ABSTRACT

JAN ELIZABETH DEWATERS. Biological Activity on Granular Activated Carbon in the Presence of Ozonated Naturally Occurring Humic Substances. (Under the direction of FRANCIS A. DIGIANO.)

Biological activity on granular activated carbon may significantly enhance the water purification process. Shifting the pathway for contaminant removal from adsorption to biodegradation results in steady state reduction across the GAC bed, and increases filter run time through regeneration of sorptive capacity for non- or slowly-biodegradable compounds. Trace contaminants present at low concentrations and/or only seasonally may not meet minimum growth and energy requirements of a biofilm population. The presence of a bulk substrate source such as humic substances could stimulate the growth of a biofilm which will, in turn, degrade trace pollutants as secondary metabolites.

A fixed-bed column reactor with a high recycle ratio was used to examine biodegradation and adsorption of ozonated humic substances (HS). The percent biodegraded at steady state increased with pre-ozonation, and with an increase in EBCT (slower flowrate) and feed TOC concentration. At an ozone dose of 1 mg $O_3$/mg TOC, EBCT = 3.9 min, and feed TOC = 7 mg/L, approximately 43% of the HS were biodegraded. Steady state biological activity, as evidenced by $CO_2$ production, was achieved within 1 day; steady state TOC removal occurred after about 160 hours. Phenol, when added at 50 µg/L to the HS system, was degraded within 60 hours. When phenol was added to an established biofilm, it was immediately biodegraded.

A method is presented for recovering viable cells from a GAC biofilm. Kinetic studies with samples recovered from an HS grown biofilm indicate that the biokinetics of phenol

utilization by these microorganisms are comparable to utilization kinetics by suspended cultures.

The enumeration of viable cells from samples recovered at different stages of biofilm growth indicates that the density of viable cells continues to increase in the GAC bed, despite the achievement of steady state biological activity. The density of viable cells in the GAC reactor bed, as enumerated by plate counts, was on the order of $10^6$ to $10^7$ cells/gm.

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# 1. INTRODUCTION

## 1.1 Background

Activated Carbon is used in water treatment processes for the removal of taste and odor causing compounds, trihalomethane precursors and trace organics which may be present in a water supply. The combination of large internal surface area (up to 100 $m^2/gm$) and surface-active sites enables adsorption of a wide range of organic molecules from solution. This also creates a favorable environment for bacterial growth. Microorganisms are attracted to the sorbed substrate, and a biofilm readily develops. The presence of this actively growing biofilm could enhance the water purification process; in addition to adsorption, organic chemicals (both sorbed and in solution) may be biologically degraded by the active microbial population.

Granular activated carbon placed in columns or filter beds takes advantage of microbial activity in the treatment process, by providing a fixed surface to which biofilm can adhere. A biologically active GAC filter offers several advantages over a purely adsorptive treatment process, as well as over a process which is purely biological, such as a slow sand filter. Biodegradation lengthens filter run time by offering continuous regeneration of sorptive sites on the GAC surface. After extended operation, a steady state removal rather than complete breakthrough of contaminants can be achieved. Adsorption offers protection during the initial stages of operation, before biological growth is

established, and also ensures the removal of slowly- or non-degradable compounds. Moreover, adsorption can remove contaminants in the event of a chemical spill, in which the microbial community may not be prepared to respond or may be damaged by toxicity.

The characteristics of a biofilm which develops within a GAC bed depend to a large extent on the nature of organic substrate in the incoming water. A water may be rich in organic matter, but if these organics are not readily biodegraded, an active biofilm may not develop. In addition, anthropogenic organic compounds present at extremely low levels (µg/L to ng/L concentrations) or for short periods may not meet minimum growth requirements of the biofilm population.

The bulk of the organic content in most potable water supplies is comprised of humic substances, which are the macromolecular decomposition products of plant material. Since humic substances are, by definition, the end products of decomposition, they are in general not easily biodegraded. Some form of pre-treatment is needed to enhance biodegradation of the humic substances, and thus to promote biofilm development. Ozonation, sometimes used as a disinfectant in water treatment, has been proposed as a method for increasing the biodegradability of humic substances and for encouraging biological activity in a GAC treatment process.

The interactions between adsorption and biodegradation at the carbon surface and the extent to which biological activity will aid in the treatment process are not fully understood. Increased removal of TOC in GAC filters due to microbial activity is variable and depends on the nature of the organic matter. Preozonation may enhance biofilm activity, by increasing the biodegradability of the natural

organics. A constant supply of biodegradable organic matter may sustain a biofilm which will, in turn, degrade trace micropollutants which may be present in a water supply seasonally and/or at low concentrations.

## 1.2 Objectives

This research is intended to develop a better understanding of biofilm activity in a flow-through GAC reactor receiving ozonated naturally occurring aquatic humic substances, and to investigate the behavior of a trace organic, such as phenol, in the presence of the humic substances-grown biofilm.

The specific research objectives are as follow:

1. Develop a method to recover viable bacteria from the adsorptive carbon surface, in order to quantify biological activity and to enable biokinetic studies of micropollutant degradation that utilize the microbial community indigenous to the GAC.

2. Study the biokinetics of phenol metabolism by a microbial community recovered from GAC colonized primarily by bacteria adapted to ozonated humic substances.

3. Observe interactions between adsorption and biodegradation of a trace amount of phenol in the presence of ozonated humic substances in a laboratory scale, fixed-bed reactor.

4. Observe development of the microbial community in a GAC filter receiving ozonated humic substances, and its ability to mineralize trace concentrations of phenol.

# 2. LITERATURE REVIEW

## 2.1 General Interest in Granular Activated Carbon

Carbon has long been known for its ability to remove contaminants from water. Filtering water through charcoal for purification is mentioned in Sanskrit writings, and ancient mariners are said to have stored their drinking water in charred wooden barrels to maintain purity (Symons, 1984).

Modern use of granular activated carbon in the U.S. began with the work of Rosen and Middleton (Braus et al., 1951), who used GAC to concentrate organic compounds from the aqueous phase for later extraction and identification. With respect to drinking water treatment, carbon was used largely for the removal of taste and odor causing compounds. Baylis conducted experiments with GAC as a water treatment aid in the late 1920's in Chicago (Baylis, 1929), and in 1929 GAC beds were installed in Bay City, Michigan (Harrison, 1940). During the 1930's, GAC filters were installed in Dundee, Michigan (Finkbeiner, 1931), in Oshkosh and Neenah, Wisconsin (Howson, 1938), and in Culver City, California (Harnish, 1937). Through the 1940's and 1950's, powdered activated carbon (PAC) largely replaced GAC as a state-of-the-art method for water purification, but during the 1960's interest in GAC was revived and by 1975 an unpublished survey by the U.S. Environmental Protection Agency (USEPA) indicated that 28 water utilities in the U.S. were routinely using GAC adsorption, while eight more were experimenting with the process. The number by 1984 had

reached 50-60 (Symons, 1984) and is expected to rise dramatically in the near future due to the 1986 amendments to the Safe Drinking Water Act, which specify GAC as the best available technology for removal of many of the synthetic organic contaminants which will require monitoring (Cook & Schnare, 1986).

Interest in biological growth on GAC filters, and in interactions between biodegradation and adsorption, is more recent than is interest in purely adsorptive mechanisms. In 1975, Eberhardt, Madsen, and Sontheimer showed that contaminants in the effluent from a GAC bed would reach steady state concentrations prior to complete breakthrough, as indicated by such parameters as UV absorbance, chemical oxygen demand (COD), and total organic carbon concentration (TOC). Biodegradation of influent substrate as well as substrate bound to the GAC surface was evidenced by measurement of $CO_2$ production and dissolved oxygen uptake across the column (Eberhardt et al., 1975). From a comparison between a sterile and a nonsterile pilot plant, Werner and co-workers (1979) conclude that microorganisms are responsible for about 60% of the total oxygen consumption and $CO_2$ production, while the remaining 40% is due to abiotic processes. They note that microbial activity increases filter efficiency and prolongs run time for GAC by continuous regeneration of sorptive sites.

The presence of bacterial and protozoan growth on GAC has been documented with the aid of scanning electron microscopy (Weber et al., 1978). Accumulation of bacteria on the GAC surface is said to follow a sigmoidal growth-type curve (Bancroft et al., 1983; Cairo & Suffet, 1979). Maximum population densities reportedly vary; Bancroft and co-workers report that populations increased to an approximate steady state of $10^8$ cells/gm of dry carbon, while $10^9$ cells/gm wet weight were measured by Latosek and

Benedek (1979). Lower values are reported, $10^6$ to $10^7$ cells/gm, for carbon used in drinking water treatment (Van der Kooij, 1976; Cairo et al., 1979). Maximum numbers attained have been described as a function of both substrate availability and influent bacterial density (Bancroft et al., 1983).

Several attempts have been made to identify microbial populations associated with GAC filters. In a study carried out at a water treatment plant on the Rhine River, Werner and co-workers found a more diverse population in the filter influent than in the effluent. The fraction of bacterial strains belonging to the genus Pseudomonas was found to increase from 55% in the raw water, to 80% in the filter effluent (Werner et al., 1979). In a 6 month pilot plant study investigating the removal of synthetic organic chemicals by GAC, bacterial analyses identified the predominant bacteria isolated from effluent and core samples as gram negative fermenters and Bacillus species; Pseudomonas aeruginosa were occassionally detected in the effluent in low numbers (Donlan et al., 1981). Research performed in a GAC treatment study on the Delaware River identified species of the genus Pseudomonas as most predominant; several Bacillus species were also detected (Cairo & Suffet, 1979).

## 2.2 Biofilm Development

Bacteria have long been known to accumulate on submerged surfaces. Zobell and Allen, in 1935, found that marine bacteria accumulated on glass surfaces and became firmly attached after only one to two hours. In 1943, Zobell determined that incubation bottles with a higher surface area-to-volume ratio increased microbial activity, especially at low substrate concentrations (Zobell, 1943).

He suggests the following advantages for bacterial accumulation at surfaces:

1. In conditions of low substrate concentration, the accumulation of nutrients at the solid/liquid interface will enhance biological growth.
2. Solid surfaces may retard the diffusion of exoenzymes away from the cell, promoting assimilation of nutrients which must be hydrolyzed extracellularly.

Additional advantages for microbial attachment include the following (Marshall, 1976; Characklis, 1973):

3. Biomass accumulated at the surface can be utilized for sustenance during periods of starvation.
4. Biomass can remain fixed within a flowing environment, and receive a constant supply of fresh nutrients without motility requirements.
5. Predation is reduced by the protection of the surrounding media.
6. Extracellular enzymes may be shared between bacteria.

The accumulation of bacteria at the solid/liquid interface results in the formation of a biofilm, which is generally a very adsorptive and porous structure, greater than 95% water. Biofilms can be a monolayer of cells or as much as 30 to 40 centimeters thick, as observed in algal mats (Characklis, 1984).

Biofilm development, as described by Characklis (1984), involves five processes:

1. transport of organic molecules to the surface;
2. adsorption of organic molecules to the wetted surface, resulting in a "conditioned" surface;

3. attachment of microbial cells to the conditioned surface;
4. metabolism by attached microbial cells resulting in more attached cells and associated material; and,
5. detachment of portions of the biofilm.

Marshall and co-workers (Marshall et al., 1971a & b; Marshall & Cruickshank, 1973; Marshall, 1976) propose that the attachment process involves two distinct phases. Phase 1 is an almost instantaneous phase, where bacterial adhesion is relatively weak and reversible. Phase 2 is irreversible film attachment, requiring an "incubation period" during which exocellular polymers are produced to achieve firm adhesion. During initial attachment, both motile and nonmotile bacteria remain in motion. At this stage the bacteria remain some small but finite distance from the surface by physical forces which result from a balance of Van der Waals attractive forces and electron double layer repulsion (Marshall et al., 1971a).

Culture age, time, temperature, and growth phase all affect Phase 1 attachment (Fletcher, 1977). Findings indicate that cells adsorb best in log growth and worst during death phases, that the number of attached cells is proportional to solution phase bacterial concentration until saturation is reached, and that the rate of adsorption is proportional to temperature. The total adsorption capacity has been found to be independent of temperature and solution phase population density. Breyers and Characklis (1982) have also observed, when feeding a biofilm reactor from a chemostat, that the rate and extent of attachment is directly proportional to growth rate in a mixed culture system.

A change in pH can cause cells to sorb or desorb due to a charge reversal phenomenon at the cell surface (Daniels,

1971). Attachment can be promoted by the addition of divalent cations to the growth media. No pH dependence was found by Werner and co-workers, however, for changes in pH from 5 to 8, nor temperature dependence between 5°C to 37°C, for bacterial loadings on carbon (Werner et al., 1979).

Phase 2 attachment, according to Marshall et al. (1971a), includes the bacterial production of exocellular polymers. Characklis (1984) has also included this phenomenon as the fourth step of biofilm development.

Zobell (1943) was first to suggest that firm attachment of bacteria to surfaces was mediated by some sort of adhesive bacterial secretion. Corpe and co-workers (Corpe, 1970a & b, 1972, 1973; Tosteson & Corpe, 1975) observed that marine bacteria attached to surfaces produced an exocellular polysaccharidic material which was found to be an acidic mucopolysaccharide composed mostly of polyanionic carbohydrates.

The relationship between the growth rate of Pseudomonas aeruginosa and the rate of exocellular polymeric substance (EPS) production under carbon limited conditions is quantified in a detailed study presented by Robinson and co-workers (Robinson et al., 1984). They investigated the extent to which growth rate affected EPS formation, and the fractionation of glucose consumption between EPS formation and purposes other than cellular reproduction. Their findings indicate that EPS was both growth and non-growth associated. Approximately 0.2 mg of polymer were formed per mg total glucose consumed; the fraction of glucose converted to EPS by P. aeruginosa was greater at lower growth rates. Others have also found that the extent of polymer formation is inversely proportional to the organism's growth rate. An important finding to note here is that neglecting polymer production by organisms such as

P. aeruginosa leads to significant overestimation of cellular yields, since such a large portion of energy and carbon is diverted to EPS formation. Differences in this study are as follows; Y = 0.6 mg cellular carbon/mg glucose carbon consumed (neglecting EPS production) and Y = 0.3 mg cellular carbon/mg glucose carbon consumed (accounting for EPS production). This is significant in light of the number of studies which use results obtained from suspended growth cultures to describe biofilm behavior (Chang & Rittmann, 1987; Namkung & Rittmann, 1986a & b; Rittmann & McCarty, 1980a & b; Speitel & DiGiano, 1987; Stratton et al., 1983).

A need for calcium and magnesium ions in secondary attachment has been observed. Marshall (1976) found that irreversible attachment failed to occur in the absence of both ions, while the addition of either was sufficient to allow attachment to proceed. Turakhia et al. (1983) observed a dramatic increase in biofilm detachment with the addition of EDTA, a calcium-specific chelant, emphasizing the importance of calcium to biofilm adhesion.

In addition to solution phase properties, characteristics of the solid surface affect bacterial attachment. Increased rates of attachment may be encouraged by a rough surface, which provides more surface area as well as some degree of protection from fluid shear, which may promote detachment (Characklis, 1984). Surfaces with lower energy have been found to retard cell adhesion; precoating exposed surfaces with a synthetic cationic polymer may significantly enhance initial biofilm attachment. Attachment in waters of low hardness, also, may be encouraged by the addition of divalent cations not only to the solution phase but to the solid surface as well.

Given that bacterial attachment and accumulation will occur at the solid surface, the film will grow to achieve a

condition of steady state which is ensured by the equilibrium maintained between cellular attachment and reproduction, and microbial decay and detachment. Characklis (1984) notes that detachment occurs from the moment of initial attachment, and results from two distinct contributions: shearing and sloughing. Shearing refers to the continuous removal of small portions of biofilm, and is highly dependent on hydrodynamic conditions within the fluid. The rate of shearing increases with biofilm thickness and fluid shear stress at the fluid/biofilm interface. Sloughing refers to the random removal of large sections of biofilm and is generally attributed to nutrient or oxygen limitations deep within the film, or to some dramatic change in the environment. Sloughing is more frequently observed in thick biofilms in a nutrient rich environment, such as a trickling filter wastewater treatment process.

Detachment may also result from chemical treatment. Oxidizing herbicides, chlorine, UV radiation, surfactants, and non-oxidizing biocides all will promote biofilm detachment to some degree.

## 2.3. Biodegradation Within the Biofilm

Biofilms are responsible for the removal of soluble and particulate contaminants from natural streams as well as in engineered treatment processes (Characklis, 1984). In natural waters, biofilms may determine water quality by influencing dissolved oxygen levels and may serve as a sink for many toxic and/or hazardous materials.

The rates of fundamental microbial processes within a biofilm are difficult to measure and are generally inferred from such lumped parameters as (1) substrate consumption,

(2) electron acceptor consumption (e.g., dissolved oxygen depletion), (3) biomass production, and (4) product formation (Characklis, 1984).

Substrate utilization within the biofilm is usually assumed to follow the Monod kinetic model:

$$\frac{dS}{dt} = \frac{k \cdot S \cdot X}{K_s + S}. \qquad (2\text{-}1)$$

where $S$ = substrate concentration within the biofilm (mass/vol)
$k$ = maximum rate coefficient (mass substrate/biomass-hr)
$X$ = biomass concentration (mass/vol)
$K_s$ = half saturation coefficient (mass/vol)
$t$ = time

(Utilization is considered to be a positive quantity.)

Utilization rates in the biofilm may be controlled by mass transfer limitations within the biofilm (Rittmann & McCarty, 1980a). Mass transport within the biofilm is governed by molecular diffusion, which is related to substrate concentration by Fick's law:

$$\text{Flux} = -D_f \frac{dS}{dz}. \qquad (2\text{-}2)$$

where $D_f$ = molecular diffusivity of substrate in biofilm ($L^2$/time)
$z$ = direction normal to surface

A material balance over an element of biofilm at steady state yields:

$$(\text{Flux in}) - (\text{Flux out}) - (\text{Reaction Rate}) = 0$$

$$D_f \frac{d^2S}{dz^2} = \frac{k \cdot X \cdot S}{K_s + S}. \qquad (2\text{-}3)$$

Utilization rates within the biofilm may also be controlled by mass transfer limitations in the bulk fluid (Characklis, 1984). At low fluid velocities, a thick mass transfer boundary layer can cause fluid phase mass transfer resistance, which will serve to decrease substrate concentrations at the fluid-biofilm interface and as a result will reduce the rate of utilization. This same phenomenon could be caused by a dilute liquid phase substrate concentration.

Substrate removal rates increase with an increase in biofilm thickness until a critical thickness is reached, beyond which removal remains constant. Trulear and Characklis (1982) observed that this critical thickness increases with bulk substrate concentration. Once the biofilm thickness exceeds the depth of substrate penetration (as determined by bulk substrate concentration and substrate utilization rates), removal rates will be unaffected by additional cells.

## 2.4 Characterizing Biofilm Behavior: Comparisons Between Free-Living and Fixed-Film Bacteria

In characterizing the kinetics of biofilm degradation, a common approach has been to use suspended cultures to determine parameters of the Monod equation for metabolism of the compound or compounds of interest (Chang & Rittmann,

1987; Namkung & Rittmann, 1986b; Rittmann & McCarty, 1980b; Speitel & DiGiano, 1987; Stratton et al., 1983). Parameters obtained in such studies are then used to predict the metabolic behavior of a biofilm grown in a flow-through system which has been seeded, usually, with organisms from the suspended population.

While the same principles of substrate utilization and microbial growth kinetics apply to both fixed-film and suspended-growth cultures, some differences in activity have been noted. Attached organisms are generally found to be more active in taking up nutrients, and are more resistant to such environmental stresses as starvation, heavy metals, and chlorine (Herson et al., 1987).

The applicability of stoichiometric data obtained in suspended culture to describe steady state biofilm processes was investigated in a study by Bakke and co-workers (1984). Their work suggests that, at a 5% level of statistical significance, there is no difference in substrate removal rates between chemostat data and combined chemostat/biofilm data. Furthermore, the biofilm growth rate data correlate well with chemostat data, supporting the use of chemostat derived kinetic coefficients to predict biofilm behavior.

Several investigators have used experimental techniques in a laboratory chemostat reactor to quantify rate and stoichiometry of fundamental processes within a biofilm (Breyers & Characklis, 1982; Trulear, 1983). Results suggest that chemostat-derived expressions may be used successfully to predict biofilm behavior in some cases. Important questions still remain, however, with respect to exocellular polymer production and microbial detachment.

Differences between the physiology of suspended-growth and fixed-film microorganisms may indeed give rise to

differences in their metabolic behavior. We might also question whether a heterogeneous population of microorganisms which colonizes a granular activated carbon filter in a water treatment process will behave similarly to cultures grown under conditions which basically select for degraders of the specific substrate or substrates under investigation. These uncertainties could be circumvented by utilizing the GAC-colonized microbial population in laboratory studies to determine biokinetic parameters. This more closely simulates conditions of the system about which predictions are to be made; thus, there should be greater confidence in the applicability of results.

In order to use the GAC biofilm population in a metabolic degradation study, the bacteria must first be removed from the carbon surface with minimal alteration of their metabolic activity. Methods have been presented in the literature for removal of viable bacteria from soil samples utilizing a variety of enzymatic, ionic, and polymeric substances in combination with blending, mixing, or homogenization (Balkwill et al., 1975, 1977; Bone & Balkwill, 1986; Macdonald, 1986). Balkwill et al. (1975) report a procedure whereby a soil sample suspended in sodium pyrophosphate solution (PPi, 0.1%) is subject to a series of blending and sonication treatments separated by slow speed centrifugation. This method, followed by additional centrifugation (15 centrifuge washes at 650xg) is said to have recovered 90% to 99% of a sample of Arthrobacter globiformis cells added to sterile and non-sterile soil (Balkwill, et al., 1977). Cells are recovered in the supernatant fraction from each centrifuged sample. In later work, Bone and Balkwill (1986) recommend the polymer polyvinyl pyrrolidone (PVP, molecular weight ranging from 10,000 to 360,000) as a releasing agent for all types of flotation studies. The material is said to release bacterial films effectively from both saturated and

unsaturated samples, although without physical agitation only 3.4% to 10.1% of the viable cells are recovered.

An entirely different procedure has been reported for removal of viable cells from colonized samples of granular activated carbon (Camper et al., 1985a). Homogenization of carbon particles at 16,000 rpm (4°C) with a mixture of Zwittergent 3-12 ($10^{-6}$ M), EGTA ($10^{-3}$ M), tris buffer (0.01 M, pH 7.0), and peptone (0.01%) recovered approximately 90% of a known concentration of Escherichia coli attached to a sample of GAC ($10^{6}$ cells per 0.1 gram carbon).

Cell recoveries in these studies have been determined by colony forming unit counts. Cell densities in supernatant samples are compared with those remaining in the pellet fraction, or with densities in the original untreated sample. While this method is preferable to a total cell count because only viable cells are enumerated, results may be biased. By definition, a viable cell plate count will only indicate the presence of microorganisms capable of utilizing nutrients present in the media chosen for growth. Cells which are viable yet unable to metabolize the media will go undetected. Extremely small colonies ("microcolonies") may develop, yet also go unobserved due to their size. Furthermore, a plate count is especially inapplicable to samples containing solid material since, while a particle may be inhabited by several bacteria, only one colony per particle is likely to be detected in the petri dish. This problem is often compensated for by introducing a normalizing factor to multiply up solid sample plate counts; this however introduces additional bias into the procedure by assuming a constant cell density per particle. The difficulties involved in quantifying biofilm bacteria raise questions regarding the actual cell recoveries reported in the literature.

## 2.5 Biodegradation - Adsorption Interactions

The presence of an adsorptive media may affect substrate utilization within the biofilm by altering solution phase concentrations, as well as by providing substrate at the biofilm/solid interface. The "biological activated carbon" (BAC) process refers to a granular activated carbon filter in which biological activity is encouraged. The mechanisms involved are similar to those of a slow sand (biological) filter, except that interactions between adsorption and biodegradation enhance contaminant removal. These interactions may be important not only during the initial stages of filter operation, before biological activity becomes significant, but also in the event of a chemical spill in that surface area is provided to adsorb non- or slowly-degradable molecules. Many compounds which may be toxic at higher levels will adsorb to the GAC surface, effectively reducing solution phase concentrations to non-toxic levels which can be managed by the microbial population. Alternatively, compounds present at concentrations below those necessary to support a community of specific degraders may sorb and be accumulated until, again, a manageable concentration is reached for biodegradation to proceed.

The influence of GAC on bacterial metabolism was investigated by Werner and co-workers (1979), using phenol as a substrate. They found that at 0.3 mg/L phenol the presence of GAC has a negative influence on utilization. At such low concentrations carbon hinders microbial metabolism. At 2.5 mg/L phenol, however, they noted utilization only in the presence of GAC. This concentration is evidently toxic, and the adsorptive surface tends to decrease solution phase concentrations to a metabolizable level.

Higher metabolic activity has been detected on GAC than on non-adsorptive media. Speitel and DiGiano (1983) present an investigation of biofilm degradation on GAC vs. that on glass beads in which GAC, preequilibrated with phenol at 0.5 to 1.2 mg/L, was used in an effort to eliminate adsorption. Their results show enhanced phenol biodegradation in the GAC system, due presumably to the availability of sorbed substrate. The same phenomenon was shown at higher concentrations by Li and DiGiano (1983). Higher specific growth rates and biodegradation rates of o-cresol, acetophenone, phenol, and benzoic acid were detected on GAC as compared to sand or glass beads. The enhanced specific growth rate increased with sorbed substrate concentration. A decrease in particle size also gave rise to increased growth rates, suggesting the importance of internal diffusion to the rate of resupply of substrate to the biofilm.

While many authors suggest interactions between the activated carbon surface and attached microorganisms, others claim that long-term contaminant removal is due not to biodegradation but to a slow adsorption process. Peel and Benedek (1983) used a dual rate adsorption kinetic model to predict purely adsorptive behavior in packed columns. The model suggests a two-step adsorption process consisting of diffusion into the macropores and then, more slowly, into the micropores of the GAC. Although biological activity was documented in their experimental column, as detected by oxygen uptake and microscopic examination, they conclude that relatively degradable organics are oxidized but that materials which resist degradation will either adsorb or pass through the process unaltered. Continued removal of substrate after long operating times, they propose, is due not to biodegradation but to the slow kinetics of adsorption into the GAC micropores.

## 2.6 Describing the Process: Biofilm Models

A great deal of interest during the past decade has been directed toward understanding the fundamental processes involved in biofilm degradation, both in the presence and in the absence of adsorption. A major thrust of the research has been to develop models which describe adsorption and biofilm degradation, for single and multiple substrate systems.

In 1976, Williamson and McCarty presented a basic biofilm model which idealizes the biofilm as a homogeneous matrix of bacteria and extracellular polymers which bind the bacteria together and to the surface (Williamson & McCarty, 1976a & b). The model takes into account mass transport of substrate from the bulk liquid phase to the biofilm surface, mass transprot within the biofilm, and substrate utilization within the biofilm. Rittmann and McCarty further developed the model by incorporating cell growth, and showed that by equating energy capture and energy expenditure in the biofilm a steady state biofilm thickness can be calculated (Rittmann & McCarty, 1980a). By equating the ratios of energy capture and expenditure, they predicted the existence of a minimum substrate concentration, $S_{min}$, below which no significant biofilm activity occurs:

$$S_{min} = \frac{K_s \cdot b}{Y \cdot k - b}. \qquad (2\text{-}4)$$

where $b$ = endogeneous decay coefficient (1/time)

$Y$ = microbial yield coefficient (mass cells/mass substrate)

Additional models for describing interactions between biodegradation and adsorption have been developed (Benedek, 1980; Chang & Rittmann, 1987; Tien, 1980; Ying & Weber, 1979; Speitel et al., 1987a). A model is presented by Speitel and co-workers (1987a) which describes simultaneous adsorption and biodegradation of a single substrate in a column system. Adsorption is described by the Fruendlich isotherm and surface diffusion in the adsorbent; Monod kinetics are used to describe biodegradation. Film transport resistance and biofilm growth and decay are also included.

Of special interest to this research is the model developed by Chang and Rittmann (1987). The model closely parallels that of Speitel et al. (1987a), except that instead of describing a column system, the model developed by Chang and Rittmann describes biofilm growth and substrate utilization in a reactor operating under a completely mixed regime (the Biofilm Activated Carbon model, BFAC). Axial concentration gradients are eliminated. The model is a combination of two component-models: the biofilm on glass beads (BFCM) model which describes biofilm degradation in the absence of adsorption, and the activated carbon in completely mixed flow (ACCMF) model which describes adsorption with little or no biodegradation. Effluent concentrations as predicted by each of the models are presented in Figure 2-1. Without adsorption, effluent concentrations are close to influent concentrations in the early stages of operation, before the biofilm is established. As microbial activity increases, effluent concentrations are reduced through biodegradation. With adsorption in the absence of biodegradation, effluent concentrations rise gradually until, eventually, there is no reduction in concentration across the filter. Biological activity in combination with adsorption offers protection during both the early stages of operation, when adsorption

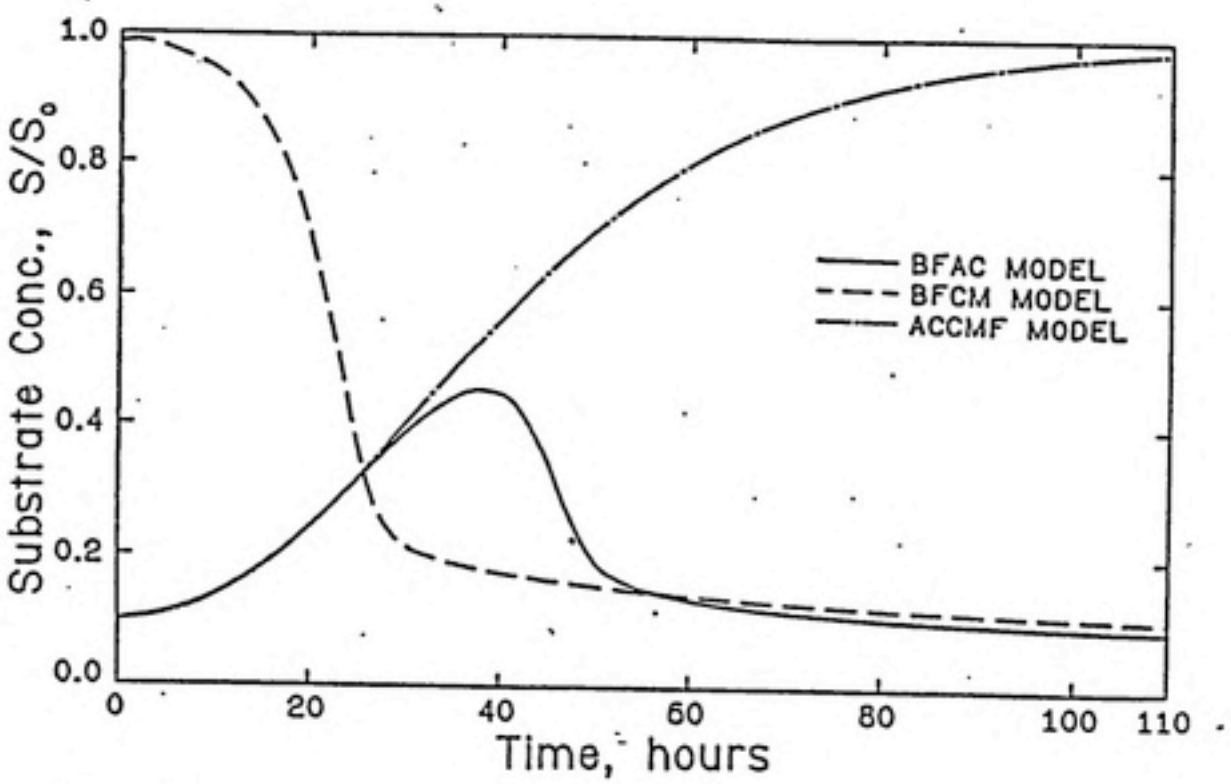



Figure 2-1. Model Predictions of Effluent Substrate Concentration from a Completely Mixed GAC Reactor (after Chang, 1985).

predominates, as well as later in the run when, although adsorptive capacity may be exhausted, contaminant removal across the bed is achieved as a result of biological activity.

## 2.7 Secondary Substrate Utilization

An expansion of the Rittmann & McCarty model has been presented which describes bisubstrate removal by biofilms (Namkung & Rittmann, 1986a & b). The model is based on a concept that substrate can be utilized by biomass which derives energy for growth and maintenance not from the substrate in question but from another substrate present. In such a way, compounds which are present at concentrations below those necessary to support growth (as determined by minimum energy requirements) may be degraded in the presence of additional compounds at concentrations greater than $S_{min}$. The utilization of a specific compound can be increased by altering the concentrations of growth-supporting substrate.

This concept, the biodegradation of compounds at concentrations below those necessary to support growth at the expense of an additional electron donor which provides energy and nutrients for growth and maintenance, is termed secondary substrate utilization. The secondary substrates do not supply energy for long term biofilm growth either because their concentrations are too low, or they are present in high concentrations but for only a short time. The model presented by Namkung and Rittmann describes steady state secondary utilization and biofilm accumulation in a bisubstrate system, as opposed to a shock loading scenario.

The biofilm model for bisubstrate utilization was tested using phenol as a target organic contaminant and acetate as representative of background metabolites (Namkung

& Rittmann, 1986b). It was found that when phenol was present at concentrations higher than $S_{min}$, an increase in acetate concentration enhanced phenol degradation. When phenol concentration was reduced below $S_{min}$, a minimum concentration of 3.3 μg acetate COD/L was required to stimulate phenol degradation; increasing acetate concentrations beyond this served to enhance phenol degradation. Further reductions in influent phenol concentrations required still higher acetate concentrations to stimulate degradation. In general they conclude that bisubstrate systems show significantly better removal than single substrate systems.

The effect of acetate addition on biological activity was also investigated by Speitel and co-workers (1987b). They added 2 mg/L sodium acetate to a GAC column receiving paranitrophenol (PNP), at 2 μg/L, in an effort to enhance biofilm growth. Degradation of PNP was less in the bisubstrate system than in a column which received 5 μg/L PNP without the supplemental acetate. The lower biological activity may have resulted from decreased PNP concentrations, or the microorganisms may have preferentially metabolized sodium acetate over the less biodegradable PNP.

Stratton and co-workers studied primary and secondary utilization of five different substrates in tubular reactors filled with glass beads (Stratton et al., 1983). Acetate, D-galactose, L-alanine, thymine, and phenol were tested as primary substrates at 3 mg/L, and as secondary substrates at 0.3 to 3.0 mg/L. They conclude from the work that, for the same test concentration, secondary utilization of a particular compound is less than primary utilization. Secondary utilization is more effective for removal of substrates at lower feed concentrations; as the secondary substrate concentration decreases, percent removal

increases. They also postulate that utilization of a permanent secondary substrate at low concentrations will usually be greater than that of a secondary substrate present in short duration.

Secondary substrate utilization of trace contaminants may occur in the presence of natural background organics (humic substances), which could serve as primary electron donors. Rittmann and co-workers, in a study of aquifer recharge in the Santa Clara Valley Water District, demonstrated that the utilization of general organics promoted and sustained the growth of a biofilm in an aquifer near the injection well. Certain individual compounds such as naphthalene and heptaldehyde were utilized as secondary substrates within the first meter of the well; other compounds were not degraded and were deemed either non- or slowly-biodegradable (Rittmann et al., 1980).

Biofilms grown on humic substances have been used to remove taste and odor causing compounds (Namkung & Rittmann, 1987c). Short-term secondary-utilization tests were performed, adding secondary substrates for two to three hours to biofilm reactors which had received 1 mg/L peat fulvic acid at 3 L/day for 290 to 360 days. Mineralization of the fulvic acids was constant at about 10%, as determined by TOC measurements. Results of the study indicate that natural humic substances are capable of supporting a biofilm which will degrade secondary compounds, both man-made (phenol, naphthalene) and of natural origin (Geosmin, 2-methylisoborneol (MIB)), which are present at very low concentrations. Best removals were noted at the lowest feed concentrations (1 μg/L for phenol and naphthalene; 100 μg/L for Geosmin and MIB).

## 2.8 The BAC Process: Contributions by Ozone

Noting the widespread occurrence of natural organic matter and its potential for serving as primary substrate for microbial growth brings us to consider a biologically active carbon filter as an effective means for controlling trace organics in a water supply. While the large majority of contaminants are present in the natural environment at concentrations below the minimum required to support growth, many may be degraded in the presence of biodegradable humic substances. Ozonation prior to a GAC filter should promote contaminant degradation, since ozone has been noted by several authors to increase the biodegradability of humic substances (Benedek, 1979; Hubele & Sontheimer, 1984; Schalekamp, 1979; Somiya et al., 1983).

Reactions between ozone and humic substances have been shown to result in the formation of carbonyl groups (chiefly COOH) among the volatiles from the humic and fulvic acids (Killops, 1986). Ozonation causes a shift in the molecular weight distribution, resulting in an increased percentage of lower molecular weight compounds (Lienhard & Sontheimer, 1979). Other effects due to ozonation include increased polarity, and a loss of double bonds and aromaticity, resulting in decreased adsorbability (Lienhard & Sontheimer, 1979).

Ozonation has been shown to increase the biodegradability of humic substances. Using a recycle reactor containing nonadsorbing anthracite coal, Hubele and Sontheimer (1984) demonstrated an increase in biodegradation with ozone dosage, from 0.18 to 1.82 gm $O_3$/gm TOC.

Preozonation has been proposed as a method for increasing biological activity in a GAC filter. In a pilot plant study, Janssens and co-workers (1984) evaluated

ozonation prior to GAC filtration with respect to organic matter removal and reduction of assimilable organic carbon (AOC). It was found that an ozone dose up to 2 mg/L could increase filter service time by 60% to 65%. Ozonation increased AOC in an amount proportional to the applied dose. Their results, in addition, suggest that ozonation may alter the pattern of the breakthrough curve due to the production of a higher proportion of biodegradable compounds as well as compounds of lower molecular weight and higher polarity, which may be less adsorbable.

In an attempt to adapt ozonation and GAC filters to produce "biological reactors", Hascoet and co-workers (1986) assert that while ozone may or may not decrease dissolved organic carbon (DOC), biodegradable dissolved organic carbon (BDOC) is always increased. In a full scale filter at Rouen-La-Chapelle, France, containing 1-year-old GAC, they determined that the dissolved organic carbon removed during filtration consists essentially of biodegradable dissolved organic carbon ($\Delta BDOC/\Delta DOC = 0.7$ to 0.9), and that this removal occurs in the top 20 to 40 centimeters of the filter.

A study was carried out on site in a potable water treatment plant for Paris which uses ozone at various points in the process train (Bourbigot et al., 1986). The experimental focus here was the role of ozone and GAC in removing mutagenic compounds and trihalomethane (THM) precursors. Samples were withdrawn at several points and tested for mutagenicity. An important finding is that, while ozone is successful in reducing mutagenicity, a sufficient dose must be applied. The number of mutants in water which received 1.5 mg/L ozone was 2.4 times higher than in the control sample, while no mutagenicity was found in water which received 3 mg/L ozone. Characteristics of the raw water were not reported. Ozone and GAC together are

reported here as one of the best methods for eliminating mutagenic compounds and for decreasing chlorine consumption and THM formation.

Preozonation has also been demonstrated to have a negligible effect on TOC removal. In a USEPA-sponsored pilot plant study at Shreveport, Louisiana, GAC beds were operated with and without preozonation at an empty bed contact time of 24 minutes (Glaze & Wallace, 1984). The organic content of the raw water ranged from 3 to 11 mg/L as TOC; ozone dosage was variable, but was usually less than 3.4 mg/L. Although microbial activity was important in both the ozonated and unozonated systems, results indicate that preozonation did not effect TOC removal at any time.

Similar studies using a different source of TOC, a shorter empty bed contact time, and a higher dose of ozone have shown improved removal of TOC with preozonation (Neukrug et al., 1984). Prechlorination of the influent water reduced the enhanced TOC removals in a parallel study, despite ozonation. This suggests that the beneficial aspects of ozonation prior to a GAC filter with respect to increased TOC biodegradation are dependent on characteristics of the raw water, as well as on the ozone dose applied.

## 2.9 Phenol Biodegradation

Phenol is a relatively degradable aromatic compound, and has been the subject of several investigations involving degradation kinetics at low substrate concentrations (Chesney et al., 1985; Jones & Alexander, 1986; Pipes, 1976; Scow et al., 1986; Shimp & Pfaender, 1985a & b; Subba-Rao et al., 1982).

Phenol utilization has been modeled as a second order process, depending equally on substrate and biomass concentrations (Paris et al., 1982), a first order process, depending only on substrate concentration (Subba-Rao et al., 1982), and also as following Monod kinetics, a mixed order model (Chang & Rittmann, 1987; Speitel & DiGiano, 1987). Jones and Alexander (1986), in a study to determine the applicability of various kinetic models to phenol mineralization in lake water, found that phenol concentration had great impact on the fit of a particular model. They determined that at 0.5 ug/L, the data fit a first order model; a Monod model without growth was best for concentrations near 1 ug/L, a logistic model at 2.0 ug/L, and logarithmic models at all higher concentrations.

The effect of additional substrates on phenol utilization has been investigated by several authors (Rozich & Colvin, 1986; Shimp & Pfaender, 1985a & b; Subba-Rao et al., 1982). Rozich and Colvin (1986) compared phenol utilization by cultures acclimated to phenol (500 mg/L) and to a phenol/glucose mixture (250 mg/L phenol, 500 mg/L glucose). Phenol removal rates were inhibited by the presence of glucose under both growth and nonproliferating conditions, indicating that the interference is occurring at the level of enzyme functioning. During the test, cells previously acclimated to phenol switched their preference to glucose. Cells may preferentially utilize compounds which will yield most rapid growth.

The addition of glucose at 10 gm/L had no effect on phenol mineralization in Cayuga Lake water (Subba-Rao et al., 1982); 78 to 96% of the phenol was mineralized in four days.

Shimp and Pfaender (1985a) investigated the influence of readily degradable carbon substrates (amino acids,

carbohydrates, fatty acids) as well as naturally occurring humic acids (1985b) on the degradation of monosubstituted phenols (m-cresol, m-aminophenol, p-chlorophenol). Phenol degradation was enhanced by adaptation to increasing concentrations of the readily degradable substrates; enhancement was the same for all three substrates, suggesting that the results are due to a general stimulation of metabolic activity.

Adaptation to increasing concentrations of humic substances, however, significantly reduced the ability of the community to degrade the monosubstituted phenols. The decrease in phenol degradation was not accompanied by a reduction in total cell counts, plate counts, or amino acid turnover times (an indication of the general metabolic state of the community), although a decrease in the population of specific compound degraders was noted. These results were unexpected; humic substances have been known to stimulate microbial activity (DeHaan, 1974, 1976; Seki, 1982). Since humic substances are relatively non-biodegradable, the community may have been starved for an additional carbon and energy source. Yet even with the addition of acetate, a readily utilizable substrate, the suppressive effect of the humic substances persisted.

It is possible that the suppressive effects noted by Shimp and Pfaender were caused by a release of heavy metals, previously bound to the humic substances, into the microbial feed solution. Another explanation may be that the humic substances interacted with bacterial enzymes. Butler and Ladd (1971) found that humic substances suppressed the activities of a number of proteases, presumably through irreversible binding of the enzyme to the humic and fulvic acid molecules. In any case, the authors emphasize that the inhibitory effects noted may be unique to the system under

investigation; the pervasiveness of such results has yet to be proven.

## 2.10 Summary

The presence of bacterial growth in granular activated carbon water filters is well documented. Numerous studies have investigated interactions between adsorption and biodegradation, and models have been developed to describe the process in single- and multiple-substrate systems.

Many investigators have used stoichiometric data obtained in chemostat or batch studies, utilizing suspended cultures, to describe biofilm behavior. Differences between characteristics of suspended-growth and fixed-film bacteria have been noted. It may be more desirable to utilize the microbial community indigenous to the GAC filter for obtaining stoichiometric data.

The interactions between adsorption and biodegradation at the carbon surface and the extent to which biological activity will aid in the treatment process are not fully understood. The growth of a biofilm on humic substances after ozonation, and the possible biodegradation of trace organic contaminants by the humic substances-grown biofilm, are both important topics for consideration.

# 3. GENERAL APPROACH TO EXPERIMENTAL WORK

The objectives of this research are directed toward obtaining a better understanding of the role played by biodegradation in a GAC filter receiving water which contains naturally occurring humic substances. We are interested in the ability of humic substances to serve as substrate for biofilm microorganisms, and in the ability of this humic substances-grown biofilm population to degrade a trace micropollutant as a secondary metabolite.

The overall experimental approach is summarized here to clarify how the different project phases are interlinked. Each phase will then be described separately in detail, including a description of experimental methods and the analysis of results.

The general experimental effort is outlined in Figure 3-1, which reiterates each of the objectives and shows how the accomplishment of one has enabled us to address the next.

Prerequisite to investigating the metabolic behavior of biofilm microorganisms is the elimination of the adsorptive system in which they have developed. The presence of an adsorptive surface such as GAC will interfere with biokinetic studies, since organic compounds will preferentially sorb to the carbon surface, altering their availability as substrate. Removing the biofilm microorganisms from the GAC surface, then, while maintaining

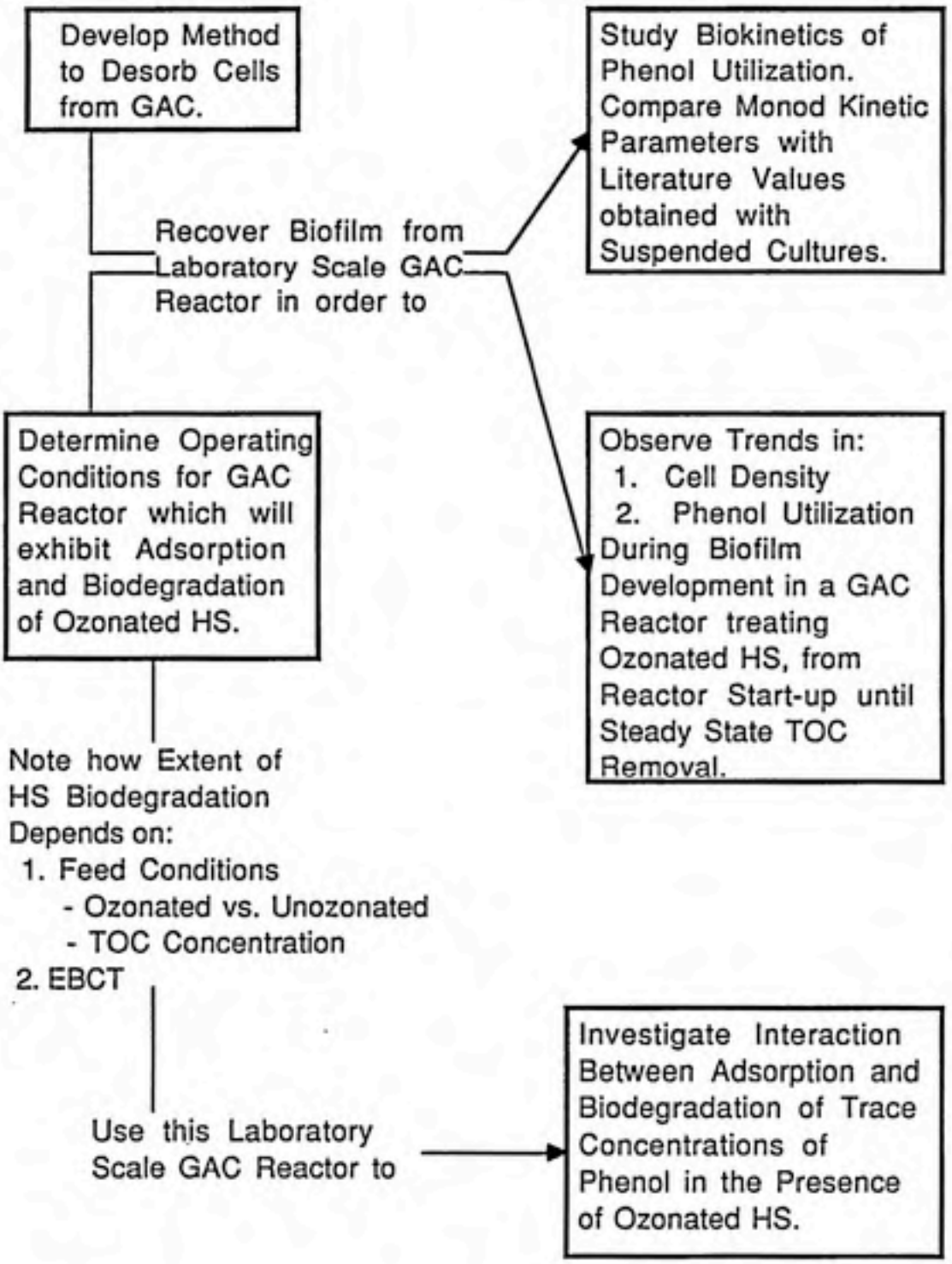



Figure 3-1. A General Overview to the Experimental Approach

their viability, was considered to be a necessary first step in the experimental design.

Also important during the initial stages of experimentation was the development of a laboratory scale column system which would exhibit adsorption and biodegradation of natural humic substances. The successful design of such a system would serve as the means for generating microbial samples to be used, in conjunction with the removal procedure, for such microbial analyses as biokinetic rate studies, and the assessment of total and viable cell densities, with cultures grown under different operating conditions. In addition, the column system would enable in situ investigations of the interactions between adsorption and biodegradation of a micropollutant in the presence of natural humic substances.

Phenol was chosen as the model micropollutant for use in this study. The compound is readily biodegradable, and in addition there is widespread information available in the literature concerning its behavior in both natural and engineered systems. While these may be good reasons for using phenol to develop and test the experimental procedures, the long-range plan for further research in this laboratory includes study of less-degradable compounds.

# 4. RECOVERY OF VIABLE CELLS FROM THE GAC SURFACE

## 4.1 Experimental Methods

Methods are presented in the literature for removing viable cells from soil samples (Balkwill et al., 1975, 1977; Bone & Balkwill, 1986; Macdonald, 1986) and from colonized granular activated carbon (Camper et al., 1985a) utilizing a variety of chemical solutions in combination with mixing, blending, or homogenization. Dobbins and Pfaender (1988) have developed a method for quantitative recovery of cells from subsurface soil samples using a solution of polyvinyl pyrrolidone (PVP-360, average molecular weight 360,000; Sigma Chemical Company, St. Louis, Missouri), a polymeric substance recommended by Bone and Balkwill (1986) as a releasing agent for microbial films, and sodium pyrophosphate buffer (PPi, $Na_2P_2O_7 \cdot 10H_2O$; Aldrich Chemical Company, Milwaukee, Wisconsin). The soil suspension is shaken vigorously on a rotary shaker, followed by slow speed centrifugation. Biomass is recovered in the liquid supernatant.

We have attempted to adapt the methods developed by Dobbins and Pfaender to the removal of viable cells from GAC. A general outline of our experimental program is presented in Figure 4-1. In an effort to optimize the procedure, we focused on the effects of physical parameters (length of time the suspension is shaken; ultrasonication vs. blending) as well as chemical parameters (three concentrations of PVP/PPi were tested, in addition to a

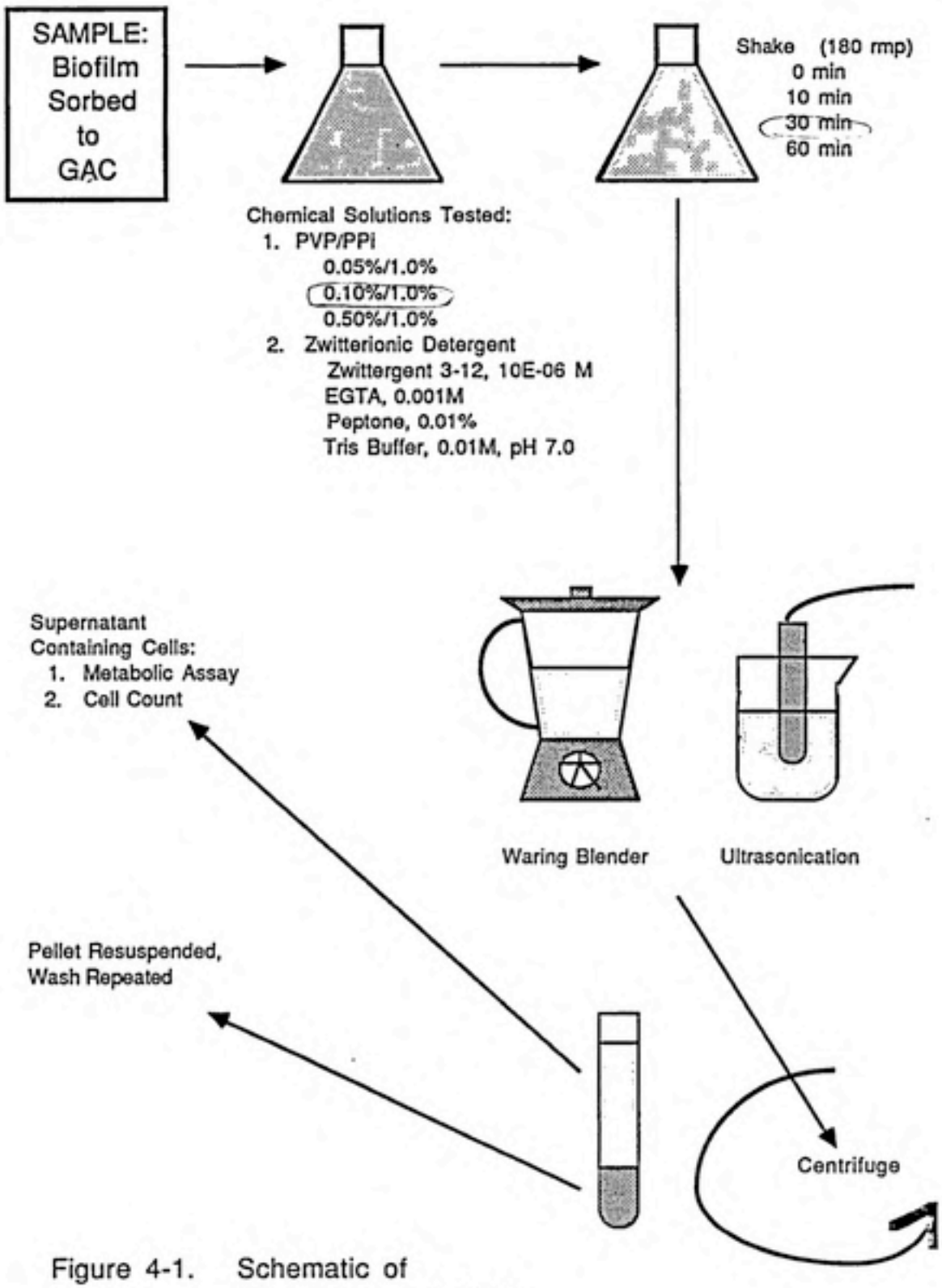



Figure 4-1. Schematic of Biofilm Removal Study

zwitterionic detergent presented by Camper et al., 1985) on the quantitative recovery of metabolically active cells.

A feasible and efficient method was needed for determining both the total number of bacteria in the original sample as well as the percent recovered into the supernatant phase. Viable cell plate counts are commonly used to quantify cell recoveries. In addition to problems noted with respect to media selectivity and the formation of "microcolonies", the method is inapplicable to solid samples; while a particle may be inhabited by several bacteria, only one colony per particle is likely to be enumerated.

We attempted, therefore, to utilize an amino acid respiration assay to quantify the general metabolic activity of the original sample as well as supernatant and pellet fractions obtained after subjecting the sample to a removal procedure. The technique involves incubation with a mixture of carbon-14 labeled amino acids and subsequent measurement of $^{14}CO_2$ evolved from solution as a result of respiration. The method could provide an unbiased measure of the recovery of metabolically active cells from the carbon surface; microscopic cell counts served as a check on the ability of this procedure to quantify cell recoveries.

Carbon Sample. Granular activated carbon was obtained from an operating GAC filter at the Sanford Water Treatment Plant in Sanford, North Carolina (5 MGD, population 18,000). The sample was collected from the top layer of the filter, packed into a clean unsterile mason jar and refrigerated at 4°C until use. Since only one carbon sample was obtained, it was necessary to store the carbon, refrigerated, throughout the duration of this phase of the project (approximately two months).

Subsamples were removed from the storage jar aseptically for each experiment, utilizing a sterile metal spatula. The carbon was rinsed three times with sterile distilled water to remove loosely attached biomass, and drained 15 minutes on sterile filter paper (Whatman # 1 qualitative filter paper). Dry weights for all GAC samples were obtained with a Metler analytical balance.

Physical Desorption Parameters. Physical agitation of a solid sample should aid in the removal of attached biomass. The extent of biofilm removal may depend on the duration as well as the intensity of physical agitation.

The influence of the duration of physical treatment was investigated by varying the length of time a sample was shaken with a solution of PVP and PPi. Since it was necessary to eliminate the effect of different chemical treatments on cell recoveries, we used a solution of 0.1% PVP/1.0% PPi (weight percent) throughout this phase of the investigation. This solution is used by Dobbins and Pfaender (1988) for desorbing biomass from soil samples. Samples of GAC (five to six grams dry weight) were suspended in sterile PVP/PPi reagent (75 ml) and shaken in sterile 200 ml beakers at 180 rpm on a rotary shaker (Jr. Orbital Shaker, Lab Line Instruments, Inc., Melrose Park, Illinois). Four samples were prepared, and were shaken for 0, 10, 30, and 60 minutes, respectively.

After shaking, each sample was blended for one minute in a sterile Waring blender (two 30 second bursts at 25 volts separated by a 30 second rest period). The mixture was centrifuged 15 minutes at 120 x g (Sorvall RC-2 refrigerated centrifuge, 4°C), enabling recovery of viable cells into the supernatant fraction. The supernatant was decanted and retained, while the pellet was resuspended in fresh PVP/PPi solution and the wash repeated. Four

successive washes were performed for each of the four treatments.

Supernatants from each of the four washes for each sample (16 supernatants total) and the carbon pellets which remained after centrifugation (four) were assayed for metabolic activity using the amino acid respiration procedure described on page 43. We used five live replicates and one dead control for each sample assayed. Dry weights of the carbon remaining from each wash and from each assay were measured and summed to enable calculation of the "amount of metabolic activity recovered per gram GAC" for each wash procedure.

In order to compare metabolic activity in supernatant and pellet fractions with that originally present in the composite (unwashed) sample, GAC (15 grams) was suspended in sterile PVP/PPi solution (200 ml) and assayed using the amino acid respiration procedure. The reason for using a PVP/PPi solution, instead of sterile distilled water, was to maintain constant conditions between the original and the treated samples. Changes in pH or solution composition could affect metabolic activity. Although the PVP/PPi solution could possibly desorb cells from the original GAC sample, whether the cells were sorbed or in solution was irrelevant; the entire solid/liquid slurry was assayed for metabolic activity.

The intensity of physical agitation was also tested for its influence on quantitative recovery of viable cells from GAC. Ultrasonication, instead of blending, was investigated as a means for a more vigorous physical treatment.

The effect of ultrasonication was examined with an activated carbon sample recovered from a laboratory column study in which an ozonated humics solution (TOC = 7 mg/L)

was fed at 2 L/day through a bed containing approximately 4 grams of GAC (Column Run No. 3, described in detail in Chapter 6). The GAC sample was split into two subsamples; both were suspended in sterile PVP/PPi solution and shaken for 30 minutes at 180 rpm (results showed that 30 minutes of shaking was optimal). One subsample was treated with the blending procedure described above, while the other was subject to ultrasonication for one minute at approximately 10 accoustic watts, pulsed at 50% duty cycle (500 Watt High Intensity Ultrasonic Processor, Tekmar Instrument Company, Cincinnati, Ohio). Both mixtures were centrifuged at 120xg; after recovering the cells with the supernatant phases, the pellets were resuspended in fresh PVP/PPi solution and the wash procedure repeated. Four successive washes were performed with each subsample. Supernatants from each wash were combined to yield one composite supernatant fraction from each treatment: blending and ultrasonication.

Supernatants from the blended and sonicated samples were subject to a metabolic rate study with phenol, as described in Chapter 5. This method was used instead of the amino acid respiration assay, to compare the treatments for recovery of metabolic activity from the GAC sample. Phenol respiration rates were measured at concentrations ranging from 1 to 5000 µg/L.

Total and viable cells were enumerated in each of the supernatant samples. Total cells were counted using a modified Acridine Orange Direct Count procedure, as described below. Viable cells were enumerated with a modified Standard Plate Count, described in Chapter 5.

Chemical Desorption Parameters. Four chemical solutions were tested for their effect in desorbing bacterial cells from the GAC surface. Three of these contained PVP/PPi in different concentrations (0.05/1.0, 0.1/1.0, and 0.5/1.0

%PVP/%PPi, respectively). The fourth solution was a zwitterionic detergent containing Zwittergent 3-12, $10^{-6}$ M (Calbiochem-Behring Corp., LaJolla, California), EGTA, $10^{-3}$ M (ethyleneglycol-bis-($\beta$-aminoethyl ether) N,N,N',N'-tetraacetic acid; Sigma Chemical Company, St. Louis, Missouri), 0.01% peptone (Difco Laboratories, Detroit, Michigan), and tris buffer (0.01 M, pH 7.0). All four solutions were prepared aseptically with sterile, particle-free distilled water.

Activated carbon was suspended in each solution (approximately 16 grams dry weight per 220 ml) in sterile 400 ml beakers. The GAC/chemical suspensions were treated with the shake-blend-centrifuge procedure described previously, using the Waring blender instead of ultrasonication. Samples were shaken for 30 minutes, which was determined optimal. During the first wash, subsamples were removed from each of the four treatments. These were assayed for total metabolic activity in the original sample as well as for total microscopic cell counts. Aliquots of the GAC/chemical slurries were pipeted from the mixtures as they were blending in the Waring blender. A total of 80 ml was removed from each sample; thus the pellets, after centrifugation, were resuspended in only 140 ml of their respective solvents for repeated washing. Six successive washes were performed on each sample.

Supernatants from each wash were combined to yield a composite supernatant fraction from each of the four chemical treatments. Supernatants and remaining carbon pellets were assayed for metabolic activity using the amino acid respiration technique. In addition, a microscopic cell count was performed on each of the four supernatant and four pellet fractions.

Potential Toxicity of PVP and PPi. Biofilm microorganisms recovered into the supernatant fraction of the GAC/chemical slurry were to be used in biokinetic studies to assess their metabolic activities. Since our intent was to relate this supernatant activity back to the original biofilm/GAC sample, it was essential that the chemicals used for desorption are not toxic or inhibitory to metabolic processes.

The effect of polyvinyl pyrrolidone (PVP) and sodium pyrophosphate (PPi) on metabolic activity was investigated by incubating carbon samples with radiolabeled amino acids in solutions of varying PVP/PPi concentrations.

A well mixed GAC/sterile phosphate buffer (0.1 M, pH 7.0) slurry was pipeted by ten ml aliquots into sterile 40 ml Pierce vials. To each of these vials, sterile PVP/PPi solutions (five ml) were added to yield the following concentrations (%PVP/%PPi): 0/0, 0/1.0, 0.05/1.0, 0.1/1.0, 0.5/1.0, 1.0,1.0, and 1.0/0.0. The samples were incubated for two hours with carbon-14 labeled amino acids and assayed for metabolic activity by quantifying the $^{14}CO_2$ produced during incubation. We prepared five live replicates and two dead controls (inhibited with sodium azide, $NaN_3$) for each PVP/PPi concentration.

Potential Adsorption of Amino Acids onto GAC. The presence of carbon fines in supernatant samples from the wash procedures could potentially complicate the amino acid respiration assay used to assess metabolic activity. Amino acids may adsorb to the carbon fines, altering solution phase substrate concentrations. Since respiration rates are a function of substrate concentration, it is important to know the precise concentration of amino acids in solution.

The adsorptive behavior of amino acids in the presence of GAC during the incubation period was investigated in two separate studies. One study was performed with a clean, untreated sample of carbon and another with carbon which had undergone several washes with PVP/PPi solution. The technique was identical for both studies.

Activated carbon, in amounts ranging from approximately 0.1 to 2 grams (dry weight), was suspended in ten ml distilled water in 40 ml Pierce vials. Metabolic activity was inhibited in each sample by the addition of sodium azide. After allowing 20 to 30 minutes to ensure inhibition, samples were injected with identical concentrations of the radiolabeled amino acid mixture as used in the respiration studies. The mixture was also injected into a vial containing only water and sodium azide to enable determination of initial amino acid concentrations.

The vials sat quiescent for 24 hours to simulate conditions of the incubation period. Following incubation, solution phases were sampled from each vial and assayed for radioactivity. In the study using GAC which had been treated with PVP/PPi solution, the vials were then acidified and shaken for 20 hours on a rotary shaker at 60 rpm. The sampling/assay procedure was repeated after shaking. Dry weights were determined for each individual sample in both studies.

The rate of amino acid adsorption was also investigated. A GAC sample (approximately three grams dry weight) was suspended in 40 ml distilled water and injected with carbon-14 labeled amino acids (final concentration again comparable to respiration studies). The supernatant phase was sampled periodically throughout the

incubation/acidification/shake procedure and assayed for radioactivity.

Determination of Total Cell Numbers. Total cell densities were determined using a modified Acridine Orange Direct Count (AODC) procedure (Hobbie et al., 1977). Supernatant, carbon pellet, and original GAC composite samples were collected in sterile 40 ml pierce vials and fixed with filtered formalin (final concentration 2%). Samples were stored at 4°C for approximately 2 weeks before counting.

Solid samples were homogenized at room temperature for approximately eight minutes (intermittent blending separated by periods of resting) using a Sorvall Omnimixer. Aliquots of the homogenized slurry were pipeted into clean, pre-weighed vials for dry weight determination.

Serial dilutions of each of the homogenized slurries and supernatant samples were prepared in triplicate and treated with Acridine Orange stain (final concentration 0.01%). Each dilution was filtered through an Iragalan Black soaked 0.2 μm Nuclepore filter and viewed through a Leitz Ortholux II epifluorescent microscope equipped with a 50 Watt A.C. mercury source, under 1250x magnification. Most cells fluoresced bright red against a field of orange, while a few were green. Ten to fifteen fields were counted per triplicate sample.

Amino Acid Respiration Technique. The amino acid respiration procedure was used to assay samples for metabolic activity. The general technique is outlined in Figure 4-2.

The assay was performed by pipeting samples in ten ml aliquots into sterile 40 ml Pierce vials. Supernatant samples were pipeted directly, while solid GAC samples were

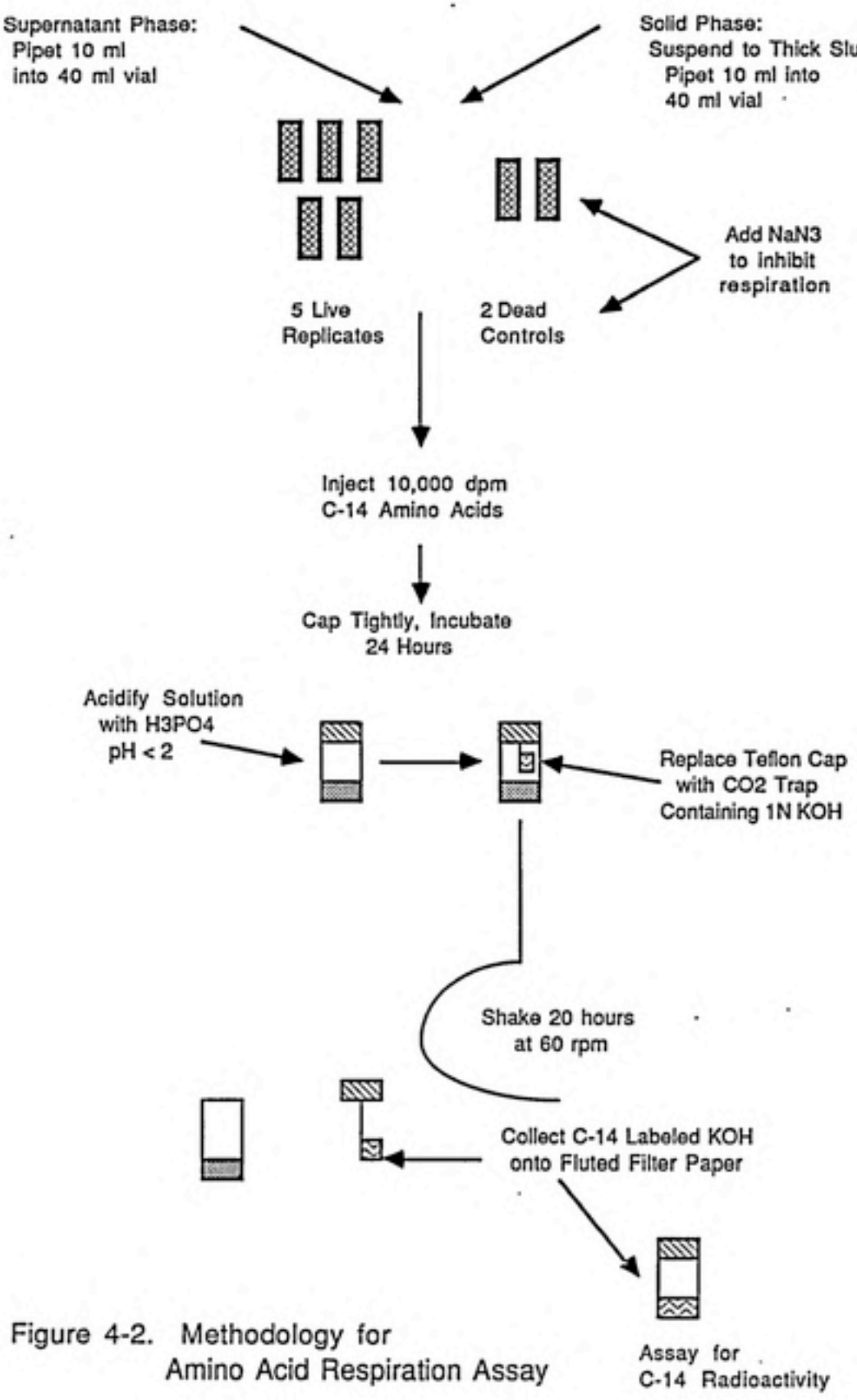



Figure 4-2. Methodology for Amino Acid Respiration Assay

suspended to a thick slurry (approximately 0.75 grams GAC per ten ml solution) in a sterile Waring blender and pipeted with a ten ml oxford pipette with an enlarged tip. For each sample assayed, six or seven vials were prepared: five served as replicate samples, while one or two were amended with sodium azide (final concentration approximately 1%) and served as "dead" controls (these are referred to as "deads", although sodium azide is a respiratory inhibitor and does not necessarily kill cells). Activities in the control vials were subtracted from the live samples to determine net respiration.

Each sample was injected with a mixture of radiolabeled amino acids which had been prepared by diluting a stock solution of carbon-14 labeled amino acids (New England Nuclear, Boston, Massachusetts; specific activity = 7.137 μg/μCi) in 50% ethanol. The volume of amino acids added to each vial was 50 to 60 μl, or approximately 0.032 μg (4.5E-03 μCi; 10,000 disintegrations per minute, dpm). Exact measurement of the initial amino acid concentration was determined by adding the mixture directly to three scintillation vials containing ten ml counting cocktail (Scintiverse II, Fisher Scientific).

Samples were capped with sterile Teflon septa and incubated for 24 hours at room temperature in the dark. This incubation time was proven sufficient to produce a measurable amount of $^{14}CO_2$ by metabolic activity. Following incubation, each vial was acidified to pH 2 with phosphoric acid (500 μl $H_3PO_4$, 20% by weight). The Teflon septa were replaced with septa equipped with a $CO_2$ trap which held 150 μl potassium hydroxide (KOH, 1N). Vials were then shaken at 60 rpm on a rotary shaker for 20 hours, driving carbon-14 labeled $CO_2$ from the acidic solution, into the base filled trap.

Carbon-14 labeled base was recovered from each of the samples onto fluted pieces of Whatman #1 filter paper, which were placed into scintillation vials with 10 ml counting cocktail. Carbon-14 activity was assayed with a Packard Tri-Carb 300 CD liquid scintillation counter.

The efficiency of $^{14}CO_2$ recovery was determined by incubating two or three additional samples with carbon-14 labeled barium carbonate ($Ba^{14}CO_3$, New England Nuclear, Boston, Massachusetts). The solution was added simultaneoulsy to two or three scintillation vials with counting cocktail to determine the amount injected (equal to 100% efficiency).

Each solid phase sample was retained for dry weight analysis. Samples were dried at 40 to 45°C for approximately two weeks, followed by dessication to ensure complete dryness.

## 4.2 Results and Discussion

The effect of PVP/PPi solutions on metabolic activity is presented in Figure 4-3. The fraction of added amino acids respired is plotted as a function of PVP/PPi concentration. An inhibitory effect of the PVP/PPi reagent is noted, as evidenced by reduced respiration. The response, however, does not necessarily depend on PVP or PPi concentration. A PVP/PPi solution concentration of 0.05%/1.0% supresses metabolic activity by 39% as compared to the untreated sample, yet at concentrations of 0.1%/1.0%, 0.5%/1.0%, and 1.0%/1.0%, activity decreases by only 22%, 25%, and 30%, respectively. A 1% solution of either PVP or PPi alone reduces activity by 28%.

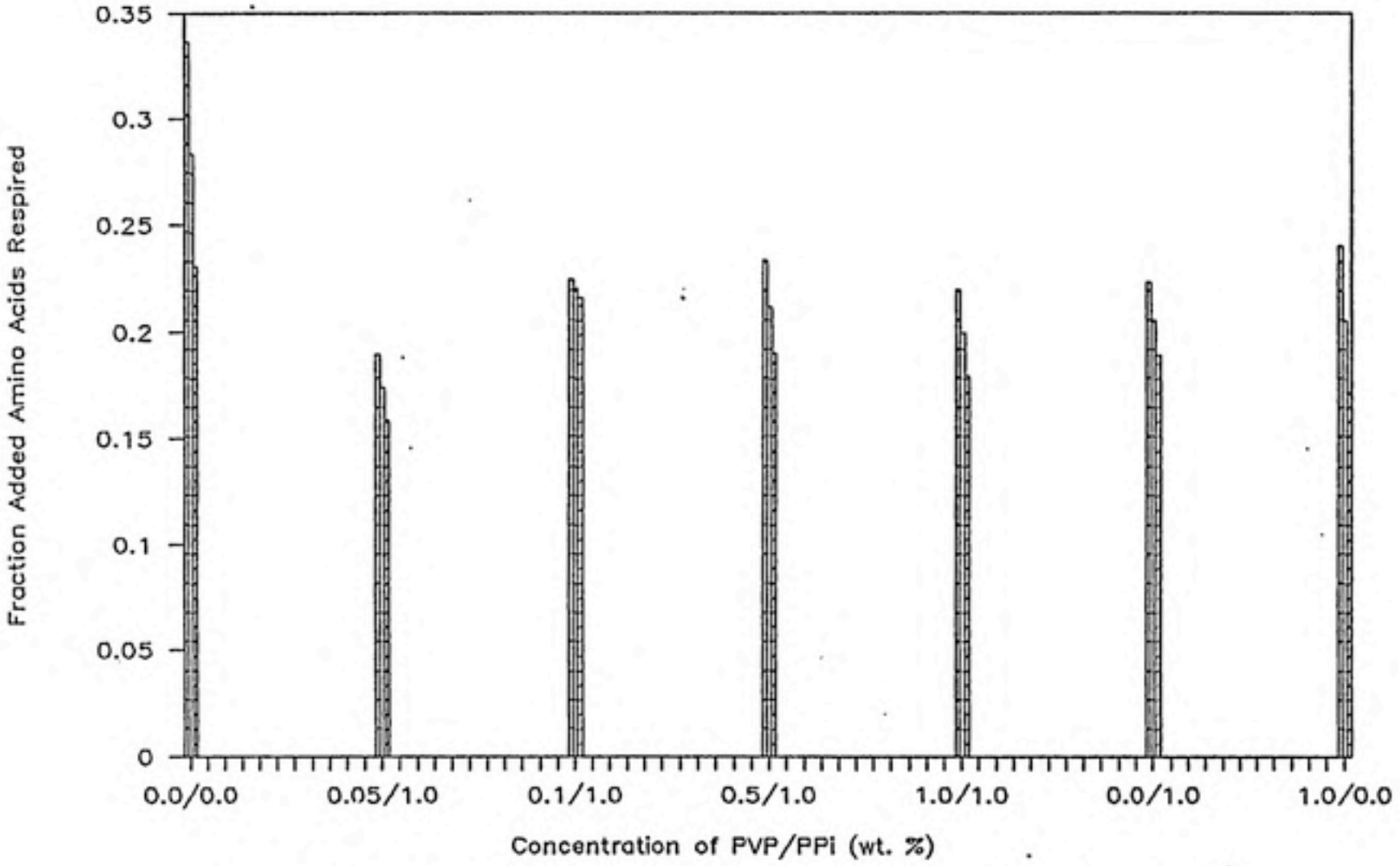



Figure 4-3. Effect of PVP/PPi Solutions on Metabolic Activity of Biofilm Microorganisms still in Contact with GAC: Fraction Added Amino Acids Respired vs. PVP/PPi Concentration. Upper and Lower Values Represent Means +/- Standard Deviations.

Results of the physical treatment study investigating the effect of mixing time on recovery of metabolic activity from the solid phase are summarized in Table 4-1 and Figures 4-4 and 4-5. Table 4-1 presents the amino acid dpm respired as well as the fraction of added amino acids respired in each supernatant sample from each wash. Values for the activity remaining in the GAC pellets from each treatment are included, as well the activity of a composite (unwashed) sample. Higher respiration indicates the presence of more metabolic activity in the sample. The fraction of added amino acids respired for each wash is expressed per gram of GAC from which the sample was obtained. Since GAC sample size varied between treatments, normalizing the results by GAC weight produced a means for comparison.

Results are plotted in Figures 4-4 and 4-5. Metabolic activities in the supernatant samples from the four washes have been summed to yield the total dpm respired for each treatment. Metabolic activity remaining in the GAC pellet is expressed as the fraction of added amino acids respired.

Recovery of metabolic activity from the solid phase into the supernatant fraction increases with an increase in length of mixing or shaking, up to a wash of approximately 30 minutes. Supernatant activity increases by 90% from a mixing time of 0 to 30 minutes, with no appreciable change thereafter (up to 60 minutes). Pellet fractions show a corresponding decrease (by about 90%) and leveling off in activity.

The recovery of significant activity even in the third and fourth washes is evident in Table 4-1. For all four samples, the fourth wash recovered 20% to 30% as much activity as the first wash (higher percentages were recovered in later washes for the 0 and 10 minute samples than for the 30 and 60 minute samples, as may be expected).

Table 4-1

Summarized Data: Effect of Mixing Time on Recovery of Metabolic Activity from GAC

| Mixing Time: | 0 min | | | | 10 min | | | | 30 min | | | | 60 min | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | dpm Resp per gm GAC | dpm/g +-S | Frac Resp (f) | f +-S | dpm Resp per gm GAC | dpm/g +-S | Frac Resp (f) | f +-S | dpm Resp per gm GAC | dpm/g +-S | Frac Resp (f) | f +-S | dpm Resp per gm GAC | dpm/g +-S | Frac Resp (f) | f +-S |
| SUP Wash | 262.6 | 215.1 | 2.39E-02 | 2.54E-02 | 197.9 | 228.6 | 2.34E-02 | 2.70E-02 | 413.7 | 484.2 | 4.89E-02 | 5.72E-02 | 401.4 | 414.3 | 4.74E-02 | 4.89E-02 |
| PER | | 190.1 | | 2.24E-02 | | 167.2 | | 1.98E-02 | | 343.2 | | 4.06E-02 | | 388.5 | | 4.59E-02 |
| RIN Wash | 61.1 | 63.4 | 7.21E-03 | 9.89E-03 | 113.5 | 136.6 | 1.34E-02 | 1.61E-02 | 120.9 | 142.2 | 1.43E-02 | 1.68E-02 | 124.6 | 130.1 | 1.47E-02 | 1.53E-02 |
| NAT | | 58.8 | | 4.53E-03 | | 90.4 | | 1.07E-02 | | 99.6 | | 1.18E-02 | | 119.1 | | 1.41E-02 |
| ANT Wash | 44.5 | 47.3 | 5.26E-03 | 5.59E-03 | 80.6 | 113.3 | 9.52E-03 | 1.34E-02 | 98.4 | 108.9 | 1.16E-02 | 1.28E-02 | 92.5 | 102.1 | 1.09E-02 | 1.20E-02 |
| AGE | | 41.7 | | 4.93E-03 | | 47.9 | | 5.66E-03 | | 87.9 | | 1.04E-02 | | 82.9 | | 9.76E-03 |
| NIS Wash | 65.8 | 73.3 | 7.77E-03 | 8.65E-03 | 72.9 | 87.6 | 8.61E-03 | 1.03E-02 | 82.4 | 95.9 | 9.74E-03 | 1.13E-02 | 106.7 | 119.7 | 1.26E-02 | 1.41E-02 |
| TS | | 58.3 | | 6.89E-03 | | 58.2 | | 6.87E-03 | | 68.9 | | 8.15E-03 | | 93.7 | | 1.11E-02 |
| Total Super. | 374.0 | 389.0 | 0.044 | 0.047 | 464.9 | 517.4 | 0.055 | 0.061 | 715.4 | 791.0 | 0.085 | 0.093 | 725.2 | 746.6 | 0.086 | 0.088 |
| | | 359.0 | | 0.041 | | 412.4 | | 0.049 | | 639.8 | | 0.076 | | 703.8 | | 0.083 |
| Pellet | 2670.5 | 3022.8 | 0.173 | 0.196 | 975.0 | 1155.6 | 0.137 | 0.162 | 707.7 | 738.4 | 0.088 | 0.092 | 867.9 | 913.8 | 0.089 | 0.093 |
| | | 2318.1 | | 0.151 | | 794.5 | | 0.111 | | 677.1 | | 0.084 | | 822.0 | | 0.084 |
| Original Composite Sample (no wash) | 4874.7 | 5258.9 | 0.402 | 0.434 | | | | | | | | | | | | |
| | | 4490.6 | | 0.370 | | | | | | | | | | | | |

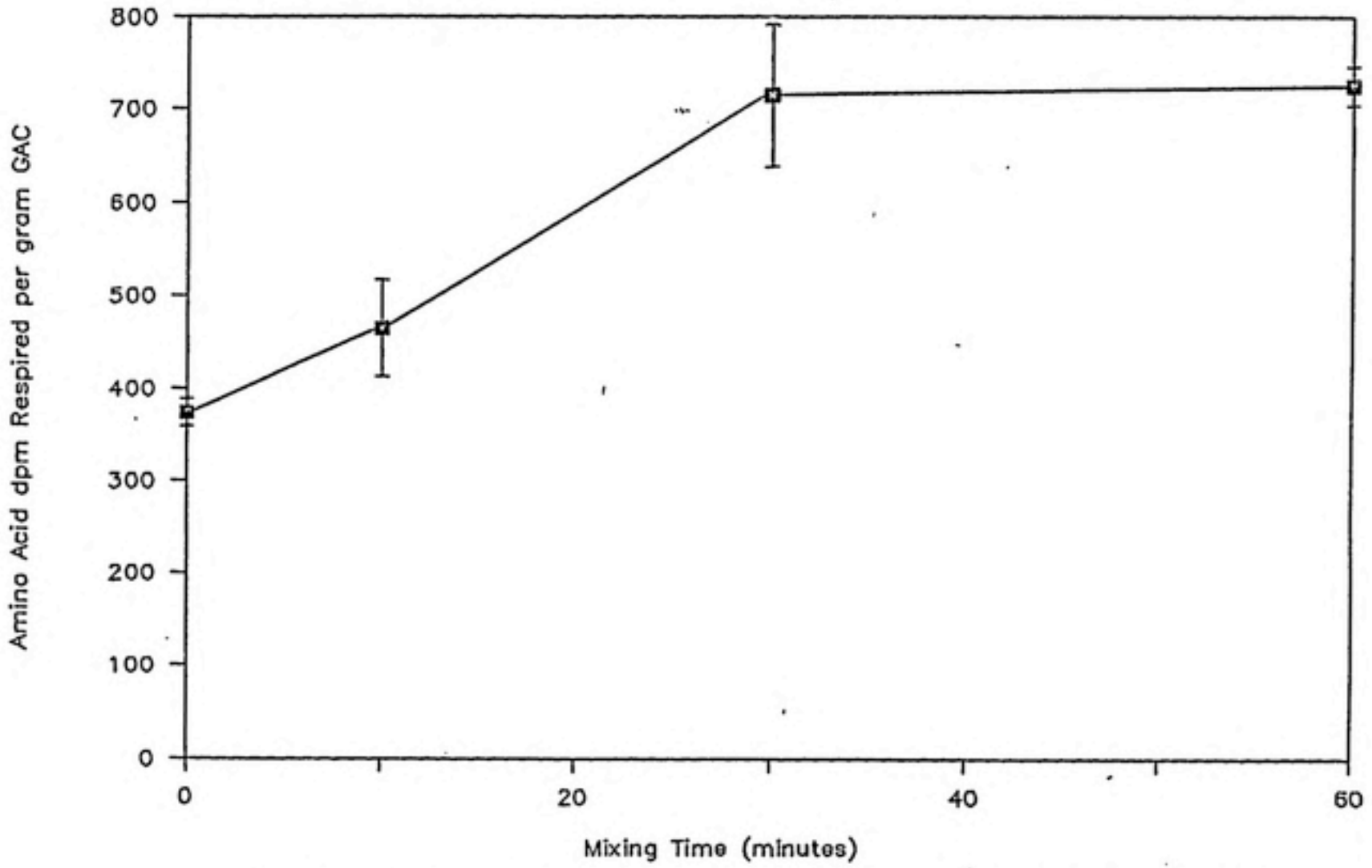



Figure 4-4. Effect of Mixing Time on Recovery of Metabolic Activity from a Biofilm into a Liquid Suspension: Amino Acid dpm Respired per gm. GAC by Supernatant Samples. Upper and Lower Values Represent Means +/- Standard Deviations.

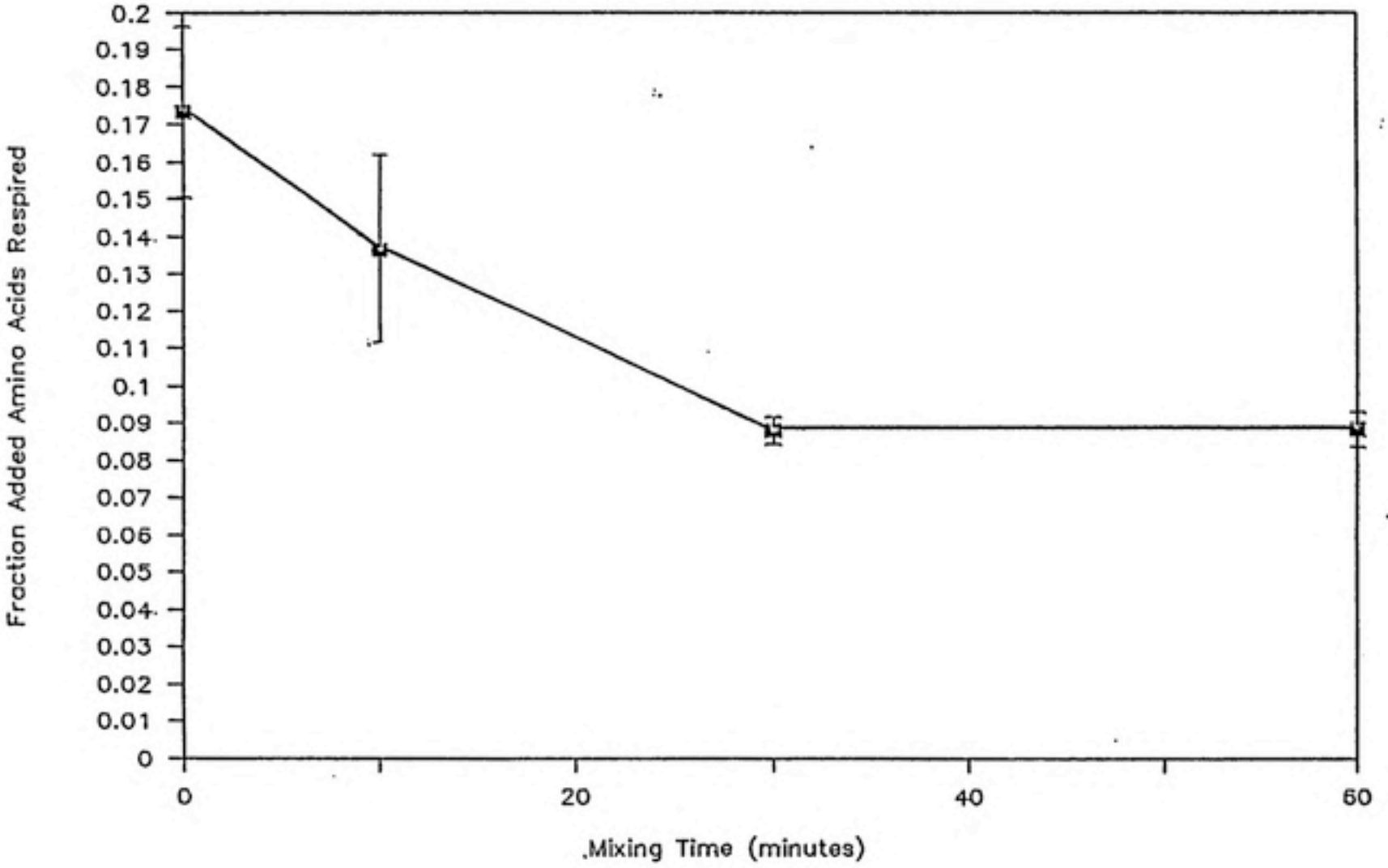



Figure 4-5. Effect of Mixing Time on Recovery of Metabolic Activity from a GAC Biofilm: Fraction Added Amino Acids Respired by GAC Pellet Fractions. Upper and Lower Values Represent Means +/- Standard Deviations.

This suggests that still more activity may have been recovered with additional washes.

These data suggest an optimum mixing time of 30 minutes with several successive washes. The percentage of metabolic activity recovered can be calculated by comparing activities presented in Table 4-1 for each of the fractions assayed from each treatment. Table 4-2 shows the percent metabolic activity recovered by each treatment, calculated in several different ways. The supernatant activity has been divided by the sum of activities in the supernatant and pellet fractions, utilizing both the "fraction of added amino acids respired (f)" (Method 1) as well as the "total amino acid dpm respired" (Method 2). In addition, percent recovery has been calculated as the difference between activity originally present in the composite sample and that remaining in the pellet fraction, divided by the original activity of the composite sample (Method 3).

It is evident from Table 4-2 that the percent recoveries of metabolic activity will vary, depending on the type of calculation used. Recoveries calculated by Method 3 are substantially higher for all four treatments than those calculated with either of the other two methods. This can be explained by noting the losses in metabolic activity between the original composite sample and the sum of supernatant and pellet fractions, after treatment. These values are also presented in the table, and exceed 50% for most samples. The inability to account for all of the original sample's metabolic activity, after treatment, implies one of two things: either the wash procedure is inhibitory to microbial respiration, or the procedure used for comparing solid (pellet) and liquid (supernatant) fractions is not valid.

Table 4-2

Calculated Results: Effect of Mixing Time on Percent Recovery of Metabolic Activity from GAC

| Mixing Time: | 0 min | 10 min | 30 min | 60 min |
|---|---|---|---|---|
| % Recovery (1) | 20 | 29 | 49 | 49 |
| % Recovery (2) | 12 | 32 | 50 | 45 |
| % Recovery (3) | 57 | 66 | 78 | 78 |
| % Loss of Activity (1) | 46 | 52 | 57 | 57 |
| % Loss of Activity (2) | 48 | 70 | 71 | 67 |

$$\%\ \text{Recovery (1)} = \frac{\text{((f) Resp. by Supernatant)}}{\text{((f) Resp. by Supernatant) + ((f) Resp. by Pellet)}} \times 100\%$$

$$(2) = \frac{\text{(dpa/ga Resp. by Supernatant)}}{\text{(dpa/ga Resp. by Supernatant) + (dpa/ga Resp. by Pellet)}} \times 100\%$$

$$(3) = \frac{\text{((f) Resp. by Original Sample) - ((f) Resp. by Pellet)}}{\text{((f) Resp. by Original Sample)}} \times 100\%$$

$$\%\ \text{Loss of Activity} = 1 - \left(\frac{\text{Met. Activity in Supernatant + Met. Activity in Pellet}}{\text{Metabolic Activity Originally Present in Composite Sample}}\right) \times 100\%$$

(1) uses the Fraction of Added Amino Acids Respired
(2) uses the Amino Acid dpa Respired per ga GAC

There are two basic problems encountered when comparing solid and liquid sample activities. One problem involves adding activities in the supernatants from each of the four washes to yield a total metabolic activity recovered by each treatment. While the procedure is straight forward for activities expressed as dpm respired per gram, adding the fractional respiration for the four washes is not so clear. The following explanation might offer help in understanding why this is so:

Let $f_i$ = the fraction of added amino acids respired by the supernatant from the ith wash of a particular treatment (i = 1 to n).

$$f_i = \frac{AA_{r,i}}{AA_{o,i}}.$$

$AA_{r,i}$= amino acid dpm respired by the supernatant from the ith wash of the treatment
$AA_{o,i}$= amino acid dpm added to the supernatant from the ith wash of the treatment.

$$\Sigma f_i = \frac{AA_{r,1}}{AA_{o,1}} + \frac{AA_{r,2}}{AA_{o,2}} + \ldots + \frac{AA_{r,n}}{AA_{o,n}}. \qquad (4\text{-}1)$$

With an infinite number of washes, all of the metabolic activity will be recovered from the biofilm into a liquid supernatant. It does not necessarily follow, though, that as i approaches infinity, f approaches 1. The fraction of added amino acids respired is a relative number used to compare metabolic activity between samples; the magnitude of f is useful in this study not as an absolute number, but for comparison purposes only.

An alternative method for adding the fractions respired in each wash can be found by considering what would have resulted had we combined the four washes to yield a composite supernatant sample, and added the entire amount of amino acids to this composite sample:

$$\Sigma f_i = \frac{AA_{r,1} + AA_{r,2} + \ldots + AA_{r,n}}{AA_{o,1} + AA_{o,2} + \ldots + AA_{o,n}}. \qquad (4\text{-}2)$$

This calculation seems more appropriate from the perspective of methodology; however, the fractional respiration obtained as such is equal to the average of the individual washes. This neglects metabolic activity recovered by additional washes.

Fractional respirations reported for the supernatant samples in Table 4-1 and used in calculating results reported in Table 4-2 were obtained by adding the fraction of added amino acids respired by each wash (f) for each treatment (equation 4-1). Neither of the methods discussed for adding the fractions are sound, yet numbers obtained with this calculation appear reasonable.

Supernatant activities expressed as dpm/gm are more reliable than those expressed as a fractional respiration, due to the uncertainties noted for adding the fraction of added amino acids respired by each wash. This reveals another problem with comparing solid and liquid samples, because for solid samples a fractional respiration more appropriately describes metabolic activity. Since the amino acid concentration is expressed per gram GAC in the solid sample, the initial substrate concentration will vary due to differences in GAC dry weights between samples. Changes in initial concentration will reflect changes in utilization kinetics. Samples with less GAC will respire at a faster rate, since more substrate is available; those with a

greater mass will respire more slowly. In other words, the difference in density of metabolically active cells in a solid sample may not reflect a change in the amino acid dpm respired, due to differences in metabolic velocities with substrate concentration. Using the fractional respiration normalizes for changes in metabolic velocities due to variation in initial substrate concentration.

Whether expressed as amino acid dpm respired per gram or as fractional respiration, the results reported in Table 4-2 indicate maximum metabolic activity recoveries of approximately 50%, with four successive 30 or 60 minute washes.

The effect of mixing intensity on desorption of biofilm microorganisms from a solid surface is shown in Table 4-3 and Figure 4-6. No attempt was made to measure metabolic activity or cell densities in the GAC pellet fractions; comparisons were made only between supernatants recovered by the two treatments. Table 4-3 presents results for total and viable cell recoveries by blending and ultrasonication. While ultrasonication apparently recovered more viable cells from the GAC (4.31E+08 vs. 9.04E+07), the results are suspect due to large standard deviations in cell densities obtained with this method. With respect to total cells, sonication recovered a substantially greater portion than did blending (4.38E+09 vs. 2.05E+09). At this point it is unclear whether or not sonication is disrupting cell membranes, resulting in the recovery and subsequent death of a larger number of cells, or whether sonication does in fact recover a higher percentage of viable cells.

Metabolic activities of the supernatants from the blended and sonicated samples are presented in Figure 4-6. The rate of phenol respiration per gram of GAC in the original sample is plotted vs. substrate concentration. The

Table 4-3

Cell Recoveries from GAC: Blending vs. Ultrasonication

| | Blended Sample | | | Sonicated Sample | | |
|---|---|---|---|---|---|---|
| Carbon Dry Wt. | 1.87 | | | 2.47 | | |
| | cells/gm (avg) | Std Dev (S) | avg +-S | cells/gm (avg) | Std Dev (S) | avg +-S |
| Viable Cells | 9.04E+07 | 4.17E+07 | 1.32E+08<br>4.87E+07 | 4.31E+08 | 8.91E+08 | 1.32E+09<br>-4.60E+08 |
| Total Cells | 2.05E+09 | 3.71E+08 | 2.42E+09<br>1.68E+09 | 4.38E+09 | 1.12E+09 | 5.50E+09<br>3.26E+09 |
| Viable/Total (%) | 4.4% | | | 9.8% | | |

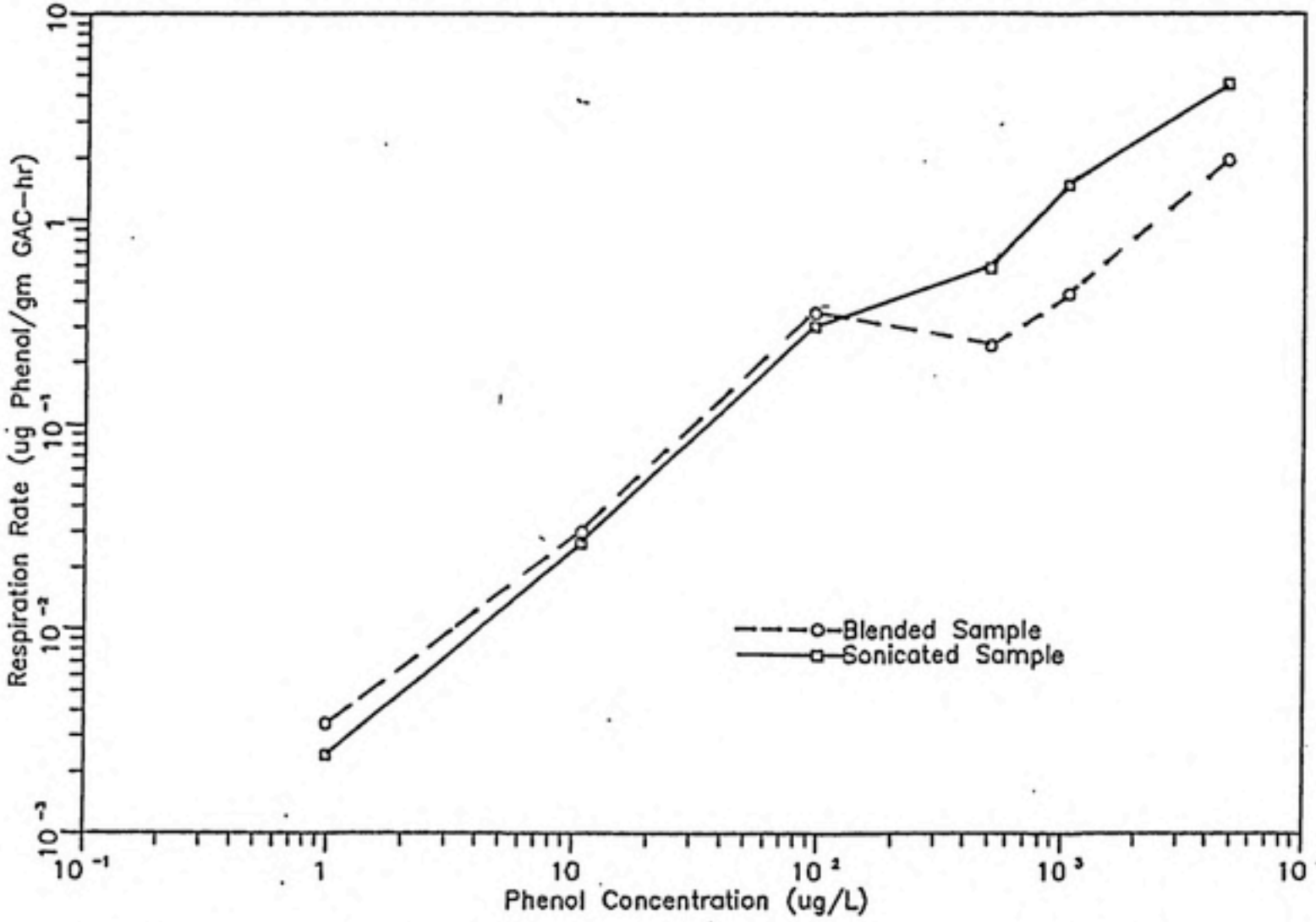



Figure 4-6. Effect of Mixing Intensity on Recovery of Metabolic Activity from a Biofilm into a Liquid Suspension: Phenol Respiration Rate vs. Concentration for Microbial Samples recovered by Blending and by Ultrasonication.

results suggest that differences in phenol respiration at lower concentrations (up to 100 μg/L) are insignificant; at higher concentrations the blended sample appears to be less active, although we should question whether or not this is a real result or an artifact of the experimental methods, as depicted by the discontinuity in rate as a function of substrate concentration for this sample.

The effect of treatment chemicals on cell recovery is summarized in Table 4-4 and Figures 4-7 and 4-8. Treatments A, B, and C refer to PVP/PPi solutions at concentrations of 0.05/1.0, 0.1/1.0, and 0.5/1.0 %PVP/%PPi, respectively; Treatment D refers to the zwitterionic detergent (formula presented in Table 4-4). Total metabolic activity has been assayed for composite samples from each of the four treatments and is presented in Figure 4-9. Metabolic activity in the composite sample decreases with an increase in PVP concentration, and is especially inhibited by the zwitterionic detergent. These potential toxic effects will tend to skew the data; cell recoveries will be masked by suppression of the total sample's metabolic activity as well as selective suppression of activity in the supernatant or pellet fractions.

Table 4-5 summarizes the percent recovery of metabolic activity by the four chemical treatments, calculated by comparing the activities presented in Table 4-4 for each of the fractions assayed from each treatment. Methods used to calculate percent recoveries parallel those used for the mixing-time experiment (Table 4-2).

Recoveries do not vary a great deal among the four chemical treatments. Expressed as fractional respiration (Method 1), the results suggest that the maximum recovery of metabolic activity was about 44%, recovered with Treatment C. Again we note losses in metabolic activity between the

Table 4-4

Summarized Data: Effect of Chemical Treatment on Recovery of Metabolic Activity from GAC

| Chemical Treatment: | Treatment A | | | | Treatment B | | | | Treatment C | | | | Treatment D | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | dpm Resp /per gm GAC | dpm +-S | Frac Resp (f) | f +-S | dpm Resp /per gm GAC | dpm +-S | Frac Resp (f) | f +-S | dpm Resp / per gm | dpm +-S | Frac Resp (f) | f +-S | dpm Resp / per gm | dpm +-S | Frac Resp (f) | f +-S |
| Supernatants | 521.7 | 570.1 | 0.057 | 0.062 | 386.9 | 381.5 | 0.040 | 0.042 | 432.1 | 461.1 | 0.047 | 0.050 | 378.6 | 476.8 | 0.041 | 0.052 |
| | | 473.4 | | 0.052 | | 352.3 | | 0.038 | | 403.1 | | 0.044 | | 280.3 | | 0.031 |
| Pellets | 2176.9 | 2433.1 | 0.094 | 0.105 | 1713.5 | 1804.7 | 0.090 | 0.095 | 1187.3 | 1301.8 | 0.061 | 0.066 | 2009.7 | 2140.7 | 0.076 | 0.081 |
| | | 1920.6 | | 0.083 | | 1622.4 | | 0.085 | | 1072.9 | | 0.055 | | 1878.8 | | 0.071 |
| Original Composite Sample | 18033.5 | 19025.0 | 0.566 | 0.598 | 14404.4 | 15519.9 | 0.523 | 0.567 | 8441.8 | 8882.3 | 0.423 | 0.445 | 1535.6 | 1652.5 | 0.122 | 0.131 |
| | | 17048.9 | | 0.535 | | 13289.0 | | 0.480 | | 8001.2 | | 0.401 | | 1418.6 | | 0.113 |

Treatment A = PVP/IPi, 0.05%/1.0%
Treatment B = PVP/IPi, 0.10%/1.0%
Treatment C = PVP/IPi, 0.50%/1.0%
Treatment D = Zwittergent 3-12 (10E-06 M)
EGTA (10-03 M)
Peptone (0.01%)
Tris Buffer (0.01 M, pH 7)

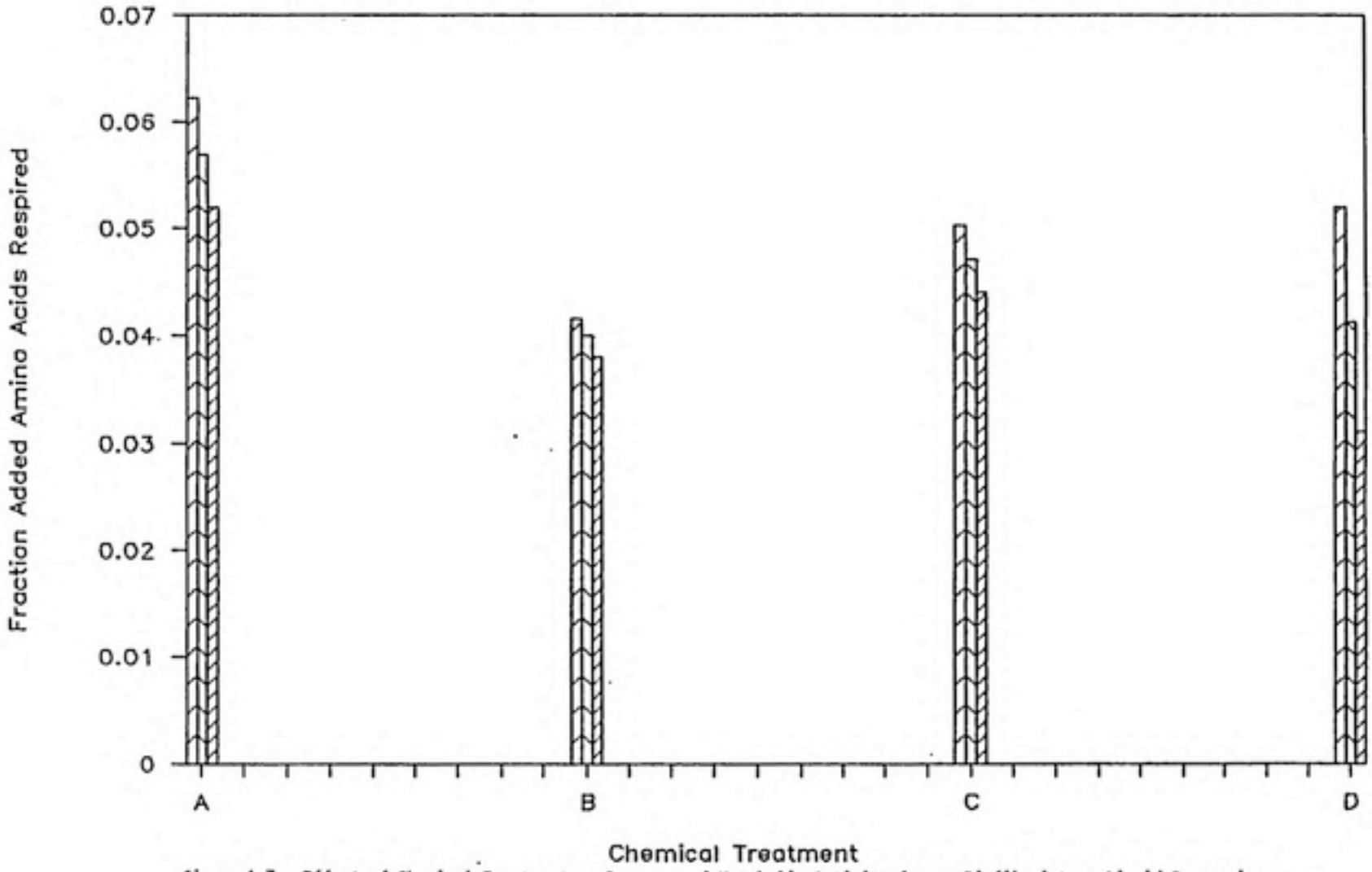



Figure 4-7. Effect of Chemical Treatment on Recovery of Metabolic Activity from a Biofilm into a Liquid Suspension: Fraction Added Amino Acids Respired per gm. GAC by Supernatant Samples. Upper and Lower Values Represent Means +/- Standard Deviations. See Table 4-4 for description of Treatments A, B, C, & D.

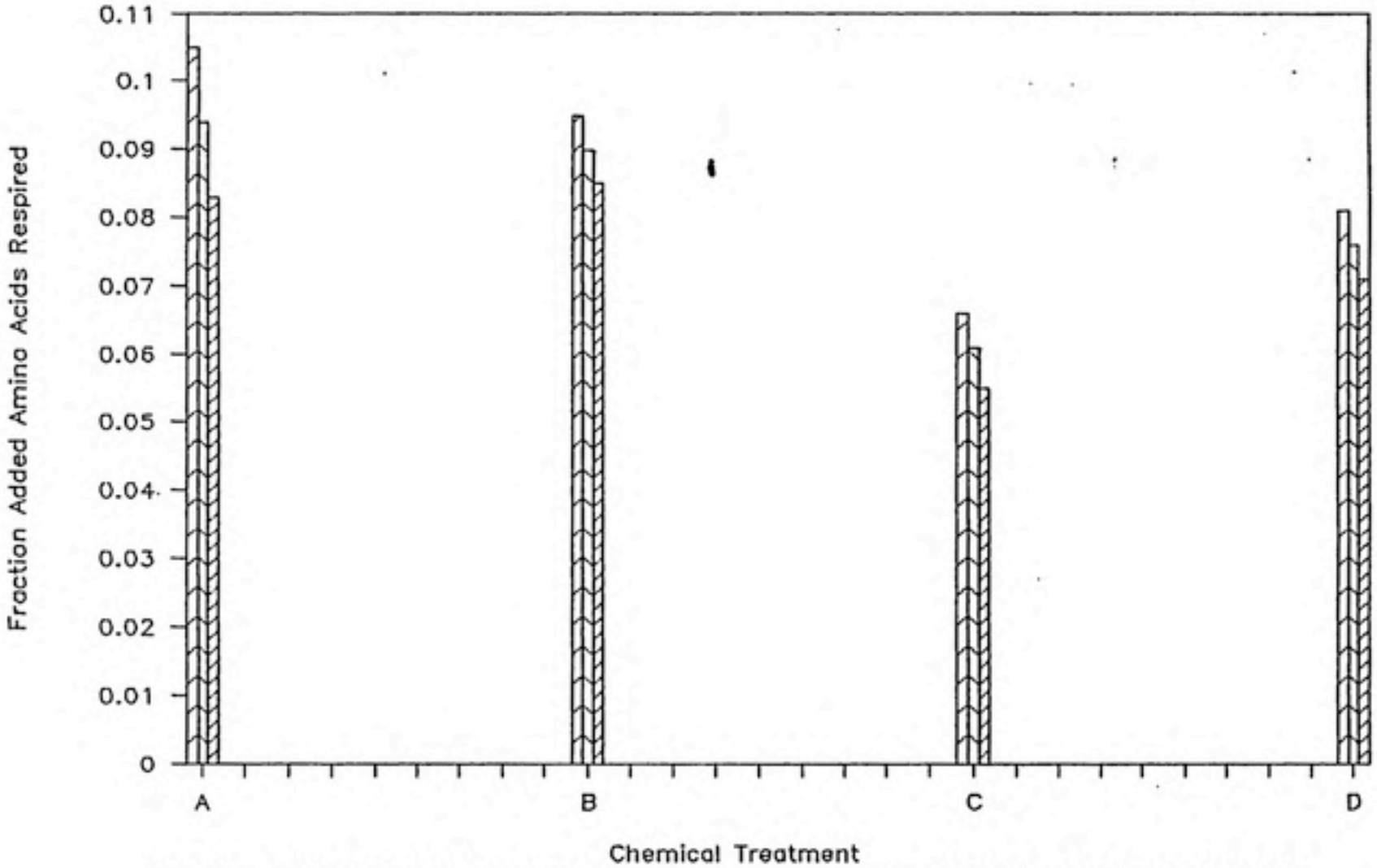



Figure 4-8. Effect of Chemical Treatment on Recovery of Metabolic Activity from a GAC Biofilm: Fraction Added Amino Acids Respired by GAC Pellet Fractions. Upper and Lower Values Represent Means +/- Standard Deviations. See Table 4-4 for description of Treatments A, B, C, and D.

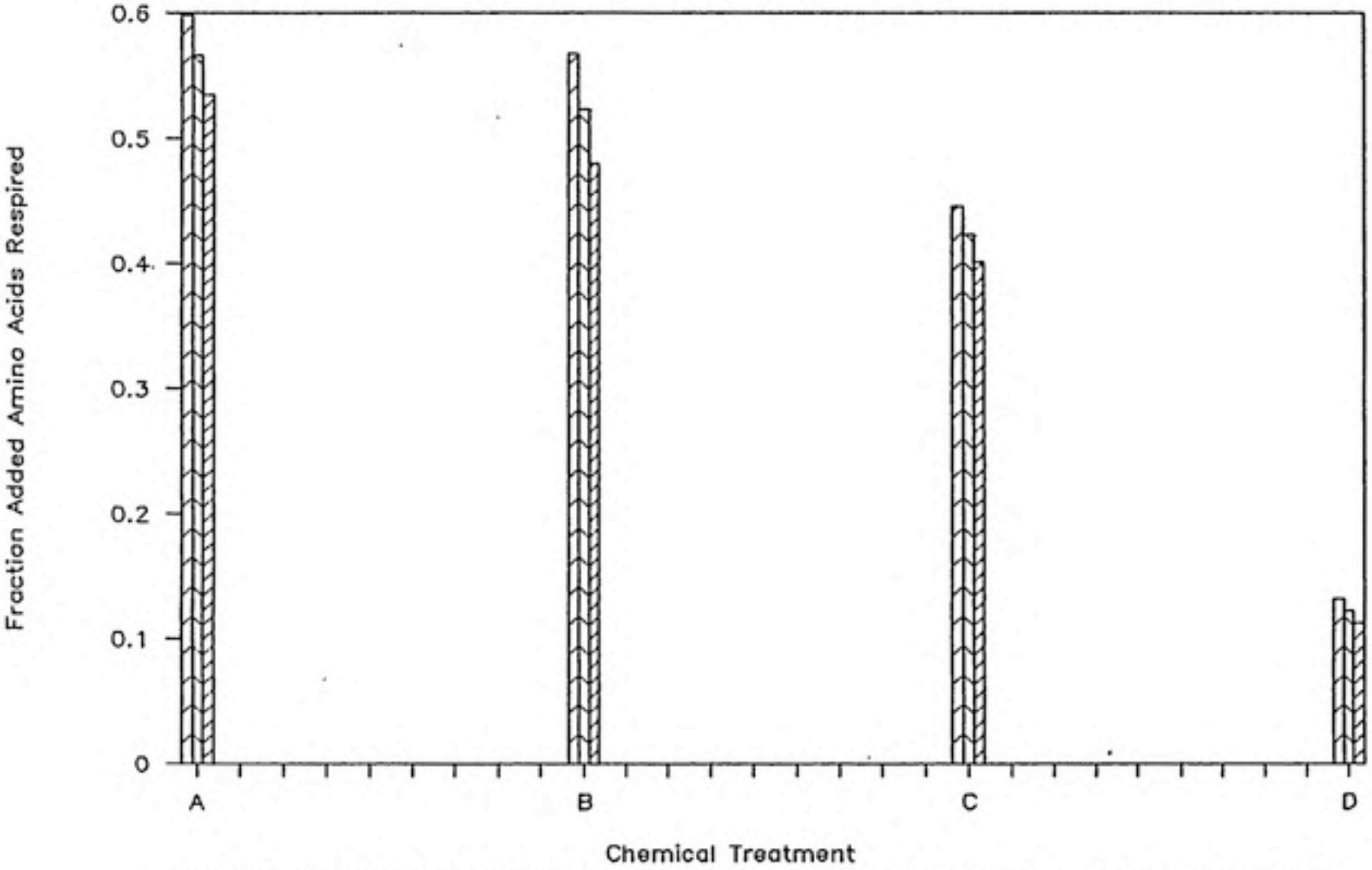



Figure 4-9. Metabolic Activity of Original Composite Samples used in Chemical Treatment Study: Fraction Added Amino Acids Respired by Composite Sample. Upper and Lower Values Represent Means +/- Standard Deviations. See Table 4-4 for description of Treatments A, B, C, and D.

Table 4-5

Calculated Results: Effect of Chemical Treatment on Percent Recovery of Metabolic Activity from GAC

| Chemical Treatment: | Treatment A | Treatment B | Treatment C | Treatment D |
|---|---|---|---|---|
| % Recovery (1) | 38 | 31 | 44 | 35 |
| % Recovery (2) | 19 | 18 | 27 | 16 |
| % Recovery (3) | 83 | 83 | 86 | 38 |
| % Loss of Activity (1) | 73 | 75 | 74 | 4 |
| % Loss of Activity (2) | 85 | 86 | 81 | -0.5* |

* The increase in activity noted for Treatment D is due to the low activity of the Original Composite Sample (see Table 4-4).

$$\%\ \text{Recovery (1)} = \frac{\text{((f) Resp. by Supernatant)}}{\text{((f) Resp. by Supernatant) + ((f) Resp. by Pellet)}} \times 100\%$$

$$(2) = \frac{\text{(dpa/ga Resp. by Supernatant)}}{\text{(dpa/ga Resp. by Supernatant) + (dpa/ga Resp. by Pellet)}} \times 100\%$$

$$(3) = \frac{\text{((f) Resp. by Original Sample) - ((f) Resp. by Pellet)}}{\text{((f) Resp. by Original Sample)}} \times 100\%$$

$$\%\ \text{Loss of Activity} = 1 - \left(\frac{\text{Met. Activity in Supernatant + Met. Activity in Pellet}}{\text{Metabolic Activity Originally Present in Composite Sample}}\right) \times 100\%$$

(1) uses the Fraction of Added Amino Acids Respired
(2) uses the Amino Acid dpa Respired per ga GAC

original sample and the sum of supernatant and pellet fractions, after treatment. The increase in activity for the sample receiving Treatment D, the zwitterionic detergent, results from the inhibition noted in the original sample (see Figure 4-9).

In addition to the amino acid respiration assay for metabolic activity, total cells were enumerated. Microscopic cell counts were performed on supernatant and pellet samples from the chemical treatment study, in an effort to corroborate the recovery of total cells with that of metabolic activity. Total cell recoveries for each of the chemical treatments are presented in Table 4-6, as determined by AODC counts; total cell density in the original composite sample was 7.98E+09 cells/gram GAC. This is comparable to but slightly higher than cell densities reported in the literature, which range from $10^6$ to $10^7$ cells/gram for GAC used in drinking water treatment (Van der Kooij, 1976; Cairo et al., 1979), to $10^9$ cells/gram wet weight (Latosek & Benedek, 1979).

A loss in total cells occurred between the original sample and the sum of supernatant and pellet fractions after treatment. Adding together the total cells enumerated in the pellet and supernatant fractions (Table 4-6), we can account for only 22%, 29%, and 29% of the cells enumerated in the original sample for Treatments A, B, and C, respectively. No count was obtained for the pellet fraction from Treatment D, due to loss of sample. The reductions in total cells between the composite sample and the sum of supernatant plus pellet fractions are comparable in magnitude to losses of metabolic activity (Table 4-5), when expressed as the fraction of added amino acid dpm respired. It can be inferred from this that metabolic activity may be correctly expressed as the fraction of added amino acids respired, rather than as total amino acid dpm respired.

Table 4-6

Summarized Data: Effect of Chemical Treatment on Desorption of Total Cells from GAC

| Chemical Treatment: | Treatment A | | | Treatment B | | | Treatment C | | | Treatment D | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | cells/gm GAC (avg) | Std Dev (S) | avg +-S | cells/gm GAC (avg) | Std Dev (S) | avg +-S | cells/gm GAC (avg) | Std Dev (S) | avg +-S | cells/gm GAC (avg) | Std Dev (S) | avg +-S |
| Supernatant Phase | 1.00E+09 | 7.83E+07 | 1.08E+09<br>9.22E+08 | 1.28E+09 | 1.66E+08 | 1.45E+09<br>1.11E+09 | 9.39E+08 | 9.08E+07 | 1.03E+09<br>8.48E+08 | 5.05E+08 | 3.89E+07 | 5.44E+08<br>4.66E+08 |
| Pellet Phase | 7.21E+08 | 1.36E+07 | 7.35E+08<br>7.07E+08 | 1.01E+09 | 9.02E+07 | 1.10E+09<br>9.20E+08 | 1.36E+09 | 1.48E+08 | 1.51E+09<br>1.21E+09 | ***** | | |

Treatment A = PVP/PPi, 0.05%/1.0%
Treatment B = PVP/PPi, 0.10%/1.0%
Treatment C = PVP/PPi, 0.50%/1.0%
Treatment D = Zwittergent 3-12 (10E-05 M)
EGTA (10E-03 M)
Peptone (0.01%)
Tris Buffer (0.01M, pH 7)

Table 4-7 summarizes the recovery of total cells and of metabolic activity by each of the four chemical treatments. As with metabolic activity, total cell recoveries do not vary a great deal among all three PVP/PPi concentrations. Comparing the recovery of metabolic activity with that of total cells by each treatment, we see that the solutions of lower PVP/PPi concentrations (Treatments A and B) recovered a higher percentage of total cells than of total metabolic activity; the effect was opposite for Treatment C, which recovered a higher percentage of metabolic activity than of total cells.

Differences between recoveries of total cells and of metabolic activity can be explained in several ways. That two samples show higher total cell recoveries while the third shows higher recovery of metabolic activity seems to indicate a flaw in one or both of the techniques. There are several weaknesses in the amino acid respiration technique, most importantly the inability to compare solid and liquid sample activities. These weaknesses, however, should affect results somewhat uniformly across all samples. Moreover, we would not expect a treatment to recover a higher percentage of metabolic activity than of total cells, expecially when the treatment seems to have an inhibitory effect on respiration (see Figure 4-9). The most likely cause for discrepancy here is, rather, an erroneously low total cell recovery for Treatment C, due to the inability to quantify cells accurately in a solid sample. Chances for error are extremely high in the counting procedure, since many cells clump together or are hidden by large black GAC particles. In addition, confidence in the accuracy of sample dry weight is low owing to variability in solution composition and slurry pipetability.

Table 4-7

Summary of the Effects of Chemical Treatment
on Percent Recovery of Total Cells and Metabolic Activity from GAC

| Chemical Treatment: | Treatment A | Treatment B | Treatment C | Treatment D |
|---|---|---|---|---|
| % Recovery of Total Cells | 58% | 56% | 41% | ***** |
| % Recovery of Metabolic Activity | 38% | 31% | 44% | 35% |

Treatment A = PVP/PPi, 0.05%/1.0%
Treatment B = PVP/PPi, 0.10%/1.0%
Treatment C = PVP/PPi, 0.50%/1.0%
Treatment D = Zwittergent 3-12 (10E-06 M)
EGTA (10E-03 M)
Peptone (0.01%)
Tris Buffer (0.01M, pH 7)

$$\% \text{ Recovery of Total Cells} = \frac{\text{(Cells/gm in Supernatant)}}{\text{(Cells/gm in Supernatant + Cells/gm in Pellet)}}$$

$$\% \text{ Recovery of Metabolic Activity} = \frac{\text{(frac. added amino acids respired by Supernatant)}}{\text{(frac. resp. by Supernatant + frac. resp. by Pellet)}}$$

Because we lack confidence in the measurement of total cell numbers for solid samples, there is additional incentive for making the amino acid respiration technique work. The ability to somehow determine correctly a percent recovery of metabolic activity into the supernatant phase, and to corroborate this recovery with a total cell count (assuming that the two will be comparable) would enable us to determine the total number of cells in the original sample simply through counting the supernatant fraction. This would circumvent problems associated with counting solid samples. In addition, we could determine the "metabolic activity per cell" in the original sample, a parameter which may be used to indicate its metabolic state for comparison purposes.

Besides potential toxicity of the removal solution, a conceivable problem associated with the metabolic assay, due directly to the respiration technique, is the potential adsorption of amino acids onto GAC during incubation. Adsorption would lower the solution phase concentration, which in turn would result in lower metabolic velocities. The effect would be greater for samples containing more carbon, since more surface area would be available for adsorption.

Addition of amino acids to GAC in the absence of the PVP/PPi solution suggests that adsorption does indeed occur (Figure 4-10). As more carbon is added, the solution phase amino acid concentration declines. At carbon concentrations comparable to those used in the respiration experiments (0.75 gm per ten ml), 10% to 15% of the amino acids added to solution are adsorbed during the incubation period. Repeating the sorption experiment using GAC which had undergone several PVP/PPi washes, however, indicates that after treatment, very little adsorption occurs (Figure 4-11). At the end of the incubation period, there is

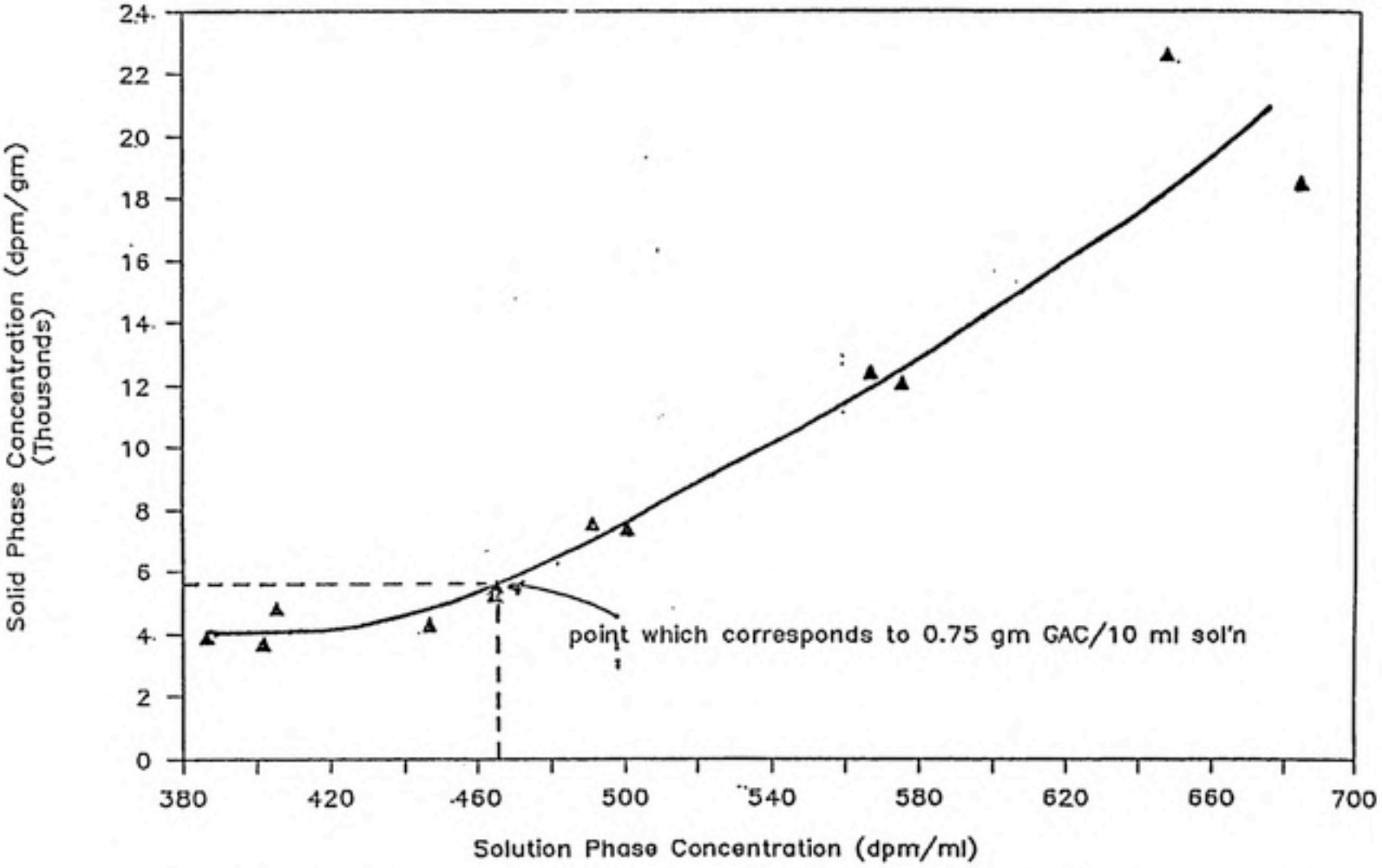



Figure 4-10. Adsorption of Amino Acids onto Virgin GAC: Solid Phase vs. Liquid Phase Concentrations after 24 hour Incubation. (Line drawn by eye.)

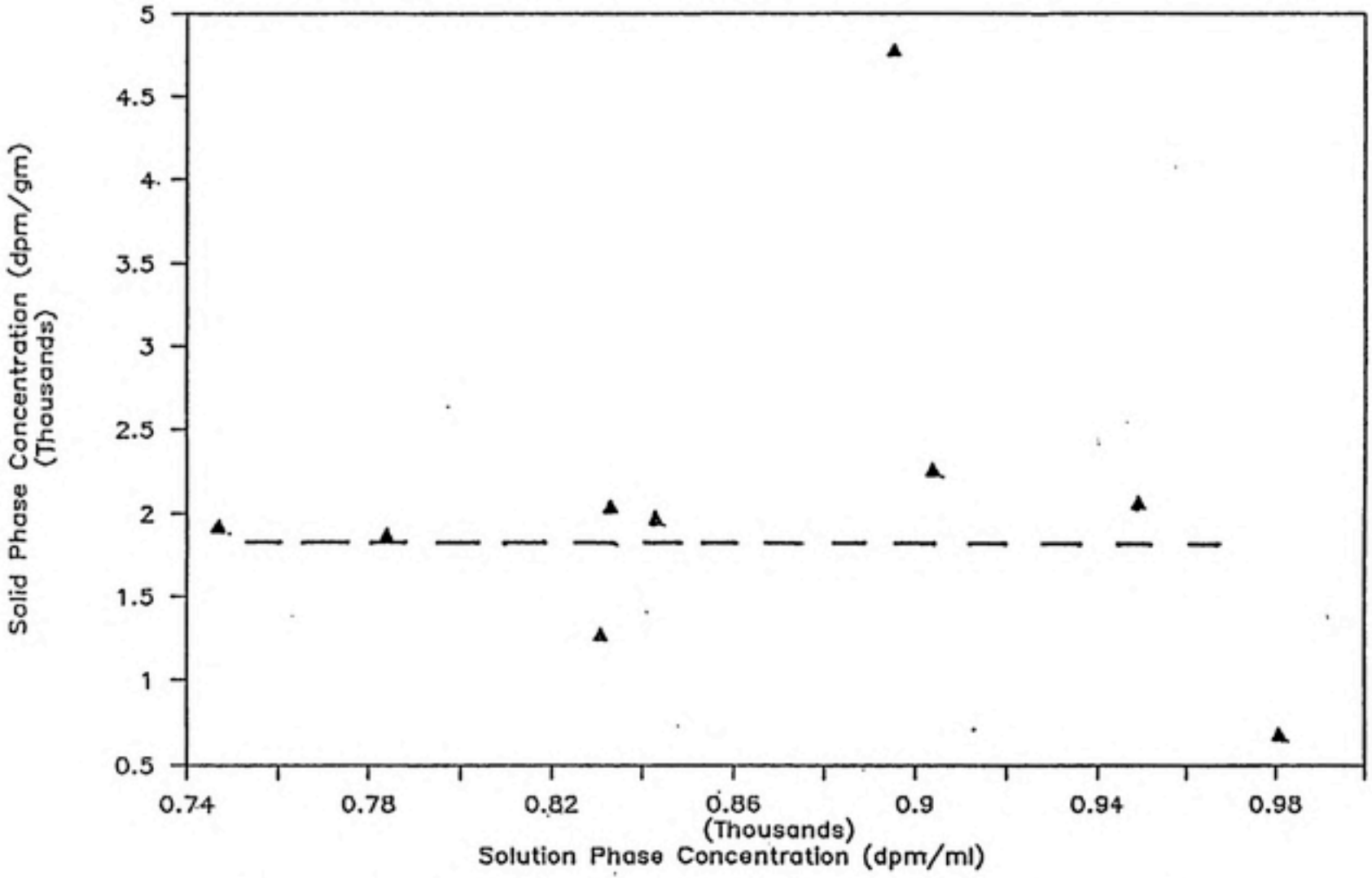



Figure 4-11. Adsorption of Amino Acids onto GAC Treated with PVP/PPI Solution: Solid Phase vs. Liquid Phase Concentrations after 24 hour Incubation. (Line drawn by eye.)

relatively little change in solution phase concentration with total GAC added. Without the addition of PVP/PPi, solid phase concentrations reached over 20,000 dpm/gm (Figure 4-10). In contrast, with PVP/PPi treatment they barely exceed 2000. Solution phase concentrations are also much higher (750 to 1000 dpm/ml) due to the lower adsorption. Apparently the PVP/PPi reagent used to treat the GAC adsorbs onto the surface, occupying sites which would otherwise have been available to amino acids in solution.

Figure 4-12 shows solid and liquid phase amino acid concentrations for the same sample of PVP/PPi treated GAC after acidification and shaking 20 hours at 60 rpm. Here we see adsorption, at magnitudes comparable to those observed with clean untreated GAC.

A profile of the adsorption process over time (using a GAC sample treated with PVP/PPi) is presented in Figure 4-13. This shows that, as expected, there is relatively little change in solution phase concentration during the incubation period. Nearly all adsorption noted for the "end-of-shake-time" sample occurs when the solution is acidified, as shown in the figure. Acidification protonates the amino acids, making them more favorable for adsorption; they preferentially displace PVP on the GAC surface.

Although Figures 4-11, 4-12, and 4-13 show nicely the changes in amino acid adsorbability with solution characteristics, we are concerned here with whether or not solution phase concentrations are altered during the incubation period due to adsorption. As seen in Figures 4-11 and 4-13, amino acids do not readily sorb onto GAC which has been exposed to PVP/PPi solution. The same result is expected for GAC treated with the zwitterionic detergent solution (Treatment D), as Zwittergent is a surfactant which

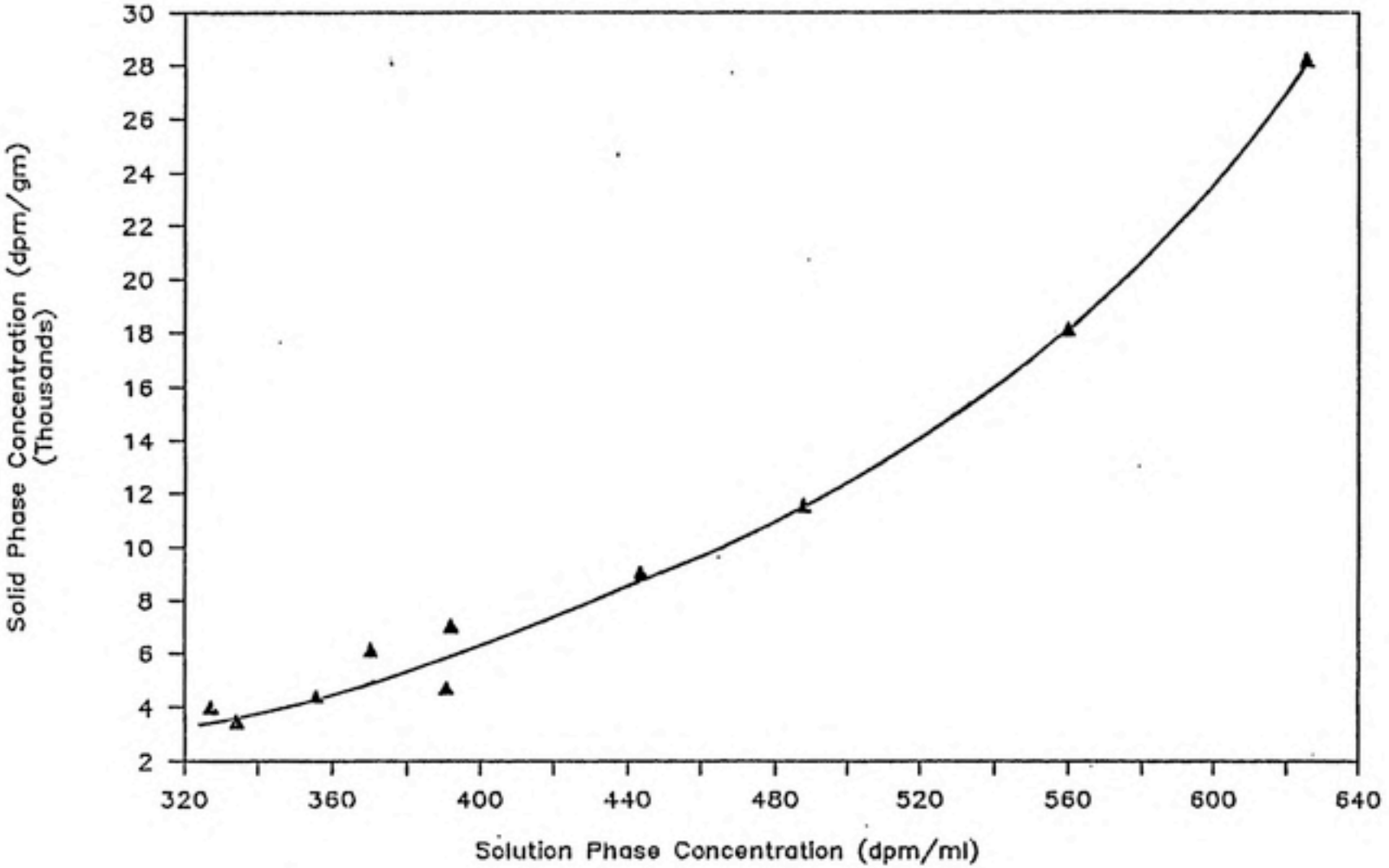



Figure 4-12. Adsorption of Amino Acids onto GAC Treated with PVP/PPI Solution: Solid Phase vs. Liquid Phase Concentrations after 24 hour Incubation, Acidification, and Shaking 18 hours. (Line drawn by eye.)

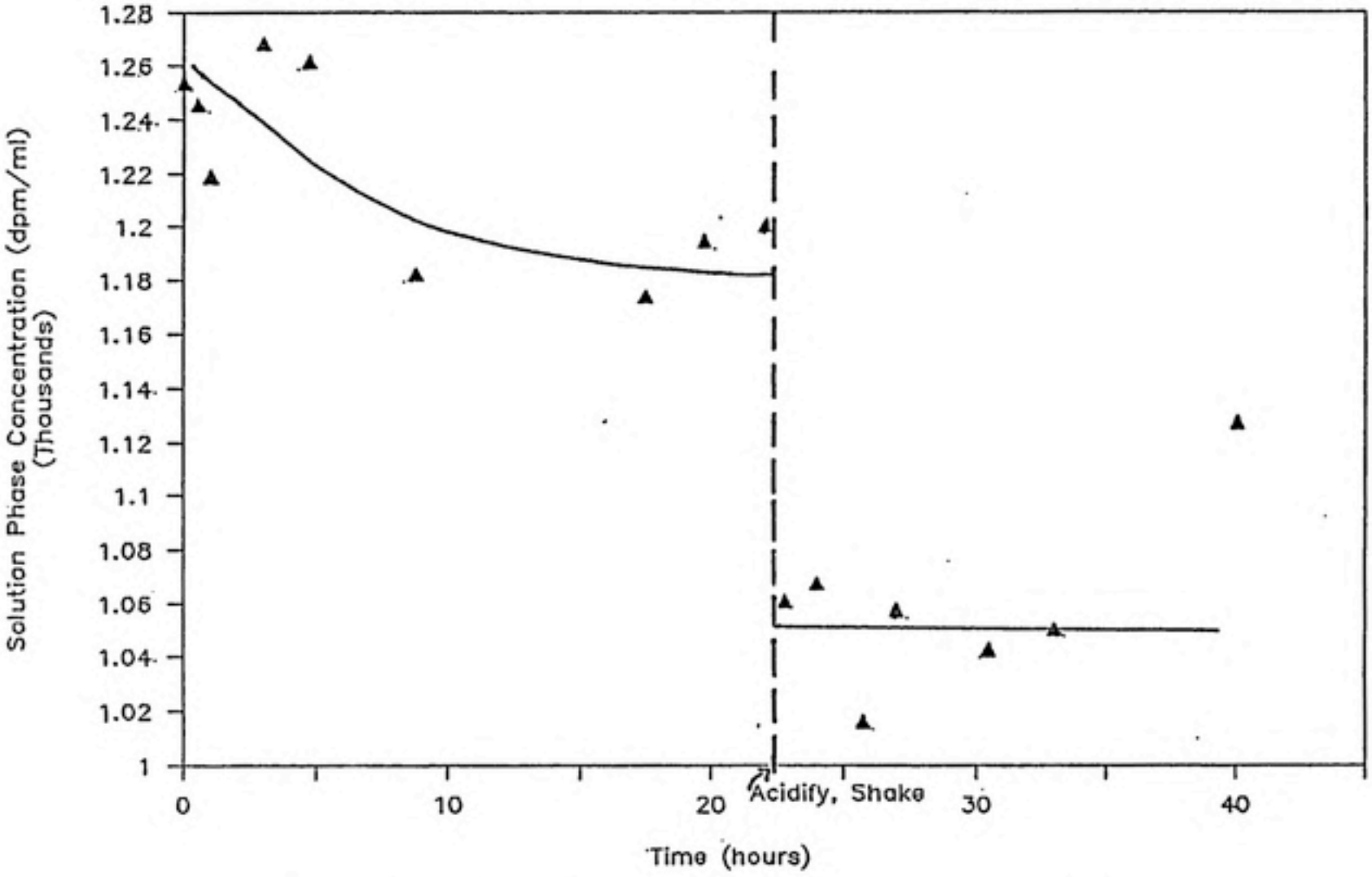



Figure 4-13. Profile of Amino Acid Adsorption onto GAC Treated with PVP/PPi Solution, throughout Incubation, Acidification, and Shake Procedure. (Line drawn by eye.)

alters the carbon surface, and peptone "prevents readsorption" of bacteria (and hence amino acids) to GAC (Camper et al., 1985).

Adsorption may, however, be more of a problem in assaying the metabolic activity of the original untreated GAC sample. As a result, activities determined for these samples may be falsely low due to a lower solution phase concentration than accounted for. This would imply that even more of the metabolic activity was lost between the original sample and the summed pellet and supernatant fractions - a finding that, instead of rectifying the method, only adds to the difficulties noted.

## 4.3 Summary

The amino acid respiration technique used here exhibits several weaknesses when used to assess the recovery of metabolically active cells from a solid sample into a liquid supernatant. Its basic shortcoming stems from an inability to compare results obtained with solid and liquid samples, as noted.

The assay does, however, seem valid for making comparisons between supernatants obtained from different types of removal treatments. With this in mind, it appears that most activity is recovered through several washes of 30 minutes each with a solution of polyvinyl pyrrolidone and sodium pyrophosphate, separated by blending for one minute in a Waring blender (two 30 second bursts separated by a 30 second rest period) and slow-speed centrifugation (15 minutes at 120xg). Solution concentration does not seem to have a significant impact on cell recoveries, although some inhibition of metabolic activity is noted.

When amino acid respiration in the combined supernatant and pellet fractions, after treatment, is compared with that in the original untreated sample, we note an apparent loss of metabolic activity due to the treatment process. Although the magnitude of this loss is comparable to losses noted of total cell counts for the same samples (ranging from 20% to 30%), the removal solutions do appear to inhibit metabolism. While PVP/PPi reagents do not exhibit a normal dose-response inhibition, there is evidence of some suppression of respiratory processes. The zwitterionic detergent, when used in the 30 minute wash procedure, is suspected to be especially toxic.

Results of the Acridine Orange cell counts indicate that six successive washes in sterile PVP/PPi solution, separated by blending and slow-speed centrifugation, recover approximately 50% of the total cells from the solid phase into the supernatant fraction. This may be comparable to recovery of metabolic activity, although results of the amino acid respiration assay are inconclusive.

Ultrasonication in place of blending appears to remove a larger portion of total cells from the carbon surface. The method may recover more viable cells and metabolic activity as well, although the results are questionable due to discrepancies in the data.

In summary, the removal technique cannot be used quantitatively to recover biofilm microorganisms for studies which will be related back to the original solid sample. We can, however, use the methods for qualitative analyses. By assuming that identical wash procedures will recover the same percentage of biofilm bacteria from different samples, the removal techniques can be used to compare biofilm densities and metabolic activities between samples obtained from different sources or subject to different treatment

parameters. The method also serves the purpose of recovering cells for other kinds of tests, such as biokinetic studies, which do not necessarily require knowledge of the actual number of micoorganisms present in the original sample or the percent recovered into suspension. In this way, we are able to study the indigenous microbial community, while avoiding possible interferences posed by the presence of the solid surface.

# 5. BIOKINETIC STUDIES: PHENOL METABOLISM BY MICROORGANISMS RECOVERED FROM A HUMIC SUBSTANCES-GROWN BIOFILM

## 5.1 Experimental Methods

Our approach in describing the biokinetics of phenol metabolism has been to characterize utilization according to the Monod kinetic model. Biomass growth is expressed as:

$$\frac{dX}{dt} = \mu \cdot X. \qquad (5\text{-}1)$$

where X = biomass concentration (μg/L)
t = time (hr)
μ = specific growth rate (μg/L-hr)

Substrate degradation is related to growth rate by:

$$\frac{dS}{dt} = \frac{1}{Y} \cdot \frac{dX}{dt}. \qquad (5\text{-}2)$$

where S = substrate concentration (μg/L)
Y = microbial yield coefficient
(μg cells produced/
μg substrate utilized).

The specific growth rate, μ, is related to substrate

concentration:

$$\mu = \frac{\mu_m \cdot S}{K_S + S}. \qquad (5\text{-}3)$$

where $\mu_m$ = maximum specific growth rate ($\mu$g/L-hr)
$K_S$ = half saturation coefficient ($\mu$g/L)

Combining equations (5-1) through (5-3), we obtain the following expression for substrate utilization:

$$\frac{dS}{dt} = \frac{1}{Y} \cdot \frac{\mu_m \cdot S \cdot X}{K_S + S}. \qquad (5\text{-}4)$$

The term ($\mu_m$/Y) is commonly referred to as the maximum rate coefficient, k ($\mu$g substrate/$\mu$g biomass-hr). Substituting this into equation (5-4), we obtain the familiar expression for substrate utilization as described by Monod kinetics:

$$\frac{dS}{dt} = -\frac{k \cdot S \cdot X}{K_S + S}. \qquad (5\text{-}5)$$

For most engineering applications three parameters, Y, k, and $K_S$, are needed to fit the model to a particular system under investigation.

The experimental methods used here to estimate kinetic parameters are a modification of the multiple concentration initial rate procedure developed by Pfaender and Bartholomew (1982). The method involves incubating a series of microbial samples with several concentrations of a radiolabeled test substrate for a short period of time, and subsequently measuring the amount of substrate assimilated

into cellular material as well as that respired to $CO_2$. The data can be used, along with measurement of biomass concentration X, to determine the desired kinetic parameters through nonlinear analysis of a plot of dS/dt vs. S (equation 5-5).

Substrate Solutions. Phenol served as the substrate in all biokinetic studies. Radiolabeled phenol (Phenol-Ring-UL-$^{14}C$, specific activity 12.22 mCi/mmol; Pathfinder Laboratories, Inc., St. Louis, Missouri) was diluted in 95% ethanol to form a stock solution, final concentration approximately 2200 dpm/µl. Working solutions were prepared by diluting aliquots of this stock with unlabeled phenol in 50% ethanol. Separate solutions were prepared for each concentration used in the rate studies; 20 µl of each were added to the samples to yield activities in the vials ranging from 10,000 to 50,000 dpm. Test substrate concentrations ranged from 1 to 10,000 µg/L.

Microbial Samples. Biomass recovered from laboratory-scale GAC reactors constituted the microbial sample for biokinetic studies. The reactors were fixed-bed columns with high recycle ratios, which approximated completely mixed-flow reactors. Biofilm growth was encouraged by feeding the reactors an ozonated solution of naturally occurring humic substances (HS). Reactor design and operation is described in detail in Chapter 6 (samples were obtained from the column specified as Run No. 2).

Biomass was recovered from the colonized GAC using the shake-blend-centrifuge procedure described in Chapter 4. The solution used to desorb the biofilm was prepared by adding PVP and PPi (0.1% and 1.0% by weight, respectively) to an aliquot of the ozonated HS used as reactor feed. The solution was filtered four times through a 0.2 µm membrane filter to approximate a particle free liquid, and sterilized

20 minutes at 20 psi prior to the addition of PVP and PPi reagents; earlier studies revealed that the PVP reagent congeals at high temperatures. The final pH was adjusted to that of the ozonated HS, pH 6.2. Column feed solutions were used for desorption in order to minimize changes in habitat for microorganisms recovered from the biofilm into the liquid suspension. Drastic changes in pH or in nutrient availability could alter their metabolic state.

Supernatants from each successive wash of the GAC for each sample contained the biomass for the biokinetic tests. These washes were combined to yield a total solution of known volume, from which aliquots were withdrawn for incubation with phenol.

Determination of Microbial Density. The biomass density, X, used for characterizing microbial degradation kinetics was determined utilizing a modified Standard Plate Count procedure (Standard Methods, 16th ed., 1985). Serial dilutions of each sample were plated onto Tryptocase Soy Agar (Difco Chemical Laboratories, Detroit, Michigan), which was prepared in the ozonated HS solution used as feed to the laboratory reactors. The media was adjusted to a final pH of 6.2.

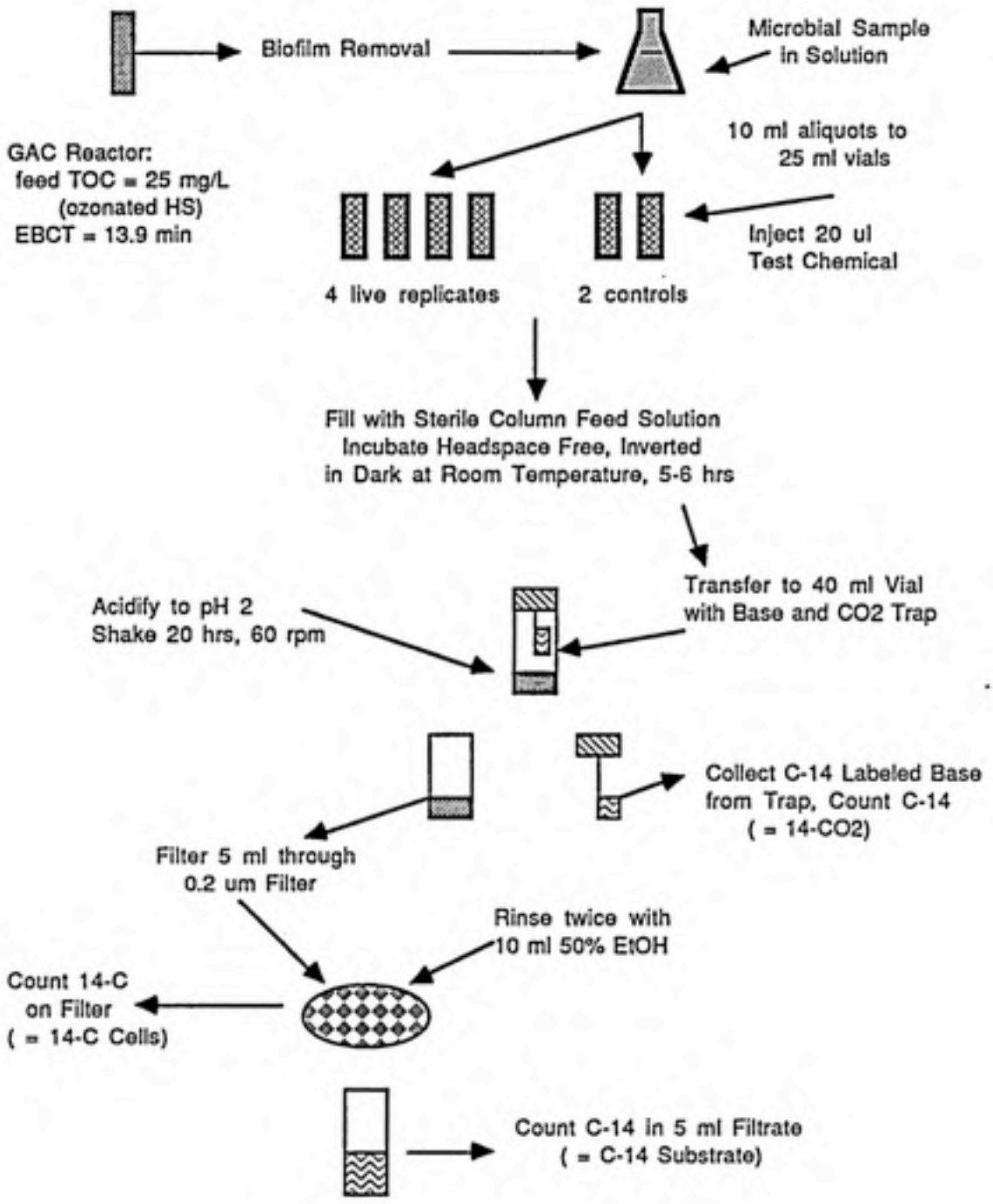
Samples were plated in duplicate at five dilutions and incubated at 27°C for 2 weeks before enumeration. This incubation temperature is given by Werner et al. (1979) as most adequate for enumerating bacteria in GAC systems. Preliminary experiments showed no apparant change in viable cell number after 14 days of incubation at this temperature.

Cell numbers were converted to biomass by assuming an average cell dry weight of 2E-10 mg/cell (Gaudy and Gaudy, 1980).

General Experimental Methodology. The general procedure for assessing microbial biokinetics is outlined in Figure 5-1. The microbial sample obtained by desorbing the biofilm from the laboratory-scale GAC reactor became the source of biomass for each substrate uptake experiment. For each phenol concentration tested, a ten ml aliquot of this biomass solution was injected into each of six sterile 25 ml vials (Wheaton Scientific, Millville, New Jersey). Two of the vials served as controls for abiotic processes and as a means for correcting results for background radioactivity; these are referred to as "dead samples" and were injected with 500 µl of 25% sodium azide, a metabolic inhibitor. The remaining four vials are designated as "live samples". After adding 20 µl of carbon-14 labeled substrate, each vial was filled to the top with a sterile HS solution (again identical to the reactor feed), capped with a Teflon lined septum and ring cap (Pierce Chemical Company, Rockford, Illinois), and inverted. Samples were incubated for five to six hours, headspace free, in the dark at room temperature.

The initial concentration of phenol was determined by injecting 20 µl of radiolabeled substrate into three scintillation vials each containing ten ml counting cocktail.

$^{14}CO_2$ Respiration. Following the incubation period, each sample was transferred to a 40 ml Pierce vial; the vials contained 150 µl of 1N KOH, to minimize $CO_2$ losses. A Teflon-lined connector cap (Wheaton Scientific, Millville, New Jersey) was used for the transfer, preventing contact of the sample with the atmosphere. In the 40 ml vial, each sample was adjusted to pH 2 by adding 1 ml 80% by volume $H_3PO_4$. The septa were replaced with base-filled traps, and $^{14}CO_2$ was recovered using the same procedure as that for the amino acids respiration assay. $^{14}CO_2$ recovery efficiencies




Net Respiration = Avg. Live CO2 dpm - Avg. Control CO2 dpm
Net Assimilation = Avg. Live Filter dpm - Avg. Control Filter dpm
= Avg. Control Filtrate dpm - Avg. Live Filtrate dpm - Net Respiration

Figure 5-1. Methodology for Biokinetic Studies

were determined by incubating five additional samples with $Ba^{14}CO_3$.

$^{14}C$ Assimilation into Cells. The uptake of carbon-14 into cellular material was determined by filtering a five ml aliquot of the acidified sample, after $^{14}CO_2$ recovery, through a 0.2 μm Gelman cellulose triacetate filter (GA-8, Gelman Sciences, Inc., Ann Arbor, Michigan). Filters were presoaked in a 1 mg/L phenol solution to saturate binding sites. Filtration was followed by two successive rinses with 10 ml 50% ethanol; preliminary experiments determined this to be the optimum procedure for recovering assimilated carbon-14.

Each filter, as well as two 5 ml portions of the combined filtrate and wash, were assayed for carbon-14 radioactivity on a Packard Tri-Carb 300 CD liquid scintillation counter.

## 5.2 Interpretation of the Data

Substrate Concentrations. By definition, an "initial rate" approach considers only initial substrate concentrations, assuming that the incubation period is sufficiently short such that changes in substrate concentration may be neglected. While this may be the case at lower substrate concentrations, metabolic velocities at higher concentrations were indeed sufficient to reduce solution phase phenol concentrations.

Because of this, average substrate concentrations have been calculated for use in describing the kinetics of the system. Substrate concentrations were calculated as

follows:

$$S_{(avg)} = S_i - (dS/dt) \cdot (\Delta t/2). \qquad (5\text{-}6)$$

Phenol Respiration. Respiration rates for phenol were calculated at each concentration as the difference between the averaged live and dead activities, correcting for $^{14}CO_2$ recovery efficiency (E) and dividing by the incubation time:

$$\text{resp. rate} = \frac{\text{(avg. live dpm)} - \text{(avg. dead dpm)}}{\text{incubation time}} \cdot \frac{1}{E}. \qquad (5\text{-}7)$$

The result obtained is in terms of "dpm/hr"; conversion to units of "μg phenol/hr" is done with a factor derived from both the specific activity of radiolabeled phenol and the ratio of labeled to unlabeled phenol in the working solution: μg phenol/dpm = (μg/L total phenol in working solution)/(specific activity of phenol (dpm/ug) x ug labeled phenol in working solution). Conversion factors were unique to each working solution prepared.

Phenol Assimilation. Phenol which has been assimilated into cellular material should be retained along with microbial biomass by a 0.2 μm membrane filter. Net assimilation of carbon-14 can be determined with two independent calculations. One method calculates differences between the radioactivity remaining on the filters from the live and the dead samples, while the other takes differences in the activities of the filtrates from the two samples. These are described as:

Method 1. assimilation = average live filter activity - average dead filter activity;
Method 2. assimilation = average dead filtrate activity - average live filtrate activity - net respiration.

Method 2 is understandable if we begin by noting that assimilation + respiration = total decrease in substrate activity due to biodegradation. The decrease in substrate activity is measured by the difference between the filtrate activity for the dead control and the live samples (the dead control filtrate will have greater substrate activity because no biodegradation has occurred). Subtracting respiration from both sides yields the equation for Method 2.

The more common approach is to use Method 1 (Pfaender and Bartholomew, 1982; Speitel and DiGiano, 1987). This method is less labor intensive since it requires that only the filters be assayed for radioactivity. Furthermore, by using only the filter activities we avoid the variation in filtrate activities caused by dilution of the filtrate with the alcohol wash. For most test concentrations, differences between the activities of live sample filters and dead sample filters gave reasonable results for net assimilation. In some cases, though, primarily at low substrate concentrations, the presence of carbon fines in the microbial sample interfered with this type of calculation. Radiolabeled phenol adsorbed to the carbon and was retained on the filter along with assimilated substrate. At low phenol concentrations, the amount of sorbed phenol in both live and dead samples was significantly greater than the net assimilation of label into cells, making differences between live and dead filter activities indistinguishable.

In an effort to remedy this problem, net assimilation was calculated at each concentration using both filter and filtrate activities, in two separate calculations. When results obtained from the two methods were in close agreement, the net assimilation reported is that obtained using the filter activities. When the two calculations gave conflicting results, a mass balance on carbon-14 in the

system usually indicated a discrepancy in the filter activities due to radiolabeled phenol associated with the carbon fines in solution. For these cases, phenol assimilation was calculated using differences in filtrate activities between live and dead samples.

Cellular Yields. The microbial yield coefficient was calculated at each substrate concentration as follows:

$$Y = \frac{^{14}C\text{-assimilated}}{^{14}C\text{-assimilated} + ^{14}C\text{-respired}} . \qquad (5\text{-}8)$$

Although cellular yields may change with nutrient concentration for substrates such as phosphate and nitrogen, the yield is much less variable when the limiting nutrient is a carbon source (Koch, 1979). Yields were calculated at each concentration and averaged to obtain an overall yield coefficient.

Application of the Monod Kinetic Model to Experimental Results. As with substrate concentration, changes in the concentration of biomass will affect substrate degradation rates. We therefore need to make another simplifying assumption in the Monod equation before applying the model to our results. This additional assumption is that changes in biomass concentration during the incubation period can be neglected.

Simkins and Alexander (1984) postulate that for conditions where the initial cell concentration is greater than an inoculum size permitting one division of active cells at a particular substrate concentration, population density can be treated as approximately constant. The actual ratio of cell density to substrate concentration necessary for making this assumption when applying data to a model depends on the specific substrate in question and on

the magnitude of the yield coefficient. We assume, for our purposes here, that our supernatant samples were sufficiently concentrated in cellular material as compared to phenol concentration, and that our incubation period is sufficiently short, such that changes in biomass concentration can be neglected.

Assuming a constant biomass density allows us to further simplify the analysis of phenol degradation kinetics. Dividing the Monod equation for substrate utilization through by X gives:

$$\frac{(dS/dt)}{X} = -\frac{k \cdot S}{K_s + S}. \qquad (5\text{-}9)$$

The terms k and $K_s$ can be estimated by nonlinear regression analysis, using (dS/dt)/X and S as the dependent and independent variables, respectively.

## 5.3 Results

Two different sources of biomass were used to measure the kinetics of phenol degradation:

1. Unexposed Population - biomass desorbed from a GAC reactor which received ozonated humic substances at a feed concentration of 25 mg/L for 19 days; the empty bed contact time (EBCT) = 13.9 minutes; and
2. Exposed Population - biomass desorbed from a GAC reactor which was fed ozonated humic substances at 25 mg/L (EBCT = 13.9 min) but, after 19 days of operation, was also fed phenol for five days at a concentration of 50 µg/L.

Degradation rates and yield coefficients for each of the experiments are presented in Table 5-1; yield

Table 5-1

Phenol Biodegradation Kinetic Data

| Concentration (µg/L) | Degradation Rate (µg phenol/µg biomass-hr) | Microbial Yield (µgC/µgC) |
|---|---|---|
| **Unexposed Population** | | |
| 1.0 | 2.30E-04 | 0.67 |
| 9.3 | 5.80E-03 | 0.68 |
| 79.0 | 1.06E-01 | 0.52 |
| 874.0 | 4.79E-01 | 0.64 |
| 9600.0 | 4.41E-01 | 0.63 |
| | | Overall Yield Coefficient = 0.63 |
| **Exposed Population** | | |
| 1.1 | 7.31E-05 | 0.48 |
| 10.8 | 4.92E-04 | 0.75 |
| 84.7 | 9.52E-02 | 0.39 |
| 968.7 | 2.27E-01 | 0.21 |
| 9783.0 | 3.44E-01 | 0.48 |
| | | Overall Yield Coefficient = 0.46 |

Unexposed Sample:

Mass of GAC = 4.12 gm
Vol. Solution into which biomass recovered = 1.0 L
Total Cell Density = 4.75E+09 cells/gm +/- 17%
Viable Cell Density = 4.88E+08 cells/gm +/- 54%
Time of Bed operation before biomass removed = 19 days

Exposed Sample:

Mass of GAC = 11.45 gm
Vol. Solution into which biomass recovered = 1.0 L
Total Cell Density = 2.94E+09 cells/gm +/- 2.7%
Viable Cell Density = 1.28E+08 cells/gm +/- 35%
Time of Bed Operation before adding phenol = 19 days
Time of Bed Operation before biomass removed = 24 days

coefficients have been calculated at each concentration as $^{14}C$ assimilated into cells divided by the total $^{14}C$ metabolized, and are presented in units of μg biomass carbon/μg phenol carbon. These units are unusual, but derive directly from the radioactivity data. Overall yield coefficients, calculated by averaging the yields at each concentration, are included in the table. Figures 5-2 and 5-3 show plots of respiration rate, uptake rate, and total degradation rate (the sum of uptake and respiration), in units of μg phenol/μg biomass-hr, vs. substrate concentration for each sample. The rates and concentrations are expressed logarithmically, in order to present all of the data for each sample in a single figure.

The Monod model was applied only to kinetic data obtained with the unexposed population (Figure 5-2). It was obvious from inspection of Figure 5-3 that a simple curvilinear function, as predicted by the Monod model, would not fit the set of data collected for the exposed population. Three methods were used to calculate values for the maximum rate coefficient, k, and the half saturation coefficient, $K_s$, in an effort to obtain a reasonable fit. The reason that three methods were tried is that an initial attempt with nonlinear regression of the data in arithmetic form (equation 5-9) appeared to give a poor fit, when plotted logarithmically. Errors at low substrate concentrations were exaggerated, due to the logarithmic scale of the plot. Another nonlinear regression was performed, on the data expressed logarithmically:

$$\log \frac{(dS/dt)}{X} = \log(k \cdot S) - \log(K_s + S). \qquad (5\text{-}10)$$

Both regressions were done with the SYSTAT subroutine NONLIN (Wilkinson, 1986). In addition, the parameters were determined by inspection of the rate curve in Figure 5-2.

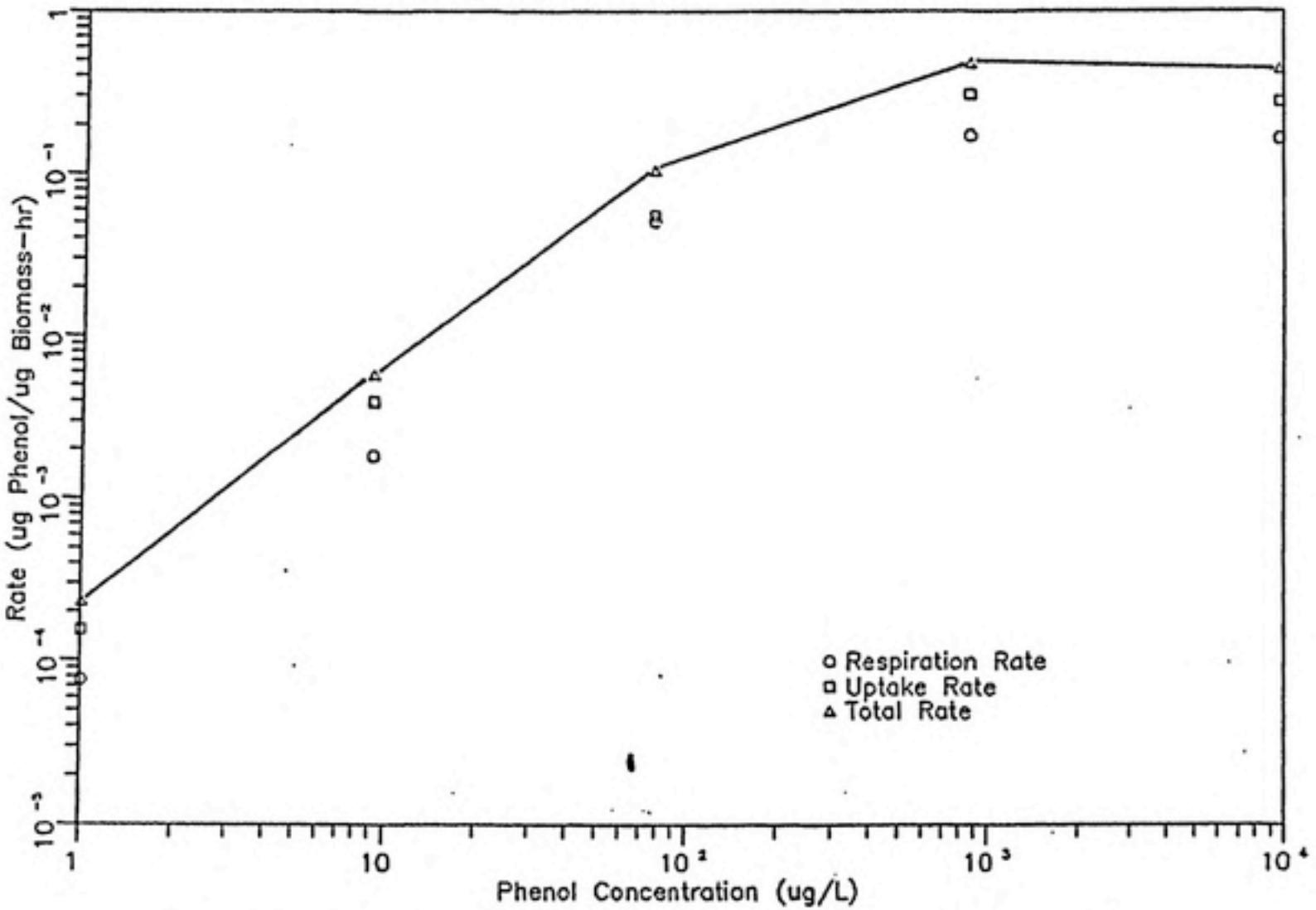



Figure 5-2. Rate of Phenol Metabolism vs. Substrate Concentration, for an Unexposed Population of Microorganisms Recovered from a Humic Substances-Grown Biofilm.

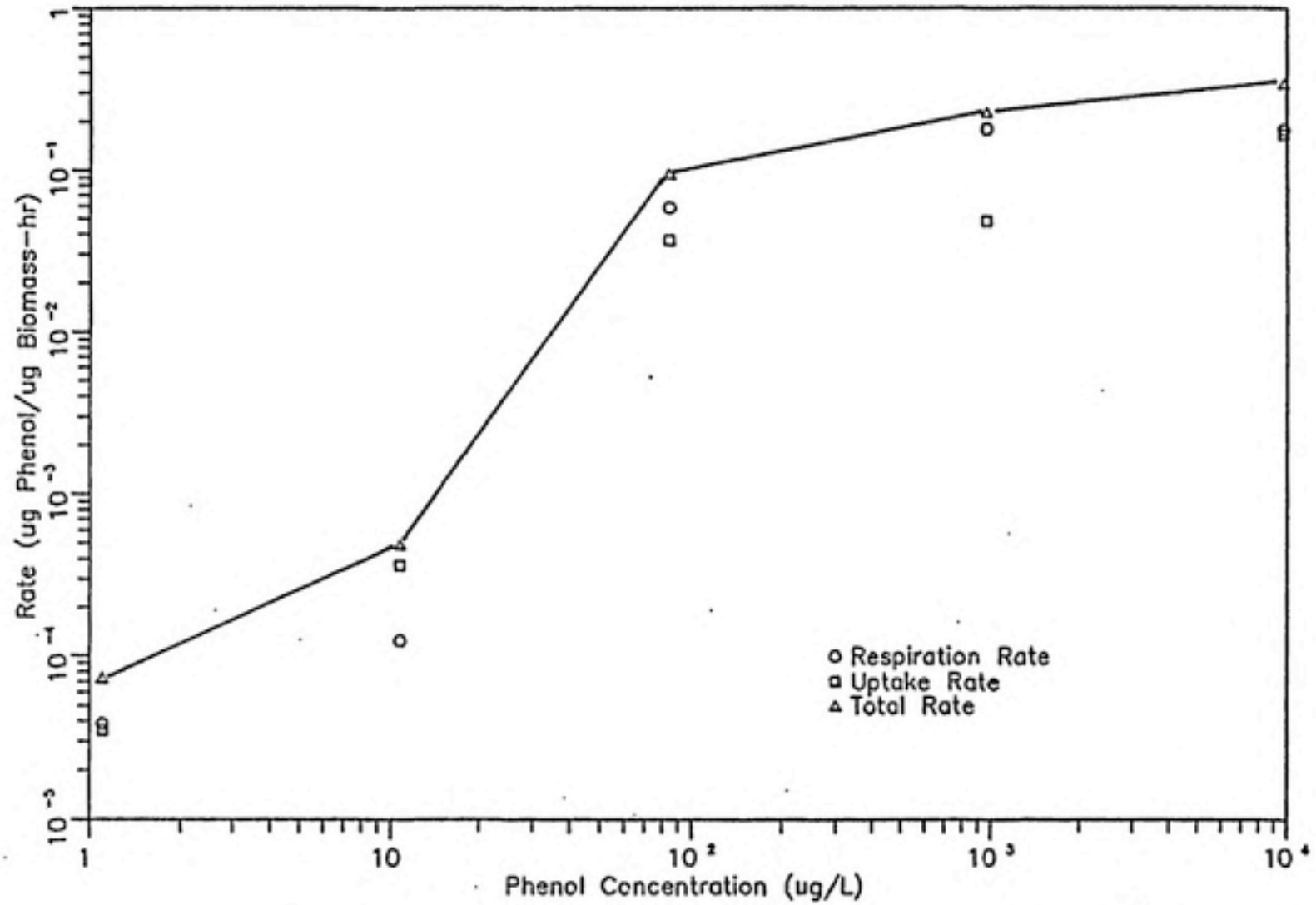



Figure 5-3. Rate of Phenol Metabolism vs. Substrate Concentration, for an Exposed Population of Microorganisms Recovered from a Humic Substances-Grown Biofilm.

At low substrate concentrations, where $S<<K_s$, $(dS/dt)/X = (k/K_s)\cdot S$ and the slope is equal to $k/K_s$. At high concentrations, where $S>>K_s$, $(dS/dt)/X = k$. This last method, although a less sophisticated approach, was employed to give a general feeling for the validity of the parameters obtained by nonlinear regression analysis.

Kinetic parameters obtained by each of the three methods are summarized in Table 5-2. Figure 5-4 shows substrate utilization rates predicted using parameters obtained with each of the three methods, and their relationship to the experimental data for the unexposed population. In general, the differences in biokinetic constants as estimated by these three methods are not considered large. The lack of a good fit of the model to the data is exaggerated in Figure 5-4 due to the use of a logarithmic scale.

## 5.4 Discussion

A unique characteristic of these biokinetic experiments is that they have utilized microorganisms recovered from a biofilm population, rather than those grown in suspended culture, to investigate phenol metabolism. Our intent was to compare biokinetic parameters obtained with the biofilm microorganisms to those obtained by other studies with suspended cultures. In this way the methods could be evaluated, assessing the benefits or drawbacks of using the biofilm population.

The Monod kinetic model has been used to describe phenol biodegradation. In order to justify applying biokinetic parameters to the Monod equation as a basis for comparing our results to the literature, we first have to

Table 5-2

Summary of Microbial Kinetic Parameters for the Unexposed Population

| Method for Estimation | k (µg Phenol/µg biomass-hr) | | $K_s$ (µg Phenol/L) | |
|---|---|---|---|---|
| | Value | Std. Dev. | Value | Std. Dev. |
| Nonlinear Arithmetic Regression (1) | 0.50 | 0.05 | 187.6 | 86.8 |
| Nonlinear Logarithmic Regression (2) | 0.55 | 0.26 | 407.0 | 248.5 |
| Inspection of Figure 5-2 | 0.46 | - | 331 | - |

$$(1) \qquad \frac{(dS/dt)}{X} = \frac{k \cdot S}{(K_s + S)}$$

$$(2) \qquad \log \frac{(dS/dt)}{X} = \log(k \cdot S) - \log(K_s + S)$$

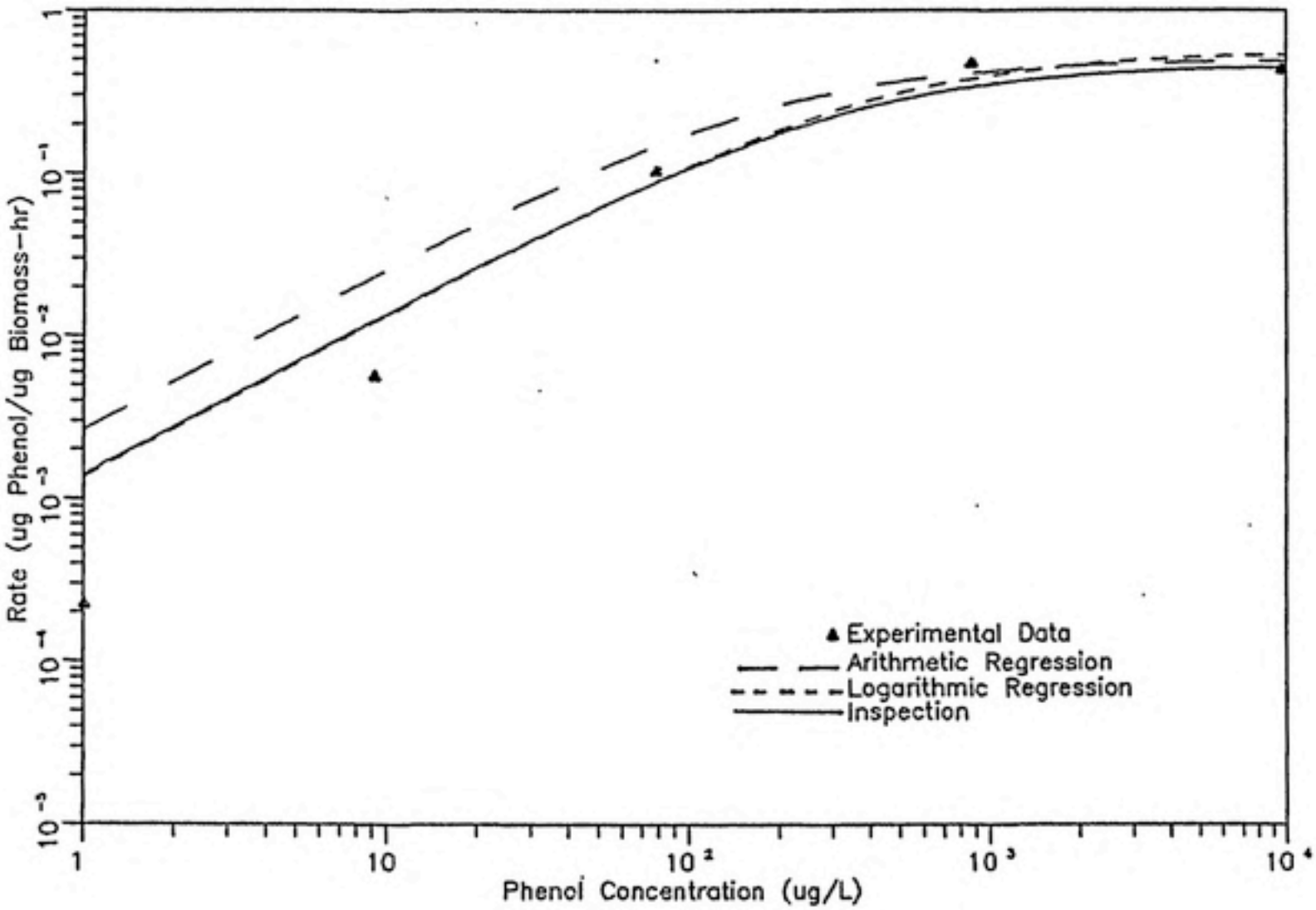



Figure 5-4. Model Predictions for Phenol Utilization Rates by the Unexposed Population of Biofilm Microorganisms.

demonstrate a satisfactory fit between model predictions and experimental data.

By inspection of Figure 5-4, it is apparent that model predictions for metabolic velocities at low substrate concentrations are higher than experimental data for the unexposed population, using kinetic parameters estimated with any of the three methods described above. At higher concentrations, where metabolism approaches a maximum rate, the predictions improve. Presented on a logarithmic scale, the prediction obtained using kinetic parameters estimated through nonlinear regression of the arithmetic expression (equation 5-9) looks especially poor. These parameters were estimated by minimizing the squared residuals about the experimental data points. The errors are distorted by the logarithmic scale; errors at low substrate concentrations are exaggerated as compared to those at higher concentrations. A nonlinear regression on the logarithmic form of the equation (equation 5-10) improves the fit somewhat at lower substrate concentrations. This prediction is nearly identical to that made using parameters obtained by inspection of the degradation curve. Although the fit looks better here, the validity of using the logarithmic form of the equation is questionable. The actual form of the model is arithmetic; data has been plotted on a logarithmic scale only as a visual aid. Thus, while the logarithmic regression may appear to give a better fit to the data, parameters obtained with this method apply to the logarithmic, not the arithmetic, form of the Monod equation.

This analysis raises questions regarding the applicability of the Monod equation for describing substrate utilization kinetics over such a wide range of substrate concentrations. It appears from these data that the model may be weak at low substrate concentrations, but that maximum utilization rates are predicted quite accurately.

The relative error at these higher substrate concentrations evidently outweighs the importance of the lower values.

The purpose here is not to present an in-depth analysis of the applicability of the Monod model to our data, but rather to estimate kinetic parameters for this model and to compare them with others reported in the literature. The biokinetic parameters listed in Table 5-3 for this research were estimated through nonlinear regression of the arithmetic expression (equation 5-9). The literature values in Table 5-3 were all obtained with suspended-growth cultures, acclimated to phenol. Those from this research were obtained using biofilm microorganisms recovered from a GAC reactor receiving ozonated HS; despite the differences in growth medium and type of culture, they fall within the range of literature reported values. This range, though, is quite large: values of k range from 0.157 to 0.57 μg Phenol/μg biomass-hr, while $K_s$ ranges from 56 to 2110 μg Phenol/L. Parameters in the table reported by Schimizn (1973) and by Neufeld & Valiknac (1979) were obtained in chemostat studies, while batch studies were used in this research. The presence of wall growth is a common problem in chemostats and may lead to erroneously high estimates for $\mu_m$, due to the accumulation of substrate at the surface of the container (Speitel, 1985). The result is a high estimate of $K_s$.

The biokinetic parameters in Table 5-3 reported by Speitel (1985) and Chang (1985) were obtained in batch rather than chemostat studies. Results from this research agree more closely with the work of Chang, the values of k and $K_s$ reported by Speitel being lower than those of Chang. One possible explaination for Speitel's lower values is the use of total organic carbon to quantify biomass concentration, X. Such a measure will include both live and dead microorganisms; the result will be a high estimate of

Table 5-3

Literature and Experimental Values for Phenol Kinetic Parameters

| Y (µgC/µgC) | k (µg Phenol/µg Biomass-hr) | $K_s$ (µg Phenol/L) | source |
|---|---|---|---|
| **This Research** | | | |
| 0.46 | -- | -- | exposed population |
| 0.63 | 0.496 | 187.6 | unexposed population |
| **Experimentally Determined by Others** | | | |
| 0.48 | 0.157 | 56 | (1) |
| 0.24 | 0.57 | 240 | (2) |
| 0.83 | 0.29 | 300 | (3) |
| 0.85 | 0.24 | 2110 | (4) |

(1) Speitel (1985)
(2) Chang (1985)
(3) Schimizn (1973)
(4) Neufeld & Valiknac (1979)

microbial density and, hence, low values for kinetic parameters. An overestimate of microbial density was avoided by Chang and in this research by using the viable cell count as a measure of biomass concentration.

It appears, then, that kinetic parameters estimated in this research with biofilm microorganisms agree reasonably well with those obtained in similar studies (batch studies) using microorganisms grown in suspended culture. What can we conclude from this regarding the value of employing biofilm microorganisms to investigate biokinetics? Had our results not agreed with literature values, there would be reason to suspect differences between the behavior of fixed-film and suspended-growth cultures with respect to phenol utilization. Such differences would warrant utilizing the GAC community in studies intended to describe biofilm behavior. These results show, however, that differences between the kinetics of phenol utilization by a suspended-growth culture and a biofilm population, after suspension, may be negligible.

This result is encouraging to those attempting to characterize biofilm behavior, since using a suspended culture avoids the necessity of desorbing viable cells from a biofilm. Caution should nonetheless be exercised in relying on results of this study to validate assumptions regarding similarities between fixed-film and suspended-growth cultures. This study was performed only once, and with a single substrate. It is possible that the behavior of these biofilm microorganisms was significantly altered upon suspension of the culture into a liquid supernatant. We have yet to confirm the applicability of these results directly to the in situ biofilm system.

As important as comparing Monod biokinetic parameters to literature values is the difference in metabolic activity

noted between the exposed and unexposed populations. Figure 5-5 presents the rate of phenol degradation vs. substrate concentration for both the exposed and the unexposed populations. At higher phenol concentrations (above S = 100 μg/L), the degradation rates are similar, but at lower concentrations (below S = 100 μg/L) the community which has been exposed to the micropollutant degrades the compound at a much lower rate than the unexposed community. A discontinuity in the relationship between degradation rate and substrate concentration as observed for the exposed population may indicate biphasic degradation kinetics. Similar behavior with respect to phenol degradation in lake water was noted by Rubin and co-workers (1982); they observed a discontinuity in the plot of phenol mineralization rate vs. concentration for three lake water samples, all at around 100 μg/L phenol. The effect, they postulate, may reflect the activity of two different types of organisms - oligotrophs, active at lower substrate concentrations, and heterotrophs (or copiotrophs), active at higher concentrations. Another possible explanation for the reduced degradation rate at low substrate concentrations by the exposed community derives from the way the data is expressed: respiration rate per viable cell. The proportion of phenol degraders to the total microbial community in the pre-exposed population is probably much greater than in the unexposed population. This leads to a lower substrate concentration per degrader and thus a lower utilization rate in the exposed community.

Another distinction to note between the exposed and unexposed populations is the difference in the microbial yield coefficient, which represents the amount of substrate incorporated into cellular material per unit substrate metabolized. As seen in Table 5-1, the yield coefficient is much higher for the unexposed than for the exposed population (0.63 vs. 0.46 μg biomass C/μg phenol C). This

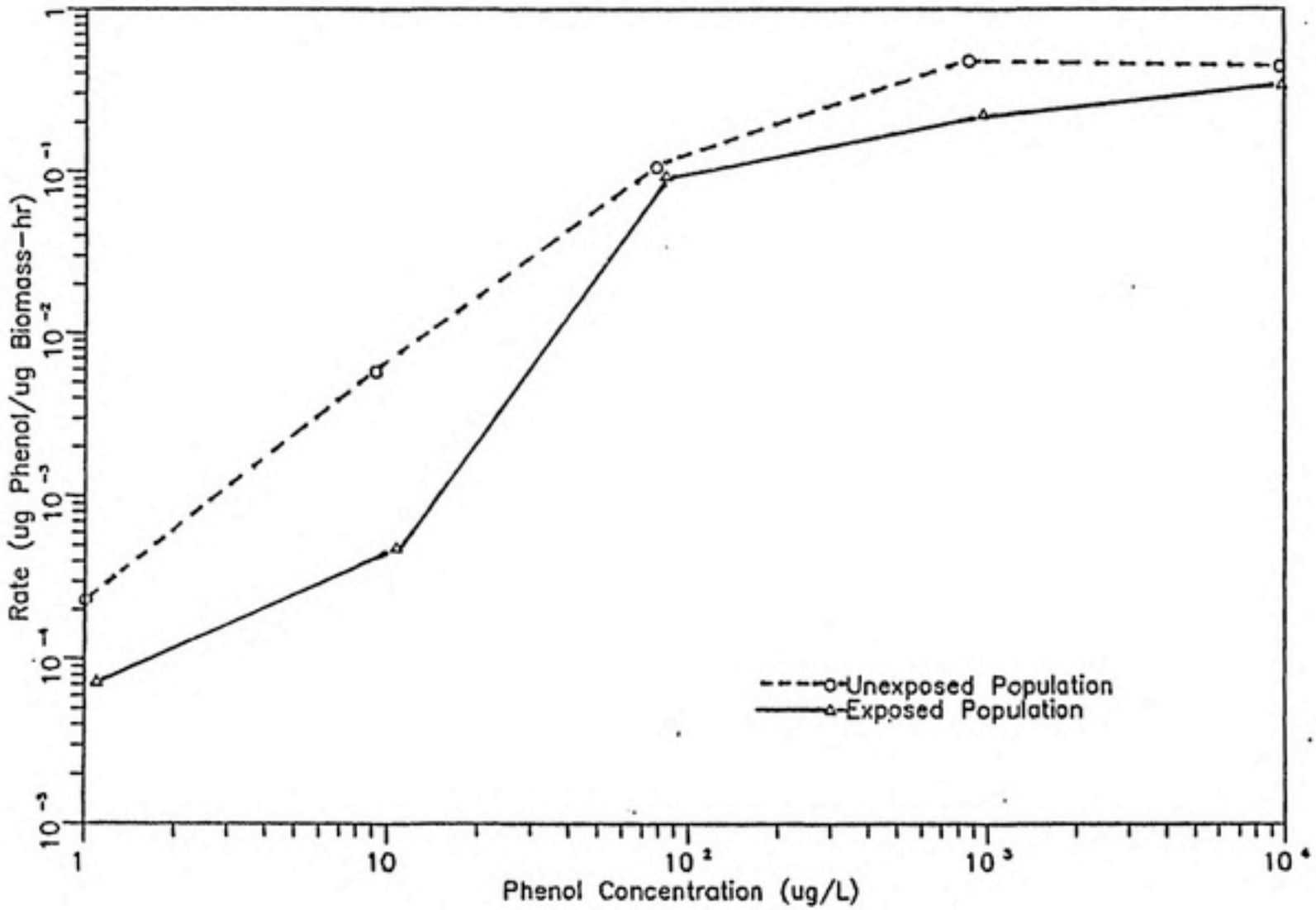



Figure 5-5. Total Phenol Utilization Rate vs. Substrate Concentration for the Exposed and Unexposed Populations.

suggests that a pre-exposed microbial community will respire a higher percentage of total substrate metabolized; an unexposed community will assimilate more material into cells. A similar result has been demonstrated by Pfaender and co-workers (personal communication, D.C. Dobbins). They have shown, with samples from pristine and from contaminated subsurface environments, that pre-exposure will lead to an increased proportion of the metabolized contaminant being respired. This indicates that the microbial community has shifted from one which maximizes cellular uptake, to one which rapidly mineralizes substrate to $CO_2$.

# 6. ADSORPTION AND BIODEGRADATION IN FLOW-THROUGH REACTORS

## 6.1 Background: Choice of Reactor Design

This phase of study was intended to investigate interactions between adsorption and biodegradation of naturally occurring humic substances (HS), as would be encountered in a GAC filter used for water treatment. We are particularly interested in the ability of HS to enhance biofilm growth, and further, in the ability of this biofilm to degrade phenol when introduced at very low concentrations and at different stages of biofilm development.

The flow-through reactors used in this study are based on a design presented by Chang and Rittmann (1987), in an investigation of the adsorption and biodegradation of phenol. Their design is a fixed-bed column with a high internal recycle ratio, which approximates a completely mixed reactor. A high recycle ratio dilutes the influent feed concentration; as a result, the change in concentration across the bed is very small though the overall change between the feed and the effluent may be quite large. Intuitively, the recycle ratio may affect the kinetics of the system, since it will control the velocity through the GAC bed. However, since by definition EBCT = (reactor volume)/(feed flowrate), the overall removal rate should be independent of the recycle ratio. With sufficient recycle ratio, the system represents a differential element in a GAC bed, having no axial concentration gradient.

This design met two important criteria for the study by Chang and Rittmann:

1. saturation of sorptive capacity occurred within a reasonable time (on the order of two weeks) for convenient laboratory investigations, and
2. axial concentration gradients were eliminated, leaving operating time as the only dependent variable for modeling considerations.

Model predictions for breakthrough curves of an adsorbable, non-biodegradable component, a non-adsorbable, biodegradable component, and the combination of the two from this fixed-bed column with high recycle ratio are presented in Figure 2-1 (see page 21).

The reactor design of Chang and Rittmann was amended to accommodate a study of biofilm growth caused by adsorption and biodegradation of ozonated HS rather than phenol. The adsorbability of HS is considerably different than that of phenol: the adsorption mass transfer zone is larger and the sorptive equilibrium capacity is much less. Both of these factors affect the choice of reactor size (hence, mass of GAC) and empty bed contact time. A larger mass of GAC, and therefore a longer EBCT, will be needed to observe a measureable (gradual) breakthrough curve for humic substances.

Another factor to be considered in modifying the reactor design is the biodegradability of ozonated HS, since removal will be due not only to adsorption but to biodegradation as well. The Monod equation for substrate utilization is helpful in understanding factors affecting the extent of substrate removal ($\Delta S$) across a reactor

element:

$$\frac{\Delta S}{\Delta t} = \frac{k \cdot S \cdot X}{K_s + S}. \qquad (6\text{-}1)$$

The change in S across the GAC bed due to biodegradation can be affected by: EBCT (since this is proportional to $\Delta t$), substrate concentration (S), and bacterial density (X). The magnitude of substrate reduction is directly proportional to the contact time in the bed as well as to biomass concentration. The relationship between $(\Delta S/\Delta t)$ and substrate concentration, S, is variable and depends on S. At low substrate concentrations, $S \ll K_s$, $(\Delta S/\Delta t)=(k \cdot S \cdot X)/K_s$ and $\Delta S$ is directly proportional to S; at higher substrate concentrations, however, where $S \gg K_s$, $(\Delta S/\Delta t)=(k \cdot X)/K_s$. Here the biodegradation rate will be unaffected by substrate concentrations in the feed solution.

Adjusting the EBCT is the simplest way to control the extent of biodegradation in the reactor. Microbial density is a result of operating characteristics and feed solutions, and is therefore beyond our control. While the feed concentration could easily be changed, we are not sure of the biodegradation kinetics (in particular, $K_s$) for ozonated humic substances; thus, the effect on utilization rate is unknown.

Two major modifications were made to the design presented by Chang and Rittmann, to meet the needs of this study. We used a larger mass of GAC (hence a larger reactor volume, as defined by the volume occupied by the fixed bed of GAC), and a longer EBCT.

## 6.2 Adsorption and Biodegradation of Humic Substances

### 6.2.1 Experimental Methods

General Design of Column System A schematic of the reactor design is shown in Figure 6-1. The fixed-bed columns were constructed of glass; two sizes were used, one with a diameter of 1.85 cm and a length of 21.5 cm (Vol = 57.8 $cm^3$), which held approximately 30 grams of GAC, and the other similar in diameter but shorter, with a length of 2 cm (Vol = 5.4 $cm^3$), which held approximately 4 grams of GAC. The two sizes were used in the process of determining the correct mass of GAC for saturating the bed with HS within a reasonable amount of time. The columns were equipped with removable end caps, which were held in place by rubber o-rings and metal clamps. GAC was retained in the bed with monofilament nylon mesh (Small Parts, Inc., Miami, Florida).

The column feed and effluent lines consisted of Teflon tubing (I.D. = 1/16 in.). The feed pump was an Ismatec peristaltic pump (Cole Palmer Instrument Company, Chicago, Illinois), which accurately delivered feed at low flowrates; pump tubing was constructed of silicon. A glass break tube was placed between the feed reservoir and feed pump to prevent back-contamination of the feed. For experimental runs which utilized more than one column, receiving identical feed solutions, the breaktube also served as a means for splitting the feed flow from a single line (from the reservoir) into several lines (one to each column).

Two types of feed reservoirs were used. For operating several columns which all received the same feed, a single reservoir (25L) constructed of autoclavable linear polyethylene (Nalge Company, Rochester, New York) was used. For runs with one or more columns receiving different feed

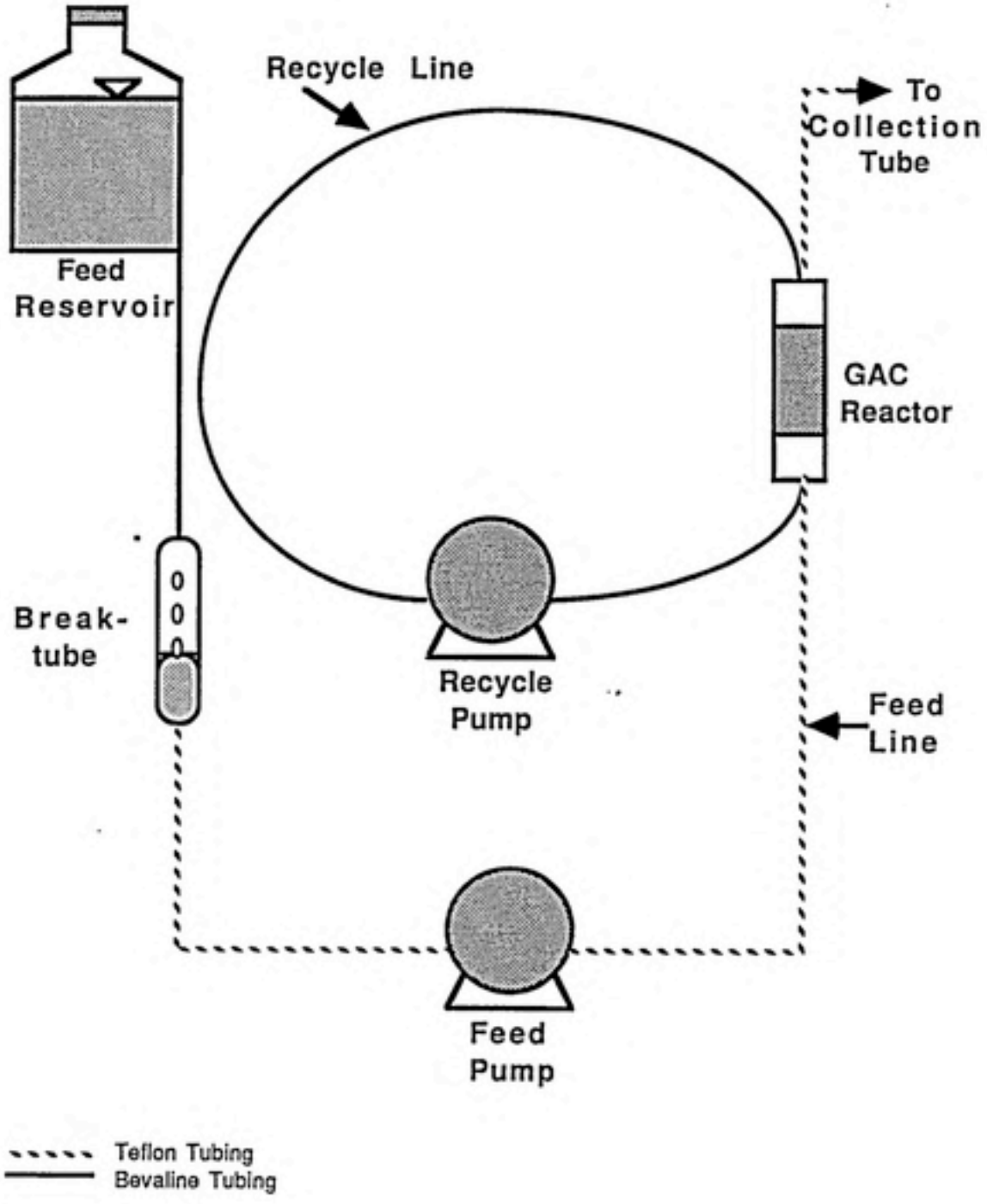



Figure 6-1. Schematic of Reactor Design

solutions, glass aspirator bottles (2L) were used. The feed reservoirs were capped with rubber stoppers equipped with holes for ventilation; glass tubing stuffed with glass wool was used to prevent atmospheric contamination of the feed.

The recycle line was constructed of Bevaline tubing (Teflon lined polyethylene tubing, I.D. = 1/4 in.; Atlantic Plastics, Raleigh, North Carolina). The recycle pump was a Masterflex peristaltic pump (Cole Palmer Instrument Company, Chicago, Illinois) which had a higher capacity than did the feed pump; recycle pump tubing, also, was silicone. The recycle ratio was set at approximately 20:1 for the first three column runs, and increased to 38:1 thereafter. Recycle ratios were set on the basis of minimizing the change in HS concentration across the reactor (to approximate a differential element), as well as to maintain a reasonable loading rate (total flowrate ($Q + Q_R$) divided by cross-sectional area) on the filter.

In all experiments, the entire column apparatus was sterilized by autoclaving for 30 minutes at 20 psi prior to initiation of the run; the Bevaline tubing, which withstands a maximum temperature of only 200°F, was sterilized in 95% ethanol. Fresh feeds were prepared daily, in freshly autoclaved feed reservoirs. Feed flowrates varied between experimental runs, ranging between 2 and 6 L/day, and were monitored daily with a graduated cylinder and stop watch.

Carbon Sample. Filtrasorb-400 GAC (Calgon Corp., Pittsburgh, Pennsylvania) served as media for all column experiments. GAC was ground with a mortar and pestel and sieved to retrieve the 30x40 mesh fraction (average particle diameter 0.5 mm). The carbon was rinsed several times with distilled deionized water to remove fines, dried at 105°C, and stored in a dessicator until use. Prior to each run,

GAC samples were rinsed again and autoclaved at 20 psi for 30 minutes.

Dry weights were obtained for GAC by drying to a constant weight at 105°C; samples were weighed on a Metler analytical balance.

Feed Solutions. Water high in natural HS concentration was obtained from Lake Drummond, a richly colored lake located in the Great Dismal Swamp in Suffolk, Virginia. The organic content of the raw water is approximately 40 mg/L as TOC; prior analyses have determined that the ratio of fulvic to humic acids is about 9:1 (personal communication, Young Seo Ko).

Water was retrieved in large quantities, pressure filtered through a 1 μm honeycomb filter (Tate Engineering, Roanoke, Virginia), and stored in 20L plastic containers at 4°C until use. For ozonated HS, the water was ozonated also in large quantities and stored. When it was desired to lower the organic content of the feed, swamp water was diluted to the desired concentration in sterile distilled deionized water.

Ozonation. The humic substances were ozonated at a dose of approximately 1 mg ozone per mg TOC. Ozone was generated with a Grace LG-2-LI laboratory ozone generator (Union Carbide, South Plainfield, New Jersey) and delivered at a gas flowrate of approximately 1 L/min, through stainless steel and Teflon tubing, to a glass reactor containing 20L of Lake Drummond water. Figure 6-2 is a schematic description of the ozonation process and Table 6-1 provides the operating parameters.

Samples were ozonated for approximately 27 minutes, followed by a 15 minute purge with nitrogen gas at a

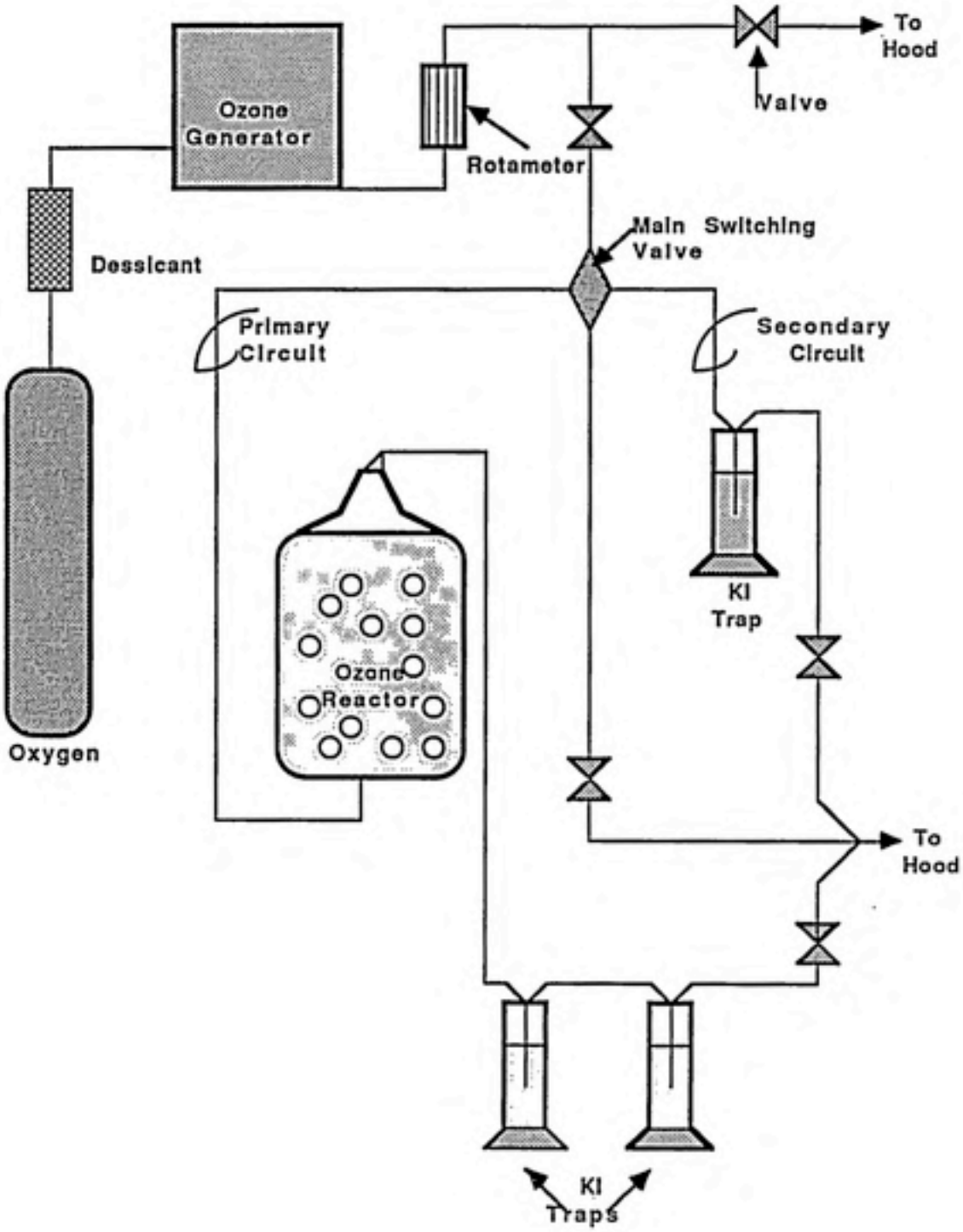



Figure 6-2. Schematic of Ozonation Apparatus

Table 6-1

Ozonation Operating Parameters

| | |
|---|---|
| $O_2$ Pressure in Generator (psi) | 15 |
| Power Supplied to Generator (V) | 120 |
| Gas Flowrate into Solution (L/min) | 1 |
| $O_3$ Supplied per Minute (mg/min) | 41 to 42 |
| Ozonation Time (min) | 27 |
| Ozone Dosage (mg $O_3$/mg TOC) | 1/1 |
| Nitrogen Purge Time (min) | 15 |
| $N_2$ Gas Flowrate (L/min) | 1 |

flowrate of 1 L/min to remove residual unreacted ozone. Generation rates were calculated by passing ozone directly to the secondary circuit KI trap for a period of two or three minutes both before and after ozonating the sample. Initial and final rates were combined to yield an average generation rate.

Ozone dosage was calculated using a material balance. The total amount generated was calculated with the average generation rate and total reaction time; unreacted ozone passed through the sample was collected in the two in-series primary circuit KI traps. No attempt was made to measure the rate of ozone decomposition. Any decomposed ozone is accounted for as reacted ozone, which may lead to calculated dosages slightly higher than those actually applied. However, the total amount of decomposed ozone as compared to that reacted should be very small at neutral pH, especially if contact time is relatively short (Sullivan and Roth, 1979). Furthermore, ozone stability increases with decreasing pH (Standard Methods, 16th ed., 1985); the pH of Lake Drummond water is approximately 6.

Ozone concentration in each KI trap was determined utilizing an iodometric titration (Standard Methods, 16th ed., 1985). Free iodine liberated by ozone from the KI solution was titrated with a standard 0.1N solution of sodium thiosulfate, using a starch indicator. Thiosulfate solutions were standardized with potassium biiodate.

Analytical Methods. Organic content was measured as total organic carbon concentration, TOC, and in some cases as dissolved organic carbon concentration, DOC. DOC samples were vacuum filtered through a 0.2 μm Gelman triacetate filter which had been pre-rinsed with distilled deionized water and approximately 20 ml of sample.

Two instruments were used to measure TOC and DOC. For the initial portion of the work, using unozonated swamp water and ozonated swamp water without dilution, TOC was measured on a Beckman Model 915-B analyzer equipped with a high sensitivity detector. These samples were acidified to pH 2 and purged with nitrogen gas to drive off inorganic carbon as $CO_2$. For all later studies a newer instrument was available; organic and inorganic ($CO_2$) carbon concentrations were analyzed with an O.I. Model 700 Total Organic Carbon Analyzer (O.I. Corporation, College Station, Texas).

### 6.2.2 Results and Discussion

Several runs were performed to find the proper combination of operating conditions (feed TOC, mass of GAC in reactor, EBCT, recycle ratio) that would yield both biodegradation and adsorption of natural humic substances, and also meet the criteria that the reactor system behave as a differential element. To be a differential element, the change in TOC across the reactor must by very small but measureable. Four runs are reported here, each differing in feed solution characteristics, empty bed contact time, or both. Operating characteristics for each column run, referred to as Run Nos. 1, 2, 3, and 4, are presented in Table 6-2.

Run No. 1 was performed to test the design for the differential element assumption. It was also used to obtain information regarding the adsorptive capacity of GAC for HS, and their biodegradability. A 57.8 $cm^3$ column containing 30 grams of GAC (dry weight) was fed untreated water from Lake Drummond, feed TOC = 25 mg/L; the breakthrough curve is presented in Figure 6-3.

Table 6-2

Operating Characteristics: Column Run Numbers 1-4
GAC Reactors Recieving Humic Substances, Varying Feed Conditions and EBCT

| Column Run No. | Carbon Dry Wt. (gm) | Bed Vol. ($cm^3$) | EBCT (min) | Feed Solution | Feed Flowrate (L/day) | Recycle Ratio ($Q_R/Q$) | Loading Rate[a] (m/hr) |
|---|---|---|---|---|---|---|---|
| 1 | 30 | 57.8 | 41.6<br>13.9[b] | TOC=25 mg/L unozonated | 2<br>6[b] | 20/1<br>20/1 | 6.2<br>19.5 |
| 2 | 33.4 | 57.8 | 13.9 | TOC=25 mg/L ozonated | 6 | 20/1 | 19.5 |
| 3 | 4.34 | 5.38 | 1.3 | TOC=7 mg/L ozonated | 6 | 20/1 | 19.5 |
| 4 | 3.97 | 5.38 | 3.9 | TOC=7 mg/L ozonated | 2 | 38/1 | 11.7 |

[a]Loading Rate = $(Q + Q_R)$/cross-sectional area of column reactor $(L^3/L^2\text{-}t)$

[b]Flowrate in Run No. 1 was increased after 9 days of operation to 6 L/day.

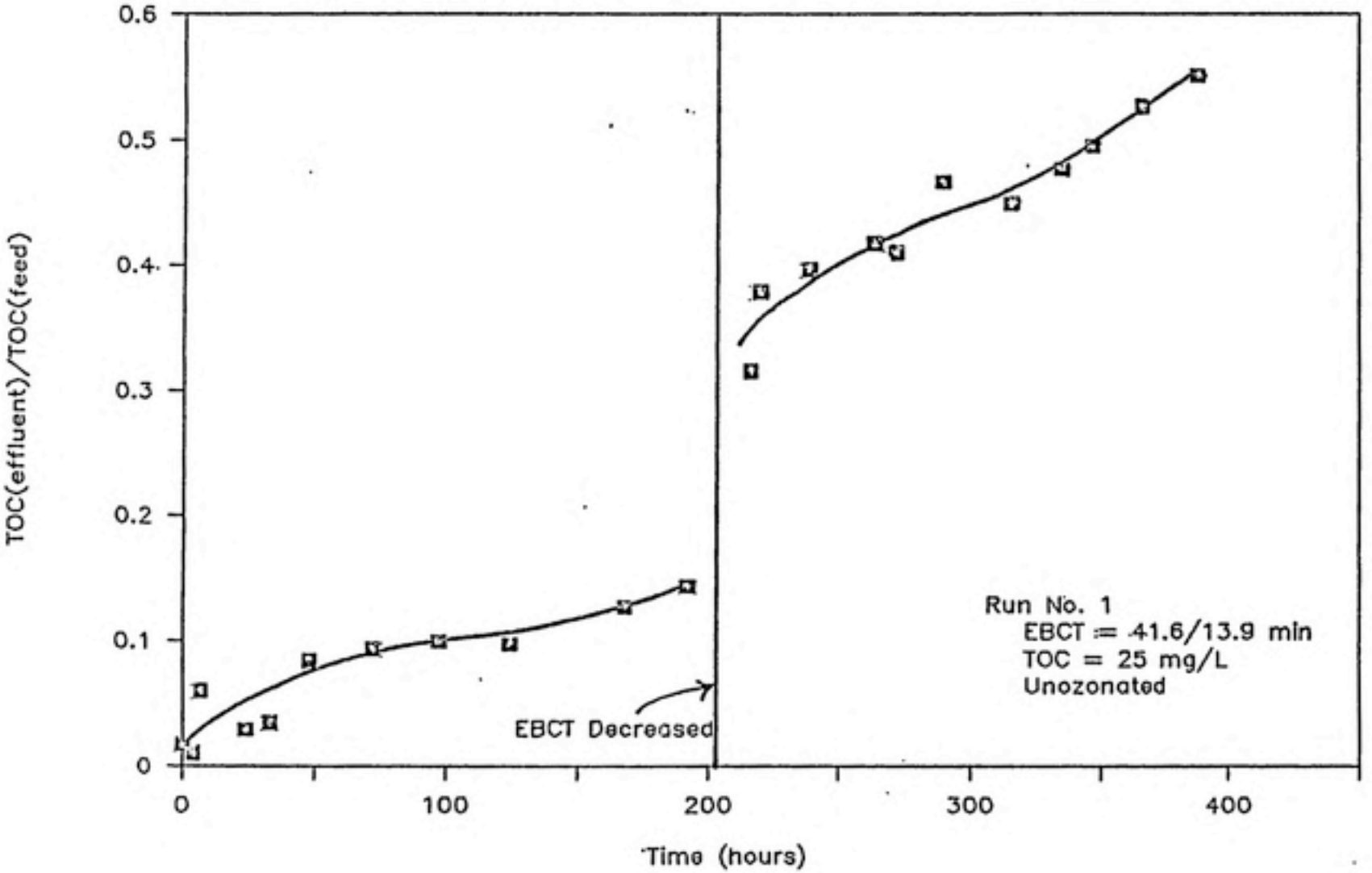



Figure 6-3. TOC Breakthrough Curve for Run No. 1: Untreated Lake Drummond Water; TOC = 25 mg/L; EBCT = 41.6 min/13.9 min.

At a feed flowrate of 2 L/day (EBCT = 41.6 min) the column exhibited roughly 10% breakthrough of HS after nine days of operation. The feed flowrate was increased to 6 L/day (EBCT = 13.9 min) after about 210 hours, in hopes of achieving a more rapid rise in effluent TOC concentrations. By 380 hours, TOC in the effluent stream had reached approximately 55% of the influent concentration and was still increasing. Thus the empty bed contact time, even at a feed rate of 6 L/day, was too long for our purposes; a convenient laboratory study requires saturation of sorptive capacity within two to three weeks. In addition, no significant biofilm growth was detected in the column, as evidenced both visually and by the continual rise in effluent TOC. This implies that without ozonation the humic substances are not easily biodegraded.

Run No. 2 was directed toward enhancing biological activity on the GAC surface. The HS solution was ozonated (1 mg $O_3$/mg TOC) and fed at 6 L/day (EBCT = 13.9 min) to a column reactor similar to that used in the unozonated system; the breakthrough curve is shown in Figure 6-4. The immediate rise and subsequent drop in effluent TOC concentrations early in the run are discussed below; after about 75 hours of operation, the effluent TOC rose continuously until reaching a steady state concentration equal to approximately 30% of the feed by 320 hours. Effluent concentrations remained at or about this level until 430 hours of operation, at which point the run was terminated.

As evidenced by the significant steady state reduction in TOC, biological activity in Run No. 2 was substantial. A small plug of carbon was removed from the influent end of the column after 330 hours of operation, and a minimum of 5E+09 cells/gm were detected on the carbon surface by epifluorescence microscopy. Upon termination of the run,

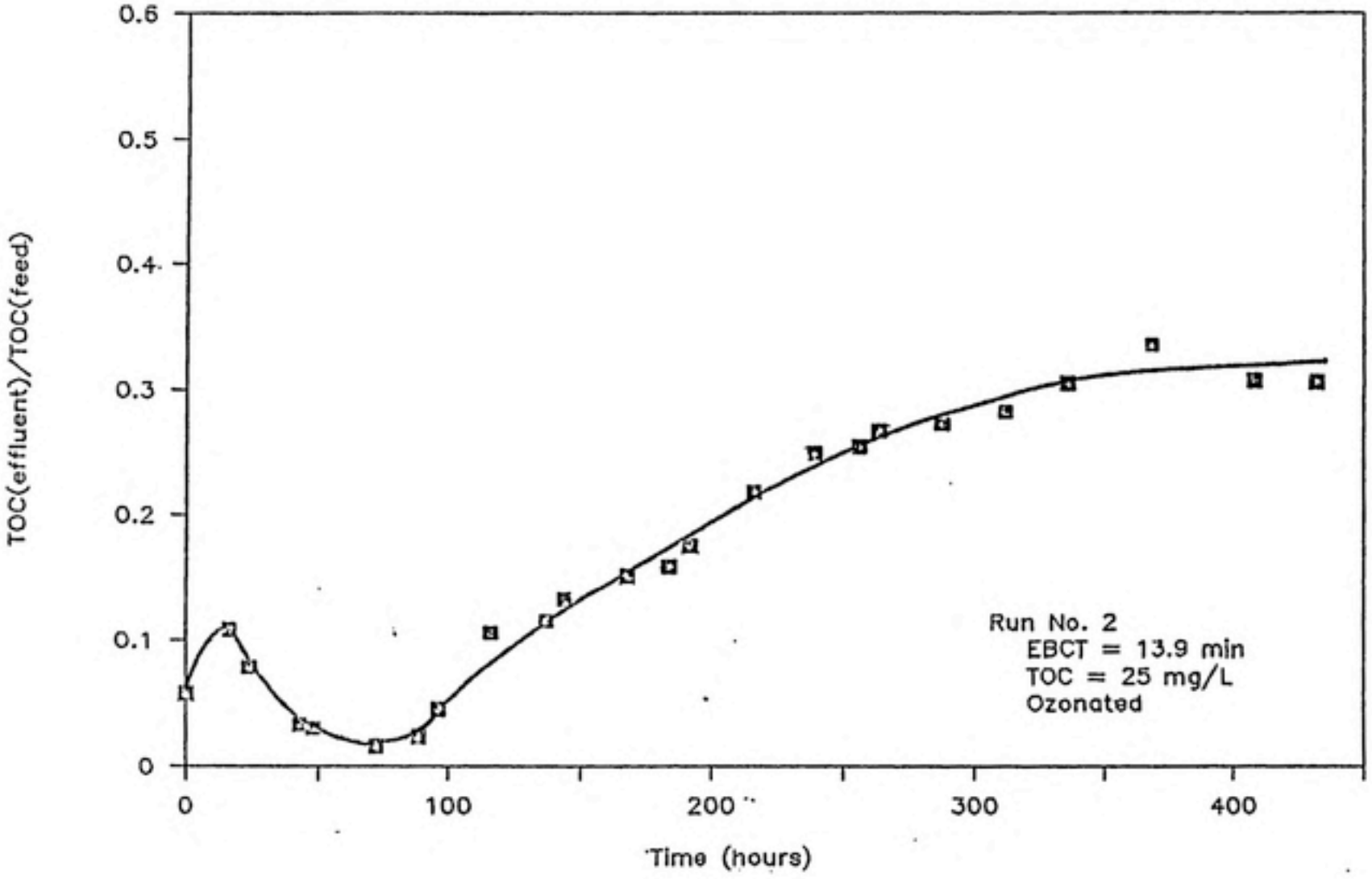



Figure 6-4. TOC Breakthrough Curve for Run No. 2: Ozonated Lake Drummond Water (1 mg $O_3$/mg TOC); TOC = 25 mg/L; EBCT = 13.9 min.

approximately 4.75E+09 cells/gm were recovered from the carbon utilizing the PVP/PPi wash procedure described in Chapter 4. About 10% of the cells, or 4.88E+08 cells/gm, were viable as indicated by a modified standard plate count. These numbers are comparable to literature reported values: total cell densities reportedly range from $10^6$ to $10^7$ cells/gm for GAC used in water treatment (Van der Kooij, 1976; Cairo et al., 1979), to $10^9$ cells/gm wet weight (Latosek & Benedek, 1979).

Extensive biological activity, in combination with the large mass of GAC, resulted in a substantial drop in TOC across the reactor in Run No. 2. The change in HS concentration across the bed was about 38%, which is nearly half of the total steady state removal noted (70%). Thus, the reactor was not operating in accord with the differential element assumption.

An interesting feature of the TOC vs. time profile shown in Figure 6-4, as previously noted, is the immediate rise and subsequent drop in effluent TOC concentrations during the first 75 hours of operation. This "hump" was also observed later in Run Nos. 3 and 4 (See Figure 6-6 and 6-7) but did not occur at the same point in time. The results in Run No. 4 suggested that the difference in time positioning of the rise and fall in TOC concentration could be related to EBCT. The ratio of the EBCT in Run Nos. 2 and 4 is 0.28, which is about the same as the ratio of operating times covered by the "hump" (0.27). Unfortunately the positioning of the "hump" in Run No. 3 did not follow the same relationship.

A possible explanation for the shape of these breakthrough curves can be found by making the assumption of a two-component mixture of humic substances. One component is biodegradable and only slightly (if at all) adsorbable,

and the other component is non-biodegradable but adsorbable. Recent modeling work by Garber in this laboratory has shown one simple way to modify the Chang and Rittmann model (1987) to account for the biodegradation and adsorption of such a two-component mixture (personal communication, J. Garber). The resulting breakthrough curves for each of the two components and that of the components added together are shown in Figure 6-5, from Garber's work. This modeling exercise suggests a "hump" in the TOC breakthrough curve when a two-component mixture is assumed. The biodegradable/slightly adsorbable component rapidly increases in concentration but then decreases again due to biodegradation; in contrast, the non-biodegradable/adsorbable component steadily increases due to exhaustion of sorptive capacity. In effect, two steady state conditions are observed in this system. A steady state with respect to biological activity is reached rather early, as depicted by the leveling off of effluent biodegradable/slightly-adsorbable TOC. However, steady state with respect to overall TOC removal across the bed is achieved only after sorptive capacity is depleted. Further evidence for early attainment of steady state with respect to biological activity will be presented later in the analysis of data for $CO_2$ production in Run No. 4 (page 125).

Differences between influent and effluent TOC concentrations after long periods of reactor operation represent the extent of steady state biodegradation within the GAC bed. The effect of ozonation on steady state biodegradation can be seen by comparing the TOC removals in Run Nos. 1 and 2. Run No. 2 received ozonated HS at a concentration of approximately 25 mg/L and an EBCT of 13.9 minutes. As seen in Figure 6-4, the effluent TOC reached only 30% of the influent at steady state. Effluent TOC for the unozonated HS used in Run No. 1, on the other hand (Figure 6-3), reached 55% of the influent concentration by

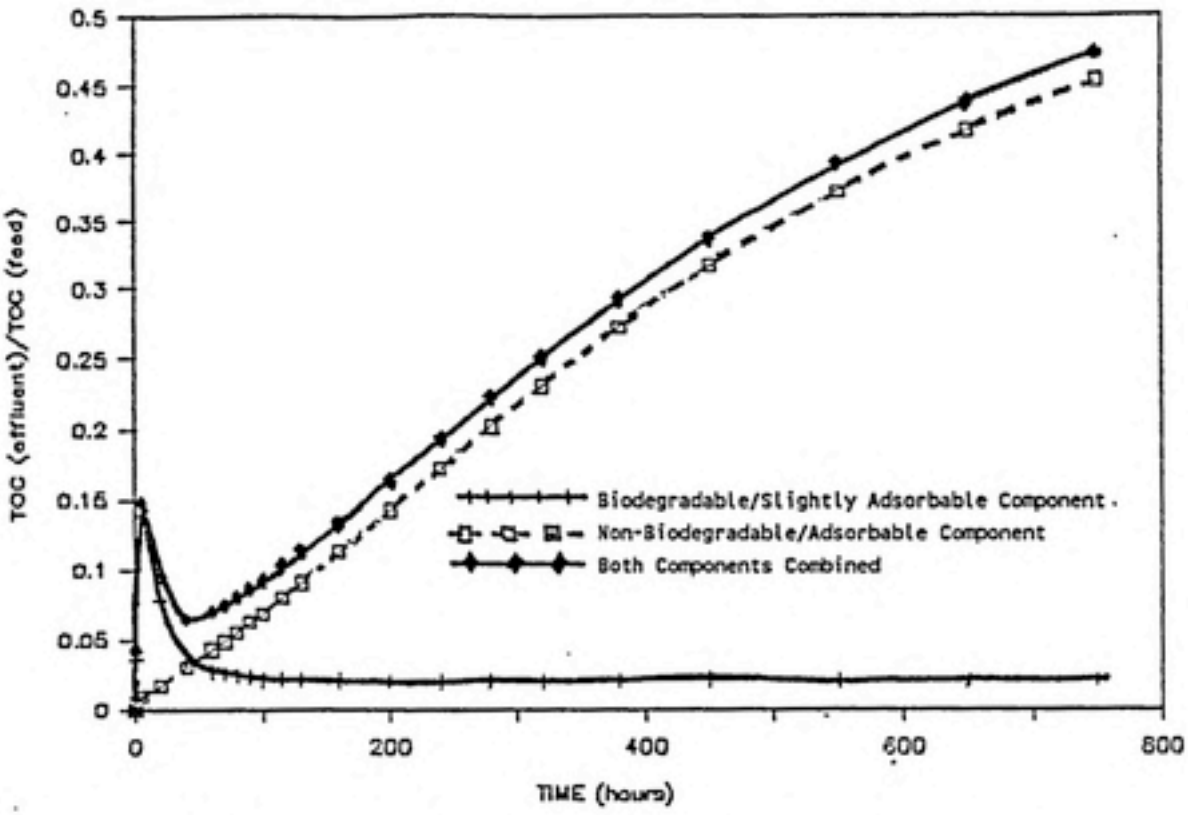



Figure 6-5. Model Predictions for TOC Breakthrough Curves of a Two-Component Mixture of Humic Substances: Slightly Adsorbable/Biodegradable and Adsorbable/Non-Biodegradable Components (Developed by Garber, 1987; Personal Communication)

380 hours and was still rising. Comparison of these results supports the idea that ozonation increases the biodegradability of humic substances. It must also be noted that the EBCT in Run No. 1 was decreased from 41.6 minutes to 13.9 minutes after 210 hours of operation. The long EBCT (41.6 min) during the first nine days would have, in fact, increased the chance for HS biodegradation compared to that in Run No. 2 (EBCT = 13.9 min). Nevertheless, we still saw less biodegradation in Run No. 1 than in Run No. 2. This reinforces our conclusion that ozonating the humic substances increases biological activity in the GAC filter bed.

The reactor design was modified after Run No. 2. Run No. 2 showed that the laboratory reactor system could be operated to produce measureable amounts of both adsorption and biodegradation. However, the reactor did not meet the criteria for behaving as a differential element, i.e., the change in TOC concentration across the reactor was too large (38%). This presented problems from a modeling standpoint. In addition, the extensive biological growth was of concern because it caused too much head loss. To overcome these difficulties, the length of the reactor was shortened in Run No.3 from 21.5 cm to 2 cm, thus reducing the EBCT from 13.9 minutes to 1.3 minutes at a feed flowrate of 6 L/day. Shortening the reactor reduces the extent of steady state biodegradation, also solving the problem of excessive head loss. In addition, the feed concentration of ozonated HS was decreased from 25 to approximately 7 mg/L (as TOC) in order to compensate for the loss in sorptive capacity incurred by decreasing the reactor length and, thus, mass of GAC.

The results of Run No. 3 are shown in Figure 6-6. The profile shows the same initial rise and fall in effluent TOC concentrations, as was noted in Run No. 2, during the first

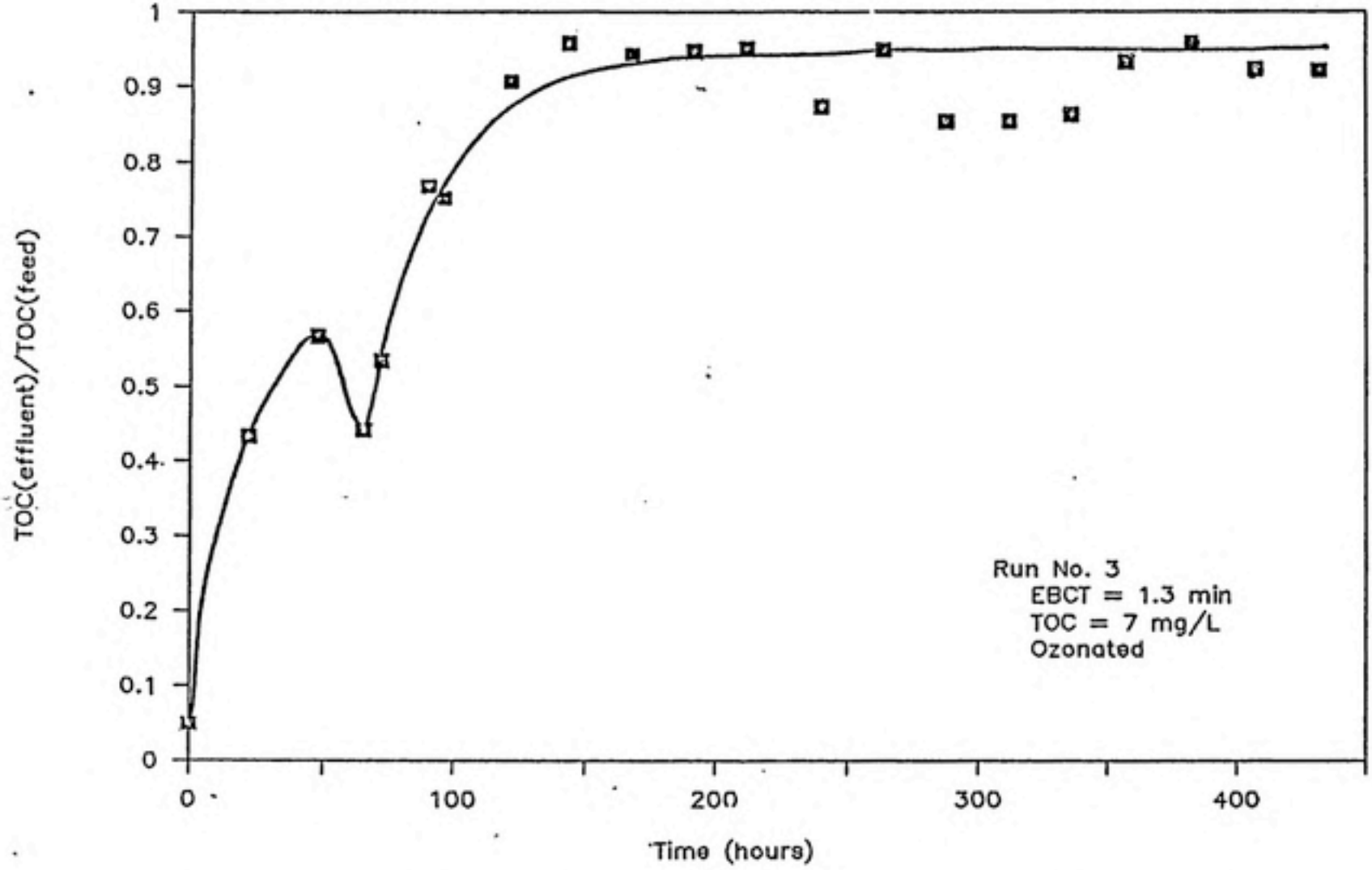



Figure 6-6. TOC Breakthrough Curve for Run No. 3: Ozonated Lake Drummond Water (1 mg $O_3$/mg TOC); TOC = 7 mg/L; EBCT = 1.3 min.

70 hours of operation. Here the early "hump" in effluent TOC extends much higher (to 57% of the feed, as opposed to 12% for Run No. 2), likely resulting from less HS biodegradation in Run No. 3 than in Run No. 2. After only 140 hours the effluent TOC had reached a steady state value, approximately 95% of the feed TOC. This steady state was maintained until 420 hours, at which time the column was disassembled.

GAC recovered from this reactor was used in the biofilm removal study, to compare the effects of ultrasonication and blending on the desorption of biofilm microorganisms (Chapter 4). A PVP/PPi wash (using the blender procedure) recovered approximately 8.53E+07 viable cells/gm from the GAC, as opposed to 4.88E+08 cells/gm recovered from Run No. 2. Assuming consistent fractional recoveries from sample to sample, this represents an 82% reduction in bacterial density at steady state (with respect to TOC removal) due to the lower EBCT and in part to the diluted feed concentration. Run No. 3 demonstrated that reducing the EBCT would, as expected, reduce the steady state removal of TOC by biodegradation. However, it was clear that biological activity was too limited in this design (only about 5% of the TOC was being removed biologically).

The EBCT was increased to 3.9 min in Run No. 4 to increase the extent of biodegradation; this was done by decreasing the feed flowrate to 2 L/day. The feed concentration was kept at 7 mg/L TOC. The resulting breakthrough curve for Run No. 4 is shown in Figure 6-7. The effluent TOC reaches a steady state, equal to approximately 57% that of the feed concentration, after about 160 hours. Thus, 43% of the feed HS was biodegraded. The "hump" in the breakthrough curve that was noted in Run Nos. 2 and 3 again occurs, in this case after about 20 hours of operation. The hump extends to an effluent TOC which

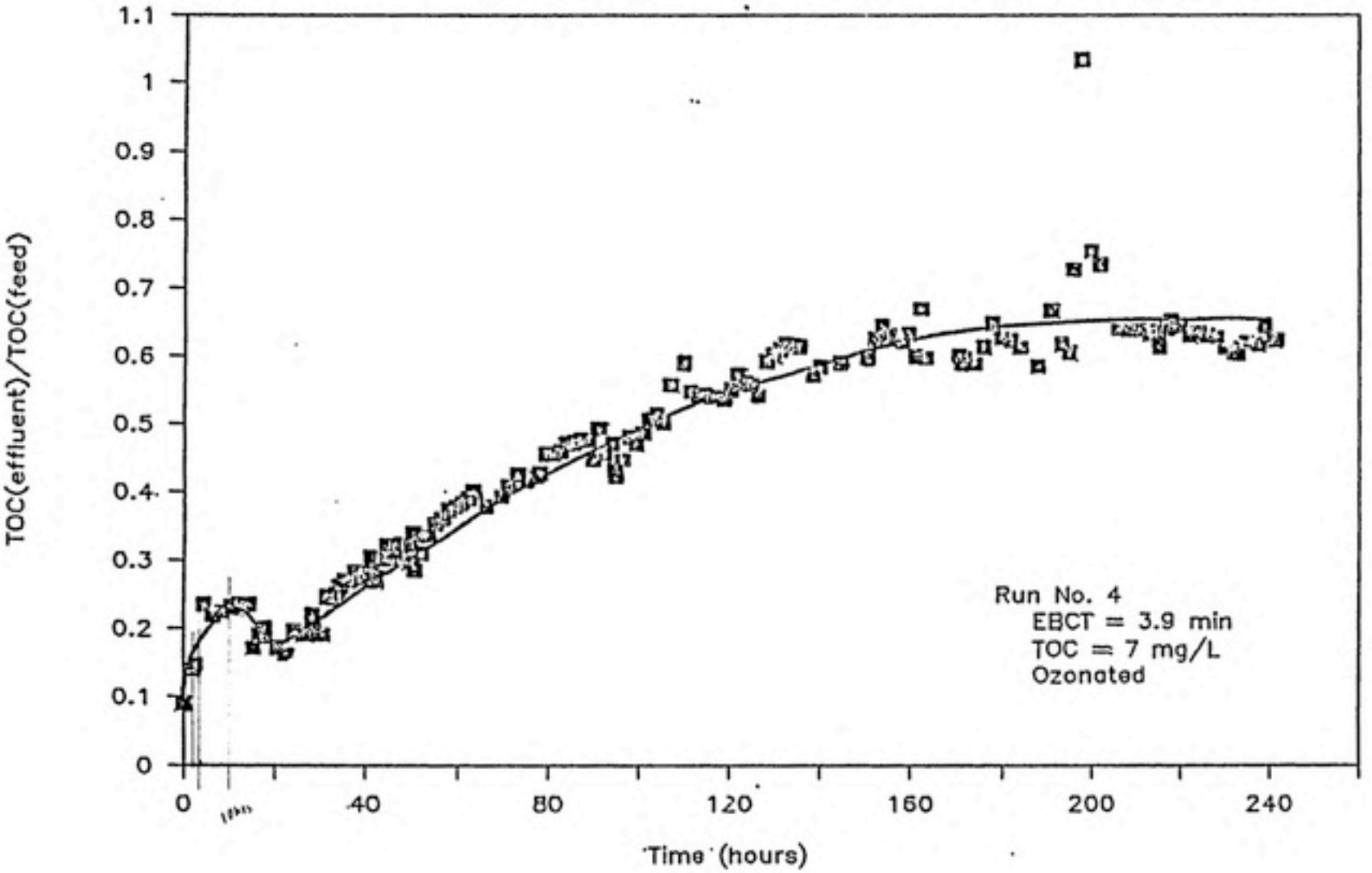



Figure 6-7. TOC Breakthrough Curve for Run No. 4: Ozonated Lake Drummond Water (1 mg $O_3$/mg TOC); TOC = 7 mg/L; EBCT = 3.9 min.

falls between the TOC reached by the hump in Run Nos. 2 and 3; in Run No. 4 the effluent TOC reaches about 25% of the feed. This suggests that the extent of HS biodegradation in Run No. 4 was greater than that in Run No. 3, but less than Run No. 2.

Figure 6-8 offers a more comprehensive description of the adsorption and biodegradation processes. Plotted together are concentrations of TOC, DOC, and $CO_2$ (in mgC/L) in the effluent stream for Run No. 4. Differences between TOC and DOC represent biomass leaving the reactor. For most of the run, the difference is very small. However, a sharp peak in TOC occurred at around 200 hours, while the DOC remained at its previous level. This implies that the loss of biofilm from the reactor is not a steady ongoing process, as is usually assumed in mathematical modeling. While this research is not aimed at modeling the results, others attempting to do so should beware of such complications. A further observation is that given the closeness of TOC and DOC during most of the breakthrough curve, either could be used to measure the concentration of HS.

Carbon dioxide in the effluent stream is an indication of microbial activity within the column. The production of $CO_2$ is due to mineralization of humic substances. Evidence for the presence of biodegradable/slightly adsorbable and non-biodegradable/adsorbable components is the leveling off of $CO_2$ concentrations after about 20 hours of operation; $CO_2$ production stabilizes within about the same operating time as is covered by the "hump" in effluent TOC. This means that biodegradation of the biodegradable/slightly adsorbable component has reached steady state while effluent TOC continues to rise, due to breakthrough of the non-biodegradable/adsorbable fraction in the HS mixture. The two steady states observed in the modeling exercise in Figure 6-5 are also noted here: a steady state with respect

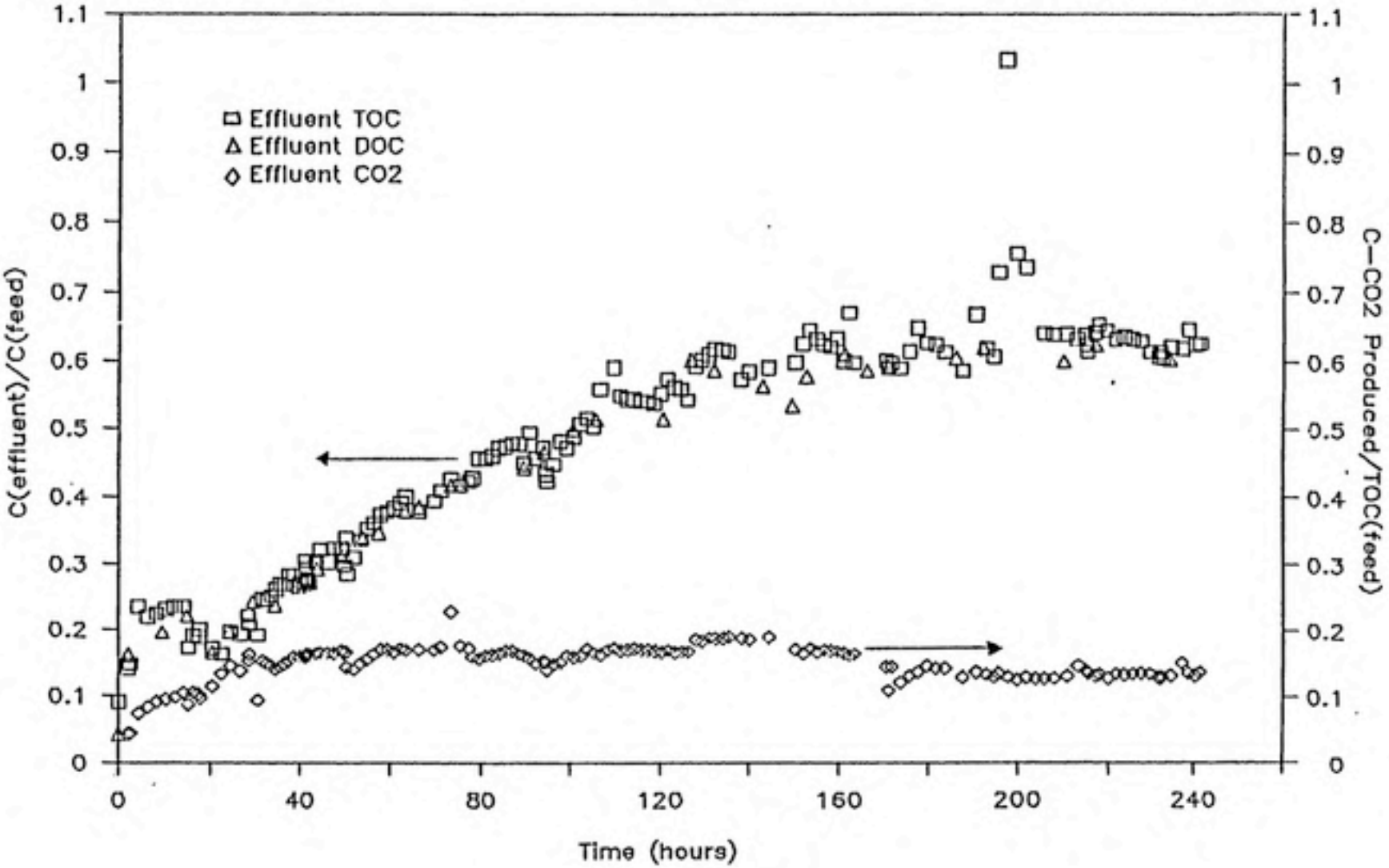



Figure 6-8. Breakthrough Curves for TOC and DOC, and $CO_2$ Production, in Run No. 4.

to biological activity, which is achieved quite early, and a steady state with respect to overall TOC removal, which is reached when the sorptive capacity of the GAC bed is exhausted.

An additional piece of information is provided by influent and effluent TOC and $CO_2$ concentrations at steady state (with respect to overall TOC removal). A material balance on carbon can be used to calculate the overall microbial yield coefficient, Y, which is equal to the carbon assimilated into cells per unit substrate carbon metabolized. For Run No. 4, the material balance is as follows:

| Feed | Effluent |
|---|---|
| TOC = 7.0 mgC/L | TOC = 4.0 mgC/L |
| $CO_2$ = 0.7 mgC/L | $CO_2$ = 1.3 mgC/L |
| Total Input = 7.7 mgC/L | Total Output = 5.3 mgC/L |

At steady state:

(Total C Input) - (Total C Output) = Carbon Assimilated in Reactor

Since, by definition, the sorptive capacity of GAC has been exhausted at steady state, the only means for assimilation of carbon in the reactor is through cellular uptake. Thus:

Carbon Assimilated into Cells = 7.7 mgC/L - 5.3 mgC/L = 2.4 mgC/L.

The Total Substrate metabolized is equal to the change in TOC across the reactor:

Total Substrate Metabolized = 7.0 - 4.0 = 3.0 mgC/L.

Finally, the yield coefficient (Y) can be calculated:

$$Y = (\text{Assimilated Carbon})/(\text{Total Carbon Metabolized})$$

$$= \frac{(2.4 \text{ mg/L biomass C})}{(3.0 \text{ mg/L substrate C})} = 0.8 \text{ mgC/mgC.}$$

### 6.2.3 Summary: Effect of EBCT and Feed Conditions on Steady State TOC Removal by Biodegradation

The results of the four column runs are summarized in Table 6-3. Comparing Run Nos. 1 and 2 shows that ozonation increased the biodegradability of the HS. Comparing Run Nos. 2 and 3 shows that decreasing the feed concentration of HS and the EBCT greatly reduced the extent of biodegradation; both can be reasoned intuitively to limit biological activity. Finally, a comparison of Run Nos. 3 and 4 shows that increasing the EBCT while holding the feed TOC concentration constant substantially increased the removal of HS by biodegradation.

The experimental design of Run No. 4 was considered the most successful; exhaustion of sorptive capacity for HS was observed in a reasonable time period (160 hours, or 6.5 days), and biodegradation could be measured conveniently without excessive head loss build-up. This became the prototype for the design of all further column experiments.

Table 6-3

Summarized Results: Column Run Numbers 1-4
GAC Reactors Receiving Humic Substances, Varying Feed Conditions and EBCT

| Run No. | Ozone dosage (mg $O_3$/mg TOC) | EBCT (min) | Feed TOC (mg/L) | Steady State Effluent TOC (mg/L) | % TOC Removed at Steady State |
|---|---|---|---|---|---|
| 1 | 0 | 41.6/13.9* | 25 | ** | ** |
| 2 | 1/1 | 13.9 | 25 | 7.5 | 70% |
| 3 | 1/1 | 1.3 | 7 | 6.6 | 5% |
| 4 | 1/1 | 3.9 | 7 | 4.0 | 43% |

* Flowrate in Run No. 1 was increased after 9 days of operation to 6 L/day.

** Steady state was never achieved in Run No. 1. After 380 hours of operation, Effluent TOC = 14 mg/L.

## 6.3 Biodegradation of Phenol in the Presence of Ozonated Humic Substances

### 6.3.1 Background

Run No. 4 established the best operating conditions for measuring adsorption and biodegradation of ozonated humic substances. The objective of this next phase of study was to measure the adsorption and biodegradation of trace levels of phenol (50 μg/L) in the presence of HS. Two questions were of particular interest:

1. Will phenol biodegradation be enhanced by the presence of ozonated HS? and
2. Will phenol be more readily biodegraded if introduced to a biofilm already developed from a feed of ozonated HS than if introduced simultaneously with HS from the beginning of reactor operation?

It is important to understand that model predictions of the interaction between adsorption and biodegradation always show that the development of a biofilm will not occur until substrate begins to accumulate outside the GAC particle, i.e., until sorptive capacity becomes limiting. This makes good sense for a single substrate like phenol: while adsorption is actively taking place, no substrate can accumulate and so the biofilm cannot grow. Dovantzis (1986) showed in his modeling work that biodegradation will not occur for several months if the feed concentration is 50 μg/L and the EBCT is 0.5 min. However, the introduction of another large supply substrate, such as ozonated HS, changes this notion. Now the biofilm can develop independent of the accumulation of phenol; the presence of this bulk-substrate-grown biofilm may alter the interactions between adsorption and biodegradation of the trace concentration of phenol.

### 6.3.2 Experimental Methods

Three columns runs were performed for this phase of research, referred to as Run Nos. 5, 6, and 7. Operating parameters (EBCT, mass of GAC, feed TOC) were identical to those of Run No. 4. Feed solution, however, was varied between runs:

Run No. 5: A 7 mg/L ozonated HS solution was fed to a GAC reactor for 210 hours before adding phenol at 50 μg/L.
Run No. 6: Both ozonated HS (7 mg/L) and phenol (50 μg/L) were fed simultaneously from the beginning of reactor operation.
Run No. 7: No HS were added to the feed; phenol at 50 μg/L was the only substrate provided.

Run No. 7 served as a "control" to determine if biofilm development would be slowed by eliminating the major supply substrate, i.e., the primary substrate. According to mathematical modeling of adsorption and biodegradation interactions, biological activity will be initiated only after substantial adsorption has occurred.

Reactor Design. The design is identical to that shown in Figure 6-1, with individual feed reservoirs for each reactor. Glass aspirator bottles (2L) were used to contain the daily supply of feed; the drip tube was eliminated to simplify column operation. In an effort to prevent microbial growth in the feed, reservoirs were replaced daily with cleaned, sterile bottles containing fresh feed solution. Effluent from each column was directed to a collection tube constructed from the tip-end of a narrow buret (total volume about 10 ml). These collection tubes were selected to minimize the loss of $CO_2$.

Feed Solutions. Feed solutions were prepared by diluting filter-sterilized nutrients and pH 6.2 buffer (filtered through a 0.2 μm Gelman triacetate filter into a sterile erlenmeyer flask) into sterile distilled deionized water. Nutrient and buffer compositions are given in Table 6-4. Ozonated humic substances were added to produce a feed TOC of approximately 7 mg/L, for Run Nos. 5 and 6; no HS were used in Run No. 7. Radiolabeled phenol was added from a stock solution, prepared in ozonated, distilled, deionized water at pH 2 (specific activity = 3,812 dpm/μL; concentration = 190.6 μg/L); the final feed concentration was 50 μg/L.

Analytical and Sampling Methodology. The HS concentration was measured by TOC with an O.I. Model 700 Total Organic Carbon Analyzer. It was also possible to measure total $CO_2$ production with the same instrument. TOC and $CO_2$ measurements are due to adsorption and biodegradation of both HS and phenol. However, the HS are present in the feed at 7 mg/L, compared to phenol at 50 μg/L. For all intents, therefore, TOC and $CO_2$ measurements reflect adsorption and biodegradation of just the ozonated humic substances.

Effluent carbon-14 radioactivity was attributed to radiolabeled phenol being fed to the reactor. This radioactivity is found in three fractions: total radioactivity, nonpurgeable radioactivity, and nonpurgeable·filterable radioactivity. The meaning of each fraction and the procedure by which each was obtained are explained below.

Total radioactivity was measured from five ml samples of effluent collected with a volumetric pipet from the collection tube for each column. Each sample was added to a scintillation vial containing two drops of Carbosorb II

Table 6-4

Nutrient and Buffer Compositions

| Constituent | Concentration (mg/L) |
|---|---|
| **Phenol Buffer** | |
| $KH_2PO_4$ | 65.0 |
| $K_2HPO_4$ | 215.0 |
| $Na_2HPO_4$ | 250.0 |
| **Nutrients** | |
| $CaCl_2 \cdot 2H_2O$ | 15.0 |
| $MgSO_4$ | 5.0 |
| $NH_4Cl$ | 3.5 |

(6003073, United Technologies, Packard Instrument Company, Downers Grove, Illinois). Total radioactivity consists of $^{14}CO_2$, $^{14}C$·biomass, $^{14}C$·substrate, and perhaps $^{14}C$·metabolic end-products.

The nonpurgeable and the nonpurgeable·filterable radioactivity were measured from approximately 20 ml effluent samples collected dropwise into a 40 ml Pierce vial. The samples were acidified to pH 2 with concentrated HCl and purged five minutes with nitrogen gas to drive off $^{14}CO_2$, thus leaving behind the nonpurgeable fraction. After removing a five ml aliquot of this nonpurgeable fraction for $^{14}C$ counting, the residual solution in the Pierce vial was filtered through a 0.2 μm Gelman triacetate filter which had been presoaked in 1 mg/L phenol to saturate binding sites. The filtrate, which excludes all radioactivity due to $^{14}CO_2$ and $^{14}C$·biomass, constitutes the nonpurgeable·filterable fraction. A five ml aliquot of this fraction was collected for $^{14}C$ counting. Scintiverse II counting cocktail (10 ml) was added to each scintillation vial prior to counting. Samples were assayed for radioactivity with a Packard Tri-Carb 300 CD Liquid Scintillation Counter.

Carbosorb II is an organic amine designed to absorb carbon dioxide for the purpose of scintillation counting; the capacity for $CO_2$ absorption is 211 mg $CO_2$/ml (Chang, 1985). Control experiments with $Ba^{14}CO_3$ indicate that the efficiency (E) of this procedure for $CO_2$ quantification is approximately 88%. The acidification and 5-minute purge was found to remove over 99% of the $^{14}CO_2$ from solution.

### 6.3.3 Data Analysis

The potential components of each of the three radioactive fractions are as follow:

$$^{14}C\cdot Total = {}^{14}CO_2 + {}^{14}C\cdot biomass + {}^{14}C\cdot substrate + {}^{14}C\cdot metabolic\ end\text{-}products$$

$$^{14}C\cdot Nonpurgeable = {}^{14}C\cdot biomass + {}^{14}C\cdot substrate + {}^{14}C\cdot metabolic\ end\text{-}products$$

$$^{14}C\cdot Nonpurgeable\cdot filterable = {}^{14}C\cdot substrate + {}^{14}C\cdot metabolic\ end\text{-}products.$$

Metabolic end-products have been ignored in this analysis, since it has been found that phenol biodegradation yields only about 2% conversion of carbon to end-products and that these are strongly adsorbed onto GAC (Schultz, 1982). Thus, the concentration of end-products in the effluent stream should be negligible and the $^{14}C\cdot nonpurgeable\cdot filterable$ fraction is equivalent to $^{14}C\cdot substrate$.

This makes the calculation of $^{14}CO_2$, $^{14}C\cdot cells$, and $^{14}C\cdot substrate$ quite simple:

$$^{14}CO_2 = (^{14}C\cdot total - {}^{14}C\cdot nonpurgeable)/E \quad \text{where } E = 88\%.$$

$$^{14}C\cdot Cells = {}^{14}C\cdot nonpurgeable - {}^{14}C\cdot nonpurgeable\cdot filterable.$$

$$^{14}C\cdot Substrate = {}^{14}C\cdot nonpurgeable\cdot filterable.$$

All of the above radiolabeled quantities refer only to phenol added in the reactor feed. We are particularly

interested in the concentration of $^{14}CO_2$, because it represents the phenol that is biodegraded in the GAC bed.

### 6.3.4 Results

Effluent TOC and total $CO_2$ production in Run Nos. 5 and 6 are shown in Figure 6-9. Effluent TOC is taken to represent HS escaping adsorption and/or biodegradation; total $CO_2$ production is caused by biodegradation of HS. The TOC breakthrough curve is similar to that observed in Run Nos. 2-4 in which only HS were added. The rise and fall of TOC during the initial stages of operation, i.e., the "hump", is quite apparent. We also note that $CO_2$ production stabilized after the "hump" is observed. This, as noted before, means that the concentration of biodegradable organics in the effluent stream was constant. The continued rise in effluent TOC is due to breakthrough of the adsorbable/non-biodegradable HS fraction. Steady state was reached after about 200 hours of operation, with approximately 40% of the applied TOC being biodegraded.

Phenol mineralization in Run Nos. 5-7, as measured by the production of $^{14}CO_2$, is presented as a function of operating time in Figure 6-10. The time scale has been shifted for Run No. 5, so that time zero corresponds to the point at which phenol addition commenced, which was actually 210 hours into the run.

The delay in $^{14}CO_2$ production for Run Nos. 5 and 6, which received ozonated HS in addition to phenol, was very brief compared to that for Run No. 7 (the control), which received only phenol at 50 µg/L. In addition, biological activity was substantially greater in Run Nos. 5 and 6 than in Run No. 7. Of the total $^{14}C$-substrate added to each reactor in Run Nos. 5 and 6, 60 to 70% was being converted

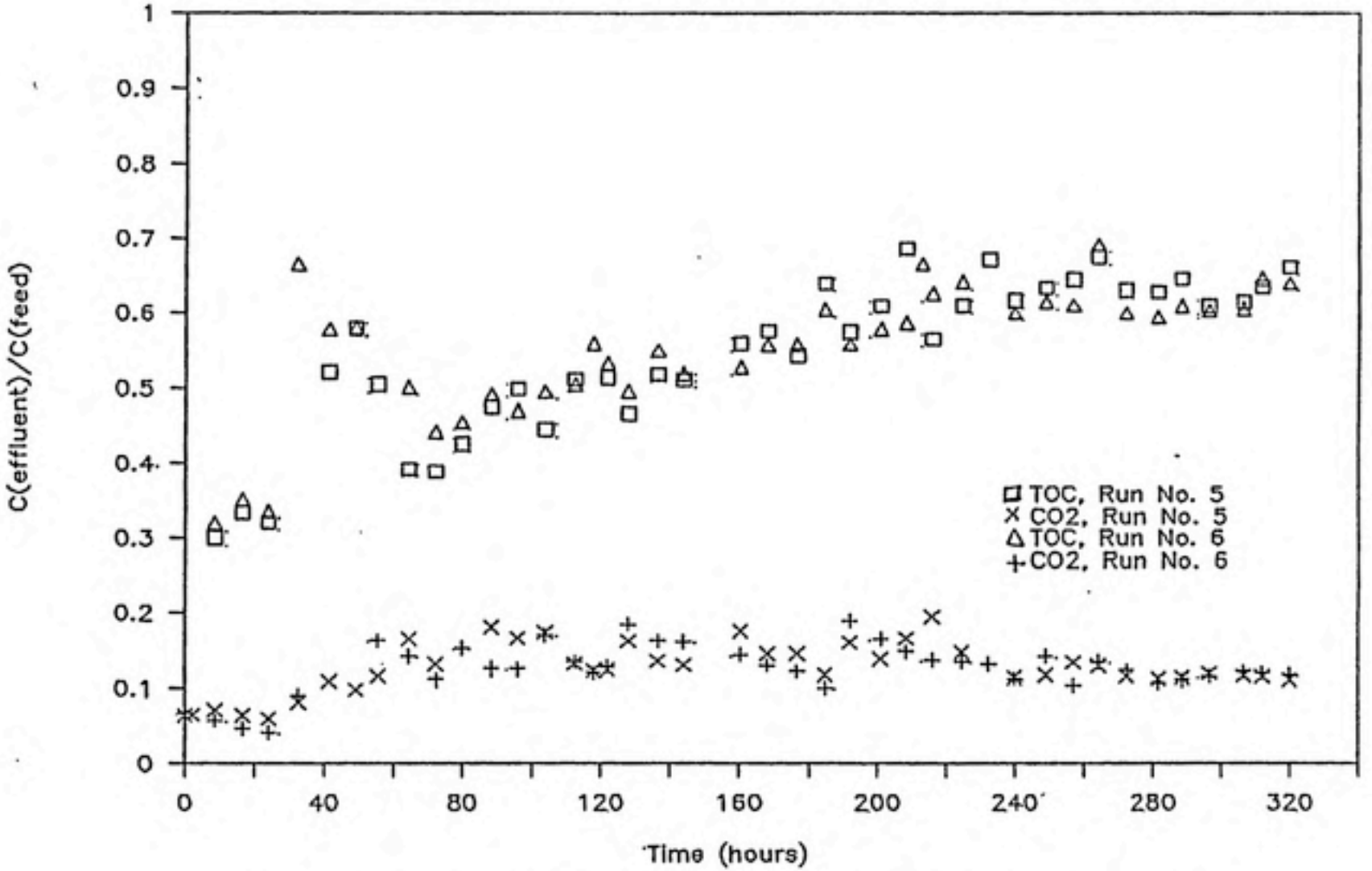



Figure 6-9. TOC Breakthrough Curves and $CO_2$ Production for Run No. 5 (HS from t=0, Phenol from t=210 hrs) and Run No. 6 (HS and Phenol from t=0): TOC = 7 mg/L; EBCT = 3.9 min.

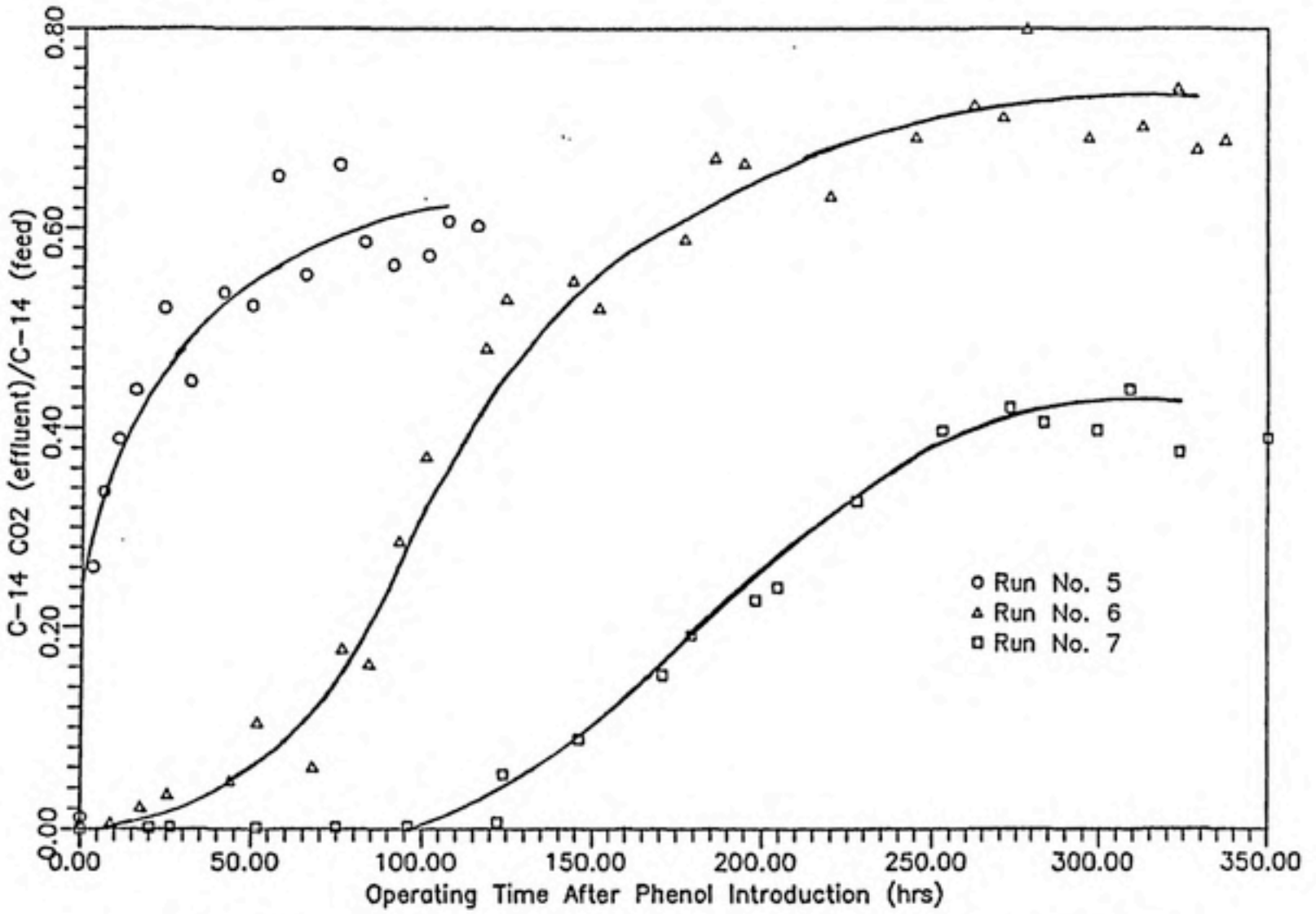



Figure 6-10. $^{14}CO_2$ Production (Phenol Biodegradation) in Run Nos. 5 (Phenol from 210 hrs), 6 (Phenol from t=0), and 7 (Phenol from t=0, no HS).

to $^{14}CO_2$ after 140 and 350 hours, respectively (Figure 6-10). Conversion of $^{14}C$·substrate to $^{14}CO_2$ in Run No. 7, on the other hand, seemed to level off at about 40%. Biological activity was not expected in Run No. 7 because the development of a biofilm should have been delayed until much of the sorptive capacity had been used and phenol began to accumulate around the GAC particle. One possible explanation for phenol biodegradation here is that the system became contaminated with microbial seed, which stimulated biological activity.

The time at which phenol biodegradation began in Run No. 6 (phenol and HS introduced simultaneously) coincides with the achievement of stable biofilm activity, as depicted by total $CO_2$ production in the column (Figure 6-9). In contrast, adding phenol to a GAC column with an established biofilm population (Run No. 5) resulted in immediate phenol degradation, as evidenced by $^{14}CO_2$ production. Thus, these results demonstrate the importance of the primary substrate (HS) in establishing microbial activity.

The shearing loss of biomass ($^{14}C$·cells) produced specifically by biodegradation of radiolabeled phenol is shown in Figure 6-11, for Run Nos. 5-7. Our earlier studies of biodegradation of HS suggested that biofilm losses occurred intermittently, rather than continuously (page 125). The same conclusion is reached here for biomass grown on phenol. In addition, we can see that the loss of $^{14}C$ labeled cells began much earlier for Run No. 5, in which phenol was added to an established biofilm. This is reasonable given the observation that biodegradation, and thus biomass growth, began earlier for this run. In contrast, biomass losses from the control, Run No. 7, were insignificant compared to Run Nos. 5 and 6 until about 330 hours of operation. This agrees with the observation that

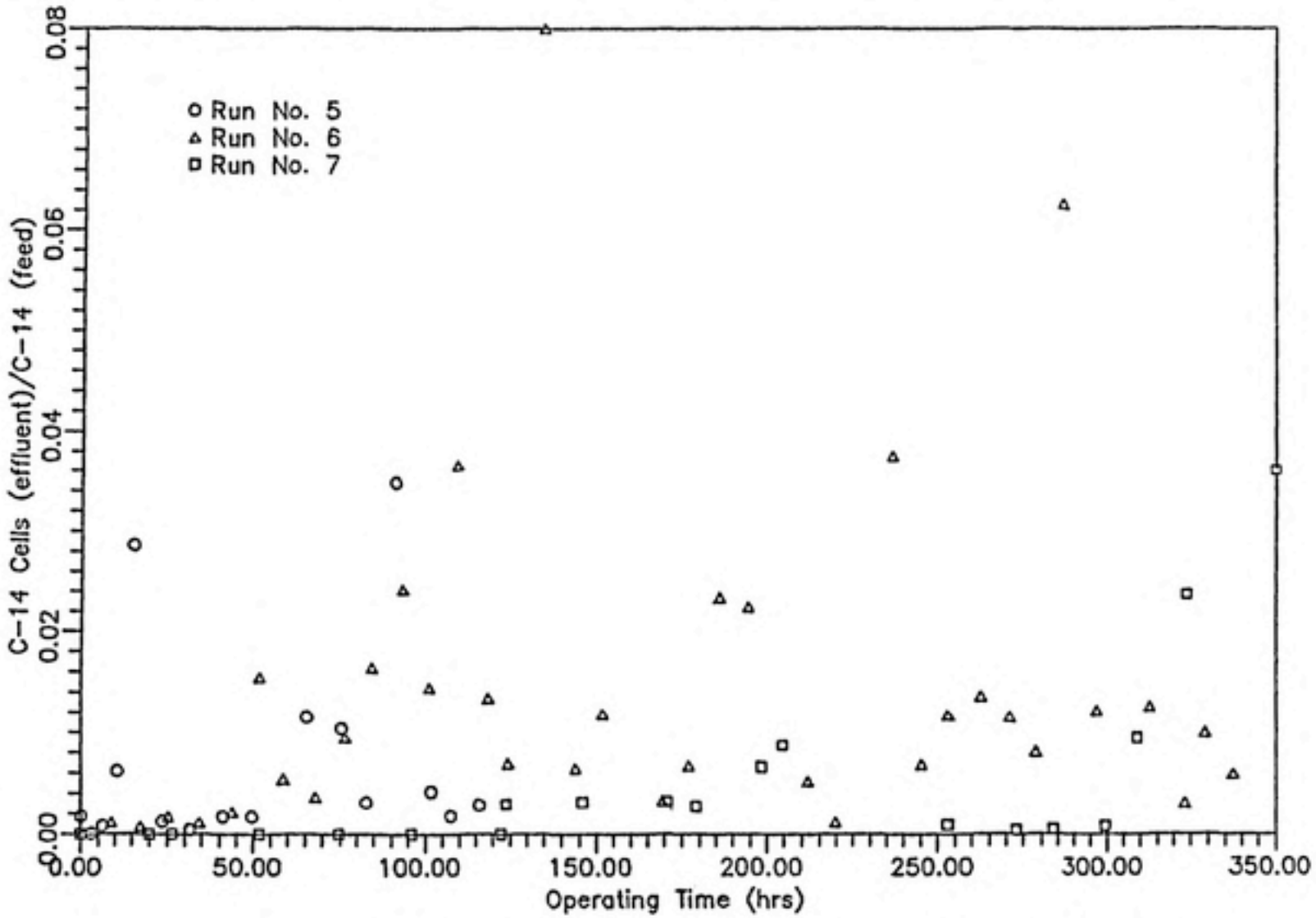



Figure 6-11. Shearing Loss of $^{14}$C-Labeled Cells in Run Nos. 5 (Phenol from 210 hrs), 6 (Phenol from t=0), and 7 (Phenol from t=0, no HS).

biomass growth, which obviously must precede biomass shear, was not as great as in Run Nos. 5 and 6.

Phenol concentration leaving the reactors, as measured by $^{14}C$·substrate, was negligible throughout all three runs. This means that phenol was either being adsorbed or biodegraded effectively throughout the time of observation. The pathway for phenol removal - adsorption vs. biodegradation - can be determined from the measurement of radiolabeled fractions. Plots of cumulative phenol biodegraded vs. phenol applied to the reactor for Run Nos. 5-7 are shown in Figure 6-12. The cumulative amount of phenol biodegraded has been calculated using $^{14}CO_2$ production, taking into account microbial yield:

$$\text{Cumulative biodegradation} = \frac{\text{Total } ^{14}CO_2 \text{ produced}}{(1-Y)}.$$

A straight line with slope equal to one would result if all the phenol applied were biodegraded. A slope less than one implies some adsorption (since no phenol left the column in solution); a slope greater than one is possible only if phenol is biodegraded from both sorbed and solution phases.

The value of the microbial yield coefficient will have a direct influence on the slope of a plot of cumulative phenol biodegraded vs. phenol applied. A value of 0.46 μg cell·carbon/μg phenol·carbon was used to prepare Figure 6-12, which is the yield coefficient determined in the biokinetic studies for the phenol-acclimated population (Chapter 5). Although the variability of this value is quite large (see Page 89), it is in reasonable agreement with prior work done in this laboratory, which determined a value of 0.48 μg cell·carbon/μg phenol·carbon (Speitel, 1985).

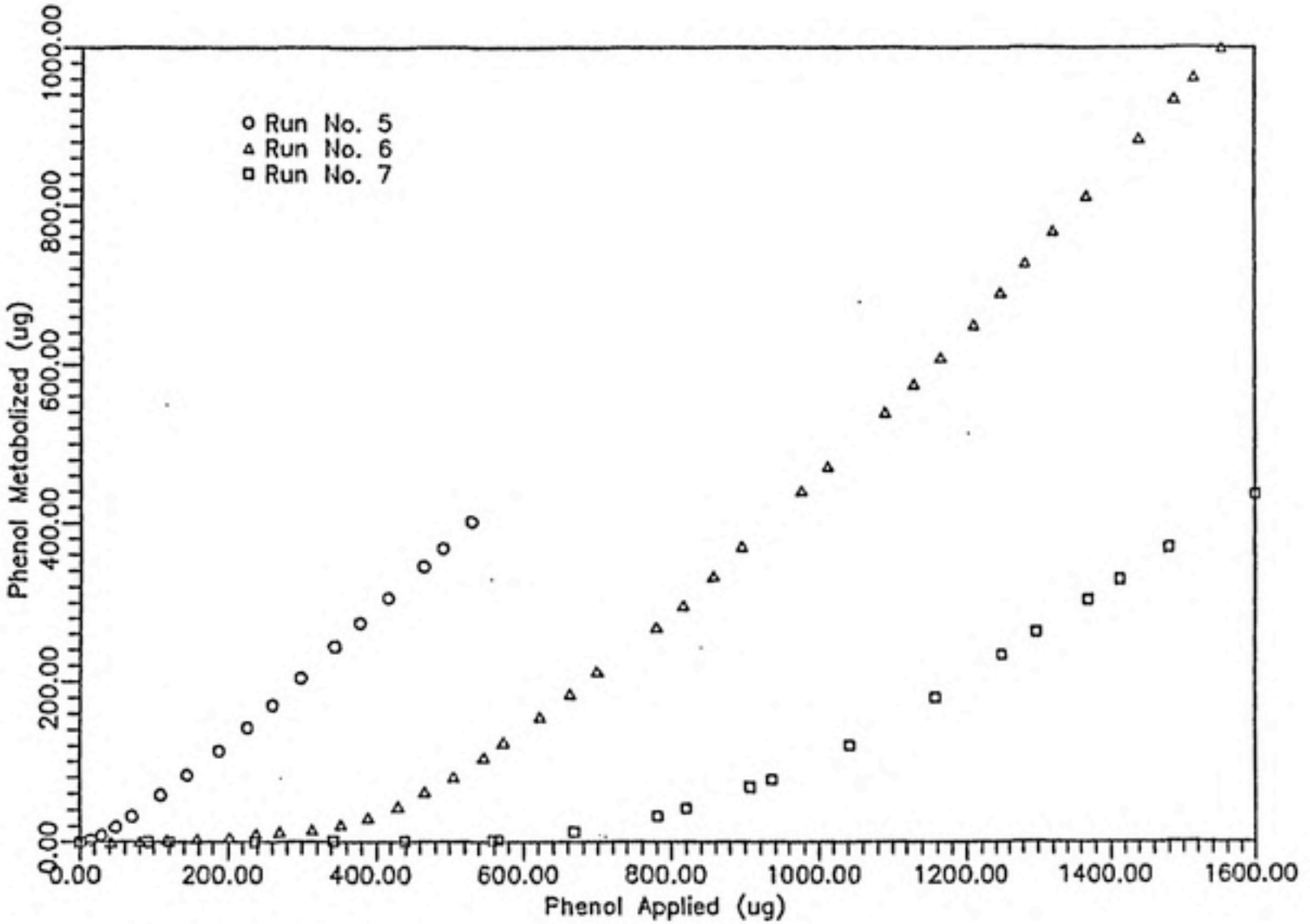



Figure 6-12. Total Phenol Metabolized vs. Total Phenol Applied, in Run Nos. 5 (Phenol from 210 hrs), 6 (Phenol from t=0), and 7 (Phenol from t=0, no HS).

### 6.3.5 Discussion

According to mathematical models of the interaction between adsorption and biodegradation for a single substrate supply, substrate must first accumulate around the GAC particle before a biofilm can develop. Thus, adsorption will delay biofilm development until sorptive sites become depleted. Aside from this explanation of delay, the phenomenon of acclimation, which cannot be included easily in a mathematical model, may also be operative.

The delay in $^{14}CO_2$ production observed in this research is extremely brief compared to what would be expected from a mathematical model. For example, Dovantzis (1986) simulated the response of a packed GAC bed having an EBCT of only 0.5 min to a feed of 50 µg/L phenol. His model results showed that adsorption dominated for the first few months of operation. The fact that a longer EBCT (3.9 min) was used in this research would suggest an even longer delay before the onset of biological activity. Therefore, the results shown in Figure 6-10 are very interesting because they show that when ozonated HS are available as primary substrate, phenol biodegradation begins within a few days (60 hours) at most, regardless of when the phenol is introduced to the system.

Similar results have been demonstrated for a purely biological system. Namkung and Rittmann (1987) showed that phenol was readily degraded at concentrations as low as 1 µg/L by an established biofilm grown in glass bead reactors which received HS at 1 mg/L as TOC. Approximately 10% of the HS were degraded at steady state; 89.6% of the applied phenol was removed by the biofilm.

Speitel et al. (1987) ran columns of virgin GAC which received 5 µg/L phenol in the presence of unozonated humic

substances at about 4 mg/L as TOC. The removal of phenol after EBCT of 0.2 and 1.4 minutes was compared. Unlike our study in which phenol biodegradation occurred after three days, biological activity (as evidenced by $^{14}CO_2$ production) was not detected until 30 days of column operation. The major difference, however, was the lack of HS biodegradation noted by Speitel et al.. That is, complete breakthrough of the humic substances occurred almost immediately at an EBCT of 0.2 minutes, and after three weeks at an EBCT of 1.4 minutes. This means that there was no biodegradation of HS and, therefore, that phenol served as the sole electron donor for the biofilm microorganisms. In our system, biological activity was encouraged by ozonating the humic substances, as well as by providing a longer empty bed contact time (3.9 minutes). We have shown, earlier in this chapter, that ozonating the humic substances enhances their susceptibility to biodegradation, and that increasing the EBCT from 1.3 minutes to 3.9 minutes increases the percent degraded at steady state from 5% to 43%.

The presence of these ozonated humic substances, then, enhances biodegradation of a low concentration of phenol. How does this enhancement effect change with operating time? When added from start-up, prior to the development of significant biological activity, phenol is adsorbed and accumulated on the GAC surface. As the biofilm becomes established, phenol degradation proceeds. The onset of phenol degradation coincides with achievement of a stable HS biofilm population, as evidenced by $CO_2$ production (Figure 6-9). It appears that phenol is degraded during these early stages as it diffuses through the biofilm toward the GAC surface. The immediate biodegradation of phenol when added to a GAC column with an established biofilm supports the idea that the phenol is degraded on its way through the biofilm from the liquid phase.

The implication of this finding is that predictions of microbial activity by mathematical models which describe interactions between adsorption and biodegradation must very carefully consider all sources of substrate that can account for biofilm development. In this case, phenol biodegradation would not have been predicted in months of operation if the HS were ignored as a substrate source.

Distinct differences in phenol removal pathways are apparent from inspection of Figure 6-12. The slope of each plot increases with column operating time as more phenol is applied. With a yield coefficient of 0.46 μg cell·carbon/μg phenol·carbon, however, the slope in no case exceeds 1.0. This is expected because a slope of 1.0 would indicate biodegradation as the sole pathway for removal. More importantly, the slope observed in Run No. 5 increases at a much faster rate than in Run No. 6. This is because the biofilm was first established with ozonated HS for 210 hours before introducing phenol and, thus, phenol biodegradation very soon became nearly 100%. In other words, phenol was not adsorbed even though sorptive capacity existed. By contrast, a very shallow slope was observed until a considerable amount of phenol was applied in Run No. 6. This suggests that adsorption, rather than biodegradation, occurred in the early stages of the run. We expect this result because the phenol and HS were introduced from the beginning of Run No. 6; biodegradation of phenol could not occur until the biofilm first became established, through biodegradation of ozonated HS.

The slope for Run No. 7, which received no additional substrate besides phenol at 50 μg/L, is much shallower than the other two runs which received ozonated HS. This means that without the HS, biological activity is substantially less and adsorption becomes the predominant pathway for phenol removal.

As more phenol is applied, the slope in Figure 6-12 gradually increases, resulting from an increase in biological activity. The slope for Run No. 5 approaches 1 (μg phenol metabolized/μg phenol applied) by the end of the run, indicating that all phenol applied to the column was being biodegraded at this point. The slope for Run No. 6 reached 0.9 μg/μg, and probably would have approached 1 had the run continued. Thus we see that, with extended operation, differences in phenol removal pathways become less apparent and the behavior of both systems receiving ozonated HS approaches that of a purely biological reactor. However, the fact that the slope in Run No. 7 increases to only 0.56 μg/μg, indicates that even after 350 hours of operation, a substantial portion of the applied phenol was removed through adsorption.

# 7. DEVELOPMENT OF A BIOFILM ON OZONATED HUMIC SUBSTANCES

## 7.1 Experimental Methods

An experiment was designed to investigate changes in biofilm characteristics from reactor start-up until steady state removal of ozonated HS was achieved. Changes in biofilm density were monitored, as well as changes in the ability of the indigenous community to mineralize phenol.

Column Operation. Four column reactors were operated in parallel, each receiving ozonated HS at a concentration of approximately 7 mg/L TOC and a feed flowrate of 2 L/day (EBCT = 3.9 min). Each reactor contained approximately 4 grams (dry weight) of GAC. The columns, referred to here as A, B, C, and D, were dissassembled in succession on Days 4 (t = 84 hours), 7 (t = 157 hours), 10 (t = 230 hours) and 15 (t = 349 hours), respectively, for microbial analyses.

Analytical Methods. Effluent DOC was measured for each reactor. These samples were obtained by vacuum filtration through 0.2 μm Gelman triacetate filters which had been rinsed with distilled deionized water and approximately 20 ml of sample. DOC was analyzed with an O.I. Model 700 Total Organic Carbon analyzer. Carbon dioxide concentration was measured in unfiltered effluent samples.

Biofilm Removal. Activated carbon was removed aseptically from each reactor and subject to the wash procedure described in Chapter 4. Columns A and B (t = 84 and 157

hours, respectively) were treated with ultrasonication; the original procedure utilizing a Waring blender was used for Columns C and D (t = 230 and 349 hours, respectively). The change in procedure, from ultrasonication to blending, was made to avoid disrupting the integrity of the cells.

Determination of Microbial Densities. Total cell densities recovered from the GAC samples were determined using a modified Acridine Orange Direct Count procedure, as outlined in Chapter 4 (Hobbie et al., 1977). Viable cells were enumerated with the plate count procedure, as described in Chapter 5.

Phenol Mineralization. The metabolic rate of phenol biodegradation was studied using biofilm microorganisms recovered from each GAC sample by the procedures described above. Our purpose was to compare phenol utilization rates at different stages of biofilm development. We therefore measured only respiration rates; cellular uptake was not measured. Since respiration is proportional to total metabolism, this measurement was sufficient for comparison purposes.

The experimental procedure for metabolic uptake study parallels that described in Chapter 5. A series of seven phenol concentrations were tested, ranging from 1 to 1,000 µg/L; samples were incubated for approximately 10 hours, headspace free at room temperature in the dark. For each of the four microbial samples, four live and two dead vials were prepared at each phenol concentration. Five additional vials were incubated with $Ba^{14}CO_3$ to determine the efficiency of $^{14}CO_2$ recovery.

## 7.2 Results and Discussion

The DOC breakthrough curves for each of the four columns, A-D, are presented in Figure 7-1. These curves should be identical because each reactor received the same DOC feed concentration and had the same EBCT. The slight differences noted are due, most likely, to variations in the actual weight of GAC in each reactor and possibly to differences in head loss through the columns. The differences seem to be most noticeable during early stages of operation. While it could be argued that these difference would lessen as the microbial community stabilizes, the number of reactors in operation also decreases according to the schedule of removing them for recovery of biofilm.

Also shown in Figure 7-1 is the production of $CO_2$ in Column D. These data are similar to those presented for earlier reactor experiments (Figures 6-8 and 6-9), showing the attainment of a steady state in biological activity after about 50 hours of operation while effluent TOC continues to rise due to further depletion of sorptive capacity for the non-biodegradable fraction.

The dry weight of GAC, total days of operation, and percent breakthrough of TOC upon disassembly of each reactor are presented in Table 7-1. The extent of HS biodegradation at steady state is seen to be about 33% and is in general agreement with results of previous experiments described with similar operating conditions.

### 7.2.1 Cell Densities

Total and viable cell densities recovered from each reactor are presented in Table 7-2. Due to a change in removal methods used between the first two and the second

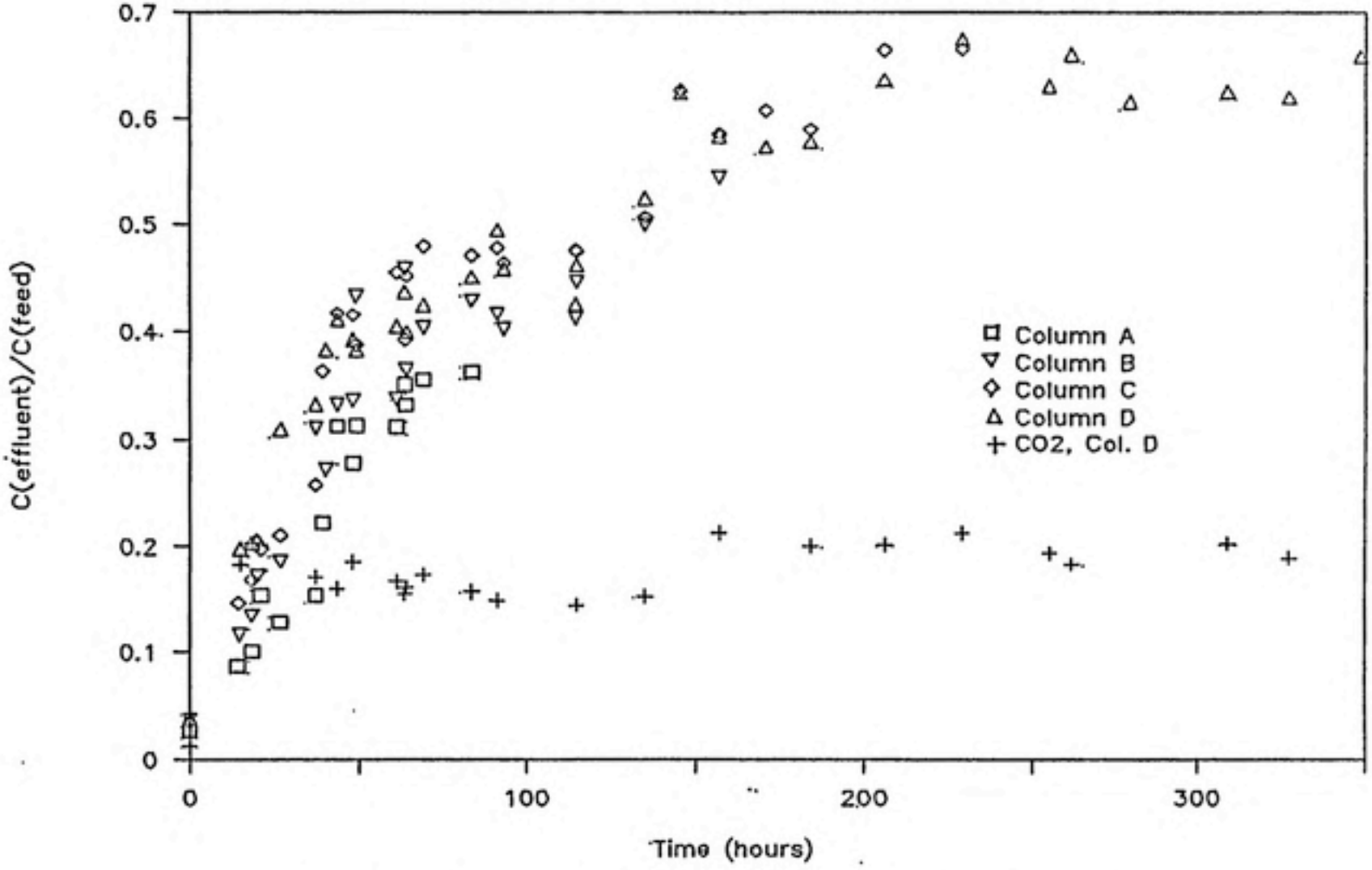



Figure 7-1. DOC Breakthrough Curves and $CO_2$ Production for Columns used in Biofilm Development Study: TOC = 7 mg/L; EBCT = 3.9 min.

Table 7-1

Operational Information: GAC Column Reactors for Biofilm Development Study

| Column | Carbon Dry Wt. (gm) | Total Hours (days) of Operation | % HS Breakthrough on Termination |
|---|---|---|---|
| A | 3.64 | 84 (3.5) | 36% |
| B | 3.63 | 157 (6.5) | 58% |
| C | 3.17 | 230 (9.6) | 67% |
| D | 3.46 | 349 (14.5) | 67% |

Table 7-2

Total and Viable Cell Densities Recovered from Column Reactors A-D

| Column | Removal Method[a] | Operating Time (hrs) | Cell Densities (cells/gm GAC) Total | Std. Dev. | Viable | Std. Dev. | % Viable/Total |
|---|---|---|---|---|---|---|---|
| A | (1) | 84 | 1.13E+09 | 1.93E+08 | 4.67E+06 | 2.20E+06 | 0.41% |
| B | (1) | 157 | 1.90E+09 | 1.02E+08 | 2.29E+07 | 9.61E+06 | 1.21% |
| C | (2) | 230 | 1.02E+09 | 2.80E+08 | 6.70E+06 | 2.34E+06 | 0.66% |
| D | (2) | 349 | 1.89E+09 | 8.71E+07 | 7.39E+06 | 6.92E+06 | 0.13% |

[a]Removal Method (1) = Ultrasonication
(2) = Blending

two samples, we are only able to use these data to compare cell densities between 84 and 157 hours, and between 230 and 349 hours, and not among all four samples. As discussed in Chapter 4, we suspect that ultrasonication removes a higher percentage of total cells and possibly a higher percentage of viable cells from the carbon surface; thus the switch from ultrasonication back to blending, between samples obtained from Columns B and C, may have resulted in the recovery of a lower percentage of cells from the surface of the last two samples.

The increase in total cells between an operating time of 84 and 157 hours was 67% (1.13E+09 to 1.90E+09 cells/gm), with a corresponding increase in viable cells of 390% (4.67E+06 to 2.29E+07 cells/gm). Between 230 and 349 hours of operation, total cell density increased by about 86% (1.02E+09 to 1.89E+09 cells/gm), while the density of viable cells in the column decreased by 64% (6.70E+06 to 2.39E+06 cells/gm). These cell densities are comparable to those observed in Run No. 2 of Chapter 6 (4.75E+09 cells/gm), being slightly lower due to the lower feed TOC and shorter EBCT.

These data also reveal some interesting trends in the ratio of viable cells to total cells during reactor operation. Between 84 and 157 hours, we see that the percentage of viable cells increased from 0.41% to 1.21%. The percentage increases if cell growth exceeds the accumulation of decaying or dead cells. In contrast, between 230 and 349 hours the percentage of viable cells decreased from 0.66% to 0.13%. Not only did the percentage decrease, but the viable cell count itself decreased while the total cells increased. Therefore, later in reactor operation decaying cells accumulated within the biofilm and resulted in an increased biomass density without a corresponding increase in viable cells.

The 64% decrease in viable cell count between 230 and 349 hours is questionable. A drop in the density of viable cells should only result from some perturbation to the system (change in pH, temperature, feed conditions, oxygen availability) which may limit microbial growth. Reduced growth, hence reduced substrate utilization, should reflect an increase in effluent TOC due to an increase in the concentration of biodegradable/slightly adsorbable fraction of HS (or a decrease in $CO_2$ production due to less activity). Neither change was observed; consequently, the noted drop in viable cells may be an artifact of the experimental procedure. This could have resulted from either a high count at t = 230 hours, or a low count at t = 349 hours.

Despite variations in total and viable cell densities with operating time, $CO_2$ production (Figure 7-1) reached a steady state within about one day from start-up. Thus, the amount of HS mineralized by the biofilm microorganisms was constant after this point, even though the density of viable cells was increasing. The rapid attainment of steady state $CO_2$ production despite further changes in cell population density may be explained using the concept of a "critical film thickness" (Trulear & Characklis, 1982). According to the theory, substrate removal rates increase with an increase in biofilm thickness until a critical thickness is reached, beyond which removal remains constant. This critical thickness refers to the depth to which substrate diffuses into the biofilm. Once the biofilm thickness exceeds the depth of substrate penetration, as determined by bulk substrate concentration and substrate utilization rates, biodegradation rates will be unaffected by additional cells which may accumulate on the surface of the film.

### 7.2.2 Metabolic Activity: Phenol Mineralization

Figures 7-2 and 7-3 show the results of a metabolic rate study performed with each biofilm sample, indicating the rate of phenol respiration as a function of substrate concentration. Figure 7-2 expresses the respiration rates per gram of GAC in the reactor from which the microbial sample was obtained. This is useful in comparing the potential for phenol utilization across the GAC bed at different stages of operation. In Figure 7-3, the rates are expressed per viable cell in the microbial sample. This normalizes for differences in the percentage of viable cells (and, hence, metabolic activity) recovered from the GAC surface by the two different methods used (blending vs. ultrasonication).

A significant reduction in metabolic activity between samples recovered with ultrasonication (t = 84 and 157 hours) and those recovered by blending (t = 230 and 349 hours) is evident in Figure 7-2. This suggests that ultrasonication may recover a higher percentage of metabolic activity from the biofilm. As a result, this figure is useful only for making comparisons of phenol utilization rates per gram of GAC between 84 and 157 hours, and between 230 and 349 hours, and not among all four samples.

Figure 7-2 suggests that an increase in the ability of the microbial community to mineralize phenol occurs during both the initial (from 84 to 157 hours) and final stages (from 230 to 349 hours) of column operation. The rate of phenol metabolism per mass of GAC within the bed should vary depending on how long the column has been in operation before the phenol is added - the rate being faster with a later addition. This is what we would expect from our study of phenol utilization in the presence of ozonated HS presented in Chapter 6. As the bed operates, a biofilm

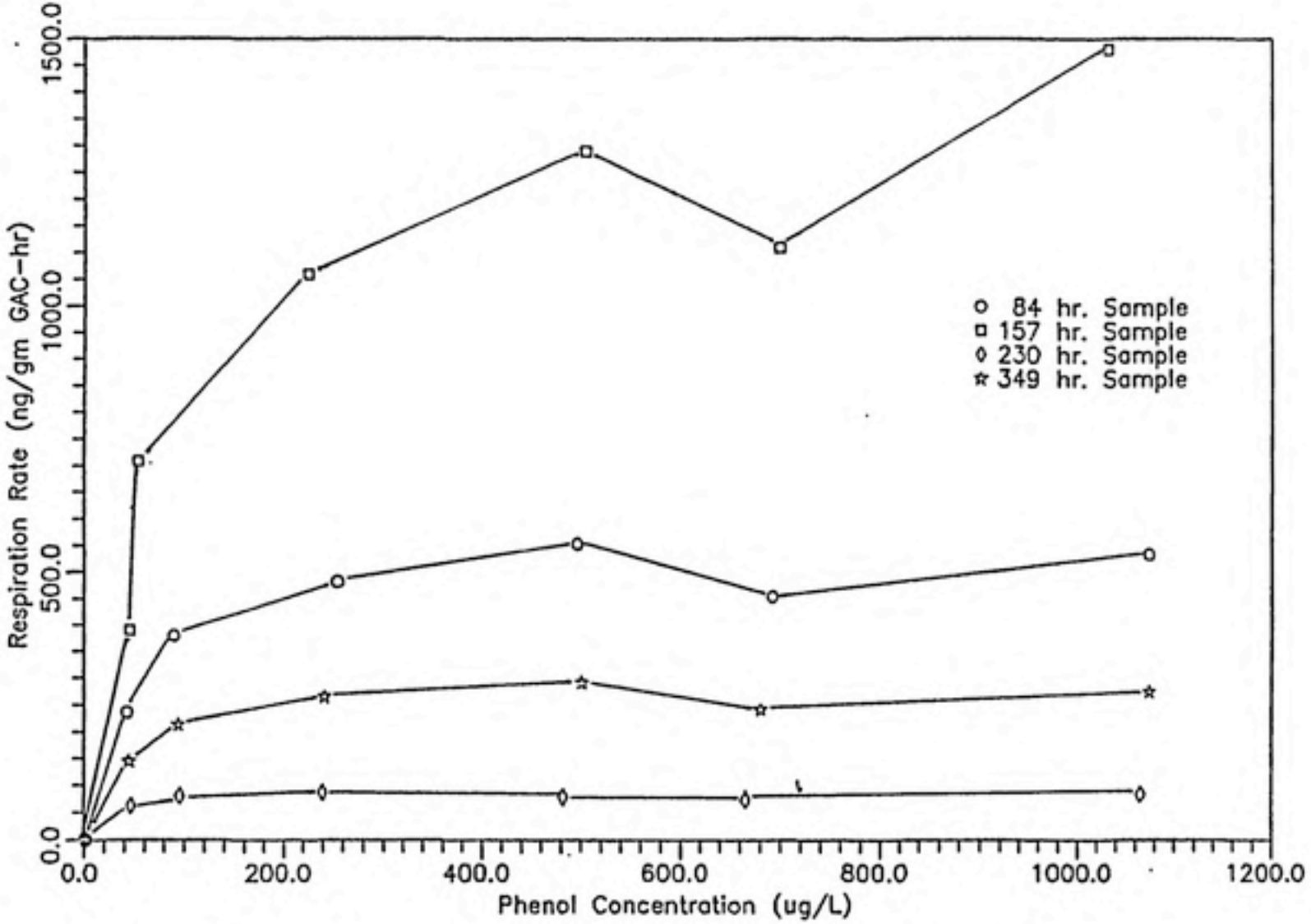



Figure 7-2. Phenol Respiration Rate vs. Substrate Concentration, by Microbial Samples Recovered from a Humic Substances-Grown Biofilm at Different Stages of Development: Rates Expressed per gm. GAC.

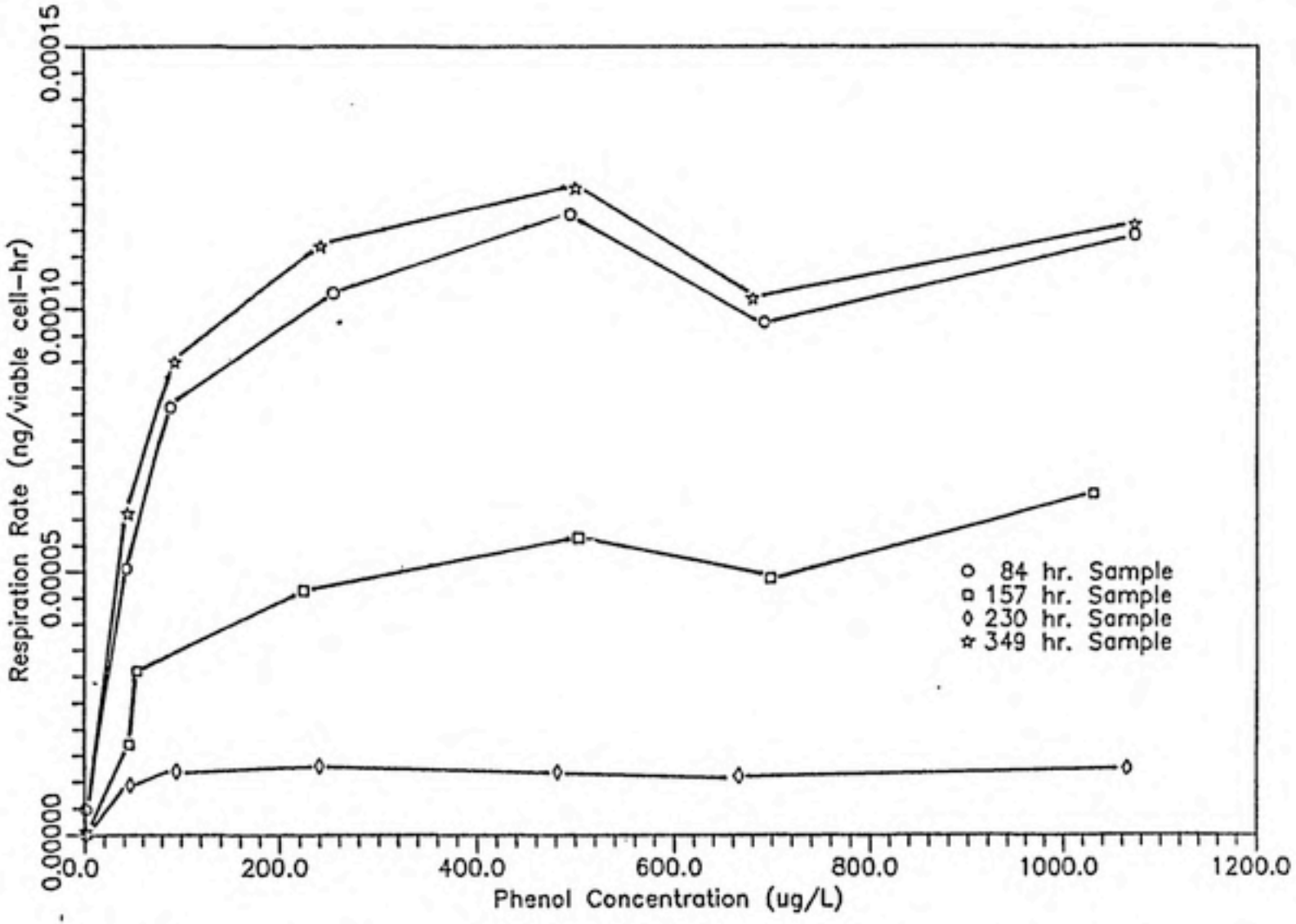



Figure 7-3. Phenol Respiration Rate vs. Substrate Concentration, by Microbial Samples Recovered from a Humic Substances-Grown Biofilm at Different Stages of Development: Rates Expressed per Viable Cell.

develops and the chances for micropollutant degradation increase. However, the increase in phenol utilization rate from 230 to 349 hours was not accompanied by an increase in viable cells. As discussed above, there may have been a problem with the viable cell plate count for either the 230 or the 349 hour sample. The increase in substrate utilization rates should reflect an increase in biomass density in the GAC bed.

The respiration rates normalized for viable cell density are shown in Figure 7-3. Presented in this way, the data suggest that unit metabolic activity per cell, with respect to phenol mineralization, decreases with operating time for the first three samples (up to t = 230 hours). The anomaly in results obtained with the 349 hour sample are unexplainable, and reinforce our suspicion in the viable cell plate count. A low count for this sample would inflate the respiration rates per cell.

This drop in phenol mineralization rate per viable cell with operating time is similar to the behavior noted with respect to HS biodegradation, where constant HS mineralization was observed (as depicted by steady state $CO_2$ production in Figure 7-1) despite an increase in the density of viable cells. We suggested the concept of a critical biofilm thickness beyond which substrate cannot diffuse as an explanation. However, no biofilm exists in studies of phenol metabolism because the cells were removed from the GAC in order to conduct the investigation. It may be that the cells which exhibited lower unit activity in the biofilm (with respect to HS degradation) retained this reduced activity even after being released from the film into a liquid suspension. The behavior is similar in character to a log-phase growth curve. Activity is high in the early stages of growth, but decreases as the community develops and less colonization sites are available. This would

explain the slowed metabolism. On the other hand, the decrease in unit activity may reflect increased competition in samples with a higher biomass density. At this point, we can only speculate. More evidence is needed for a conclusive explanation.

## 7.3 Summary

This biofilm development study suggest that although viable cell density continues to increase during the initial stage of column operation (up to at least 157 hours, or 6.5 days), the rate of humics mineralization in the column stabilizes after only one day of operation. A decrease in unit activity per cell might be explained using the concept of a critical biofilm thickness, at which point the film thickness is equivalent to the depth of substrate penetration (Trulear & Characklis, 1982). Once this critical thickness is reached, substrate removal rates will be unaffected by additional cells. The critical biofilm thickness for a reactor operating under conditions used here is reached during the first day of operation.

A decrease in unit activity per cell with column operating time was also noted during phenol mineralization. Biofilm microorganisms, when recovered from the film into a liquid solution, exhibit decreased unit activity per cell as the biofilm develops. Although mineralization rates per cell decrease, there is an increase in total phenol mineralization rates (hence, total activity in the GAC bed with respect to phenol degradation) with operating time during both the initial and later stages of filter operation.

# 8. CONCLUSIONS AND RECOMMENDATIONS

## 8.1 Conclusions

1. Viable cells can be desorbed from the surface of colonized GAC using a solution of polyvinyl pyrrolidone (PVP) and sodium pyrophosphate (PPi). Successive 30 minute washes on a rotary shaker at 180 rpm in PVP/PPi, separated by blending and slow-speed centrifugation (120xg), recovered approximately 50% of the total cells from the biofilm into a liquid supernatant. The amount of metabolic activity recovered cannot be determined.

Microorganisms recovered from a biofilm using this PVP/PPi wash procedure can be used in biokinetic studies. Results of a study which measured the kinetics of phenol degradation using biomass samples recovered from a GAC bed indicate that biokinetic parameters obtained using biofilm microorganisms are comparable to literature reported values, obtained with suspended cultures.

2. Differences in phenol metabolism between an exposed and an unexposed population of biofilm microorganisms reflect a change in response due to pre-exposure. The biodegradation rate was noted not to be a continuous function of substrate concentration for the exposed population, i.e., a sudden jump occurred at around 100 µg/L Phenol. In addition, pre-exposure resulted in a higher proportion of the metabolized phenol being respired to $CO_2$.

3. Ozonated humic substances are capable of supporting biofilm growth in a GAC reactor. The extent to which biological activity develops depends on:

   1. Pretreatment of HS: ozonation at a dose of 1 mg $O_3$/mg TOC enhanced biological growth.
   2. EBCT: increasing the EBCT resulted in a larger percent TOC removal by biodegradation.
   3. Feed HS Concentration: a higher feed TOC concentration encouraged biofilm growth.

4. A hump which occurred very early in the breakthrough curve for ozonated HS suggests that the organics may be comprised of two components: one which is biodegradable and only slightly adsorbable, and another which is adsorbable but non-biodegradable. The effluent concentration of the biodegradable/slightly adsorbable component rapidly increased after reactor start-up, but then decreased again due to biodegradation. In contrast, the non-biodegradable/adsorbable component steadily increased due to exhaustion of sorptive capacity. Carbon dioxide production stabilized within about the same operating time as was covered by the hump in effluent TOC, meaning that a steady state with respect to biological activity was achieved quite early in the run.

5. The density of viable cells within the laboratory-scale GAC reactor continued to increase despite the attainment of steady state $CO_2$ production. Microorganisms recovered from a GAC bed at different stages of operation exhibited a reduced unit metabolic activity per cell with respect to phenol mineralization, as the biofilm developed.

6. The delay in $^{14}CO_2$ production in a GAC reactor (EBCT = 3.9 min) after adding carbon-14 labeled phenol, at 50 µg/L, was very brief when ozonated HS were provided (at 7 mg/L). When phenol and HS were added simultaneously from start-up,

the commencement of $^{14}CO_2$ production coincided with the achievement of stable biofilm activity, as evidenced by steady state $CO_2$ production (presumably from HS biodegradation). Adding phenol to an established biofilm resulted in immediate phenol biodegradation.

In either case, phenol biodegradation began within 60 hours. By 300 hours (12 days) the pathway for phenol removal across the GAC bed was predominantly through biodegradation. The implications of these results are that mathematical models which describe interactions between adsorption and biodegradation must very carefully consider all sources of substrate which can account for biofilm development. By way of example, a typical mathematical model result given by Dovantzis (1986) showed that no biodegradation occurs within the first few months of operation if the feed is phenol at 50 µg/L and the EBCT is 0.5 min. The presence of a much larger, less adsorbable substrate source, such as ozonated HS, allows much more rapid biofilm development and subsequently more rapid biodegradation of phenol.

## 8.2 Recommendations

1. The fixed-bed recycle reactor design of this research should be used further to test model predictions utilizing the theory of a 2-component HS mixture (biodegradable/slightly adsorbable and non-biodegradable/adsorbable components).

2. The study should be repeated utilizing a compound which is less susceptible to biodegradation than phenol; substituted phenols like P-nitrophenol should be considered.

3. The design of the fixed-bed recycle reactor should be modified to obtain more information. A shorter EBCT would

be helpful to enable observation of micropollutant breakthrough, yet the EBCT must be sufficient for HS biodegradation. Differences between the behavior of ozonated HS and of trace micropollutants in a GAC bed pose difficulties to the design of a system which will enable observation of adsorption and biodegradation of both components. Thought should be given to amending the design for further study of micropollutant adsorption and biodegradation.

4. With refinement, the techniques presented here for desorption of biofilm microorganisms could provide a means for investigating biological activity associated with an operating GAC filter. The methods would enable an assessment of the potential for contaminant biodegradation in a particular GAC bed, by performing biokinetic studies with the microbial community indigenous to the filter. Further refinement should focus on removing carbon fines from the supernatant into which biomass is recovered, and also on developing a means for relating results back to the GAC sample. A method is needed for determining the percent recovery of biologically active cells from the biofilm into the liquid suspension.

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