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Two-tank suspended growth process for accelerating the detoxification kinetics of hydrocarbons requiring initial monooxygenation reactions

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Abstract

An experimental evaluation demonstrated that suspended growth systems operated in a two-tank *accelerator/aerator* configuration significantly increased the overall removal rates for phenol and 2,4-dichlorophenol (2,4-DCP), aromatic hydrocarbons that require initial monooxygenations. The accelerator tank is a small volume that receives the influent and recycled biomass. It has a high ratio of electron donor (BOD) to electron acceptor (O_2). Biomass in the accelerator should be enriched in reduced nicotinamide adenine dinucleotide (NADH + H^+) and have a very high specific growth rate, conditions that should accelerate the kinetics of monooxygenation reactions. For the more slowly degraded 2,4-DCP, the average percentage removal increased from 74% to 93%, even though the volume of the two-tank system was smaller than that of the one-tank system in most experiments. The average volumetric and biomass-specific removal rates increased by 50% and 100%, respectively, in the two-tank system, compared to a one-tank system. The greatest enhancement in 2,4-DCP removal occurred when the accelerator tank comprised approximately 20% of the system volume. Biomass in the accelerator tank was significantly enriched in NADH + H^+ when its dissolved oxygen (DO) concentration was below 0.25 mg/L, a situation having a high ratio of donor to acceptor. The accelerator biomass had its highest NADH + H^+ content for the experiments that had the highest rate of 2,4-DCP removal. Biomass in the accelerator also had a much higher specific growth rate than in the aerator or the system overall, and the specific growth rate in the accelerator was inversely correlated to the accelerator volume.

Introduction

For many hydrocarbons, the initial rate-limiting step in their biodegradation is catalyzed by a monooxygenase enzyme and requires two cosubstrates: molecular oxygen (O_2) and a reduced electron carrier, such as reduced nicotinamide adenine dinucleotide NADH + H^+ (Gottschalk 1986; Rittmann et al. 1994; Rittmann & McCarty 2001; Sáez & Rittmann 1993). In principle, the kinetics for the transformation of a slowly biodegraded hydrocarbon can be accelerated if the intracellular concentrations are increased for the monooxygenase and/or either cosubstrate.

The objective of this research is to evaluate a means to accelerate the rate of monooxygenation reactions of slowly degraded hydrocarbons while also maintaining good BOD removal in a suspended growth reactor system. In this paper, we describe an experimental program used to evaluate a novel two-tank suspended growth system, illustrated in Figure 1, that we hypothesize can achieve our objective. Wastewater and recycled sludge enter a small *accelerator tank* that is designed to accelerate monooxygenation kinetics by increasing the cells' specific growth rate and intracellular level of reduced electron carrier. This is accomplished by the accelerator tank's small volume, high BOD loading, and carefully poised DO

concentration. Mixed liquor then flows to the *aerator tank*, which has a normal DO concentration for a suspended growth system and is used to ensure complete BOD removal, biomass synthesis, and a proper solids retention time (SRT) (Rittmann & McCarty 2001; Grady et al. 1999).

We present and interpret results showing that the two-tank system can significantly increase the removal of 2,4-DCP. A companion paper (Dahlen and Rittmann, companion) provides a mechanistic explanation for why the two-tank system is successful.

Background

Monooxygenation and phenols

Biological transformations of hydrocarbons can proceed rapidly once the microorganisms have made an "investment" of electrons to convert a low-reactivity compound into a more reactive species (Rittmann et al. 1994). Subsequent oxidation of the more reactive species allows the cells to gain electrons and carbon, thus replenishing the electron investment. The classic example is a monooxygenation reaction, in which a monooxygenase enzyme catalyzes the investment of two electrons from NADH + H^+ to insert one oxygen atom from O_2 into the substrate (Hayaishi 1969). The benefit is that a carbon-hydrogen bond is substituted by a carbon-oxygen bond (Nishinaga et al. 1988), which converts the reduced, low-reactivity compound into a more oxidized, reactive, and soluble product (Tung et al. 1992).

Figure 2 shows the first two steps for the transformation of phenol, one of the substrates used in this study and a good example of monooxygenation. In the first step, catalysis by a monooxygenase results in the insertion of one oxygen atom of O_2 into the hydrocarbon, oxidizing it by two electron equivalents to form an activated hydrocarbon, catechol (Gottschal et al. 1981). The second oxygen atom in O_2 is reduced by two electrons (from NADH + H^+) to form H_2O. Catechol is further broken down by ortho-cleavage of the aromatic ring (Rittmann et al. 1994) through the action of a dioxygenase enzyme that inserts both oxygens to form the dicarboxylic acid, *cis*, *cis*-muconic acid. Subsequent steps lead to tricarboxylic acid (TCA) cycle intermediates (Madigan et al. 1997; Rittmann & McCarty 2001). It is the electron-releasing oxidation of the intermediates that regenerates the reduced electron carriers invested in the monooxygenation step (Woo & Rittmann 2000; Van Briesen 2001).

Oxygenation reactions also are important initial steps in the biodegradation of hydrocarbons substituted with halogens. Similar to phenol biodegradation, the initial step in the biodegradation is catalysis by a monooxygenase to form a catechol (Spain & Gibson 1991; Doong & Wu 1992; Steiert & Crawford 1985; Reineke 1984). Figure 3 shows the biodegradation of 2,4-DCP, the halogenated substrate in this research. Monooxygenation products are similar to those of phenol degradation, but differ in two key ways. First, one chlorine substituent is removed as HCl. Second, four electrons, needed to reduce both oxygen molecules in O_2, must come from two NADH + H^+ molecules, because the carbon from which the chlorine leaves does not change oxidation state. Chlorocatechol is subsequently degraded via an ortho-cleavage pathway, releasing the second chloride during further metabolism of the intermediates. Further breakdown of lactone leads to TCA intermediates and regeneration of the NADH + H^+ consumed in the monooxygenation.

The monooxygenase enzyme used to degrade 2,4-DCP probably is the same as the monooxygenase used for phenol. The presence of the electron-withdrawing halogens reduces the electron density in the aromatic ring and causes the rate of attack by the monooxygenase to be slower (Knackmuss 1981; Schwarzenbach et al. 1993). However, the enzymes that are used for the chlorocatechol – namely, the dioxygenase, cycloiomerase II, and the hydrolase – are different and tend to prefer chlorinated to nonchloriated substrates (Valo et al. 1984). They are induced by growth in the presence of the chlorinated substrates, but not by their nonhalogenated analogs (Reineke 1984). Thus, only a subset of microorganisms capable of mineralizing phenol have the enzymes to carry out the mineralization of 2,4-DCP (Joshi & Walia 1996).

When microorganisms are able to mineralize 2,4-DCP, they are able to gain electrons, energy, and carbon to support biomass synthesis. Dahlen and Rittmann (2000), using a chemostat and a mixed culture enriched on a mixture of acetate, phenol, and 2,4-DCP, found that all three substrates were utilized simultaneously and supported synthesis. The true yield values (Y, in g volatile suspended solids per g chemical oxygen demand) for each substrate were proportional to the number of electrons removed in nonoxygenation reactions. The kinetics for 2,4-DCP were much slower than for phenol, and the main reason was that the half-maximum-rate coefficient (K_s, in mg chemical oxygen demand per liter) for 2,4-DCP

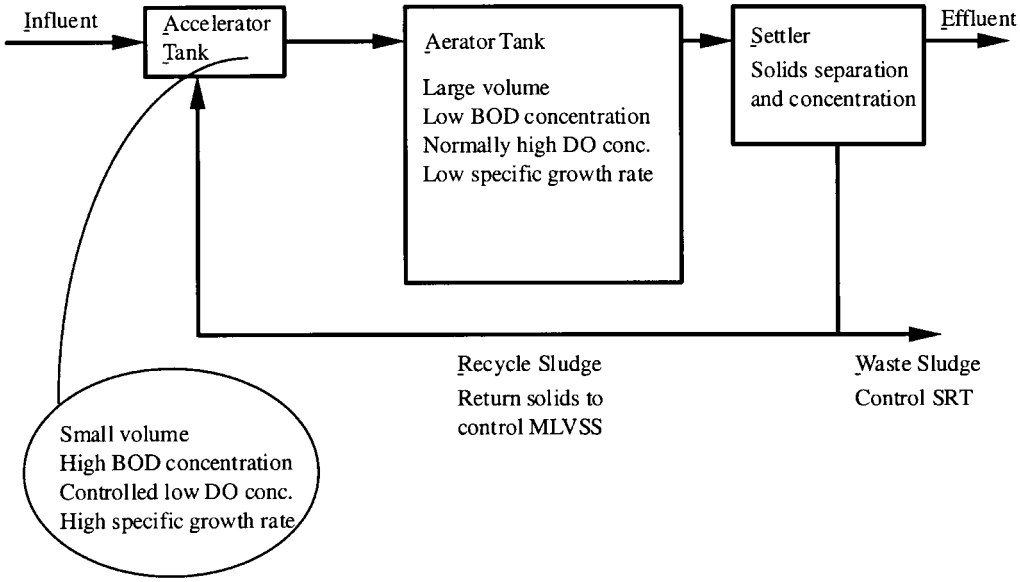

Figure 1. Schematic of the two-tank system to accelerate detoxification involving initial monooxygenation reactions.

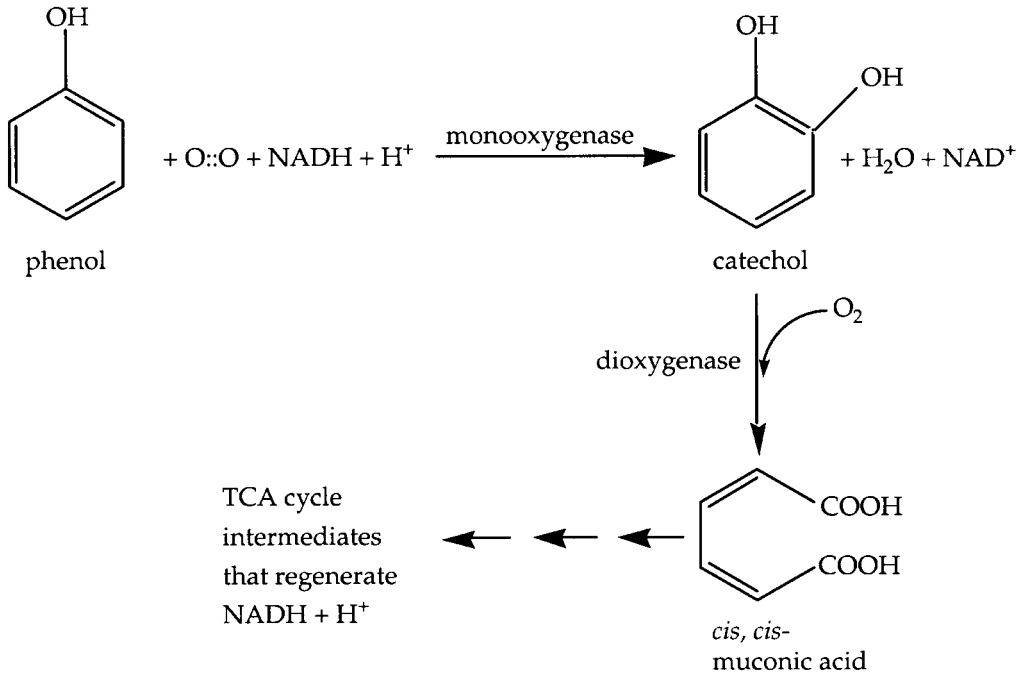

Figure 2. Initial oxygenation reactions for phenol.

was 19 times larger. Thus, 2,4-DCP, phenol, and acetate comprised a multi-component primary substrate (Namkung & Rittmann 1987a, b), but each substrate has unique yield and utilization parameters.

Effects of dissolved oxygen and intracellular reduced cofactors

DO plays two roles when oxygenations are involved, as illustrated in Figures 2 and 3. The first role, as the primary electron acceptor, is necessary for respiration and energy generation (Madigan et al. 1997; Rittmann

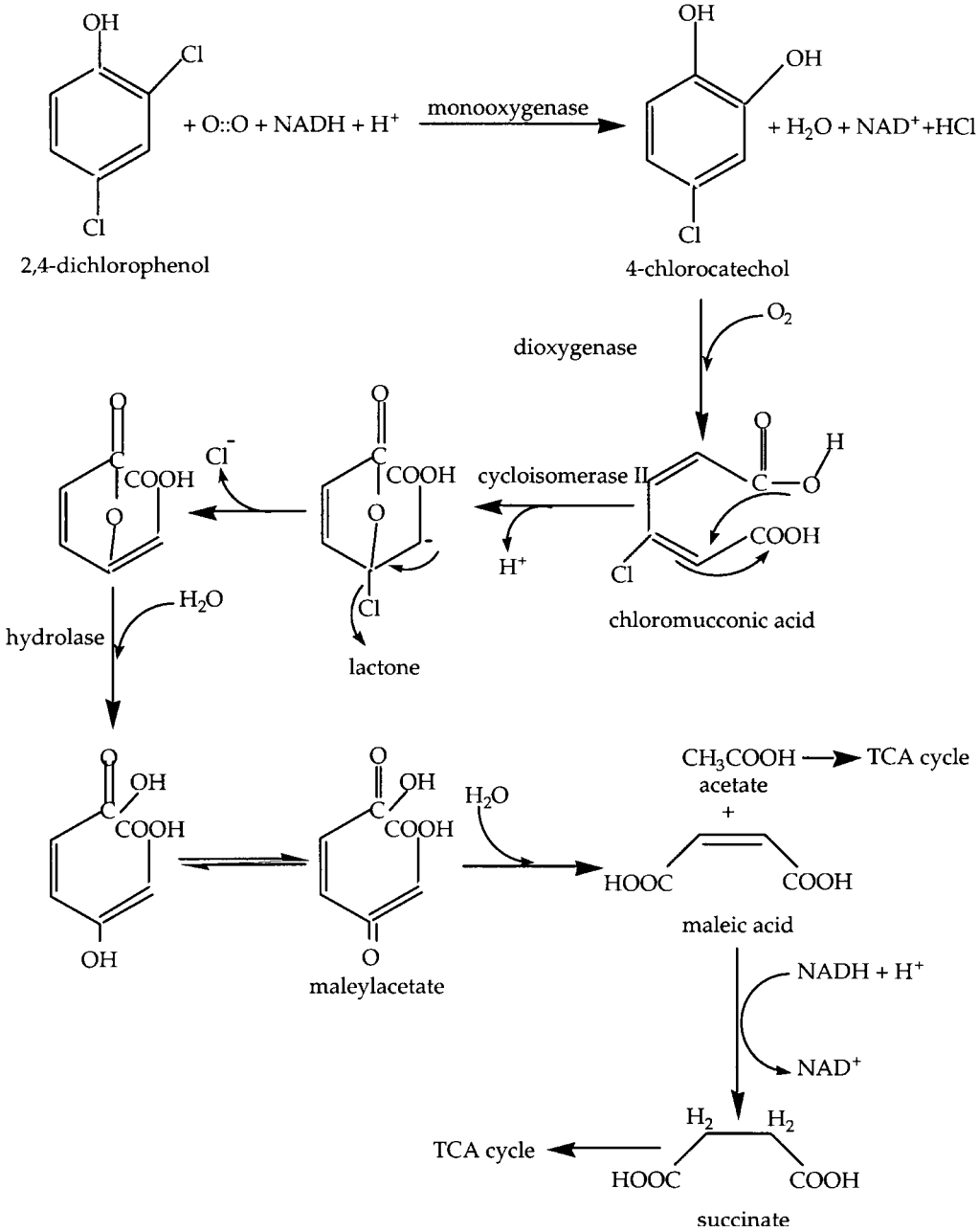

Figure 3. Initial degradation reactions for 2,4-dichlorophenol (adapted from Rittmann et al. 1994).

& McCarty 2001). DO's second role is as a direct cosubstrate in critical activation reactions (Rittmann et al. 1994).

In systematic studies, Bae and Rittmann (1996a, b) documented that the intracellular NADH + H^+ concentration can be increased by manipulating the concentrations of externally supplied primary electron donor and acceptor substrates. Such manipulations should have direct effects on the rates of monooxygenation, since NADH + H^+ is a co-substrate. On the other hand, reducing the DO concentration in order to increase the intracellular level of NADH + H^+ has a limit, because O_2 also is a co-substrate for the monooxygenation reaction.

Past work suggests that increasing the intracellular concentration of NADH + H^+ can significantly

increase rates of reactions that require NADH + H^+ as a co-substrate: For example, Sáez & Rittmann (1993) saw very large increases in the rate of biotransformation of 4-chlorophenol when phenol was being oxidized as a primary donor substrate. In addition, Loh & Wang (1998) enhanced the biodegradation rates of phenol and 4-chlorophenol by *Pseudomonas putida* ATCC 49451 by augmenting the medium with additional electron-donating sources, such as sodium glutamate and glucose.

Enzyme regulation by specific growth rate

Past work suggests that the production of catabolic enzymes, including monooxygenases, is up-regulated by a high specific growth rate. Bally et al. (1994) studied nitrilotriacetic acid (NTA)-monooxygenase regulation in the NTA-*Chelatobacter heintzii* system. They showed that enzyme activity and expression levels were proportional to specific rates of growth and NTA degradation. Baloo & Ramkrishna (1991) analyzed a multi- substrate system (glucose and xylose) and demonstrated that cells synthesized xylose- degrading enzymes faster when the specific growth rate was increased by a shift in the dilution rate. Xu et al. (1994) demonstrated that, as the specific growth rate of *E. coli* YMC21 increased, so did the concentration of glutamine synthetase, a key enzyme in nitrogen metabolism. Fleming et al. (1993) measured catabolic gene expression in PAH-contaminated soils. They quantified the levels of mRNA for the NAH7 napthalene dioxygenase (*nahA*) gene, which encodes for enzymes necessary for the degradation of naphthalene (Yen & Serdar 1988). The *nahA* transcript levels correlated positively with naphthalene mineralization rates, which, presumably, translated to a higher specific growth rate.

Specialized zones in activated sludge

The hypothesis underlying this research is that creating a specialized zone – the accelerator tank – will increase the kinetics for monooxygenation reactions while maintaining the traditional goal, good BOD removal. Creating specialized zones in suspended growth treatment systems has a long and successful history for achieving a range of different objectives. Other specialized zones include the selector tank for controlling sludge bulking (Rittmann & McCarty 2001; Gabb et al. 1991; Chudoba et al. 1985; Daigger et al. 1985), an anaerobic tank for enhanced biological phosphorus removal (Rittmann & McCarty 2001; Comeau et al. 1986; Muyima et al. 1995; Sorm et al. 1996), an anoxic tank for pre-denitrification (Rittmann & McCarty 2001; Barnard 1973), and a contact tank for colloid sorption and reducing process size (Rittmann & McCarty 2001). Each of the other specialized zones relies on unique biochemical, ecological, or physical mechanisms, and none exploits the mechanisms hypothesized for the accelerator tank.

Materials and methods

Materials

The reactor system was inoculated with biomass originally obtained from the activated sludge process at the Northside Water Reclamation Plant, located in Skokie, IL. Acclimation of this community to the specific substrates was described by Dahlen & Rittmann (2000), and the experiments reported here were carried out with the same community after the experiments in Dahlen & Rittmann (2000) were completed.

Three substrates were used to evaluate the systems designed to accelerate monooxygenation reactions. The first substrate was acetate, an easily degradable carbon source that is fully mineralized without oxygenation reactions. The second and third substrates were phenol and 2,4-DCP, both of which are attacked by an initial monooxygenation reaction. The kinetics of 2,4-DCP utilization are much slower than for phenol (Dahlen & Rittmann 2000); thus, the need to accelerate 2,4-DCP's biodegradation is accentuated. The community utilized the three substrates simultaneously to form a multi-component primary substrate (Dahlen & Rittmann 2000).

Figure 4 is a drawing showing the components of the reactor system that was designed to provide the essential features of the two-tank suspended growth system at the bench scale. The two-stage reactor system was comprised of the accelerator and aerator tanks, followed by a solids-settling zone. The mineral medium (Dahlen 1999; Dahlen & Rittmann 2000) was similar to that described by Martin & Gottschal (1976) and employed successfully by Sáez & Rittmann (1993), who studied similar phenolic substrates. The medium included Buffer and Phosphorus source solution (20 mL/L), nitrogen source solution (20 mL/L), calcium and magnesium solution (20 mL/L), and a trace elements solution (20 mL/L). To minimize the possibility of deterioration of influent quality, we supplied phenol + acetate, nutrients,

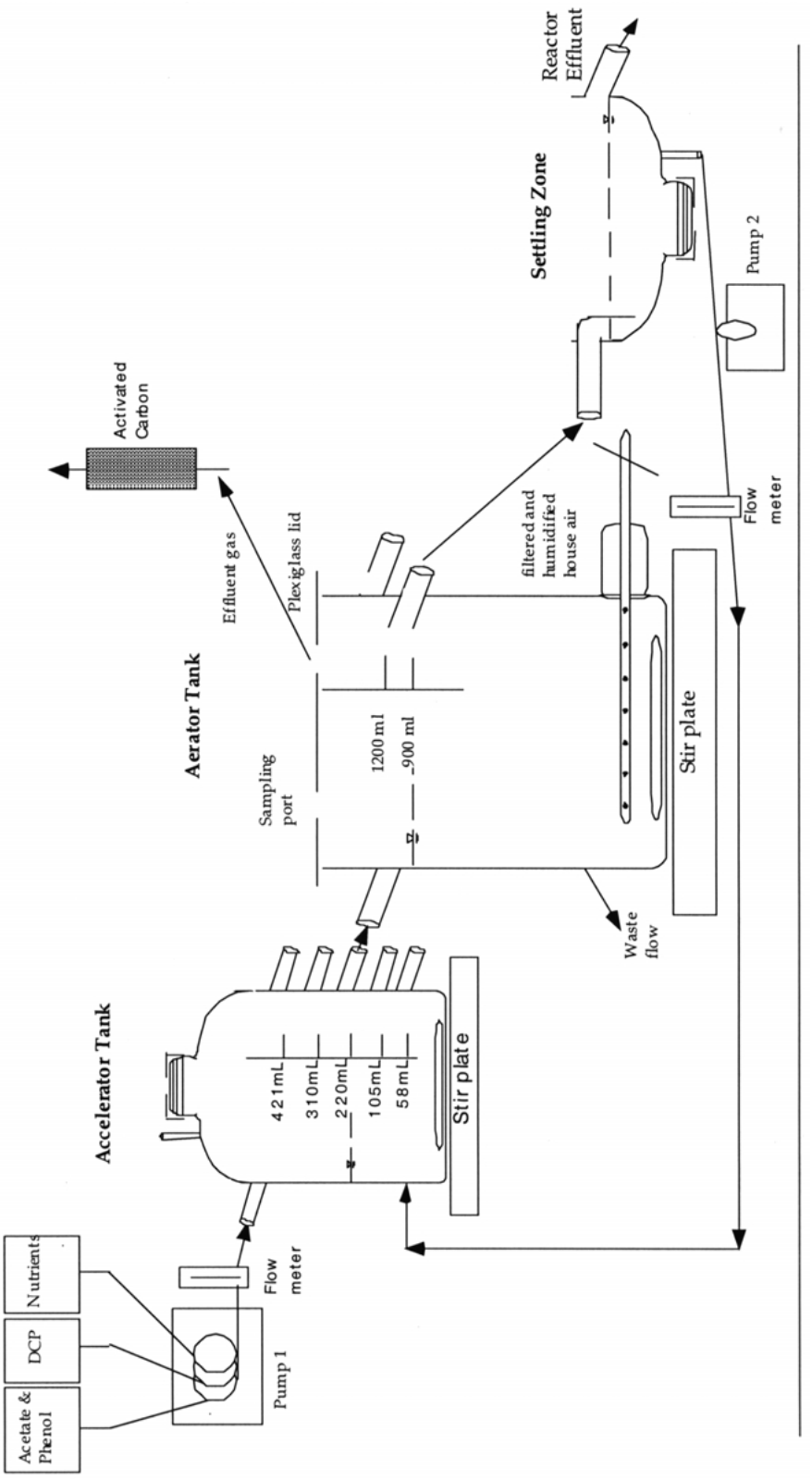

Figure 4. The bench-scale two-tank system.

and 2,4-DCP independently by peristaltic pumps from three separate reservoirs.

The accelerator and aerator tanks were made of glass and, with the exception of Tygon pump tubing, the tubing throughout the system was made of Teflon to minimize abiotic losses, such as by absorption to the tubing walls. The tubing directly attached to the feed bottles, prior to a size reduction, was neoprene, which was flexible enough to be clamped and could be sterilized in an autoclave. Because acetate, phenol, and DCP are nonvolatile and do not sorb strongly, we needed no other precautions to prevent uncontrolled losses to the gas phase or the biological solids (Schwarzenbach et al. 1993; Sáez & Rittmann 1993).

Samples were drawn from the accelerator and aerator with a syringe through the port in the wall of the tank. The volume of samples removed routinely from the accelerator tank did not exceed 5 ml per sampling period. No more than 10% of the total tank volume (around 13 ml) was removed at any one time. Samples taken from the aerator tank were the same size as those from the accelerator. Filtered and humidified compressed air was supplied to the aerator to maintain a concentration of 4 mg/L or greater. We sampled the DO concentration in the accelerator and aerator by pumping the reactor liquid into the flow cell of a DO meter (described below), where it made contact with the DO probe before being cycled back into the accelerator tank. The fluid was pumped at 130 mL/s, according to the manufacturer's recommendations.

We included a solids-settling zone to provide a small amount of solids separation and concentration to be fed back into the accelerator tank, but the solids in the recycle line were not as concentrated as in systems containing a true settling tank. Solids wasted from the accelerator (mainly in the effluent) controlled the SRT, which we computed from (Rittmann & McCarty 2001),

$$\mathrm{SRT} = \frac{X_{\mathrm{aer}} V_{\mathrm{aer}} + X_{\mathrm{acc}} V_{\mathrm{acc}}}{(Q - Q_w) X_e + Q_w X_w}, \qquad (1)$$

where X_{aer} and X_{acc} are the biomass concentrations (mg VSS/L) in the aerator and accelerator, V_{aer} and V_{acc} are the volumes (L) of the aerator and accelerator, Q and Q_w are the flow rates (L/d) for the influent and biomass wasting, and X_e and X_w are the biomass concentrations (mgVSS/L) in the effluent and biomass wasting. Because the solids- settling zone allowed some concentration of the recycled biomass, the solids- concentration ratio (SRT/θ) was between 1.05 and 1.12 for the two-tank system; it was 1.13 for the one-tank system. In a full-scale suspended growth treatment system, the solids-concentration factor is in the order of 20 (Rittmann & McCarty 2001). Thus, the bench-scale reactor system did not replicate the solids recycle system found in full-scale operation.

Experimental strategy

We ran a series of five sets of experiments that were distinguished by the ratio of the total system volume contained in the accelerator tank: 6, 10, 20, 26, or 32 percent. We also ran one series of experiments with no accelerator tank; this set is called the one-tank series. We carefully controlled donor substrate and DO concentrations in the accelerator tank in order to manipulate the cells' specific growth rate and content of NADH + H^+. A high BOD concentration was achieved by feeding the influent substrates directly to the accelerator tank, which contained only a small fraction of the total system volume (6–32%). We controlled the DO concentration in the accelerator to low levels (from 0.01 to 2.2 mg/L) by vacuum degassing the air space in the feed bottles and in the accelerator tank. Each experiment was operated to a steady-state condition, determined by consecutive biomass measurements with no significant change in concentration, at which time we took the samples providing the data reported herein.

Operating parameters for the five series of experiments performed on the two-tank system are summarized in Table 1. The five series are distinguished by different retention-time ratios ($\theta_{\mathrm{acc}}/\theta$, where θ_{acc} is the hydraulic retention time of the accelerator tank and θ is the total hydraulic retention time), while experiments within each series had different DO concentrations in the accelerator tank. Other physical parameters, such as flow rate (2.4 mL/min), aerator volume (900 mL), recycle rate (0.4 $\pm$ 0. mL/min), pH (7.0 $\pm$ 0.1), and temperature (23.5 $\pm$ 0.9 °C in the accelerator and 24.0 $\pm$ 0.9 °C in the aerator) were stable and monitored. Experimental series 6, which was distinguished from series 1 to 5 in that it contained no accelerator tank, allowed us to compare the performance of the two-tank system to a one-tank system similar in all ways except for the accelerator. The volume of the aerator tank in series 6 was 1200 mL. For comparison, the total volume in the two-tank experiments ranged from 957 mL (series 4) to 1320 mL (series 5). We performed a total of 34 experiments on the two-tank reactor and four experiments on the one-tank system. The changes in accelerator volume

were not in one direction, but alternately increased and decreased in order to prevent any experimental bias (Table 1).

The DO in the accelerator tank was a critical parameter controlled in the two-tank experiments (Table 1). It ranged from 0.01 to 2.23 mgDO/L, with an overall average of 0.35 mgDO/L throughout the two-tank experiments. The DO concentration in the aerator was kept high in all experiments. It varied from 3.9 to 10 mgDO/L, with an average of 6.4 mgDO/L throughout the 6 series of experiments.

Analytical methods

Biomass concentration was assayed as volatile suspended solids (VSS) by drying the sample at 180 °C overnight and igniting it at 550 °C, according to the method 2540 E in *Standard Methods* (APHA 1992). Glass microfiber filters (Whatman type GF/C) were used to retain the solids. The aggregate concentration of organic electron donors was measured as chemical oxygen demand (COD) after filtration through a 0.45-μm membrane filter (APHA 1992). We employed HACH COD vials, reagents, and digester. The difference between the measured COD and the measured phenol and 2,4-DCP concentrations converted to COD gave the influent acetate concentration. The conversion factors for phenol and 2,4-DCP are 2.383 g COD/gphenol and 1.178 g COD/gDCP. The Cl^- concentration in the reactor was monitored using a standard Cl^- probe (Accumet, 13-620-527) calibrated against standard concentrations. The pH of the system was measured using a standard pH probe calibrated against standard buffers. DO was measured very precisely at μg/L-levels using a high resolution, digital DO meter and probe (Martek Instruments, Inc., Model Mark XVIII) (Bae & Rittmann 1996a).

We measured concentrations of the phenolic substrates by high performance liquid chromatography (HPLC) of membrane-filtered aqueous samples (Häggblom & Young 1990). The column was a Whatman C-18, 4.7-mm I.D. by 235 mm. Detection was by diode array. The solvent system was methanol:water:acetic acid (60:38:2 % volume) at a flow rate of 1.0 mL/min. In addition to measuring phenol and 2,4-DCP, the HPLC method allowed us to measure concentrations of catechol, chlorocatechol, and chlorophenol (multiple isomers) to assess oxidative transformations along a monooxygenation pathway. During the preliminary experiments in the chemostat study (Dahlen & Rittmann 2000), we observed build-up of chlorocatechol and catechol prior to reaching steady state. During the two-tank experiments, we observed no intermediates at any time.

The intracellular electron carriers, NAD^+ and $NADH + H^+$, were measured by applying a highly sensitive cycling method that combined the oxidation of $NADH + H^+$ with the production of a reduced spectrophotometrically detectable product, P_{red} (Bae & Rittmann 1996a; Cartier 1968; Bernofsky & Swan 1973; Nisselbaum & Green 1969). NAD^+ is reduced to $NADH + H^+$ in the presence of excess ethanol (10%) and alcohol dehydrogenase (50 units/mL) at the expense of ethanol oxidation to acetaldehyde. $NADH + H^+$ then reduces 5-ethylphenazinium ethyl sulfate (PES, present at 0.06%) to its reduced form, PES-H_2, which finally reduces the oxidized reactant, thiazolyl blue (0.5 M; denoted R_{ox}), to its spectrophotometrically detectable product, P_{red}, at 570 nm. Since the reduction and oxidation of $NAD(H + H)^+$ are cyclic, the rate of P_{red} formation is proportional to the concentration of NAD^+ or $NADH + H^+$, provided sufficient amounts of ethanol and R_{ox} are present. $NADH + H^+$ was assayed by extracting the sample in 0.75 N NaOH, in which only the $NADH + H^+$ is stable, whereas NAD^+ was analyzed after acid extraction (0.75 N HCl). A Hitachi U2000 was employed for the spectrophotometric determination.

Further details on the methods, such as their calibration and detection limits, can be found in Dahlen (1999).

Results and discussion

Measurements from the accelerator tank are noted with subscript acc and from the aerator with subscript aer. The average influent concentrations and operating parameters for each of the six series of experiments and the retention times are shown in Table 2. In general, the influent concentrations were relatively constant in the ranges of 236–310 mg COD/L for total COD, 138–172 mg COD/L for acetate, 82–121 mg COD/L for phenol, and 12–17 mg COD/L for 2,4-DCP. However, several experiments in series 6 were operated with elevated concentrations of acetate and phenol in order to test if increased loading of non-halogenated substrates affected removal of 2,4-DCP. Therefore, the averages for series 6 are higher than for series 1 to 5. The system SRT averaged 0.36 day, with a range of 0.31 to 0.43 d. These values document that the system SRT was stable and was not a factor con-

Table 1. Summary of experimental operating parameters

Experimental Order	Number of experiments	Accelerator volume (mL)	Retention time ratio	Range in accelerator DO (mg DO/L)	Average accelerator DO (mg DO/L)
Series 1	6	20	0.20	0.01–1.20	0.33
Series 2	8	105	0.10	0.08–0.40	0.21
Series 3	7	310	0.26	0.07–0.36	0.20
Series 4	6	58	0.06	0.10–1.30	0.50
Series 5	7	421	0.32	0.15–2.23	0.54
Series One-tank	4	Aerator Volume: 1200 mL	0.0		Average DO in aerator: 6.3 ± 1.8

trolling system performance among the experiments. All values of SRT were small, compared to the more typical values around 5 days common for full-scale systems (Rittmann & McCarty 2001). The small SRT values were a consequence of the marginal capability of the bench-scale solids-settling zone.

Table 3 summarizes the concentrations of the substrates in the accelerator and aerator tanks. The effluent concentration equaled the concentration in the aerator tank. Significant removal of 2,4-DCP was achieved, particularly in the two-tank experiments. Figure 5, which compares the molar mass of DCP removed from each tank versus the molar mass of chloride ion measured in solution, confirms that both moles of chloride ion were released for each mole of DCP removed.

Removal of 2,4-DCP appears to be significantly higher in the two-tank experiments than in the one-tank experiments. To compare directly the performance of the two-tank system to that of the one-tank system, we calculated the overall removal percentages and the rates of substrate removal based upon experimental measurements. We computed the percentage removals from Equation (2).

$$\%\ \text{Substrate Removal} = \frac{[S]_{\text{in}} - [S]\text{aer}}{[S]_{\text{in}}} 100\%. \quad (2)$$

The substrate-concentration terms, indicated by $[S]$, can represent phenol, 2,4-DCP, acetate, or total COD. We computed volumetric rates of substrate removal from Equation (3).

$$\text{Volumetric Substrate-Removal Rate} = [S]_{\text{in}} - [S]_e)/\theta). \quad (3)$$

We also determined the rate of substrate utilization per unit biomass by dividing the calculated rate per unit volume by the biomass concentration in the volume considered.

Figure 6 is a plot of the average removal percentages for each series of experiments performed on the two-tank reactor system and the average for the one-tank system. The average 2,4-DCP removal over all experiments in the two-tank system was 93%, compared to 74% in the one-tank system. The average removal of phenol was 99% in the two-tank system, compared to 93% in the one-tank system. Soluble-COD removals (not shown) were 95 and 83 percent in the two-tank and one-tank systems, respectively. Improved removals of the phenolic substrates (and soluble COD) occurred when the total volume of the two-tank system was smaller than the volume of the one-tank system, as was the case in the three series with retention time ratios less than 0.25.

The volumetric and biomass-specific removal rates were higher in the two-tank reactor system for phenol and 2,4-DCP. Figure 7 shows that the rates of substrate removal per unit biomass were higher for every experiment with the two-tank reactor system. The removal rates confirm the conclusion obtained with percentage removals: The two-tank system was more efficient in removing the phenolic substrates, which require initial monooxygenations.

The results shown in Figures 6 and 7 suggest a maximum overall removal rate at a retention-time ratio between 0.1 and 0.2, where the highest percentage removal and the highest rate of utilization were observed for 2,4-DCP. We performed a Student's T test (Guttman et al. 1971) to compare the rates of 2,4-DCP removal at the retention time ratios 0.1 and 0.2 to the

Table 2. Operating data for the two-tank and one-tank reactor systems

Retention time ratio	Hydr. retention time, θ days	Solids retention time, SRT days	Solids concentration factor SRT/θ	$[Ac]_{in}$ mg COD/L	$[Ac]_{in}$ mg COD/L	$[PH]_{in}$ mg COD/L	$[DCP]_{in}$ mg COD/L
0.20	0.32	0.36	1.12	236	138	82	16
0.10	0.29	0.31	1.08	301	172	113	17
0.26	0.35	0.37	1.05	310	172	121	17
0.06	0.28	0.31	1.11	274	159	103	12
0.26	0.38	0.43	1.11	264	144	108	12
One-tank (ranges)	0.35	0.39	1.13	441 (286–507)	267 (163–331)	157 (105–228)	17 (12–20)

Table 3. Average concentrations for the two-tank and one-tank reactor systems

Retention-time ratio	SRT (days)	$[COD]_{acc}$	$[Ac]_{acc}$	$[DCP]_{acc}$ (mg COD/L)	$[COD]_{aer}$	$[Ac]_{aer}$	$[PH]_{aer}$	$[DCP]_{aer}$ (mg COD/L)	
0.20	0.36	119	46	62	10.5	7.9	5.4	1.85	0.67
0.10	0.31	205	111	85	9.0	6.6	1.24	0.77	
0.26	0.37	247	135	99	12.2	22.4	12.8	7.76	1.84
0.06	0.31	204	108	88	8.0	8.9	7.6	0.61	0.71
0.26	0.43	189	94	87	7.7	18.6	17.3	0.40	0.89
One-tank (ranges	0.39					76 (37–113)	42 (20–66)	30.2 (0.71–90)	4.24 (3.77–4.88)

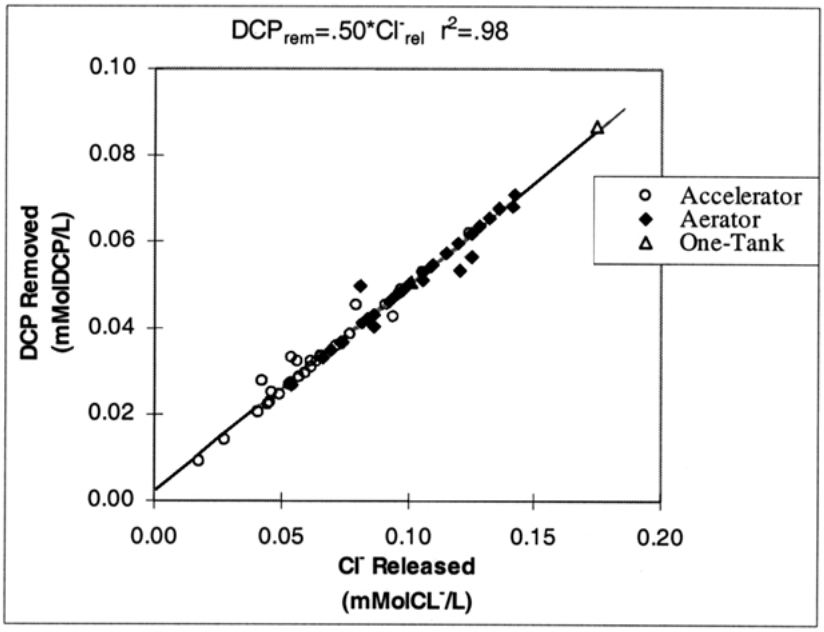

Figure 5. Relationship between the mass of 2,4-DCP removed and the mass of chloride ion measured in the two-tank and one-tank reactor systems.

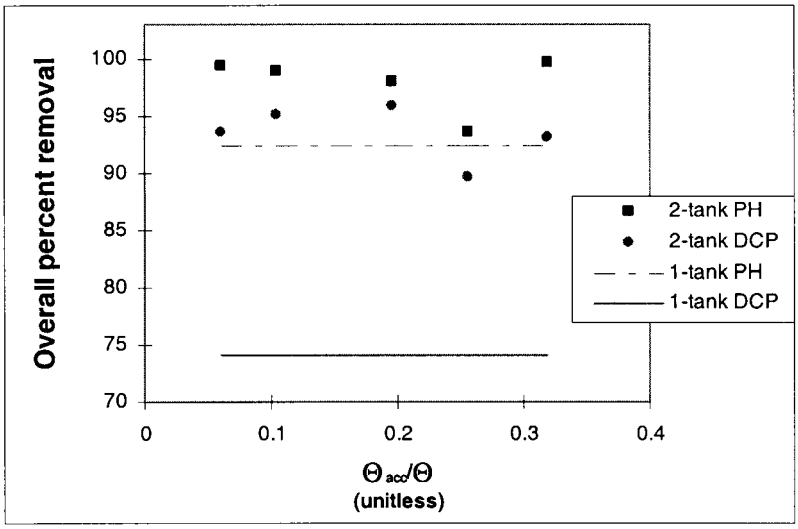

Figure 6. Average percent removals of phenolic substrates in the two-tank and one-tank systems.

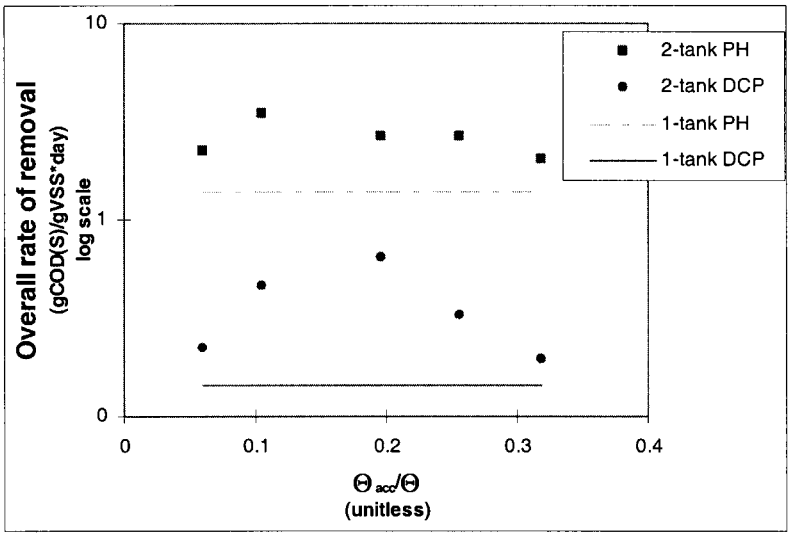

Figure 7. Comparison of the rates of removal per unit biomass for the two-tank and one-tank systems.

remaining three retention times to determine if they were statistically higher. The comparison resulted in a 2.4-percent likelihood that the rates were different. Thus, the statistical analyses support that the removal rate was maximized for retention time ratios of 0.1 and 0.2 when the DO was kept low in the accelerator.

The hypothesis underlying the two-tank design states that the rates of the monooxygenation reactions should be higher in the accelerator tank. To evaluate this hypothesis, we computed the rates of phenol and 2,4-DCP utilization for each tank of the two-tank system. Table 4 tabulates the removal rates per unit biomass for phenol and 2,4-DCP for each of the five series of experiments performed on the two-tank reactor system and in the one-tank system. The rates of 2,4-DCP and phenol utilization were significantly higher in the accelerator tank compared to the aerator and one-tank system, thus supporting the underlying hypothesis.

The average concentrations (with standard deviations) for the concentrations of the reduced, oxidized, and total forms of NAD(H) are summarized in Table 5 for the aerator tank and the one-tank series. The average concentrations were similar in the aerator and the one-tank system and are comparable to measurements made by others. The total carrier concentrations

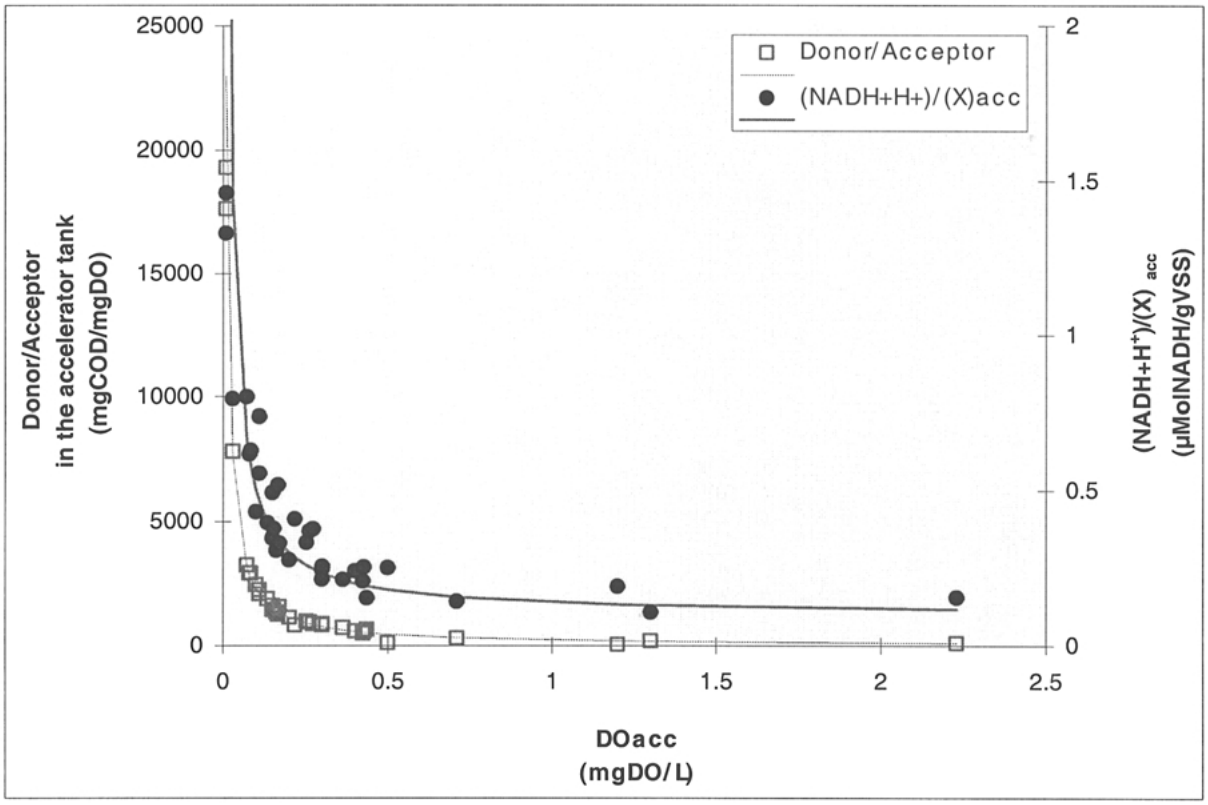

Figure 8. The ratio of donor to acceptor and the corresponding normalized NADH + H^+ concentrations versus the DO concentration in the accelerator tank.

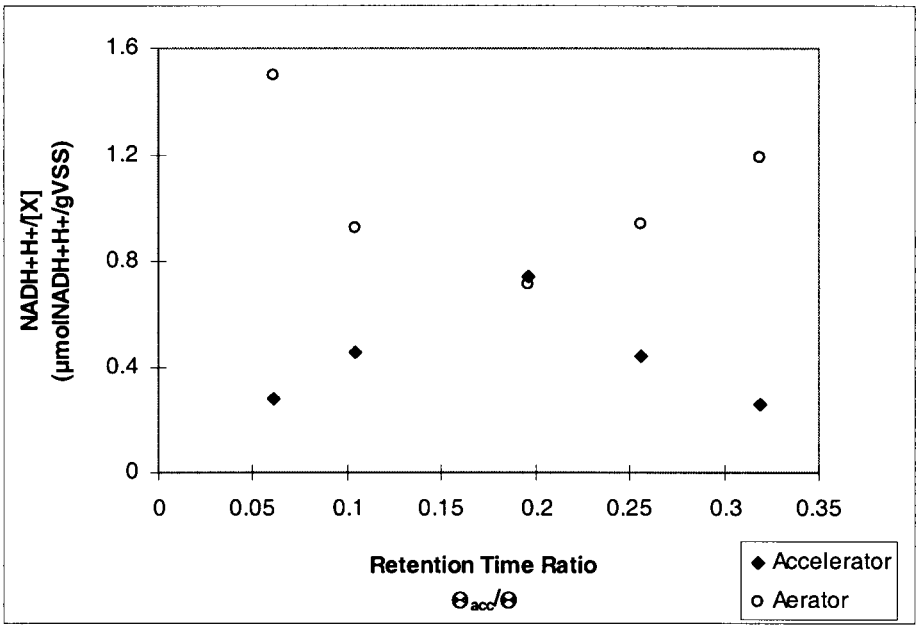

Figure 9. Comparison of the average normalized NADH + H^+ results in the accelerator and aerator tanks versus the retention-time ratio.

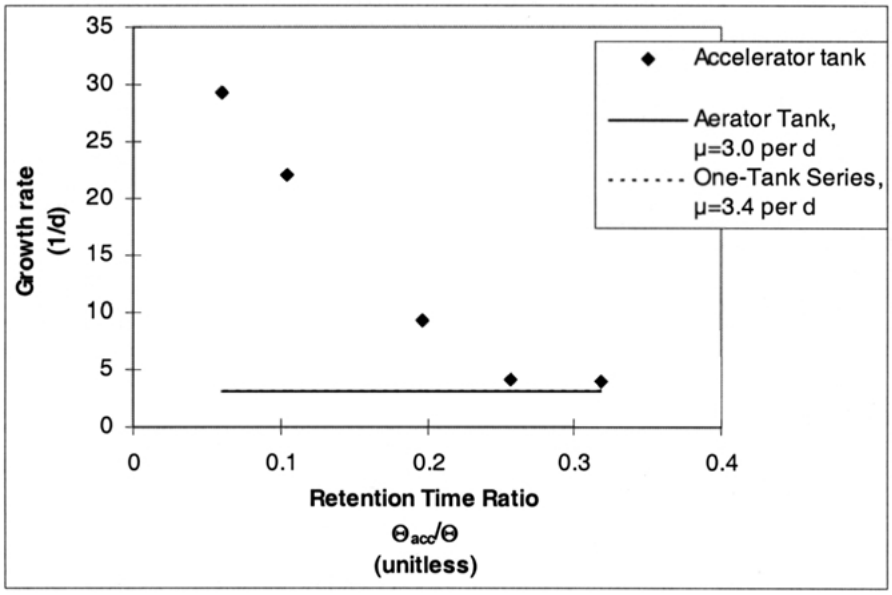

Figure 10. Average specific growth rates (μ) versus retention-time ratio.

Table 4. Comparison of the rates of phenol and DCP utilization in the accelerator and aerator tank and in the one-tank system

	Accelerator				Aerator		
Units Series	Retention time ratio $\theta_{acc}/\theta_{total}$	Rate of phenol utilization	Rate of DCP utilization		Rate of phenol utilization	Rate of DCP utilization	
			g COD/g VSS*day				
Two-tank system							
4	0.06	15.4	5.0		2.3	0.2	
2	0.10	10.2	4.0		3.5	0.5	
1	0.20	8.1	2.9		2.7	0.5	
3	0.26	4.4	1.4		2.8	0.3	
5	0.32	2.1	0.7		2.0	0.2	
Averages			8.1	2.8		2.7	0.4
One-tank system					3.0	0.3	

averaged 3.37 and 3.39 μMol/g VSS in the aerator and one-tank system, respectively, which compare to 3.53 μMol/g VSS for the chemostat experiments (Dahlen & Rittmann 2000) and to 3.35 μMol/g VSS from Bae & Rittmann (1996a). The reduced form averaged 1.06 and 1.53 μMol/g VSS, respectively, in the aerator tank and one-tank system. These values are similar to the chemostat experiments with the same substrates (0.98 μMol/g VSS, Dahlen & Rittmann 2000), as well as to other reported values (Bae & Rittmann 1990; Wimpenny & Firth 1972).

The reduced-carrier concentrations in the accelerator tank varied significantly with changes in the DO concentration. The production and consumption of NADH $+H^+$ are directly related to the flow of electrons in the cell, and this flow depends upon the externally supplied electron-donor and electron-acceptor substrates. The ratio of electron donors to acceptors defines a potential for electron flow within the cell. In the accelerator tank, the acceptor concentration was maintained at a low concentration, but was changed from one experiment to the next. We calcu-

Table 5. Comparison of the average reduced and oxidized intracellular electron carrier concentrations in the aerator and one-tank series of experiments

Experimental System	$[NADH + H^+]/[X]$ μMol/NADH + H^+/g VSS	$[NAD^+]/[X]$ μMol NAD^+/g VSS	$[NAD(H)]_{total}/[X]$ μMol NAD(H)/g VSS
Aerator tank	1.06 ± 0.52	2.31 ± 0.57	3.37
One-tank series	1.53 ± 0.25	1.86 ± 0.32	3.39

lated the ratio of available electron donor to acceptor in the accelerator tank using Equation (4).

$$\frac{\text{Donor}}{\text{Acceptor}_{acc}} = \left(\text{COD}_{acc} - \left(\frac{4}{20}\right)\text{DCP}_{acc}\right)/\text{DO}_{acc}. \quad (4)$$

We subtracted 4/20th of the COD of the 2,4-DCP from the total COD, because the first two steps of DCP degradation do not generate NADH + H^+ (Figure 3). However, products of 2,4-DCP after muconic acid contribute to NADH + H^+. The DO_{acc} concentration refers to the measured DO concentration in the accelerator tank. The measured donor-to-acceptor ratio versus the DO concentration in the accelerator is shown in Figure 8. This figure shows that restricting the availability of DO in the accelerator below about 0.5 mg/L caused a buildup of electron donors over electron acceptors and accentuated the conditions desired for building up NADH + H^+ in the accelerator. The effect was very dramatic for DO less than about 0.25 mg/L. Also shown in Figure 8 are the measured NADH + H+ contents of the biomass in the accelerator for the same conditions. Figure 8 demonstrates that the cells were enriched in the reduced carrier when the accelerator conditions strongly favored the electron donor over the acceptor, and the condition was affected by a very low DO concentration.

The cells' total content of intracellular electron carrier ($NADH_{total}$) was almost identical throughout the reactor system: averages of 3.41 μ Mol/g VSS for the accelerator and 3.38 μ Mol/g VSS for the aerator. Thus, the changes in NADH + H^+ for varying DO in the accelerator were caused by shifts between NAD^+ and NADH + H^+. The average NADH + H^+ concentration in the aerator was higher than in the accelerator: 12% in the accelerator versus 31% in the aerator. For comparison, Bae & Rittmann (1996a) reported 22 percent NADH + H^+. While a shift in the NAD(H) partitioning between the accelerator and the aerator tank confirms that the environmental conditions manipulated the cell accumulation of NADH + H^+ in the accelerator tank, the average shift was opposite to the trend that should increase the rate of monooxygenation in the accelerator.

While the average NADH + H^+ concentration in the accelerator was lower than in the aerator, there were significant differences among the five series and the individual experiments within a series. The average normalized NADH + H^+ concentrations for each experimental series are shown in Figure 9. The trend shown for the NADH + H^+ concentration in the accelerator varied in the same manner as the rates of 2,4-DCP removal (Figure 7). The highest average NADH + H^+ concentrations were measured in the accelerator tank for the experimental series performed at the retention time ratio of 0.20. This is the same experimental series that had the highest 0.21. average rates of phenol and 2,4-DCP removal and the only series of experiments in which the average NADH + H^+ concentration in the accelerator tank was greater (albeit slightly) than that in the aerator tank. Figure 9 shows that, as the concentration of NADH + H^+ in the accelerator tank increased, the corresponding concentration measured in the aerator tank decreased.

We calculated the biomass specific growth rate, μ (day^{-1}), in the accelerator and aerator tanks from mass balances on each tank. The mass balance equations are shown in Equations (5) and (6) and were solved for the specific growth rate in Equations (7) and (8).

For the accelerator:

$$0 = (\mu_{acc})X_{acc}V_{acc} + Q_{rec}X_{rec} - (Q + Q_{rec})X_{acc}. \quad (5)$$

For the aerator:

$$0 = (\mu_{aer})X_{aer}V_{aer} + (Q + Q_{rmrec})X_{acc} - (Q + Q_{rec})X_{aer} \quad (6)$$

$$\mu_{armacc} = \frac{(Q + Q_{rec})X_{acc} - Q_{rec}X_{rec}}{V_{acc}X_{acc}} \quad (7)$$

$$\mu_{aer} = \frac{(Q + Q_{rec})}{X_{aer} V_{aer}} (X_{aer} - X_{acc}), \qquad (8)$$

where subscript rec refers to the solids recycle flow.

Figure 10 plots the average specific growth rate in each experimental series and shows that it varied with the retention-time ratio in the accelerator tank. The specific growth rate in the accelerator tank varied from 4 to 30 per day, while in the aerator tank and in the one-tank series, the rate was nearly constant at 3.0 and 3.4 per day, respectively. In all cases, the specific growth rates in the accelerator tank were significantly higher than in the aerator and one-tank series. As the retention-time ratio decreased below 0.25, the specific growth rate increased dramatically. Although the aerator kept the specific growth rate for the entire system near 1/SRT, the accelerator was a specialized zone of high μ.

Conclusions

The experimental evaluation demonstrated that a suspended growth system operated in the two-tank *accelerator/aerator* configuration significantly increased the overall removal rates for phenol and 2,4-DCP, aromatic hydrocarbons that require initial monooxygenations. For the more slowly degraded 2,4-DCP, the average percentage removal increased from 74% to 93%, even though the volume of the two-tank system was smaller than that of the one-tank system in most experiments. The average volumetric and biomass-specific removal rates increased by 50% and 100%, respectively, in the two-tank system, compared to a one-tank system. The greatest enhancement in 2,4-DCP removal occurred when the retention-time ratio was approximately 0.2.

Biomass in the accelerator tank was significantly enriched in NADH + H^+ when its DO concentration was below 0.25 mg/L, a situation having a high ratio of donor to acceptor. The accelerator biomass had its highest NADH + H^+ content when the retention-time ratio was 0.2, which correlated to the highest rate of 2,4-DCP removal. Biomass in the accelerator also had a much higher specific growth rate than in the aerator or the system overall. The specific growth rate in the accelerator was inversely correlated to the retention time-ratio.

The experimental results support our hypothesis that a small accelerator tank can significantly accelerate the degradation kinetics for aromatic hydrocarbons, which require an initial monooxygenation reaction. Although the trends in NADH + H^+ and specific growth rate are generally consistent with the mechanisms underlying our hypothesis, a more detailed evaluation is required to establish the exact causes for the accelerated monooxygenation kinetics. The companion manuscript (Dahlen and Rittmann, companion) provides that detailed evaluation.

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