The fates of three polar organic solvents in a microcosm constructed wetland wastewater treatment system
by Janet Lyn Kowles

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering
Montana State University

Abstract:
The EpiCenter project at Montana State University proposed to create a national showcase for environmentally sustainable building design. The proposal included a greenhouse-contained constructed wetland to treat waste from lavatories and undergraduate chemistry laboratories. The goal of this study was to evaluate the capability of natural constructed wetland systems to treat this mixed waste to a safe and legal discharge level.

Fifteen 4-inch diameter planted column batch reactors were used to study the fate of several polar organic solvents in a constructed wetland system. Twelve of the columns were variously planted with four wetland plant species - Juncus effusus, Carex lurida, Iris pseudacorus and Pondeteria cordata, while three remain as implanted controls. The three solvents being studied - acetone, tetrahydrofuran (THF) and 1-butanol - were chosen because they are hydrophilic, moderately degradable, and commonly used in organic chemistry laboratories. The solvents were added at 100 mg/l to post-primary wastewater from the Bozeman municipal wastewater treatment plant in order to simulate a mixed waste stream. Preliminary experiments with jars showed that sorption and direct volatilization were not major removal pathways.

All three solvents were largely removed from the batch systems within the 14 day incubation period. 90% removal of l-butanol typically took less than three days. 90% removal of acetone required from 5 to 10 days, and 90% removal of THF required at least ten days. In winter incubations, 90% removal of THF was frequently not achieved. ¦ Planted columns performed dramatically better than unplanted columns, with Juncus effusus standing out as an exceptional treatment plant. Seasonal effects were observed, but were less in the planted columns than in the unplanted controls. The plant effects are believed to be at least partly due to higher microbial metabolic activity in a more oxygenated environment. This is confirmed by lower sulfide production in the planted columns.

The amount of evapotranspiration occurring in planted columns correlated significantly with the amount of solvent removed. A deterministic model, based on a prediction of plant uptake of nonionic dissolved chemicals, suggests that as much as 28% of the tetrahydrofuran in solution could have been removed through plant transpiration. Based on this model, plant-assisted vaporization of solvents accounts for some, but not all, of the plant treatment effects observed.

THE FATES OF THREE POLAR ORGANIC SOLVENTS IN A MICROCOSM CONSTRUCTED WETLAND WASTEWATER TREATMENT SYSTEM

by

Janet Lyn Kowles

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Environmental Engineering

MONTANA STATE UNIVERSITY
Bozeman, Montana

June 2001

APPROVAL

of a thesis submitted by

Janet Lyn Kowles

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

Otto R. Stein, Ph. D. Otto R Stein (Signature) 6/20/01 Date

Approved for the Department of Civil Engineering

Donald A. Rabern, Ph. D. Don Rabern (Signature) 6/25/01 Date

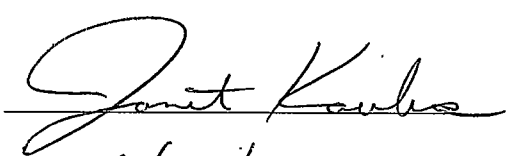
Approved for the College of Graduate Studies

Bruce McLeod, Ph. D. Bruce R. McLeod (Signature) 7-10-01 Date

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library.

If I have indicated my intention to copyright this thesis by including a copyright notice page, copying is allowable only for scholarly purposes, consistent with "fair use" as prescribed in U.S. Copyright Law. Requests for permission for extended quotation from or reproduction of this thesis in whole or in parts may be granted only by the copyright holder.

Signature 

Date 6/20/2001

ACKNOWLEDGEMENTS

I would like to acknowledge the Montana State University Epicenter Project for providing the impetus and part of the funding for this project, and the National Science Foundation Graduate Research program for funding the second year of the project.

I would like to thank my advisor, Dr. Otto Stein, for his guidance and suggestions on this project, as well as my other committee members Dr. Anne Camper and Dr. Warren Jones. Working with them in a spirit of collaboration was truly a joy.

Special thanks goes to John Neuman, for his invaluable help in the laboratory. Others deserving of thanks include laboratory assistant Kelly Jacques, CAT scan operator Mike Edens for his noble efforts and Julia Sharp for statistical consultations.

Lastly, I would like to thank my officemate Debbie Borden, whose friendship made work more of a pleasure and Bozeman more of a home.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The EpiCenter project at Montana State University proposed to create a national showcase for environmentally sustainable building design. The proposal included a greenhouse-contained constructed wetland to treat waste from lavatories and undergraduate chemistry laboratories. The goal of this study was to evaluate the capability of natural constructed wetland systems to treat this mixed waste to a safe and legal discharge level.

Fifteen 4-inch diameter planted column batch reactors were used to study the fate of several polar organic solvents in a constructed wetland system. Twelve of the columns were variously planted with four wetland plant species – Juncus effusus, Carex lurida, Iris pseudacorus and Pondeteria cordata, while three remain as unplanted controls. The three solvents being studied – acetone, tetrahydrofuran (THF) and 1-butanol – were chosen because they are hydrophilic, moderately degradable, and commonly used in organic chemistry laboratories. The solvents were added at 100 mg/l to post-primary wastewater from the Bozeman municipal wastewater treatment plant in order to simulate a mixed waste stream. Preliminary experiments with jars showed that sorption and direct volatilization were not major removal pathways.

All three solvents were largely removed from the batch systems within the 14 day incubation period. 90% removal of 1-butanol typically took less than three days. 90% removal of acetone required from 5 to 10 days, and 90% removal of THF required at least ten days. In winter incubations, 90% removal of THF was frequently not achieved. Planted columns performed dramatically better than unplanted columns, with Juncus effusus standing out as an exceptional treatment plant. Seasonal effects were observed, but were less in the planted columns than in the unplanted controls. The plant effects are believed to be at least partly due to higher microbial metabolic activity in a more oxygenated environment. This is confirmed by lower sulfide production in the planted columns.

The amount of evapotranspiration occurring in planted columns correlated significantly with the amount of solvent removed. A deterministic model, based on a prediction of plant uptake of nonionic dissolved chemicals, suggests that as much as 28% of the tetrahydrofuran in solution could have been removed through plant transpiration. Based on this model, plant-assisted vaporization of solvents accounts for some, but not all, of the plant treatment effects observed.

INTRODUCTION

As humankind begins to take seriously the search for more ecological, less destructive ways to live on the earth, the field of waste management is increasingly drawing inspiration from natural systems. This was the impetus behind the Epicenter project at Montana State University. The project proposed to create a new building to house part of the chemistry department. The building would provide a healthy working and learning environment, utilize rainwater and sunlight efficiently, and recycle nearly all of its waste. The building would feature a wetland-contained treatment system to handle the combined wastewater load from lavatories and laboratories in the building. This research project was undertaken in order to evaluate the feasibility of a greenhouse-contained constructed wetland system for this application, and to suggest what limitations a wetland treatment system would impose upon the laboratories operating in the building.

This research focused on non-halogenated polar organic solvents – a class of chemicals which is generally highly water soluble, of low to moderate toxicity, and biodegradable. Of all chemical classes used in laboratories, this group appears to have the highest potential for effective removal in a wetland type system. The three solvents chosen to represent this class are acetone, tetrahydrofuran (THF) and 1-butanol, or normal butyl alcohol.

Acetone is a natural fermentation product, and is a common industrial solvent. It is universally used in chemistry laboratories for the cleaning of glassware because it evaporates quickly when not dissolved in water. Acetone is regulated under the Resource

Conservation and Recovery Act (RCRA) at a level of 0.28 mg/l for land disposal of hazardous wastewaters (RCRA, 1997).

THF is a simple cyclic compound, used industrially as a solvent for polyvinylchlorides and other polymers (Verschueren, 1996). Under RCRA land disposal restrictions, hazardous waste streams regularly containing THF must be subject to wet air oxidation or chemical oxidation followed by carbon adsorption or incineration (RCRA, 1997). No acceptable concentration is given.

1-butanol was chosen to represent the primary alcohol chemical class. Toxic environmental effects of 1-butanol have been reported at concentrations as low as 8 mg/l (Verschueren, 1996). It is regulated by RCRA at a level of 5.6 mg/l for land disposal (RCRA, 1997).

Constructed wetlands offer a great deal of promise as an effective, adaptable and low cost treatment alternative, yet they are not necessarily appropriate for all applications. By sketching the fates of acetone, tetrahydrofuran and 1-butanol in a wetland system, it is hoped that the capabilities and limitations of these systems can be better delineated and understood.

A study to this end was divided into two phases. In the first phase, a synthetic wastewater containing the three solvents was placed into jars filled with a gravel medium which had previously been used in a mesocosm wetland system. In the second phase, wastewater was placed into planted mini-column wetlands, to explore the effects of the plants on the solvents and also of the solvents on the plants.

The goals of the jar experiments were threefold. The first goal was to gain a general idea of what would happen to the three solvents of concern in a wetland media.

The second goal was to look at different levels of background organic matter to see what effect that would have on solvent degradation. The third goal was to compare the response of an otherwise-identical sterile system to a biologically active system to determine what fraction of the solvent disappearance observed could be credited to biological degradation.

The mini-column experiments were used to compare treatment effectiveness among four wetland plant species and unplanted controls. The effects of temperature and seasonal cycles of plant growth were also explored. By looking at redox indicators and evapotranspiration in the columns, it was also possible to make some speculative conclusions about treatment mechanisms at work within the wetland systems.

Due to the novel and interdisciplinary nature of this study, the Literature Review presents a summary of literature from several disciplines, including microbiology, plant science and organic chemistry as well as environmental engineering. The Materials and Methods section describes the procedures followed in both experimental phases. The findings of both studies are discussed separately, and eventually integrated in the Results and Discussion. The final section gives Design Implications and Conclusions.

LITERATURE REVIEW

Constructed Wetlands for Wastewater Treatment

The popularity of constructed wetlands for improving water quality has been booming around the world. More than 650 natural and constructed wetlands have been used to treat wastewater in North America (Brockson, 1998). In small communities and rural areas, wetlands offer an effective treatment alternative at a relatively low cost. They also offer additional benefits, including wildlife habitat and aesthetic values. Brix (1997) specifically mentions *Iris pseudacorus*, or yellow flag, as a potential treatment wetland plant with an attractive appearance.

In a subsurface flow wetland, the water flows below the surface of the wetland media (usually gravel) rather than ponding on top. This eliminates mosquito problems, increases wastewater contact with active biological surfaces, and provides some insulation against cold winter temperatures (Reed et al., 1995). Reed also estimates that the largest wastewater flow at which subsurface flow wetlands are economically feasible is about a million gallons per day (1 MGD or 4000 m^3/day). For larger systems, the large requirements of land area and gravel media offset the savings in technical equipment and operational costs.

In municipal-type wastewater applications, wetland performance is primarily judged by ability to remove organic carbon, usually measured as oxygen demand (chemical-COD or biological-BOD), as well as the nutrients nitrogen and phosphorous. Design models for treatment wetlands are not based on the processes functioning in the

wetland (Burgoon et al., 1995), but rather predict treatment based on either regressions of data collected from existing systems, areal rate constants or 1st order plug flow kinetics (Reed et al., 1995). Kadlec and Knight (1996), observing that even effluent from natural wetlands has some oxygen demand, suggest a modified first order model where concentration approaches not zero, but rather an irreducible background concentration, C*. For BOD, they recommend a value of C* equal to 3.5 + 0.053 times the influent BOD.

Although functioning treatment wetlands are generally designed as plug flow systems, many microcosm studies have attempted to use batch reactors to study wetland mechanisms and kinetics. Brix and Schierup (1990) suggested that draining and filling a batch reactor will cause air to become entrained, and therefore performance will over-estimate the through-flow systems they are intended to mimic. Burgoon et al. (1995) showed that the drain and fill process did not improve waste treatment in a gravel-media batch reactor, and calculated that the amount of oxygen entrained by the gravel biofilm could oxidize less than 2% of the carbonaceous BOD in the wastewater. Biederman (1999) emphasized that the time-for-space substitution made when batch systems are used to model plug flow kinetics does not account for the differences in microbial ecology between the two systems. Burgoon et al. (1995) found that first order removal rates were similar for batch and plug-flow systems. Biederman (1999) found that plug flow systems performed better than batch, suggesting that a batch system is a conservative predictor of flow-through performance, at least for oxygen demand.

Plant Effects – Oxygen Transport and Seasonal Variation

In nature, plant tissue serves as a vital conduit for oxygen transport to roots and for escape of waste gases, including CO_2 and methane, to the atmosphere (Kadlec and Knight, 1996). The ability of plants to flourish in flooded environments is largely dependent on their capacity to transport oxygen to their root systems. Some of this oxygen escapes into the rhizosphere, where it performs other functions important to the plant, including detoxifying H_2S and reduced forms of iron and manganese (Reddy et al., 1989). Some plants release more oxygen to the root zone than others due to a variety of factors, including different root respiratory rates, varying structural ability to transport gases, and differences in root wall composition that affect how easily oxygen can diffuse out (Reddy et al., 1989).

In oxygen-deprived environments, which includes the substrates of almost all natural and constructed wetlands, there is competition for available oxygen between respiring root tissues and the microbial and chemical processes in the rhizosphere. Based largely on contradictory results from Brix and Shierup (1990), Kadlec and Knight (1996) warn against inferring oxygen transport from COD reduction alone, and suggest that plants transfer oxygen only to support root respiration. However, Brix (1997) later reemphasizes the role of macrophytes in oxygenating the root zone in treatment wetlands. The amount of oxygen which escapes from the root is largely a function of the oxidation-reduction level of the surrounding sediments (Sorrel and Armstrong, 1994).

Early experiments tried to directly measure the amount of free oxygen released into oxygen-depleted nutrient solutions by wetland plants. This quantity is disappointingly small, because this sort of laboratory solution does not mimic the high

oxygen demand and low redox potential of natural soils (Sorrell and Armstrong, 1994). For example, a study by Moorhead and Reddy (1988) calculated average plant-to-rhizoshere oxygen transport rates ranging from less than zero (oxygen uptake) to as high as 3.95 g O_2 h^{-1} per kg root mass (dry weight) for a series of floating and emergent macrophytes in nutrient solutions. In contrast, a study of *Juncus ingens* showed that, while transport to an oxygen-depleted solution was negative or very small, transport rates to an oxygen-scavenging titanium citrate solution averaged 40 g O_2 kg^{-1} h^{-1} (Sorrell and Armstrong, 1994).

Reddy et al. (1989) quantified oxygen transport by nine species of floating and emergent plants using the reduction in 5-day biological oxygen demand (BOD_5) in municipal waste as a measure of oxygen supplied by the plants. An impermeable barrier prevented atmospheric oxygen from diffusing into the solution. While this method may not provide the true transfer rate, relative rates for different species may be pertinent. Their top performing emergent plants were *Canna flaccida*, *Scirpus validus* and *Pondeteria cordata*, with average transport rates of 0.81, 1.08, 1.01 and 0.78 g O_2 kg^{-1} h^{-1}, respectively. Common cattail (*Typha latifolia*) and *Scirpus pungens* performed significantly worse at 0.16 and 0.18 g O_2 kg^{-1} h^{-1}, respectively, suggesting that even different species within a specific genus can have different transport rates.

To conceptualize the oxygenation processes in a wetland, where free oxygen is usually not detectable, the image of the oxidation-reduction, or redox ladder is useful. The chemical redox couple or couples which control the electron activity in a solution roughly determine what the electrochemical redox potential (E_H) of the solution will be. In complex systems, redox potential is best thought of as a quantitative measure of the

redox couples, O_2/H_2O is the highest on the redox ladder. Theoretically, a standard mixture of H_2O and O_2 should have an E_H of 812 mV. Figure 1 shows how common environmental redox couples compare on the redox ladder, down to CO_2/CH_4 at -244 mV (Stumm and Morgan, 1996). As the oxygen becomes depleted from a nutrient and organic-rich medium, its redox potential drops, until oxygen can no longer be detected at around 333 mV (Steinberg, 1994).

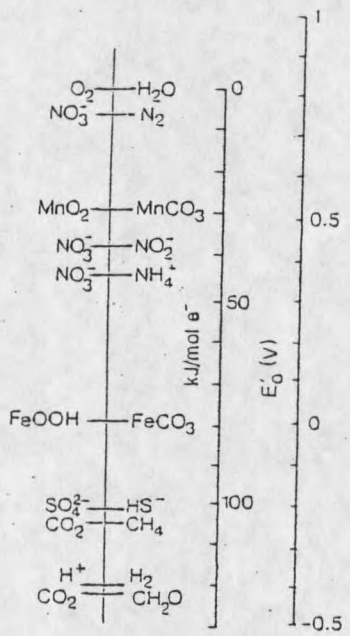


Figure 1. Relative potential of prominent redox couples in natural aquatic systems. Fenchel and Finlay, 1995

Experiments done at Montana State University from 1996 to 1999 monitored E_H in 10-inch diameter gravel media planted columns filled with a synthetic municipal wastewater with an average influent COD of 470 mg/l. After wastewater was added, E_H in all columns consistently dropped to around –200 mV within 24 hours. Columns planted with *Typha latifolia* (cattail) and unplanted columns achieved significant removal

planted with *Typha latifolia* (cattail) and unplanted columns achieved significant removal of COD, especially in the first 24 hours, but redox potential remained near –200 mV throughout the 20-day incubations. In columns planted with *Scirpus acutus* and *Carex rostrata*, which demonstrated superior COD removal, redox levels tended to recover over the incubation period, to average final values of 230 and 330 mV, respectively. The measured solution COD level at which increases in E_H began to appear was 30 mg/l (Allen, 1999).

Due to oxygen competition between root respiration and reduced sediments, temperature and season have a major impact on oxygen transport, which in turn affects treatment. Several studies were able to measure oxygen release from roots only at lower temperatures. *Shoenoplectus lacustris* demonstrated positive O_2 release only at 5 or 10°C, while a *Phragmites australis* released oxygen at 5°C, not at 20°C (Sorrell and Armstrong, 1994). Allen (1999) reported that differences in COD removal and redox potential among plant species were greater at low temperatures (4°C versus 24°C). With *Typha latifolia* and unplanted controls, 6-day COD removal decreased at cold temperatures. However, in *Carex rostrata* and *Scirpus acutus* columns, 6-day COD removal increased at colder temperatures. Recovery in E_H value was also most pronounced in these species at lower temperatures.

Properties of Acetone, THF and 1-Butanol

The three representative polar organic solvents in this study were acetone, 1-butanol and tetrahydrofuran (THF). Some basic properties of these three chemicals are summarized in Table 1.

Property	Acetone	THF	1-Butanol
Chemical formula	CH_3-CO-CH_3	C_4H_8O-cyclical	CH_3-CH_2-CH_2-CH_2OH
Molecular weight	58.08	72.10	74.12
Aqueous solubility	miscible	miscible	77,000 mg/l (a)
Dimensionless Henry's Constant (H)	1.52×10^{-3} (b)	4.50×10^{-3} (c)	3.47×10^{-4} (a)
Octanol/Water Partition Coeff. (K_{OW})	0.58 (a)	5.4 (c)	7.6 (a)

Table 1. Properties of acetone, THF and 1-butanol.
Notes: (a) Vershueren, 1996 (b) Schwarzenbach et al, 1993 (c) Bhattacharya et al, 1996

The dimensionless Henry's Constant (H) is defined as the chemical concentration in air (mg/m^3) divided by chemical concentration in water (mg/m^3) at equilibrium. These solvents are of only moderate volatility, and their H values range within one order of magnitude of each other. Solvents with low H values were chosen for this study because air stripping rather than biological treatment is often the most economical method of removal for chemicals with high H values.

The Octanol/Water Partition Coefficient (K_{OW}) is the ratio of solvent concentration in octanol to the concentration in water in an equilibrated system. A higher K_{OW} indicates the chemical has a higher affinity to octanol, and to organic material in general, as compared to its affinity to water. The K_{OW} has been correlated with diverse environmental processes including soil adsorption, biological uptake, lipophilic storage and biomagnification (Verschueren, 1996).

The biodegradability of the three solvents has been quantified in the literature using several standard or modified standard techniques. One technique is to conduct a biological oxygen demand (BOD) test and report the oxygen used as a percent of

theoretical oxygen demand (%ThOD). ThOD is calculated from a balanced oxidation-reduction reaction where the solvent is mineralized to CO_2 and H_2O. The standard inoculum for this test is dilute municipal sewage. BOD tests can be conducted over any period of time, although 5 days is standard.

According to the Handbook of Environmental Data on Organic Chemicals (Verschueren, 1996), the 5-day BOD of acetone can range from 14% ThOD to as high as 65%. After 10 days, measured values collected from the literature range from 55% to 74%. For 1-butanol, the 5-day BOD ranges from 33% ThOD to as high as 79% (Verschueren, 1996).

THF was once classified as "not readily biodegradable" (Kohlweyer et al., 2000), however there are organisms known to use it as a food source. Delay times before degradation begins can be greater than 25 days in unacclimated systems, leading to dramatic ranges in standard test results. One study reported a half-life for THF of 4.2 to 8.7 days in soil (Verschueren, 1996).

Bacteriology and Degradation Pathways for Solvents

Researchers have demonstrated the biological degradation of all three solvents of concern in this study. Degradation pathways and mechanisms have also been proposed.

There exists a fairly large body of work on the microbial metabolism of acetone, which many bacteria are able to utilize as a growth substrate (Clark and Ensign, 1999). In aerobic wastewater treatment, acetone is regarded as easily degradable (Platen and Schink, 1989). Several degradation pathways have been studied in aerobic organisms, including hydration to 1,2 propanediol (CH_3-CHOH-CH_2OH) or oxygenase catalysis to acetol (CH_3-CO-CH_2OH), which can easily be broken apart into C_1 and C_2 fragments

(Bonnet-Smits et al., 1988). More recently, several organisms have been isolated which are capable of degrading acetone anaerobically, using a variety of electron acceptors including nitrate (Platen and Schink, 1989; Bonnet-Smits et al., 1988), sulfate (Platen et al., 1990; Janssen and Schink, 1994) and CO_2, which is reduced to methane (Platen and Schink, 1987). A fermentative culture which completely degrades acetone to methane and CO_2 has also been studied (Platen et al., 1994). Most of these organisms were originally isolated from anaerobic digesters at community wastewater treatment plants. Some of the anaerobic acetone degraders employ a unique degradation pathway, where acetone is first carboxylated with available CO_2 to form four-carbon acetoacetate (CH_3-CO-CH_2-COO^-) (Platen et al., 1990; Platen et al., 1994).

As would be expected, the rate of degradation is much faster the higher an organism is operating on the redox ladder. For example, a denitrifying bacterial strain growing on acetone under optimal conditions had a doubling time of 5.7 to 6 hours (Platen and Schink, 1989). By contrast, a sulfate reducing bacterium, *Desulfobacterium cetonicum* grew on acetone with a doubling time of 69 hours (Janssen, 1995), while a doubling time of 2.8 to 3.5 days was calculated for a methanogenic enrichment culture growing on acetone (Platen and Schink, 1987).

Bernardt and Diekmann (1991) isolated six aerobic *Rhodococcus* strains capable of growth on tetrahydrofuran. The organisms were able to completely degrade 8 mM THF (576 mg/l) in less than 4 days, during which time the optical density of the culture nearly tripled. Above 10 mM (720 mg/l) the lag phase of growth became prolonged, although growth was not entirely inhibited. They proposed a mechanism whereby a carbon adjacent to the oxygen on the THF ring is hydrated, then oxidized to a cyclic

ether. The ring can then be cleaved into 4-hydroxybutyric acid - a straight chain molecule with an alcohol on one end and a carboxylic acid on the other, which is easily metabolized. Another THF degrader, a *Pseudonocardia* species, was isolated from sludge from a community wastewater treatment plant in Germany (Kohlweyer et al., 2000).

1-Butanol is degraded by a wide variety of organisms. In general, a primary alcohol is oxidized to its respective fatty acid: ethanol goes to acetate, propanol goes to propanoate, etc (Eichler and Schink, 1984). 1-Butanol can be oxidized in two steps to n-butyrate (CH_3-CH_2-CH_2-COO^-) which can be used as a growth substrate by many organisms (Poelarends et al., 2000; Arp, 1999). A kinetic study of primary alcohol utilization in *Acholeplasma* and *Mycoplasma* species concluded that, at any particular substrate concentration, oxidation rate decreased with increasing molecular mass of the alcohol, (Abu-Amero et al., 1999). A study of an *Acetobacterium* species oxidizing primary alcohols under methanogenic conditions concluded that while butanol and pentanol led to much slower growth than ethanol or propanol, they also formed more methane. This suggests that organisms growing on longer-chain alcohols are more efficient in hydrogen scavenging (Eichler and Schink, 1984). Branching in alcohols generally make them even more difficult to degrade (Mormile, et al., 1994) and the ternary form *tert*-butanol has proved especially resistant to biodegradation (Henry et al., 1996).

The biodegradation of secondary alcohols is intimately connected with the respective ketones. In particular, 2-propanol can be oxidized to acetone according to the thermodynamically unfavorable reaction (Terzis, 1994):

$$4\ CH_3\text{-}CHOH\text{-}CH_3 = 4\ CH_3\text{-}CO\text{-}CH_3 + 4H_2 \quad \Delta G°_{RXN} = +98.8\ kJ \qquad \text{(Eq. 1)}$$
(2-propanol) (acetone)

Terzis proposed that oxidation to acetone is the first step in the anaerobic degradation of 2-propanol.

Solvent Degradation in a Variety of Engineered Treatment Systems

Numerous studies have explored the fates of various organic solvents in a variety of treatment systems. A study by Bhattacharya et al. (1996) looked at compounds regulated under the Resource Conservation and Recovery Act (RCRA, 1997) in a pilot-scale activated sludge system. Municipal wastewater was spiked with 11 solvents including acetone and THF, each at a concentration of 0.25 mg/l. Their expectation, confirmed by their results, was that while chlorinated VOC's are primarily removed by an air stripping mechanism, nonchlorinated VOC's mainly biodegrade. While this conclusion might be expected based on physical parameters such as H and K_{OW}, they found that the extent to which individual solvents are adsorbed, stripped or degraded is not predictable based on these or other standard parameters. The Bhattacharya study included seven sampling "events" over a period of as many weeks. They measured adsorption and volatilization directly, and inferred solvent degradation from the unaccounted-for fraction. In their first sampling event, 95.7% of acetone was removed, with 94.2% biodegraded. 54.8% of THF was removed, with 15.6% stripped and 37.2% biodegraded. Sorption was not a major mechanism for either solvent. Over the seven events, average acetone removal was 94.1% (σ= 6.1). Average THF removal over six events was 89.1% (σ= 17.2). Although the authors fail to comment on it, their THF removal appears to improve consistently over the course of the study, with 100% removal

reported for events 6 and 7. This could indicate a gradual adaptation by the microbial community.

Recognizing that often a less expensive technology can be adequate to achieve desired treatment goals, Hannah et al. (1986 and 1988) compared the VOC removal obtained by six alternative treatment systems to a pilot activated sludge system. Their general conclusions were that activated sludge or a standard rate trickling filter were most effective at removing VOC's. Facultative lagoons with long retention times came next, followed by aerated lagoons with shorter retention times, then by high rate trickling filters. Chemical precipitation and direct filtration were effective treatment methods only on substances that sorb strongly to the solid phase. Although none of the three compounds used in the present study was used in Hannah's, ethylbenzene, used in the Hannah study, behaved very similarly to acetone in the Bhattacharya study. Greater than 95% removal of ethylbenzene was achieved in activated sludge, standard rate trickling filters and facultative lagoons. The direct filter achieved 71% removal, while the high-rate aerobic lagoon had 70% removal of ethylbenzene.

Another branch of wastewater treatment research has focused on the feasibility of anaerobic (usually methanogenic) treatment systems for industrial solvent waste streams. Butanol and acetone are both listed as "amenable to anaerobic biotechnology" (Speece, 1983). Anaerobic systems function as complex populations of different types of organisms (Terzis, 1994). Monod kinetics, which are used to design activated sludge systems, are also considered appropriate for describing methanogenesis (Rajagopalan et al, 1998; Terzis, 1994). Many wastewater treatment texts, such as Grady, Daiger and Lim (1999), include full descriptions of Monod kinetics. Rajagopalan et al. (1998)

emphasize the difference between extant kinetics – the in-situ capability of biomass – and intrinsic kinetics – the capability of biomass under ideal conditions and with a full enzyme complement. Any effort to quantify microbial kinetics ex-situ is likely to achieve a compromise between these two ideal extremes. Speece (1983) also emphasizes the importance of trace elements, particularly iron, cobalt and nickel, without which anaerobic bioreactors are bound to fail.

One implication of Monod kinetics is of a minimum substrate concentration, S_{MIN}, below which biomass death and waste rates exceed growth. Data compiled by Terzis (1994) on the anaerobic treatment of 2-propanol yields an S_{MIN} of 120 mg COD/l, or 50 mg/l 2-propanol. Terzis concludes that anaerobic treatment is, at best, a first treatment phase with aerobic treatment as a necessary second step to meet water quality standards. In the context of this present research on constructed wetlands, it serves as a reminder that the many different processes in a wetland have different limitations and fulfill different niches.

Solvents and Wetlands

Most of the constructed wetland literature is focused either on bulk parameters such as BOD and COD, on nutrient removal, or on metal-laden waste streams. Studies of individual organic chemicals are few. Many petroleum companies, and some paper mills, have wetland projects underway for wastewater polishing, although much of this information is proprietary. Studies have also looked at surfactants, food processing wastes, pesticides, and naphthoic acid (Kadlec and Knight, 1996). Kadlec and Knight reported a zero order areal rate constant for several chemicals – 0.55 $g/m^2/day$ for phenol and 0.044 $g/m^2/day$ for naphthoic acid.

Kadlec and Knight also provide an extremely concise summary of the relevant mechanisms for solvent removal operating in wetland systems:

> The major routes for removal of hydrocarbons from wetland waters are (1) volatilization, (2) photochemical oxidation, (3) sedimentation, (4) sorption, and (5) biological (microbial) degradation. Three types of microbial processes can contribute: fermentation, aerobic, and anaerobic respiration.

In a subsurface flow wetland, photochemical oxidation is not an important mechanism. Sorption and sedimentation are also not expected to be important mechanisms with the highly hydrophilic, low molecular weight solvents chosen for this study.

In previous research using batch reactors (Allen, 1999) it was assumed that plants uptake only pure water and some nutrients, with all COD remaining in the bulk solution. Based on this assumption, it was reasoned that the water lost to the system due to evapotranspiration would be perfectly replaced by the inflow of clean water, and the net effect on the mass balance of COD would be negligible. In the context of this present research with solvents, this assumption was called into question.

For a dissolved component to enter into the tissue of a plant, it must pass through a waxy barrier called the Casparian strip, then desorb into the aqueous solution within the xylem tissue of the plant. How easily a solute can make this transition depends largely on the water solubility and membrane retention of the solute (Cunningham et al., 1996). A good estimator of these properties is the octanol-water partition coefficient, or Kow, which measures the lipophilicity of a compound. Shone and Wood (1974) defined a transpiration stream concentration factor (TSCF), which is equal to the ratio of solute concentration within the xylem sap to that in the external solution. Based on a study

looking at methylcarbamoyloxime and phenylurea pesticides, Briggs et al. (1982) found TSCF to be a nonlinear function of Kow, according to Equation 2.

$$TSCF = 0.784 \exp\left[\frac{(\log K_{OW} - 1.78)^2}{-2.44}\right] \qquad \text{(Eq. 2)}$$

Precise results are expected to vary among plant species, particularly depending upon the composition of lipids in the roots, however the work of Briggs et al. (1982) and Hsu et al. (1990) supports the general validity of this relationship over a variety of plant species, compounds and experimental techniques (Cunningham, 1996).

MATERIALS AND METHODS

Overview

This research consisted of two experimental phases. In the first phase, solvent degradation was studied in gravel-filled jars using a synthetic wastewater containing the three solvents of concern – acetone, tetrahydrofuran and 1-butanol. The results from these simple experiments were used to design and interpret the second phase, in which degradation of the same solvents was studied in experimental planted mini-column wetlands. First, the analytical methods used in both research phases will be described, then the materials and methods of these two experimental phases will be described separately.

Analytical Methods

The concentrations of all three solvents plus 2-propanol were measured on a gas chromatograph (GC) using a ten foot (3.048 m) by 1/8 inch (3.175 mm) outer diameter stainless steel column with 3% SP1500 on 80/120 Carbopak B packing (Supelco 1-2592). A carrier gas of helium was used at a flow rate of 25 ml/min and a pressure of 42 psi (289.6 kPa).

All samples for GC analysis were filterered through a 0.2 micron syringe filter into 2 mL amber glass vial with a TFE-lined septa. Vials were stored in a refrigerateor for up to a week before before being analyzed. To analyze samples, 2 μL of the aqueous solution were injected, and oven temperature was increased from 100 to 180° C at a rate of 15 degrees per minute. The inlet temperature was maintained at 220° C. A flame ionization detector was used, with a temperature of 275° C. The entire process was

automated with a Hewlett Packard Model 5890, Series II GC and autosampler. A standard curve was generated for all three solvents from 1 to 100 PPM approximately every two months, and a linear fit was generated using least squares regression. Several 50 PPM standards were run during every sample batch to ensure that the method maintained calibration to within 10%.

The COD of samples was measured using a potassium dichromate colorimetric Hach test. Samples from jar experiments were filtered through a 0.2 micron syringe filter, while mini-column COD samples were not filtered, although care was taken to avoid visible clumps of sloughed biomass. Other colorimetric Hach tests were used to measure ammonia, total nitrogen, total phosphorous and sulfide. Samples of each of the three solvents of concern (plus 2-propanol) dissolved in deionized water were individually measured for COD using the colorimetric Hach test. This provided an experimental value of chemical oxygen demand per solvent mass. By combining this information with the measured COD and solvent concentrations in each wastewater sample, it was possible to estimate the amount of COD accounted for by the solvents.

The anions nitrate, phosphate, and sulfate were measured on a Dionex Model LC10-20 ion chromatograph.

Jar Experiments

Experimental Design

Three experimental treatments were established, each in three replicates. The first, labeled H for high COD, was designed to mimic a high-strength municipal wastewater spiked with 100 mg/l of each of the three representative solvents. The second treatment, labeled L for low COD, consisted only of the three solvents at 100 mg/l each

in tap water. The third treatment, labeled C for control, was identical to the H treatment except that it was conducted under sterile conditions.

The synthetic wastewater used in the H and C treatments consisted of 928 mg/l of ground-up Excel dog food and 230 mg/l of ammonium chloride dissolved in tap water. This mixture has approximantely 450 mg/l COD and 110 mg/l total Kjeldahl nitrogen. This is double the strength of synthetic wastewater which had been used in previous research to mimic a domestic wastewater (Blicker, 1997).

The experimental setup consisted of nine glass jars filled with biofilm-coated gravel, 1.5 to 2.0 cm diameter, taken from existing wetland mesocosms in a greenhouse at Montana State University. These mesocosms had previously been used with a wastewater designed to mimic the Butte, Montana Metro Storm Drain – high metal loads and high sulfate – with 250 to 500 mg/l COD added as sucrose (Sturm, 2000). The jars containing the H and L treatments were standard, 2-liter Wheaton wide mouth jars, 122 mm diameter. The three control jars were made of autoclavable borosilicate glass, with a capacity of 2.2 liters and a diameter of 110 mm. All jars had a single air vent with a 0.2 micron bacterial filter, to allow transfer of gases with the atmosphere without microbial contamination of the control jars. Each jar had a sampling port of FEP-coated Tygon tubing lashed to a stainless steel rod, with the end located approximately 8 cm from the bottom of the jar. In the autoclavable control jars, the Tygon sampling tube was connected to a fitting in the jar lid, with a rubber septum that enabled samples to be drawn through a syringe without opening the jar to the atmosphere.

To prepare the C jars for their experimental incubation, synthetic wastewater was first mixed without solvents. This wastewater, additional tap water, and the three C jars

containing gravel were separately autoclaved for one hour. To prevent vaporization, solvents were added aseptically to the wastewater after autoclaving, and this mixture was then transferred to the gravel-filled jars. The additional sterile tap water was used to replace the wastewater that vaporized in the autoclave, restoring the mixture to its original volume. Once the C jars were sealed, they remained sealed over the entire incubation, with samples drawn through the rubber septa using a sterile syringe. The H and L jars were opened for sampling. Incubations were conducted at room temperature of approximately 20°C.

The first jar incubation was begun on August 30, 1999. That experiment ended on September 28. Due to analytical difficulties, the next incubation was not begun until January 22, 2000, ending on February 20th. The third incubation was begun on March 24th and ran until April 22nd. The first two incubations differed from the third in two ways. During the first two incubations, the concentration of synthetic wastewater in the L jars was one quarter that of the H jars, rather than zero. It was reduced to zero for the third incubation because differences between the L and H jars had not been significant in the first two incubations. Also, in the first two incubations, the experimental technique for establishing and sampling the C jars aseptically had not been perfected, so removal effects characteristic of biological degradation were observed. The data from the first two incubations are not reported, and results are based on the third incubation.

Sampling and Analysis

Samples were drawn from each jar through the sampling tube using a glass syringe. Samples were taken first after 1 hour, then after 1, 2, 3, 5, 7, 9, 12, 16, 20 and 25 days. All samples were filtered through a 0.2 micron syringe filter. COD samples were

digested immediately, while solvent samples were refrigerated until they could be analyzed according to the protocols described in the Analytical Methods section.

Planted Mini-columns

Physical Apparatus

The centerpiece of this research project consisted of planted mini-columns used as microcosms of constructed treatment wetlands. Twenty-four columns were constructed of 4 inch (10 cm) inner diameter PVC pipe, ASTM D-2729, cut to a length of 18 inches (46 cm) and capped on one end (see Figure 2), though only 15 were used in the final experimental design. This small size had two perceived advantages. The first was that enough wastewater to fill the fifteen columns used in the study could be easily transported. Secondly, this size is the largest that could physically fit in a CAT scanning device maintained at the Civil Engineering Department at Montana State University. The intent was to monitor plant growth and sediment build-up non-destructively using the CAT scan device. Unfortunately, the CAT scan was unable to produce useful images of the inside of the columns, because the high level of radiation required to penetrate the gravel made it impossible to distinguish between plant matter and water.

Each column was given a bottom drain plug hole and a tube fitting 3.5 inches (9 cm) from the bottom through which the water level was maintained. Fifteen columns were placed into a rectangular support array, and connected to a fluid level maintenance manifold with opaque flexible tubing. The manifold had a continuous drip of Bozeman tap water from a peristaltic pump, which maintained an overflow level 18 inches (45.7 cm) above the base of the columns.

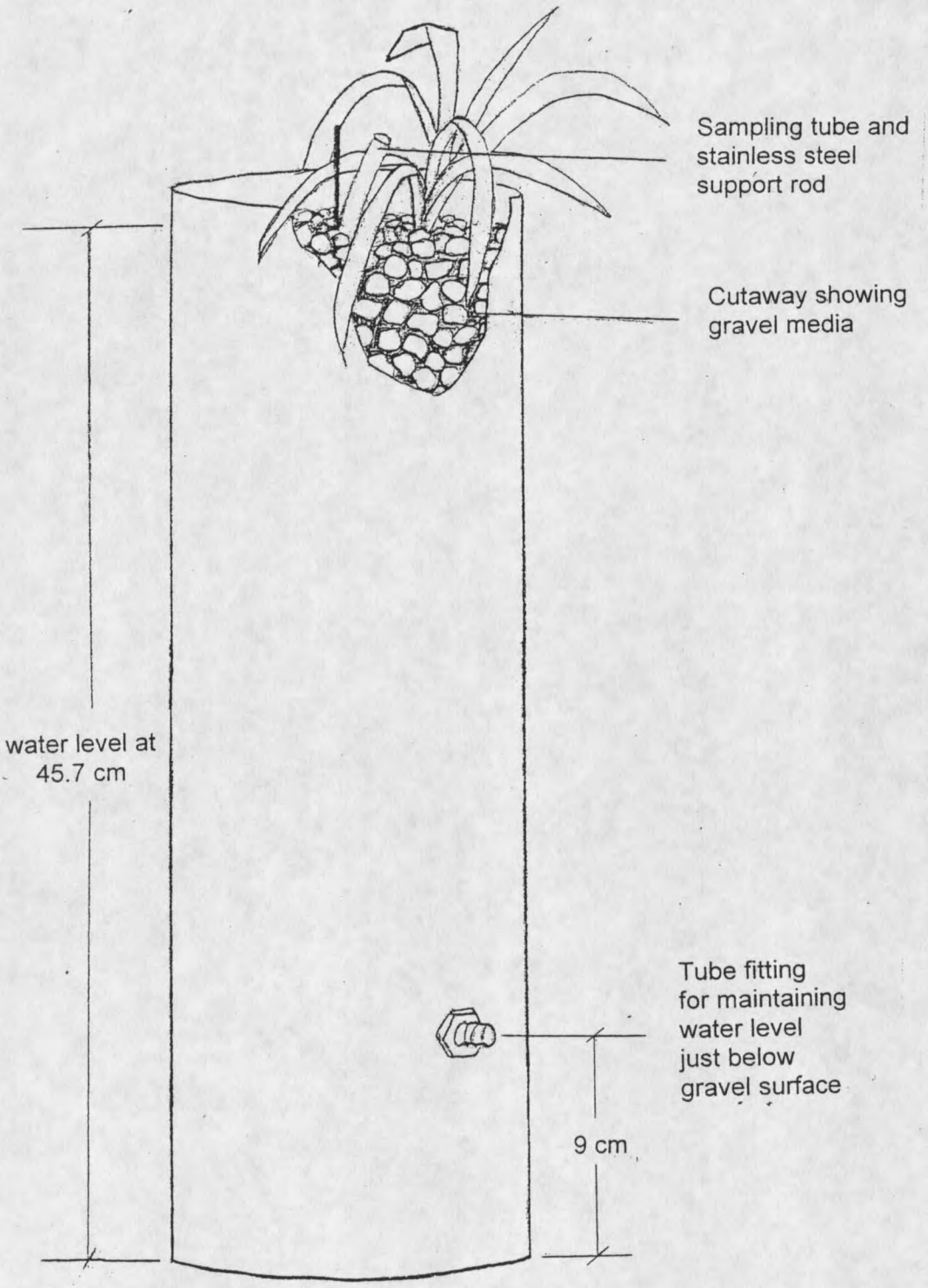


Figure 2. Schematic diagram of mini-column apparatus.

The gravel media used was washed pea gravel 1/8 to 1/2 inch (0.4 to 1.3 cm) diameter. This media supported a stainless steel rod to which was attached a 1/8 inch (3.2 mm) inner diameter sampling tube of FEP-lined Tygon. The tubing extended from above the gravel surface to a point approximately eight inches (20 cm) above the bottom. Previous studies at MSU (Allen, 1999) have shown that measured distribution of chemical wastewater parameters does not vary significantly with depth in similar batch systems.

Plants

Four plant species were studied in the mini-column experiments. Each species was initially planted in 5 columns, in order to allow for transplant failure. Propagules were shipped in early January, 2000 from Wetland Supply, Inc. of Apollo, Pennsylvania. All species were chosen specifically for a greenhouse-contained treatment application, and were expected to exhibit a shorter senescent period than wild Montana native plants. The four species are summarized in Table 2.

Scientific Name	Common Name	Characteristics
Carex lurida	Shallow sedge	Grass-like with high capacity to oxygenate the root zone. (Allen, 1999)
Juncus effusus	Soft rush	Aerenchymous, hardy and dense-growing
Iris pseudacorus	Yellow flag	Aerenchymous flowering perennial
Pondeteria cordata	Purple pickerelweed	Broadleaf flowering perennial

Table 2: Summary of plant species planted in mini-column microcosms.

Upon receipt, each propagule was rinsed to remove soil particles and placed inside a column. Pea gravel was filled in around the plant roots to within 2 centimeters of the top of the column. Four columns were left unplanted. All columns were filled with Peter's 50 PPM N 20-10-20 GP (The Scott's Company) fertilizer solution to provide

nutrients for plant growth, and the water level was maintained near the gravel surface. Columns were drained and refilled with fertilizer water approximately once per month until mid May, when they received their first dose of wastewater. During this initial establishment period, there were several occasions when leaks in the columns or plugs in the drip line caused trauma to the growing plants. Of the five *Pondeteria* propagules originally planted, two failed to grow at all and a third fell victim to drought trauma, therefore only two *Pondeteria* columns were used in experimental data collection. The fifteen experimental columns consisted of four *Carex*, three *Juncus*, three *Iris*, two *Pondeteria*, and three unplanted control columns. These columns were randomly arranged in a rectangular array, with columns placed 25 cm on center.

Wastewater

The specific goal of this research project was to explore the fates of polar organic solvents in a constructed wetland treatment system. As with the jar experiments, wastewater was prepared with approximately 100 PPM each of acetone, tetrahydrofuran (THF) and 1-butanol. Departing from previous MSU research that has used synthetic wastewater, solvents were added to post-primary municipal wastewater in order to best duplicate the microbial ecology expected to develop in a functional treatment wetland.

Wastewater was collected from the Bozeman Wastewater Treatment Plant immediately after the junction where supernatant streams from the twin primary clarifiers combine. Wastewater was manually scooped using a pole sampler and collected in two 20-liter Nalgene carboys. Solvents were measured volumetrically and added to the carboy by pipette. Wastewater was then mixed and siphoned into the mini-columns. According to Bozeman treatment plant operational records, the average influent BOD is

152 mg/l, with a standard deviation of 29, based on daily measurements in the year 2000. On average, 19% of influent BOD is removed during primary clarification, based on post primary BOD measurements taken approximately once per week in 2000. The average post-primary BOD of 123 mg/l has a standard deviation of 22. Ninety percent of recorded measurements were between 91 mg/l and 147 mg/l. According to Metcalf and Eddy (1991) a BOD of 110 mg/l is considered weak, while 220 mg/l is considered medium strength. Based on the conclusions of the preliminary jar experiments (see Results and Discussion), low oxygen demand wastewater is appropriate for this treatment system. Samples of Bozeman post-primary wastewater were measured for COD, ammonia, nitrate, phosphate, sulfate, total nitrogen and total phosphorous. Specific procedures are described in the Sampling and Analysis section.

Experimental Design

As explained in the Plants section, the period from mid January until mid May 2000 was devoted to plant establishment. From mid May until late July, columns were drained and refilled with fresh Bozeman post-primary wastewater four times in order to begin establishment of an attached-growth microbial community. The last two wastewater doses also included the three solvents of concern at approximately 100 PPM.

Starting in July, 2000, six 14-day incubations of municipal wastewater containing solvents were conducted in the fifteen mini-column system described above. Seasonal variations were expected to have a dramatic impact on wetland performance. For this reason, incubations were conducted under two different conditions – three simulated summer greenhouse conditions, and three simulated winter. During summer incubations, which were conducted from July through October, 2000, the greenhouse controls were set

to maintain a temperature of 24° C during the day and 16° C at night. During the winter incubations, which were conducted from November 2000 through January 2001, controls were set for 13° C during the day and 7° C at night. Actual temperatures were much more variable due to limitations of the greenhouse temperature control systems. The system automatically recorded the mean, high and low temperatures every hour.

Table 3 summarizes the experimental schedule for the mini-columns. All experimental incubations were conducted using all three solvents of concern, unless otherwise indicated. Incubations ET1 and ET2 were for evapotranspiration studies, as described in the section by that name.

Sampling and Analysis

For each incubation, wastewater was sampled prior to transfer to the columns, then samples were drawn periodically from the sampling port in each column. The first sample was drawn 1 hour after the wastewater was introduced, then subsequently after 1,2,3,5,7,10, and 14 days. In four of the incubations, an 8-hour sample was added to this original sampling regimen.

Samples were drawn from the sampling port tubes using a glass syringe. The Teflon luer tip on the syringe created an airtight seal with the Tygon tubing. For each sample, 2 mL were first drawn and discarded, in order to flush the volume of liquid which had been stagnant inside the sampling tube. Samples of 5 to 6 mL were collected in test tubes for later analysis in the laboratory, except for periodic sulfide analysis, which was done on site due to the high volatility of sulfide. Samples were stored in a refrigerator for up to two days before initial processing.

Mid-January 2000	Propagules planted in columns, nourished with fertilizer
May 16, 2000	Columns filled with Bozeman post-primary wastewater
June 6, 2000	Columns drained and refilled with wastewater
June 22, 2000	Columns drained and refilled with wastewater plus three solvents
July 11, 2000	Columns drained and refilled with wastewater plus three solvents
July 28, 2000	Incubation 1 begun (no THF in *P. cordata*)
August 18, 2000	Lines flushed. Incubation 2 begun (no THF in *P. cordata*)
September 20, 2000	Columns filled with wastewater (no solvents). Incubation ET1 begun
October 11, 2000	Incubation 3 begun.
October 30, 2000	Columns filled with wastewater (no solvents) Greenhouse set to winter temperature regimen
November 15, 2000	Incubation 4 begun.
December 8, 2000	Incubation 5 begun.
December 23, 2000	Columns filled with wastewater (no solvents)
January 16, 2000	Incubation 6 begun
January 30, 2000	Columns filled with wastewater (no solvents). Incubation ET2 begun.

Table 3. Experimental timeline for mini-column experiments.

All samples were measured for COD and for the concentrations of the three solvents according to protocols described in the Analytical Methods section. Selected samples were also measured for ammonia, nitrate, phosphate, sulfate, sulfide, total nitrogen and total phosphorous. Samples for COD analysis were not filtered, unlike samples in the jar experiments.

Evapotranspiration Study

At both summer and winter temperatures, measurement of evapotranspiration was made in the wetland columns in order to model plant uptake and transpiration as a possible removal pathway, and to look for correlations between evapotranspiration and removal efficiency. Because of the continuous drip mechanism employed during degradation incubations, it was necessary to measure evapotranspiration independently, as shown in the experimental timeline. A small divot was created in the gravel in each column, large enough to allow an ink marking to be made on the column wall below the main gravel surface. The automatic drip was turned off, and once or twice per day water was manually added to each column up to the marked level, and the amount of water added to maintain a constant water level was recorded. Because no leaks or other removal mechanisms were apparent, daily water loss is attributed solely to evapotranspiration.

Statistical Methods

Differences among treatments were analyzed using the General Linear Model protocol in Minitab v. 13. Differences among treatments were evaluated using Tukey 95.0% simultaneous confidence intervals, or $\alpha = 0.05$.

RESULTS AND DISCUSSION

Jar Experiments

Solvent Overview

Synthetic wastewater containing approximately 100 mg/l each of acetone, tetrahydrofuran (THF), and 1-butanol was added to wetland gravel-filled glass jars and sampled periodically over a 26 day incubation. There were three treatments, each with three replicates. Treatment L had no additional COD or nitrogen, treatment H had approximately 450 mg/l background COD in the form of ground up dog food and 110 mg/l total Kjeldahl nitrogen from dogfood and added ammonium chloride (Blicker, 1997). The control treatment C was identical to treatment H except that it was conducted under sterile conditions.

The jar experiments confirmed what had seemed apparent from the literature, namely that of the three solvents of concern, 1-butanol was the most easily degraded, followed by acetone, with THF being the most recalcitrant (see Figure 3). Comparison between the sterile control treatment and biologically active H and L treatments show that the major removal mechanism for all solvents can be attributed to biological activity.

In the biologically active jars, 90% removal of 1-butanol was obtained in 5-6 days. Ninety percent removal of acetone required 8-12 days, but was complicated by an interesting redox-related transformation to 2-propanol (see Carbon Load Effects). Both solvent removal rates are comparable to, or exceed, the highest removal rates reported in 5 and 10 day BOD test data available in the literature (Verschueren, 1996). THF was

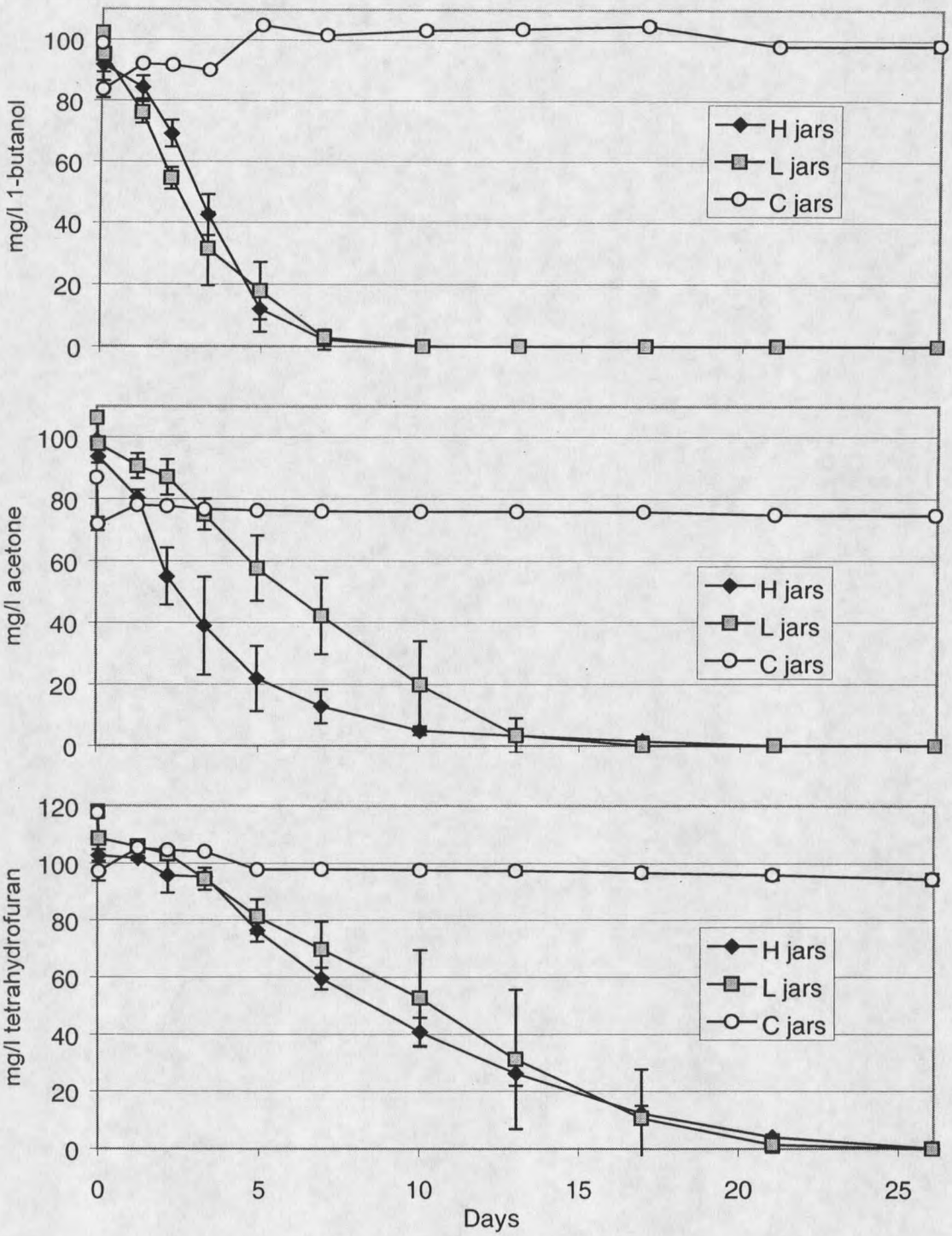


Figure 3. Concentrations of 1-butanol, acetone and tetrahydrofuran in 26-day jar incubations with gravel wetland media. Error bars indicate ± one standard deviation among three replicates of each treatment. The first point shown is of concentration prior to pouring wastewater into jars.

90% removed after 15 to 20 days. The measured half-life was 8 to 12 days, which is several days longer than the values reported in the Verschueren handbook for soil systems. A half-life does not appear to describe the system well, however, as there appears to be a lag period of approximately three days followed by a decay which resembles zero order more than first order.

For all treatments, significant solvent losses of up to 20% were measured within one hour after filling the jars, which could be due to sorption to the gravel medium, volatilization during transfer, or other unaccountable experimental error. After the first day, solvent concentrations in control jars remained quite constant. The largest observed loss over the remaining 25 days of the incubation was 10.4% of THF, which is just outside the GC margin of error. Volatilization, sorption, spontaneous chemical breakdown or some other abiotic process do not appear to be significant removal pathways for any of the solvents in gravel bed systems after initial transfer loss.

Oxygen Demand and Carbon Load Effects

Significant differences between high-carbon H treatments and L treatments with no carbon added were observed in the removal of 1-butanol on days one and two. In general, the L treatment outperformed the H treatment, but overall differences were not significant at $\alpha = 0.05$. Differences in THF degradation were not significant at any time in the incubation.

Because all solvents present in solution contribute to chemical oxygen demand, measured COD is a bulk parameter which includes the amount of solvent plus the background soluble COD supplied by dog food and biomass. To determine the

background COD concentration at any time, the oxygen demand of the solvents must be subtracted from the measured COD.

The chemical oxygen demand of each of the three solvents and 2-propanol was independently measured over a range of concentrations varying from 10 to 100 ml. The results are summarized in Table 4. Measured chemical oxygen demand of the solvents is generally less than the theoretical values, assuming total oxidation to CO_2 and H_2O. The discrepancy is larger for the more recalcitrant solvents acetone and THF than for either of the alcohols measured. One possible reason for the discrepancy is volatilization of solvents under the high-temperature conditions of the COD test.

Oxygen Demand (mg O_2 / mg solvent)	Acetone	Tetrahydrofuran	1-Butanol	2-Propanol
Measured COD	1.93	2.19	2.51	2.40
Theoretical	2.21	2.44	2.60	2.40
Percent Measured	87%	90%	97%	100%

Table 4. Measured and theoretical COD of four solvents. Measured COD values are based on a linear regression of measurements at 10, 50 and 100 mg/l.

To compute the non-solvent COD, the concentration of each solvent measured on the GC for each sample is multiplied by the measured COD/solvent ratio in Table 4, and these values are subtracted from the total measured COD. Non-solvent COD values for each treatment are plotted on Figure 4. As expected, the non-solvent COD in the L treatments with no added non-solvent carbon hovered around zero throughout the duration of the experiment. Variations are likely due to experimental error, as well as COD from microbial byproducts and solvent degradation intermediates. The calculated non-solvent COD in the control treatment also remained relatively constant near 360 mg/l. In the high-carbon H treatment, non-solvent COD after 1 day averaged only 10

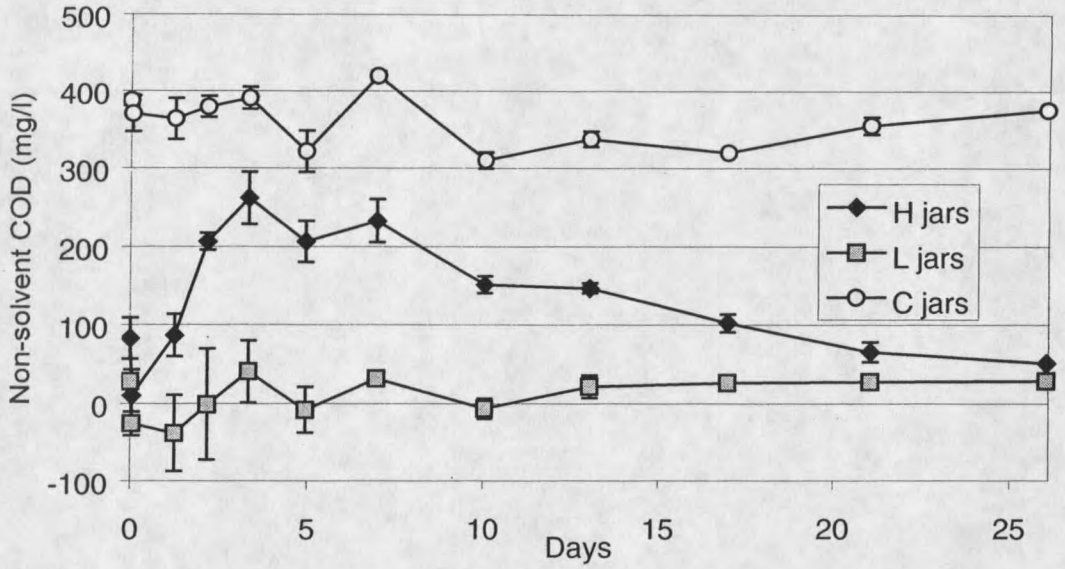


Figure 4: Measured COD in jars adjusted for theoretical oxygen demand of three solvents plus 2-propanol. Error bars indicate ± one standard deviation among three replicates of each treatment.

mg/l, but increased to 262 mg/l by day 3, then decreased to a final average COD of 52 mg/l on day 26. Because these COD samples were filtered, the most likely explanation for this behavior is that the COD in the synthetic wastewater was initially in particulate form, and had to be hydrolyzed to a soluble form by microbial exoenzymes. Only soluble COD is thought to be available for microbial metabolism (Grady et al., 1999). The COD in the control treatment wastewater apparently became solubilized in the autoclave. The non-solvent soluble COD in the control treatment gives an approximation of the initial total COD in the biologically active high carbon treatment. Over the 26-day incubation, microbial processes appear to have utilized over 300 mg/l of non-solvent oxygen demand in the high-carbon H treatment, as compared to none, or nearly none, in the treatment with no carbon added. Because oxygen demand is really a demand for

electron acceptors, the difference between these two treatments could be expected to have measurable effects on all redox processes in the system.

From day 1 through day 10, acetone concentration in the high carbon treatment is significantly less than the treatment without carbon (see Figure 3). However, GC analysis revealed an additional peak gaining prominence in the high carbon treatment as the acetone disappeared. Potential degradation intermediates such as propanediol, acetol, or acetoacetate (Bonnet-Smits et al., 1988; Platen et al., 1990; Platen et al. 1994) did not explain the peak. Instead, the peak was confirmed by mass spectrometry to be 2-propanol, or isopropyl alcohol – a reduction product of acetone. A different carbon effect on acetone degradation is revealed by summing molar concentrations of acetone and 2-propanol (see Figure 5). By day 3, over 40% of the acetone in each H treatment had been converted to 2-propanol, while less than 15% of the acetone in each L treatment had been converted. By day 10, the total of the two solvents in the L treatments was significantly less than in the H treatment. On day 17, no acetone or 2-propanol was detected in the L treatment, while all three H replicates maintained a residual on day 21.

According to Terzis (1997), acetone is an intermediate of 2-propanol degradation, not the other way around. In the reduced environment of the high-COD H jars, it appears that acetone is being used by the microbial community as an electron acceptor for oxidation of solvents and non-solvent carbon. The standard hydrogen electrode potential, $E°_H$ of the acetone/2-propanol redox couple can be calculated according to Equation 3.

$$E_H^0 = -\Delta G^0 / nF \qquad \text{(Eq. 3)}$$

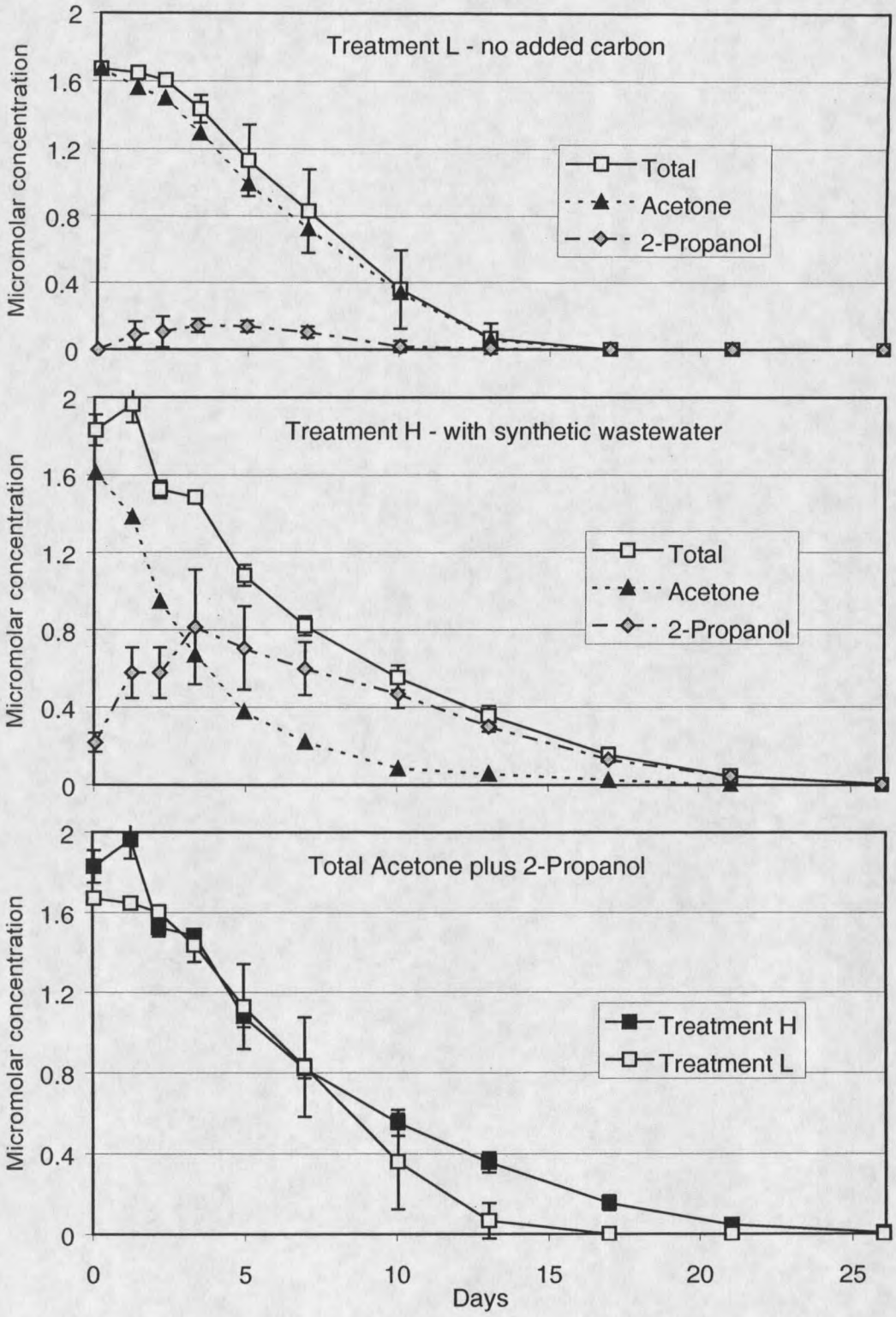


Figure 5. Average concentrations of acetone and 2-propanol and the total of the two in treatments L and H. Error bars on 2-Propanol and Total show ± one standard devation among three replicates of each treatment.

where n is the number of electrons transferred, in this case 2, and F is the Faraday constant (Stumm and Morgan, 1996) Using a $\Delta G°$ of –24.7 kJ, found by normalizing the relationship in Terzis (1994) to a single mole of acetone, a value of 128 millivolts is calculated, which places acetone on the redox ladder well below oxygen and nitrate, but in a favored position relative to sulfate and CO_2 (see Figure 1). When oxygen and nitrate are not available for microbial metabolism, it appears that acetone is the next favored electron acceptor.

The net effect of the acetone/2-propanol interaction is a slower rate of their combined removal. Acetone disappears as it is converted to 2-propanol, but as the E_H of the solution recovers as carbon degrades, this 2-propanol must eventually be converted back to acetone to be oxidized and degraded. In the mini-column experiments, acetone and 2-propanol concentration have been added together for consideration of treatment effects.

Planted Mini-Columns

General Trends

Bozeman municipal post-primary wastewater, inoculated with approximately 100 mg/l of each of the three solvents of concern, was placed into 15 mini-column wetlands, twelve of which were planted with species of *Carex, Juncus, Iris* and *Pondeteria*, while three remained unplanted as controls. The experiment consisted of six 14-day incubations, three each simulating summer and winter greenhouse operating conditions.

In the unplanted mini-columns, 90% removal of butanol generally occurred in one to three days. During winter incubations 4, 5 and 6, one or two days longer was generally required to reach the same removal level as summer incubations (Figure 6).

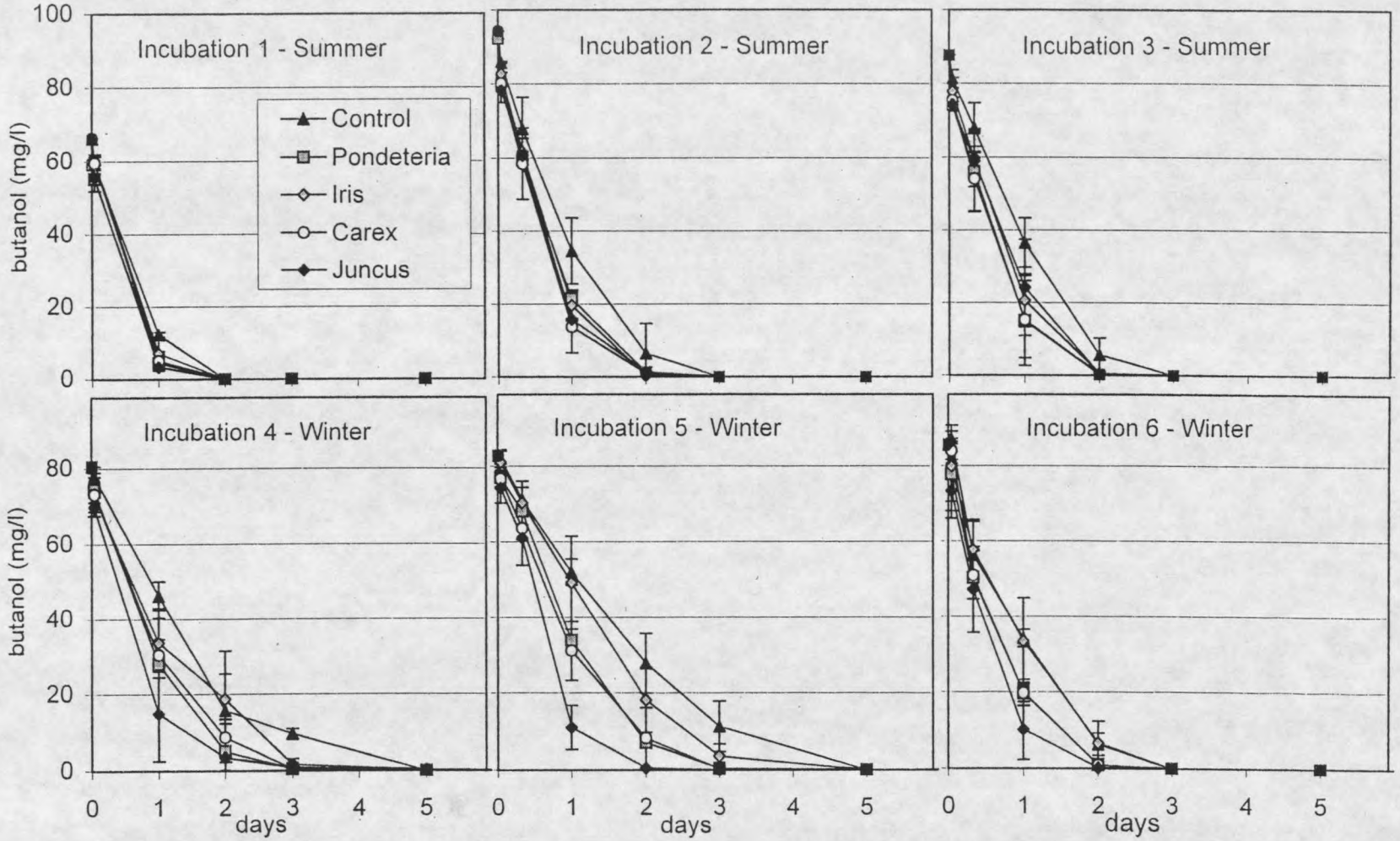


Figure 6. 1-butanol degradation in mini-columns, by incubation and treatment. Data points show averages of all replicates of each treatment within each incubation. Error bars represent ± one standard deviation.

Planted treatments typically achieved 90% removal in less than one day, and nearly all outperformed the unplanted controls.

Removal of coupled acetone and 2-propanol varied considerably with both season and plant type (Figure 7). Ninety percent removal generally occurred in 5 to 7 days in planted treatments, while control columns typically took 7 to 10 days for the same level of treatment. However, in the colder incubations in November and December, many individual columns failed to achieve 90% removal by the end of the 14 day incubation.

Ninety percent removal of THF was achieved within the 14 day incubations only in the most vigorous of the planted columns (Figure 8). In unplanted control columns, 50 to 70% removal in 14 days was typical, with planted columns generally performing better than controls.

COD measurements over each incubation show the general patterns for COD removal (see Figure 9), however much of this COD consists of the four solvents, which have already been measured. A clearer picture of the COD removal trends emerges when COD attributed to the solvent is subtracted from the measured total COD, as described in the jar experiment results (see Figure 10). With the exception of incubation 4 (November), non-solvent COD in all columns was reduced to less than 50 mg/l by the end of the fourteenth day. All incubations except the first show an initial reduction in non-solvent COD, followed by a plateau of several days, then a gradual decline. This may be due to the appearance of solvent degradation intermediates, such as organic acids, which contribute to COD but are not measured in GC analysis.

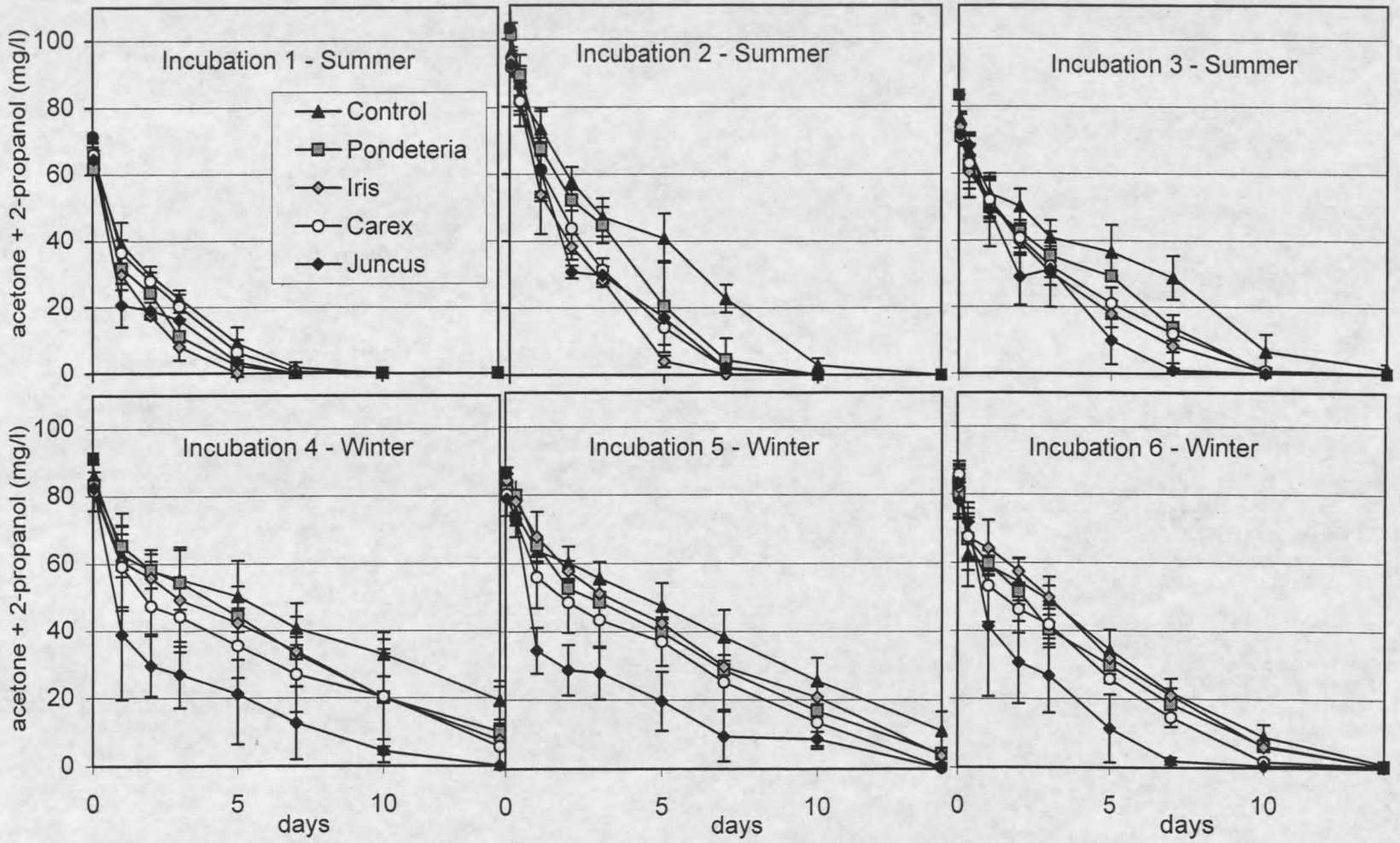


Figure 7. Acetone plus 2-propanol degradation in mini-columns, by incubation and treatment. Data points show averages of all replicates of each treatment within each incubation. Error bars represent ± one standard deviation.

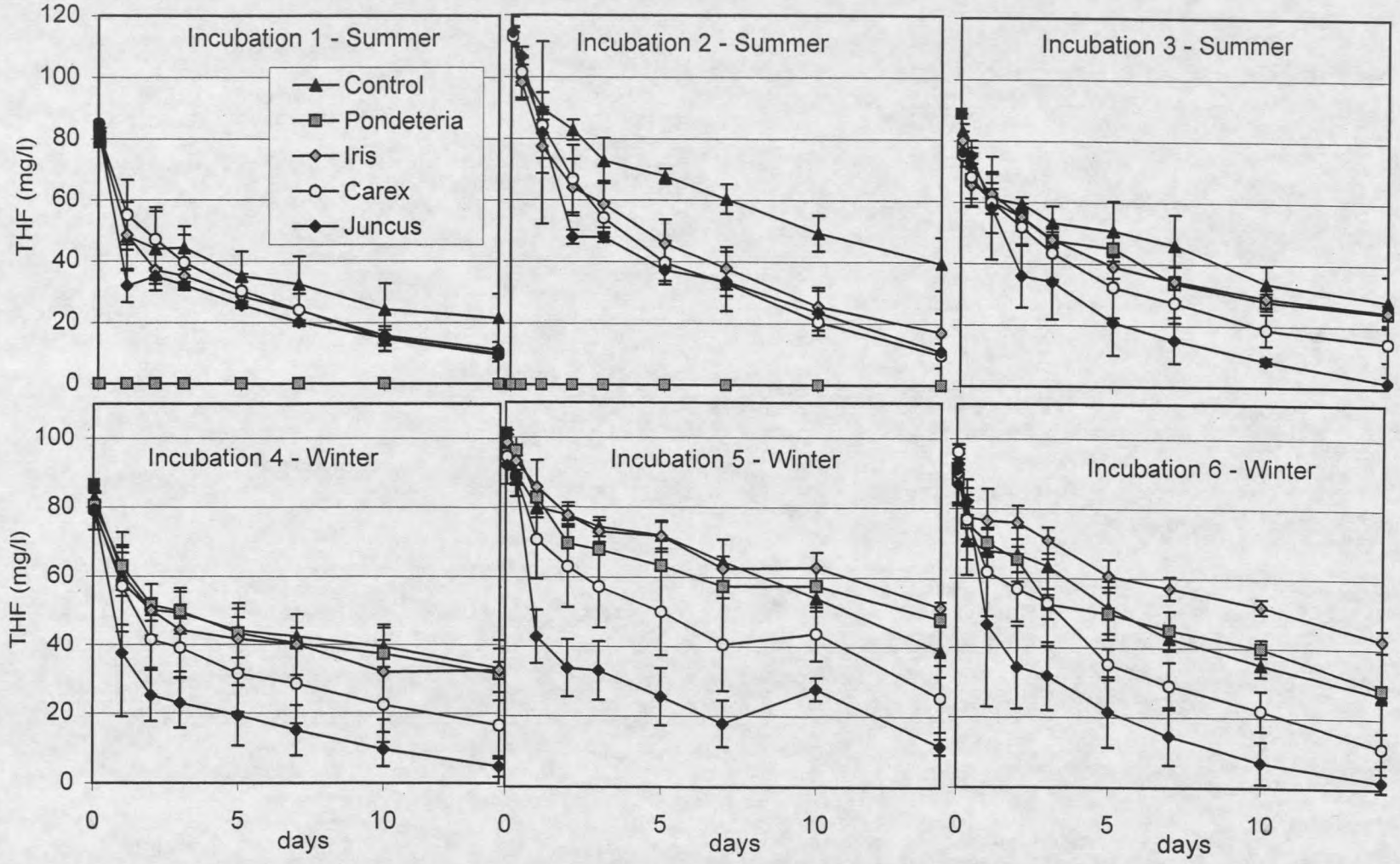


Figure 8. Tetrahydrofuran degradation in mini-columns, by incubation and treatment. Data points show averages of all replicates of each treatment within each incubation. Error bars represent ± one standard deviation.

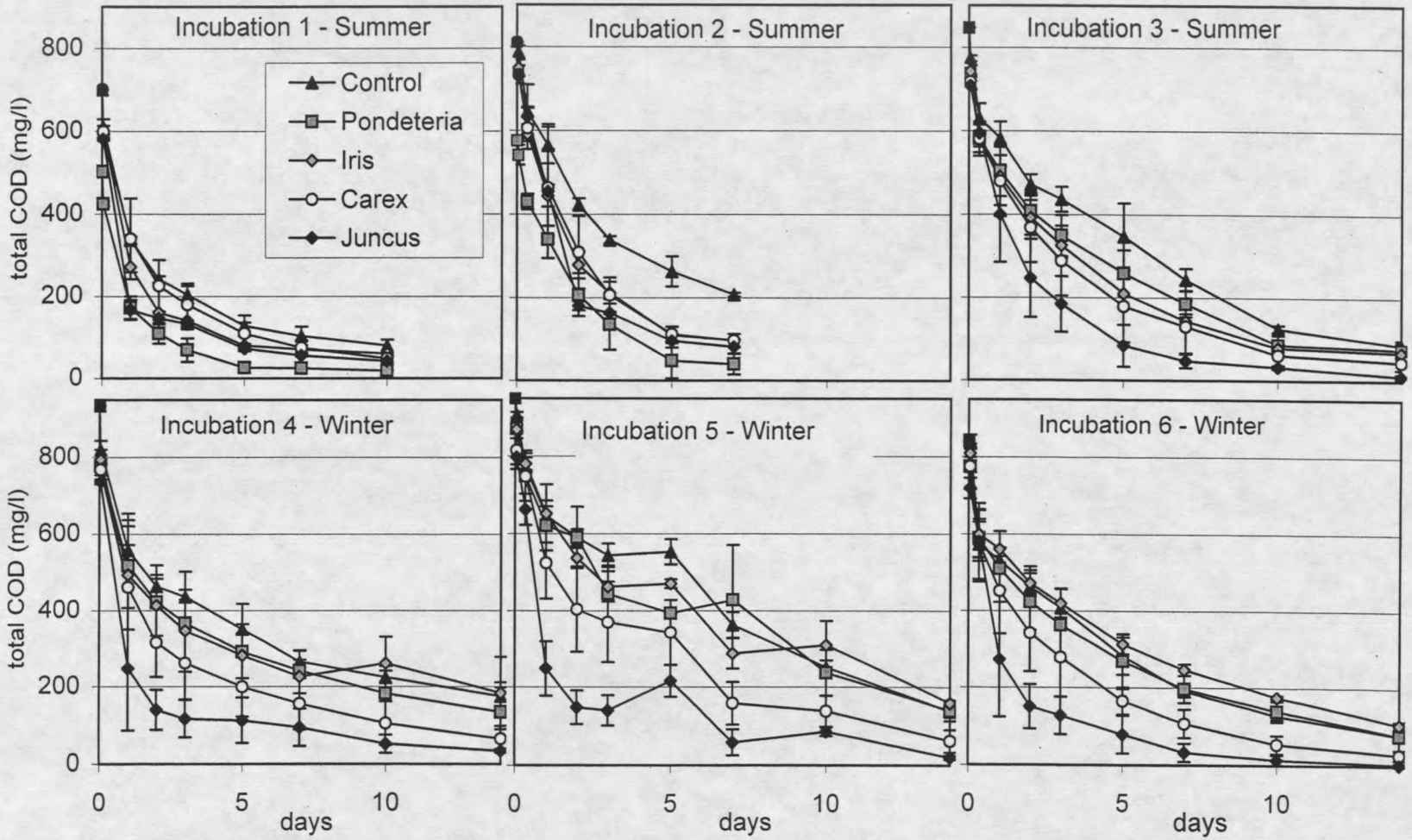


Figure 9. Total chemical oxygen demand in mini-columns, by incubation and treatment. Data points show averages of all replicates of each treatment within each incubation. Error bars represent ± one standard deviation. COD was not measured for day 14 in incubations 1 and 2 or for day 10 in incubation 2.

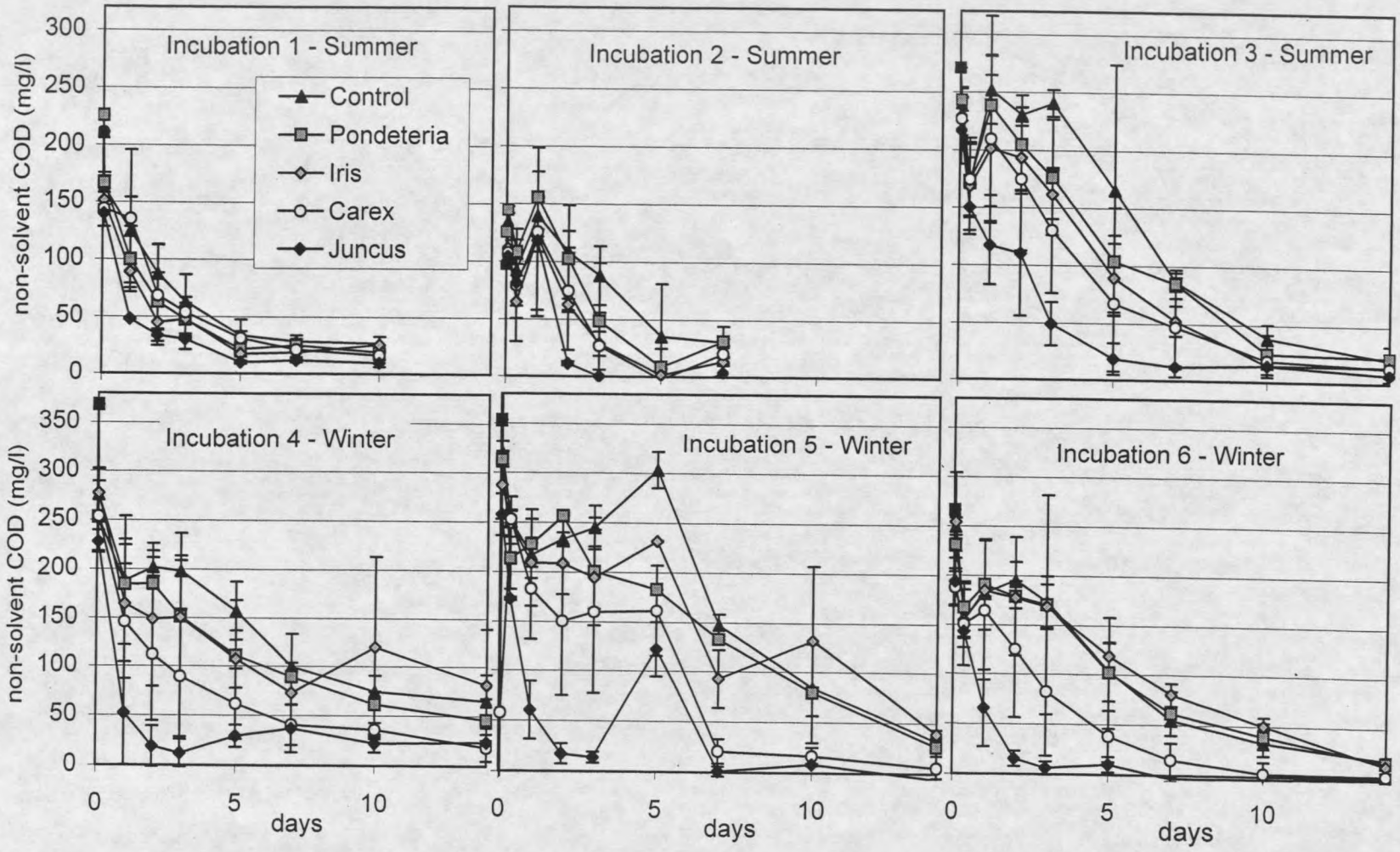


Figure 10. Measured chemical oxygen demand, minus the experimentally calibrated oxygen demand of the measured solvents in mini-columns, by incubation and treatment. Data points show averages of all replicates of each treatment within each incubation. Error bars represent ± one standard deviation.

Plant Effects

Comparison Procedures

In order to compare the solvent removal efficacy of the mini-column treatments, key time intervals were chosen at which to compare them. Because of the historical significance of 5-day BOD test, and because a 5 day retention time is typical in many treatment wetlands, five days was used as a time snapshot for comparing percent removal of COD and acetone plus 2-propanol. A 14 day snapshot was used to compare THF percent removal, since no column achieved complete removal within the incubation period. A one day snapshot was used for 1-butanol, as many columns achieved complete removal in two days.

Observation of solvent removal curves for COD, acetone and THF suggests that the removal mechanism is not the same over the duration of the incubation – there is a rapid removal in the first one to two days, followed by a shallower removal curve which could conceivably fit either zero or first order kinetic removal models. Percent removal of COD and acetone plus 2-propanol at day one and percent removal of THF at day two approximates the transition between the initial rapid removal period and the slower long-term process. Removal comparisons at these times were used to judge whether significant differences between treatments emerge during the initial rapid removal period, or whether they emerge only over the long run.

In order to compare treatment effectiveness, each of the five plant treatments was separated into winter and summer seasons. Within each season, data from the 15 columns and 3 incubations was sorted into 5 treatments of 6 to 12 repetitions each (two to four

columns, times three repetitions, minus data missing due to experimental procedural errors).

It should be noted that, although greenhouse temperature settings were changed abruptly on October 30th, actual seasonal trends followed a gradual curve. Seasonal effects are not a simple function of temperature, but are also tied to insolation and plant senescence. Dividing the data into two "seasons" for comparison is somewhat arbitrary, but still serves to highlight some significant seasonal differences.

Comparison Results

Long and short term treatment comparisons, as described in the preceding paragraphs, are shown in Tables 5 and 6, respectively. Useful comparisons can be made among plant species within the same season, and also between the summer and winter performance of each treatment.

Long Term Removal – Table 5

All four plant species averaged greater than 80% removal of acetone plus 2-propanol in the first five days during the three summer incubations. The differences among the species were not significant in summer, although all plants except *Pondeteria* performed significantly better than the unplanted controls. All treatments except *Juncus* demonstrated significantly better performance in summer over winter, though the trend was identical for *Juncus*. In winter, *Juncus* performed significantly better than all other treatments, which were statistically similar.

Five-day trends for total COD removal were similar to the acetone removal trends. The only significant difference among treatments in summer is between *Juncus* and Control. *Juncus* and *Carex* both performed significantly better than controls in

	Fraction COD Removed Day 5				Fraction Acetone plus 2-Propanol Removed Day 5				Fraction THF Removed Day 14				Fraction 1-Butanol Removed Day 1			
Summer																
Control	0.69	(0.11)	a	x	0.68	(0.16)	a	x	0.71	(0.08)	a	x	0.68	(0.12)	a	x
Pondeteria	0.85	(0.13)	ab	x	0.81	(0.15)	ab	x	NA				0.84	(0.10)	ab	x
Iris	0.83	(0.07)	ab	x	0.92	(0.10)	b	x	0.82	(0.08)	b	x	0.82	(0.08)	ab	x
Carex	0.83	(0.07)	ab	x	0.84	(0.09)	b	x	0.88	(0.05)	bc	x	0.87	(0.09)	b	x
Juncus	0.89	(0.04)	b	x	0.89	(0.07)	b	x	0.95	(0.05)	c	x	0.82	(0.13)	ab	x
Winter																
Control	0.57	(0.13)	a	y	0.49	(0.10)	a	y	0.65	(0.06)	a	x	0.47	(0.11)	a	y
Pondeteria	0.66	(0.07)	ab	y	0.56	(0.06)	a	y	0.62	(0.09)	a		0.67	(0.11)	bc	x
Iris	0.62	(0.09)	ab	y	0.61	(0.17)	a	y	0.55	(0.08)	a	y	0.53	(0.15)	ab	y
Carex	0.74	(0.10)	bc	x	0.62	(0.09)	a	y	0.81	(0.10)	b	x	0.67	(0.09)	b	y
Juncus	0.85	(0.08)	c	x	0.80	(0.12)	b	x	0.94	(0.05)	c	x	0.85	(0.10)	c	x

Table 5. Mean removal results for chemical oxygen demand and three solvents by season and treatment. Numbers in parenthesis are standard deviations. Differences between treatments within season are not different at $\alpha = 0.05$ if followed by the same letter a-c. Differences between seasons within treatment are not different at $\alpha = 0.05$ if followed by the same letter, x-y.

winter, while *Juncus* was also significantly better than *Iris* or *Pondeteria*. *Juncus* and *Carex* both behaved similarly in summer and winter, while winter performance was significantly poorer in other treatments.

Fourteen day values for percent THF removal showed distinct trends by treatment. In summer, only four treatments were compared, as THF was not added to the *Pondeteria* columns for incubations 1 and 2. The three planted treatments all averaged better than 80% THF removal, and all performed significantly better than the unplanted controls. *Juncus* also performed significantly better than *Iris*. In winter, *Juncus* performed significantly better than *Carex*, which in turn performed significantly better than the other three treatments. *Juncus, Carex* and controls had no significant differences between summer and winter performance, however *Iris* showed dramatically less treatment in the winter, achieving treatment worse than even the control columns, although not significantly so.

In the summer, all four species of planted columns averaged better than 80% removal of 1-butanol in the first 24 hours, though only *Carex* consistently performed well enough to be judged significantly better than the controls. In winter, *Juncus, Carex* and *Pondeteria* all performed significantly better than the controls, with *Juncus* significantly better than all other treatments except *Pondeteria*. All columns performed significantly worse in the winter over the summer except *Juncus* and *Pondeteria*. *Juncus* actually averaged better in the winter than in the summer, though not significantly so.

Short Term Removal – Table 6

Removal of acetone plus 2-propanol in the first day was greatest in *Juncus* columns, averaging 50% or greater in both seasons. *Carex* tended to be the next best

	Fraction COD Removed Day 1				Fraction Acetone plus 2-Propanol Removed Day 1				Fraction THF Removed Day 2			
Summer												
Control	0.38	(0.11)	a	x	0.37	(0.08)	a	x	0.39	(0.11)	a	x
Pondeteria	0.49	(0.14)	a	x	0.44	(0.06)	a	x	NA			
Iris	0.50	(0.10)	a	x	0.48	(0.10)	a	x	0.48	(0.08)	ab	x
Carex	0.46	(0.12)	a	x	0.50	(0.18)	a	x	0.44	(0.08)	a	x
Juncus	0.57	(0.18)	a	x	0.50	(0.18)	a	x	0.60	(0.07)	b	x
Winter												
Control	0.36	(0.06)	a	x	0.29	(0.05)	a	x	0.31	(0.10)	ab	x
Pondeteria	0.40	(0.10)	a	x	0.26	(0.12)	a	x	0.27	(0.13)	a	
Iris	0.37	(0.10)	a	x	0.26	(0.12)	a	y	0.27	(0.13)	a	y
Carex	0.47	(0.12)	a	x	0.35	(0.11)	a	x	0.42	(0.12)	b	x
Juncus	0.72	(0.18)	b	x	0.56	(0.17)	b	x	0.67	(0.10)	c	x

Table 6. Mean removal results for chemical oxygen demand and two solvents by season and treatment. Numbers in parenthesis are standard deviations. Differences between treatments within season are not different at $\alpha = 0.05$ if followed by the same letter a-c. Differences between seasons within treatment are not different at $\alpha = 0.05$ if followed by the same letter, x-y.

treatment, but the only statistically significant separation was between *Juncus* and all other treatments during winter incubations. Seasonal variation was also minimal as only *Pondeteria* (worse in winter) showed statistical difference. COD removal was statistically very similar to acetone, with only the *Juncus* treatments in winter showing improved removal.

THF removal in the first two days averaged greater than 60% in the *Juncus* columns for both seasons. No other treatment had better than 50% removal. In the summer, *Juncus* performed significantly better than all treatments except for *Iris*. In

winter, *Iris* and *Pondeteria* performance was similar to control columns, and significantly worse than *Carex*, which in turn was worse than *Juncus*. Only *Iris* displayed a significant decrease in performance in winter.

In all columns, it is clear that a large fraction of total removal is occurring in the short term. For all three measures in each treatment and season, the average short term removal was greater than 40% of the long term removal, as reported in Table 5. Most were greater than 50%. While the mechanisms of short term removal are not known, they are clearly significant.

Trends in Plant Effects

The plant effect patterns that emerge from this analysis are very clear. By nearly every measured parameter in both seasons, columns planted with *Juncus* performed better than all others. *Juncus* especially stands out in the winter because, unlike other treatments, its winter performance does not decline significantly relative to summer. *Carex* is typically the next best treatment, especially over the longer time periods summarized in Table 5. It displays a greater performance decrease in winter over summer than *Juncus*, but less than the other treatments. It does not have the dramatic initial treatment efficacy observed in *Juncus* in the first one or two days of each incubation (Table 6). The long-term differences between *Juncus* and *Carex* do not appear to be as great as the short-term differences, suggesting that *Carex* is narrowing the gap. *Carex* may actually be equally or more effective than *Juncus* after the initial rapid removal period.

Average performance of *Iris* and *Pondeteria* is consistently better than controls during the summer, although only a few of these differences are significant. In winter,

differences are smaller still, with these two treatments performing worse than controls for THF removal. While both *Juncus* and *Carex* maintained some vegetative growth during the winter months, *Iris* and *Pondeteria* became fully senescent with new shoots emerging in the spring. It appears that, when dormant, *Iris* and *Pondeteria* have little or no beneficial effect on treatment processes. Differences between winter and summer were most dramatic in the *Iris* treatment.

Seasonal Effects

Although grouping treatments into winter and summer "seasons" makes it easy to compare large numbers of data points, it does not accurately reflect the reality of gradual seasonal shifts. Although the shift from summer to winter greenhouse conditions is still apparent, Figure 11 reveals that even automatically controlled greenhouse temperatures were influenced by actual weather patterns. July incubation 1 was warmer than incubation 3 in October, and December incubation 5 was colder than incubation 6 in January.

In addition to changing temperatures, the microcosms were exposed to seasonal patterns of changing day length. The seasonal blooming of the flowering plants, in particular, demonstrated that natural seasonal processes were still in force.

The actual variations in greenhouse temperature are directly reflected in the treatment trends for the four parameters of concern, as shown in Figure 12. Standard deviation error bars are not shown on this figure in order to preserve visual clarity.

Seasonal patterns are very distinct in the control treatment. The patterns for 1 day 1-butanol removal and five day acetone plus 2-propanol removal in the control columns

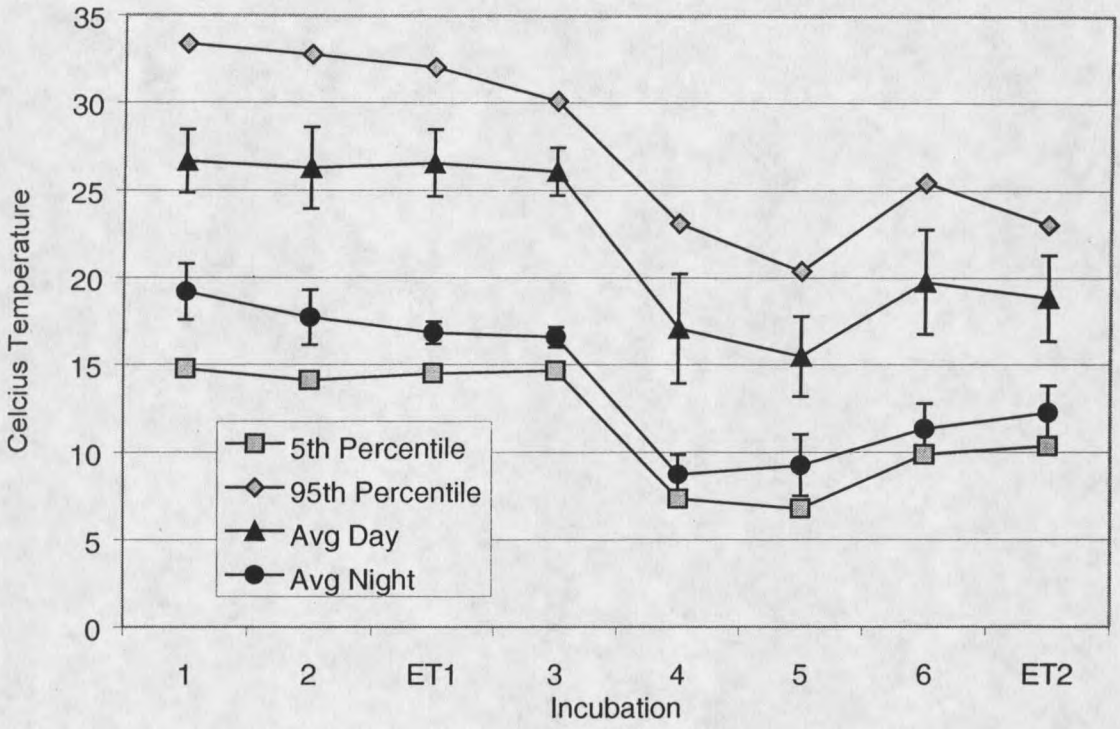


Figure 11. Statistical trends in actual greenhouse temperatures for all time intervals when treatment or evapotranspiration measurements were taken. Average temperatures during daylight and nighttime hours are shown. Error bars indicate standard deviation. The 5th and 95th percentiles of all measurements are also shown. Greenhouse temperature settings were changed on 10/30 (between incubations 3 and 4) from 24°C in the day and 16°C at night to 13°C during the day and 7°C at night. Average day is based on 12 hours of daylight in summer and 9 in winter. Average night is based on 10 hours of night in summer and 11 in winter. The remaining hours are transition periods between the daytime and nighttime temperatures.

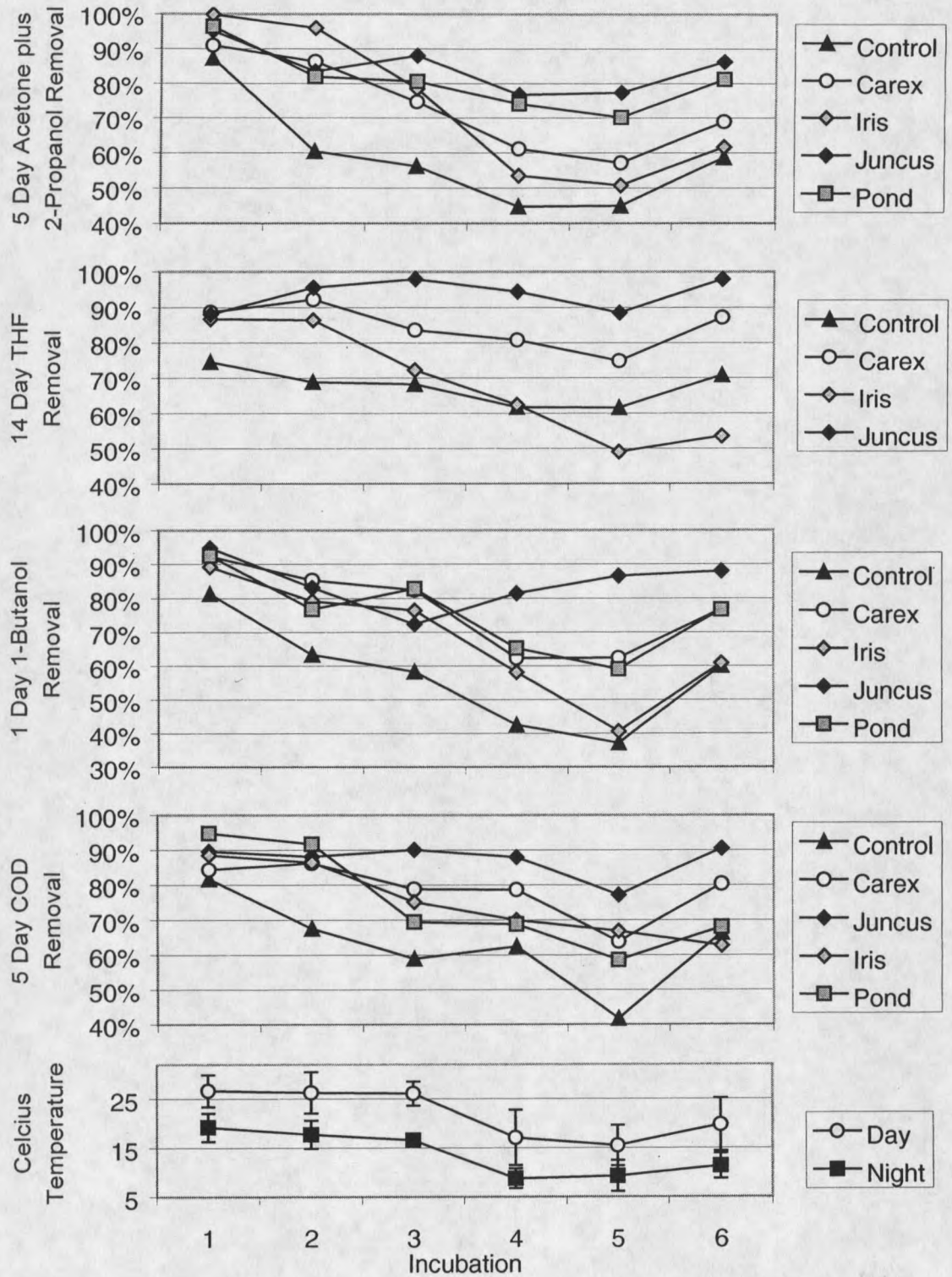


Figure 12. Seasonal variation of solvent and COD removal on selected days. Data points show mean values for each treatment. Bottom panel shows actual greenhouse temperatures, with error bars showing one standard deviation

show a steady drop in performance through the summer runs, a performance low in November and December (incubations 4 and 5), and a dramatic improvement in January (incubation 6). The trend is similar but less dramatic for the long term THF removal.

As suggested by the statistical comparisons, some species of plants tend to exert a leveling effect on these seasonal trends, while others exacerbate them. *Juncus*, the top-performing plant overall, was least effected by seasonal trends. Indeed, aside from a small dip in performance in December (incubation 5), hardly any trend is apparent for 5-day COD removal and 14 Day THF removal in *Juncus*. The data for acetone and 1-butanol show more seasonal variation. The seasonal trends are most pronounced in the *Iris* columns. The dramatic differences between summer and winter performance appear not as a sudden shift but as a smooth trend, with performance clearly at its worst in November and December (incubations 4 and 5). For some treatment measurements, such as 5-day acetone removal, the performance improvement between incubation 4 in November and incubation 6 in January is striking.

An explanation of the differing seasonal effects of different plants, where *Juncus* mediates seasonal differences while *Iris* exacerbates them, is suggested by the different mechanisms at work. Both Sorrell and Armstrong (1994) and Allen (1999) have reported greater transport of oxygen by plants to the root zone at lower temperatures. In the absence of oxygen transport, however, the net effect of plants is to provide an additional source of organic matter and, hence, oxygen demand to the media. The death of plant material contributes sugars, starches, and low weight cellulose compounds to the biological oxygen demand of the system, while less degradable humic substances are not generally broken down by soil microorganisms but do elevate the COD (Kadlec and

Knight, 1996). *Pondeteria* and *Iris* shoots and stems became dry and brittle in the winter months. Aerenchymous cavities in *Iris* appeared flattened, brown and bent, while *Pondeteria* stalks twisted and withered. Although previous research (Allen, 1999) had shown *Carex* and *Scirpus* species to be effective at transporting oxygen in their senescent states, the oxygen-transport pathways in the flowering perennial plants in this study do not appear to remain effective through the plant dormancy period.

The research of Allen (1999) suggests that any seasonal trends observed in the first year of a treatment system are suspect. Although very clear trends of plant treatment effects emerged in that research project, they did not emerge clearly until the second winter of the project. The maturation of wetlands includes vegetative fill-in, root and rhizome development, and the establishment of a stable microbial community. Full scale functioning wetlands have observed adaptation trends as long as three years from project start-up, with adaptation periods of one to two years more common (Kadlec and Knight, 1996).

Comparisons Between Mini-Column and Jar Experiments

In general, all three solvents appeared to degrade more rapidly in the mini-columns than in the jars – even when the unplanted control columns are used for comparison – however performance in the mini-columns was also much more variable. Comparisons of solvent degradation among the unplanted mini-column incubations and the two jar incubations are shown, for acetone plus 2-propanol, 1-butanol and tetrahydrofuran (THF) in Figures 13, 14 and 15, respectively. For all three solvents, the mini-columns show a much more rapid initial solvent removal than the jars.

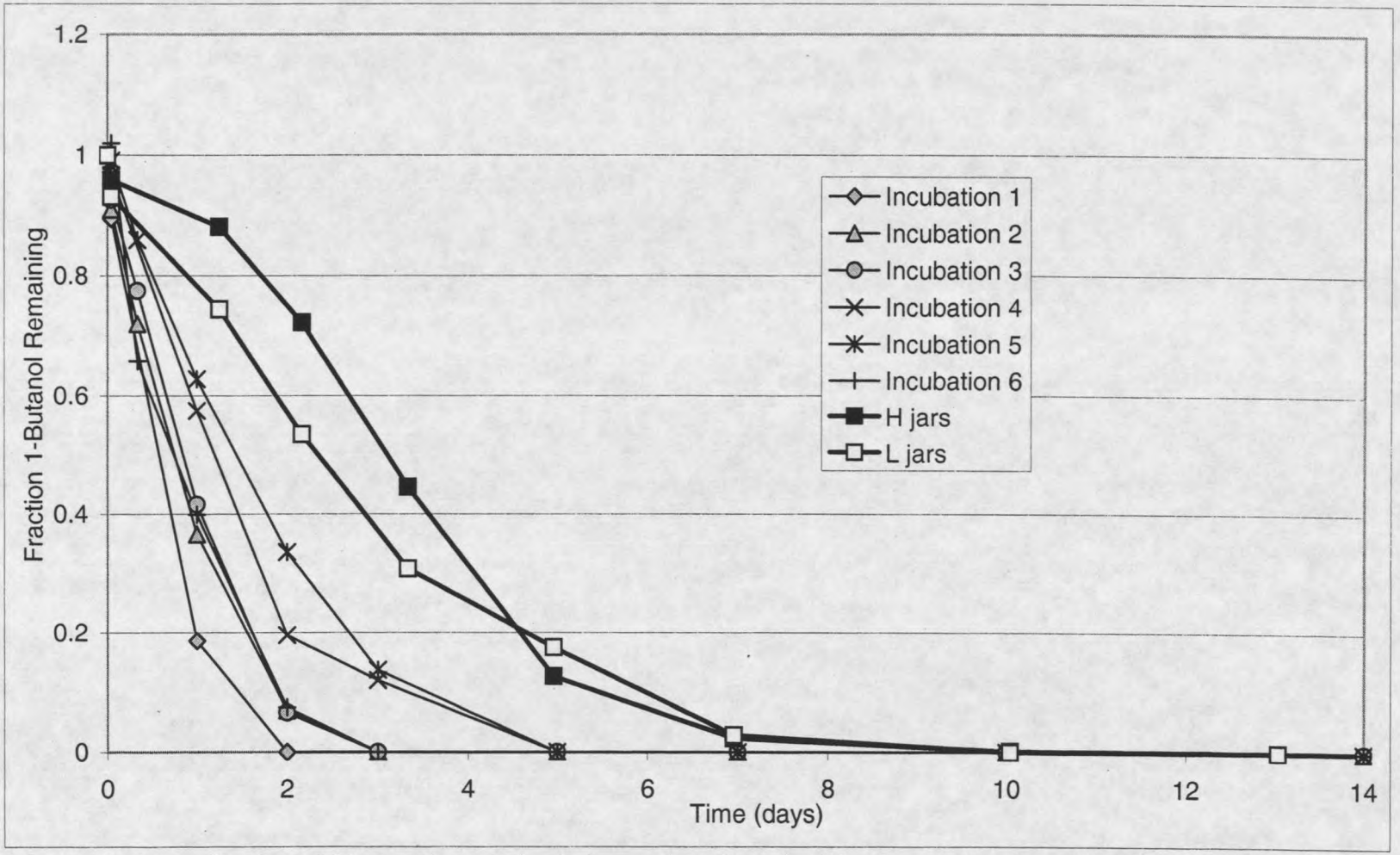


Figure 13. Mean fraction of 1-butanol remaining in jars and unplanted mini-columns over time. Values have been averaged among three replicates of each treatment.

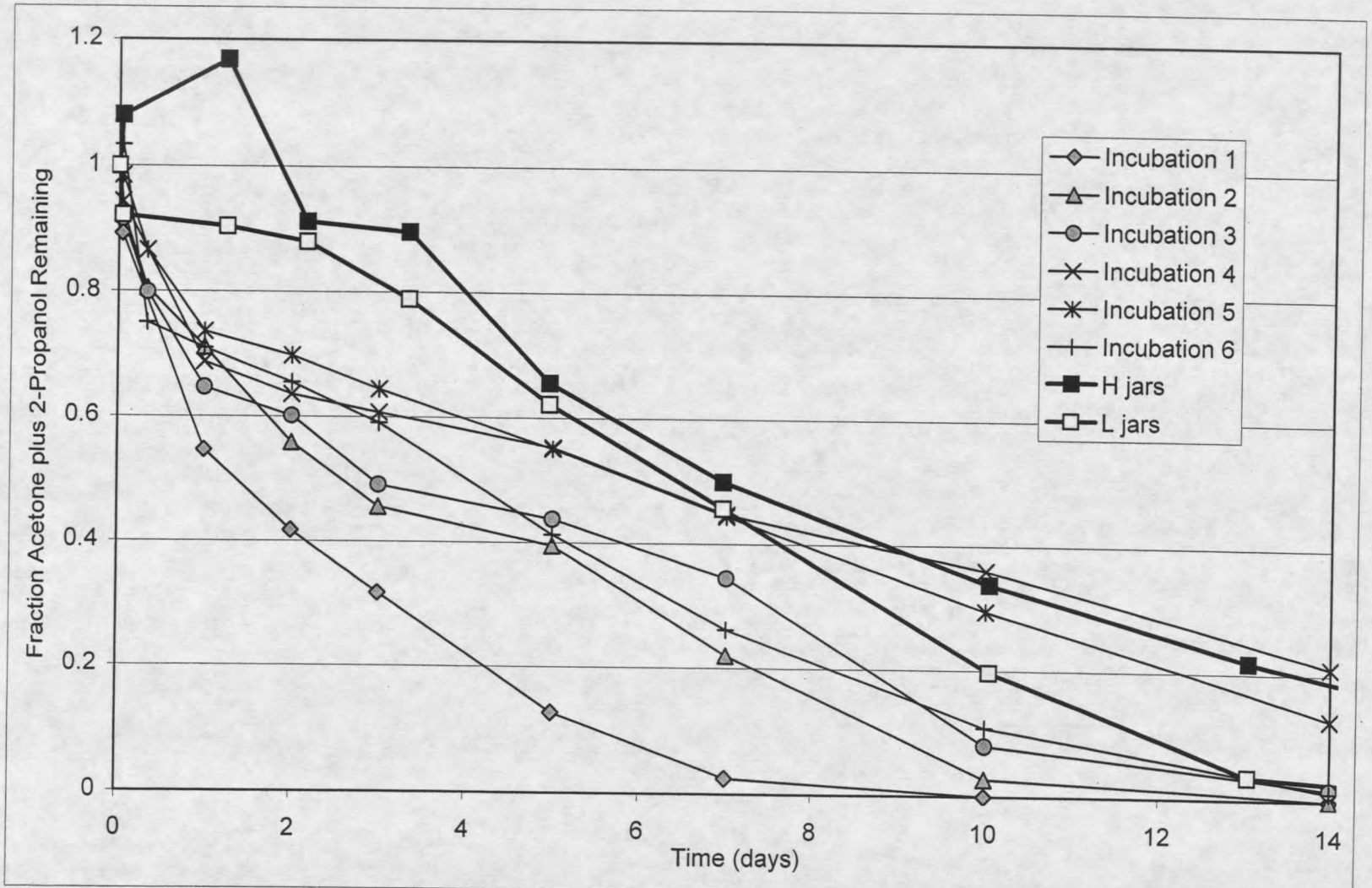


Figure 14. Mean fraction of acetone plus 2-propanol remaining in jars and unplanted mini-columns over time. Values have been averaged among three replicates of each treatment.

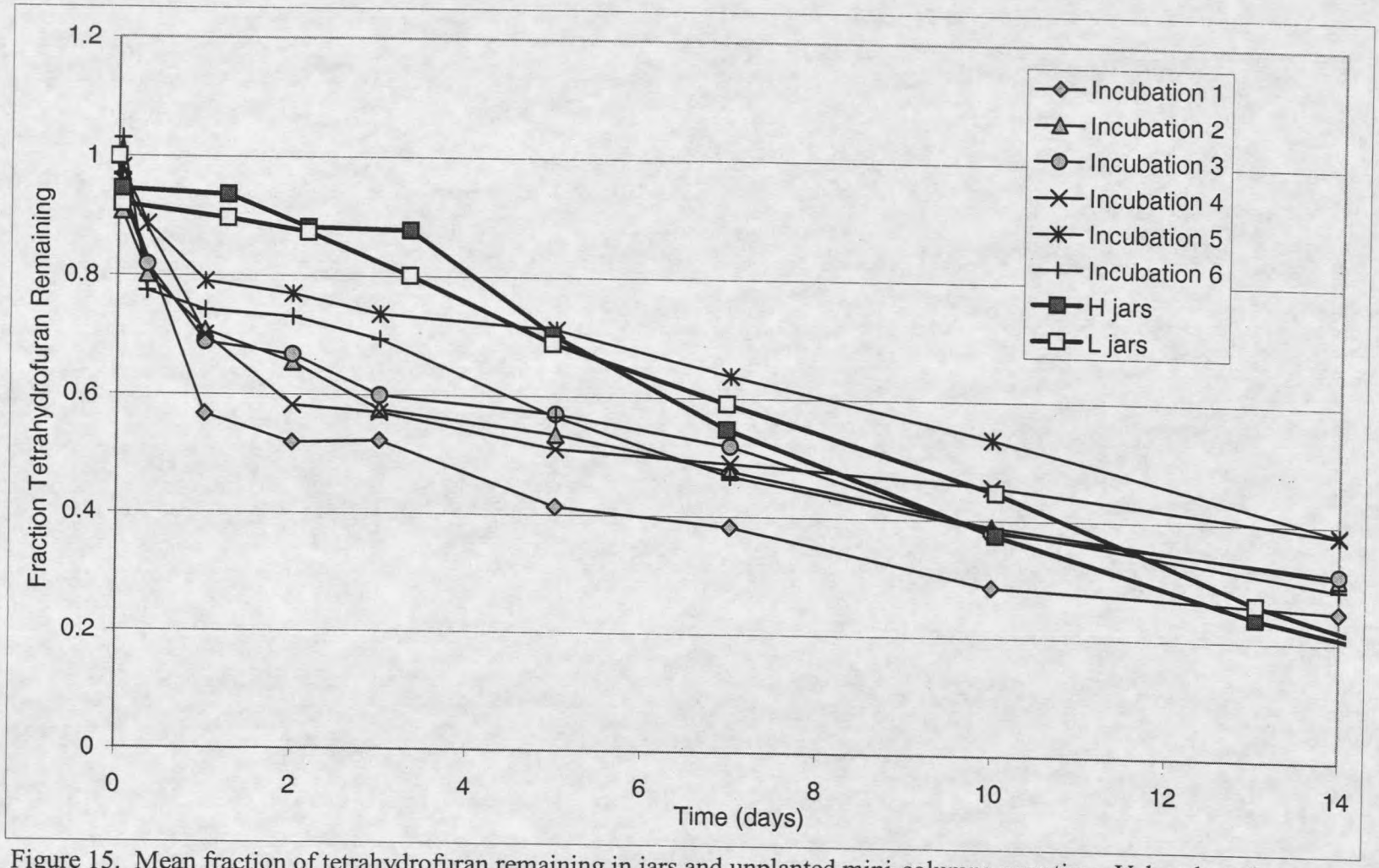


Figure 15. Mean fraction of tetrahydrofuran remaining in jars and unplanted mini-columns over time. Values have been averaged among three replicates of each treatment.

While indoor jar experiments were conducted at room temperature of approximately 20°C, Greenhouse temperatures varied greatly, as shown in Figure 11. Summer average daily temperatures were above 25°C, while winter daily temperatures were generally less than 20°C. Considering these differences, it is not surprising that control columns outperformed jars in the summer months (incubations 1, 2 and 3), but it is interesting that columns in incubations 4, 5 and 6 also tended to outperform jars.

Besides temperature, several other experimental design differences between the jars and columns might have factored into observed treatment differences. These include the smaller gravel size in the columns, the replenishing flow of tap water to columns, and the character of the wastewater used.

Because the time intervals over which the differences were observed was short (frequently less than one day), it seems unlikely that the tap water replenishment played a role in these differences, especially in the control columns where evaporation was very small. More likely, use of a diverse and microbially active wastewater in an environment with higher surface area had a dramatic effect in improving treatment efficiencies, particularly within the first day of each incubation.

Evapotranspiration Data

Evapotranspiration (ET) data, as measured during incubations ET1 (September) and ET2 (February) are shown in Figure 16. As expected, summer ET is greater than winter in every column except *Carex* 2, which showed less ET than any other *Carex* column in either season. Differences in ET among columns in the same treatment appear to be largely accounted for by observable differences in plant vitality.

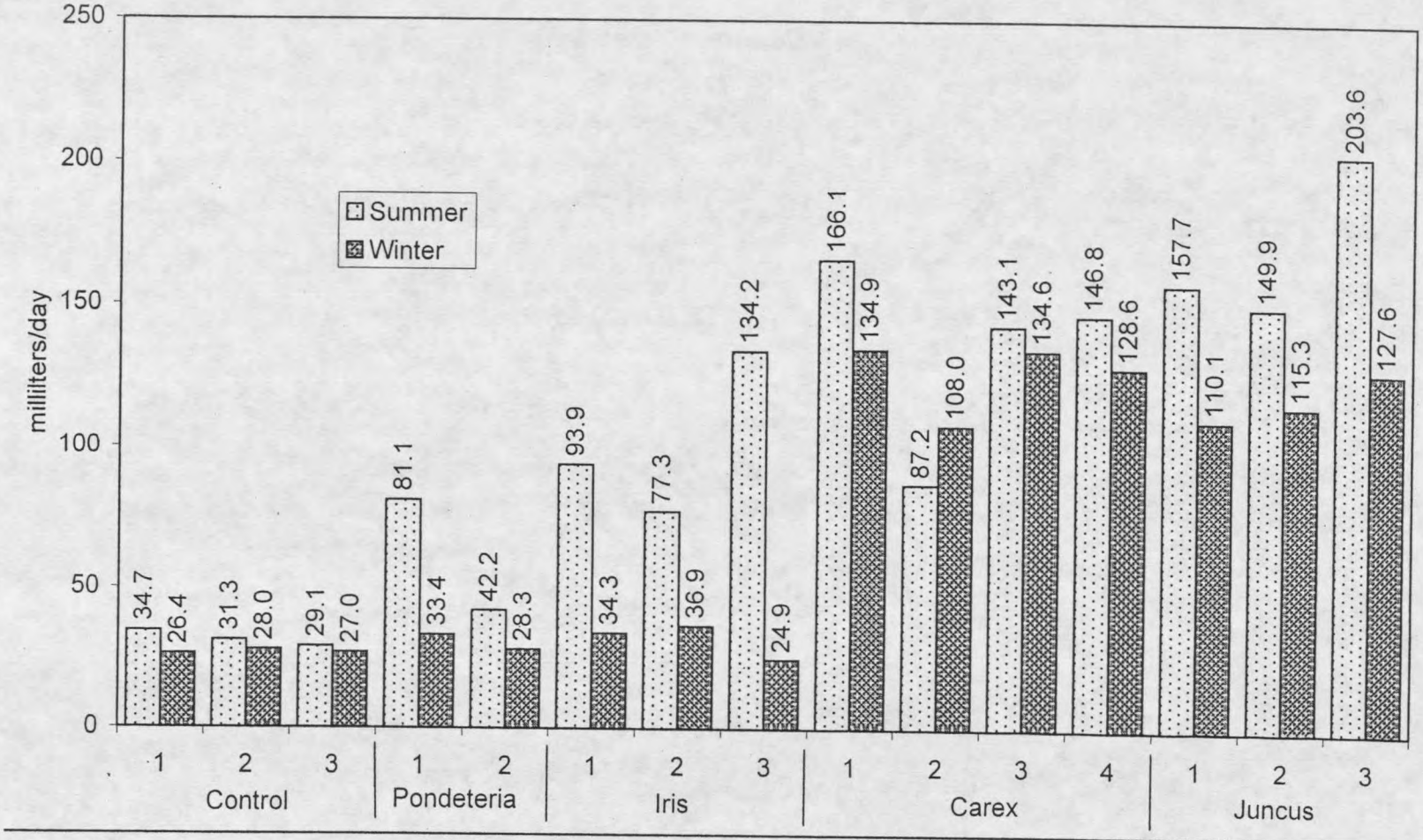


Figure 16. Average daily evapotranspiration as measured in each mini-column during winter and summer ET incubations. X-axis numbers refer to individual replicates.

Large differences were observed between evapotranspiration in daylight hours versus over night, and on sunny versus cloudy days. The values given in Figure 16 and used in the model below are averaged over the entire ET incubation, in order to best represent typical conditions of that season. Both ET incubations encompassed a variety of weather conditions.

Transpiration Modeling

Statistical Correlations

There is a clear statistically significant positive correlation between the quantity of water transpired and the treatment achieved, as typified by Figure 17. This led to the

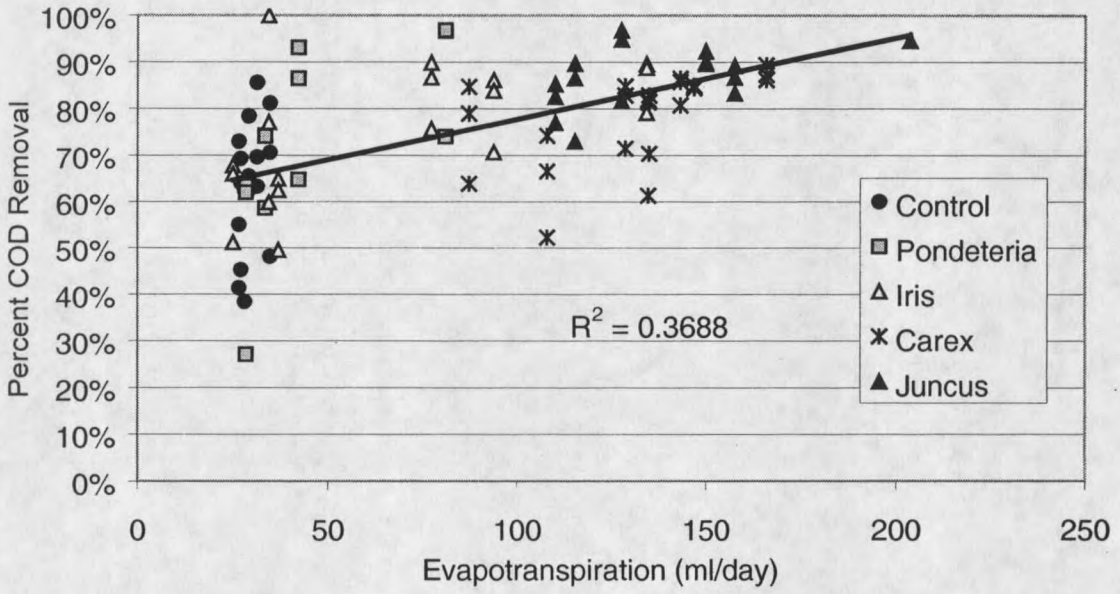


Figure 17. Scatter plot showing positive correlation between 5-day percent COD removal and evapotranspiration for each individual column and incubation. The five treatments are distinguished symbolically. The bold line shows a least squares linear regression line with an R^2 value of 0.3688.

question of whether high transpiration and effective water quality improvement are both unrelated measures of plant vitality, or whether increased transpiration represents a

mechanism which is responsible for the higher treatment efficacy observed. It is suggested that the truth incorporates a little bit of both possibilities.

Explanation of Theoretical Model

The uptake of nonionic dissolved components into the tissue of plants is a process of high complexity. The most extensive quantitative studies are taken largely from the pesticide literature. Equation 2 describes a transpiration stream concentration factor (TSCF) as a function of the octanol-water partition coefficient (K_{OW}). This was calibrated by Briggs et al. (1992) based on the behavior of pesticides, which are markedly unlike the low molecular weight solvents of concern in this study. Nevertheless, the phenomenological basis for the relationship, and the lack of any other quantitative predictors for plant uptake of solvents, justifies the exploratory use of the Briggs relationship in this study. Results of modeling based on Equation 2 should be treated as extremely rough estimates as to the ultimate fate of the solvents. A true deterministic model would need to be calibrated for the low weight polar organic compounds and the wetland plants used in this study.

Transpiration of water and solvents was modeled separately for each individual column. Each average daily ET value in Figure 16 was applied to all three incubations for that column and season.

Average transpiration values for each column were obtained by subtracting estimated evaporation from the measured ET. To estimate pure evaporation, the calculated daily ET values were averaged among the unplanted control columns for each season. This evaporation value was assumed to be the same for all planted and unplanted columns for that season.

The transpiration stream concentration factor (TSCF) was calculated for acetone and tetrahydrofuran based on Equation 2 and the K_{OW} values given in Table 1. The calculated TSCF is 0.147 for acetone and 0.499 for THF. Multiplying the TSCF of a solvent by the concentration of that solvent in the bulk solution, one obtains the quantity of solvent which will be taken up by a plant per unit of water the plant transpires. This can be expressed in mathematical form according to Equation 4.

$$dX = TSCF \ \frac{X}{V} dT \qquad \text{(Eq. 4)}$$

where X is the quantity of solvent in the column, V is the liquid volume of the column, and dT is the liquid flux due to transpiration.

To estimate the total amount of solvent transpired over the entire incubation, ΔX_{total}, each incubation was divided into n discrete time intervals of length t_i, corresponding to the times between sampling events. The total solvent lost was then estimated according to Equation 5, where $(X/V)_i$ is the concentration of solvent at the

$$\Delta X_{total} = \sum_{i=1}^{n} \left[TSCF \ \left(\frac{X}{V} \right)_i T \times t_i \right] \qquad \text{(Eq. 5)}$$

beginning of time interval i and T is the transpiration per unit time. Values for $(X/V)_i$ were determined from measured concentrations. This method of approximating solvent loss assumes that the concentration of solvents in the column is constant over each time interval, giving a small but consistant overestimation of transpiration loss. Because transpiration losses are slightly exagerated in this way, the model will be conservative in assessing whether there truly are significant differences among columns due to other removal mechanisms.

Model Results

The model described above was used to compare the acetone and THF lost to transpiration to that lost to biological degradation, evaporation and other mechanisms over both winter and summer incubations. Transpirative 1-butanol loss was not modeled, as it disappeared so quickly that transpiration could not have been a major factor. Figure 18 compares removal pathways for THF removal at day 14. Results of the model are averaged by treatment and season, with the average amount lost to ET shown in lighter gray.

For acetone, the model predicts that, by day five, the greatest percent of acetone was lost to transpiration in *Juncus* columns in the winter, at 3.8% of initial acetone. *Iris* and *Pondeteria* in summer and *Juncus* and *Carex* in both seasons all lost between one and four percent of their initial acetone concentrations to transpiration. Transpiration by itself clearly does not solely account for the greater efficacy of these treatments (see Table 5).

With the longer treatment period and higher TSCF of THF, the percentages lost through transpiration are greater. The model estimates that up to 28% of initial THF in solution was lost to evapotranspiration in *Juncus* columns in summer. The remaining solvent removal is attributed to biodegradation and evaporation. With transpiration losses accounted for, the differences observed between treatments are lessened. For example, the percent of THF lost to other mechanisms in *Iris, Carex* and *Juncus* in the summer and also in *Carex* in the winter is very close to the percent lost in the control columns. For these planted treatments, the transpiration pathway could potentially explain why these columns obtained greater treatment in this study. However,