1 **Effect of Substrate Characteristics on Microbial Community Structure, Function, Resistance,**
2 **and Resilience; Application to Coupled Photocatalytic-Biological Treatment**

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ABSTRACT: Advanced oxidation (AO) coupled with biodegradation is an emerging treatment technology for wastewaters containing biologically recalcitrant and inhibitory organics, including those containing chlorinated aromatic compounds. The composition of the AO effluent organics can vary significantly with reaction conditions, and this composition can affect the performance of subsequent biodegradation. Three synthetic effluents were used to mimic varying degrees of AO of 2,4,5-trichlorophenol: 4-chlorocatechol to mimic light transformation, 2-chloromuconic acid to mimic moderate transformation, and acetate to mimic extensive transformation. The substrates were fed to identical chemostats and analyzed at steady state for removal of chemical oxygen demand (COD) and dissolved organic carbon (DOC), biomass concentration, and bacterial diversity. The chemostat fed acetate performed best at steady state. The 2-chloromuconic acid chemostat was next in terms of steady-state performance, and the 4-chlorocatechol reactor performed worst, correlating with degree of AO transformation. A spike of 100 μ M 2,4,5-trichlorophenol was then applied to each chemostat. The chemostat fed 4-chlorocatechol exhibited the best resistance to the perturbation in terms of maintaining consistent community structure and biomass concentration, whereas the performance of the acetate-fed chemostat was severely impaired in these categories, although it quickly regained capacity to remove organics near pre-perturbation levels demonstrating good resilience. The opposing trends for steady-state versus perturbed performance highlight tradeoffs inherent in coupled chemical-biological systems.

1 - Introduction:

Combined chemical-biological treatment is a developing technology for wastewaters containing strongly recalcitrant or inhibitory organics. These wastewaters are not amenable to treatment by biological processes alone, while advanced oxidation processes (AOPs) alone are not efficient for complete mineralization of the organic contaminants. Using an AOP to partially degrade or transform the toxic organics into more benign products and then feeding this effluent into a biological post-treatment provides an efficient and robust technology that overcomes the shortcomings of the individual processes. Combined systems have been developed utilizing a variety of chemical pretreatments for a range of contaminants, including UV/H₂O₂ (Esplugas et al. 2002, Scott and Ollis 1995, Zhang et al. 2010) ozone (Aghapour et al. 2015, Hong and Zeng 2002, Moussavi and Mahmoudi 2009, Tuin and Bos 2004, Vaiopoulou et al. 2015), Fenton type reagents (Al Momani et al. 2004, Lapertot et al. 2008, Molina et al. 2010, Monsalvo et al. 2015, Ribordy et al. 1997, Sarria et al. 2003, Wu et al. 2013), and photocatalysis (Li et al. 2011, Ma et al. 2015, Marsolek et al. 2014, Marsolek et al. 2008, Oller et al. 2007, Parra et al. 2002, Zhang et al. 2012, Zhou et al. 2015). Several review papers summarize the technology (Guieysse and Norvill 2014, Oller et al. 2011, Scott and Ollis 1995).

Regardless of the pretreatment scheme used, altering the reaction conditions allows some control over the composition of the organics delivered to the biological process. For example, certain reaction conditions produced biodegradable and non-toxic constituents, while other conditions generated an effluent containing toxic and non-biodegradable organics (Alaton et al. 2004, Hörsch et al. 2003, Lapertot et al. 2008, Marsolek et al. 2014, Wang et al. 2003).

One aspect of a high quality effluent for biological post-treatment is that its organic matter is readily biodegradable and not toxic. In this case, chemical oxygen demand (COD) and dissolved organic

carbon (DOC) removals are high, and biomass is readily accumulated. A second aspect is that the community in the post-treatment must be resistant and resilient to any residual toxic compounds not transformed in the pretreatment or produced as by-products in the pretreatment. *Resistance* is defined as the ability of a community to immediately withstand the negative impact of the stress, whereas *resilience* refers to the ability to recover from process upsets after the stress has passed (Fernandez et al. 2000, Girvan et al. 2005, Hashsham et al. 2000, Marsolek et al. 2014, McKew et al. 2011, Wertz et al. 2007). Resistance and resilience gain heightened importance when the loading of toxic or difficult-to-degrade materials spikes over time, as is the reality in many practical situations. Few previous studies document anything about the impact of pretreated wastewater on microbial community composition, and they do not agree. Ballestoros Martin et al. (2011) found little impact on the microbial community of an MBR when fed Fenton pre-treated wastewater. Marsolek et al. (2014) found that different effluent compositions from photocatalytic oxidation of 2,4,5-trichlorophenol (TCP) produced unique microbial communities. Although not using Fenton reagent as a pretreatment, Jho et al. (2014) documented that shocking a microbial community with Fenton reagent could lead to loss of chloroaliphatic-degradation capacity.

A biological post-treatment whose influent normally contains none of the toxic or difficult-to-degrade organics will achieve good COD removal, but it may not be well suited to handle toxic spikes, resulting in poor resistance and resilience. At the other extreme, an influent containing only toxic or difficult-to-degrade organics will not give consistent biodegradation, leading to poor treatment for all conditions. Therefore, a balance must be achieved between providing organic compounds that are sufficiently easy-to-biodegrade, but also maintain a microbial community that is robust in the face of toxic inputs.

The target compound in this work is 2,4,5-trichlorophenol (TCP). TCP has been measured in the environment throughout the world and in various media. It was detected in tap water in Finland from 0.17-0.3 μM , in various Dutch rivers at less than 3 μM , in leachate from a German landfill at a maximum of 560 μM , and in soil samples ranging from 0.3 $\mu\text{g/kg}$ to 3 g/kg (Hazardous Substances Data Bank, HSDB #4067). TCP is highly bio-recalcitrant at concentrations greater than 40 M and inhibits respiration of non-adapted communities in direct proportion to its concentration (Marsolek et al. 2007). Photocatalytic degradation of TCP yields a variety of products (Agrios et al. 2004, Hurum et al. 2004), ranging from similarly difficult-to-degrade chlorinated aromatic compounds to readily biodegradable non-chlorinated aliphatics (Agrios et al. 2003, Marsolek et al. 2014). Altering the photocatalytic reaction conditions can shift the effluent composition towards either of these extremes.

Previous work used actual effluents from photolytic and photocatalytic degradation of TCP (Marsolek et al. 2014). Four different photocatalysis conditions produced four unique photocatalytic effluents with varying concentrations of residual COD and TCP, as well as varying degrees of chlorination and aromaticity. The four photocatalytic effluents were fed to continuous aerobic biological reactors (chemostats) and assessed for TCP removal, COD removal, microbial diversity via denaturing gradient gel electrophoresis (DGGE), and resistance and resilience to a spike of TCP. The results indicated that the photocatalytic effluent giving the best steady-state biodegradation did not yield a microbial community with strong resistance and resilience to a TCP spike, although the most resistant and resilient community did not achieve nearly as good removal of COD during steady state.

The photocatalytic effluents in Marsolek et al. (2014) were chemically complex, increasing the ambiguity of direct comparison of substrate composition to community diversity and bioreactor performance. Here, we use single compounds to mimic the varying stages of photooxidation of TCP (structures are shown in Figure 1): 4-chlorocatechol (4CC) to mimic a light transformation of TCP, 2-chloro muconic acid (2CMA) to mimic a moderate degree of transformation, and acetate (AC) to

mimic extensive transformation (Agrios et al. 2003, Górska et al. 2009, Hurum et al. 2004). The use of single compounds of known chemical characteristics makes it possible to gain more cause-and-effect understanding of the tradeoffs inherent to using advanced oxidation as a pre-treatment to biodegradation; together with the previous work, use of defined compounds gives a well-rounded assessment of the tradeoffs inherent in coupled photocatalysis and biodegradation.

We fed each of the substrates continuously to aerobic, suspended-growth chemostats inoculated with identical biomass sources. The substrate concentrations fed to the bioreactors were normalized to have the same input COD loading, and the reactors were maintained with stable conditions until they came to steady state. We sampled the reactors for substrate concentration, COD, DOC, and biomass concentration (by optical density at 600 nm), and we extracted DNA to monitor the microbial community through denaturing gradient gel electrophoresis (DGGE). After the chemostats reached steady state, we perturbed each of them with a 100- M spike of TCP in order to gauge the resistance and resilience of each of these communities to the toxic parent compound. We correlated the impact of this perturbation on biomass, COD, DOC, and community diversity to the substrate and steady-state community structure.

2- Experimental/Methods:

2.1 - Substrates

Table 1 shows the different substrates used, the identifier for them, their concentrations, and their input COD and DOC concentrations. The influent COD was set to 103 mg/L for each. Because CMA is more oxidized than 4CC and AC, its DOC concentration was higher. AC is the only substrate with no chlorine substitution.

2.2 – Medium

The medium for all experiments consisted of 3.6 mM NH_4Cl , 45.0 M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.6 M FeCl_3 , 4.2 M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.6 M $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, and 24.3 nM Na_2MoO_4 , prepared in MilliQ water and autoclaved. 1 M phosphate buffer (0.4 M KH_2PO_4 , 0.6 M K_2HPO_4) was filter sterilized and added to the cool medium for a final concentration of 10 mM with a final pH of 7.0-7.2. The TCP stock solution had a concentration of 1.0 mM and was filter sterilized prior to adding aseptically.

2.3 – Inoculum

Activated sludge from the Stickney Wastewater Treatment Plant (Calumet City, IL) was frozen in 10-mL aliquots at -80 °C in a 1:1 mixture (10 mL inoculum:10 mL glycerol solution) with 50% glycerol (vol/vol). Prior to inoculating a reactor, the frozen inoculum was thawed, and centrifuged at 5000 rpm for 5 minutes. Then 18 mL of supernatant was removed and replaced with 18-mL of clean medium. This process was repeated three times to remove the glycerol and extraneous carbon compounds from the inoculum.

2.4 - Reactor set-up

Continuous bioreactors (chemostats) were 1-L Erlenmeyer flasks fitted with an outlet at the 500-mL level, resulting in a 325-mL liquid volume in the reactor during shaking. Calibrated Minipuls 2 pumps with Viton pump tubing were used to pump medium to the reactors. All tubing from the media bottle to the reactors, excepting the pump tubing, was red (opaque) Teflon to minimize light and tubing effects. The reactors were covered in aluminum foil and were operated aerobically at room temperature (20-25 °C) and under continuous shaking (Innova 2100, New Brunswick Scientific, Edison, NJ) at 125 rpm.

To allow adaptation after start up, the reactors were operated in batch mode for 1 week with 50 M of each organic substrate. After this week, the reactors were operated with continuous feeding at a flow rate of 3 mL/hr, giving a hydraulic residence time of 4.5 days and a steady state dilution rate of 0.222/day.

2.5 – Sampling

Samples were removed from the mixed reactors three times per week. Once the reactors reached steady performance they were operated for two more weeks prior to perturbation. For purposes of this study, steady-state is defined as a two-week interval during which the ratio of 95%-confidence-interval/average for OD, COD, and DOC was less than 20%. For all three reactors and all three measures, this ratio was in actuality less than 15% for the chosen two-week interval, except for acetate DOC values, where this measure was 23%. However because of the quality of OD and COD measures for this duration and since the criteria was nearly met, we proceeded with the perturbation experiment. The AC and 4CC reactors had seven steady-state samples for OD, COD, and COD in the two-week interval, and the CMA reactor had nine samples. Mean and 95% confidence intervals for steady-state performance are based on these samples. Bioreactors were monitored for bacterial growth via optical density (600 nm) using a spectrophotometer (Spectronic 20 Genesys). High performance liquid chromatography (HPLC) was used to measure chemical concentrations of the substrates. Samples were first filtered through a 0.2- m membrane filter (Whatman #6872-2502, PVDF membrane). HACH closed-reflux method COD digester tubes were used to monitor COD, a Teknar-Dohrmann Apollo 9000 TOC analyzer to measure DOC, and a Hitachi D-7000 HPLC system having a Supelco SupelcoSil LC18 column (25 cm x 4.6 mm) for HPLC analysis of 4-chlorocatechol, and a BioRad Aminex 87H column for monitoring acetate and 2-chloromuconic acid. The HPLC elution solvent was 70% pure HPLC-grade methanol plus 30% acetic acid in water (2% vol/vol) for the C18 column (retention time for TCP – 11.1 minutes, 4CC – 4.0 minutes), and it was dilute phosphoric acid

in water (0.04% vol/vol) for the Aminex column (retention time for AC – 9.8 minutes, CMA – 27 minutes). A standard curve for each compound was generated, and peak area was used to quantify sample concentrations.

2.6 - DNA Extraction

15-mL samples were centrifuged at 6000 rpm for 5 minutes, leaving approximately 2 mL of concentrated biomass. The DNA was extracted using a kit (Ultraclean Soil DNA Kit, MoBio Laboratories, Inc., Solana Beach, CA) according to manufacturer instructions and DNA was stored at –20 °C.

2.7 - Polymerase Chain Reaction

A portion of the 16S rRNA gene was PCR-amplified for DGGE analysis. Primers were GM5FGC and 907r (Teske et al. 1996). In all cases, PCR reactions were carried out in 50- L volumes using 1 L template DNA and a kit (PCR Master kit, Roche Diagnostics Corporation, Mannheim, Germany) according to manufacturer instructions. Primer concentrations in the reactions were 1 M. A touchdown PCR method (Teske et al. 1996) was used for amplification.

2.8 – DGGE

DGGE analyses were performed with a Bio-Rad universal mutation detection system. 2% acrylamide gels were prepared according to manufacturer instructions and 200 ng of DNA sample was loaded per well. The DNA concentration (ng/ L) was quantified through agarose gel electrophoresis and calibration against mass standards (Invitrogen Low Mass Ladder, 10068-013) using the Bio-Rad gel-doc 2000 camera system and accompanying software. Gels were run for 16 hours and stained for 1 hour in SYBR Green I nucleic acid stain (Molecular Probes, S7567). Gel images were captured with a Kodak DC-120 digital camera, and banding patterns analyzed with Bio-Rad Quantity-One gel

analysis software using the rolling disk method with the following parameters: background radius of 20, band sensitivity of 4.6, band minimum density of 0, band filter of 2.7, band shoulder of 21.3, and band size of 4. Similarity indices were also calculated with Quantity-One software using the Dice Coefficient, with intensity weighting on. Evenness values were calculated following Pielou's Evenness Index (Pommier et al. 2010). The steady-state data represent averages of the three DNA samples collected during the two-week steady-state interval. The post-perturbation similarity indices reported are the average for each post-perturbation profile (2, 4, 8, and 14 days) to the three-steady state profiles. For example, at 2 days post-perturbation, the similarity to the three steady state profiles was averaged into one number and plotted in Figure 7a at time point 2 days. Means were compared via T-test using equal or unequal variances, which was determined by an F-test, with a significance criterion of 95%.

3 - Results and Discussion:

3.1 - Steady-state performance and community structure:

An average of the final two weeks of steady-state data were used to assess bioreactor performance.

Figure 2 shows the average steady-state OD and effluent concentrations of COD and DOC for each of the reactors. All reactors had 103 mg COD/L in the influent, DOC and substrate concentration varied as shown in Table 1. All acetate, 2-chloromuconic acid, and 4-chlorocatechol was completely biodegraded in each reactor.

The OD results show that biomass accumulation was higher for non-chlorinated and more oxidized organics. The CMA reactor produced an OD only 43% as high as that of the AC reactor, while the 4CC reactor was only at 39%. This trend correlates with the degree of substrate chlorination and the number of oxygenation reactions required for catabolism. Because aerobic oxidation of mono- and di-chlorophenols is generally initiated through monooxygenase or dioxygenase reactions (Dahlen and Rittmann 2002a, b, Häggblom 1992), complete oxidation of chlorinated aromatics requires several oxygenation steps that cost the cells electrons and energy by oxidizing the substrate without net generation of NADH₂. Because oxygenations require an electron investment, they compete with other electron sinks, including respiration and synthesis. The consequence is decreased biomass yield (Dahlen and Rittmann 2002a, b). Additionally, some chlorinated compounds exhibit intrinsically slow kinetics or inhibition, which decrease the rate of transformation (Hughes and Parkin 1996). In contrast, acetate requires no oxygenation reactions, has intrinsically fast kinetics, and exhibits no inhibition at relevant concentrations.

Figure 2 also shows that output COD and DOC increased with degree of substrate chlorination. The AC reactor removed 80% of input COD and 83% of input DOC, whereas the CMA reactor removed 74% and 84%, respectively, and the 4CC reactor 68% and 60%, respectively. These measurements

correlate with the OD data: The AC and CMA reactors performed best at steady state, whereas the 4CC reactor performed worst.

The steady-state microbial community was assessed through richness and evenness, shown in Figure 3. The AC reactor generated the greatest richness, having an average of 21 operational taxonomic units (OTUs) for 16S ribosomal DNA, while the CMA and 4CC reactors presented only 14 and 13 OTUs, respectively. The AC reactor's diversity was significantly greater ($p = 0.010$ for AC vs. CMA, $p = 0.042$ for AC vs. 4CC) than each of the other communities, while the difference between the CMA and 4CC communities was not significant ($p = 0.795$). The AC reactor had much more diversity than the other reactors possibly because almost all heterotrophic strains are able to biodegrade it. Unlike richness, evenness showed no significant differences among the microbial communities (Fig. 3), and none of the substrates led to domination by one microbial type.

Table 2 summarizes the similarity indices for each community profile. The banding patterns were unique for each community, despite starting with the same inoculum in each case. One caveat is that the 4-CC community structure was more volatile during steady state than the AC and CMA reactors; the three banding patterns during steady-state for the AC reactor had a composite similarity index of 64.9 ± 10.4 (the average similarity of each of the steady-state profile to the other two steady-state profiles; if there was no variability during steady-state the composite similarity index would be 100), CMA was 70.6 ± 2.2 , but 4CC 34.4 ± 28.6 .

3.2 - Resilience and Resistance

We perturbed the AC, CMA, and 4CC reactors with a 100- M spike of TCP and monitored TCP, original substrates, COD, DOC, biomass accumulation, and community diversity for 14 days

immediately following the spike. The other components of the influent remained constant at their steady-state levels throughout the perturbation test.

Figure 4 shows the TCP concentration versus time compared to the predicted washout value, assuming no degradation in the continuously stirred tank reactor (the TCP addition to the CMA reactor was only 92 μ M, while it was 100 μ M for the AC and 4CC reactors). Past work (Marsolek et al. 2007) indicated that TCP concentrations greater than 40 μ M are biorecalcitrant, but TCP is biodegradable by adapted communities at concentrations below this threshold. Biomass sorption is minimal, as evidenced by the similarity of the washout curve to experimental data for the AC reactor. The TCP concentration never deviated from the washout curve for the AC reactor until day 11, when the TCP concentration was small and relative error is greater, and the CMA reactor showed only small divergence through day 8. In contrast, the 4CC reactor exhibited consistent divergence from the predicted washout line beginning on day 2.

Figure 5 shows the effect of TCP perturbation on removal of the normal substrate. The AC reactor again showed a dramatic negative trend: After 12 hours, 32% of the influent acetate (by concentration) was in the effluent. The CMA reactor had a smaller impact -- 14% of the influent CMA passing through the reactor --, but the 4CC reactor showed no leakage of 4-chlorocatechol at any time after the TCP perturbation. By 2 days, however, the original substrates were again being 100% biodegraded in all cases.

Figure 6 shows the OD, COD, and DOC data leading up to and after the perturbation. The AC reactor demonstrated an immediate and sustained decrease in OD, stabilizing at an OD value 65% of that from pre-perturbation levels. The CMA and 4CC reactors had opposite behaviors. The OD in the CMA reactor decreased initially, and then it increased until it stabilized at 92% of pre-

perturbation levels at 14 days. OD data for intermediate times were unreliable and are not included. The 4CC reactor had a 2% decrease in OD for the first 12 hours, but it subsequently increased, reaching 152% of its pre-perturbation level at 14 days. The COD and DOC data showed similar trends. In both cases, the AC reactor demonstrated low resistance, with short-term COD and DOC rising dramatically, but had good resilience by reclaiming steady-state performance by day 4 post perturbation. The CMA reactor demonstrated similar resistance as AC, but even better resilience, stabilizing near steady state performance by day 2. The 4CC reactor demonstrated the best relative resistance, but it exhibited elevated levels of DOC and COD until day 14. An important point is that, even after perturbation, the performance of the AC reactor still surpassed the CMA and 4CC reactors in terms of effluent COD and DOC concentration.

Community structure, assessed via similarity indices (scored relative to their steady-state profiles), was most stable for 4CC, followed by CMA and then AC. Figure 7a shows the similarity indices for each of the time points relative to the steady-state profile. Similarity comparison is difficult for the 4CC reactor, since it did not reach a stable profile at steady state, but averaging the three steady state profiles offers a comparison point and shows that variability remained consistent.

Figure 7b shows the impact of perturbation on the richness of the three communities. The richness of the AC community decreased to 52% of its steady state magnitude by day 11. In contrast, the richness of the CMA community was hardly affected, while the 4CC community fluctuated, but neither increased nor decreased significantly by day 14. The strong shift in community structure of the AC reactor correlates with the sharp decrease in OD, but does not correlate with COD and DOC removal. This indicates a shift in community structure towards a more ‘condensed’ community that could utilize AC at a similar rate to its predecessor.

These data, which were derived from experiments with single substrates to model varying degrees of photocatalytic oxidation of TCP, show similar trends to earlier work (Marsolek et al., 2014) that used actual photocatalytic effluents with varying degrees of oxidation of TCP. In both cases, the most degraded substrate (or substrate mixture in Marsolek et al., 2014) demonstrated the best steady-state performance, but relatively poor resistance and resilience to perturbation. In contrast, a less-degraded substrate (or mixture) performed relatively poorly at steady-state, but was more resistant to perturbation. Finally, this work showed that moderately oxidized photocatalytic effluents, modeled by CMA here, could combine adequate steady-state performance and resistance to perturbation. The community-diversity data and leakage of the normal substrate after perturbation show the fragility of the AC community to the toxic spike, indicating the value of some moderately and lightly degraded compounds such as CMA and 4CC to prime the community against toxic shocks.

4 - Conclusions

This work demonstrated that three unique substrates mimicking varying degrees of photocatalytic oxidation of 2,4,5-trichlorophenol produced divergent microbial communities with large variability in steady-state performance and resistance to perturbation. The bioreactor fed acetate performed best at steady state but its community was most impaired as a function of perturbation by TCP. However, COD removal in the acetate reactor was still greater than that of the other reactors post-perturbation and showed good resilience. The reactor fed 4-chlorocatechol, mimicking a lightly photodegraded effluent, performed poorly at steady state and demonstrated mixed resistance but strong resilience to perturbation in terms of organics removal; its community was least impaired by the perturbation. The reactor fed 2-chloromuconic acid, mimicking a moderately photodegraded effluent, performed adequately at steady-state, demonstrated similar resilience to perturbation in terms of organic removal as the acetate reactor, but with slightly better community resistance. The results agree well with previous work and suggest that photoeffluents containing easily biodegradable organics augmented

340 with some moderately and lightly degraded products could provide good steady state performance
341 while still priming the community against process upsets.

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Graphical Abstract

Three substrates were chosen to mimic varying degrees of photocatalytic oxidation of 2,4,5-trichlorophenol (TCP): acetate (AC), 2-chloromuconic acid (CMA), and 4-chlorocatechol (4CC). Chemostats fed these substrates were compared at steady-state (a), and after perturbation **from a spike addition of 100 μ M TCP** (b), both in terms of biomass growth and organics removal (**shown here for steady state**) and community structure (**shown here after perturbation**). These figures demonstrate the tradeoffs inherent between steady-state performance and resistance to perturbation, with AC performing well at steady-state, but suffering after perturbation by the toxic spike. (OD = optical density, COD = chemical oxygen demand, DOC = dissolved organic carbon, Similarity = measure of changes in microbial community structure)

Table 1

Table 1: Substrates used and their influent characteristics

Experiment ID	Substrates	Substrate Concentration (μM)	Input COD (mg/L)	Input DOC (mg/L)
AC	Sodium Acetate	1614	103	38.7
CMA	2-Chloromuconic Acid	646	103	46.5
4CC	4-Chlorocatechol	538	103	38.7

Table 2

Table 2: Similarity indices of the steady-state banding patterns for all pairwise comparisons. Similarity between three steady-state samples is shown in the diagonal (with 95% confidence interval). All of the communities were unique, despite starting from the same inoculum, underscoring the impact of substrate on community structure.

	AC	CMA	4CC
AC	64.9±10.4	14.2	16.3
CMA	14.2	70.6 ±2.2	10.9
4CC	16.3	10.9	34.4±28.6

Figure 1

Figure 1: Structures of the parent compound, 2,4,5-trichlorophenol (TCP), and three model photocatalysis products: 4-chlorocatechol (4CC), 2-chloromuconic acid (CMA), and acetate (AC).

Figure 2

Figure 2: Comparison of reactor steady-state performance in terms of optical density, effluent COD, and effluent DOC. Error bars indicate 95% confidence limits. All acetate, 2-chloromuconic acid, and 4-chlorocatechol was biodegraded.

Figure 3

Figure 3: Comparison of richness (number of OTUs) and evenness for each reactor at steady state. The AC reactor demonstrated significantly more diversity, but evenness was similar among all reactors. Error bars indicate 95% confidence limits of the final three DNA samples during steady state.

Figure 4

Figure 4: TCP concentration after the 100 μM TCP perturbation (92 μM for CMA) (a) and ratio of the measured TCP concentration vs the concentration predicted for washout of a conservative tracer (b).

Figure 5

Figure 5: Effect of TCP perturbation on removal of the normal substrate. Substrate leakage was zero for all of the substrates prior to the perturbation. After the perturbation, the AC reactor was the most significantly impaired, while the CMA was slightly impaired, and the 4CC reactor exhibited no effect.

Figure 6

Figure 6: The effect of a 100 μ M spike of TCP on the biomass accumulation (a), COD (b), and DOC (c) in the AC, CMA, and 4CC reactors. The AC reactor had the worst resistance, and poor resilience in terms of OD. The 4CC reactor had good resistance but poor resilience, while CMA demonstrated good resilience in terms of OD, COD, and DOC.

a)

a)

Figure 7

Figure 7: a) Similarity indices and b) richness for each of the three communities relative to the steady-state profiles. The time-zero point represents the average similarity and richness of the steady-state communities for each reactor. Error bars indicate 95% confidence interval comparing community profile to three steady-state community profiles. The CMA and 4CC communities demonstrated greater resistance to perturbation than the AC reactor, although resilience was similar based on richness.