



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  
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**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.

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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.

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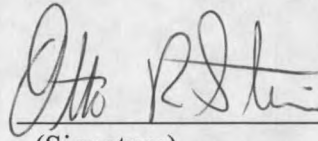
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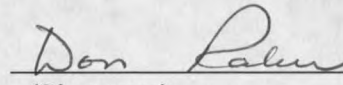
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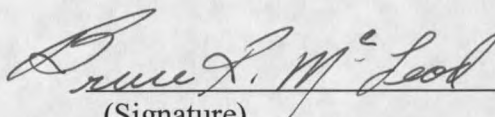
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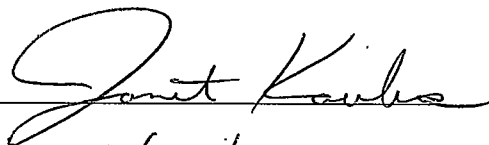
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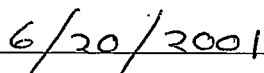
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## ABSTRACT

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 *Pondetaria 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<sup>3</sup>/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 1<sup>st</sup> 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 overestimate 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<sub>2</sub> 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<sub>2</sub>S 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-rhizosphere oxygen transport rates ranging from less than zero (oxygen uptake) to as high as  $3.95 \text{ g O}_2 \text{ h}^{-1}$  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  $40 \text{ g O}_2 \text{ kg}^{-1} \text{ h}^{-1}$  (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 ( $\text{BOD}_5$ ) 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 *Pondetaria cordata*, with average transport rates of 0.81, 1.08, 1.01 and  $0.78 \text{ g O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ , respectively. Common cattail (*Typha latifolia*) and *Scirpus pungens* performed significantly worse at 0.16 and  $0.18 \text{ g O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ , 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).

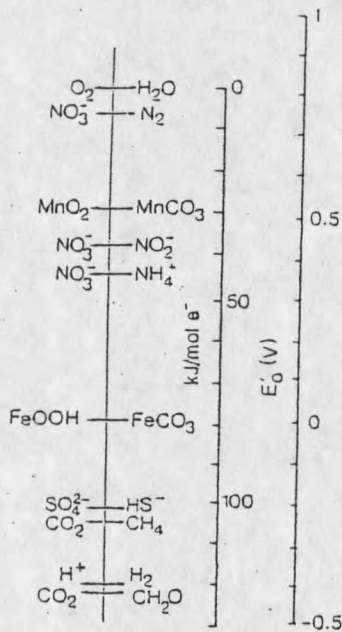


Figure 1. Relative potential of prominent redox couples in natural aquatic systems. Fenchel and Finlay, 1995

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  $10^\circ\text{C}$ , while a *Phragmites australis* released oxygen at  $5^\circ\text{C}$ , not at  $20^\circ\text{C}$  (Sorrell and Armstrong, 1994). Allen (1999) reported that differences in COD removal and redox potential among plant species were greater at low temperatures ( $4^\circ\text{C}$  versus  $24^\circ\text{C}$ ). 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 <sub>3</sub> -CO-CH <sub>3</sub>	C <sub>4</sub> H <sub>8</sub> O-cyclical	CH <sub>3</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> OH
Molecular weight	58.08	72.10	74.12
Aqueous solubility	miscible	miscible	77,000 mg/l (a)
Dimensionless Henry's Constant (H)	1.52 X 10 <sup>-3</sup> (b)	4.50 X 10 <sup>-3</sup> (c)	3.47 X 10 <sup>-4</sup> (a)
Octanol/Water Partition Coeff. (K <sub>OW</sub> )	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<sup>3</sup>) divided by chemical concentration in water (mg/m<sup>3</sup>) 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<sub>OW</sub>) is the ratio of solvent concentration in octanol to the concentration in water in an equilibrated system. A higher K<sub>OW</sub> indicates the chemical has a higher affinity to octanol, and to organic material in general, as compared to its affinity to water. The K<sub>OW</sub> 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  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . 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 ( $\text{CH}_3\text{-CHOH-CH}_2\text{OH}$ ) or oxygenase catalysis to acetol ( $\text{CH}_3\text{-CO-CH}_2\text{OH}$ ), which can easily be broken apart into  $\text{C}_1$  and  $\text{C}_2$  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  $\text{CO}_2$ , which is reduced to methane (Platen and Schink, 1987). A fermentative culture which completely degrades acetone to methane and  $\text{CO}_2$  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  $\text{CO}_2$  to form four-carbon acetoacetate ( $\text{CH}_3\text{-CO-CH}_2\text{-COO}^-$ ) (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).

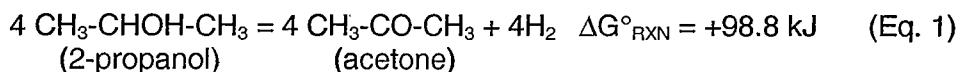
Bernardt and Diekmann (1991) isolated six aerobic *Rhodococcus* strains capable of growth on tetrahydrofuran. The organisms were able to completely degrade 8 mM THF (576 mg/l) in less than 4 days, during which time the optical density of the culture nearly tripled. Above 10 mM (720 mg/l) the lag phase 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 ( $\text{CH}_3\text{-CH}_2\text{-CH}_2\text{-COO}^-$ ) 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):





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% ( $\sigma = 6.1$ ). Average THF removal over six events was 89.1% ( $\sigma = 17.2$ ). 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<sup>2</sup>/day for phenol and 0.044 g/m<sup>2</sup>/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  $K_{ow}$ , 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 methylcarbamoxyloxime and phenylurea pesticides, Briggs et al. (1982) found TSCF to be a nonlinear function of  $K_{ow}$ , according to Equation 2.

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

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 Carbowax 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 filtered through a 0.2 micron syringe filter into 2 mL amber glass vial with a TFE-lined septa. Vials were stored in a refrigerator for up to a week before being analyzed. To analyze samples, 2  $\mu$ L 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 approximately 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 20<sup>th</sup>. The third incubation was begun on March 24<sup>th</sup> and ran until April 22<sup>nd</sup>. 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.



























































































































