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# Application of a Redox Gradostat Reactor for Assessing Rhizosphere Microorganism Activity on Lambda-Cyhalothrin

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**Abstract** Bacterial activity on pesticides can lead to decreased toxicity or persistence in aquatic systems. Rhizosphere activity is difficult to measure in situ. To mimic rhizosphere properties of the soft rush, *Juncus effusus*, a single-stage gradostat reactor was developed to study cycling of lambda-cyhalothrin by rhizobacteria and the effects of Fe(III) and citrate, both common in wetland soil, on lambda-cyhalothrin degradation. Redox gradient changes, greater than ±10 mV, were apparent within days 5–15 both in the presence and absence of ferric citrate. Through the production of a redox gradient ($p < 0.05$) by rhizobacteria and the ability to measure pesticide loss over time ($p < 0.05$), reactors were useful in expanding knowledge on this active environment.

**Keywords** Wetlands · *Juncus effusus* · Pyrethroid insecticide

Synthetic pyrethroids such as lambda-cyhalothrin are commonly used in the southern United States to control cotton insect pests, have very low water solubility and an affinity to solid surfaces. As a result, they are moderately persistent in soils, especially in those with large amounts of organic matter. During storm or irrigation runoff events, these bound residues may be transported from the field into subsequent aquatic receiving systems. Such action is detrimental to both vertebrates and invertebrates in aquatic ecosystems (Bennett et al. 2005; Budd et al. 2007; Werner et al. 2010). Researchers have focused on a variety of best management practices that can be implemented to mitigate potential negative impacts to receiving aquatic systems. Use of aquatic plants, such as the soft rush *Juncus effusus*, in conjunction with drainage ditches as filters has been investigated to prevent runoff contamination of surface waters (Moore et al. 2001; Bennett et al. 2005). While this particular practice has demonstrated success, few studies have examined the effect rhizosphere microbial communities have on the fate of synthetic pyrethroids, such as lambda-cyhalothrin.

Considering the strong affinity of lambda-cyhalothrin to solid surfaces (He et al. 2008), this study emphasized bacterial action on lambda-cyhalothrin present within the rhizosphere of the soft rush, *J. effusus*, a prominent aquatic emergent plant.

Pesticide effects on soil microbial communities have been extensively studied (Imfeld and Vuilleumier 2012). The current study took the opposite approach by asking what effect bacterial communities have on the presence of pesticides around plants. Typically dominated by a Gram-negative microbial community, the rhizosphere is an area of increased microbial activity and biomass of plants (Morgan et al. 2005). Anderson et al. (1994) reported $4.2 \times 10^5$ microbial constituents in rhizosphere soil, but an order of magnitude fewer microbial constituents ($3.5 \times 10^4$) were present in non-vegetated soil. This study also observed enhanced herbicide (atrazine, metolachlor, and trifluralin) degradation in the rhizosphere (Anderson et al. 1994).

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Xenobiotic degradation within the plant rhizosphere is generally attributed to the presence of a microbial consortium, rather than one particular microbial member (Gilbert et al. 2003).

In an effort to assess the loss of lambda-cyhalothrin as a result of bacterial activity within the rhizosphere, bioreactor system methodology was investigated. Bioreactors have been constructed to assess rhizosphere and rhizobacteria associations concerning nitrogen fixation reactions and degradation of organophosphate insecticides (Fritzsche and Niemann 1990; Wimpenny et al. 1992; Ueckert et al. 1995). The primary devices utilized in these studies were the gradostat and bioreactor flasks. Gradostats were constructed as a series of in-line chemostats with bidirectional pumps that fed different nutrient types, ultimately resulting in a nutrient gradient. A simpler system was later developed and termed the directly coupled gradostat; designed to study growth in chemical gradients (Wimpenny et al. 1992). In the current study, a modified gradostat was constructed that retained only the ability to create a gradient (i.e. nutrient, redox).

In nature, Fe(III) is a common electron acceptor in subsurface and aquatic environments with an important role in redox gradient formation by oxidizing organics and subsequently being reduced to Fe(II). In conjunction with the prevalence of Fe(III) in subsurface environments (i.e. wetlands), citrate is a common wetland plant exudate that can serve as a carbon source for microorganisms inhabiting rhizoplane and rhizosphere environments (Kamilova et al. 2006). As a result, this study also examines the influence, of ferric citrate upon the biodegradation of lambda-cyhalothrin via a co-metabolic affect (Hazen 2010).

## Materials and Methods

The modified gradostat used a closed system with both a single reservoir and a single discharge end, creating a one-way flow-through system (Fig. 1). Media flow was gravimetrically controlled. A solid inert matrix was used to simulate surrounding soil and root surface for adhesion interactions. Instead of imposing superficial gradients upon the system, rhizobacteria were allowed to impose their own redox gradients based upon their ability to biochemically cycle lambda-cyhalothrin as their only carbon source and in the presence of ferric citrate.

Chromaflex low-pressure chromatography columns (three, 60 cm length × 2.5 cm I.D., Kontes Glass Co.) with 11 custom-made 6.35 mm holes at 5 cm apart the length of the columns were used as the reactor. Teflon-faced butyl septa (13 × 6.35 mm Kimble Glass Inc.), were placed in each of the eleven column holes and used as sampling locations. Column tops and bottoms were sealed

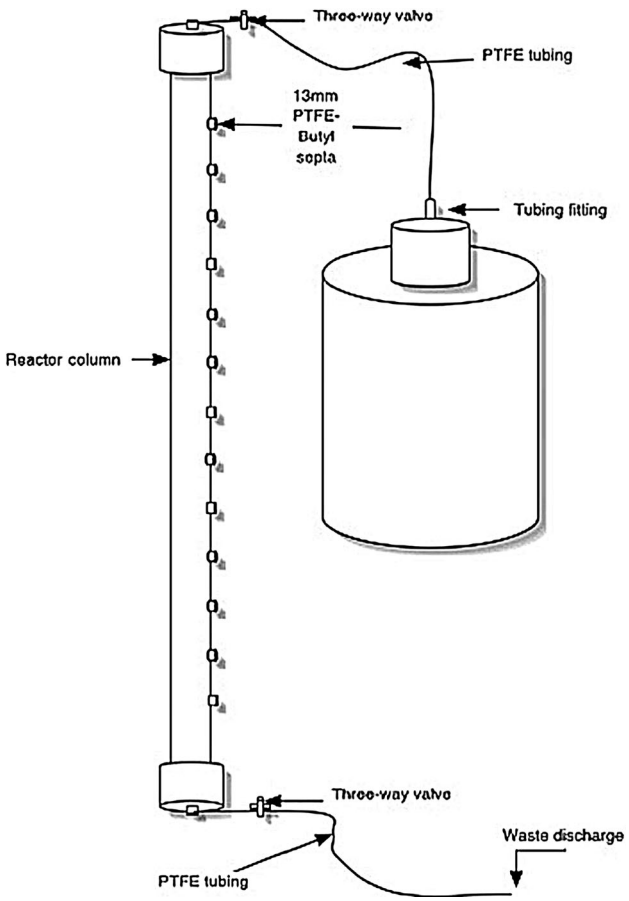


**Fig. 1** Diagram of a single-stage redox gradostat reactor

with the following fittings (after being autoclaved with the matrix inside the column): two screw end caps (2.5 cm I.D., Kontes Glass Company), two Teflon end fittings (2.5 cm OD, Kontes Glass Company), two Teflon bed supports (20 μm fritted discs Kontes Glass Company), two Teflon reducer fittings (Kontes Glass Company), two Teflon nut line fittings (Kontes Glass Company), two line ferrules (Kontes Glass Company), and two, 3.05 m lengths of Teflon tubing (Kontes Glass Company). Mystic White quartz sand (#90 fine sand, New England Silica Inc., CT) was used as reactor matrix due to its low fluorescing properties and inertness. Sand was used to fill the column to the neck resulting in a headspace of approximately 1.5 cm when columns were fully assembled. Three-way valves (Cole-Parmer) with male luer lock connections were spliced into Teflon tubing leading into and out of reactors. The top valve was used to inoculate the column aseptically by closing off the feed media line and injecting 10 mL of inoculum onto the top of the reactor matrix via a 10 mL sterile syringe (Becton–Dickinson) with a female luer lock connection. Fluorinated 18-L Nalgene™ carboy lids were fitted with a 1/4-28 Teflon nut line fitting from Kontes to

accommodate 1/8 OD Teflon line, enabling the carboy lids to remain sealed. Carboys were then placed at a height of 40 cm and the reactor was clamped onto a ring stand with three-finger clamps. The bottom three-way valve at the effluent end was used to control reactor flow rate.

Minimal media for enrichment was a mixture of a mineral salt stock consisting of 20 mM $NH_4Cl$ and 2 mM $KH_2PO_4$ buffered to pH of 7 with 20 mM HEPES, a modified Hutner's mineral base, and 5 % lambda-cyhalothrin (Karate™, Syngenta Technologies, 13.1 % active ingredient) in ethanol. Final prepared media contained 6.7 µL of sterile mineral salt stock, 1 µL of filter sterilized (Sterile Acrodisc, 0.2 µm, Pall-Gelman), 5 % lambda-cyhalothrin (3.82 mL of 0.131 g $mL^{-1}$ lambda-cyhalothrin in 6.18 mL of 1 % ethanol), and 10 mL of standard sterile mineral base stock. Solution components were prepared per liter with sterile deionized water. Liquid enrichment media was so that one carboy contained modified Hutners mineral base with 0.111 µM lambda-cyhalothrin and one carboy contained 5 mM ferric citrate $FeC_6H_5O_7{\cdot}5H_2O$ (Sigma), modified Hutners mineral base and 0.111 µM lambda-cyhalothrin. Before addition of lambda-cyhalothrin, all carboys were autoclaved for 3 h and sterile media was allowed to flow through the column to saturation for 24 h. Solid media, 18 g $L^{-1}$ of Noble Agar (Difco Media) was added to liquid enrichment media as prepared above and sterilized. Lambda-cyhalothrin, 0.111 µM, was added to cooled sterilized media, and enrichment plates were poured and allowed to cure at room temperature for 24 h before subculturing using primary enrichment culture isolates. Final inoculum density was 0.1 optimal density (OD) in a 10 mL inoculum volume.

The column was then drained to determine void volume, 110 mL, and 10 % of this volume was used to inoculate the column. Flow rate was reestablished to an initial rate of 0.52 mL $min^{-1}$ and checked every third day to ensure consistency. Before inoculation, the three-way valve was closed at the effluent end. Samples from each gradostat port, 1–11, were taken daily for a period of 30 days and redox potential (mV) measured in triplicate using a Corning combination redox (mV), temperature (°C) probe with meter equipped with a 10 mL syringe. Samples were then injected into 1 mL auto-sampler vials for extraction and analysis of lambda-cyhalothrin using the method outlined by Bennett et al. (2000). Statistical analysis was performed using ANOVA single factor within the Microsoft Excel statistical analysis package.

## Results and Discussion

Changes in redox potential (mV) were detected through the course of the experiment in all reactors. The first significant ($p < 0.05$), changes in redox (greater than ±10 mV), occurred between days 5 and 15 (Tables 1, 2).

Production of a redox gradient within the rhizosphere microenvironment is a measure of metabolic activity (Fritzsche and Niemann 1990; Morgan et al. 2005). Production and reduction of redox potential (mV) within a system suggests biotic activity upon a variety of substrates. Data suggest rhizosphere microbial flora are capable of biochemically cycling lambda-cyhalothrin as a sole carbon source (Table 1). Natural assemblages' ability to produce a redox gradient within the reactor suggests their ability to utilize lambda-cyhalothrin as an electron donor (no other carbon/electron donor was furnished with the minimal medium) and create a gradient ($n = 25$, $p < 0.05$). Likewise, it would support microbes at different depths (per port number) as expected in actual rhizosphere niches supported by a variety of gradients including redox. Loss of extractable lambda-cyhalothrin from within the column is additional evidence that production of the redox gradient is a result of lambda-cyhalothrin utilization as a sole source of carbon and electrons. Results agree with Meyer et al. (2013), who utilized soil slurries in batch culture and observed approximately 45 % loss of compound in 28 days. However, Meyer et al. (2013) used whole soil, which can introduce abiotic factors that influence accessibility of lambda-cyhalothrin to metabolism (Sukul and Spiteller 2001). Additionally, use of whole soil may introduce soil organic matter and electron acceptors, which influence metabolic activity of the microorganisms present. This is a plausible explanation of the observed differences in percentages of lambda-cyhalothrin lost.

Alternate electron acceptors such as Fe(III) are key to the biodegradation of many anthropogenics, including agricultural chemicals, and there exists a relationship between organic carbon oxidation and subsequent reduction of Fe(III) (Nealson and Saffarini 1994). With the prevalence of Fe(III) in many types of aquatic and subsurface environments, the oxidation of organics, both natural and anthropogenic, have the potential to be coupled to Fe(III) reduction, which has been shown in the bioremediation of organic contaminants including aromatic hydrocarbons and pesticides (Cozarelli et al. 1995; Chang et al. 1998; Lovely and Anderson 2000). The production of a variety of exudates, such as the organic

**Table 1** Gradostat I with redox potentials (mV) at central ports over time (days) without addition of ferric citrate and with 50 µg $L^{-1}$ lambda-cyhalothrin at pH 6.8

| Day | Port 1 (mV) 5 cm depth | Port 6 (mV) 30 cm depth | Port 11 (mV) 55 cm depth |
|---|---|---|---|
| 5 | 256 | 240 | 244 |
| 10 | 231 | 206 | 185 |
| 15 | −9.30 | 1.60 | −1.60 |
| 20 | −2.20 | 1.30 | 1.50 |
| 26 | 1.80 | 0.40 | 0.30 |

**Table 2** Gradostat II redox potentials (mV) at central ports over time (days) with addition of 5 mM ferric citrate and 50 μg $L^{-1}$ lambda-cyhalothrin at pH 3.0

| Day | Port 1 (mV) 5 cm depth | Lambda-cyhalothrin concentration (μg $L^{-1}$) | Port 6 (mV) 30 cm depth | Lambda-cyhalothrin concentration (μg $L^{-1}$) | Port 11 (mV) 55 cm depth | Lambda-cyhalothrin concentration (μg $L^{-1}$) |
|---|---|---|---|---|---|---|
| 0 | 10 | – | 5.5 | – | 17 | – |
| 5 | −1.7 | 8.03 | −5.4 | 0.40 | −8.5 | BD |
| 10 | −0.2 | 67.7 | −3.2 | 2.45 | −23 | 1.16 |
| 15 | −29 | 2.01 | −37 | BD | −36 | BD |
| 20 | −33 | 1.16 | −37 | BD | −37 | BD |
| 25 | −46 | 0.22 | −38 | 0.35 | −34 | 4.89 |
| 30 | −32 | – | −28 | – | −27 | – |

*BD* below limit of detection (0.2 μg $L^{-1}$)

– Indicates no sample

acid citrate, produced by the plants and excreted into the rhizosphere could potentially serve as an alternate electron donor and increase the degradation rate of anthropogenics (Chang et al. 1998; Singh et al. 1999; Luu and Ramsay 2003). While anthropogenics may not serve as the sole source of carbon and electrons, they could potentially be degraded through co-metabolism via the degradation of citrate (Chang et al. 1998; Singh et al. 1999). The production of redox gradients in the presence of ferric citrate and corresponding pesticide degradation (Table 2) demonstrate the capacity of the isolates to produce a redox gradient ($n = 31$, $p < 0.05$) that is concomitant with degradation. Similar results, demonstrated by Meyer et al. (2013), also suggest this relationship as the redox values in differing sediments correlates to loss of pesticides, including lambda-cyhalothrin, over time. The current study offers further evidence of the importance of microbially-mediated pesticide remediation in the rhizosphere of vegetation. With this knowledge, science can begin to target more efficient and effective remediation practices.

**Acknowledgments** Mention of equipment, software or pesticide trade names does not constitute an endorsement for use by the USDA. All programs and services of the USDA are offered on a nondiscriminatory basis without regard to race, color, national origin, religion, sex, marital status, or handicap.

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