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Polar organic solvent removal in microcosm constructed wetlands

Janet Kowles Grove[a], Otto R. Stein[b,*]

[a]*Landworks Consulting and Design, Missoula, MT 59807, USA*
[b]*Department of Civil Engineering and Center for Biofilm Engineering, Montana State University, Bozeman, MT 59717, USA*

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Abstract

Three polar organic solvents, acetone, tetrahydrofuran (THF) and 1-butanol, were added at 100 mg/l each to post-primary municipal wastewater in order to simulate a mixed waste stream. This mixture was applied to an experimental microcosm subsurface constructed wetland system consisting of replicates of *Juncus effusus*, *Carex lurida*, *Iris pseudacorus*, *Pondeteria cordata* and unplanted controls in a series of 14-day batch incubations over a yearlong period simulating a summer and winter season. 90% removal of 1-butanol typically took less than 3 days. 90% removal of acetone required from 5 to 10 days in summer and 10 to 14 days in winter. 90% removal of THF required at least 10 days and was frequently not achieved during the 14-day incubations. Initial experiments confirmed that the majority of solvent removal was via microbial bioremediation. Solvent removal was typically better in planted replicates, especially *Juncus*, regardless of season. The removal rate of all solvents was slower in winter, but the seasonal effect was most pronounced in the unplanted control replicates and least in the *Carex* and *Juncus* replicates. Plant and seasonal effects are believed to be due, in part, to variation in metabolic pathways induced by plant and seasonal variation in available root-zone oxygen. Variation in transpiration also influenced species and seasonal effects on THF removal, but not the other more biodegradable solvents. A model based on a prediction of plant uptake of nonionic dissolved chemicals suggests that as much as 39% of the THF in solution could have been removed through plant transpiration.

Keywords: Bioremediation; Evapotranspiration; Industrial; Plant; Season; Treatment

1. Introduction

Potentially toxic chemicals such as hydrocarbons, pesticides and solvents may be introduced to individual household and municipal wastewater treatment systems as an occasional shock load or may be more continuously discharged through a variety of commercial enterprises employing their use. Several studies have looked at the fates of various toxic organic compounds in wastewater facilities, including Bhattacharya et al. (1996) and Hannah et al. (1986, 1988). In general, removal of these compounds in activated sludge systems was high: greater than 94% for 9 out of 11 compounds in the Bhattacharya study. The alternative wastewater treatment systems studied by Hannah were less effective than activated sludge at removing similar compounds, suggesting a greater concern for the fate of toxics in

Abbreviations: CW, Constructed wetland; ET, Evapotranspiration; GC, Gas chromatograph; SD, Standard deviation; THF, Tetrahydrofuran.

*Corresponding author. Tel.: +1 406 994 6121; fax: +1 406 994 6105.
E-mail address: ottos@ce.montana.edu (O.R. Stein).

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wastewater treatment systems employing constructed wetlands (CWs). In addition, CWs are often a preferred alternative for the treatment of agricultural and isolated industrial waste streams that likely contain toxic compounds. However, only a few studies have looked at the fates of specific toxic compounds, especially chlorinated solvents, in wetlands. Some are summarized in Kadlec and Knight (1996), who suggest volatilization, photochemical oxidation, sedimentation, sorption, and microbial degradation as five possible removal mechanisms in CWs.

An impetus for our study on solvent removal was a proposed indoor CW system to treat mixed lavatory and chemistry laboratory wastewater for non-potable reuse within a new building on the Montana State University campus. A survey of existing laboratory practices revealed that dozens of potentially toxic chemicals might be disposed in laboratory sinks, at least accidentally. However non-halogenated polar organic solvents, especially acetone, are routinely used for glassware washing, and thus comprise on a mass basis the vast majority of potentially toxic compounds in the laboratory waste stream. Non-halogenated polar organic solvents also display low to moderate toxicities and moderate volatilization from water; thus their presence might not preclude use of an indoor CW system to treat the mixed waste stream. A few CW efficacy studies have focused on phenols, glycols and linear alkylbenzenesulfonates (e.g. Polprasert et al., 1996; Revitt et al., 2001; Thomas et al., 2003), but we could find none dealing specifically with acetone. This study was developed to examine the general efficacy of CW systems for the treatment of domestic wastewater mixed with relatively high concentrations of three non-halogenated polar solvents and the ultimate fate of the solvents.

The aromatic tetrahydrofuran (THF) and the primary alcohol 1-butanol were chosen, in addition to the simple ketone acetone, in order to study a range of solvent types. Selected chemical properties for these solvents are given in Table 1. All are regulated for land disposal of wastewater in the United States under the Resource Conservation and Recovery Act (RCRA, 1997) and, as summarized below, have been shown to be completely biodegradable under ideal conditions, and at least partly degradable by both aerobic and anaerobic pathways.

A fairly large body of research exists 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). In addition, 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, 1995) and carbon dioxide, which is reduced to methane (Platen and Schink, 1987). 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 5.7–6 h (Platen and Schink, 1989). In contrast, a sulfate reducing bacterium, *Desulfobacterium cetonicum* grew on acetone with a doubling time of 69 h (Janssen and Schink, 1995), while a doubling time of 2.8–3.5 days was calculated for a methanogenic enrichment culture growing on acetone (Platen and Schink, 1987).

In general, a primary alcohol is oxidized to its respective fatty acid: ethanol goes to acetate, 1-propanol goes to propanoate, etc. (Eichler and Schink, 1984). 1-Butanol can be oxidized by a wide variety of organisms to *n*-butyrate (CH_3–CH_2–CH_2–COO^-) which can be used as a growth substrate by many organisms (Arp, 1999; Poelarends et al., 2000). In contrast, the biodegradation of secondary alcohols is intimately connected with the respective ketone. In particular, 2-propanol can be oxidized to acetone according to the thermodynamically unfavorable reaction (Terzis, 1994)Terzis proposed

$$\underset{\text{(2-propanol)}}{4CH_3-CHOH-CH_3} \rightarrow \underset{\text{(acetone)}}{4CH_3-CO-CH_3} + 4H_2, \quad \Delta G^{\circ}_{RXN} = +98.81\,\text{kJ}. \tag{1}$$

Table 1
Selected properties of acetone, THF and 1-butanol

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)

Data from (a) Verschueren (1996), (b) Schwarzenbach et al. (1993), (c) Bhattacharya et al. (1996).

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

Once considered "not easily biodegradable", Kohlweyer et al., (2000) demonstrated that THF can be degraded by a *Pseudonocardia* species isolated from anaerobic wastewater treatment sludge. 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 and then oxidized to a cyclic ether. The ring can then be cleaved into 4-hydroxybutyric acid, which is easily metabolized.

Based on this literature it is clear that biodegradation of these particular solvents is at least possible in sub-surface CWs regardless of the wetland redox state. Of the other potential removal pathways identified by Kadlec and Knight (1996), photochemical oxidation is unlikely and sedimentation, sorption and direct volatilization are probably minor for these hydrophilic, low molecular weight solvents in sub-surface CW systems. However, volatilization at rates at least equal to that of evaporation of free water and preferential uptake and volatilization through plant tissues are possible. For a dissolved component to enter into the tissue of a plant, it must pass through a waxy barrier called the Casparian strip and 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 K_{ow}, 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 K_{OW}, according to Eq. (2).

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

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 et al., 1996).

We conducted our study of solvent degradation in CWs in two experimental phases. The first, dubbed "jar experiments", was designed to test the hypothesis that solvent biodegradation in sub-surface CWs was (a) possible and (b) influenced by the load of readily degradable organic matter typical of domestic wastewater. The second, "plant experiments", was designed to determine the influence of plant species and seasonality on overall solvent removal efficacy and the contribution of all loss pathways in a model CW system.

2. Materials and methods

2.1. Jar experiments

These experiments were designed to determine the rate of simultaneous biodegradation of the three solvents with and without the presence of an additional labile organic carbon load. We compared the change in concentration of the solvents with time in reactors containing a biologically active gravel medium with and without additional labile organic carbon and with additional carbon, but under sterile conditions.

Three experimental treatments were established, each in three replicates. A high-COD treatment was designed to mimic a high-strength municipal wastewater amended with 100 mg/l of each of the three solvents (acetone, 1-butanol and THF). A low-COD treatment consisted only of the three solvents at 100 mg/l each in tap water. The control treatment was identical to the high-COD treatment, except that it was conducted under sterile conditions. The synthetic domestic wastewater used in the high COD and control treatments consisted of 928 mg/l of pureed dog food and 230 mg/l of ammonium chloride dissolved in tap water. This mixture had approximately 450 mg/l COD and 110 mg/l total Kjeldahl nitrogen.

The nine experimental reactors were wide-mouthed glass jars with an approximate 2-l capacity that were filled with a biofilm-coated gravel, 1.5–2.0 cm diameter, taken from well-established wetland mesocosms used in other studies (Borden et al., 2001; Stein et al., 2003). All jars were sealed, but had a single air vent with a 0.2 μm bacterial filter, to allow equilibration of gases with the atmosphere while preventing microbial contamination. Each had a sampling port of FEP-coated Tygon tubing, which terminated 8 cm from the bottom of the jar. The control jars containing gravel and the wastewater (without solvents) were separately sterilized in an autoclave prior to the experimental incubations. Appropriate quantities of solvents were then added aseptically. A fitting in the control jar lid contained a rubber septum enabling samples to be drawn through a syringe without opening the jar to the atmosphere.

Three 26-day incubations were conducted at a room temperature of approximately 20 °C, though due to contamination and other unforeseen experimental difficulties subsequently eliminated, only the last incubation produced reliable results. Samples were drawn using a glass syringe from the wastewater mixture prior to filling and from each jar at 1 h, and at 1, 2, 3, 5, 7, 9, 12, 16, 20 and 26 days after filling. Each sample was filtered through a 0.2 μm syringe and measured for COD using a potassium dichromate colorimetric Hach test. Solvent and 2-propanol concentrations were measured on a gas chromatograph (GC) using a 3.048 m × 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 289.6 kPa. All samples for GC analysis were filtered through a 0.2 μm syringe filter into a 2 ml amber glass vial with a TFE-lined septa. Vials were stored in a refrigerator for up to a week before being analyzed. The experimental method maintained calibration to within ±10%. Additional details of the experimental design, infrastructure and methodologies can be found in Kowles (2001).

2.2. Plant experiments

These experiments were designed to estimate the capacity of a CW system to remove the three selected solvents when mixed collectively with municipal wastewater. We compared the relative efficacy of four species and an unplanted control during simulated summer and winter seasons by measuring changes in chemical concentrations with time in model CW batch reactors. Plant and season effects were compared, and the amount of solvent removal attributable to biological degradation as opposed to other mechanisms was estimated.

Fifteen CW reactors (columns) were constructed of 10 cm inner diameter PVC pipe, cut to a length of 46 cm and capped on one end. Columns were filled with a medium of washed pea gravel, 0.4–1.3 cm diameter, to within 1 cm of the top. Each column had a bottom drain plug and a tube fitting 9 cm from the bottom connected to a constant head tank filled with tap water to maintain water level just below the gravel surface during experiments. Samples were drawn through a tygon tube permanently inserted through the gravel with an opening 20 cm from the bottom of each column. Twelve columns were planted with emergent plant species; three with *Juncus effusus* (soft rush), three with *Iris pseudacorus* (yellow flag), four with *Carex lurida* (shallow sedge) and two with *Pondeteria cordata* (pickerelweed); and three remained as unplanted controls. The plant species were purchased from a greenhouse supply company and transplanted in January 2000, 7 months before experiments began. The experimental system was operated in a controlled environment greenhouse using natural sunlight conditions for 45°N latitude.

Five times in 5 months columns were drained and filled with 50 ppm of a commercial 20–10–20 fertilizer solution to aid plant establishment. Four times in the following 2 months columns were drained and filled with post-primary wastewater collected from the local wastewater treatment plant to stimulate biofilm growth on plant roots and gravel. Treatment plant records report an average 123 mg/l BOD (SD = 22 mg/l) for this water. During the third and fourth times the three solvents were added at 100 mg/l (153 kg/ha) each, to acclimate the microbial community to the mixed-stream wastewater. This mixture also was used for experimental incubations.

Six 14-day experimental incubations were conducted over the ensuing 7 months (August–February). During the first three (and during the preceding establishment and acclimation periods), greenhouse air temperatures were set to 24 °C during the day and 16 °C at night to simulate summer conditions. On October 30, 2000, temperatures were changed to 13 °C during the day and 7 °C at night to simulate mild winter conditions. Outside weather influenced air temperature at specific times, but the mean day/night values of individual incubations were within 5 °C of set temperatures and our "seasons" induced typical plant growth and dormancy responses. During each season an additional incubation was conducted without solvents and with the water replacement mechanism disabled to measure the seasonal evapotranspiration (ET) rate. Instead, the volume of water required to return the water surface to a constant level was measured on 12–24 h intervals over a 14-day period. These ET measurements were considered indicative of the entire season in which they were taken.

During each experimental incubation, samples were drawn through the sampling tube with a glass syringe at 1 h, and 1, 2, 3, 5, 7, 10 and 14 days after filling the columns with the wastewater mixture. In four incubations, an 8-h sample was also included. All samples were measured for COD, solvent and 2-propanol concentrations with procedures identical to the jar experiments except that COD samples were unfiltered. Additional details of the experimental design, infrastructure and methodologies can be found in Kowles (2001).

3. Results

3.1. Jar experiments

Observed changes in the three solvent concentrations with time are shown in Fig. 1. An average loss of up to 20% was measured within 1 h after filling the jars, which could be due to sorption, volatilization during transfer, or unaccountable experimental error. After the first day,

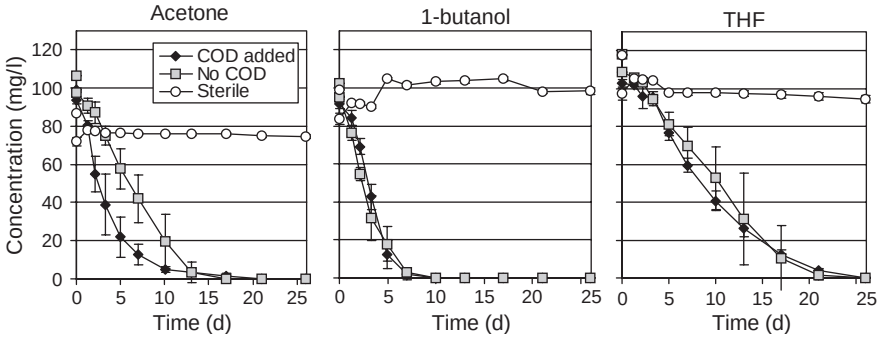

Fig. 1. Concentrations of 1-butanol, acetone and tetrahydrofuran in 26-day jar incubations with gravel wetland media. Error bars indicate ±1 SD among three replicates of each treatment. The first point shown is of concentration prior to pouring wastewater into jars.

solvent concentration in the sterile control jars was nearly constant, confirming that the additional loss observed in the non-control jars was biologically mediated. In the biologically active jars, 90% removal of the solvents required 5–6 days for 1-butanol, 8–12 days for acetone and 15–20 days for THF. 1-Butanol and acetone removal rates are comparable to, or exceed, the highest removal rates reported in available 5- and 10-day BOD test data, but the THF was slightly slower than that reported for in situ soil-based systems (Verschueren, 1996).

Comparing the biologically active treatments, the low-COD treatment had significantly (at $\alpha = 0.05$) less 1-butanol than the high-COD treatment on days 1 and 2. This trend continued (but not significantly) until removal was nearly complete in both treatments at 5 days. Differences in THF degradation between these treatments were not significant at any time in the incubation. Contrarily, the data suggest that acetone was removed more quickly in the high-COD treatment. However, GC analysis revealed an additional peak gaining prominence in the high-COD treatment as solvents were degraded. The peak did not correspond to potential degradation intermediates of acetone such as propanediol, acetol or acetoacetate (Bonnet-Smits et al., 1988; Platen et al., 1990, 1994). Instead, the peak was confirmed by mass spectrometry to be 2-propanol, or isopropyl alcohol. As shown by Terzis (1994), 2-propanol can be anaerobically oxidized to acetone with a Gibbs free energy (ΔG^{o}_{RXN}) of +98.8 kJ (Eq. (1)). Using the Nernst equation (Eq. (3), with n equal to the number of electrons transferred and F the Faraday constant), the standard hydrogen electrode potential, E^{o}_{H}, of this redox couple equals 128 mV. This places acetone well below oxygen and nitrate, but in a favored position relative to sulfate or carbon dioxide as an electron acceptor in microbial metabolism. Apparently, the high-COD treatment favored opportunistic microorganisms capable of utilizing acetone as an electron acceptor in the oxidation of the readily metabolized dog food and/or the other solvents. As overall COD levels dropped, likely increasing the redox level, 2-propanol was likely oxidized either by first converting it to acetone or by another mechanism.

$$E^{\circ}_{H} = \frac{-\Delta G^{o}}{nF}. \quad (3)$$

When molar concentrations of acetone and 2-propanol are summed together, it is clear that the overall removal of this couple is somewhat slower than for acetone alone and, more importantly, the low-COD treatment had equal or more rapid removal than the high treatment (Fig. 2). This suggests that given a fixed but unknown oxygen transfer rate, variation in the overall COD level of a mixed domestic/toxic wastewater stream, and corresponding changes in redox level during treatment, alters the degradation pathways of specific sources of COD including solvents. While this conclusion may be intuitive, the ramifications for solvent removal in batch operated CWs in which redox level can be altered by carbon load, plant species selection and season (Hook et al., 2003; Stein and Hook, 2004) may be profound. These issues are explored in the next section.

3.2. Plant experiments

In line with the jar experiments, removal of 1-butanol was more rapid and complete than the removal of acetone, which was in turn more rapid and complete than THF removal, regardless of plant type and season. Due to the observed acetone/2-propanol degradation couple in the jar experiments, acetone removal is reported as the change in the sum of acetone and 2-propanol concentrations, a more conservative measure than acetone alone. Mean butanol removal approached 90% within 1–3 days depending on treatment type, while the same removal rate for acetone plus 2-propanol required 5–10 days. At the end of the 14-day incubations

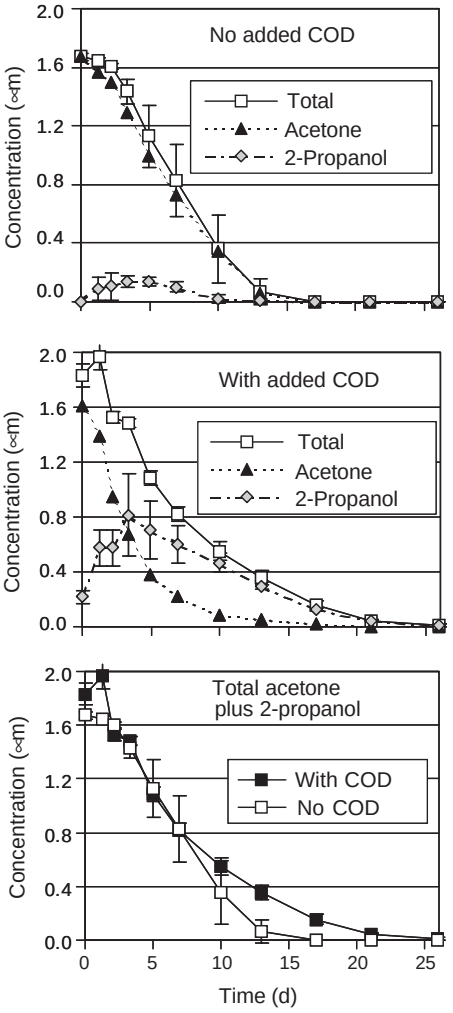

Fig. 2. Average millimolar concentrations of acetone and 2-propanol and the total of the two in low- and high-COD treatments. Error bars on 2-propanol and total show ±1 SD among three replicates of each treatment.

THF was only 50–90% removed depending on treatment (Fig. 3). Species and season demonstrated statistically significant effects on the removal of all three solvents. In general, removal was greater for planted treatments than unplanted ones and was better in summer than in winter. Due to the obvious variability in removal efficacy between solvents, we make species and seasonal comparisons at 14 days for THF, 24 h for butanol, and 5 days for acetone and overall COD. At these times, removal was significant but not complete. Overall COD includes the organics found in the post-primary municipal wastewater as well as the solvents and thus changes in solvent concentration influence COD. Mean removal values at these times for each parameter as a function of species and season are shown in Table 2.

Fourteen-day values for THF removal showed distinct trends by treatment. In summer, the three planted treatments (THF was not added to the *Pondeteria* columns in two summer incubations) 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 removal in the winter, removal was poorer than the control columns, although not significantly so.

All planted treatments averaged better than 80% removal of 1-butanol within 24 h in the summer, though only *Carex* was significantly better than unplanted 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 than summer except *Juncus* and *Pondeteria. Juncus* actually removed more butanol in the winter than in the summer, though not significantly so.

All planted treatments averaged greater than 80% removal of acetone plus 2-propanol within 5 days during summer incubations. Differences among species were not significant in summer, although all plants except *Pondeteria* performed significantly better than the unplanted controls. In winter, *Juncus* performed significantly better than all other treatments, which were statistically similar. All treatments except *Juncus* demonstrated significantly better performance in summer over winter, though *Juncus* demonstrated the same seasonal trend.

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 unplanted control. *Juncus* and *Carex* both performed significantly better than unplanted controls in 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.

Average measured evapotranspiration (ET) is shown in Table 2. As expected, evaporation from unplanted controls was less than ET from planted treatments in both seasons, and summer ET was greater than winter for all treatments. Species differences correlate with general observations of plant growth; *Carex* and *Juncus* had greater ET loss regardless of season but less seasonal variation than *Pondeteria* and *Iris*. Therefore, potential solvent loss by transpiration could be larger, but less seasonally variant, in *Carex* and *Juncus.*

To estimate the effect of transpiration on solvent removal, we applied the model of Briggs et al. (1992)

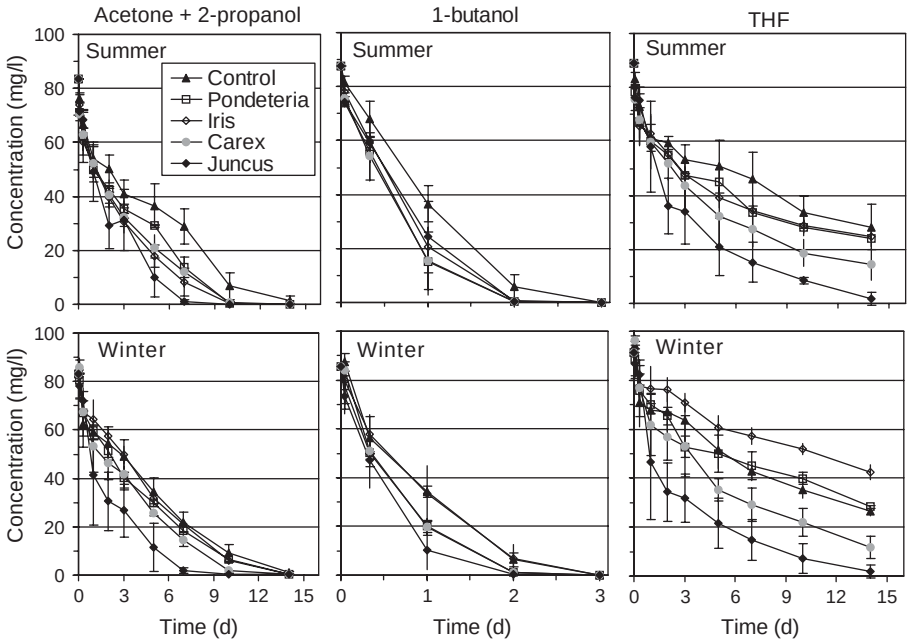

Fig. 3. Average concentrations of 1-butanol, acetone plus 2-propanol, and tetrahydrofuran for each plant species in representative summer (3) and a winter (6) incubations. Data points show averages of all replicates of each treatment. Error bars indicate ±1 SD of the replicates.

Table 2
Removal ratio of solvents and COD at specified times and evapotranspiration by season and treatment

	THF (day 14)		1-Butanol (day 1)		Acetone (day 5)		COD (day 5)		ET(ml/day)
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Summer									
Control	0.71ax	0.08	0.68ax	0.12	0.68ax	0.16	0.69ax	0.11	32
Pondeteria	NA		0.84abx	0.10	0.81abx	0.15	0.85abx	0.13	62
Iris	0.82bx	0.08	0.82abx	0.08	0.92bx	0.10	0.83abx	0.07	102
Carex	0.88bcx	0.05	0.87bx	0.09	0.84bx	0.09	0.83abx	0.07	136
Juncus	0.95cx	0.05	0.82abx	0.13	0.89bx	0.07	0.89bx	0.04	170
Winter									
Control	0.65ax	0.06	0.47ay	0.11	0.49ay	0.10	0.57ay	0.13	27
Pondeteria	0.62a	0.09	0.67bcx	0.11	0.56ay	0.06	0.66aby	0.07	31
Iris	0.55ay	0.08	0.53aby	0.15	0.61ay	0.17	0.62aby	0.09	32
Carex	0.81bx	0.10	0.67by	0.09	0.62ay	0.09	0.74bcx	0.10	127
Juncus	0.94cx	0.05	0.85cx	0.10	0.80bx	0.12	0.85cx	0.08	118

Differences between plant treatments within season are not different at $\alpha = 0.05$ if followed by the same letter, a–c. Differences between seasons within plant treatments are not different at $\alpha = 0.05$ if followed by the same letter, x–y.

(Eq. (2)) for the transpiration stream concentration factor (TSCF). Though this model has been best calibrated for large molecular weight pesticides and not the solvents or the specific plant species used in our study, the phenomenological basis for the relationship, the data of Cunningham et al. (1996) and the lack of another quantitative relationship rationalized use of Eq. (2). Based on data in Table 1, the calculated TSCF, the ratio of transpired water to pore water solvent concentration, is 0.499, 0.147 and 0.563 for THF, acetone and 1-butanol, respectively.

Transpiration of water and solvents was modeled separately for an individual column and then averaged by treatment and season. ET values measured during the

seasonal ET incubation were applied to the other three incubations conducted during that season. Transpiration values were obtained by subtracting average evaporation measured from the unplanted control columns from the measured planted column ET value; thus we assumed the presence of plants affected only transpiration. To estimate the total mass of solvent lost via transpiration M, we multiplied the TSCF by the measured pore water concentration of each solvent at each sampling time C_i, the change in time t_i between n sampling events and the estimated transpiration rate T (Eq. (2)). An example calculation is shown in Table 3.

$$M = \sum_{i=1}^{n} \mathrm{TSCF}\, C_i\, t_i\, T. \quad (4)$$

Fig. 4 compares removal pathways for THF at day 14. Summer transpiration loss values ranged between 16% and 28%, and winter values between 1% and 25%. Plant treatment and seasonal effects closely follow differences in measured ET. Differences in transpiration losses account for the majority of seasonal and plant species differences in measured overall removal of THF. Due to the combined effect of a relatively low TSCF and relatively rapid decrease in concentration, the maximum estimate of acetone lost via transpiration at day 5 was 3.8% for the *Juncus* columns in winter. Comparing this loss to the overall removal ratios in Table 2, it is clear that mechanisms other than transpiration were responsible for the vast majority of acetone removal. Because removal approached 90% within 24 h, 1-butanol loss via transpiration is insignificant.

4. Discussion

Removal of all three solvents was more rapid in the plant experiments at any season than in the jars even when the unplanted control columns, tending to be the poorest performing (Table 2), are used for comparison (Fig. 5). Temperature cannot explain the differences as greenhouse summer temperatures were marginally higher than the room-temperature jar experiments, but greenhouse winter temperatures were considerably cooler. The quantity of additional organic carbon is also not a factor as the COD of municipal wastewater used in the columns was about 250 mg/l, midway between the low and high carbon jar treatments. The differences are not large at 1 h, a time period we attribute to sorption and/or volatilization at transfer in the jar experiments, but

Table 3
Example transpiration stream calculation for column *Carex* C2, incubation 3

TSCF for THF	0.499
Liquid volume of column	1218 ml
Average calculated transpiration for this column and season	4.8 ml/h
Measured THF concentration (C_i) after 24 h (beginning of time increment 4)	59.0 mg/l
TSCF × C_i × T × t_i for increment 4 (duration = 24 h)	3.39 mg
Predicted concentration at end of time interval 4 due to transpiration losses only	56.2 mg/l
Measured concentration at end of time interval 4	46.5 mg/l

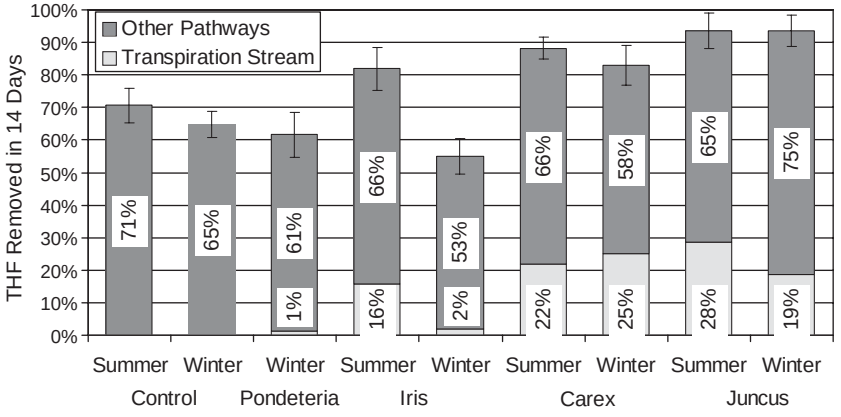

Fig. 4. Day-14 THF removal attributed to transpiration and other removal mechanisms. Error bars indicate 95% confidence intervals for non-transpired fraction.

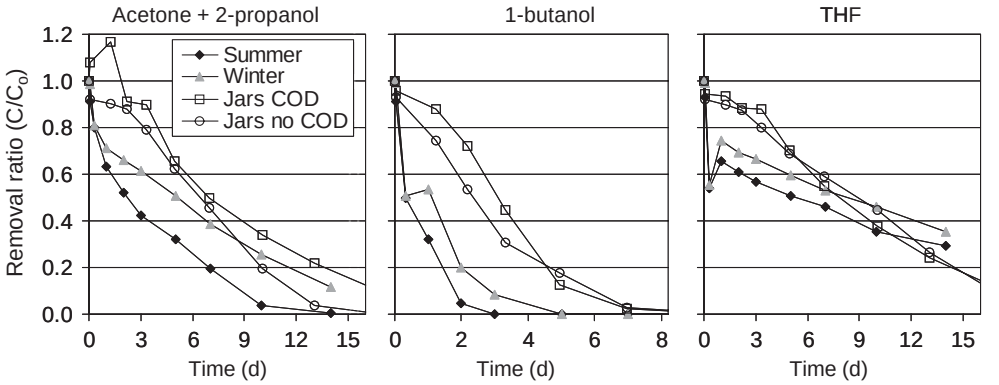

Fig. 5. Comparison of average solvent concentration with time in the unplanted wetland columns in summer and winter to the gravel "jar experiments" with and without additional COD loading (approximately 450 mg/l). Additional COD loading in the unplanted columns was approximately 250 mg/l.

are most apparent by 24 h. The most striking difference is that unlike the jars, the control columns do not display a lag in removal during the initial 24–72 h (depending upon the particular solvent). Either the sorption capacity of the smaller column gravel is larger but slower, or the different additional organic carbon composition influenced the initial solvent degradation rate, or the column biofilm was better able to initially sequester and ultimately degrade the solvents. Allen et al. (2002) reported similarly large initial (<24 h) removal of simulated domestic wastewater COD in similar CW columns. Stein et al. (2003) speculated this was due to biosequestration induced by a high initial redox level due to batch operation. Presumably the open-topped columns have a larger oxygen transfer rate, hence a higher redox level than the jars, which were only vented to the atmosphere. Despite differences at early times, the shape of the curves at times greater than 24–72 h and the ultimate removal efficacy in the two experiments are remarkably similar.

It is clear from the plant experiments that plant species has a strong effect on performance. *Juncus* performed better than all others for nearly every measured parameter in both seasons. *Juncus* especially stands out in winter because, unlike other treatments, its winter performance does not decline significantly relative to summer, especially for COD and THF. *Carex* is typically the next best treatment, especially in winter. It displays a greater performance decrease in winter over summer than *Juncus*, but less than the other treatments. Average performance of *Iris* and *Pondeteria* is consistently better than controls during the summer, although differences were typically insignificant statistically. In winter, differences between the planted *Iris* and *Pondeteria* treatments and unplanted controls are even smaller; unplanted controls had better THF removal. *Juncus* and *Carex* tended to dampen seasonal variation compared to unplanted controls, while *Iris* and *Pondeteria* demonstrated greater seasonal variation. Both *Juncus* and *Carex* had much greater above ground biomass and maintained some vegetative growth during the winter months; *Iris* and *Pondeteria* became fully senescent with new shoots emerging in the spring.

The evapo-transpiration pathway is a significant component of plant effects for the more recalcitrant THF requiring longer treatment times, but cannot explain species effects for other solvents. Though other removal pathways such as direct volatilization, plant and microbial uptake and storage might be influenced by plants and probably contributed to the observed removal of these solvents (Kadlec and Knight, 1996), we attribute non-ET related solvent removal to gravel sorption and biodegradation. Gravel sorption, which contributed up to 20% of the observed removal based on jar experiment results, should be independent of plant species and season. Thus species differences are likely due to plant effects on biodegradation. Specific aerobic and anaerobic microorganisms using a variety of electron acceptors have been identified as capable of degrading all three solvents (e.g. Platen and Schink, 1989; Bernardt and Diekmann, 1991; Verschueren, 1996; Kohlweyer et al., 2000; Kowles, 2001). Some of these studies suggest that the higher the organism operates on the redox ladder, the faster the rate of degradation. Allen et al. (2002) and Hook et al. (2003) have shown that some plant species are capable of increasing wetland redox values during winter conditions. Increased redox levels correlated with increased COD removal and Stein and Hook (2004) argued that the increased winter redox level mitigates the negative influence of reduced temperature on biodegradation. They identified *Carex utriculata* as better than *Schoenoplectus acutus* which was better than *Typha latifolia* at

raising winter redox levels and improving winter COD removal. Our *Juncus* and *Carex* treatments displayed less seasonal variation in overall removal than the other treatments (Table 2). Indirect indices of redox level such as the sulfate/sulfide couple and the acetone/2-propanol couple suggest these treatments had higher redox levels than the other treatments (Kowles, 2001). Based on general plant morphology parameters, *Juncus* probably behaves more similar to *Carex* than to *Pondeteria* or *Iris* species (which were selected in our study for ornamental rather than treatment purposes). Considering just biodegradation (Fig. 3), *Juncus* still displayed statistically significant better THF removal than other treatments in winter. Thus it seems likely that the improved treatment observed in *Juncus* and *Carex* is due, at least in part, to greater biodegradation due to a higher redox level.

5. Conclusions

This research demonstrates that batch loaded constructed wetland systems can be expected to remove large quantities of three classes of non-halogenated polar organic solvents from municipal wastewater streams. The three classes are a ketone, aliphatic alcohol and an aromatic represented by acetone, 1-butanol and tetrahydrofuran (THF), respectively. Biodegradation is identified as the dominant removal mechanism in our model systems but as much as 20% of the total removal may be attributed to sorption. Total mass removal and removal rate is consistent with published information on solvent biodegradability, with 1-butanol > acetone > THF. The effects of season and plant species were similar regardless of the type of solvent; removal was generally better during summer than winter and varied between plant species such that a general ranking is *Juncus* > *Carex* > *Iris* = *Pondeteria* > unplanted control. Detailed statistical comparisons are made in Table 2. We infer that differences in overall efficacy between species are due, in large part, to the effect of plants on the redox state and appurtenant degradation pathways. Due to its relatively high lipophilicity and relatively low biodegradability, plant transpiration contributed between 25% and 30% of the total THF loss in all planted treatments in summer and *Carex* and *Juncus* in winter.

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References

Allen, W.C., Hook, P.B., Biederman, J.A., Stein, O.R., 2002. Temperature and wetland plant species effects on wastewater treatment and root zone oxidation. J. Environ. Qual. 31, 1010–1016.

Arp, D.J., 1999. Butane metabolism by butane-grown *Pseudomonas butanovora*. Microbiology 145, 1173–1180.

Bernardt, D., Diekmann, H., 1991. Degradation of dioxane, tetrahydrofuran and other cyclic ethers by an environmental *Rhodococcus* strain. Appl. Microbiol. Biotech. 36, 120–123.

Bhattacharya, S.K., Madura, R.L., Dobbs, R.A., Angara, R.V.R., Tabak, H., 1996. Fate of selected RCRA compounds in a pilot-scale activated sludge system. Water Environ. Res. 68 (3), 260–269.

Bonnet-Smits, E.M., Robertson, L.A., van Dijken, J.P., Senior, E., Kuenen, J.G., 1988. Carbon dioxide fixation as the initial step in the metabolism of acetone by *Thiosphaera pantotropha*. J. Gen. Microbiol. 134, 2281–2289.

Borden, D.J., Stein, O.R., Hook, P.B., 2001. Seasonal effects of supplemental organic carbon on sulfate reduction and zinc sulfide precipitation in constructed wetland microcosms. In: Proceedings, International Ecological Engineering Society Meetings, 26–29 November 2001, Lincoln University, New Zealand, pp. 296–300.

Briggs, G.G., Bromilow, R.H., Evans, A.A., 1982. Relationships between lipophilicity and root uptake and translocation of non-ionized chemicals by barley. Pesticide Sci. 13, 495–504.

Clark, D.D., Ensign, S.A., 1999. Evidence for an inducible nucleotide-dependent acetone carboxylase in *Rhodococcus rhodochrous* B276. J. Bacteriol. 181 (9), 2752–2758.

Cunningham, S.D., Anderson, T.A., Schwab, A.P., Hsu, F.C., 1996. Phytoremediation of soils contaminated with organic pollutants. Adv. Agron. 56, 55–114.

Eichler, B., Schink, B., 1984. Oxidation of primary aliphatic alcohols by *Acetobacterium carbinolicum* sp. nov., a homoacetogenic anaerobe. Arch. Microbiol. 140, 147–152.

Hannah, S.A., Austern, B.M., Eralp, A.E., Wise, R.H., 1986. Comparative removal of toxic pollutants by six wastewater treatment processes. J. Water Pollut. Control Fed. 58 (1), 27–34.

Hannah, S.A., Austern, B.M., Eralp, A.E., Dobbs, R.A., 1988. Removal of organic toxic pollutants by trickling filter and activated sludge. J. Water Pollut. Control Fed. 60 (7), 1281–1283.

Hook, P.B., Stein, O.R., Allen, W.C., Biederman, J.A., 2003. Plant species effects on seasonal performance patterns in model subsurface wetlands. In: Mander, Ü., Jenssen, P.D. (Eds.), Constructed Wetlands for Wastewater Treatment in Cold Climates. Advances in Ecological Sciences, vol. 11. WIT Press, Southhampton, UK, pp. 87–106.

Hsu, F.C., Marxmillar, R.L., Yang, A.Y.S., 1990. Study of root uptake and xylem translocation of cinmethylin and related compounds in detopped soybean roots using a pressure chamber technique. Plant Physiol. 93, 1573–1578.

Janssen, P.H., Schink, B., 1995. Metabolic pathways and energetics of the acetone-oxidizing, sulfate-reducing bacterium, *Desulfobacterium cetonicum*. Arch. Microbiol. 163, 188–194.

Kadlec, R.H., Knight, R.L., 1996. Treatment Wetlands. CRC Press, Boca Raton, FL.

Kohlweyer, U., Thiemer, B., Schräder, T., Andreesen, J.R., 2000. Tetrahydrofuran degradation by a newly isolated culture of *Pseudonocardia* sp. strain K1. FEMS Microbiol. Lett. 186, 301–306.

Kowles, J.L., 2001. The fates of three polar organic solvents in a microcosm constructed wetland wastewater treatment system. MS Thesis, Montana State University.

Platen, H., Schink, B., 1987. Methanogenic degradation of acetone by an enrichment culture. Arch. Microbiol. 149, 136–141.

Platen, H., Schink, B., 1989. Anaerobic degradation of acetone and higher ketones via carboxylation by newly isolated denitrifying bacteria. J. Gen. Microbiol. 135, 883–891.

Platen, H., Temmes, A., Schink, B., 1990. Anaerobic degradation of acetone by *Desulfococcus biacutus* spec. nov. Arch. Microbiol. 154, 355–361.

Platen, H., Hanssen, P.H., Schink, B., 1994. Fermentative degradation of acetone by an enrichment culture in membrane-separated culture devices and in cell suspensions. FEMS Microbiol. Lett. 122, 27–32.

Poelarends, G.J., Kulakov, L.A., Larkin, M.J., van Hylckama Vlieg, J.E.T., Janssen, D.B., 2000. Roles of horizontal gene transfer and gene integration in evolution of 1,3-Dichloropropene- and 1,2-Dibromomethane-degradative pathways. J. Bacteriol. 182 (8), 2191–2199.

Polprasert, C., Dan, D.P., Thayalkumaran, N., 1996. Application of constructed wetlands to treat some toxic wastewaters under tropical conditions. Water Sci. Technol. 34 (11), 165–171.

RCRA, 1997. The Hazardous Waste Consultant, vol. 15, issue 5: RCRA Land Disposal Restrictions: A Guide to Compliance. Elsevier Science, Inc., New York, NY.

Revitt, D.M., Worrall, P., Brewer, D., 2001. The integration of constructed wetlands into a treatment system for airport runoff. Water Sci. Technol. 44 (11–12), 469–476.

Schwarzenbach, R.P., Gschwend, P.M., Imboden, D.M., 1993. Environmental Organic Chemistry. Wiley, New York, NY.

Shone, M.G.T., Wood, A.V., 1974. A comparison of the uptake and translocation of some organic herbicides and a systemic fungicide by barley. J. Exp. Bot. 25 (85), 390–400.

Stein, O.R., Hook, P.B., 2004. Temperature, plants and oxygen: how does season effect constructed wetland performance? J. Environ. Sci. Health A. 40 (6–7), 1331–1342.

Stein, O.R., Hook, P.B., Biederman, J.A., Allen, W.C., Borden, D.J., 2003. Does batch operation enhance oxidation in subsurface constructed wetlands? Water Sci. Tech. 48 (5), 149–156.

Terzis, E., 1994. Anaerobic treatment of industrial wastewater containing organic solvents. Water Sci. Tech. 29 (9), 321–329.

Thomas, R., Freeman, C., Rehman, N., Fox, K., 2003. Removal of linear alkylbenzenesulfonate (LAS) in constructed wetlands. In: Vymazal, J. (Ed.), Wetlands—Nutrient, Metals and Mass Cycling. Backhuys Publishers, Leiden, The Netherlands, pp. 35–47.

Verschueren, K., 1996. Handbook of Environmental Data on Organic Chemicals, third ed. Van Nostrand Reinhold, New York, NY.