

Characterization of organic substrates used for anaerobic bioremediation of mining impacted waters

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Abstract

The specification of organic substrates for anaerobic bioremediation of mining impacted waters has been based on descriptive characteristics (e.g., leaf compost and hardwood chips). This study investigated the characterization of organic substrates based on operationally defined measures of the organic composition (e.g., neutral detergent soluble fraction). Ten organic materials and two inoculum sources were investigated. The organic materials are grouped into two categories: agricultural and industrial products (alfalfa, beet pulp, brewery waste, corncob, and walnut hull), woods (maple, oak, pine, poplar, and walnut) and inoculum sources (dairy manure and wetland sediment). Pine, oak, corncobs and alfalfa were used in column experiments with manure as the inoculum. Metal removal and sulfate reduction rates were evaluated in columns receiving synthetic mine water (50 mg-Zn/L, 50 mg-Mn/L and 1000 mg-SO₄²⁻/L) for up to 145 days. Short-term (the first 40 days) metal and sulfate removal were not correlated to the measures of organic composition used. Effluent zinc, manganese and sulfate were higher during the first 40 days for alfalfa and corncobs columns compared to pine and oak columns. After 50 days, the alfalfa and corncob columns exhibited lower effluent sulfate than the pine and oak columns. The rate of sulfate

reduction after 49 days was correlated to the ethanol and neutral detergent soluble fractions measured in the four organic materials. Operationally defined characteristics of organic materials are useful in understanding long-term sulfate reducing rates in anaerobic biozones.

Key words: mine drainage, metal, treatment, sulfate reduction, anaerobic

1 Introduction

Mining impacted waters (MIW) may contain elevated concentrations of sulfate or dissolved metals that exceed aquatic toxicity or drinking water limits. Conventional mine water treatment processes involve continuous maintenance, and require accessibility and infrastructure not practical in remote areas. Passive treatment systems (PTS) are a low operation and maintenance alternative better suited to remote sites.

Some PTS employ an anaerobic biozone to remove metals from MIW; these include constructed wetlands, bioreactors, and permeable reactive barriers. PTS are a low cost alternative because they require no power and no chemicals after construction (Gusek and Wildeman 2002). The lack of continuous upkeep allows PTS to be implemented in areas where conventional treatments are not practical. However, long-term effectiveness of PTS has been variable.

Anaerobic biozones rely on the reduction of sulfate to sulfide by sulfate reducing bacteria (SRB) to facilitate the indirect precipitation of metals as metal sulfides in the reducing environment found in anaerobic microbial PTS. Another product of SRB is bicarbonate that can participate in the neutralization of acidic MIW. SRB couple the reduction of sulfate with the oxidation of organic compounds (e.g., lactate or ethanol) and thus require a continued source of appropriate organic compounds to grow.

Previous studies have evaluated individual and mixtures of organic materials to assess the potential to promote and sustain sulfate reduction in anaerobic biozones. Gilbert et al., (1999) reported that the highest sulfate reduction rate was produced from a mixture of 25% dairy manure, 10% sawdust, and 10% alfalfa. This mixture is descriptively different from the 20% poultry manure, 30% leaf compost, 3% wood chips mixture used by Cocos et al., (2002), and the mixture of 10 % sewage sludge, 10% sheep manure, 5 % leaf compost, 12.5% wood chips, and 12.5 % sawdust reported by Waybrant et al., (1998).

Currently organic substrates are specified based on qualitative descriptors using experience and batch or column tests to determine sulfate

reduction rates. Quantitative measures of organic components in agricultural residuals are used by the National Renewable Energy Laboratory (NREL) Biomass to Energy Program to assess the usable fraction of different agricultural residues for biological ethanol production. The protocol at NREL requires highly skilled technicians and expensive equipment to assess specific organic components (e.g., glucans or mannans). A simpler method based on operationally defined fractions (e.g., ethanol, neutral detergent and acid solubility) was used in this study to characterize the components of organic substrates typically used for anaerobic biozones. Figure 1 shows the operational fractions discernable by the selected method.

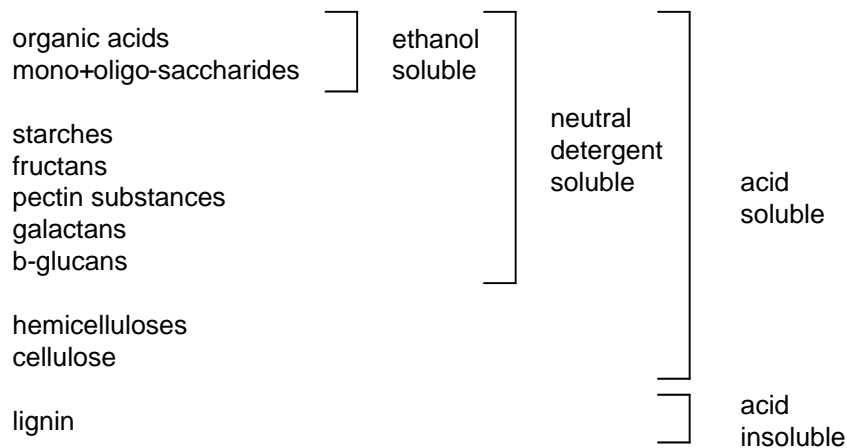


Fig. 1. Operationally defined fractions of plant material as measured by the relative solubility in ethanol, neutral detergent and acid.

Complicating the comparison of organic composition with sulfate reduction rate is the interrelationship between sulfate reducers and other members of the anaerobic microbially community. Ultimately sulfate reducers rely on microorganisms that can hydrolyze solid phase organic materials (e.g., protein, cellulose and lignin) to produce soluble organic compounds (e.g. glucose) that are further degraded to appropriate substrates (e.g., lactate) for sulfate reducers. Figure 2 is a simplified schematic of key microbial processes and substrates in an anaerobic biozone.

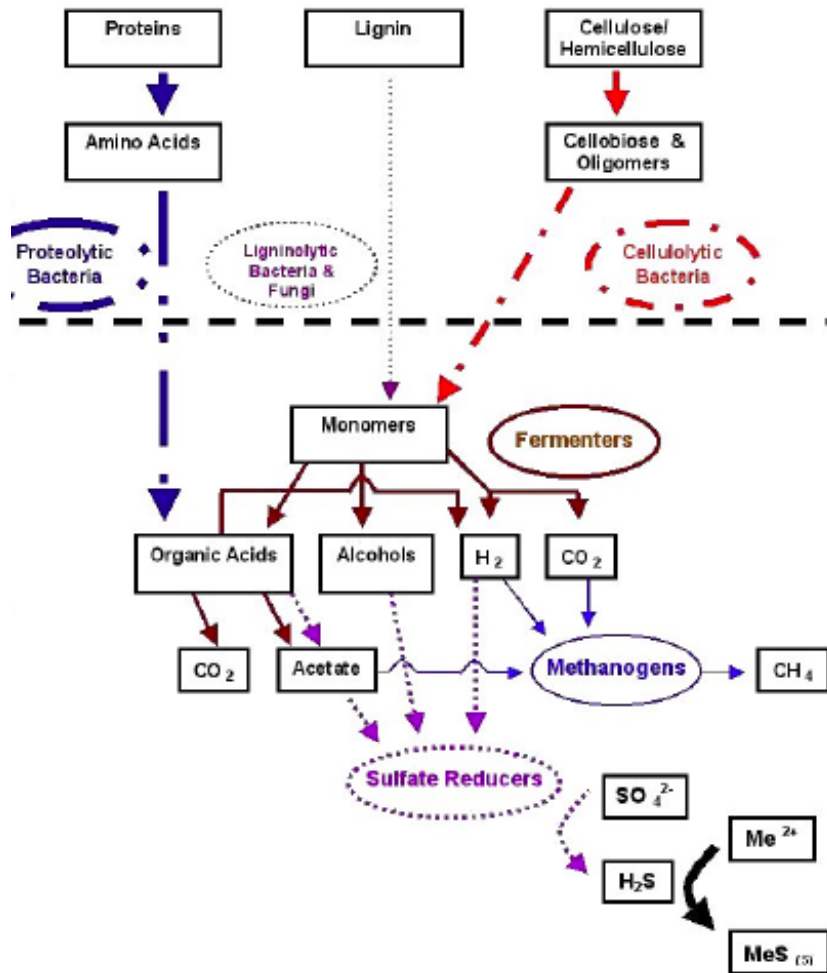


Fig. 2. Simplified schematic of anaerobic degradation of complex organic compound and associated microbial processes.

The working hypothesis of this study was that operationally defined measures of organic composition are related to sulfate reduction rates (SRR) in anaerobic biocolumns. The specific objectives were:

- 1) To use relatively simple operationally defined methods to characterize organic compositions of organic materials used in anaerobic biozones and
- 2) To determine which of the above organic fractions (e.g., organic acid) are correlated to the rate of sulfate reduction in anaerobic biocolumns.

2 Methods

2.1 Organic Material Characterization

For all experiments, the organic material was milled through a 4-mm sieve. The following methods were performed using the neutral detergent-soluble carbohydrates nutritional relevance and analysis (NDSCNRA) laboratory manual developed by Hall (2000) unless otherwise noted. All tests were performed in duplicate unless otherwise noted.

Moisture, total solids and volatile solids were determined in accordance with Standard Methods (1998). The total carbon and nitrogen percent of each organic material was performed using an Exeter Analytical, Inc. C, N, H CE-440 Elemental Analyzer (USGS laboratory in Boulder, CO) run in accordance with the manufacturer's operating procedure.

The Neutral Detergent-Soluble Carbohydrates Nutritional Relevance and Analysis (NDSCNRA) (Hall 2000) uses differences in solubility to separate and quantify carbohydrate fractions found in organic materials (Figure 1). The 80% ethanol extraction is able to solublize low molecular weight mono- and oligosaccharides, organic acids, and a small amount of crude protein found in organic material. The saccharide content of the 80% ethanol solution is measured by a colorimetric assay and the organic acid fraction can then be estimated by difference. Starch can be measured in the ethanol insoluble residue by an enzymatic/colorimetric assay. The neutral detergent soluble fiber (NDSF) (fructans, pectic substances, β -glucans, and other non-starch polysaccharides) is estimated by subtracting the ethanol insoluble and starch fraction from the neutral detergent soluble carbohydrates (NDSC). A neutral detergent/ α -amylase extraction will solublize all carbohydrate fractions except hemicellulose, cellulose, and lignin. By performing an acid hydrolysis on the neutral detergent insoluble fraction, one can fractionate the lignin from the hemicellulose and cellulose. Water solubility analysis was performed using the ethanol soluble fraction analysis method. Nanopure water was substituted for the 80% ethanol throughout the method.

2.2 Compositional Analysis Calculation

The neutral detergent soluble fiber fraction (NDSF), organic acid fraction (OA), and cellulose/hemicellulose fraction (CHF) of the organic materials were calculated as the difference in mass among residues of known composition. The CHF was calculated by subtracting the lignin fraction

from the NDISF. The NDSF and OA fractions were defined by the following equations (all fractions are shown in percents):

$$OA = (ESF - ESFC - (TP - EISFP))$$

$$NDSF = ((EISF - EISFP) - (NDISF - NDISFP) - Starch)$$

A combination of NDSF + Starch fraction was estimated (due to problems quantifying the starch fraction) from the percent solubility of organic materials in ethanol (ESF) and neutral detergent (NDS).

$$NDSF + Starch = (NDS - ESFC - OA - (TP - EISFP))$$

The abbreviations used for the operationally defined fractions are listed below.

NDSF = neutral detergent soluble fiber fraction

OA = organic acid fraction), and cellulose/hemicellulose fraction

CHF = cellulose/hemicellulose fraction

NDISF = neutral detergent insoluble fraction

ESF = ethanol soluble fraction

ESFC = ethanol soluble fraction carbohydrates

TP = total protein

EISFP = ethanol insoluble fraction protein

NDISFP = neutral detergent insoluble fraction protein

NDS = neutral detergent soluble

2.3 Column Design

30 cm long by 5 cm diameter glass columns were used during the column experiments. Each column had four 1-cm threaded side sample ports located at 6 cm increments from the base of the column. All sample ports, except the effluent port, were sealed with a rubber gasket and a screw-on cap. A 75 mm rubber stopper served as the top to the column as well as aiding in the release of gas. A plastic cap fitted with rubber o-rings was placed over the bottom of the columns. A screen was placed on the inside of each end-cap to prevent the flow of bulk material exiting the column.

An Isometric peristaltic pump was set at level 7 and using a tube diameter of 0.59 mm provided a sustained flow of 30 ml/day. The effluent tube was arched over a support bar ~40 mm higher than the effluent port, (producing a static water level) and then placed into a collection bottle. A schematic of the columns is presented in Figure 3.

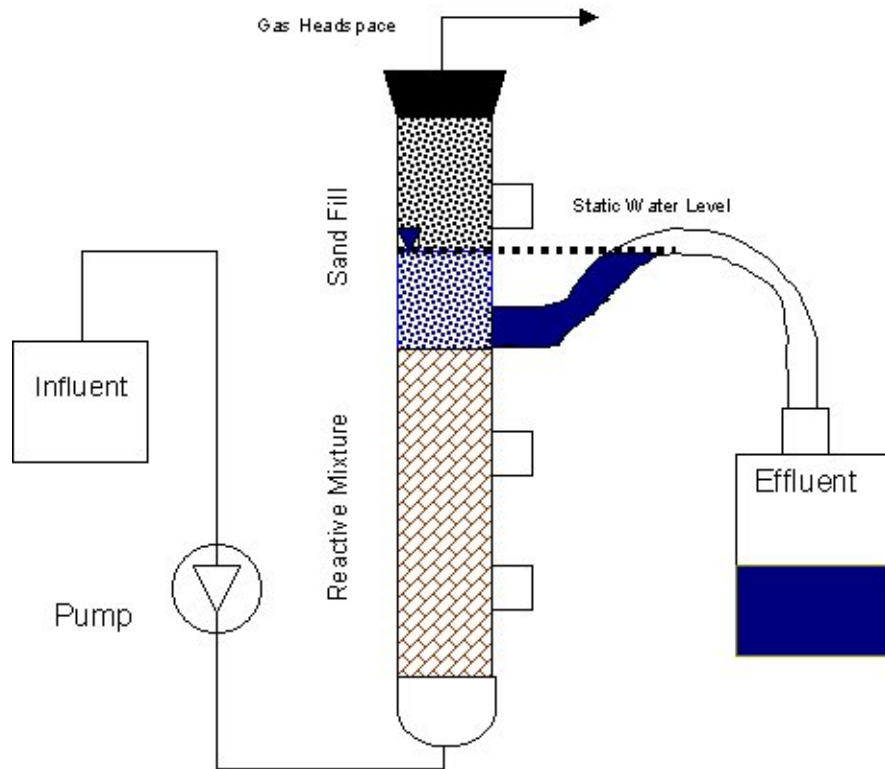


Fig. 3. Anaerobic biocolumn schematic

2.4 Column Mixture and Packing Procedures

150 grams of reactive mixture was weighed out (dry wt. basis) for each column and mixed in separate 3.8 Litre Ziploc bags. The reactive mixture comprised of 45% # 8 mesh silica sand, 5% #10 mesh limestone, 10% fresh dairy manure, and 40% selected organic material (either alfalfa, corncobs, oak, or pine) on a weight basis. Duplicate columns were constructed for each selected organic material. All columns were packed wet using a solution of 1000 mg/l SO_4 . All columns were packed to below the second highest sampling port (average pack volume = $380 \text{ cm}^3 \pm 90 \text{ cm}^3$). A screen was placed on top of the mixture to prevent the flow of bulk material. The difference between the total and packed volume was then filled with silica sand. The packed columns were allowed to sit for 7 days with no pumping, and then an influent with only 1000 mg/l SO_4 was pumped through the columns for another 7 days. The influent composition and flow rates for the column experiment are shown in Table 1. The

effluent was collected in sample bottles and stored in a refrigerator at 4°C prior to analysis. Table 2 shows the chemical analyses run on all effluent samples.

Table 1. Synthetic mine water influent composition and flow

Constituent	Day 7-14	Day 15-end
Sulfate (mg/L)	1000	1000
Zinc (mg/L)	0	50
Manganese (mg/L)	0	50
Iron (mg/L)	0	0
pH	6.0	6.0
Flow (mL/d)	30	30

Table 2. Chemical analyses

Analyte	Method
Metal and total sulfur	Perkin Elmer ICP-AES
Alkalinity	Hach titration
pH	Orion pH probe and meter
Conductivity	Probe and meter

3 RESULTS

3.1 Organic Material Characterization

The purpose of the organic material analysis was to use relatively simple operationally defined methods to characterize organic materials, and then correlate the resulting organic fractions to SRR. The different organic materials chosen for investigation can be categorized into three groups: agricultural and industrial products (AIP), woods, and microbial inocula sources. The AIP includes: alfalfa, brewery waste, corncobs, sugar beet pulp, and walnut hulls. The different woods tested were maple, oak, poplar, walnut, and pine. The two inocula sources tested were fresh dairy cow manure (manure) and the sediment from a constructed wetland (wetland). The results of the total nitrogen, carbon, protein, and organic fractions are presented in Table 3. The solubility in ethanol and water of each organic material are also shown in Table 3.

Table 3. Characteristics of dried samples presented as average value \pm standard deviation. Protein estimated as 6.25 times the mass of nitrogen

Sample ID	%Nitrogen	%Protein	%C	%Organic	%ESF	%WSF
Alfalfa	3.8 \pm 0.01	23.8	45.0 \pm 0.29	87.6 \pm 0.18	23.1 \pm 1.27	32.1 \pm 0.09
Brewery Waste	3.7 \pm 0.01	23.3	46.9 \pm 0.12	93.3 \pm 0.47	19.4 \pm 0.34	26.8 \pm 0.70
Beet Pulp	1.5 \pm 0.00	9.7	45.0 \pm 0.07	90.5 \pm 0.55	12.3 \pm 0.95	24.7 \pm 0.80
Corn cob	1.1 \pm 0.03	6.9	42.8 \pm 4.67	83.7 \pm 0.66	12.5 \pm 1.59	17.2 \pm 2.67
Wetland	0.6 \pm 0.05	3.6	9.3 \pm 0.74	13.3 \pm 1.10	3.7 \pm 0.28	10.1 \pm 0.64
Manure	2.0 \pm 0.08	12.8	29.2 \pm 1.40	42.1 \pm 4.96	3.7 \pm 2.67	8.9 \pm 1.16
Maple	0.4 \pm 0.00	2.5	49.7 \pm 0.01	100 \pm 0.00	0.8 \pm 0.75	3.4 \pm 3.43
Oak	0.4 \pm 0.00	2.6	50.0 \pm 0.02	99.9 \pm 0.06	5.7 \pm 1.16	3.7 \pm 1.21
Pine	0.3 \pm 0.00	2.0	50.3 \pm 0.03	100 \pm 0.00	2.3 \pm 0.77	3.7 \pm 0.22
Poplar	0.3 \pm 0.01	1.7	48.3 \pm 0.16	99.8 \pm 0.20	0.0 \pm 0.00	1.7 \pm 0.25
Walnut Hull	1.5 \pm 0.00	9.3	49.9 \pm 0.13	96.9 \pm 0.01	0.5 \pm 0.25	4.0 \pm 0.06
Walnut	0.3 \pm 0.01	1.7	48.7 \pm 0.14	99.8 \pm 0.00	4.0 \pm 0.54	2.9 \pm 1.93

The nutritional analysis data (Table 4) presents the carbohydrate fractions in order of lowest (i.e. organic acids) to highest (i.e. lignin) structural complexity (from left to right). The starch content could not be analyzed following the method outlined 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. Column 4 in Table 4 therefore gives the combined % of NDSF and starch.

Table 4. Summary of organic material carbohydrate characterization using NDSCNRA as percent of dry mass except carbohydrate/organic ratio

Sample ID	1	2	3	4	5	6	7	8	9
	Organic Acids	Mono/Oligo-saccharides	NDSF & Starch	NDSC	Hemi-/Cellulose	Lignin	Total Carbo-hydrate	Total Organic	Carbo-hydrate/Organic
Alfalfa	10.9	3.0	33.4	47.3	28.5	8.2	84.0	90.0	0.93
Brew Waste	4.7	11.5	16.4	32.7	38.3	15.5	86.5	93.3	0.93
Beet Pulp	1.8	9.6	39.2	50.6	32.5	3.6	86.6	93.0	0.93
Corn cobs	9.2	0.8	15.5	25.5	56.4	11.5	93.3	83.7	1.11
Wetland	3.5	0.0	ND*	3.5	6.0	2.7	12.2	13.3	0.92
Manure	3.5	0.2	0.0	3.7	27.7	5.1	33.0	42.1	0.78
Maple	0.0	0.7	7.2	7.9	68.5	21.2	97.6	100.0	0.97
Oak	3.6	1.6	11.6	16.8	59.7	20.7	97.2	99.9	0.97
Pine	1.2	1.0	9.1	11.4	64.5	22.8	98.6	100.0	0.99
Poplar	0.0	0.4	14.1	14.5	66.2	17.5	98.1	99.8	0.98
Walnut Hulls	0.5	0.5	23.2	24.2	40.8	26.6	91.6	96.9	0.95
Walnut	2.0	1.7	6.8	10.5	67.1	19.9	97.5	99.8	0.98

3.2 Column Study

Four organic materials were chosen as the dominant organic substrate for the column study. The effluent sulfate concentrations of the columns with time are shown in Figure 4. Column performance is also presented in terms of average sulfate reduction rate (SRR), Figure 5 with time. This is illustrated graphically in Figure 5, and summary data are given in Table 5. Zinc was completely removed once sulfate reduction was established. Manganese was removed initially but effluent manganese increased with time. Effluent alkalinity and conductivity decreased with time for the alfalfa and corncob columns and stabilized around 50 days of operation. Effluent pH was between 7 and 8 after 50 days for all columns.

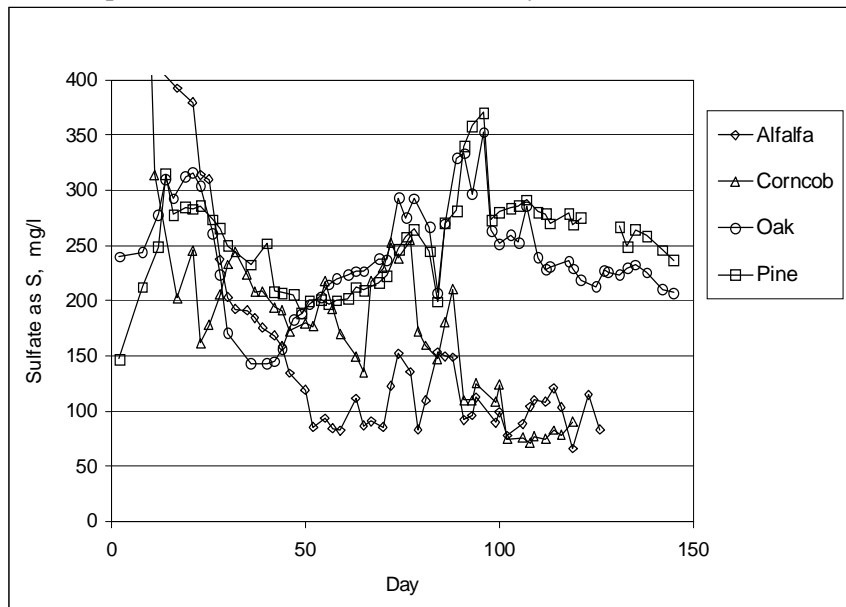


Fig. 4. Average effluent sulfate as S with time for anaerobic biocolumns. Influent sulfate was 334 mg/L as S

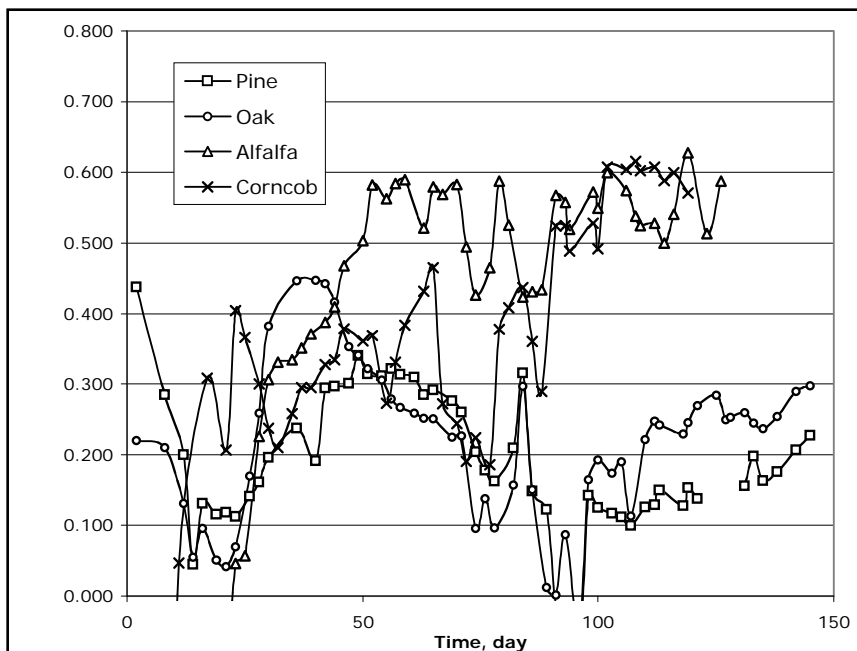


Fig. 5. Average sulfate reduction rate (mol S per m³ of reactive volume per day) with time for anaerobic biocolumns.

Table 5. Summary of average SRR and standard deviations for selected periods

Column	Day 15-50		Day 50-100		Day >100	
	avg.	st. dev.	avg.	st. dev.	avg.	st. dev.
Alfalfa	0.25	0.21	0.53	0.06	0.55	0.04
Corncob	0.31	0.06	0.37	0.11	0.59	0.04
Oak	0.27	0.16	0.18	0.11	0.23	0.05
Pine	0.20	0.08	0.20	0.13	0.15	0.04

4 Discussion

Organic materials were analyzed for operationally defined measures of organic composition. Organic composition characteristics were then correlated to SRR from anaerobic biocolumn studies. The calculated SRR before 50 days of operation for alfalfa and corncob columns was affected

by the leaching of sulfur. Additionally, zinc and manganese were observed in the effluent of the alfalfa and corncob columns until the leaching of inorganic components ended (around 50 days). One gram of these substrates leached 0.5 to 0.7 mg of sulfur into 100-mL of water over a 24-hour period in a batch assessment. The amount of water soluble (leachable) inorganic components from organic substrates is important in determining the potential for inorganic contaminant release from organic substrates.

Organic carbon content of mixtures has been used as an estimate of available substrate to the microbial communities. Waybrant et. al, (1998) found that in anaerobic batch studies mixtures with the lowest carbon content (9-11%) had the lowest sulfate reduction rates ($0.14 \text{ mg L}^{-1} \text{ day}^{-1} \text{ g}^{-1}$). Mixtures with the highest carbon content (35-47%) were found to also have the highest sulfate reduction rates (SRR) ($1.52\text{-}4.23 \text{ mg L}^{-1} \text{ day}^{-1} \text{ g}^{-1}$). However, one mixture with 40% carbon did not follow the pattern of higher SRR with higher organic content. The data summaries presented in Tables 4 and 5 suggest that higher SRR are not always associated with higher organic carbon content. In fact, both alfalfa and corncob (% organic carbon of 42.8 and 45, respectively) had higher SRR values than pine and oak with 50% organic carbon. This suggests that the SRR depends on nutritional characteristics other than organic carbon.

Waybrant et. al, (1998) observed that one mixture studied, that had high organic carbon content and lower SRR values, also had a low total nitrogen level. A comparison between the protein values of the organic materials and the SRR from our experiments (Tables 3 and 5) suggests that protein content is not related to SRR.

The ESF is used in the NDSCNRA to separate the organic acids and mono/oligo-saccharides from the more structurally complex fractions. SRB and methanogens can use some organic acids as energy sources (e.g. lactate for SRB and acetate for SRB and methanogens). The anaerobic degradation of mono/oligo-saccharides typically results in organic acids. In a batch study comparing cellulosic organic materials for the remediation of uranium laden mine waters, alfalfa was found to have a higher initial SRR than that of sawdust (Thombre et al., 1996). A comparison of the ESF analysis shows that alfalfa has a 23% ESF while the average for the woods was only 2.6%. The SRR results from the anaerobic biocolumn experiments suggests that higher SRR are correlated to both ESF and NDSC.

The NDSC plus acid solubility analysis offers a simpler but still valuable, alternative to performing all the tests required for the NDSCNRA. The NDSC can be performed in any lab with a hot plate, standard reagents, and a vacuum filter apparatus. The NDSC fraction is a

combination of the ESF, starch, and detergent soluble polysaccharides fractions. The test separates the carbohydrates into the fractions that are degraded through hydrolysis from the non-hydrolyzed components. Acid solubility can then be used to partition the NDISC into hemicellulose/cellulose and lignin fractions.

Organic materials like alfalfa and sugar beet pulp have close to 50% of their carbohydrates in the more highly degradable fraction. Woods are on the opposite end with NDSC fractions ranging from 8% for maple to 17% for oak. Walnut hulls, corncobs, and brewery waste (24, 26, and 32% respectively) could provide the highly degradable fraction initially for adequate SRB growth, but will also have a larger quantity of the more slowly consumed carbohydrate source than the alfalfa and sugar beets.

Benner et al., (1984) studied the anaerobic degradation rates of organic materials having high and low NDSC fractions (grass and hardwoods). After 294 days 30 % of polysaccharides found in grass were mineralized, while only 4.1% of polysaccharides were mineralized for hardwoods. The current study suggests that for the organic materials tested, high NDSC tends to result in a greater extent of overall degradation. Benner et al. (1984) also observed differences between the lignin degradation rates of organic materials. Over the same time period, 1.5% of the hardwood's lignin was mineralized compared to 16.9% of grass's lignin. Benner observed that the biodegradation rates of the lignin component of organic materials with high NDSC fractions are much higher than those with low NDSC fractions. This suggests that even the slowly degraded fractions of organic materials with high NDSC fractions are consumed faster than those materials with low NDSC fractions.

Previous studies (batch to full-scale treatment) have reported that the use of a mixture of different types of organic substrates provides the highest sustainable SRR (Pinto et al., 2001; Gilbert et al., 1999; Waybrant et al., 1998; Kuyuck et al., 1994; Wildeman et al., 1994). These studies suggest that balanced mixtures of organic materials containing high organic acid and saccharide fractions (quickly consumed by SRBs or fermenters), with those comprised of mainly lignocelluloses (slowly degraded source of carbohydrates), result in a sustained SRR. The NDSCNRA method, or proposed simplification, allows one to chemically analyze organic materials and develop mixtures based on rapidly measured parameters.

The sum of the carbohydrate fractions from the NDSCRA method relative to the organic content varied from 0.92 to 0.99 for most of the materials tested. Only manure (0.78) and corncobs (1.11) exhibited relative fractions that differed by greater than 10%. Difficulties were encountered

with these two materials relative to filtering when separating soluble and insoluble fractions.

5 Summary

Sulfate reduction rate in the columns appears to increase with neutral detergent soluble compounds (NDSC), water-soluble fraction (WSF) and ethanol soluble fraction (ESF) once the water driven leaching of inorganic from substrates had subsided. Thus, NDSC, WSF and ESF appear to be good measures of the relative degradation rates of the organic material over a period of up to 150 days. Organic carbon and total organic content did not show this apparent relationship to SRR and thus were poor measures of the relative degradation rates of organic material.

The amount of water soluble (leachable) components from organic substrates is important in determining the potential for organic substrate and inorganic contaminant release from organic substrates. The water soluble components may be removed from the column by advective flux and result in unacceptable effluent concentrations of organics and inorganics.

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