The Utilization of Cellulosic Biomass in treating AMD and the Subsequent Generation of Fermentable Sugars

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ABSTRACT

The drainage from closed and abandoned mines is often acidic with elevated heavy metal concentrations. The interaction between acid mine drainage (AMD) and the environment has a significant impact. Whilst temporary remediation can lessen this impact, complete amelioration is only possible using active and/or passive treatment. Active technologies are often costly and can produce a secondary waste stream which may require additional treatment. In this project we explored a passive option combining different technologies. We recognise AMD as a resource of sulphuric acid which we used as a pre-treatment on cellulosic feedstock. Following this, we enzymatically digested the partially hydrolysed cellulose to produce fermentable sugars (which could be used to produce bioethanol). Concurrently, the pH of the AMD increased from 2.11 to 5.46 and 62% of the iron was removed from solution. Previous research has shown that sulfate is also remediated (primarily through dissimilatory sulfate reduction [DSR]). In this research we were successfully able to combine two separate, yet established technologies and were able to remediate AMD with a cellulosic feedstock through DSR and Fe precipitation, whilst being able to use the cellulose residue as a feedstock for bioethanol production.

Keywords: Cellulose, AMD, Fermentation Feedstock

INTRODUCTION

Acid mine drainage (AMD) refers to the outflow of acidic water from (usually abandoned) metal and coal mines. It is characterized by low pH and high concentrations of sulfate and heavy metals (Acid mine Drainage (AMD) South Africa, 2011). It is considered the most important mining industry related pollution problem (Rob and Robson, 1995; Johnson and Hallberg, 2005; Kuyacak *et al*, 2011). AMD occurs when sulfide ores are exposed to the atmosphere, which can be magnified through mining and milling activities, where oxidation reactions are initiated (Jennings *et al*., 2008; Zipper *et al.*, 2011; McCathy, 2011; Johnson and Hallberg, 2005). Upon exposure to oxygen and water, pyritic minerals oxidise to form acidic, iron and sulfate rich drainage. The oxidation of the pyritic minerals is the primary mechanism by which acid is released into AMD (Ackil and Kodas, 2006, Neculita *et al*., 2007, Sangita *et al*., 2010). The reactions involved in the formation of AMD are a multistep process involving oxygen-independent and oxygen dependant reactions. A general equation for the process is (Equation 1):

$$FeS_2(s) + 14Fe^{3+}(aq) + 8H_2O = 15Fe^{2+}(aq) + 2SO_4^{2-} + 16H^+(aq) \qquad (1)$$

The net effect of these reactions is to release H^+, which lowers pH and maintains the solubility of ferric ions (Blodau 2006).

AMD treatment can be achieved through active, semi-passive or passive methods (Greben *et al*., 2009). A broad range of passive and active treatment approaches are available for dealing with AMD. Neutralisation (pH control) is the AMD treatment mechanism for both passive and active systems. By increasing pH to create alkaline conditions, the solubility of most metals is significantly decreased by precipitation (Taylor *et al*., 2005).

In previous work, biomass has been successfully used to treat AMD. The results indicated that grasses hold promise for remediating AMD, as a maximum of 99% iron removal, 80% sulfate removal, and a final pH of 8.5 was achieved from initial conditions of 2000mg/l iron, 6000mg/l sulfate, and a pH of 3. (Sheridan *et al*., 2013).

In this study, it is hypothesised that the lignin fraction of grasses and other cellulosic feedstocks contain numerous reactive groups which bind to and remove metals from AMD. Simultaneously the sulfuric acid in the AMD hydrolyses hemicellulose and exposes the cellular structure of cellulose whereby it can be subjected to a subsequent enzymatic hydrolysis step. In this study, we assess the potential use of the acidity present in AMD to (at least) partially hydrolyse cellulose. This would result in partially remediated AMD as well as a fermentable feedstock which could be utilised to produce, for example, bioethanol.

Materials

Two different cellulosic feedstocks were tested in this study: sugarcane bagasse and switch grass. Sugarcane bagasse was originally obtained from Illovo Sugar Company based in Kwazulu Natal. The switch grass, *Panicum virgitum* (an indigenous perennial grass) was obtained milled from the laboratory of the School of Chemical and Metallurgical Engineering. The second batch of switch grass was collected from the African Leadership Academy garden. It was obtained already dry. The enzyme (cellulase) was supplied by Yakult Pharmaceutical Ind. Co., Ltd from Tokyo, Japan. The enzyme was obtained in powdered form. The product name of the enzyme is Cellulase "Onozuka" FA. The enzyme was composed of cellulase 12% and lactose 88%.

METHODOLOGY

The AMD feed was exposed to different cellulosic feedstocks for a period of 14 weeks. The resulting materialwas subsequently enzymatically digested with cellulase. The pH of the AMD exposed to the biomass was measured using a bench top pH meter. The iron concentration was analysed using the Merck test kit number 114761 and the Merck spectroquant Pharo 300 according to the manufacture's manual. Reducing sugars were measured using a 1200 series Agilent HPLC. Scanning electron microscopy was done using an FEI Quanta 400 E-SEM.

RESULTS

Dissolved iron and pH changes

Figure 1 and Figure 2 show the increase in pH and the decrease in the dissolved iron concentration during treatment of AMD using different lignocellulosic biomass over the 14 week experimental period.

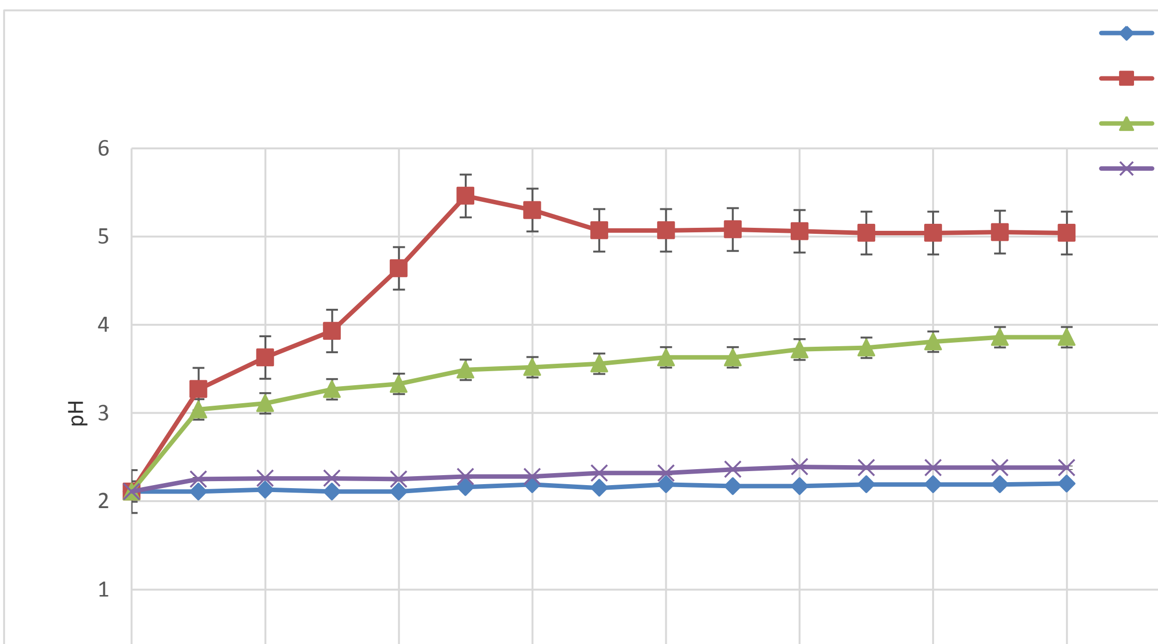

Figure 1 Changes in pH as effected through different types of lignocellulosic biomass

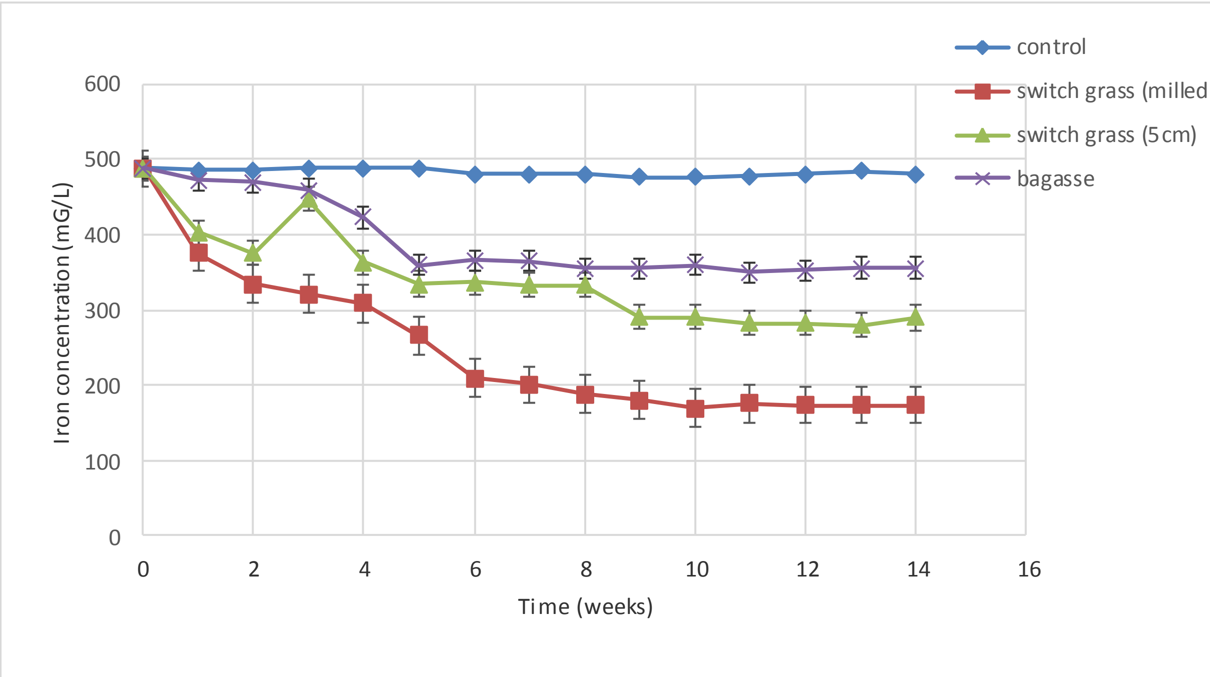

Figure 2 Changes in Iron concentration as effected by different types of lignocellulosic biomass

The data presented in Figures 1 and 2 indicate that the three sources of cellulosic biomass were able to effect some remediation of AMD. In all three cases dissolved iron concentration was reduced and pH was increased. The milled switch grass (≤2mm) was the most effective in the remediation of AMD. The milled switch grass was able to remove 65.2% of dissolved iron and increase the pH from 2.11 to 5.30. The maximum pH was obtained in a relatively shorter period of time (5 weeks). The switch grass (5cm) was able to remove 42% of dissolved iron and increased the pH from 2.11 to 3.86. Sugarcane bagasse had the least remedial effect on AMD. Sugarcane bagasse removed 28% of iron from AMD and increased the pH from 2.11 to 2.36; this was achieved in 14 weeks. The pH and the iron concentration of the control which contained only AMD did not change much during the experimental period, indicating that the biomass (switch grass and bagasse) caused the increase in alkalinity and the decrease in dissolved iron concentration. Similar results were obtained by Ramla (2012). In Ramla's investigation, two indigenous grass species, *Hyparrheia hirta* and *Setaria spacenta*. Maximum pH values of 8.48 and 99% percentage removal of iron from the AMD solution was reported in that study.

These values are comparable with the ones reported in literature for the biosorption and precipitation of metals on switch grass and other biomasses. According to literature the main mechanisms of metal ion removal in bioreactors are precipitation in the form of sulfides, hydroxides and carbonates and sorption mechanisms such as adsorption (Neculita *et al.*, 2007). The greatest effect on remediating the AMD was observed for the milled switchgrass. The decrease in dissolved iron in solution can be explained by the presence of a large number of active sites on the milled switch grass due to the increased surface area caused by milling. Functional groups capable of metal sorption in biomass include phenolic, hydroxyl and carboxylic groups. Milling exposes these functional groups to the dissolved ions. These functional groups in the biomass are protonated at higher pH and presumably available for binding dissolved metals (Neculita *et al*., 2007). This explains the fact that the increase in pH is accompanied by a marked decrease in dissolved iron concentration (Figure 5.1).Increases in pH of acidic water effectively removes some metals due to the precipitation of metals in the form of hydroxides (Goatham, 2013).

Microbial activity also plays a role in the remediation of the AMD. Microbial activity was observed in the two switch grass series with the milled switch grass seeming to support more growth. IT is most probable that this microbial population is comprised of sulfate reducing bacteria (SRB). As explained by Ramla (2012), the increase in pH can be directly related to SRB population growth. The substrate may have been providing nutrients to sulfate reducing bacteria which contributed to the growth of the microbial population. The SRB activity reduces sulfate to sulfide and produces hydrogen carbonate (Equation 2). The hydrogen carbonate partially neutralises the AMD, while the hydrogen sulfide reacts with dissolved metals in the AMD, resulting in the precipitation of metals as metal sulfides (Equation 3) (Taylor *et al*, 2005).

$$SO_4^{2-} + 2CH_2O + 2H^+ \rightarrow H_2S + 2H_2CO_3 \qquad \text{(Equation 2)}$$

$$H_2S + M^{2+} \rightarrow MS + 2H^+ \qquad \text{(Equation 3)}$$

Where CH_2O are the sugars and M^{2+} represents metal ion.

The release of glucose during the treatment of AMD using milled switch grass (≤2mm)

The release of glucose was observed only in the milled switch grass, suggesting that milling was a significant factor in the generation of glucose. Milling reduces cellulose crystallinity and disrupts the lignin-carbohydrate complexes. This aids the acid treatment process and the subsequent enzyme hydrolysis (Mais *et al*., 2002: Carmen *et al*., 2013). The change in concentration of glucose is shown in Figure 3. Glucose started to appear in the third week of treatment where a rapid increase was observed. The maximum amount of glucose obtained was 8.4mg/ml in the seventh week. Thereafter the concentration decreased to 4.9mg/ml after 14 weeks. The decrease in glucose concentration may be explained by the consumption of glucose by sulfate reducing bacteria (SRB). The generation of glucose and other reducing sugars during AMD treatment is attributed to acid catalysis, where the sulfuric acid in the AMD catalyses the breakdown of cellulose and hemicellulose to release glucose, xylose and other degradation products. Hemicellulose, comprised mainly of mannan and xylan, accounts for up to a third of the total carbohydrate in many lignocellulosic materials (Pingali *et al*., 2010). In a study by Roman (2004), much higher levels of reducing sugars (306g/l) were produced. In that study, production was also followed by consumption. The consumption was accompanied by an increase in pH from 5.8 to 7. The increase in pH was found to be related to sulfate reduction and the production of alkalinity within the bioreactor.

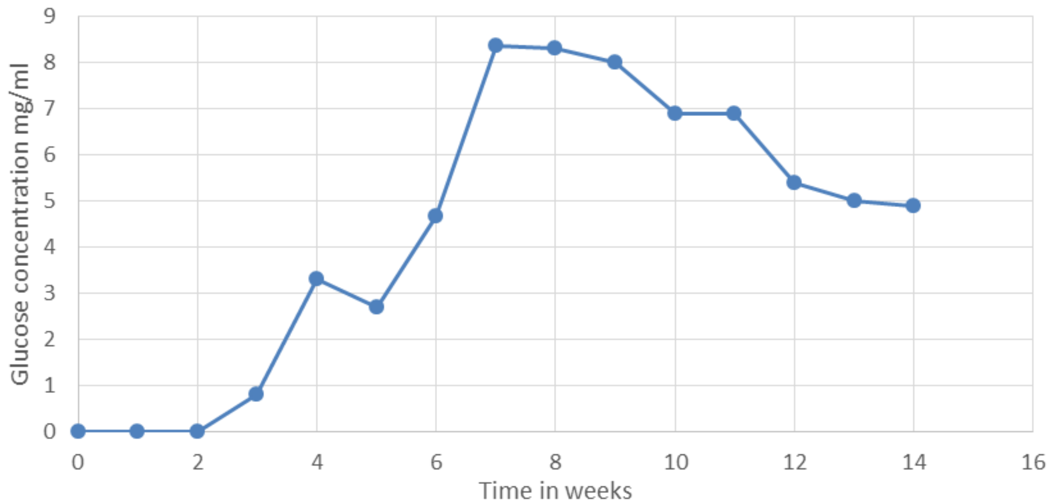

Figure 3 The release of glucose during treatment of AMD using milled switch grass

Scanning Electron Microscope (SEM) analysis of switch grass treated by water and AMD before and after enzymatic hydrolysis.

SEM was used to study the morphological features and surface characteristics of the switch grass treated by water and AMD before and after enzyme hydrolysis (Figure 4).

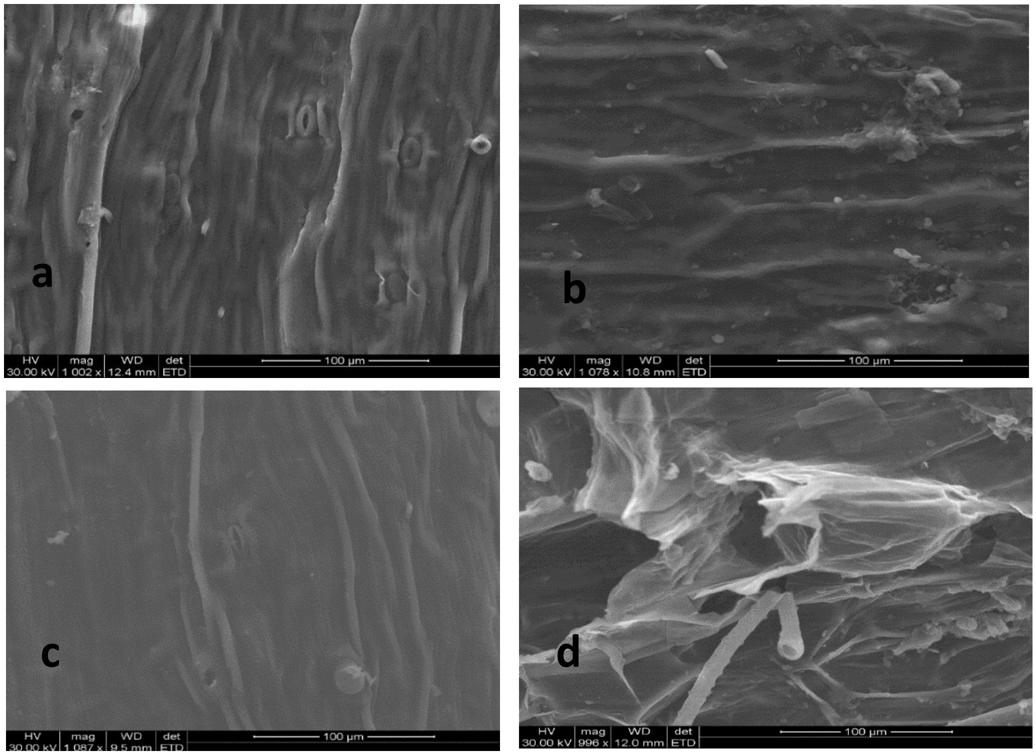

Figure 4 Scanning electron microscope images of switch grass (a) water treated, (b) AMD treated, (c) water treated followed by enzyme treatment and (d) AMD treated followed by enzyme treatment.

The SEM images reveal that pre-treatment induced significant morphological changes in the switch grass. Water treated switch grass exhibited rigid and highly ordered fibrils (Figure 4a). The fibres of the AMD treated switch grass appear to be distorted with some holes evident on the surface (Figure 4b). This renders the switch grass more accessible to cellulase, resulting in higher enzymatic hydrolysis rates and cellulose digestibility (Moxley *et al.*, 2008: Remli *et al*., 2013). The molecular structure of the water pre-treated and enzyme treated switch grass remained intact, showing no digestion occurred during the hydrolysis of the water treated switch grass (Figure 4c). The molecular structure of the switch grass which was pre-treated by AMD followed by enzyme digestion was completely destroyed (Figure 4d). The improved/altered morphological properties caused by AMD pre-treatment of switch grass appeared to be the primary source for the enhancement of enzymatic hydrolysis (Goshadrou *et al*., 2011). These micrographs are in agreement with the HPLC results shown in Table 1, which show high digestibility of AMD treated switch grass and no or very little digestibility in the water treated switch grass.

Enzymatic hydrolysis of AMD treated and untreated switch grass

Table 1 shows the concentrations of xylose and glucose after the enzymatic hydrolysis of the water treated and AMD treated switch grass as analysed using HPLC.

Table 1 Enzymatic hydrolysis of water treated and AMD treated switch grass.

	Water treated		AMD treated	
Enzyme %	**Xylose mg/ml**	**Glucose mg/ml**	**Xylose mg/ml**	**Glucose mg/ml**
0	**0**	**0**	**0**	**0**
0,5	**0**	**0**	**1,9**	**0**
2,5	**0**	**0**	**3,3**	**3,8**
5	**0**	**0**	**0**	**4,8**
10	**1,4**	**0**	**3,4**	**3,7**

The water treated sample was our control for this experiment. It is noted from Table 1 that the glucose yield for the water treated switch grass was zero for all the enzyme concentrations. The xylose yield was zero except for the 10% enzyme concentration which had xylose concentration of 1.4 mg/ml. The SEM images (Figure 4) showed that the water treated switch grass displayed no obvious signs of mechanical disruption of the general surface morphology. This rigid and compact structure of untreated lignocellulosic biomass hinders the effectivity of cellulase and prevents it from digesting the grass. The enzymatic digestion of the AMD treated switch grass show the concentrations of glucose and xylose in mg/ml. The maximum sugar concentration achieved with the milled switch grass were 4.8 mg/ml glucose at 5% enzyme concentration and 3,4 mg/ml xylose at 10% enzyme concentration. The enzyme concentration had a significant effect on the amounts of glucose and xylose obtained: there is a general increase of the glucose and xylose concentration with the increase in enzyme concentration. Small quantities of enzymes were ineffective in digesting the treated switch grass. The AMD treatment of the switch grass was therefore able to significantly improve the accessibility of the cell walls to enzymatic digestion, leading to greater sugar yields compared with the water treated switch grass.

DISCUSSION AND CONCLUSION

Switch grass has been shown in this study to have the capacity to effectively remove iron and acidity from AMD. The results indicate that milled switch grass (2mm) was more effective in remediating AMD than sugar cane bagasse or the large particle size switch grass (<5cm). The potential of switch grass, sugarcane bagasse and other lignocellulosic material to remove metals by adsorption is expected since such materials contain functional groups such as phenolic, hydroxyl, carboxyl and carboxylic (Harman *et al*., 2007). This theory is supported by Minawar and Riwandi (2010) who attributed the decrease in dissolved iron in the first two weeks of AMD treatment to iron retention by the organic matter rather than iron precipitation. In our study the milled switch grass which had a higher surface area was able to retain more iron resulting in lower dissolved iron concentration. Zagury *et al*., 2006 (cited in Munawar and Riwandi, 2010) explained that metal removal tends to occur sooner than to sulfate reduction, through adsorption of metals to organic matter. Once reducing conditions are established metal precipitation rather than adsorption becomes the predominant mechanism of removal. Munawar and Riwandi (2010) reported the decrease of sulfate concentration in the third week of their study, using chicken manure mixed with AMD. This was attributed to sulfate reduction to metal sulfides such as iron sulfide. In our study microbial activity seemed to dominate in the third week of AMD treatment supporting Munawar and Riwandis' findings that sulfate reduction occurs at a later stage of AMD treatment. SRB activity produces hydrogen carbonate which increases the alkalinity of the AMD.

The milled switch grass was able to increase the pH of the AMD from 2.11 to 5.30 and to remove dissolved iron from the AMD by 65.2% by the fifth week. Both the pH and the dissolved iron content started to stabilise thereafter. To further increase the alkalinity and to reduce the dissolved iron concentration, periodic substrate loading may be considered in future for the long term remediation of AMD (Ramla 2012).

Santos *et al*., (2004) worked with different biomass concentrations and showed that an increase of the biomass (grape stalks) concentration up to 30g/L leads to a 20% reduction of the total content of dissolved iron in the AMD. In a similar study Greben *et al*., (2008) used cut grass serving as a carbon and energy source for continuous sulfate removal and obtained between 80 and 90% removal efficiency by adding cut grass loadings every two weeks. The increase in grass loadings provides new surface area for adsorption of metal ions, further reducing their concentration.

In our study the residue switch grass obtained from the AMD treatment process was prepared for a further enzymatic hydrolysis step. The preparation included washing to remove metal ions adsorbed to the surface. SEM was used to study the effects of the AMD on the switch grass. The results showed that AMD induced morphological changes in the switch grass (Figure 4). These changes are due to the hydrolysis of hemicellulose and to a lesser extent cellulose by the sulfuric acid in the AMD. The hemicellulose fraction is converted to its monomeric sugars which include pentose and glucose. This treatment is analogous to the pre-treatment of lignocellulosic biomass using acid. In literature dilute acid pre-treatments of lignocellulosic material are done at higher temperatures and pressure and are able to recover relatively higher amounts of fermentable sugars from the hemicellulose fraction of lignocellulose. Barrier et al., (1985) reported the conversion and recovery of sugars from the hemicellulose fraction treated with dilute acid to be more than 90% efficient. Dilute sulphuric acid in the range 0.5-1.5% and temperatures above 160 has been found the most favoured for industrial applications. Under these conditions high sugar yields from hemicellulose are obtained (Hisham and Mageed, 2008). In our study the conditions are mild (room temperature and pressure) and hence we expected reduced rates.

Glucose was detected during the treatment of AMD in the course of this study (Figure 3). A maximum glucose concentration of 8.4 g/ml was obtained in the seventh week of the study. After AMD treatment it is necessary to recover some of the sugars from the AMD/sugar liquor. The sugars in solution in the AMD are highly contaminated with metal ions and would need purification if they are to be recovered and to be used for further bioprocessing. It is difficult to recover the hemicellulose fraction of sugars from the AMD/sugar solution. The AMD/sugar liquor can be fermented to produce ethanol which can be recovered by distillation, or alternatively the AMD/sugar liquor could be used as a carbon and energy source for SRB in subsequent treatments. The remaining cellulose residue, instead of being discarded, could be further processed by additional enzymatic hydrolysis as the cellulose residue contains the bulk of the glucose. Cellulase enzymes were used in our study to recover some of the sugars in the lignocellulose, with maximum values of glucose and xylose at 4.8 mg/ml and 3.4 mg/ml respectively. The xylose produced in the processing may also be fermented or used to produce other value added products. One possible use of the unconverted xylose is to produce fodder yeast (single cell protein) for animal feed (Barrier *et al*., 1985). This study has evaluated the potential of AMD treated switch grass in the production of fermentable sugars. From this study it can be concluded that:

a) Lignocellulosic material has the capacity to treat AMD,
b) The AMD treated switch grass has the potential to produce fermentable quantities of glucose upon enzymatic hydrolysis,
c) AMD is not only remediated but assists in a bioprocess to prepare second generation feedstock for industrial biotechnology.

The research is, however, at a very early stage of development. Substantial additional work is required to optimise the production of fermentable sugars as well as understanding the rates of AMD treatment. There is also still much work needed into developing a process which could be used commercially. This is the subject of ongoing research.

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