CHARACTERIZING AND TRACKING REACTIVE MIXTURE ALTERATIONS: NEW TOOLS FOR PASSIVE TREATMENT SYSTEM DESIGN AND MONITORING[1]

Daphne L. Place [2], Linda Figueroa [2], Thomas Wildeman[2], and David Reisman[2]

Abstract. Microorganisms within passive reactive zones (e.g. wetlands, SR bioreactors, PRB's, etc.) utilize organic substrates in the process of reducing sulfate and immobilizing metals in mine drainage waters. The rate and extent of substrate utilization influences treatment longevity and performance. Tracking substrate utilization is a quantitative tool for planning and design, accomplished by characterizing alterations in reactive mixtures temporally and/or spatially. Characterizing alterations is applicable to selecting substrates, developing microbial utilization rates and rate curves over time, and assessing temporal and spatial variations within treatment zones. The characterization of alterations is determined using a method adapted from a sequential extraction technique used to determine carbohydrate and lignin composition in agricultural products. This paper presents the results of an evaluation of alterations in the reactive mixture composition of two bench-scale bioreactors and a field-scale passive treatment system. Interpretation of these results supports the use and applicability of characterizing and tracking alterations as a new tool for designing and monitoring passive treatment systems. The four selection criteria for determining the appropriate reactive mixture to use in the system are: total organic, cellulose content, lignin content and cellulose to lignin ratio. Using total carbon to predict the longevity of passive treatment systems is insufficient because it does not detail if the carbon is in a bioavailable form for the microbial community. Interpretation of the results from these bioreactors shows how tracking substrate alterations over time can provide information on microbial utilization rates and generate rate curves. This paper evaluates the application of tracking substrate alterations at various locations within a field-scale passive treatment system, and describes how microbial utilization of organic substrate varies spatially. These results suggest that spatial tracking provides valuable insight for the monitoring of passive reactive treatment zones, thereby helping manage the sustainability of the passive treatment system by indicating when the reactive mixture needs to be refreshed.

Additional Key Words: bioreactors, field—scale systems, passive reactive barriers (PRBs), passive reactive zones (PRZ), Peerless Jenny King, performance and monitoring, reactive mixtures, remediation, sulfate reducing bacteria (SRB), sulfate reducing bioreactors (SRBR), substrate utilization, sustainability, treatment, wetlands.

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Introduction

Passive reactive zones (wetlands, sulfate (SO_4^{-2}) reducing bioreactors, permeable reactive barriers) are treatment systems designed to remediate mine drainage waters. These waters may or may not be acidic, but usually contain both dissolved metals and SO_4^{-2}. The goal of the system is to cultivate an anaerobic microbial community capable of the sustained reduction of SO_4^{-2} to sulfide and subsequent oxidation and precipitation of dissolved metals as metal sulfide complexes. In order to cultivate such a community, these systems employ reactive mixtures that contain a built in microbial ecosystem and organic substrate. These organic substrates serve as the first source in the microbial food chain. Each group of microorganisms in the community provides food for another group of organisms. The first group of key organisms is the cellulose degraders. These organisms hydrolyze the organic substrate, providing food for the fermenting bacteria. The next group of key organisms, fermenting bacteria, then conducts metabolic processes providing food for the target organism, SO_4^{-2} reducing bacteria (SRB). SRB are the microbes capable of generating the sulfide that precipitates the dissolved metals from the drainage waters. A breakdown in the microbial utilization of substrate, such as not making enough food for the next microbial functional group, can cause the chain to collapse leading toward treatment system failure. In this manner, rate and extent of substrate utilization controls the performance and longevity of the passive treatment system.

An effective substrate is sufficiently biodegradable to support the metabolic needs of the microbial community, and is sustainable over the expected life of the passive treatment system. Kalin (2004) acknowledges that this has been difficult to do. "The engineering challenge...[has been to] design systems capable of operation for years to decades with little or no maintenance" (Kalin, 2004 page 229). Opinions vary as to what length of time constitutes the expected life of passive treatment systems. Long-term performance of passive bioreactors (Gusek et al., 1998) and passive reactive barriers (Blowes et al., 2000), treating inorganic contaminants suggest longevity between 12 and 20 years, respectively. Unfortunately, the majority of passive treatment systems are not meeting this expected life. The life expectancy calculation is based on the following reaction equation:

$$CH_2O + SO_4^{-2} + 2H^+ \rightarrow H_2S + 2H_2O + 2CO_2$$

This chemical reaction shows the utilization of organic material (CH_2O) in the reduction of sulfate to produce sulfide. To project the life of the passive treatment system, the total organic mass of the reactive mixture and the SO_4^{-2} concentration are used along with kinetic data together with the equation above. Other research that considered substrate utilization, such as Waybrant et al. (1998), Cocos et al. (2002), use total C, total N and/or total P to characterize alterations in the solid phase organic substrate in the reactive mixture of passive treatment systems. Looking solely at total C is not enough. The C must be in a form that is available to the microbial community, which is less than the total C. Longevity expectations based on total C will likely overestimate the sustainability of the system because some C cannot be utilized by the microbial population.

The technique employed in this research is capable of characterizing the usable forms of C and tracking these forms throughout the life of the treatment system. Tracking alterations in the organic substrate of these passive treatment systems may improve overall performance and

treatment system sustainability by helping with organic substrate selection and providing an additional means of monitoring the system over time.

The following pages provide a description of the method for characterizing the usable forms of C present in reactive mixtures. In addition, the results of using this method to analyze bench-scale bioreactors and a field-scale passive treatment system are presented. The resulting data are discussed and interpreted in the context of substrate selection, as well as substrate utilization, in both temporal and spatial scenarios.

Treatment Systems Analyzed

Bench-scale Bioreactors

In early 2003, eight bench-scale bioreactors were constructed as part of a research effort to determine the startup performance of four organic based reactive mixtures (Figueroa et al., 2004). At the end of 2003 the startup research was completed but the bioreactors continued to operate. In mid 2004, six of the eight bioreactors were dismantled and a sample of the reactive mixture was retained for analysis. The other two bioreactors continued to operate until mid 2005, when they were dismantled and the entire reactive mixture was retained for analysis. The bioreactors were glass columns, 30 cm long and 5 cm in diameter, with four 1 cm threaded side ports distributed at 6 cm increments up from the base of the column. Each column contained 150 grams of reactive mixture, on a dry weight basis having a packed volume of 380 $cm^3 \pm 90$ cm^3. This reactive mixture was composed of 45% #8 mesh silica (67.5g), 40% specific organic substrate, 4 mm size (60g), 10% fresh dairy manure (15g), and 5% #10 mesh limestone (7.5g). Mesh data was provided as a specification of the lab grade silica and limestone used in the experiment. The specific organic substrate was oak, alfalfa, pine, or corn stover, packed in duplicate. All columns received an influent of synthetic mine water containing 1000 mg/L sulfate, and 50 mg/L zinc and manganese. The influent was delivered by an isometric peristaltic pump, set to 30 mL/day. The pH of the influent was 6. As part of the previous study, effluent samples were collected and analyzed for sulfate, zinc and manganese concentrations. The early data (first 3 months) indicated that the alfalfa and corn stover based columns allowed for a higher reduction in sulfate, with effluent concentrations between 75 and 125 mg/L. Comparatively, effluent concentrations for oak and pine based bioreactors were between 200 and 300 mg/L. From this data, it was concluded that alfalfa and corn stover supported faster initial reduction of sulfate in the treatment system as designed. Analysis presented in this paper focuses on the alfalfa and corn stover based bioreactors. Information about the other four bioreactors is presented in Place et al. (2005).

Peerless Jenny King

The Peerless Jenny King (PJK) passive treatment system was constructed in 2003 on the Ten Mile Creek EPA Superfund site located near Helena, Montana. It is a two phase treatment system, using a baffled constructed wetland followed by a series of four serpentine sulfate reducing bioreactor (SRBR) treatment cells. The influent water comes from the PJK adit and contains levels of Fe and Zn that exceed water quality standards. The treatment system is currently successful in reducing the iron and zinc concentrations to meet water quality standards. Fig. 1 provides a design schematic of the biogeochemical system.

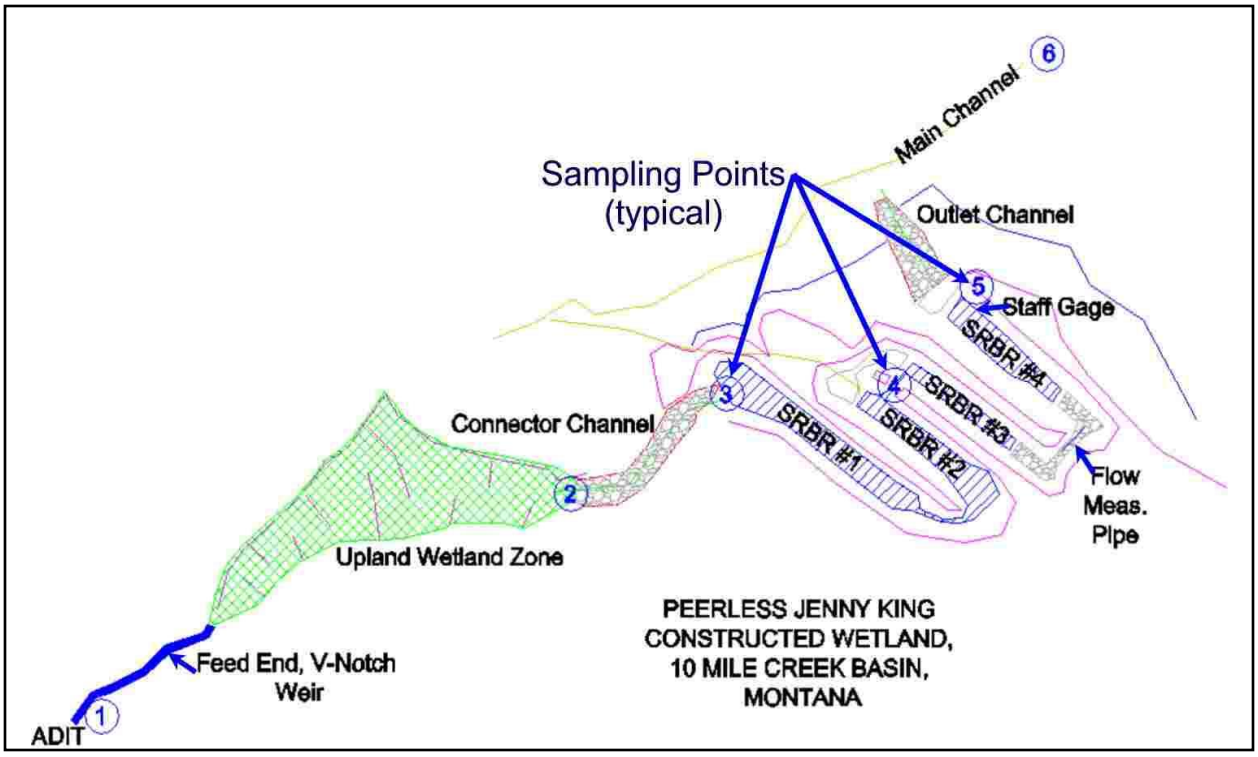

Figure 1. Design schematic of Peerless Jenny King passive biogeochemical treatment system (Gusek, 2005).

Samples of reactive mixture were taken at various locations along the treatment path of this system during two sampling events: June 2005 and August 2005. The four samples are from the June 2005 sampling event and are located in SRBR #1 and SRBR #3, referred herein as Cell 1 and Cell 3. Sampling was achieved by excavating into the cell and exposing a vertical surface to depth profile of the reactive mixture. Samples were collected at vertical locations along the profile. Several samples were obtained from each of these cells, however only four samples are presented. The two samples located in Cell 1 are from the front (influent) end of the cell at the bottom of the sampling profile and from the middle of the cell at the bottom of the sampling profile. The two samples located in Cell 3 are from the middle of the treatment cell, one at the top of the sampling profile and the other at the bottom of the sampling profile. One of the goals of analyzing these samples is to determine if there is any difference between the utilization of the reactive mixture in the beginning (Cell 1) and middle (Cell 3) of the reactor. The other goal is to determine if there is any difference between the utilization of the reactive mixture at different horizontal and vertical locations within a treatment cell.

Method of Characterizing Substrate

The technique for characterizing substrate alterations is synthesized from a method, by Hall, for determining the carbohydrate composition in agricultural products (Hall, 2000) and a method for determining acid insoluble lignin in biomass (Templeton and Ehrman, 1995). According to these techniques organic plant material can be subdivided into four operationally defined categories: (1) organic acid, mono- and oligosaccharide; (2) polysaccharide, starch, fructan, pectin substances and β-glucans; (3) cellulose and hemicellulose; and (4) lignin (Hall, 2000;

Templeton and Ehrman, 1995). The proportion of each category present in organic material is determined through a successive extraction process that is shown in Fig. 2. The insoluble portion of an extraction is the initial sample for the next extraction. The substrate sample is subjected to ethanol extraction, separating the ethanol soluble (category 1) from ethanol insoluble fraction. Next, a sample of the ethanol insoluble fraction is subjected to neutral detergent extraction, separating the neutral detergent soluble (category 2) from the neutral detergent insoluble fraction. A sample of the neutral detergent insoluble fraction is then subjected to the acid hydrolysis extraction, separating the acid soluble (category 3) from acid insoluble fractions. The acid insoluble fraction will contain both organic and inorganic components (Templeton and Ehrman, 1995). Therefore, the lignin (category 4) is the organic component of the acid insoluble fraction (Templeton and Ehrman, 1995).

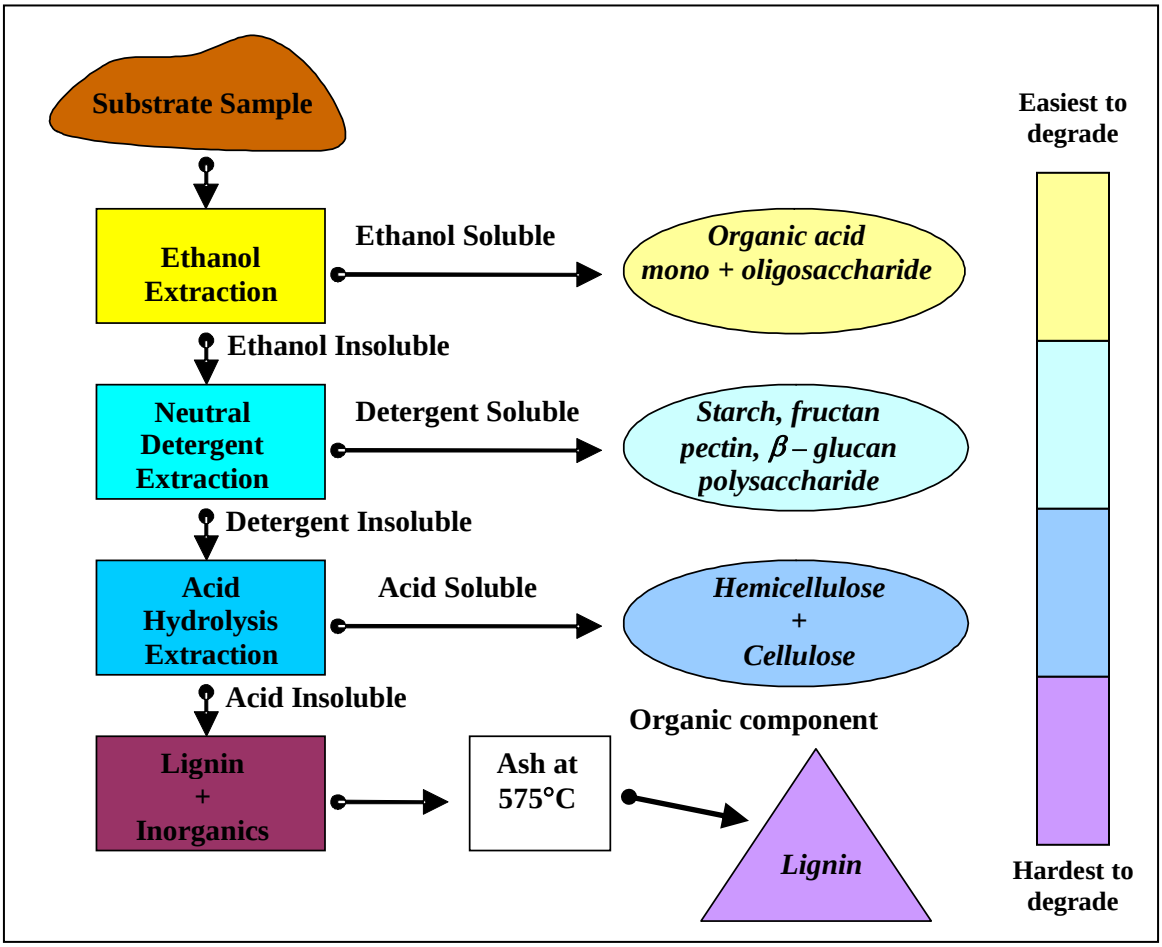

Figure 2. Diagram of sequential extraction process with fractions and categories (Place et al., 2005).

Ethanol Extraction

The purpose of the first phase of the sequential extraction is to separate lower molecular weight mono- and oligosaccharides from polysaccharides present in the solid phase organic substrate. The procedure requires a sample to ethanol ratio of 1.0 ± 0.04 g : 200 mL. An 80%

(by volume) ethanol with DI water solution is used for all extractions, as called for in the procedure by Hall (2000). Once the ethanol and reactive mixture are combined, the extraction mixture is agitated, by shaker table or stir plate, overnight or about 18 hours. After being agitated the mixture is filtered under a vacuum using Whatman 541 filter paper, having a nominal pore size of 20-25 μm. The portion of the mixture that passes through the filter is the ethanol soluble fraction, which consists of mono- and oligosaccharides. The portion of the mixture that is retained on the filter is the ethanol insoluble fraction, which is used in the neutral detergent residual method. The filters are dried and pre-weighed. The ethanol insoluble fraction, with filter, is dried and weighed (Hall, 2000). A sample of the insoluble fraction is ashed to determine the organic content.

Neutral Detergent Residue Method

The purpose of this next phase of the sequential extraction is to separate polysaccharides, starch, fructans, pectin substances, and β-glucans from cellulose and hemicellulose. This separation is accomplished by using the neutral detergent residual method. The procedure requires that one gram of sample be added to 100 mL of neutral detergent solution and 0.2 mL of heat-stable α-amylase. The one gram sample is from the ethanol insoluble fraction produced above. The neutral detergent is a mixture of 1.8 L deionized water, 54 g sodium lauryl sulfate, 26.3 g EDTA (ethylenediaminetetra acetic acid), 7.2 g sodium hydroxide, 12.26 g sodium borate, 8.21 g disodium hydrogen phosphate, and 18 mL triethylene glycol, prepared using the method presented in Hall (2000). The heat stable α-amylase is added to ensure the dissolution of the starch present in the sample. This mixture is heated to a rolling boil for 1 hour, swirling every 15 minutes. Once boiled, the mixture is filtered under a vacuum using a Whatman 541 filter paper, like that used for the ethanol extraction. The portion of the mixture that passes through the filter is the neutral detergent soluble fraction, which consists of polysaccharides, starch, fructans, pectin substances, and β-glucans. The portion of the mixture that is retained on the filter is the neutral detergent insoluble fraction, which is used in the acid-insoluble lignin determination. The filters are dried and pre-weighed. The neutral detergent insoluble fraction, with filter, was dried and weighed (Hall, 2000). A sample of the insoluble fraction was ashed to determine the organic content.

Acid-Insoluble Lignin Determination

The final phase of the sequential extraction is to separate cellulose and hemicellulose from lignin. This is accomplished using the acid-insoluble lignin determination (Templeton and Ehrman, 1995). The procedure for this phase requires that 0.30 ± 0.01 grams of reactive mixture be combined with 3 mL of 72% (by weight) sulfuric acid and placed in a 30°C water bath to hydrolyze for 2 hours, stirring every 15 minutes. The sulfuric acid hydrolyzes the cellulose and hemicellulose, but does not affect the lignin, which is mostly insoluble in mineral acids (Templeton and Ehrman, 1995). After hydrolysis is complete, the mixture is diluted to a 4% sulfuric acid solution by bringing the volume to 84 mL, using deionzed water. It is then placed in an autoclave at 121°C for 1 hour. The mixture is filtered under a vacuum through a pre-weighed fritted crucible. The portion of the mixture passing through the crucible is the acid soluble fraction, which consists of cellulose and hemicellulose. The portion of the mixture retained in the crucible is the acid insoluble fraction, which consists of lignin, inorganic materials, and proteinaceous materials (lignin-like substances). The crucible and contents are dried at 105°C until constant weight is reached. The crucible is then placed in a 575°C muffle oven for at least 3 hours. The crucible is cooled and weighed. The material left in the crucible is

the acid insoluble ash. The acid insoluble fraction less the acid insoluble ash is the lignin content (Templeton and Ehrman, 1995).

Organic Content Determination

Samples of the reactive mixture from these treatment systems will contain inorganic materials like sand, gravel or metals. In order to properly analyze the substrate alterations, the organic content of the raw sample and each insoluble fraction must be determined. This determination is accomplished by placing the sub sample in a muffle oven at 575°C for 3 hours. The sub-sample to be analyzed for organic content is first dried at 105°C. This removes any moisture present. The sampled is then weighed and placed in a pre-weighed crucible. The sub-sample is then ignited over a Bunsen burner to prevent the sample from flaming when it is in the muffle oven, which may cause some of the sub sample to leave the crucible. The crucible with sub-sample is placed in the muffle oven for 3 hours. The crucible and sample are cooled in a desiccator and weighed. The mass lost during this process represents the organic content of the sub-sample.

Results

Bench-scale Bioreactors

Each analysis for the characterization was completed in a least duplicate for both the alfalfa based and corn stover based bioreactors, depending on the amount of sample available. The data shown is an average of those values. Table 1 shows data for the alfalfa based reactive mixture and corn stover based mixture at three points in time: initial (early 2003), 18 months (mid 2004) and 30 months (mid 2005). To establish a mass basis for comparison, the percent composition data was converted to mass data by assuming that lignin was not significantly degraded. This assumption is supported by research conducted in anaerobic composting where lignin was found to have a degradability of 0% (Haug, 1980). This is further supported by the use of lignin content as a part of an empirically based calculation for determining the biodegradable fraction of volatile solids in organic waste components, where high lignin content correlates with low biodegradability (Tchobanoglous et al., 1993).

Peerless Jenny King

Four samples were analyzed from the PJK passive treatment system. These samples came from two different cells within a four cell treatment system and at two different spatial locations within these two cells. The first set of samples is from Cell 1. One sample comes from the front of the cell at the bottom of the sampling profile. The other comes from the middle of the cell at the bottom of the sampling profile. The second set of samples is from Cell 3. Both samples are from the middle of the cell. One is from the top of the sampling profile and the other is from the bottom of the sampling profile. Table 2 shows the percent carbohydrate fraction for the four samples.

Table 1. Mass and percent carbohydrate fraction for alfalfa based and corn stover based bench-scale bioreactors at three points in time (initial data from Figueroa et al., 2004).

Sample with Condition	Organic Acids Mono + Oligosaccharides (g)		Starch Fructan, Pectic Substances, b-Glucans and other non-starch Polysaccharides (g)		Hemicellulose Cellulose (g)		Lignin (g)		Organic Mass of Column (g)
Alfalfa (Initial)	8.9	11.9%	20.0	26.7%	21.3	28.3%	5.7	7.6%	55.9
Alfalfa (18 months)	0.5	2.7%	1.2	6.9%	8.9	49.7%	5.7	31.9%	17.8
Alfalfa (30 months)	0.1	0.5%	2.7	19.9%	5.0	37.3%	5.7	42.3%	13.4
Corn Stover (Initial)	6.6	8.7%	9.3	12.4%	38.0	50.7%	7.7	10.2%	61.5
Corn Stover (18 months)	0.5	1.3%	16.6	42.7%	12.4	31.8%	7.7	19.7%	38.9
Corn Stover (30 months)	0.1	30.0%	5.4	19.1%	15.1	53.5%	7.7	27.2%	28.2

Table 2. Percent carbohydrate fraction for sample locations in the Peerless Jenny King passive treatment system obtained June 2005.

Sample	Organic Acids Mono + Oligosaccharides	Starch Fructans, Pectic Substances, ß-Glucans and other non-starch Polysaccharides	Hemicellulose Cellulose	Lignin
Cell 1 front bottom	3.8%	9.7%	61.6%	24.9%
Cell 1 middle bottom	1.0%	13.1%	68.5%	17.5%
Cell 3 middle top	5.1%	13.9%	38.6%	42.4%
Cell 3 middle bottom	4.1%	13.6%	31.0%	51.1%

Discussion and Interpretation

Selection Criteria

Performing substrate analyses on potential reactive mixtures provides a quantitative tool for selecting the most appropriate mixture for inclusion in a passive treatment system. There are four key criteria to consider when selecting a reactive mixture during the design phase. These

criteria are: total C (organic), cellulose content, lignin content, and the ratio of cellulose to lignin (C:L). Fig. 3 shows the mass of these four criteria in the initial condition for pine, alfalfa, and corn stover based reactive mixtures used in bench-scale bioreactors (Figueroa et al., 2004). As stated previously, cellulose provides the food for the initiating microbe in the community food chain that ultimately supports the target organism, sulfate reducing bacteria. Considering C:L while selecting a suitable substrate suggests the bioavailability of the cellulose.

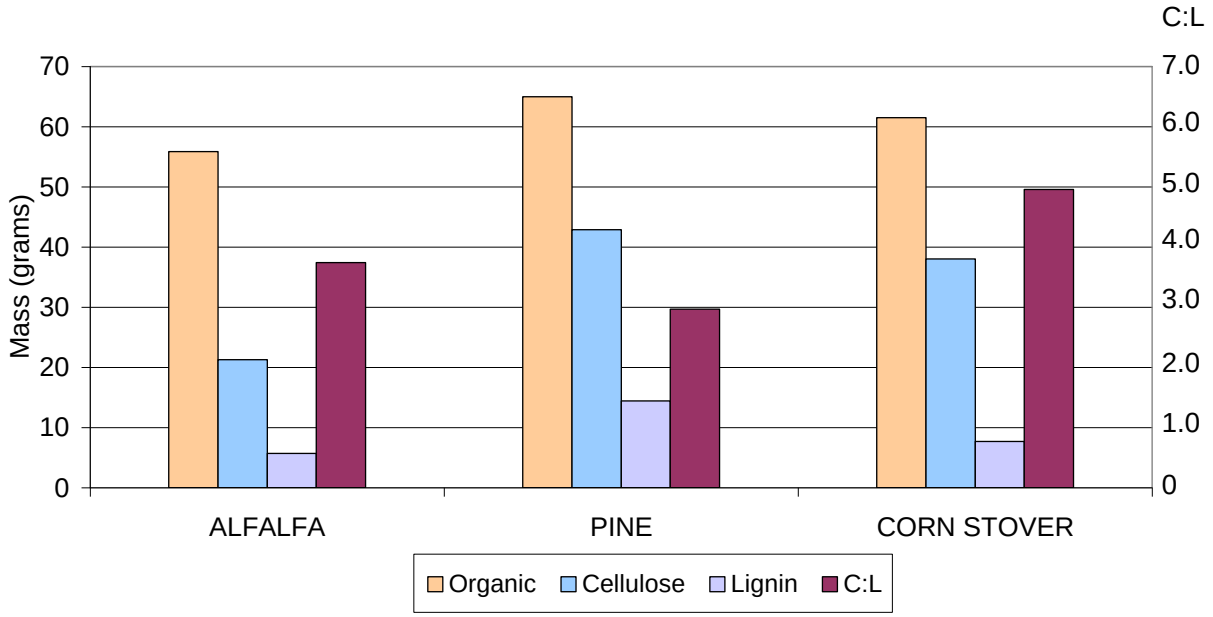

Figure 3. Initial organic mass, cellulose (category 3), lignin (category 4) and C:L for the reactive mixtures of three bench-scale bioreactors. (Figueroa et al., 2004)

As can be seen in Fig. 3, the pine based reactive mixture had the highest organic and cellulose contents. However, the lignin content of this reactive mixture was higher than the other two mixtures. This translates into the pine based reactive mixture having the lowest C:L. In contrast, the alfalfa based reactive mixture had the lowest organic, cellulose and lignin mass, but it had the second highest C:L. Comparatively, the mass of organic, cellulose and lignin for the corn stover based reactive mixture fell between the wood and alfalfa based reactive mixtures. However, this reactive mixture possessed the highest C:L, more than 1.5 times the pine based reactive mixture. This suggests that the cellulose in the corn stover based reactive mixture is more bioavailable than the cellulose present in the other reactive mixtures. When selecting a reactive mixture it is useful to consider these four criteria as they indicate how much of the total carbon is in a form that can be utilized by the microbial community.

Substrate Utilization

Tracking substrate alterations temporally or spatially can indicate microbial utilization rates for the reactive mixture and differences in utilization at various locations within a treatment system. To follow is a discussion on the application of tracking substrate utilization in temporal and spatial scenarios for both bench-scale bioreactors and PJK treatment system.

Temporal Tracking. From temporal tracking, rates of utilization of organic mass or a particular category of organic substrate within the reactive can be calculated. Figure 4 shows data for

alterations in the reactive mixture of the alfalfa and corn stover based bioreactors at three points in time. Recall that the duplicates of these bioreactors operated one year longer than the other bioreactors. Ideally sampling for substrate utilization analysis occurs at greater frequency than that represented by these bioreactors. More points in time with which to compare the utilization would allow for the development of rate curves that show startup utilization rates and sustainable utilization rates.

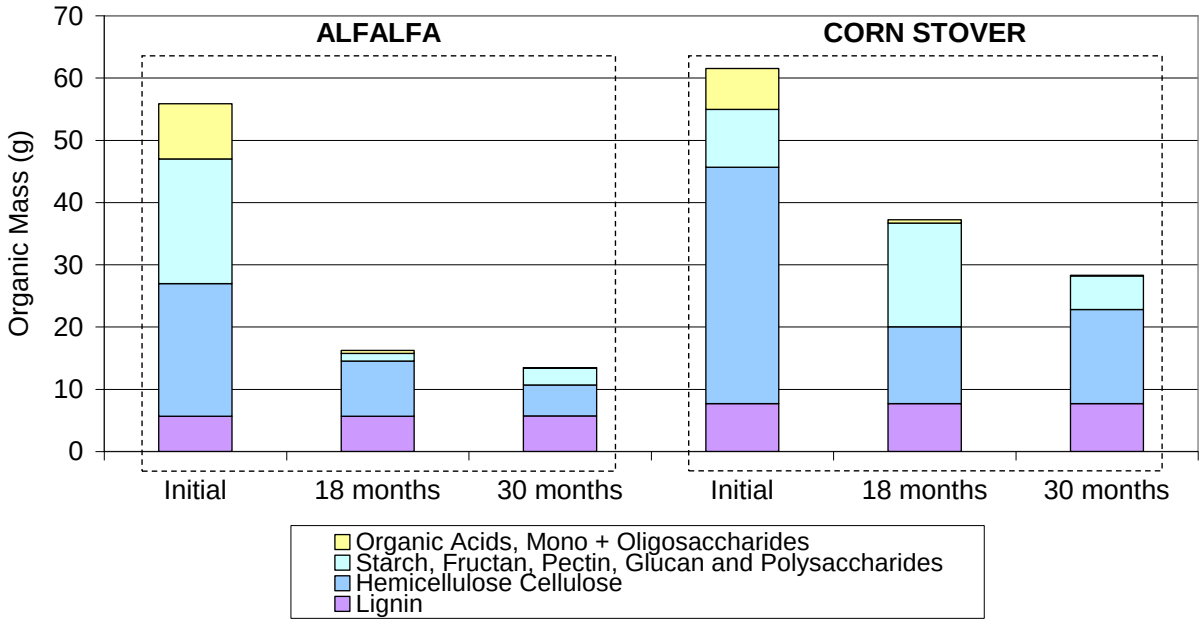

Figure 4. Temporal data for alfalfa and corn stover based reactive mixtures in bench-scale bioreactors at three points in time.

Temporal data for the alfalfa based reactive mixture showed a decrease in the rate of utilization over time. During the first 18 months the microbial community utilized 12.4 grams of cellulose and 38.1 grams of total organic. The rates for the first increment of time were 0.7 g / month of cellulose utilization and 2.1 g / month of organic utilization. During the final 12 months the microbial community utilized 3.9 grams of cellulose and 4.4 grams of total organic. The rates for the final increment of time were 0.3 g / month of cellulose utilization and 0.4 g / month of total organic utilization. Temporal data for the corn stover based reactive mixture showed a more modest decrease in the rate of utilization over time. The data indicates a gain in the mass of the cellulose fraction (category 3). The gain of mass in the cellulose fraction is likely due to starch fraction being retained in the neutral detergent insoluble fraction, as the corn stover based reactive mixture was difficult to filter. However, the total organic mass loss is unaffected by this interference. During the first 18 months the microbial community utilized 22.6 grams of total organic at a rate of 1.3 g / month. For the last 12 months the microbial community utilized 10.7 grams of total organic at a rate of 0.9 g / month.

Another way of looking at temporal data is within the context of the four selection criteria described above. Figure 5 shows the changes in the four selection criteria at three points in time for the alfalfa based bioreactor and the corn stover based bioreactor.

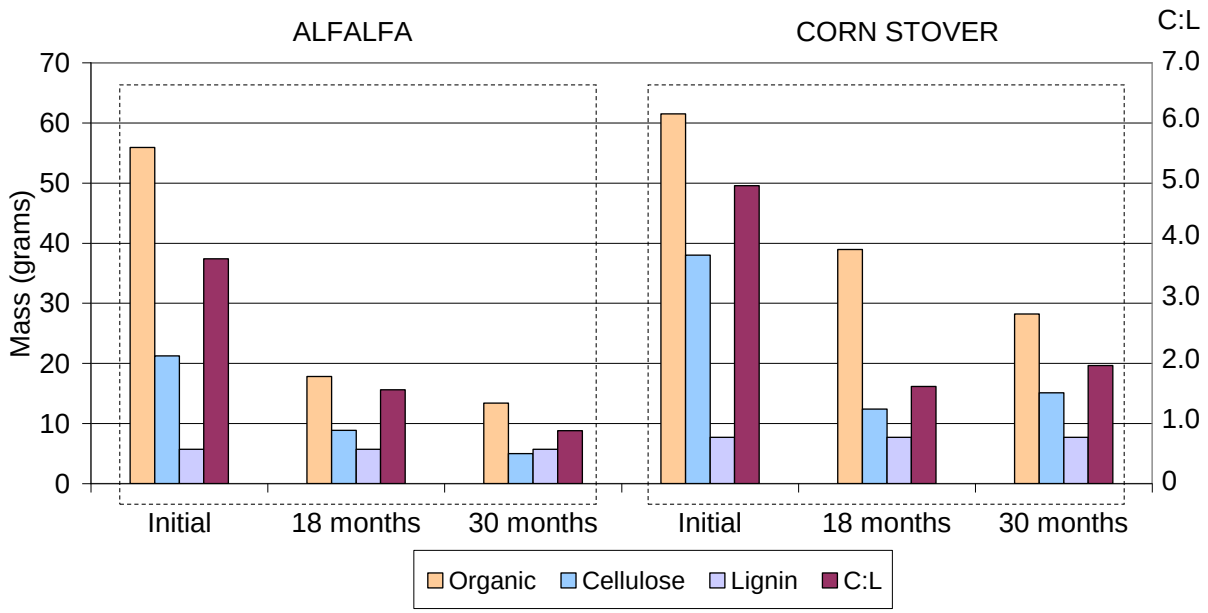

Figure 5. Temporal data showing the changes in organic mass and C:L in the four selection criteria for alfalfa and corn stover based reactive mixtures in bench-scale bioreactors at three points in time.

Viewing the data in this way highlights the utilization of the total organic and cellulose, and subsequent decrease in the C:L. If this data were compared with sulfate removal performance it may explain declines in performance and help determine if the decline is due to the system reaching a sustainable operation status or due to a depletion of usable organic material.

Spatial Tracking. The results of the substrate analysis for the PJK treatment system are presented in terms of percent of organic sample (Table 3). To better illustrate the variation in total organic content of the four samples from PJK, the data was normalized to the same mass, 100 grams. Figure 6 shows the results as they apply to a 100 gram sample of the reactive mixture. Recall that the sample of reactive mixture contains both organic and inorganic components; therefore the organic content is less than 100 grams. As stated earlier, all four cells in this treatment system were constructed with the same reactive mixture.

The total organic content of the two samples located in Cell 1 differ by 8.6%, based on total sample mass. The sample from the influent end (front) of the cell has less organic material. The difference in cellulose between these two samples is 10.3%, based on total sample mass. This difference may be caused by more microbial utilization occurring at the front of the cell than at the middle. If a substrate analysis had been performed on the reactive mixture in its initial condition, an explanation for the difference might be clearer.

The difference between the samples in Cell 3 is less than in Cell 1. The total organic content of the samples located in the middle of Cell 3 differ by 3.9%, based on total sample mass. Similarly, the amount of cellulose between the two samples differs by 3.8%. Because the differences are small and similar it is concluded that there is no difference between the utilization of the reactive mixture at the top and bottom of the sample profile in Cell 3.

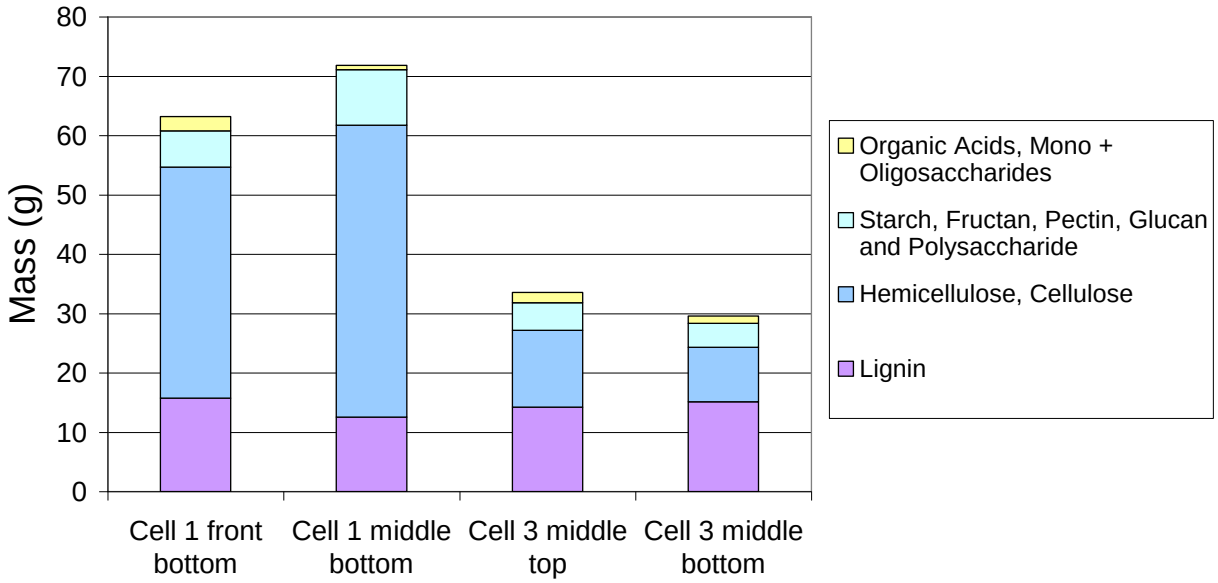

Figure 6. Spatial data of the organic categories for the four sample locations at the Peerless Jenny King passive treatment system during the June 2005 sampling event.

A 100 gram sample of the reactive mixture from the locations within Cell 1 contains more organic material than a 100 gram sample collected from locations within Cell 3. This difference could be because the sample locations are in areas of heterogeneity and cannot necessarily be compared to each other or be considered representative of the cell. However, it is more likely due to differences in substrate utilization between Cell 1 and Cell 3. Figure 7 shows the changes in the four selection criteria at the four sampling locations.

The C:L for the two samples from Cell 1 is 2.5 to 3.9 and for the two samples from Cell 3 is 0.6 to 0.9. Based on the alterations seen in the bench-scale bioreactors, the decrease in C:L strongly suggests a utilization difference. To further support the theory that the differences between the 2 samples in Cell 1 and between samples Cell 1 to Cell 3 are due to microbial utilization, consider the lignin content of all four samples. The lignin content of each sample is very similar, differing by no more than 3.2%, based on total sample mass. Recall the all cells in the PJK treatment system were filled with the same reactive mixture so it makes sense that the lignin content of the four samples is similar. Unlike for the bench-scale bioreactor analysis, there are no assumptions made about the mass of lignin. Therefore, it appears that there has been more microbial utilization occurring in Cell 3 than in Cell 1. The data also suggests that more microbial utilization has occurred at the front of Cell 1 than at the middle. Tracking substrate utilization in the PJK treatment system over time will provide a clearer understanding of the activity within these treatment cells.

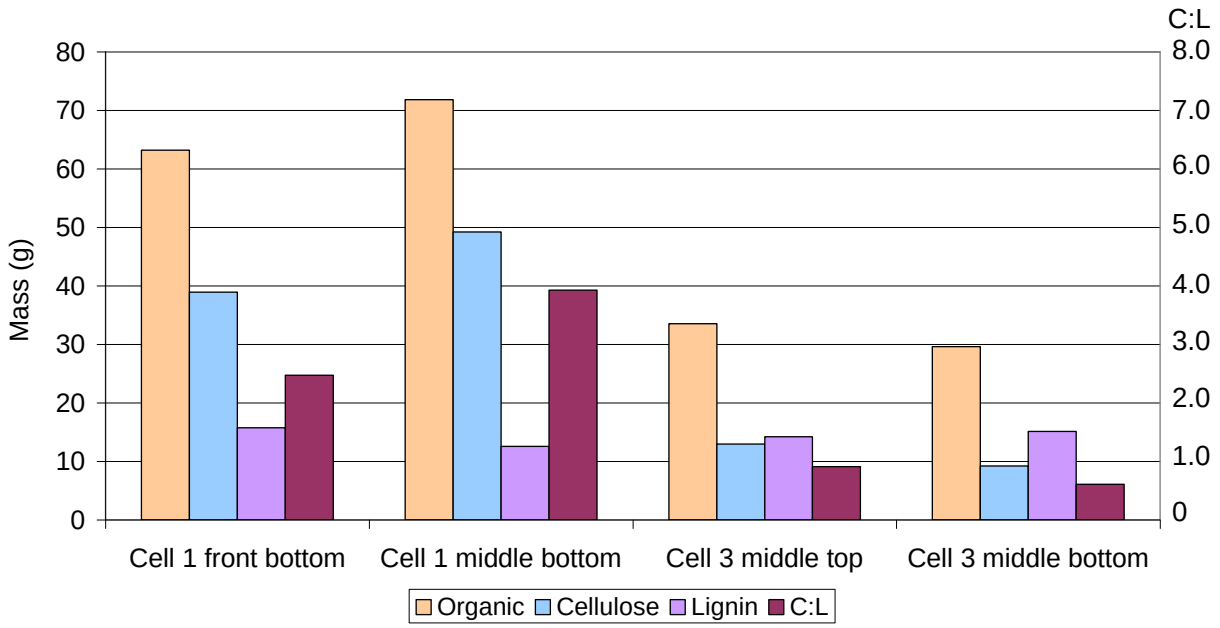

Figure 7. Spatial data showing the differences in organic mass and C:L in the four selection criteria for four samples locations at the Peerless Jenny King passive treatment system during the June 2005 sampling event.

Conclusions

Characterizing reactive mixtures focusing on total organic, cellulose content, lignin content, and C:L provides a quantitative tool for reactive mixture selection. In addition, tracking substrate alterations over time can be used to develop rates and rate curves for the microbial utilization of the organic fractions of reactive mixtures. Based on the results of the analysis of the field-scale passive treatment system, application of this quantitative tool provides measurable differences in the utilization of substrate at different locations within a treatment cell and between treatment cells.

In conclusion, characterizing reactive mixtures and tracking substrate alterations temporally and spatially is a new tool for selecting reactive mixtures and monitoring the sustainability of the substrate and microbial community over the operating time of the passive treatment system. Other research that considered substrate alterations used total carbon, total nitrogen and/or total phosphorus. As stated previously, looking solely at total carbon is not enough. Total C will not show the loss of specific carbohydrate groups utilized by the microbial community in supporting the SRB community. A key to the biodegradability of a substrate is a function of both the amount of lignin relative to the amount of cellulose and the arrangement of the lignin within the cellulose. Noting the mass loss of the four carbohydrate categories can be critical in the monitoring and maintenance of a successful passive treatment system. Comparing performance data with the substrate utilization data will help explain the activity of the system. Even more importantly it gives another tool that will help understand and anticipate changes in function, so appropriate actions can be taken to maintain the usefulness and longevity of passive treatment systems.

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