon buffering system can achieve two advantages in operation of biofilters subjected to intermittent loading: 1) the magnitude of peak loading to the biofilter can be decreased, leading to more complete removal of contaminants in the biofilter; and 2) because contaminant loading to the biofilter is more uniform as a function of time, diminished performance due to starvation conditions as frequently encountered in conventional biofilters can be minimized. Because of competitive adsorption, however, the degree of load equalization achieved by a passively operated GAC system for multi-component contaminants with different physicochemical properties can vary markedly, and this would need to be
accounted for when designing GAC buffering systems for biofilters treating waste streams containing multiple contaminants.
CHAPTER 8  OVERALL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

8.1. Overall Conclusions

Several studies were conducted to evaluate two different design modifications/operating strategies for improving performance of biofilters treating unsteady-state VOCs loadings. In the first approach that was tested, activated carbon was incorporated into the biofilter packing medium and a novel periodic operating strategy, Sequencing Batch Biofilter (SBB) operation, was experimentally tested under “normal” and “shock” loading conditions treating methyl ethyl ketone (MEK) contaminated air streams. The feasibility of SBB operation for treating waste air streams was initially tested using laboratory-manufactured polyurethane foams impregnated with powdered activated carbon as biofilter packing media. To determine the effect of SBB operating strategy on biofilter performance, the SBB operating strategy was further tested in comparison to a conventional continuous flow biofilter (CFB). In these later studies, commercially available activated carbon coated polyurethane foam cubes were used as the packing medium because of their higher sorption capacity compared to those used in initial SBB experiments.

Data presented in Chapters 3 and 4 established that SBB operation is a feasible technology for treating air streams contaminated by low concentrations of MEK. The laboratory-scale SBB was able to achieve greater than 99% contaminant removal when subjected to a loading of 106 ppm, MEK with EBRTs ranging from 15 to 30 seconds. The SBB systems exhibited stable long-term performance when nutrients were added on a monthly or biweekly basis. During transient periods of elevated contaminant loading, the SBB system was able to accumulate MEK during the FEED period and subsequently degrade the accumulated organics during the following REACT period, even after long-term operation. Once an appropriate operating strategy was selected, the SBB was able to remove more than 99% of the influent
MEK at a transient loading rate of 380 g·m⁻³·h⁻¹ and 83% of the influent MEK at a transient loading rate of 760 g·m⁻³·h⁻¹. The operational flexibility of the SBB system facilitated selection of operational conditions that led to higher overall removal efficiency and higher minimum instantaneous removal efficiency than were achieved in the CFB. It was demonstrated that application of an active control strategy (e.g., simultaneously loading more than one biofilter in a multiple-biofilter system), made possible by SBB operation, can result in more complete contaminant removal during the transient period of elevated contaminant loading than would have otherwise occurred. This provides an effective alternative for removing contaminants during transient periods of elevated contaminant loading in cases where on-line instrumentation or sufficient process knowledge exists to allow implementation of process control decisions.

As described in Chapter 5, a DGGE “DNA fingerprinting” technique was successfully applied to evaluate microbial communities in biofilters subjected to continuous flow and sequencing batch loading strategies. Both the SBB and CFB biofilter had stratified microbial community structures along the height of each column. There was a significant difference in the degree of microbial diversity between the bottommost two sections and the upper four sections of the column subjected to continuous loading (i.e., the CFB). On the other hand, there was no significant difference in the degree of microbial diversity along the height of the SBB column. Microbial populations differed more in terms of relative abundance of each species rather than in terms of the species richness (i.e., number of species present) along the height of each column as well as between these two biofilter columns. DGGE analysis suggests that operating strategies imposed on the biofilters imparted a sufficiently large selective pressure to influence microbial community structures.
In the second approach studied for improving biofilter performance, an activated carbon column was placed in series before a biofilter to assess the potential of the integrated system to effectively treat VOC mixtures generated on a discontinuous basis. Abiotic fixed-bed sorption experiments and numerical modeling were conducted to assess the degree of load-equalization achieved by GAC columns for gas flows containing intermittent concentrations of acetone and toluene present as single-component contaminants and as a mixture. Results presented in Chapter 6 demonstrate that the GAC column was able to effectively attenuate dynamic loadings of both acetone and toluene when they were present as single-components in a synthetic waste gas stream. This load equalization effect for single-contaminant waste gases was demonstrated under a wide range of influent concentrations. Because of competitive sorption, however, the degree of load equalization achieved for different constituents in multi-contaminant gas streams can vary markedly. Acetone was much less attenuated in the presence of toluene than it was when toluene was absent. Under the discontinuous contaminant loading conditions, the only driving force necessary for contaminant desorption was the naturally occurring decrease in influent contaminant concentration. The contaminant mass temporarily accumulated in the GAC column can be desorbed within a sufficiently short time interval (i.e., during each loading cycle) to be of practical benefit as a load dampening mechanism. Regeneration of the GAC column through other means (e.g., heating) was not necessary for successful load dampening.

Results presented in Chapter 6 demonstrate that the PSDM model can be calibrated and validated successfully using experimental data by optimizing only a single parameter such as the film transfer coefficient $K_f$. The calibrated PSDM was successful in qualitatively and quantitatively predicting the pattern of VOC concentrations exiting the GAC column for both single and multi-component gas streams under discontinuous loading conditions. This model
may be useful in developing a design basis for activated carbon load dampening systems for biofilters treating dynamically varying VOC concentrations.

As described in Chapter 7, treatment performance of a GAC-biofilter integrated system was experimentally evaluated in comparison to a conventionally designed biofilter (i.e., one lacking an activated carbon column) to determine whether activated carbon load dampening can improve performance of biofilters treating multi-component mixtures of acetone and toluene. Under the discontinuous contaminant loading conditions imposed on the system, the GAC column applied prior to biofilter treatment effectively dampened the dynamic toluene loading to a reasonably stable concentration throughout the entire operation under GAC column EBCTs ranging from 2.5 to 10 seconds. The dynamic loading of acetone was also buffered by the GAC column but to a lower degree than toluene, and the dampening decreased as EBCT decreased.

The GAC-biofilter integrated system could remove 100%, 99%, and 100% of toluene and 100%, 98%, and 87% of acetone when subjected to progressively increasing discontinuous VOC loading rates of 185, 370, and 740 g m$^{-3}$ d$^{-1}$ for toluene and 506, 1012, and 2024 g m$^{-3}$ d$^{-1}$ for acetone, respectively. In contrast, the unbuffered biofilter removed only 40.5%, 58.1%, and 54% of toluene and 93%, 67.6%, and 80.4% of acetone when subjected to progressively increasing discontinuous VOC loading rates of 185, 370, and 618 g m$^{-3}$ d$^{-1}$ for toluene and 506, 1012, and 1686 g m$^{-3}$ d$^{-1}$ for acetone, respectively. The buffered system exhibited more stable and higher contaminant removal efficiencies than did the unbuffered biofilter throughout the various phases of operation. This clearly demonstrates that a passively-operated GAC load-dampening system installed in series before a biofilter is feasible and offers advantages in treating dynamically varying concentrations of acetone and toluene mixtures in contaminated air.
Overall, results presented in Chapters 6 and 7 demonstrate that a passively-operated activated carbon buffering system can achieve two advantages in operation of biofilters subjected to intermittent loading: 1) the magnitude of peak loading to the biofilter can be decreased, leading to more complete removal of contaminants in the biofilter; and 2) because contaminant loading to the biofilter is more uniform as a function of time, diminished performance due to starvation conditions as frequently encountered in conventional biofilters can be minimized. Because of competitive adsorption, however, the degree of load equalization achieved by a passively operated GAC system for constituents with different physicochemical properties in multi-contaminant gas streams can vary markedly. Therefore, such competitive adsorption would need to be accounted for in designing GAC buffering systems for biofilters treating waste streams with multiple contaminants.

Further research is necessary to fully evaluate the relative merits of the two design modifications evaluated in this dissertation (i.e., SBB operation or use of a GAC load dampening system). Each is likely to have advantages in different applications, and in some cases, it may be desirable to use a combination of both technologies (i.e., a passively operated GAC buffering system followed by SBB treatment). Decisions about whether to implement one or both of these design modifications will depend on the characteristics of the specific waste air stream, its treatment goal, regulatory compliance standards, availability of trained operators, and cost. In cases where air pollution control regulations require a specified removal efficiency (e.g., 90%) on a continuous basis, SBB operation may be well suited for minimizing contaminant emissions during transient loading conditions by temporarily sorbing spikes during FEED periods and subsequently degrading the accumulated contaminants during REACT periods. This may allow expanded use of biofilters to cases where they could not otherwise meet treatment goals, and this
may result in cost savings because biofilters can be less expensive than alternative treatment technologies such as catalytic thermal oxidation. An SBB system, however, is more complicated than conventional biofilters (both in construction and operation) and the cost of implementing such an alternative is not yet clear.

A passively operated GAC column could achieve much the same goal without the need for a sophisticated control system, but its ability to meet a specific removal efficiency on a continuous basis will depend to some extent on the potential regulatory monitoring requirements at a given site. The location where contaminants are measured for assessing removal efficiency may be potentially important. Because a GAC column can shift the time at which peak biofilter loading occurs, peak biofilter loading and therefore peak biofilter contaminant emission may occur at a time when the contaminants entering the treatment system is low or even zero. For example, this was the case during Phase 1 loading condition described in Chapter 7. The peak biofilter loading (i.e., peak contaminant exiting the GAC column) occurred at a time when the concentration entering the GAC column was zero. If the system’s removal efficiency was assessed on the basis of contaminant concentrations entering the GAC column and exiting the biofilter on an instantaneous basis, then the removal efficiency would actually be negative (outlet higher than inlet) if there is any contaminant emission from the biofilter. This scenario may be problematic for an integrated GAC buffered biofilter, even if the overall removal efficiency averaged over a longer time interval (e.g., one day) is quite high (e.g., 99%). On the other hand, if the system’s removal efficiency on a continuous basis is assessed by measuring RE in terms of contaminant concentration entering and exiting the biofilter (as opposed to entering GAC column and exiting the biofilter), then meeting regulatory compliance could likely be more easily
achieved. Thus a thorough evaluation of the cases where each of these design modifications would be applicable depends in part on regulatory requirements.

In general, an SBB system can be favorably considered for handling transient loading conditions where contaminants exhibit relatively slow degradation or when a high degree of kinetic inhibition/catabolic repression occurs for a waste stream with multiple contaminants. Because high relative humidity adversely affects GAC sorption capacity (Crittenden et al., 1988; Ramakrishnan et al., 2004), a GAC-biofilter integrated system may work best when applied to relatively dry air streams. Because a GAC-biofilter system is somewhat simpler in operation compared to an SBB system, the GAC-biofilter integrated system may be more appropriate in cases where trained operators are not available or personnel costs are high.

8.2. Recommendations for Future Research

Some additional experiments could be conducted to obtain further information about implementation of the two design modifications/operating strategies tested in this study. For example, tests could be performed to assess whether SBB operating strategy has superior capability compared to conventional biofilters for contaminant removal in cases where kinetic inhibition and/or catabolic repression exist for multiple-contaminant waste gas streams. Another test of interest is to determine whether there are likely scale-up issues in full-scale implementation of either SBB operating strategy or the GAC-biofilter integrated system by testing them in a pilot-scale system.

In the study described in Chapter 6, single-component adsorption of toluene or acetone was experimentally tested under only one fixed EBCT and superficial velocity, while binary mixture adsorption of toluene and acetone was experimentally tested under only one concentration level at fixed EBCT and superficial velocity. Although the model showed excellent agreement with the experimental data, because of the limited data used in model
calibration and validation, it is not possible to fully assess whether the model can accurately predict emissions from GAC columns subjected to loading conditions with a different EBCT, superficial velocity, periodicity of dynamic loading, or influent contaminant concentrations. Consequently, it is recommended that further experimental data be collected before applying the model to loading conditions markedly different from those experimentally tested in this research. It may also be useful in developing a design methodology as a guidance for reactor sizing and operating parameter estimation for the GAC-biofilter integrated system, based on the combined experimentally determined and modeled data of load dampening achieved under a wide range of GAC column empty bed contact times (EBCTs) and influent contaminant concentrations.

Differences in the microbial community structures between the GAC-biofilter buffered system and the unbuffered system could also be analyzed using a molecular approach in an attempt to establish a link between the composition of the microbial population structure and overall treatment performance. For the buffering system placed in series prior to the biofilter, a different adsorbent such as zeolite or resin or mixtures of different adsorbents could be tested to assess the effectiveness of load dampening for multi-component contaminants. Finally, it is recommended that future research be conducted to develop effective strategies to limit excess biomass accumulation in either the SBB or GAC-biofilter systems in order to ensure stable long-term performance.
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APPENDIX A SUPPLEMENTAL DATA FROM EXPERIMENTS DESCRIBED IN CHAPTER 5

Table A.1: Relative quantity of bands in DGGE fingerprints from replicate B (gray color denotes presence of band).

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Table A.2: Relative quantity of bands in DGGE fingerprints from replicate C (gray color denotes presence of band).

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Figure A.1: Dendrograms revealing the relatedness of PCR-DGGE fingerprints from the SBB (left) and CFB (right) generated from dice index similarity matrix based on presence/absence of band for Replicates A, B, and C, respectively. Vertical axis denotes unweighted similarity ($C_s$).
Figure A.2: Dendrograms revealing the relatedness of PCR-DGGE fingerprints from the SBB (left) and CFB (right) generated from dice index similarity matrix based on band relative quantity for Replicates A, B, and C, respectively. Vertical axis denotes weighted similarity ($C_{sw}$).
Figure A.3: Dendrograms revealing the relatedness of PCR-DGGE fingerprints from both the SBB and CFB generated from dice index similarity matrix based on band relative quantity for Replicates A, B, and C, respectively. Vertical axis denotes weighted similarity ($C_{sw}$).
APPENDIX B  VALIDATION OF DNA EXTRACTION PROTOCOL USED IN CHAPTER 5

BACKGROUND

As described in Chapter 5, a PCR-DGGE method was used to study the microbial population diversity between two biofilters operated using different loading strategies. In DGGE analysis, the generated banding pattern is considered as an “image” of the whole bacterial community. The number of band types (reflecting species richness) and band relative intensity (reflecting relative quantity of species) from the various samples were used to compare the microbial diversity along the height of each biofilter as well as between two biofilters. Several assumptions were presented in Chapter 5 regarding interpretation of the DGGE fingerprinting patterns. One of the assumptions is that there was no bias in the DNA extraction. The extracted DNA was assumed to reflect the predominant bacterial populations in the biomass grown attached to the biofilters’ foam cubes. The validity of this assumption is particularly important with respect to one’s ability to draw conclusions based on the resulting data. Therefore, the soundness of the DNA extraction method used in the analysis was tested prior to further application.

Three potential concerns arise from the procedures for DNA extraction used in the studies described in Chapter 5. The first is that in the procedure utilized, biomass was removed from the foam cube by vortexing for 5 minutes to separate biomass for use in DNA extraction. A visual inspection of the foam cubes after biomass was removed using the vortexing procedure revealed that for the foam cubes removed from biofilter sections closest to the column inlet (where the highest biomass concentration existed), there was still a substantial amount of biomass attached to the foam cube even after the 5-minute vortexing. Biomass could be observed from the brownish color appearing on the surfaces of the black foam cube. Thus, the biomass
suspension used for DNA extraction contained only part of the microbial population of the foam sample. This is a matter of great concern because the microbial population distribution in the foam cube was unknown. If, for example, there was a significant difference in the microbial population between the inside and outer surfaces of the foam cube, then it is likely that some microbial species unique to the inner part of the foam cube would not be captured in the suspension. In addition, some cell types are probably more tightly bound to the foam surface than others. These effects could result in misleading interpretation of microbial diversity in the biofilters because the PCR-DGGE approach would reflect the diversity of the population structure in the biomass removed by the vortexing procedure rather than the structure of the entire population originally attached to the foam cubes.

The second concern is whether the DNA extraction method described in Chapter 5 was successful at extracting DNA from all of the predominant microbial populations present in the biomass suspension. It is not uncommon for different extraction methods to have different extraction efficiencies (Burlage, 1998), because a range of lysis treatment could be used in different protocols, including enzymatic (lysozyme, proteinase K) treatment, sodium dodecyl sulphate (SDS), microwave, sonication, freeze/thaw, and bead beating, as well as different combinations of these treatment (Trevors and van Elsas, 1995). A detailed discussion of the advantages and disadvantages of different lysis methods can be found in Chapter 5.

The third concern is about the representativeness of the samples taken along the height of each biofilter column. In Chapter 5, the question of whether there was difference in the bacterial diversity at different height of the biofilter as well as between two columns was assessed by analysing whether there was difference in the bacterial diversity in the samples taken. A significant variation in the microbial diversity in the random samples collected from the same
location would make interpretation of the data difficult. Therefore, it is also desirable to know whether there is much variation in the microbial diversity for random samples collected from the same height of the columns.

In order to determine whether the biomass removal and DNA extraction methods described in Chapter 5 were able to capture all the possible microbial diversity as reflected by the species types, species richness and species relative abundance, an alternative extraction method was tested for comparison purposes. The alternative extraction technique used direct lysis of biomass from the foam cube samples, thereby circumventing the possible experimental error due to incomplete biomass removal from foam cube before extraction. It also used a series of physical/chemical methods for DNA extraction. A PCR-DGGE approach was used to compare bacterial community patterns obtained with 1) the vortexing procedure and subsequent application of Mo Bio Ultraclean™ Microbial DNA isolation kits (see Chapter 5), and 2) a rigorous direct lysis method. Samples used for these extractions were collected from the same height of a biofilter column in order to assess the variations in microbial diversity due to random sampling.

MATERIALS AND METHODS

DNA Extraction

A total of 5 foam cubes (designated cubes I, II, III, IV, and V, respectively) were removed from the same height of the second section (second from inlet) of the continuous flow biofilter on day 320. Using sterile microtome blades, Foam cubes D and E were each cut into 8 pieces of approximately the same dimensions (sliced into half along the length, width, and height of the foam cube). The DNA was extracted in three scenarios, depending on how samples were handled and the extraction method used.
In the first extraction scenario, foam cubes I, II, and III were each processed in the same way the samples were processed for the purpose of the microbial diversity comparison between the two biofilters using Mo Bio DNA isolation kit: before the extraction was performed, each cube, processed separately, was immediately placed in a 15 mL sterile centrifuge tube containing 5 mL of cold (4°C) sterile TE buffer (10 mM Tris, 1 mM EDTA, pH=8). Each sample was vortexed for 5 minutes to remove biomass from the foam packing to the liquid suspension. The polyurethane foam was then removed from the centrifuge tube and discarded. Samples of the remaining bacterial suspension were vortexed for one minute prior to DNA extraction using the Mo Bio Ultraclean™ Microbial DNA isolation kits with modifications in the manufacturer’s protocol as described in Chapter 5. Briefly, cell pellets obtained after centrifugation were resuspended with micro bead DNA solution containing guanidine isothiocyanate, resuspended cells were transferred to MicroBead tube, a solution containing SDS was added to the tube, cells were then lysed by a bead beating method. Both guanidine isothiocyanate and SDS protected the released nucleic acid from environmental nucleases and aid in lysis. The lysate was then centrifuged through a spin filter. DNA was bound to spin filter due to the high salt nature of the binding buffer. The filter was washed with solutions containing ethanol and sodium chloride to remove any residual salt from the nucleic acid pellet. Finally, the DNA was released into DNA free 10 mM Tris elution buffer. Samples extracted were designated E1-I, E1-II, and E1-III, respectively, with “E1” denoting the first extraction scenario.

In the second extraction scenario, two 1/8 slices of foam cube IV and two 1/8 slices of foam cube V were processed in the same way that samples were processed for the first extraction scenario as described above. The extracted DNA from two slices of cube IV and cube V was
designated E2-IV.1, E2-IV.2, E2-V.1, and E2-V.2, respectively, with “E2” denoting the second extraction scenario.

In the third extraction scenario, another two 1/8 slices of foam cube IV and two 1/8 slices of foam cube V were extracted using direct lysis. The extracted DNA from these 4 slices of foam cubes were designated E3-IV.3, E3-IV.4, E2-V.3, and E2-V.4, respectively, with “E3” denoting the third extraction scenario. The rationale for applying different 1/8 slice cubes which were cut from the same foam cube within the same extraction method as well as between different extraction methods during the second and third extraction scenarios was to minimize random sampling error.

For the third extraction technique, the 1/8 slice of foam cube was placed in 5 mL SET buffer (200 g/L sucrose, 0.05 M EDTA, 0.05 M Tris HCl, pH=7.6) in a 50 mL centrifuge tube. Then, 500 µL of a lysozyme solution (made by adding a pinch of lysozyme to 1mL of chilled water) was added to the sample. The sample was incubated in a water bath at 37ºC for 2 hours. It was shaken regularly (every 5-15 minutes). 500 µL of 10% SDS (filter sterilized by passage through a 0.2 mm filter) was added to the sample to denature the cellular materials. Three freeze/thaw cycles were performed (-80ºC for 5 minutes or until frozen, 70ºC for 5 minutes). The samples were centrifuged at 5000 rpm for 10 minutes so that the polyurethane foam and cell debris could be removed. The supernatant was transferred to a new 50 mL centrifuge tube and refrigerated at 4ºC until further use.

The pellet was resuspended in 5 mL of 0.12 M Na₂HPO₄ and 50 µL of Proteinase K for further extraction. The resuspended pellet was incubated in a 37ºC water bath for 30 minutes. It was shaken at regular intervals (every 5-15 minutes). The resuspended pellet was then incubated in a 65ºC water bath for 1 hour during which time it was shaken at regular intervals (every 5-15
minutes). The resuspended pellet was then centrifuged at 5000 rpm for 30 minutes. The new supernatant was added to the refrigerated supernatant in the earlier step. The combined supernatant was centrifuged at 8000 rpm for 30 minutes, and transferred to a new tube avoiding any pellet. 10 mL PEG (30% w/v Polyethylene Glycol 8000 in dH₂O) was added and mixed by shaking, 1.5 mL 5 M NaCl solution was slowly added, the sample in the tube was mixed well and refrigerated at 4°C overnight. The sample was centrifuged at 7000 rpm for 30 minutes, with supernatant discarded. The precipitated DNA in the pellet was resuspended in 4 mL TE buffer and vortexed, four aliquots of 1 mL sample were transferred to 1.5 mL centrifuge tubes. 500 μL phenol was added to each tube, the samples were vortexed and centrifuged for 10 minutes at 13,000 rpm. The aqueous phase was transferred to new tubes with the organic layer (bottom layer) discarded. 500 μL of chloroform was added to each tube, the samples were vortexed and centrifuged for 10 minutes at 13,000 rpm. The aqueous phase was transferred to new tubes with the organic layer (bottom layer) discarded. The extracted DNA was then purified using an UltraClean™ PCR clean-up kit (Mo Bio Laboratories, Inc., Solana Beach, CA).

**PCR Amplification**

The PCR primers, reagent, and PCR protocols were exactly the same as described earlier in Chapter 5.

**DGGE**

Duplicate DGGE gels were made and loaded with PCR products from the 11 DNA extractions. Correspondingly, each gel contained 11 lanes representing the 11 DNA extractions using 3 different sample handling and extraction scenarios. Whenever possible, the duplicate gels were loaded with the exact same PCR products for each corresponding lane in order to
check the reproducibility of the gel. DGGE was performed and images were captured exactly as described previously in Chapter 5.

**Analysis of DGGE Bands**

Digital images of the resulting DGGE gels were analyzed using Quantity One software (Bio Rad). The software performs a density profile through each DGGE lane, detects the bands, and calculates the relative quantity of each band to the total band signal in the lane after applying a rolling disk as background subtraction. Bands with a relative intensity of less than 0.2% were discarded. The different lanes from the same gel were compared and the bands occupying the same position in the gel were identified. Non-weighted Dice index similarity matrix based on the presence/absence of bands, weighted Dice index similarity matrix based on band relative quantity, and Shannon-Weaver diversity index were calculated as described in Chapter 5. A dendrogram based on the weighted similarity matrix was constructed by UPGAMA algorithm in cluster analysis using SAS 8.0 (SAS Institute Inc., Cary, NC).

**RESULTS AND DISCUSSION**

**Comparison Based on Unweighted Dice Similarity Coefficients**

The duplicate DGGE fingerprints for the three extraction scenarios are shown in Figure B.1, and the identified bands are shown in Figure B.2. The overall patterns of the two replicate gels were quite similar but not identical. Individual bands identified for each lane of the two gels are shown in Table B.1 for Replicate A and Table B.2 for Replicate B. In these two tables, grey shading denotes the presence of band, and white indicates absence of band. The number listed in grey-shaded boxes indicates the relative quantity of that band relative to the total detected in the lane.
Figure B.1: DGGE fingerprints for different extraction scenarios.
Figure B.2: DGGE band matching.

The Dice index similarity matrix based on presence/absence of bands for each gel, and the average similarity matrix calculated from the two individual matrices are shown in Table B.3.
As can be seen from the figures and tables, for both gels, there were very similar band patterns among all the 11 lanes, with the number of detectable bands ranging from 18 to 22. Some bands (for example, bands 5, 6, 19, 21, and 22) are absent in certain lanes, while present as very faint bands in the other lanes. Band type similarity was very high ranging from 87.2% to 100% for replicate A, and 92.3% to 100% for replicate B.

Table B.1: Relative quantity of bands in DGGE fingerprints for three extraction scenarios for Replicate A (gray color denotes presence of band).

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The unweighted Dice indices were calculated as a pairwise comparison of the same lanes between the two gels (e.g., the band pattern for lane 1 in replicate A was compared to lane 1 in replicate B) (see Table B.4). The unweighted Dice similarity indices ranged from 94.7 to 100 with an average of 97.0 and a standard deviation of 2.3. Thus, the banding patterns based on
presence/absence of bands for the same PCR products on different DGGE gels were very reproducible.

### Table B.2: Relative quantity of bands in DGGE fingerprints for three extraction scenarios for Replicate B (gray color denotes presence of band).

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The unweighted Dice indices calculated for comparing different lanes on the two replicate gels were quite similar. The percentage difference between the Dice Indices for the two gels (calculated as the absolute value of the weighted Dice Index calculated for replicate A minus that calculated for replicate B divided by that calculated for replicate A multiplied by 100%) ranged from 0 to 11.7%, with an average of 2.8% and a standard deviation of 2.6. Thus, the DGGE process (including image analysis) was quite reproducible with respect to analyzing the same PCR products on replicate gels.
Table B.3: Dice index similarity matrix based on presence/absence of band.

a. Replicate A

<table>
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<th>similarity</th>
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<th>E1-III</th>
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<th>E2-IV.2</th>
<th>E2-V.1</th>
<th>E2-V.2</th>
<th>E3-IV.3</th>
<th>E3-IV.4</th>
<th>E3-V.3</th>
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b. Replicate B

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c. Average of duplicate gels

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235
Table B.4: Unweighted and weighted Dice indices for pairwise comparison of the same lane positions (loaded with identical PCR products) between the two gels.

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Summary

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<th>Range: (94.7,100)</th>
<th>Range: (65.8, 92.3)</th>
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<tr>
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<tr>
<td>Std:</td>
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<td>8.3</td>
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In the direct lysis method (third extraction scenario), in which case cells were lysed in the presence of the environmental matrix (i.e., the polyurethane foam cube), a combination of chemical lysis (by lysozyme and SDS) and mechanical lysis (by freeze/thaw) was used to lyse the cells that may have remained attached to the foam cube in the other lysis procedures. Therefore, the direct lysis protocol described here promised to be a rigorous extraction strategy that captured the microbial diversity of the original sample to the greatest extent possible. Since biomass removal by vortexing followed by the Mo Bio extraction method captured the microbial population with essentially the same species richness compared to the direct lysis extraction method, this implies that the biomass removal by vortexing combined with Mo Bio extraction used in Chapter 5 essentially captured the whole microbial population richness of the original foam cube samples. In other words, the bacterial population in the biomass remaining attached to the foam cubes in the vortexing procedure was the same, or at least sufficiently similar to, the microbial population in the biomass that was removed by vortexing, thereby the unweighted Dice similarity coefficients were not appreciably different. The high unweighted similarities
between different samples further inferred that there is no significant heterogeneity in the species richness in the different samples collected from the same height of the column. Therefore, random sampling is not expected to contribute to significant bias in the microbial analysis.

**Comparison Based on Weighted Dice Similarity Coefficients**

The weighted Dice index similarity matrix based on band relative quantity for each gel, and the averaged weighted similarity matrix calculated from the two matrices are shown in Table B.5. Dendrograms based on these weighted similarity matrices were constructed to graphically illustrate the similarities of microbial diversities between the extracted samples (see Figure B.3). From the weighted similarity matrices and the dendrograms, it was found that the similarity values based on band relative quantity was lower than the similarity values based on only presence/absence of bands. The weighted similarity values ranged from 59.1% to 93.7%, with most similarity values in the range of 65% to 85%.

To further ascertain why the differences were larger for the weighted vs. non-weighted approach, weighted Dice indices were calculated for a pairwise comparison of the same lanes between the two gels (e.g., the band pattern for lane 1 in replicate A was compared to lane 1 in replicate B) (see Table B.4). The weighted Dice similarity indices ranged from 65.8 to 92.3 with an average of 84.5 and a standard deviation of 8.3. Thus, the weighted banding patterns for the same PCR products on different DGGE gels were somewhat different. Although a comparison of Tables B.1 and B.2 (or even a visual inspection of the replicate gels shown in Figure B.1) reveals that bands that were more intense on one gel (those with higher relative quantity) were generally more intense (higher relative quantity) on the other and that faint bands (those with lower relative quantity) on one gel generally followed the same trend on the other, some variability exists, and this is reflected in the weighted Dice index comparison of lanes between the two gels. This suggests that at least some of the differences in the weighted similarity matrix
are due to the DGGE process and/or image analysis rather than in differences in microbial population composition, cell lysis efficiency, or PCR biases.

**Table B.5: Dice index similarity matrix based on band relative quantity.**

### a. Replicate A

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<th>E1-III</th>
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Figure B.3: Dendrograms revealing the relatedness of PCR-DGGE fingerprints from the three extraction scenarios based on band relative quantity. Vertical axis denotes similarity ($C_{sw}$).
The percentage difference between the weighted Dice indices calculated to compare the various lanes on the two gels (calculated as the absolute value of the weighted Dice Index calculated for replicate A minus that calculated for replicate B divided by that calculated for replicate A multiplied by 100%) ranged from 0 to 39.0%, with an average of 8.3% and a standard deviation of 9.4. Thus, the DGGE process (including image analysis) was less reproducible with respect to analyzing the same PCR products on replicate gels than was the case for the unweighted Dice Index.

In spite of the limitations noted above, the weighted similarity matrix based on band relative abundance was used for further assessment of whether the biomass removal combined with the Mo Bio DNA extraction method described in Chapter 5 was able to capture all the possible microbial diversity as reflected by the species types, species richness, and species relative abundance. The dissimilarity of the relative quantity of bands between different samples as reflected in Table B.5 and Figure B.3 could be due to numerous causes including: (1) Differences in staining and image exposure; (2) Different efficiencies of the DNA extraction methods; (3) Heterogeneity in microbial populations for different foam cubes at the same height of the column; and (4) Heterogeneity in microbial populations at different spatial locations even within a single foam cube.

From Table B.5, for the samples cut from the same foam cubes in the third extraction scenario using direct lysis method, sample IV.3 and sample IV.4 had a similarity of 93.7% (average of replicates A, 95.7%, and B, 91.8%) in band relative quantity, and sample V.3 and sample V.4 had a similarity of 83.5% (average of replicates A, 96.6%, and B, 70.3%). Because the samples were extracted using the same direct lysis method and the biomass vortexing procedure was circumvented, the dissimilarity between these samples could only be accounted
for by DGGE bias, heterogeneity in the microbial distribution throughout the foam cube, or a combination of both factors. The sizable difference in comparison of samples V.3 and V.4 between the two replicates (A, 96.6%, and B, 70.3%) in the third extraction scenario, suggests that the dissimilarity may be due entirely to DGGE bias. This is consistent with data shown in Table B.4 which indicates that in pairwise comparison of the weighted data for samples V.3 and V.4 between replicates A and B, the similarity was 74.1% and 81.4%, respectively.

In the second extraction scenario, the two samples cut from the same foam cube IV had a weighted similarity value of 67.2% (average of replicates A, 67.0%, and B, 67.4%), and the two samples cut from the same foam cube V had a weighted similarity value of 64.4% (average of replicates A, 66.4%, and B, 62.4%). This is consistent with data shown in Table B.4 which indicates that in pairwise comparison of the weighted data for samples V.3 and V.4 between replicates A and B during the second extraction scenario, the similarity was 81.3% and 88.0%, respectively, and it suggests that the DGGE process itself was responsible for at least a portion of the calculated difference.

Cluster analysis based on the averaged weighted similarity matrix (see Figure B.3) showed that the microbial diversities of all the samples captured using different extraction methods could be classified into two main clusters. All the samples extracted using the third extraction scenario formed one cluster, the samples extracted using the first and second extraction scenarios formed the other cluster. The microbial diversities of the samples captured by the third extraction scenario had relatively higher similarities than those captured by the first and second extraction scenarios. These two clusters had a similarity value of 67%. The cluster analysis, thus, suggests that biomass removal by vortexing followed by extraction using the Mo Bio method may have led to a difference in the weighted comparison to the direct lysis method.
Because the direct lysis technique was not used to extract DNA from biomass removed from the foam cube by vortexing, which would have allowed a direct comparison to the Mo Bio method in terms of cell lysis, it is impossible from the data collected to ascertain how much (if any) of the observed difference was caused by differences in microbial community composition in the biomass that remained attached to the foam versus difference in cell lysis efficiency.

A considerable advantage of using the Mo Bio DNA isolation kit was that it was relatively rapid to perform (extraction took approximately 20 minutes to perform), whereas the direct lysis as an alternative was time consuming and laborious (extraction took approximately 2 days to perform). The incomplete biomass removal from the foam cube by vortexing prior to the Mo Bio extraction, however, potentially makes this extraction method less desirable option. Use of a direct lysis DNA extraction method would avoid this potential bias in future studies on microbial diversity of biofilms attached to solid support media.

Finally, it has to be noted that the above interpretation of band relative quantity analysis is based on the assumption that band relative quantity is directly related to the species relative abundance. Unfortunately, the validity of this assumption has been a subject of controversy itself, as band intensities are also influenced by 16S rDNA gene copy numbers, differential DNA extraction, PCR biases, or by comigration of two or more sequence types as described in Chapter 5. Therefore, future tests should be conducted to check the validity of this assumption and quantify the bias from DGGE approach itself before a sound comparison of the different DNA extraction method is established.

Based on the analysis presented above, it was concluded that although the weighted Dice index comparisons may be of use in assessing large differences in microbial populations, the approach will likely be of limited use in assessing relatively small differences in microbial
populations because of the reproducibility of the DGGE process itself. Differences on the order of 30% were observed in the weighted Dice Index even for identical PCR products.

CONCLUSIONS

The unweighted Dice Index comparison revealed that there is very high similarity in the microbial richness in the different samples collected from the same height of the column. Therefore, random sampling is not expected to contribute to significant bias in the microbial analysis. Furthermore, biomass removal by vortexing followed by the Mo Bio DNA extraction method captured the microbial population richness in a manner essentially indistinguishable from the direct lysis extraction method.

Because of variability in the relative quantity of bands calculated for identical PCR products analyzed in replicate DGGE gels, the weighted Dice Index approach is not able to accurately assess minor differences between microbial populations. Variation in band intensity inherent to the method indicates that some variability (on average, approximately 15% difference) was observed even for PCR products that were identical. Nevertheless, this method may be useful for assessing large differences in microbial populations because although not completely reproducible, the technique yielded results that followed a clear trend.

REFERENCES


APPENDIX C  SENSITIVITY ANALYSIS, DETAILED MODEL CALIBRATION AND VALIDATION FOR PSDM MODELING DESCRIBED IN CHAPTER 6

INTRODUCTION

As described in Chapter 6, abiotic fixed-bed adsorption/desorption experiments and numerical simulation were conducted to evaluate the range of load dampening expected for GAC column subjected to various inlet concentrations of acetone and toluene as intermittently loaded single-component contaminants and as a two-component mixture. The pore and surface diffusion model (PSDM) described by Crittenden et al. (1986) was used for simulation of contaminant breakthrough behavior through the GAC bed. Simulations using the PSDM were performed using AdDesignSTM software (Michigan Technological University).

In calibrating the model, it was desirable to obtain a qualitative and quantitative appreciation of the influence of various parameters on the overall GAC column dynamic adsorption and to examine the level of accuracy required in estimation of parameters employed for adsorption modeling. Therefore, sensitivity analysis of the model was performed prior to model calibration to provide guidance for optimization of parameters to achieve model calibration. The parameters investigated in the sensitivity analysis as described herein were the Freundlich isotherm parameters and kinetic parameters. The sensitivity analysis was performed using loading conditions identical to the first loading conditions of toluene or acetone single-component adsorption described in Chapter 6. A portion of the results of the sensitivity studies are presented in the following section.

Following the sensitivity analysis, alternate model calibrations were performed by adjusting single parameters such as the kinetic and Freundlich isotherm parameters. For the sake of brevity, only the model calibration and validation by optimizing the film mass-transfer coefficient alone was described in Chapter 6. Data presented herein include a detailed output of
model calibration and validation obtained by optimization of other parameters as well as the film mass-transfer coefficient.

**METHODOLOGY**

The five model input parameters selected for the sensitivity analysis were the kinetic parameters ($K_f, \ D_s, \ D_p$), and the Freundlich isotherm parameters ($K$ and $1/n$). The base values of the kinetic parameters $K_f, \ D_s, \$ and $D_p$ were $2.55 \text{ cm/s}$, $8.75 \times 10^{-6} \text{ cm}^2/\text{s}$, and $8.24 \times 10^{-2} \text{ cm}^2/\text{s}$ for toluene, and $2.55 \text{ cm/s}$, $2.54 \times 10^{-5} \text{ cm}^2/\text{s}$, and $1.07 \times 10^{-1} \text{ cm}^2/\text{s}$ for acetone, calculated based on the Gnielinski correlation, Sontheimer correlation, and Wilke-Lee modification of the Hirschfelder-Bird-Spotz method as described in Chapter 6, respectively. The base values of Freundlich $K$ and $1/n$ were $305 \ (\text{mg/g}) \ (\text{L/mg})^{1/n}$, $0.107$ for toluene, and $82 \ (\text{mg/g}) \ (\text{L/mg})^{1/n}$, $0.291$ for acetone, respectively, generated using Calgon Carbon’s proprietary model. During the sensitivity analysis, one such parameter was varied singularly from its base value, and the modeled breakthrough curve for each case was plotted. The first loading condition of single-component adsorption experiments (i.e., the first 12 and 8 days of sorption data for toluene and acetone at 868 and 960 ppm, influent concentration loaded 8 hr/day, respectively) were used as influent input for sensitivity analysis. To facilitate visual display of the output and at the same time better discern subtle differences when the parameters were changed, data from both the experimental measurements and model simulations were plotted showing only a 24-hour period of breakthrough at quasi-steady state conditions. The 24-hour data output from either the experimental or model simulations were the average of breakthrough curves from several days during which quasi-steady states were reached. Data were selected and plotted such that time zero corresponds to the point at which minimum daily concentration was reached. Measured data depicted in the figures are the average concentrations measured during the last three days of
the first loading condition. Data from the first loading condition for each single-compound adsorption experiment described in Chapter 6 were used for model calibration, correspondingly, data from the last two loading conditions were used for model validation. Criteria for model calibration and validation were the same as described in Chapter 6.

RESULTS AND DISCUSSION

Initial Modeling of Toluene and Acetone Adsorption Using Base Values of Kinetic and Freundlich Isotherm Parameters

Initial model simulations, conducted for each single-component adsorption using base values of the kinetic and Freundlich isotherm parameters as described above, predicted the same general pattern of breakthrough curves as were experimentally measured (see Figures C.1 and C.2 for toluene and acetone adsorption, respectively). Peak effluent concentrations, however, were over-predicted and minimum effluent concentrations were under-predicted, in such initial model simulations. Such discrepancy in the magnitude of effluent concentrations between the measured and initially modeled data was greater for acetone adsorption in comparison to toluene adsorption.

Figure C.1: Experimental measurement and initial modeling of toluene adsorption using base values of kinetic and Freundlich isotherm parameters under three discontinuous loading loadings (EBCT = 5.5 sec).
Figure C.2: Experimental measurement and initial modeling of acetone adsorption using base values of kinetic and Freundlich isotherm parameters under three discontinuous loading loadings (EBCT = 6.1 sec).

Sensitivity Analysis of Kinetic Parameters

The film transfer coefficient was varied ranging from 0.05 to 2 times its base value $K_f$ (2.55 and 2.99 cm/sec for toluene and acetone adsorption, respectively). As depicted in Figure C.3, increasing $K_f$ to as high as twice its base value does not significantly change the shape or magnitude of the breakthrough curves. On the other hand, the modeled peak concentration is more attenuated when $K_f$ was decreased from its base value. The overall pattern of the predicted breakthrough curve agreed well with the measured data when the film transfer coefficient was decreased to 0.4 and 0.15 times its base value for toluene and acetone, respectively. However, further decrease of $K_f$ resulted in model prediction of a much more attenuated breakthrough curve than was experimentally measured.

In further sensitivity analysis, the surface diffusion coefficient was varied at values ranging from 0.01 to 10 times its base value $D_s$ ($8.75 \times 10^{-6}$ and $2.64 \times 10^{-5}$ cm$^2$/sec for toluene and acetone adsorption, respectively). As can be seen from Figure C.4, the model is only weakly sensitive to variations in $D_s$. There is slight increase in the predicted peak effluent concentrations when $D_s$ was increased to as high as 10 times its base value. For toluene
adsorption, an obvious decrease in the maximum effluent concentration was predicted as \( D_s \) was reduced to 0.1 times its base value. When eventually reduced to 0.01 \( D_s \), the predicted toluene breakthrough curve approximates that of the measured data. For acetone, however, the predicted peak effluent decreased only by 10% when the surface diffusion coefficient was reduced from its base value to 0.01 \( D_s \), which was still substantially different from the measured acetone breakthrough curves.

In still further sensitivity analysis, the pore diffusion coefficient was varied at values ranging from 0.01 to 10 times its base value \( D_p \) (8.24×10^\(-2\) and 0.107 cm^2/sec for toluene and acetone adsorption, respectively). As shown in Figure C.5, no appreciable difference in the shape or magnitude of the predicted daily breakthrough curve was produced even at a large variation in the \( D_p \).

![Figure C.3: Sensitivity analysis of the film transfer coefficients for toluene adsorption (a) and acetone adsorption (b).](image)

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Figure C.4: Sensitivity analysis of the surface diffusion coefficients for toluene adsorption (a) and acetone adsorption (b).

Such changes in the adsorber profiles with respect to an increase or decrease in the kinetic parameters clearly indicate that the model is very sensitive to a decrease of the film transfer coefficient, the model is moderately sensitive to the surface diffusion coefficient at significantly decreased values, and the model is relatively insensitive to change in the pore diffusion coefficient.

The variation in model predictions due to varied kinetic parameters was further interpreted by investigating the relationship between some dimensionless parameters. Relationships between dimensionless parameters have been used to characterize the relative importance of various mechanisms as well as to identify the specific controlling mechanism (Sontheimer et al., 1988). The biot number for surface and pore diffusion-controlled
intraparticle diffusion describes the ratio of the film transfer rate to the intraparticle diffusion rates as follows:

\[ Bi_s = \frac{S_t}{Ed_s} \]  \hspace{1cm} (C.1)

\[ Bi_p = \frac{S_t}{Ed_p} \]  \hspace{1cm} (C.2)

Where: \( S_t \) = Stanton number, \( Ed_s \) = surface diffusion modulus, and \( Ed_p \) = pore diffusion modulus. If both \( Bi \) numbers are high (i.e., greater than 5, for the case of gas phase adsorption), then intraparticle diffusion resistance will control the mass transfer rate into the aggregate. Specifically, the faster of the two diffusion mechanism (either by surface or pore diffusion) will control the transport rate because intraparticle transport takes place by either surface or pore diffusion which act in parallel. For example, if \( Ed_s \) or \( Bi_p \) is much larger than \( Ed_p \) or \( Bi_s \), respectively, then surface diffusion will control the intraparticle transport rate, and the converse is also true. On the other hand, if either \( Bi \) number is low (i.e., lower than 5, for the case of gas phase adsorption), then film transfer resistance controls the transport rate (Crittenden et al., 1986; Mertz et al., 1999).

Take toluene adsorption, for example, in the initial toluene model simulation using all the base values of the kinetic and Freundlich parameters, the Stanton number is equal to 5.6, the surface diffusion modulus, \( Ed_s \), is 9.2 and the pore diffusion modulus, \( Ed_p \), is 0.57. \( Bi_s \) or \( Bi_p \) number is 0.6 and 9.8, respectively. In running the initial model, a surface to pore diffusion flux ratio (SPDFR) value of 16 was used as suggested by Crittenden et al. (1988), with the underlying reason that for gas phase applications, the impact of pore diffusion on the intraparticle adsorption rate is negligible and surface diffusion is predominant. This results in \( Ed_s \) 16 times that of \( Ed_p \) and the surface diffusion being the controlling diffusion mechanism inside the particle. The surface Biot number \( Bi_s \) is 0.6, therefore, the initial model predicts the film transfer to be the
rate-limiting step and is the overall controlling mechanism. This account for the fact that during the sensitivity analysis, the model output is more sensitive to variation in film transfer coefficient than it is to variation in surface or pore diffusion coefficient. The assumption that surface diffusion is predominant for intraparticle transport was also justified by the observation that the model was insensitive to variations in $D_p$. The predicted surface Biot number $B_{is}$ increased to 6.05 and 60.5 when the surface diffusion coefficient was decreased to 0.1 $D_s$ and 0.01 $D_s$, respectively. At such low surface diffusion rate, the model predicts the surface diffusion becomes the overall rate limiting step instead, leading to a significant change of the predicted toluene breakthrough.

Figure C.5: Sensitivity analysis of the pore diffusion coefficients for toluene adsorption (a) and acetone adsorption (b).
Sensitivity Analysis of Freundlich Isotherm Parameters

For toluene adsorption, the Freundlich capacity parameter $K$ was varied at values ranging from 0.5 to 1.5 times its base value, 305. For acetone adsorption, the Freundlich capacity parameter was varied at values ranging from 0.5 to 1.95 times its base value $K$, 82. The results shown in Figure C.6 indicate high sensitivity of the model to variations in $K$. A decrease in $K$ causes the predicted quasi-steady state breakthrough curve to have a steeper slope and higher peak concentration. Conversely, increasing $K$ reduced the peak concentration greatly and the predicted effluent concentration was more attenuated. However, before effluent toluene concentration reaches quasi-steady state (data not shown), such a change in $K$ at the same time causes a significant shift in the initial breakthrough time (i.e., as an indicator of the relative adsorptive capacity of the bed, a lower $K$ value predicts a lower bed adsorption capacity and shorter time to reach initial breakthrough). Conversely, an increase in $K$ resulted in model prediction of a greater bed adsorption capacity and longer time to reach initial breakthrough.

![Figure C.6](image_url)

**Figure C.6:** Sensitivity analysis of the Freundlich $K$ for toluene adsorption (a) and acetone adsorption (b).
For toluene adsorption, the Freundlich intensity parameter 1/n was varied at values ranging from 0.5 to 1.8 times its base value, 0.107. For acetone adsorption, the Freundlich intensity parameter was varied at values 0.5 to 2.7 times its base value 1/n, 0.291. As shown in Figure C.7, a slight decrease of the 1/n would sharpen the predicted breakthrough curve, whereas a slight increase in 1/n predicts a more attenuated breakthrough. This suggests that the model is highly sensitive to variations in 1/n as well.

![Figure C.7: Sensitivity analysis of the Freundlich 1/n for toluene adsorption (a) and acetone adsorption (b).](image)

**Model Calibration for Single-component Adsorption**

The above sensitivity analysis suggests that the model could be calibrated to more closely fit the experimental data by adjusting single or multiple kinetic or isotherm parameters. Numerous model simulations were conducted with various values of $K_f$, $D_s$, $K$, and 1/n for the first loading condition for each single-component adsorption. In these simulations, only one parameter was varied from the base case (i.e., the initial model) per simulation. It was found that
for toluene adsorption, the shape and magnitude of the breakthrough curve fit the measured breakthrough profiles reasonably well when the model was run separately at the following adjusted parameters in comparison to their corresponding base values: 0.4 $K_f$, 0.01 $D_s$, 1.3 $K$, or 1.35 $(1/n)$. For acetone adsorption, the model agreed well with the experimental data when the model was run separately at the following adjusted parameters in comparison to their corresponding base values: 0.15 $K_f$, 1.7 $K$, or 2.7 $(1/n)$. The selected model calibration breakthrough curves are shown in Figures C.8 and C.9 for acetone and toluene adsorption, respectively. Subsequently, the adjusted models were further evaluated as described in the following section.

Figure C.8: Model calibration of the first loading conditions of acetone. Parameters varied singularly from the base case are film mass-transfer coefficient $K_f$ (a), Freundlich $K$ (b), and Freundlich $1/n$ (c).
Figure C.9: Model calibration of the first loading conditions of toluene. Parameters varied singularly from the base case are film mass-transfer coefficient $K_f$ (a), surface diffusion coefficient $D_s$ (b), Freundlich K (c), and Freundlich $1/n$ (d).

Model Validation for Single-component Adsorption

- **Toluene Adsorption.** By using the above four adjusted parameters individually for toluene adsorption (i.e., at a finally adjusted $K_f$, $D_s$, $K$, and $1/n$ value of 1.02, $8.75 \times 10^{-8}$, 396.5, and 0.144, respectively) model simulations were conducted and the model output was then compared with the experimental data from the entire 25-day period of step decrease of influent...
concentrations under discontinuous loading conditions as described above. Figure C.10 shows the measured data compared to model output with different adjustment during the entire 25 days.

Figure C.10: Model validation of toluene adsorption. Parameters varied singularly from the base case are film mass-transfer coefficient $K_f$ (a), surface diffusion coefficient $D_s$ (b), Freundlich $K$ (c), and Freundlich $1/n$ (d).
In order to scrutinize the difference, the averaged 24-hour quasi-steady state breakthrough curves from either the predicted or measured data during the second loading condition only (i.e., a discontinuous influent toluene concentration of 488 ppm,) were plotted as shown in Figure C.11, with each curve beginning with minimum daily effluent concentration.

![Figure C.11](image_url)

Figure C.11: Comparison of the 24-hour breakthrough curves for toluene adsorption model validation. Data reported are the 24-hour averaged breakthrough curve during the second loading condition under quasi-steady state.

Data in Figures C.10 and C.11 showed that under both of the last two loading conditions, all of the predicted breakthrough curves with the above adjusted \( K_f \), \( D_s \), \( K \) or \( 1/n \) fit the measured data pretty well once quasi-steady state was reached. It is noticeable that an increase in \( K \) by 30% predicts the initial breakthrough time approximately 3 days longer than that experimentally measured. Model simulations by adjustment of the kinetic parameters \( K_f \) or \( D_s \) seem to be more in phase with the measured data. From a model fit perspective, either of the two calibration techniques may be reasonable. Likewise, a model calibration in which multiple parameters were adjusted would also likely result in a good fit with the measured data in this case.
Acetone Adsorption. By using the above three adjusted parameters individually for acetone adsorption (i.e., at a finally adjusted $K_f$, $K$, and $1/n$ value of 0.4485, 139.4, 396.5, and 0.7857, respectively) model simulations were conducted and the model output was then compared with the experimental data from the entire 21-day period of step decrease of influent concentrations under discontinuous loading conditions as described above. Overall differences between the measured and simulated data under all loading conditions were shown in Figure C.12. The averaged 24-hour quasi-steady state breakthrough curves from either the predicted or measured data during the second loading condition only (i.e., a discontinuous influent acetone concentration of 544 ppm$_v$) were plotted as shown in Figure C.13, with each breakthrough curve beginning with minimum daily effluent concentration.

Figure C.12a shows that adjusting $K_f$ generates the best fit during the second and third loading conditions of acetone adsorption. Although adjusting the Freundlich $K$ also generates a good fit once quasi-steady state is reached, it predicts a much longer initial breakthrough time (see Figure C.12b). Figure C.12c shows that by adjusting the $1/n$ value, even though the predicted breakthrough curve was adjusted to nicely match the measured data at an influent concentration of 960 ppm$_v$ during the model calibration period, the model under-predicted the buffering capacity at lower influent concentrations under the two other loading conditions. Therefore, adjustment of $1/n$ was determined to be a less desirable candidate for model calibration. Using an adjusted $K_f$ value was deemed to be the most favored approach for model calibration of acetone adsorption.

From the above experimental and model simulation of single-component adsorption of either toluene or acetone, it is clear that by optimizing one of the $K$, $1/n$, $K_f$, or $D_s$ values to get the best fit of the experimental data under certain loading conditions, the PSDM model was
successful in qualitatively and quantitatively predicting the pattern of VOC concentrations exiting the GAC column for single-component adsorption under other loading conditions (i.e., at other inlet concentration levels). Before applying the model to conditions outside the range of those experimentally evaluated, however, it is recommended that further experimental testing and a more rigorous validation procedure be established.

![Figure C.12: Model validation of acetone adsorption. Parameters varied singularly from the base case are film mass-transfer coefficient $K_f$ (a), Freundlich K (b), and Freundlich $1/n$ (c).](image-url)
CONCLUSIONS

Results of sensitivity analysis showed that the predicted breakthrough curves using the PSDM model were highly sensitive to variations in the Freundlich isotherm parameters $K$ and $n$, and the film transfer coefficient ($K_f$); the model was less sensitive to variations in the surface diffusion ($D_s$) and pore diffusion ($D_p$) coefficients.

By optimizing one of the $K$, $1/n$, $K_f$, or $D_s$ values to get the best fit of the experimental data under certain loading conditions, the PSDM model was successful in qualitatively and quantitatively predicting the pattern of VOC concentrations exiting the GAC column for single-component adsorption of toluene or acetone under other loading conditions.
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Congna Li was born in Rizhao, Shandong, China, in February 1976. Congna received her Bachelor of Science degree in water supply and drainage engineering from Jiangsu Institute of Petrochemical Technology, China, in July 1997. Afterwards, she began her graduate studies in College of Civil Engineering, Southeast University, China, and obtained her Master of Science degree in environmental engineering in April 2000. In June 2000, Congna joined the doctoral program of the Department of Civil and Environmental Engineering, Louisiana State University, under the direction of Dr. William M. Moe, developing and testing operating strategies for bioreactors treating volatile organic compounds (VOCs) contaminated air. She anticipates receiving her doctoral degree at the Fall Commencement, 2004.