EFFECT OF SOLID PHASE ORGANIC SUBSTRATE CHARACTERISTICS ON SULFATE REDUCER ACTIVITY AND METAL REMOVAL IN PASSIVE MINE DRAINAGE TREATMENT SYSTEMS[1]

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Abstract. This paper is a progress report on studies whose objectives are to determine methods of analysis that will rate metal sorption and sulfate reduction activity of organic materials for use in passive treatment systems (PTS). Substrates tested include agricultural residues (alfalfa pellets, sugar beat pulp pellets, brewery waste, corncobs, and walnut hulls), inoculums (dairy manure and wetland inoculum), and a variety woods (maple, oak, pine, poplar, and walnut). Characteristics targeted include moisture, organic and nutrient content; water, ethanol and acid soluble and insoluble fractions and metal sorption capacity. The short-term and long-term effects of organic substrate characteristics on metal removal and sulfate reduction rate are being evaluated in batch and column experiments receiving mine water. These data are not presented in this paper but will be included in the oral presentation. Measured values of moisture and organic content ranged from 5.5 to 65% and 7.4 to 95% relative to raw sample weights, respectively. The water-soluble fractions and protein content ranged from 0 to 32% and 2 to 23% relative to dried samples, respectively. Low concentration zinc sorption studies were described well by Freundlich isotherms. Using a wider range of concentrations, manganese sorption to substrates was more closely modeled by Langmuir isotherms. The highest manganese sorption was observed for manure, corncobs, walnut hulls and wetland inoculum (8-13 mg Mn / gram substrate at an equilibrium concentration (C_e) = 50 mg/L Mn). Corncobs and walnut hulls can be included in substrate specifications to target manganese removal. Moisture and organic content are important parameters in the specification of organic substrates as a significant portion of the raw organic substrate weight can be inorganic. A high soluble fraction should correlate with a rapid startup of SRB activity and thus is an important element in substrate specification. All substrates have some capacity for metal sorption and their quantification is essential for use in PTS.

Additional Key Words: mine drainage, metal treatment, passive treatment, organic substrate, lignin, polysaccharide

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Introduction

Acid mine drainage (AMD) is primarily caused by exposure of pyrite to air and water via natural and mining activities. This exposure causes the contaminated waters to have increased acidity, and elevated concentrations of heavy metals, sulfate, and other total dissolved solids (Stumm & Morgan 1996). The production of AMD persists well after the mining activity ends and may result in long-term mitigation activities that can be costly. For this reason an effort has been made to find a low-cost and long-term solution.

The most common method to mitigate the effects of low pH and metal in AMD is chemical neutralization and precipitation. This active process is not practical for numerous remote mining sites that produce mine drainage. "Passive" treatment alternatives such as constructed wetlands, anaerobic bioreactors and permeable reactive barriers (PRBs) have been employed which are characterized by lower operation and maintenance. These passive biotreatment systems (PTS) use a mixture of organic and inorganic materials to sustain microbial populations. Anaerobic microbial PTS rely on sulfate reducing bacteria (SRB) to produce sulfide from the sulfate found in AMD. This reaction allows for the removal of heavy metals such as Ag, Cd, Cu, Fe(II), Pb, Ni, and Zn through precipitation as metal sulfides. The PTS promotes the growth and expansion of SRB by providing a source of organic material in an anaerobic environment.

The success of PTS applied to metal contaminated waters is mixed. One aspect of PTS design that has been identified as problematic is the qualitative specification of organic materials used. PTS are typically constructed of complex mixtures of both organic and inorganic materials all serving different functions. PTS have been constructed with a mixture of 0 to 85% gravel with the remainder organic material. The type, size, and quantity of gravel used primarily affects permeability and neutralization if limestone is used. The composition of the organic material determines the composition of the microbial community and relative rates of reaction.

In the past, selection of the organic substrates placed in PTS has been based on descriptive characteristics (i.e. Chicken vs. Cow manure, or leaf vs. municipal compost) (Gilbert et. al., 1999; Pinto et. al. 2001). These systems exhibited high levels of sulfate and metal reduction in the initial operating period but lacked long-term sustainability. Examples of substrates used include: chitin, leaf compost, various types and ages of manure, walnut hulls, municipal compost, different types of wood shavings, sewage sludge, hay, walnut shells, and pecan shells.

(Waybrant et. al., 1998; Benner et. al., 1997; Gilbert et. al., 1999; Wildeman & Updegraff 1997) Identification of SRB rates associated with specific fractions of organic matter will allow for specification of organic substrate combinations that provide rapid startup and long sustainability. This paper is a progress report on studies whose objectives are to assess characterization methods for organic matter and the relationship between different fractions and observed SRB rates.

Important characteristics of organic matter may include moisture content, organic fraction, nutrient content and the composition of the organic fraction. Analysis of individual organic components (e.g., glucan) is cost prohibitive. However, operationally defined characteristics may be correlated to SRB rates and sustainability. The major pools of carbon that make up organic matter include lipids, protein, simple sugars, organic acids, cellulose/hemicelluloses, and lignin. Soluble proteins, simple sugars, organic acids and lipids can be separated and categorized by using various solvents. These soluble components are readily available to the microbial community. Ethanol and water can be used for extractions and the extracts are designated ethanol-soluble fraction (ESF) or water-soluble fraction (WSF), respectively. The residues are termed the ethanol-insoluble fraction (EIF) and water-insoluble fraction (WIF), respectively. The EIF and WIF represent fractions that must be hydrolyzed before they are available to the microbial community. Hydrolysis is typically the rate-limiting step in anaerobic environments supported by solid phase organic matter. These insoluble fractions are further divided into the acid-soluble (e.g., protein and cellulose/hemicellulose) and acid-insoluble fraction (e.g., lignin). Figure 1 illustrates key microbial processes in an anaerobic environment (e.g., PTS) and shows the different organic matter fractions associated with these processes.

Another important characteristic of substrates is the metal sorption capacity. Batch experiments can be used to estimate the equilibrium capacity of substrates. Column studies can be used to estimate kinetic effects and the sorption zone. The sorption capacity of the substrate is important during the start-up period of the microbial populations and may be the primary mechanism for metal removal for some metals (e.g., manganese).

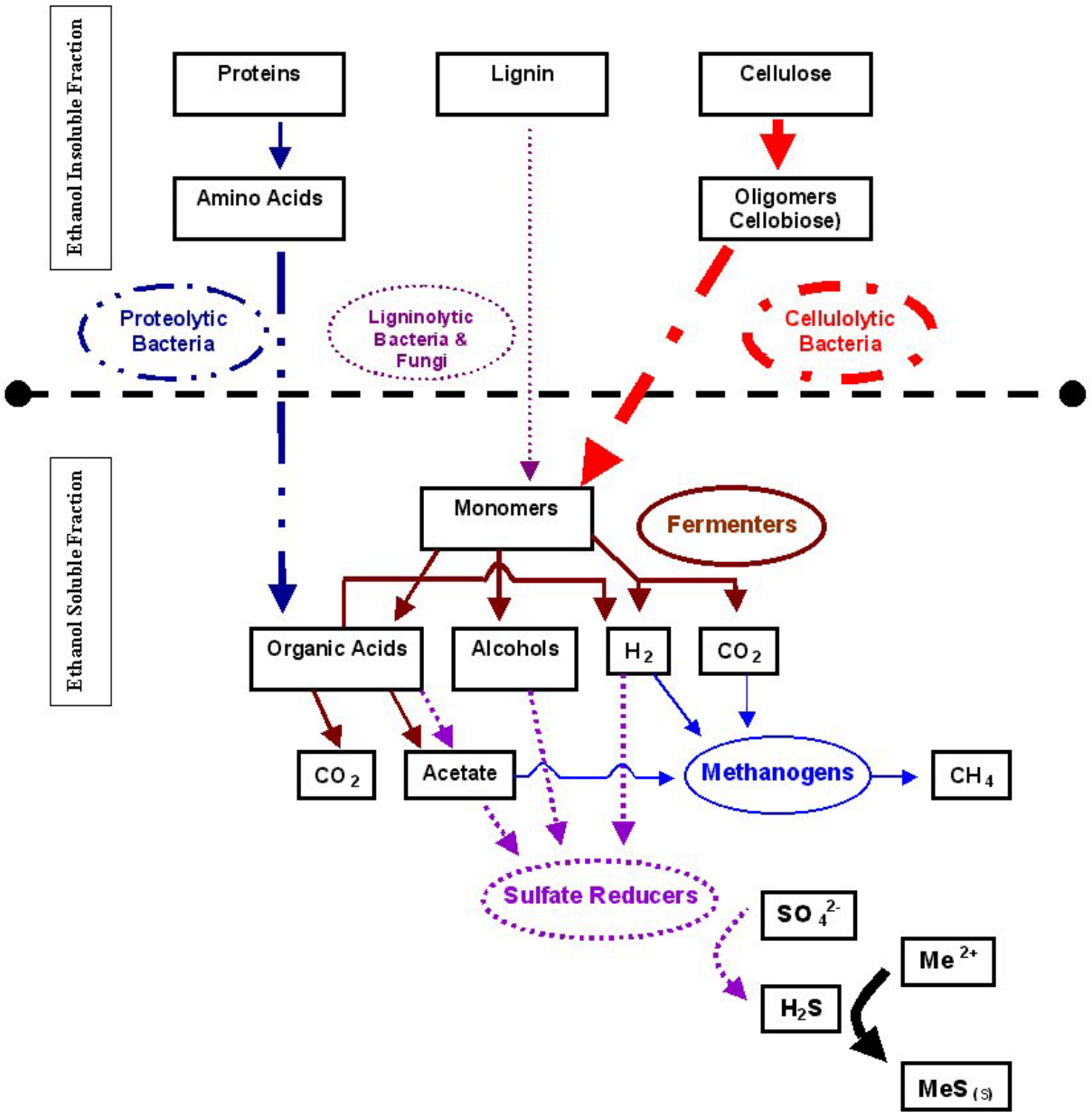

Figure 1. Conceptual Microbial Process Model.

The sustainability of sulfate reduction in PTS by solid phase organic substrates is dependent on the composition of the substrate. In this study we will use relatively simple analytical techniques in order to characterize a substrate's composition. We will then assess the metal removal and SRB reduction rate for different substrates. We expect to develop specifications for substrate selection based on easy to measure characteristics of the substrate that are related to target metals concentration/loading and performance.

Methods

Substrate Characterization

Volatile solids are determined and are an estimate of organic solids. Total and volatile solids determinations are done following protocols from Standard Methods (1995). Total carbon and nitrogen analysis is performed by using HACH kits (Loveland, CO) and a C, N, H analyzer (USGS, Boulder). Crude protein is estimated from the N concentration.

The organic fraction of each substrate is analyzed by using food chemistry protocols (Hall 2000). First, a batch 24 hour 80% ethanol extraction is used to separate the solid phase substrate into an ethanol-soluble fraction (ESF) and ethanol-insoluble fraction (EIF). A similar approach is used to separate dried sample into the water-soluble fraction (WSF) and water-insoluble fraction (WIF). The soluble fractions from the extractions are analyzed for soluble carbohydrates using a phenol sulfuric acid test, total organic carbon using a liquid TOC analyzer and nitrogen using a HACH kit. The insoluble fractions will undergo an amylase/amyloglucosidase enzymatic reaction that provides starch content plus an additional organic faction and protein analysis. A separate neutral detergent/amylase extraction for cellulose content, and 72% H_2SO_4 acid hydrolysis to quantify lignin content are run on the original sample.

The organic acid fraction and the neutral detergent soluble fiber (NDSF) fraction (i.e. fructans, pectic substances, galactans, β-glucans) will be derived by following the procedures found in *Neutral Detergent-Soluble Carbohydrates Nutritional Relevance and Analysis* (NDSCNRA) (Hall 2000). These fractions have considerable amounts of variability and are more easily derived than characterized. The ethanol extraction removes organic acids, ethanol soluble simple carbohydrates (ESC), as well as some soluble proteins. The organic acid fraction is then calculated by knowing the % ESF and subtracting the % ESC plus the % soluble protein. A neutral detergent solution will solublize starches, NDSF, ESC, organic acids, and some protein. The NDSF is calculated in a similar fashion to the organic acid fraction.

Substrates were selected from readily available waste products. For all experiments each substrate was milled in a Wiley mill with a 4-mm sieve. This milling eliminates any effects that could be caused by differences in particle size. A number have been previously used in PTS and others have been characterized by the National Renewable Energy Laboratory (NREL) for

bioenergy production. The substrates and expected relative characteristics based on literature information are presented in Table 1.

Table 1. Substrates analyzed and expected characteristics.

Substrate	**Expected characteristics**
Alfalfa	high soluble and nitrogen fractions
Brewery Waste	high soluble and nitrogen fractions
Beet Pulp	high soluble and intermediate nitrogen fractions
Corncobs	balanced soluble and insoluble fractions
Wetland*	high insoluble fraction
Manure*	high protein and insoluble fraction
Maplewood	high insoluble fraction
Oak wood	high insoluble fraction
Pine wood	high insoluble fraction
Poplar wood	high insoluble fraction
Walnut Hulls	high insoluble fraction
Walnut wood	high insoluble fraction

*used as an inoculum source

Metal Sorption

The metal sorption capacity is quantified in batch studies. Differing amounts of substrate are placed into solutions with known metal concentrations and shaken for 24 hours at room temperature. These samples are then centrifuged, filtered, and acidified prior to analysis on the ICP. Freundlich and Langmuir isotherm models are fit to the data. The simplest model that provides a good fit is subsequently used. Metal sorption is also assessed in mini-columns to assess kinetic effects.

Columns

The glass columns used were 5 cm in diameter and 30cm long. The preliminary columns were packed loosely with 185 grams of material. These columns contained 45% sand, 5% limestone, 20% manure, 5% wetland inoculum, 10% alfalfa, and 15% walnut wood on a mass basis. Influent water for the first set of experiments was obtained from the Dinero Tunnel, Leadville, Colorado. The water contained 37 mg/L Mn, 10 mg/L Zn, 4 mg/L Fe and 125 mg/L SO_4 at pH 6.0. The preliminary column experiment used a flow rate of 220 ml per day.

The second set of columns used 150 grams of material and were packed very tightly. These columns were comprised of 45% sand, 5% limestone, 10% manure, and 40% substrate on a mass

basis. Each column was wet packed using a 1000 mg/l SO_4 solution and was then given a 5 day startup period during which no flow occurred. Influent water was comprised of 1000mg/l of SO_4, 50mg/l of Mn and 50mg/l of Zn. A flow rate of 30ml/day was then maintained throughout the experiment. The residence time based on tracer studies is between 4-5 days. After two weeks of receiving only 1000 mg/l SO_4 solution, the influent was switched to 1000mg/l of SO_4, 50mg/l of Mn and 50mg/l of Zn. This delay allows the establishment of an active SRB community.

For all column studies the effluent is collected in iced sample bottles and is analyzed for metals & total S on an ICP, sulfate on an ion chromatograph and carbon on an organic carbon (TOC) instrument. Figure 2 is a schematic for the column setup.

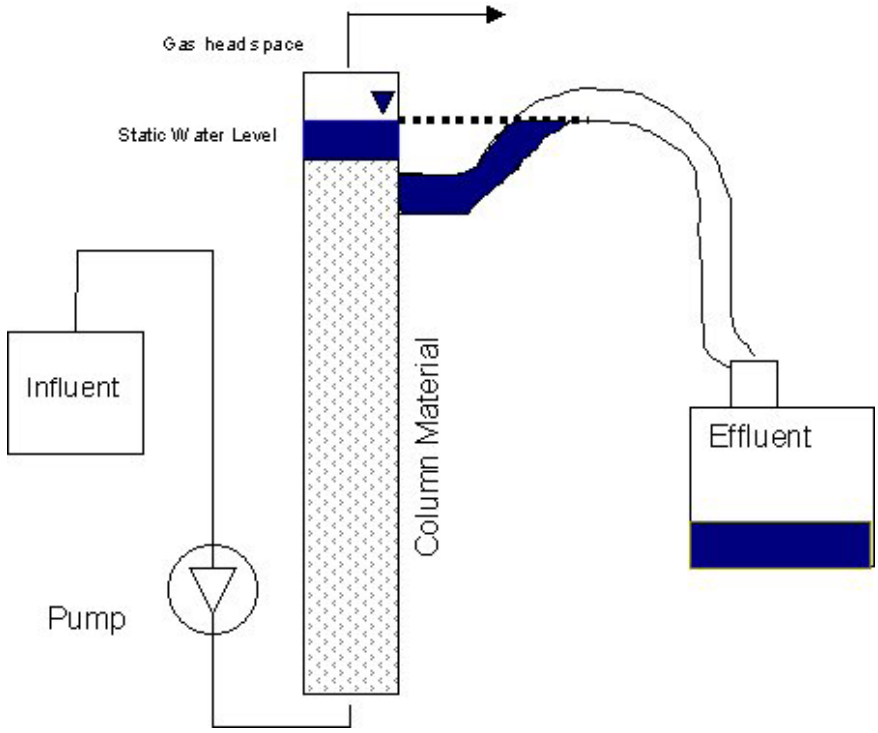

Figure 2. Schematic of Column Setup.

Mini Columns

Mini-columns with a volume of 40 cm^3 were used to assess column sorption. Mini-columns were packed with a mixture containing 5 grams of substrate (dry weight), 5% manure & wetland inoculum, 5 grams of limestone and the remaining volume was filled with sand. Walnut hulls, corncobs, brewing waste and walnut wood were the selected substrates used in this experiment. The flow rate was 24 ml per day and a simulated mine water comprised of 50 mg/L Fe, Mn, and Zn, and 1400 mg/L SO_4 at pH 6.0 was used.

Results and Discussion

Substrate composition

Simple physical characteristics measured are useful in the characterization of potential substrates, Table 2. Field weight measurements are done on the raw sample and most compositional analysis is reported on a dry weight basis. Thus, it is important to know the relative moisture content to calculate the actual composition of the emplaced substrates. For woods and pelletized alfalfa and brewing waste, the error of neglecting moisture content is less than 10%. However, for the wetland inoculum and manure the water content is greater than 40% and variable (data not shown) and errors on the order of 50% may be propagated.

Chemical characteristics of the dried sample (C, N, protein, organic) are presented in Table 3. Nitrogen analysis for both HACH total nitrogen test and the CNH analyzer are presented. The HACH method was only used on four substrate types. The HACH method is a technique that is easy to do in a minimally equipped laboratory. The HACH method produced N values that were 60 to 100% of the values measure with the CNH analyzer. The HACH method is sensitive to particle size and grinding of the sample prior to analysis should improve the nitrogen yield. The protein content reported is based on 6.25 times the nitrogen content. This method has been used by NREL in their biomass to ethanol fuel program for biomass characterization. Their mass balances have closed to within 1% using this estimate for protein.

Table 2. Physical characteristics relative to raw sample weight.

Sample ID	Moisture %	Solids %	Organic %
Alfalfa	5.9	94	82.5
Brewery Waste	6.4	94	87.4
Beet Pulp	9.5	90	81.9
Corncob	6.5	93	78.2
Wetland	44.4	56	7.4
Manure	65.4	35	14.6
Maple	5.2	95	94.8
Oak	5.6	94	94.4
Pine	8.1	92	91.9
Poplar	5.4	95	94.4
Walnut Hull	13.5	86	83.8
Walnut Wood	5.5	94	94.3

Table 3. Chemical characteristics of dried sample.

Sample ID	N(HACH) %	N(Analyzer) %	Protein %	C %	Organic %
Alfalfa	3.2	3.8	23.4	45.1	87.6
Brewery Waste	2.8	3.6	22.3	46.8	93.3
Beet Pulp		1.6	9.7	44.8	90.5
Corncob		1.0	6.1	37.5	83.7
Wetland	0.6	0.6	3.5	8.3	13.3
Manure	1.2	2.0	12.5	27.8	42.1
Maple		0.4	2.5	49.7	100.0
Oak		0.4	2.7	50.0	99.9
Pine		0.3	1.9	50.5	100.0
Poplar		0.3	1.7	48.4	99.8
Walnut Hull		1.4	8.7	49.9	96.9
Walnut Wood		0.4	2.4	48.7	99.8

Soluble fraction characteristics are presented in Table 4. The ESF and WSF values of the wood materials are relatively low as expected, 0 to 6%. The non-wood substrates (excluding the inoculum) all show high ESF and WSF values but vary significantly in the percent sugars and protein.

Table 4. Soluble fraction characteristics relative to dried sample.

Sample ID	**ESF** %	**ESF** sugar %	**WSF** %
Alfalfa	23.1	3.0	32.1
Brewery Waste	19.4	11.5	26.8
Beet Pulp	12.3	9.6	24.7
Corncob	12.5	0.8	17.2
Wetland	3.7	0.0	10.1
Manure	3.7	0.2	8.9
Maple	0.8	0.7	3.4
Oak	5.7	1.6	3.7
Pine	2.3	1.0	3.7
Poplar	0.0	0.4	1.7
Walnut Hull	0.5	0.5	4.0
Walnut Wood	4.0	1.7	2.9

Figure 3 shows the NDSCNRA breakdown of plant carbohydrates into ethanol soluble carbohydrates (ESF), neutral detergent soluble carbohydrates (NDSC), neutral detergent soluble fiber (NDSF), neutral detergent insoluble fiber (NDF), and acid detergent fiber (ADF). Table 5 provides the results from the NDSCNRA analysis of our substrates.

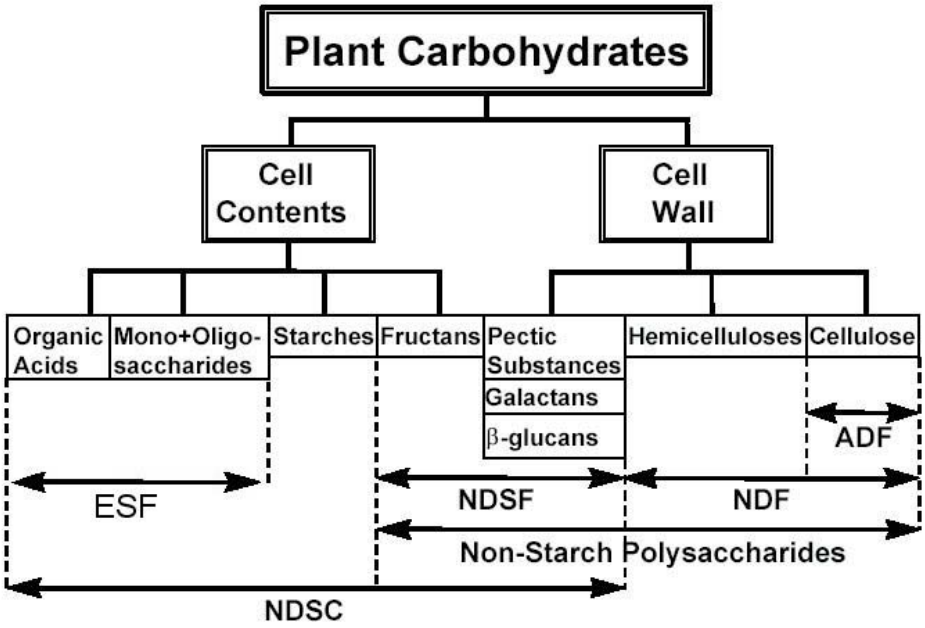

Figure 3. Plant carbohydrate diagram (Hall 2000).

Table 5. Substrate carbohydrate characterization results

Sample ID	% Organic Acids	% Mono+Oligo-saccharides	% NDSF & Starch**	% NDSF	% Hemicelluloses/ Cellulose	% Lignin	Summed Total***	% Organic
Alfalfa	10.9	3.0	33.4	18.3	28.5	8.2	84.0	90.0
Brew Waste	4.7	11.5	16.4	2.0	38.3	15.5	86.5	93.3
Beet Pulp	1.8	9.6	39.2	36.9	32.5	3.6	86.6	93.0
Corncobs	9.2	0.8	15.5	ND*	56.4	11.5	93.3	83.7
Wetland	3.5	0.0	ND*	1.4	6.0	2.7	12.2	13.3
Manure	3.5**	0.2	0.0	0.0	27.7	5.1	33.0	42.1
Maple	0.0	0.7	7.2	7.2	68.5	21.2	97.6	100.0
Oak	3.6	1.6	11.6	11.7	59.7	20.7	97.2	99.9
Pine	1.2	1.0	9.1	8.5	64.5	22.8	98.6	100.0
Poplar	0.0	0.4	14.1	14.4	66.2	17.5	98.1	99.8
Walnut Hulls	0.5	0.5	23.2	21.8	40.8	26.6	91.6	96.9
Walnut Wood	2.0	1.7	6.8	6.9	67.1	19.9	97.5	99.8
Sugar Beet (Hall 2000)	0.4	12.8	30.0	30.0	Combined value = 44.6		87.8	91.1

* No data available **Values were derived via a mass balance based on Figure 3. ***Summed Total (Column 7) represents the total of column (1,2,3,5, &6)

The NDSCNRA analyses provided valuable differences within the agricultural residues and the woods. Corncobs and sugar beet pulp had similar % ESF values (10 & 11 respectively) but the NDSCNRA analysis shows that 90% of the corncob's ESF is organic acids while 90% of sugar beet pulp's ESF is simple sugars. Although the woods category may seem similar, the hemi/cellulose vs. lignin values ranged from 2.8 – 3.8 and oak was found to have the largest ESF (5.7% compared to poplar's 0.4%). SRB activity is a factor of available substrate as well as the active population of SRB. Manure has a low fraction of soluble components yet has also been credited with high SRB activity. However, the high numbers of SRB found in manure are subsequently not sustained long term by the available substrates. In our current and future column/batch studies we hope to determine the effects each organic fraction has on SRB activity.

The % starch values could not be analyzed following the method found in the NDSCNRA because the amyloglucosidase required is no longer in production. A substitute amyloglucosidase was used but it contained 10-12% starch and interfered with the quantification of starch in the sample. A column presenting the combination of %NDSF & Starch was calculated for the maximum combined value (Table 5). When compared to a batch of sugar beat pulp tested and reported by Hall (2000), our own sugar beet pulp analysis resulted in difference of less than 2% for the Summed Total and the % Organic (see Table 5). The disparity in the other fractions can be attributed to seasonal, lot, and manufacturing differences between the two batches of sugar beet pulp tested. Also included is a Summed Total column, which shows the total of all the calculated organic fractions. When compared to the % Organic, all values are within a 10 percent difference. All of these tests were originally made for analyzing wood, and within our wood group the difference between the Summed total column and the % organic column is 1-3 percent.

Metal Sorption

Data for zinc sorption to walnut wood shavings, walnut hulls, and brewery waste are presented in Figure 4. A linear Freundlich isotherm ($q_e=K*C_e^{1/n}$ where n = 1 and K values are shown on the graphs in Figure 4 & 5) fit the sorption data well (r = 0.82 to 0.98). Zinc sorption capacity to manure was the highest of the substrates tested, Figure 5. The heterogeneity of the manure resulted in greater variability in replicate samples and a lower r^2 (0.69) for the fit of the Freundlich isotherm. Additional experiments are required to generate equilibrium zinc values

(C_e) of at least 50 mg/L to generate sorption capacity data that is appropriate for the column experiments.

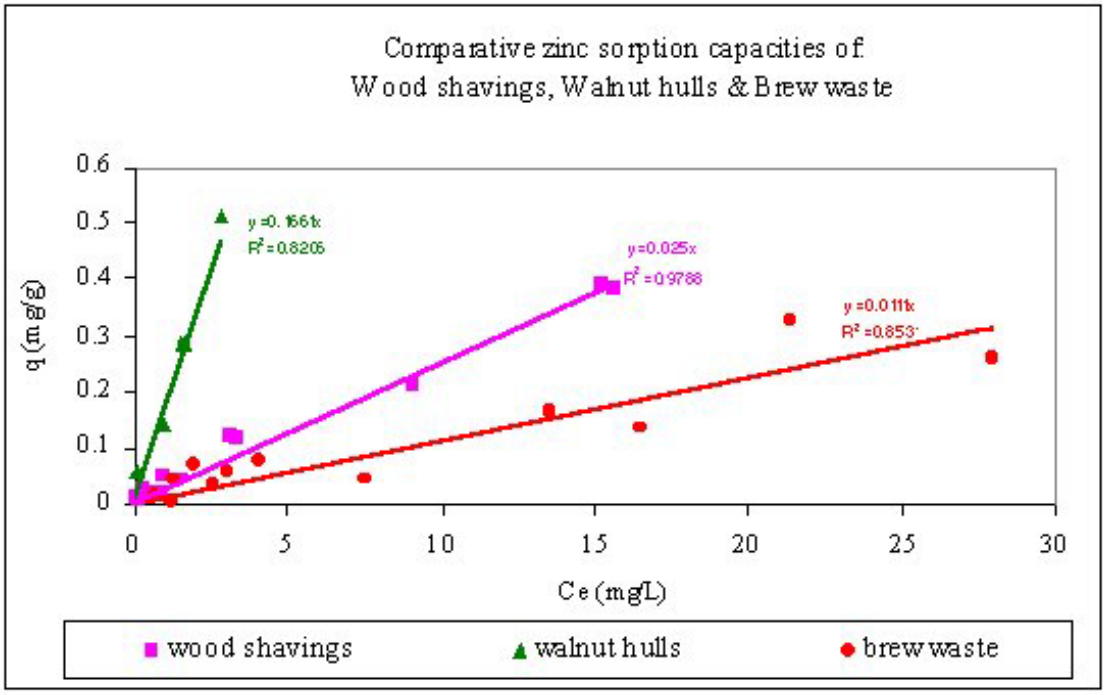

Figure 4. Zinc sorption per unit mass of sorbent versus zinc concentration for walnut wood, walnut hulls and brew waste.

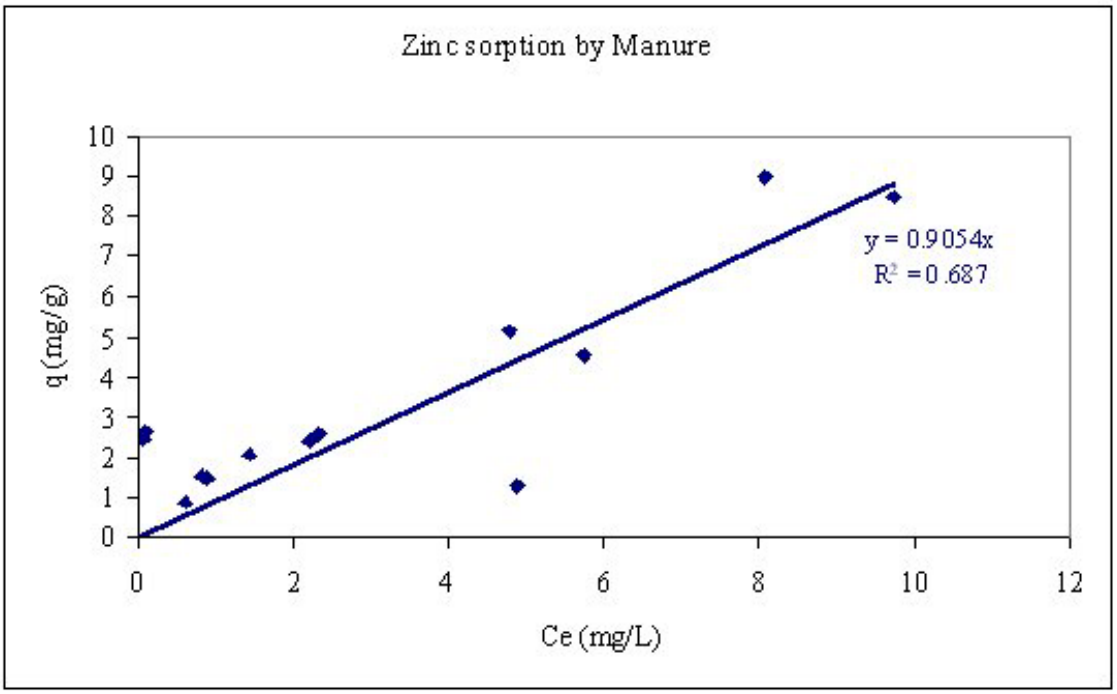

Figure 5. Zinc sorption per unit mass of sorbent versus zinc concentration for manure

Walnut hulls and corncobs show complete removal of manganese during the study period (see Figure 6) in mini-columns. These results suggest that walnut hulls and corncobs have potential to remove manganese through sorption from mine waters. Following this discovery, additional batch sorption studies were performed on all substrates identifying their manganese sorption capacity (see Figure 7). In this study, equilibrium manganese values were generated for values greater than 50 mg/L. All sorption curves except for that of manure reached a maximum sorption capacity. In addition a Langmuir isotherm was modeled and fit to each curve. The "a" and "b" values used in the model ($qe = a*C_e / 1+ b*C_e$), as well as the r^2 values, are presented in Table 6. Figure 8 shows the sorption graph of walnut hulls with its isotherm.

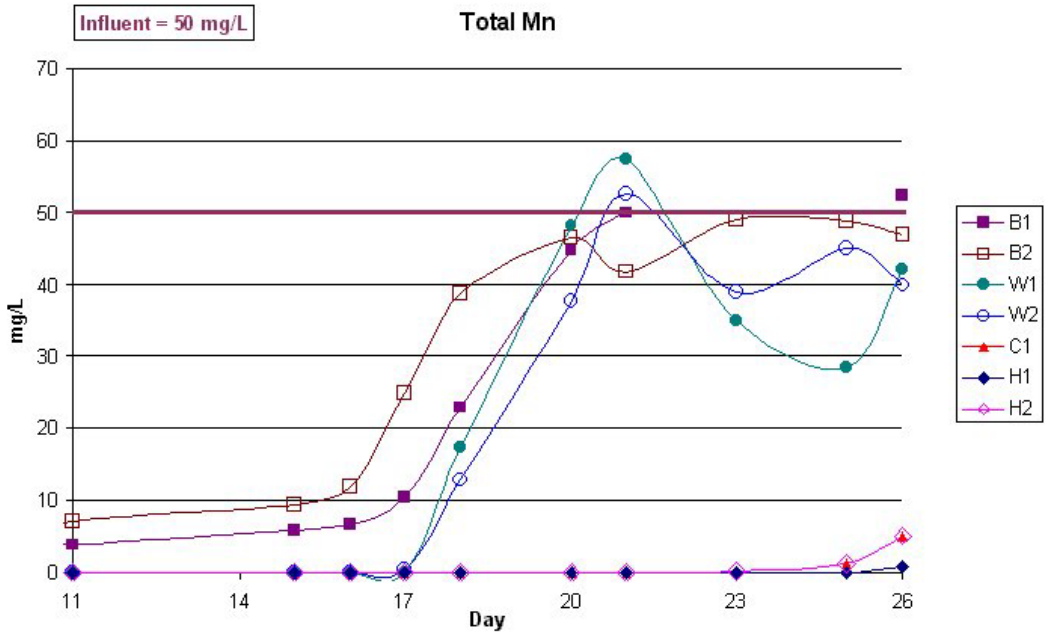

Figure 6. Mn sorption in mini-columns by brewery waste (B), walnut wood (W), corncobs (C) and walnut hulls (H).

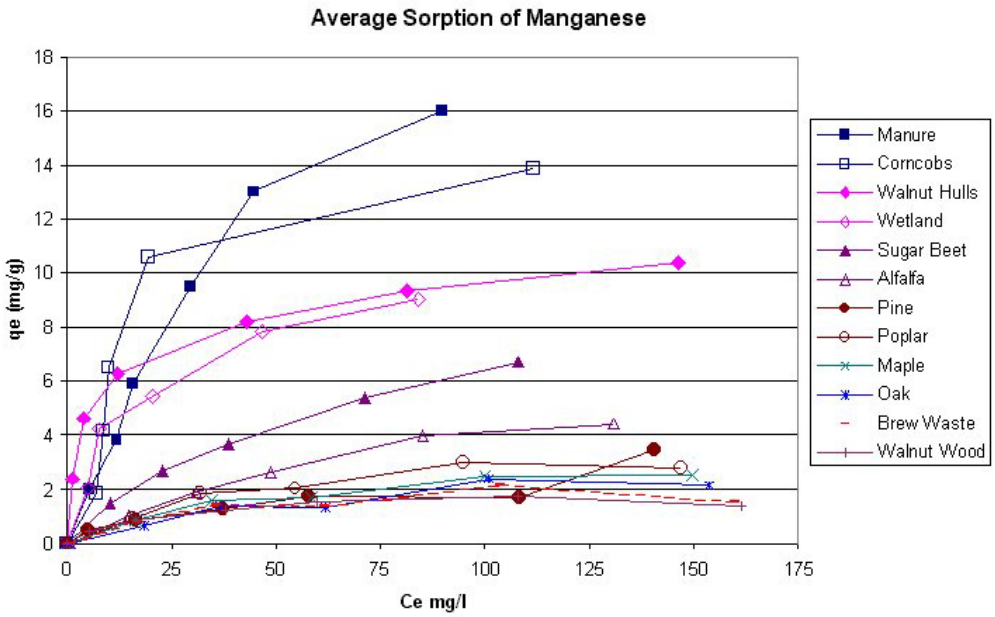

Figure 7. Results of the batch manganese sorption study.

Table 6. a, b, and r^2 values for the Freundlich isotherms of each substrate

Sample ID	a	b	r^2
Alfalfa	0.084	0.011	0.99
Brew Waste	0.103	0.051	0.88
Beet Pulp	0.149	0.013	1.00
Corncobs	0.857	0.051	0.91
Wetland	0.658	0.062	0.98
Manure	0.486	0.018	0.99
Maple	0.072	0.021	0.98
Oak	0.053	0.016	0.93
Pine	0.041	0.007	0.85
Poplar	0.093	0.024	0.97
Walnut Hulls	1.859	0.187	0.98
Walnut Wood	0.115	0.066	0.95

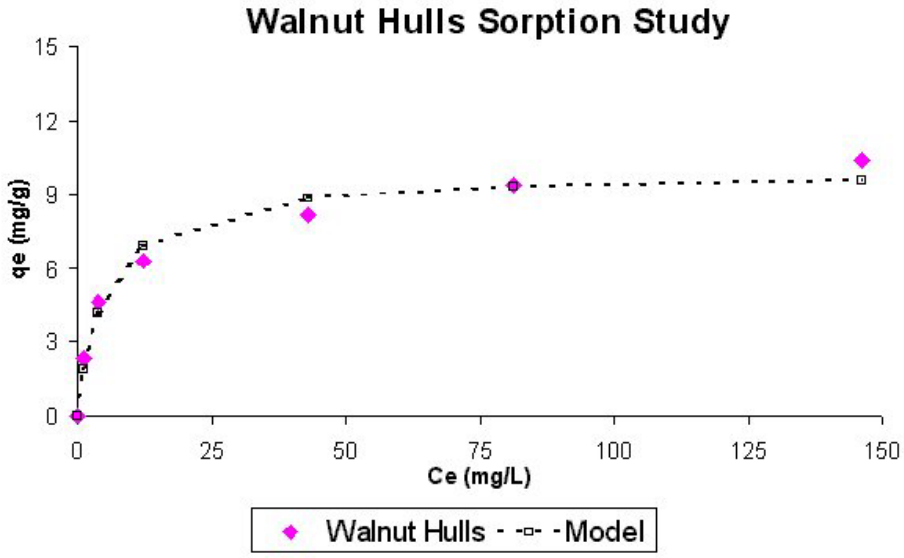

Figure 8. Walnut manganese batch sorption study and isotherm.

With this sorption information we can calculate the maximum quantity of manganese each column is capable of removing via sorption. Manure, corncobs, walnut hulls, and wetland inoculum showed a significantly higher affinity (8–13 mg of Mn/ gram of substrate at C_e= 50 mg Mn/L) for the sorption of manganese than the other substrates. Based upon these results, corncob was selected as a substrate for the larger column tests.

Column Experiments

The first column experiments involved replicate columns packed with a mixture similar to those found in the literature. Based on the substrate analysis, this mixture contained a 36% organic fraction, N:C ratio of 1:20, 5.6% of the "organic" substrate added was water soluble and conversely 94.4% of the substrate was water insoluble. The rate of sulfate reduction versus time for the first column study is presented in Figure 9. The first column took two to three weeks to reach the maximum rate of sulfate reduction, which was sustained for only three weeks. The sulfate reduction rate then decline over a period of 8 to 10 weeks and reached a lower sustained rate. The organic fraction of 36% was less than the targeted organic fraction of 50%, which was based on dry weight measurement alone. A significant error in the original estimate of organic content resulted from not correcting the substrate weight for the inorganic fraction. The N:C ratio is well in excess of the theoretical requirements for heterotrophic sulfate reducers and

methanogens, 1:53 and 1:85 respectively. Thus, nitrogen limitation was probably not the reason for the observed decline in sulfate reduction rate. The water-soluble fraction could account for a fast startup but a relatively short longevity as this fraction would be washed out of the column over the first several weeks of operations. The majority of the available carbon was in the insoluble phase. Thus, hydrolysis reactions (which are relatively slow in anaerobic systems) were probably the rate-limiting step in the column. Similar patterns of rapid initial sulfate reduction rates followed by declines to significantly lower sulfate reduction rates also have been seen in other column experiments and pilot wetlands (Wildeman and Updegraff 1997; Clayton et. al. 1998; Wildeman et. al. 1997).

Additional column experiments will be completed in March through May of 2003 and these data will be incorporated into the presentation in June 2003. The new columns are packed with a single target organic substrate supplemented by the inoculum. Alfalfa was chosen because it has the highest protein content and percent water solubility. Corncob was selected due to its high manganese sorption potential and organic acid content. Pine is the target softwood as it is widely used for lumber and paper and is very abundant. The pine column will also determine if it contains any characteristics that inhibit microbial growth. The substrate characteristics of hardwoods were similar and therefore oak (having the highest ESF and being more abundant than the other woods) was chosen as our target hardwood.

We hypothesize that the fate of operationally defined fractions in our column experiments will progress as shown in Figure 10. Each fraction is hypothesized to have a characteristic rate of degradation correlated to the key microbial processes presented in Figure 1. Rapid degradation is expected for the water-soluble fraction. The consumption of this fraction should cause a rapid increase in sulfate reducing activity found in a PTS. Once the entire soluble fraction is consumed, cellulolytic degradation becomes the driving factor in SRB activity. Because this degradation rate is much slower, the SRB activity will diminish and maintain itself for a longer period of time. Finally when the cellulose fraction has been depleted, the remaining lignin will drive all following reactions. The degradation of lignin is even slower than that of cellulose, and therefore the SRB activity should be reduced yet again. Because of its degradation rates, the lignin phase of the system is expected to persist much longer than that of water-soluble and "cellulose" phases. The superposition of the three curves yields an overall pattern of activity similar to the data presented in Figure 10. Based on the key microbial processes presented in

Figure 1 and the differences in reaction rates, we expect to have similar results to the graphs below (Figure 9&10).

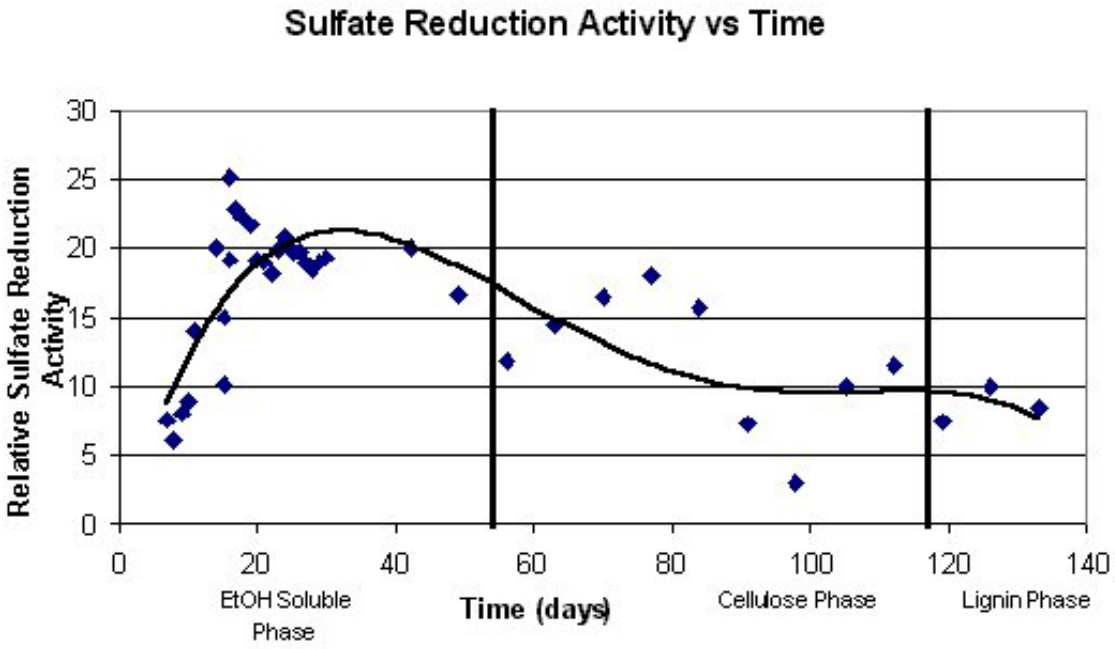

Figure 9. Sulfate reduction activity versus time in the preliminary column experiment.

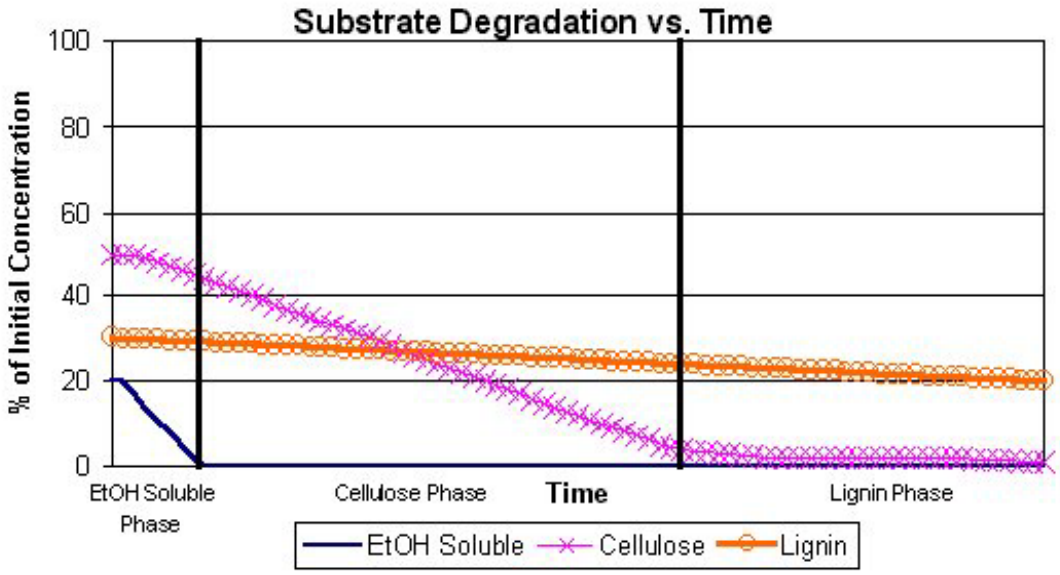

Figure 10. Estimated degradation pattern of water soluble, acid soluble (cellulose) and acid insoluble (lignin) fractions in a passive treatment system.

Conclusions

Moisture and organic content are important parameters in the specification of organic substrates for PTS. Measured values of moisture and organic content ranged from 5.5 to 65% and 7.4 to 95% relative to raw sample weights, respectively. The highest soluble fraction content should correlate with the most rapid startup of SRB activity, and thus is an important element in substrate specification. The water-soluble fractions ranged from 0 to 32% relative to dried samples. The Hall (2000) method produced distinct and valuable compositional differences between all substrates tested. The NDSC values for the wood samples varied from 8 to 18% with the ESF fraction ranged from 0 to 6%. For the agricultural residues, the NDSC varied from 26 to 51% and the ESF fraction varied from 10 to 16%. The importance of the compositional differences related to sulfate reduction will be determined in the on going column experiments. Isotherm models fit both a zinc sorption study and a manganese sorption study. The low concentration zinc sorption study was described well by a linear Freundlich isotherm. While the more extensive manganese sorption experiments were better modeled by using a Langmuir isotherm. These isotherm models can be used to estimate the maximum sorption capacity of the organic substrate used in PTS.

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