Chemical characterisation of organic electron donors for sulfate reduction for potential use in acid mine drainage treatment

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Introduction

The production of effluents containing high amounts of sulfate, heavy metals and low pH is a worldwide problem. These effluents are known as acid mine drainage (AMD) and the primary source is coal mining, where AMD is formed when sulphide ores undergo chemical and biological oxidation processes (Christensen et al. 1996). An oxidising environment is established in which sulphuric acid is formed and heavy metals associated with the particular ore are mobilized (Feng et al. 2000). AMD leads to various environmental problems, killing aquatic life as well as economic problems due to the fact that it is highly corrosive.

AMD may be remediated by neutralising the effluent and removing the sulfate and metals. Various active sulfate removal technologies are available including chemical treatment with mineral precipitation, membranes, ion-exchange and biological sulfate removal (Hulshoff Pol et al. 2001; LOREX 2003). Among the processes that use chemical treatment with mineral precipitation, the limestone/lime and SAVMIN process appear to be the most suitable for sulfate removal from AMD (LOREX 2003). However, large amounts of sludge are produced during these processes (LOREX 2003). Membrane and ion-exchange technologies produce high quality water that can be used as drinking water but the major disadvantage is the production of brine that requires disposal at additional costs (LOREX 2003). Widespread application of these active AMD treatment processes has not yet been seen. This could be attributed to the high costs involved as well as the degree of maintenance and supervision required.

Several researchers have undertaken investigations using organic electron donors to drive biological sulfate reduction including:

- Monitored sulfate reduction using 10 cm columns packed with different organic electron donors (hay, straw, leaf compost, manure and compost, Rabenhorst et al. 1992).
- Sulfate reduction using municipal compost, composted yard waste and sawdust as substrates (Eger 1991).
- Sulfate reducing bacteria (SRB) having been grown with a variety of different complex organic electron donors e.g. fishmeal, wastewater sludge (Pipes, 1960) and cellulose as well as sawdust (Maree et al. 1985; Tuttle et al. 1969).
- Nanninga & Gottschal (1986) using waste water of a potato-starch production factory in an anaerobic purification process.
- Chicken litter and rice hulls having been tested in bench and field pilot plants to drive sulfate reduction (Eger 1991).
- Several other experiments using primary sewage sludge, waste activated sludge molasses, rubber waste effluent and cheese whey (Chang et al. 2000; Kaufman et al. 1996; Obarsky et al. 1984; Oleskiewicz et al. 1986; Pipes 1960).

There is an increasing demand for inexpensive, environmental friendly technologies to remediate AMD. One such a potential technology is the so-called passive treatment system defined as a water treatment system utilising naturally available energy sources such as microbial metabolic energy, photosynthesis, chemical energy, the use of natural topographical gradients to regulate flow and requires regular but infrequent maintenance (Pulles et al. 2003). Sulfate reducing bacteria drive sulfate reduction in passive treatment systems and utilise organic compounds, if a suitable electron donor is available for the reduction of sulfate to sulphide (Dill et al. 1994).

One of the limitations of passive treatment systems is the availability of readily available carbon source to drive sulfate reduction over the design life of the passive treatment system (~20 years). Studies performed on passive treatment systems indicated that substrate availability became the limiting factor in terms of sulfate reduction after a period of 9 months (Coetser 2003; PHD 1999).

To develop efficient passive AMD treatment systems, the anaerobic sulfate reduction process must be optimised, by selecting the most appropriate organic electron donors for sulfate reduction, or a mixture. The effectiveness in the remediation of AMD is dependant on developing and maintaining optimal environments for desirable microbial populations. These microbial populations would therefore generate preferred modes of degradation and/or that of sulfate reduction. The degradation of the organic electron donors supplied for biological sulfate reduction may well lead to the release of residual organics and nutrients, which in turn will be removed by the SRB.

The standard method for evaluating the capacity of a carbon source or mixture of various organic electron donors to drive sulfate reduction would be to set-up a pilot plant using different mixtures of organic electron donors and AMD (Dill et al. 1994). These bioreactors however need to be operated for periods of at least 9 months to establish the sustainability of the organic electron donors to drive sulfate reduction. If the chemical characterisation of a carbon source can be correlated with the capacity to drive sulfate reduction one could use this to predict the capacity of a carbon source to derive sulfate reduction as a substitute for expensive, time consuming continuous bioreactor set-ups.

The most cost-effective and available organic electron donors are of plant material (wood chips, silage, grass etc.) or industrial and/or municipal waste (sewage sludge etc.) (Howard et al. 1989; Weider et al. 1989). The chemical composition of these organic electron donors is mainly lignin, hemicellulose, cellulose, proteins and carbohydrates. The difference in the degradation of the different organic electron donors, due to their differences in their chemical composition would distinguish effective organic electron donors for use in the passive treatment of AMD.

Lignocellulose is the term used to describe the composite of the predominant polymers of plants. The main constituents of lignocellulosic materials are cellulose, hemicellulose and lignin (Malherbe & Cloete 2002). These components are present in different quantities in different plant species and the ratio of the components would determine the biodegradability of the material. The in situ degradation of lignocellulose is limited by the intricate nature of this structure as well as the physical and chemical properties of the polymers themselves (Malherbe 2000). Lignin is the most recalcitrant of the three polymers (Betts et al. 1991; Deobald & Crawford 1997; Malherbe 2002; Paul & Clark 1989). Various studies have been performed on the biodegradation of lignin involving fungi, actino-mycetes and bacteria (Malherbe 2000). Lignocel-lulose biodegradation by prokaryotes is essentially as slow process characterised by the lack of powerful lignocellulose degrading enzymes, especially lignin peroxidases (Malherbe 2002). Most fungi are capable of cellulose degraders. However, their ability to facilitate rapid lignocellulose degradation attracted attention from scientists and entrepreneurs alike (Malherbe 2002). White-rot fungi comprise powerful lignin degrading enzymes that enable them in nature to bridge the lignin barrier and, hence, overcome the rate-limiting step in the carbon cycle (Malherbe 2002). Cellulose and hemicellulose represents readily biodegradable organic electron donors that can be utilised by natural microbial consortia (Malherbe & Cloete 2002). Other constituents of organic electron donors include proteins, peptides and amino acids which are readily metabolised in soil (Kuzyakov 1997). Aerobic and facultative aerobic bacteria are equally capable of oxidising proteins and amino acids. These organisms are able to utilise amino acids as their sole source of carbon and energy (Doelle 1975). The end products are simple compounds that can be oxidised to carbon dioxide and water in any of the oxidative metabolic pathways (Doelle 1975). Therefore, free proteins represent easily utilisable substrates for most microorganisms.

Other constituents of organic electron donors are triglycerides, which are enzymatically degraded by lipases to yield fatty acids and glycerol (Malherbe & Cloete 2002). Lipids can therefore serve as a source of energy for most microorganisms and lipids and related compounds are readily utilised once they become available (Malherbe 2002).

Therefore, in the biochemical decomposition of organic matter, facultative and anaerobic bacteria of a wide variety hydrolyse and convert the complex materials to low-molecular-weight compounds. Among the low- molecular-weight compounds formed, the short-chain fatty acids are important components depicting biodegradation.

The primary objectives of this study were to:

- classify different organic electron donors according to their chemical composition
- correlate chemical composition of different organic electron donors to sulfate reduction capacity for potential use of the organic electron donors in AMD treatment

Methodology

Chemical composition analysis

The selection of organic electron donors for sulfate reduction used during this study depended on criteria such as availability and cost (Table 1).

Table 1. Organic electron donors for sulfate reduction subjected to chemical characterisation

Wood products	Grasses	Other products
• Fresh pine chips	• Dry Buffalo grass	• Molasses
• Old pine chips	• Fresh Buffalo grass	• Bagasse
• Fresh pine prunings	• Dry *Eragrostis* grass	
• Fresh blue gum chips	• Fresh *Eragrostis* grass	
• Old blue gum chips	• Dry wild type grass	
• Fresh blue gum prunings	• Fresh wild type grass	
• Wattle chips		
• Fresh wattle prunings		
• Old wattle saw dust		

Table 2. Chemical analyses performed on the carbon sources

Chemical analyses	Method/Reference
Dry matter	Samples are dried overnight at 100 ^{o}C to determine dry matter (Harris 1990)
Ash content	Ash content is measured after incinerationovernight at 550 ^{o}C and then for 2 h at 900 ^{o}C (Harris 1990)
Total Kjeldahl nitrogen/Crude protein	Crude protein can be estimated as Kjeldahl N X 6.25 using a LECO FP-2000 protein analyser (LECO AFRICA Pty(Ltd))
Lignin, cellulose and hemicellulose	The gravimetric method of Goering and van Soest (1970) is used to determine the concentration of hemicellulose, cellulose and lignin
Crude fat	Crude fat is determined through ether extraction (AOAC 1984)
Crude fibre	% Crude fibre is calculated as follows: $\%\ \text{Crude fibre} = \frac{\text{RCD-RCA}}{\text{original sample mass}} \times 100$ where: RCD = residue in crucible after drying RCA = residue in crucible after ashing (AOAC 1984)
In vitro digestability	It involves a 48 h fermentation by rumen microorganisms in a buffer solution followed by a 48 h pepsin digestion after acidifying with hydrochloric acid (Tilley & Terry 1963)
Water-soluble carbohydrates	Carbohydrates measured spectrophotometrically against standards (Harris 1990)
Total non-structural carbohydrates	Digestion of sample with amylase enzymes and carbohydrates measured spectrophotometrically against standards (Faichvey & White 1983)
Starch	Glucose concentrations of a sample is determined and the values multiplied by the factor 0.912 in order to convert it to % starch (AOAC 1984)

Before analysis, the undefined organic electron donors were ground using a cyclone mill, screened through a ca. 100 mm sieve and dried in an oven at 85 °C for approximately 2 h. The Agricultural Research Council (ARC; Irene, South Africa) performed all of the chemical analyses as indicated in Table 2.

Sulfate reduction studies

Unplastised poly vinylidine chloride (uPVC) pipes (diameter of 200 mm) and uPVC fittings were used to construct pilot plant bioreactors with a volume of 22 l. Bioreactors were packed with monotypes of organic electron donors (Table 1) and inoculated with 1.1 l of a mixture of equal parts of anaerobic digestor sludge, supernatant of the digestor and anoxic digestor sludge (Daspoort Water Purification Works, Pretoria, South Africa). Bioreactors were then fed with a synthetic AMD mixture with a theoretical sulfate concentration of 2000 mg l^{-1} (Table 3). Technical grade chemicals and tap water were used and the pH was adjusted to 7 using industrial grade sodium hydroxide and hydrochloric acid. Operational periods for each bioreactor is presented in Table 4 and represents the time during which the bioreactors were fed with AMD and evaluated.

Various chemical analysis were performed on the influent and effluent of the bioreactors. However, for the purpose of this article, only sulfate concentrations determined using ion chromatography (IC) (Methrom 790 personal IC, Swisslab) will be used. Sulfate reduction was determined by subtracting the measured effluent sulfate concentration (mg l^{-1}) from the measured influent sulfate concentration (mg l^{-1}) and the average sulfate reduction calculated over the operational period of the different bioreactors. Sulfate reduced per mass of organic electron donor was also calculated by incorporating the mass of carbon packed each bioreactor.

Table 3. Chemical composition of synthetic AMD

Chemical	Concentration ($g\ l^{-1}$)	Sulfate ($mg\ l^{-1}$)	Other ($mg\ l^{-1}$)
$MgSO_4\ 7H_2O$	1.22	475.0	[Mg] = 120
$MnSO_4\ 4H_2O$	0.04	17.5	[Mn} = 10
$Al_2(SO_4)_3\ 16H_2O$	0.017	53.4	[Al] = 10
Na_2SO_4	2.15	1454.1	

Table 4. Operational period and average sulfate reduction of the different bioreactors

Organic electron donor	Operational period (days)	Average sulfate reduced ($mg\ l^{-1}$)	Average sulfate reduced ($mg\ kg^{-1}$ carbon)
Fresh wild type grass	371	540	180
Bagasse	252	512	233
Dry wild type grass	357	511	352
Fresh blue gum prunings	369	511	102
Fresh pine prunings	364	468	117
Molasses	140	414	734
Fresh blue buffalo grass	371	408	146
Fresh wattle prunings	364	398	88
Dry blue buffalo grass	357	390	300
Fresh blue gum chips	355	346	63
Old blue gum chips	369	335	84
Fresh *Eragrostis* grass	288	330	120
Wattle sawdust	350	318	57
Fresh pine chips	251	290	50
Dry *Eragrostis* grass	253	284	189
Old wattle chips	209	205	38
Old pine chips	217	140	23

The organic electron donors were categorised into low (0–4%), intermediate (4–10%) and high protein content (>10%) for discussion purposes. The same was done for cellulose content e.g. low (0– 20%), intermediate (20–40%) and high (>40%).

Results and discussion

Several biological sulfate reduction studies where performed using various organic electron donors. Due to the fact that each study were performed under different operating conditions e.g. some batch, some continuous for different periods of operation and that results obtained are reported in different formats, it makes it very difficult to compare results obtained between different studies. The purpose of this study was not to focus on sulfate reduction as such but to compare the different chemical characteristic of the organic electron donors with the sulfate reduction obtained between bioreactors operated under similar conditions. Detailed analysis on the performance of the different bioreactors was further undertaken by Coetser (2003).

Percentage dry matter

The percentage dry matter of the organic electron donors evaluated ranged between 92.2% and 96.4% (Table 5). The small variance in percentage dry matter is due to the fact that all of the organic electron donors tested were solids. The grass types however such as dry wild type grass and dry blue buffalo grass, had the highest percentage dry mass and the wood types such as fresh blue gum prunings and fresh pine chips, the lowest percentage dry matter. No correlation could be drawn between percentage dry matter of a carbon source and sulfate reduction. Parameters other than percentage dry matter play a more important role when it comes to selecting organic electron donors to drive sulfate reduction.

Percentage ash content

No correlation could be drawn between percentage ash content and the sulfate reduction capacity of a carbon source

(Table 5). Ash content should therefore not be used as one of the parameters to select suitable organic electron donors for sulfate reduction.

Percentage crude fat

All three types of wood prunings had a high crude fat content, ranging between 3.65% and 3.86% (Table 5). This was ascribed to the waxes present on the leaves of the trees. These organic electron donors led to higher sulfate reduction than the wood chips containing low crude fat content indicating that crude fat content increases sulfate reducing capacity (Table 5). Organic electron donors containing high crude fat content are preferred over those containing low crude fat content to drive sulfate reduction.

Total non-structural carbohydrates (TNC) and water-soluble carbohydrates (WSC)

Only molasses, bagasse, wattle chips and fresh *Eragrostis* grass were analysed in terms of TNC and WSC (Table 5). The TNC for the tested organic electron donors was higher than the WSC (Table 2). Except for bagasse, it seems that the higher the TNC, the higher the sulfate reduction (Table 5). Organic electron donors containing

Table 5. Summary of the carbon characterisation results for the various organic electron donors for sulfate reduction

Substrate	% Dry matter	% Ash	% Crude protein	% Crude fat	% Crude fibre	% NDF	% ADF	% ADL	% Hemi-cellulose	% Cellulose	% Lignin	% *In vitro* digestability	Starch	WSC	TNC
Fresh wild type grass	95.1	6.16	3.45	1.55	43.4	80.2	51.1	8.35	29.1	42.8	2.19	49.9	ND	ND	ND
Bagasse	94.8	11.9	2.79	1.59	42.0	70.2	59.2	17.4	11.1	41.8	5.49	42.3	1.53	2.39	10.1
Dry wild type grass	96.4	3.99	8.33	1.61	35.5	75.5	41.9	6.32	33.6	35.6	2.33	44.7	ND	ND	ND
Fresh blue gum prunings	92.5	5.4	8.12	3.65	37.3	62.3	52.3	17.5	9.97	34.8	12.1	43.0	ND	ND	ND
Fresh pine prunings	93.6	3.33	4.51	3.86	48.5	75.1	63.1	31.2	12.0	31.9	27.9	26.0	ND	ND	ND
Molasses	ND	2.44	0.35	0.14	ND	0.27	0.00	0.00	0.27	0.00	-2.44	ND	0.00	63.6	74.7
Fresh blue buffalo grass	95.3	9.28	4.85	0.63	44.0	78.5	52.1	9.53	26.4	42.6	0.25	43.3	ND	ND	ND
Wattle prunings fresh	93.7	5.78	15.8	4.76	25.8	57.1	42.5	27.6	14.6	14.9	21.8	30.3	ND	ND	ND
Dry blue buffalo grass	96.0	7.12	4.71	0.93	43.5	76.3	47.1	7.02	29.2	40.1	-0.10	36.5	ND	ND	ND
Fresh blue gum chips	94.0	2.07	2.02	0.58	66.8	86.1	75.9	21.4	10.2	54.5	19.3	18.5	ND	ND	ND
Old blue gum chips	93.9	1.51	1.87	1.02	69.5	86.9	77.9	18.9	8.98	59.0	17.4	19.0	ND	ND	ND
Fresh *Eragrostis* grass	95.3	4.08	9.03	2.01	40.4	81.6	48.1	8.55	33.5	39.5	4.47	28.8	0.00	4.30	11.8
Wattle sawdust	93.8	1.97	2.47	0.65	63.8	89.2	74.9	21.9	14.3	53.0	20.0	17.0	ND	ND	ND
Fresh pine chips	92.2	2.88	1.57	0.90	70.6	89.7	77.8	29.5	11.9	48.3	26.6	19.5	ND	ND	ND
Dry *Eragrostis* grass	94.1	7.60	4.66	1.13	38.6	73.6	45.0	5.21	28.7	39.7	-2.39	65.1	ND	ND	ND
Wattle chips	95.1	0.75	1.71	0.27	71.0	88.7	52.4	17.6	36.3	34.8	16.8	ND	0.00	0.64	5.27
Old pine chips	93.9	2.16	2.81	1.06	70.2	88.4	76.2	27.2	12.2	49.0	25.0	15.6	ND	ND	ND

NDF = Neutral detergent fibre.
ADF = Acid detergent fibre.
ADL = Acid detergent lignin
WSC = Water-soluble carbohydrates
TNC = Total non-structural carbohydrates
ND = Not determined

high TNC would be suitable to drive sulfate reduction.

Protein and nitrogen content

Classically, the pathway of anaerobic degradation of organic matter involves the hydrolysis of large molecular weight compounds such as proteins, nucleic acids, carbohydrates and lipids to lower molecular weight products such as organic acids and alcohols (Gibson 1990). These may then be fermented into volatile fatty acids and gasses. Terminal oxidative processes are then able to degrade these substrates further. In environments with a plentiful supply of sulfate, it is during these terminal stages that SRB have a major role in the metabolism of organic detritus (Gibson 1990).

The following organic electron donors were classified as having low protein content (Table 5): molasses, bagasse, fresh pine chips, old pine chips, fresh blue gum chips, old blue gum chips, wattle chips, wattle sawdust and fresh wild type grass (Table 5). Normally, wood chips, sawdust, bagasse and molasses are products of mature plants. Hence, the low protein and high fibre content (Table 5).

Fresh pine prunings, fresh blue gum prunings, fresh buffalo grass, dry buffalo grass and dry wild type grass were classified as organic electron donors containing intermediate protein levels (Table 5).

It has been indicated by Waksman (1952) that the older the plant becomes, the less is the proportion of its proteins, water-soluble (sugars and amino acids) and mineral constituents and the greater is the concentration of cellulose, lignin and, to some extent, the hemicellulose as can be seen when comparing the results obtained from pru-nings of a wood to the results obtained form the wood chips (Table 5). Only fresh wattle prunings were classified as having high protein content (Table 2).

The ratio of % crude protein: % crude fibre was low for organic electron donors with higher sulfate removal efficiencies and high for organic electron donors with lower sulfate removal efficiencies (Figure 1). Thus the higher the % crude fibre in comparison to the % crude protein the lower the sulfate reducing capacity of the carbon source. This was expected, as crude fibre represent the more recalcitrant fraction of the organic electron donors.

Proteins make up 1–20% of all plant residues (Waksman 1952). Proteins are complexes of amino acids. Proteins contain, on average, 50–55% carbon, 15–19% nitrogen, 6–7% hydrogen, 21–23% oxygen, and small amounts of phosphorus and sulphur. During hydrolysis, the specific enzymes or by chemical reagents, the proteins are first split into various polypeptides and finally into simple amino acids. The latter are further attacked by a great variety of bacteria and fungi, giving rise to

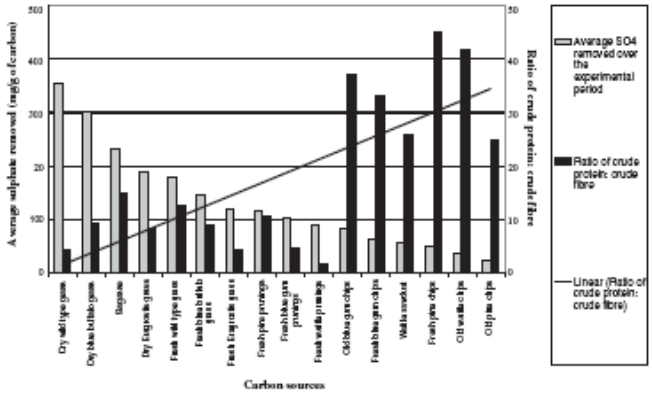

Figure 1. Sulfate removed (mg l^{-1}) versus ratio of % crude protein: % crude fibre (1% crude protein: x% crude fibre) of the different organic electron donors where x is reported on the graph.

ammonia, carbon dioxide, and various organic acids and alcohols (Barry et al. 1995). Waksman (1952) also indicated that the amount of ammonia liberated depends upon the abundance of the protein and also upon the other constituents of the plant material, especially the carbohydrates. The lack of fermentable substrates favours bacterial lyses which releases proteins into the fermentative medium (Krishnamoorthy et al. 1991).

Lignocellulose content

Molasses and fresh wattle prunings were the only organic electron donors containing low cellulose (Table 5). The low cellulose content could be due to the low fibre content of fresh wattle prunings (Table 5). Dry and fresh *Eragrostis* grass, dry wild type grass, fresh blue gum prunings, wattle chips and fresh pine prunings were categorised as having intermediate cellulose content (Table 5). The rest of the organic electron donors had cellulose percentages higher than

40% (Table 5). No direct correlation could be drawn between cellulose content and a carbon source's ability to drive sulfate reduction (Table 5). It seems as if the presence of other more readily available carbon constituents play a larger role in the initial phases of driving sulfate reduction.

The % hemicellulose of the organic electron donors evaluated ranged between 0.27% and 36.3% (Table 5). Wattle chips and the different grass types had the highest hemicellulose content ranging between 26.4% and 36.3% (Table 5). Molasses had the lowest % hemicellulose at 0.27% (Table 5). Once again, no direct correlation could be drawn from the studies conducted in terms of the hemicellulose content and the sulfate reducing capacity of a carbon source (Table 5). The presence of other constituents plays a larger role in driving the initial phases of sulfate reduction.

The highest lignin content observed was 27.9% (Table 5). The different pine wood varieties had the highest lignin content followed by the different wattle wood varieties (Table 2). The different grasses had the lowest lignin content (Table 5). As previously indicated, lignin is the most recalcitrant of the three polymers of lignocelluloses. In plants, lignin is chemically bonded to hemicellulose and wraps around fibers composed of cellulose. It was concluded that the lower the lignin content, the more biodegradable the substrate (Figure 2).

Figures 2 and 3 summarises sulfate removed versus % lignin and % in vitro digestibility.

When plotting trend lines for % lignin versus sulfate reduction it is clear that the higher the lignin concentration, the lower the sulfate reduction (Figure 2). When plotting % in vitro digestibility, the higher the % in vitro digestibility, the higher the sulfate reduction (Figure 3). These results were expected as the recalcitrant nature of lignin would decrease the digestibility of a carbon source.

The degradation of lignocellulose is limited by its intricate nature of its structure as well as the physical and chemical properties of the polymers themselves. Lignocellulose comprises three groups of polymers: cellulose, hemicellulose and lignin

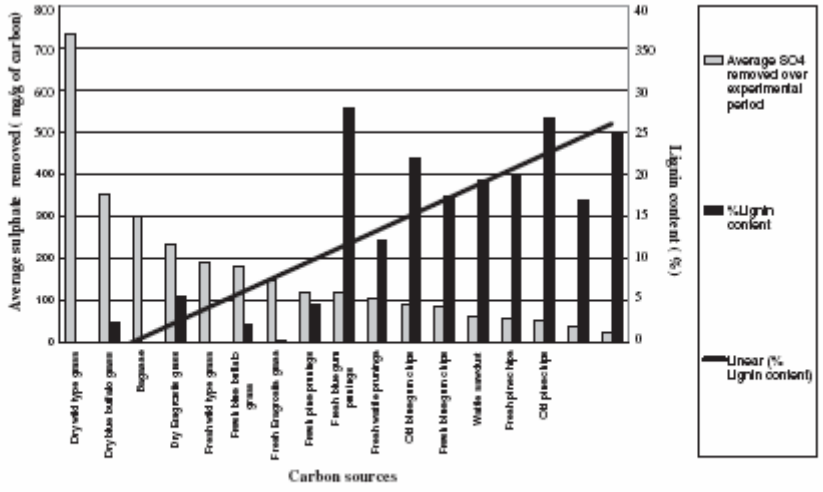

Figure 2. Sulfate removed versus % lignin content of the different organic electron donors.

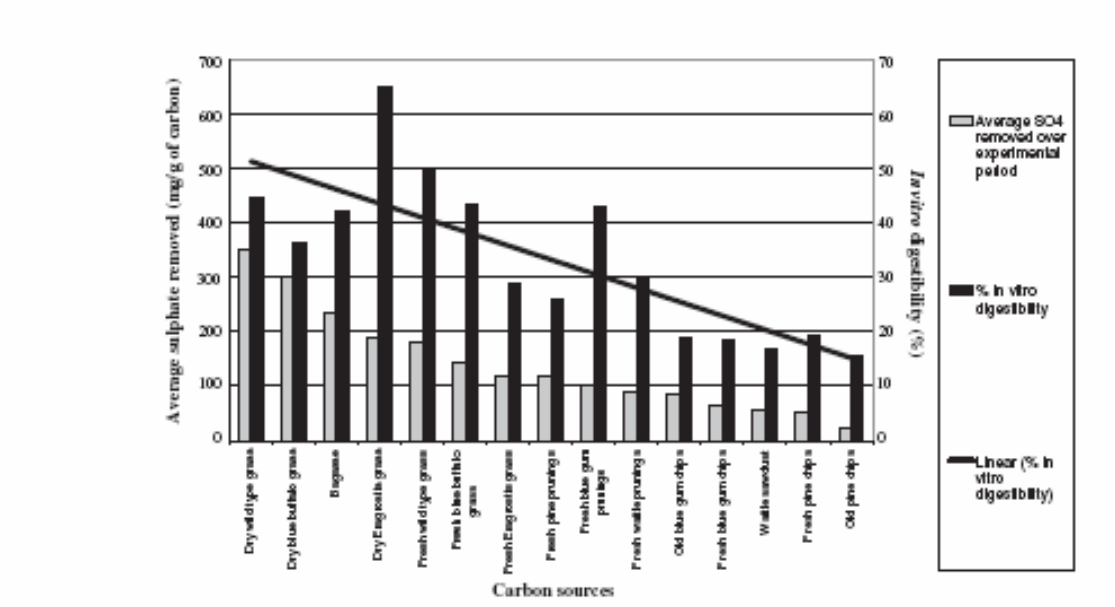

Figure 3. Sulfate removed versus % *in vitro* digestibility of the different organic electron donor

Cellulose is linear polymers of *b*-1,4-linked sugar residues, susceptible to hydrolysis by acid and enzymes. The first step in hydrolysis is the hydrolytic cleavage of the cellulose molecule exposing the long chain ends (Rampersad et al. 1998). The cellobiose is further degraded to glucose monomers.

Hemicelluloses are more variable and chemically complex than cellulose, because they represent a great variety of chemical compounds, usually divided into polysaccharides and poly-uronides or mixtures of sugars and sugar acids (Van Soest et al. 1991). The designation of individual hemicelluloses is based on the sugar produced on their hydrolysis by acids or enzymes (Van Soest et al. 1991). Hemicellulose is attacked by a great variety of bacteria and fungi.

Lignin is the most recalcitrant of the three polymers of lignocellulose. Lignin comprises three types of aromatic monomers linked by a range of stable intermonomeric linkages, non-hydrolysable carbon sources and relatively inert ether bonds. This polymer is highly irregular, insoluble and is made up of phenyl propane subunits with no chains of regular repeating units. Under aerobic conditions lignin is not absolutely resistant to decomposition but can be gradually oxidised (Evans et al. 1994).

Percentage crude fibre

The higher the percentage crude fibre, the lower the sulfate removal capacity (Figure 4). Organic electron donors with lower crude fibre content were more suitable to drive sulfate reduction.Chemical characterisation of carbon should not be seen in isolation. Subjecting organic electron donors to different types of microbial degradation, environmental conditions and hydraulic dynamics are only a few of the other parameters that affect the biodegradability of a carbon source.

In terms of sulfate reduction, it is suggested to use mixed layers of organic electron donors (Pulles et al. 2003). The more readily available organic electron donors (e.g., molasses) would be responsible for initiating not only sulfate reduction but anaerobic metabolic processes to condition environmental conditions by removing oxygen from the system and reducing redox potentials and thereby optimise environmental conditions for SRB's. Different grass types, which are not as readily biodegradable as molasses yet not as recalcitrant as wood chips, will be responsible for sulfate reduction over a short-term period. The more recalcitrant types of organic electron donors e.g. wattle chips will be responsible for maintaining sulfate reduction over the long term.

Conclusions

Understanding different carbon sources in terms of their capacity to drive sulfate reduction assisted with the development of mixtures of carbon sources for passive treatment systems able to sustain effective sulfate reduction over extended periods (Pulles et al. 2003, 2004). Pilot plant sulfate reducing studies of different carbon sources together with the chemical characterisation of the

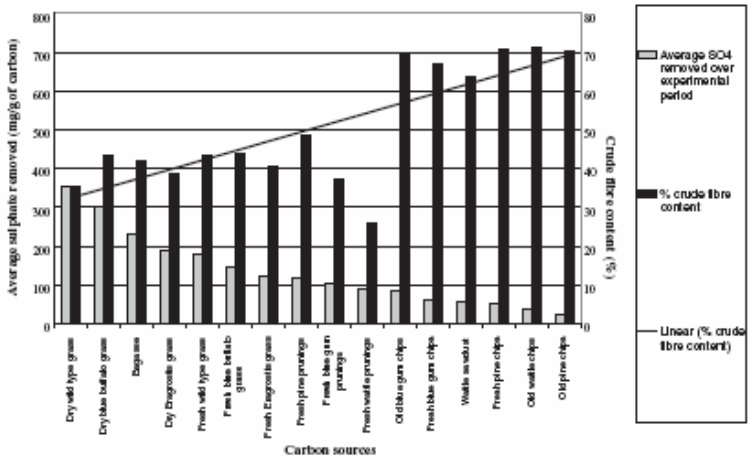

Figure 4. Sulfate removed versus crude fibre content of the different organic electron donors.

carbon sources not only improved our understanding of the capacity of different carbon sources to drive sulfate reduction but also correlated chemical characteristics of a carbon source with the capacity to drive sulfate reduction.

The following conclusions were made from this study:

- The most readily biodegradable carbon source would be high in protein content and low in lignin content e.g. different grass types.
- The higher the carbohydrate content and crude fat content of a carbon source, the higher the capacity to drive sulfate reduction.
- The higher the crude fibre content of a carbon source, the lower the capacity to drive sulfate reduction.
- Chemical characterisation can be used to assist in predicting sulfate reduction capacity of a carbon source and the selection of organic electron donors for potential use in AMD treatment.

Acknowledgements

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