VOLATILE FATTY ACID FORMATION AND UTILIZATION IN ANAEROBIC SULPHIDOGENIC BATCH REACTORS

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Abstract

Acid Mine Drainage needs to be treated, before it is recharged in rivers and dams. The biological treatment of AMD can be applied using degradation products of cellulose, e. g. Volatile Fatty Acids as carbon sources. The aim of this study was to demonstrate that microbes originating from compost and rumen are able to ferment Grass Cuttings to produce Volatile Fatty Acids which are utilized during the biological sulphate removal process. Two studies were conducted: 1) Four stirred batch-test reactors (2 ℓ) were operated, fed with artificial SO_4 rich (1700 mg/ℓ) feed water and tap water (controls). The reactors received sulphate reducing bacteria, compost bacteria and grass cuttings. The experimental period was 25 days, the operating temperature was 20 to 22 ˚C. 2) Two anaerobic reactors (2.5 ℓ) were operated at 37-39 ˚C and at pH of 6.7 - 6.9 to accommodate the rumen organisms. The test reactor contained SO_4 rich water and the control reactor tap water, as well as SRB, rumen bacteria and grass cuttings. The duration of study 2 was 32 days. In both studies SO_4 reduction could be observed (from ≈ 2000 to 0 mg/ℓ over 8 days). The Volatile Fatty Acids results showed that both butyrate and propionate were produced and subsequently utilised for the sulphate reduction and that a clear relationship existed between the organic acids concentration and sulphate reduction. It was concluded that the compost and rumen microorganisms could degrade grass to high concentrations of Volatile Fatty Acids resulting in continuous SO_4 removal.

Key words: Cellulose, compost & rumen microbes, fermentation, sulphate

INTRODUCTION

Acid Mine Drainage (AMD) mainly originates from coal mines both in South Africa as in mining areas all over the world. AMD is formed when the mineral pyrite, the most common sulphite mineral, comes into contact with oxygen and water to form sulphate and acidity, resulting in a low pH. AMD needs to be treated, either by chemical or by biological means or through the integrated treatment methods (1), before discharge in receiving water bodies. In this study the focus is on the *biological* treatment of AMD, using a cost effective carbon source in the form of degradation products of cellulose. Cellulose is

the major constituent in plant biomass, forming an important component in the carbon cycle. Plant biomass is a sustainable source of energy when cellulose is utilised in the anaerobic degradation and composting to produce Volatile Fatty Acids (VFA) (2). This process involves many species of fermentation bacteria, including the Sulphate Reducing Bacteria (SRB) (3). SRB participate in the degradation of the polymers and monomers to produce VFA. Greben & Baloyi (4) showed that the anaerobic degradation of grass cuttings (GC) to VFA was enhanced when a SRB mixture was added to the fermentation process, even when no sulphate was present. The utilisation of propionic acid by SRB in the absence of sulphate was shown by Harmsen (5).

The digestion of cellulose occurs through the activity of special microbial populations originating from the rumen of the ruminants (6). Cellulose in the ruminant feed are converted into microbial cells and into acetic, propionic and butyric acids, which acids are food for the ruminant. The rumen is inhabited with billions of bacteria and protozoa which can efficiently execute the anaerobic degradation of plant material as these organisms produce fibre degrading enzymes (7). Recent work published by Sonakya (8) showed the use of digested cattle feed to produce VFA from grass cuttings.

The aim of this study was to demonstrate that microbes originating from compost and rumen fluid can ferment grass cuttings to produce VFA which are utilized during the biological sulphate removal process.

MATERIALS AND METHODS

Study 1: Compost Bacteria

Feed water and reactors

The feed water for two stirred batch-test reactors, (R1 and R2) consisted of artificial sulphate rich feed water of which the SO_4 concentration ($NaSO_4$) was approximately 1 700 mg/ℓ, while tap water was used for two control stirred batch-test reactors, R3 and R4. All feed waters were supplemented with both macro nutrients (75 mg/ℓ ammonia-N and 15 mg/ℓ ortho-phosphate-P) and micro nutrients (100 µg/ℓ Fe, 210 µg/ℓ Co, 0.28 µg/ℓ Mn, 0.44 µg/ℓ V, 0.25 µg/ℓ Ni, 0.48 µg/ℓ Zn, 0.40 µg/ℓ Mo, 0.18 µg/ℓ B, 0.37 µg/ℓ Cu). Grass cuttings (50 g) were added to the feed water as the carbon and energy source. Glass bottle reactors R1 to R4 (volume 2 ℓ) were operated under anaerobic conditions. All reactors received 250 mℓ SRB mixture, obtained from the CSIRosure reactor (Witbank, South Africa), of which the VSS was 11.9 g/ℓ and 70 mℓ biomass isolated from compost (VSS was 25.5 g/ℓ). Samples (50 mℓ) were taken daily from the bottom of the reactor, which volume was replaced by sulphate rich water (R1 and R2) and tap water (reactors R3 and R4). The experiments were carried out at room temperature (22 °C).

Experimental Conditions

R1-R4 were operated for 25 days, during which time the contents were stirred using magnetic stirrers. Daily samples were taken to monitor the VFA and

COD production in conjunction with the sulphate reduction. Reactors R3 and R4 were the controls for R1 and R2 in respect to COD and VFA production. After 8 days of operation, when it was noticed that the sulphate concentration was < 200 mg/ℓ in R1 and R2, fresh sulphate was added to the reactors. At the same time (day 8), sulphate was added to R4 (control reactor) with the aim to investigate whether the accumulated VFA in R4 could serve as the carbon and energy source for the SRB present in R4. On day 21, 30 gram fresh grass cuttings were added to all four reactors.

Study 2: Rumen bacteria

Experimental Conditions

Two anaerobic reactors: L1 and L2 were operated at 37-39 ˚C and at pH of 6.7 - 6.9 to accommodate the rumen organisms. The experimental data is given in Table 1. The duration of study 2 was 32 days.

Table 1.   The experimental conditions

Reactor	Contents
L1	1500 mg/ℓ SO_4 + 30 g/ℓ GC + 250 mℓ RB + nutrients
L2	Tap water + 30 g/ℓ GC + 250 mℓ RB + nutrients

Analytical

Determinations of sulphate, COD, pH, mixed liquor suspended solids (MLSS) and volatile suspended solids (VSS) were carried out according to standard analytical procedures as described in *Standard Methods* (15). With the exception of the MLSS, VSS, sulphide and feed COD, all analyses were carried out on filtered samples (Whatman #1). The COD samples were pre-treated to eliminate the sulphide contribution to the COD concentration.

All VFA analyses were done using a gas chromatograph (Hewlett Packard. HP 5890 Series II) equipped with a flame ionisation detector (GC/FID), while the data analyses were done using the Chem Station software package, supplied by Hewlett Packard, The column used was a HP-FFAP, 15 m x 0.530 nm, 1 micron. An outline of the GC/FID programme used is depicted in Table 2. The N_2 flow rate was set at 1 mℓ/min.

**Table 2: The GC/FID programme for the detection of VFA**

Parameter	Setting
Initial oven temperature (˚C)	30
Initial time (Min)	2
Temperature programme: (˚C)	80
Rate (˚C/min)	25
Final temperature (˚C)	200
Final time (min)	1
FID temperature (˚C)	240

RESULTS AND DISCUSSION

Study 1

Sulphate removal as a function of the butyrate concentration.

The sulphate and the butyric (C4) acid concentrations in R1-R4 are shown in Figures 1 and 2.
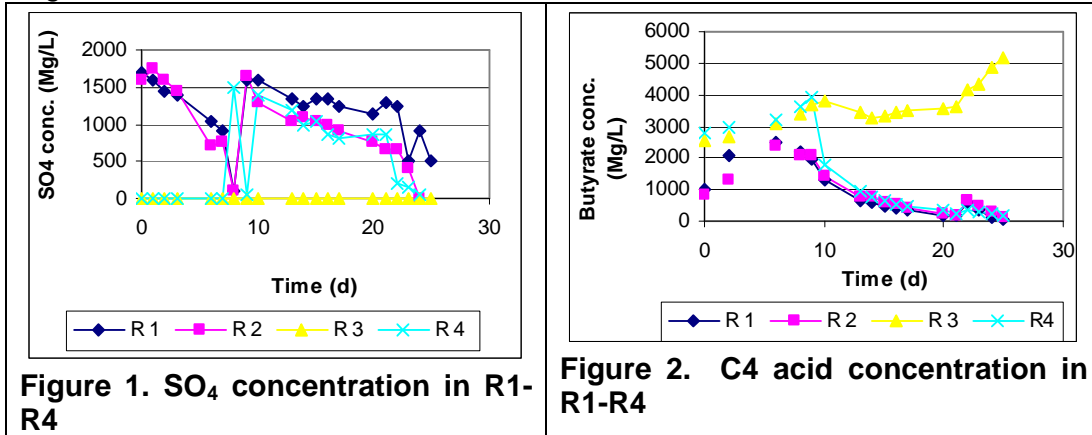
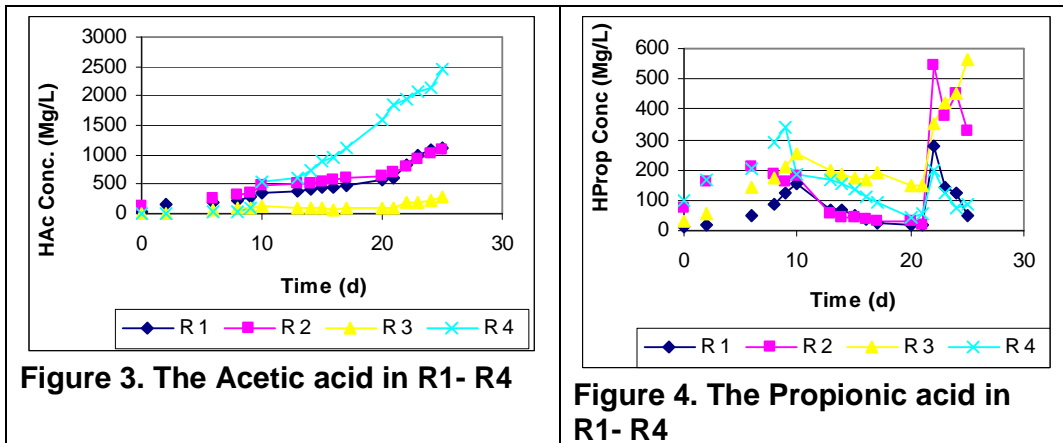**Figure 1. SO$_4$ concentration in R1-R4**

Figure 2. C4 acid concentration in R1-R4

The SO$_4$ concentrations in R1 and R2 decreased from 1700 to 0 mg/ℓ over 8 days. On day 8, fresh sulphate was added to R1 and R2. From day 8 - 25, the sulphate concentration decreased again, but at a slower rate than from day 1-8. This can be ascribed to the lower butyrate concentration in the reactors (Fig.2). Fresh grass (30 g) was added to the 4 reactors on day 21, which showed hardly any effect on the sulphate reduction and on the butyrate production. It can be observed from Figures 1 and 2 that the decrease in sulphate concentration coincided with a decrease in butyric acid. These results indicate that the available butyric acid is utilized for the biological sulphate reduction. The graphs in Figure 2 show that the butyric acid (C4) increased in the control reactors. When on day 8, sulphate (1500 mg/ℓ) was added to R4, both the sulphate concentration (Fig.1) as well as the butyric acid concentration decreased sharply (Fig 2). The SO$_4$ concentration decreased to 0 mg/ℓ within 24h, while the butyric acid decreased from 4000 to 2000 mg/ℓ. The sulphate removal is a function of the decrease in butyric acid concentration: the SRB utilized the butyric acid to reduce the available sulphate. The butyric acid concentration in all 4 reactors indicated that the compost micro organisms could degrade the cellulose in grass to VFA. SRB can utilise propionic and butyric acid for the reduction of sulphate. The acetate and propionic acid concentrations in R1-R4 reactors are presented in Figure 3 and 4.

VFA concentration in R1-R4

The acetic acid concentration is higher in the sulphate reducing reactors than in the control reactors, which can be ascribed to the fact that when 1 mole of butyric acid is used for the biological sulphate reduction, 2 moles of acetate are formed. Thus when sulphate is reduced and butyric acid is utilized, the acetic acid concentration increased. This can be seen in the graphs depicted

in Figure 3, especially when compared to the graphs representing the control reactor (R3). Initially, R4 was a control reactor, till day 8, when sulphate was added to the reactor
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Figure 3. The Acetic acid in R1- R4

Figure 4. The Propionic acid in R1- R4

The graphs in Figures 1 and 2 showed that the sulphate was removed during 12 h and that the butyric acid concentration decreased during the same period. The graph representing the acetic acid concentration in R4, showed a similar increase in the acetate concentration. The biological SO_4 removal utilizing butyrate is presented in equation 1.

$$Butyrate^- + \tfrac{1}{2}\,SO_4^{2-} \quad \rightarrow \quad 2\;Acetate^- + \tfrac{1}{2}\,HS^- + \tfrac{1}{2}\,H^+ \qquad [1]$$

The graphs in Figure 4 show that propionic acid was produced as well, al be it in much lower concentrations than the butyric acid production. It can be observed from the graphs in Figure 4 that the propionic acid concentration in R4 was the highest (340 mg/ℓ) and that this concentration decreased sharply, coinciding with the addition and removal of sulphate and with the decrease of the C4 acid. This result indicated that propionic acid was also used for the sulphate reduction, according to equation 2.

$$Propionate^- + \tfrac{3}{4}\,SO_4^{2-} \quad \rightarrow \quad Acetate^- + HCO_3^- + \tfrac{3}{4}\,HS^- + \tfrac{1}{4}\,H^+ \qquad [2]$$

The propionic acid concentration in R3 (control reactor) increased daily and was 565 mg/ℓ on day 25. Fresh grass cuttings were added on day 21, which resulted in a propionic acid increase in the four reactors. It seems that after an acclimatisation period, the VFA production shifted from butyric acid to propionic acid. However, in the control reactor, both butyric and propionic acids were produced.

When cellulose in grass is degraded by fermenting bacteria, the degradation pattern (Figure 5) indicates that all short chain VFA can potentially be produced together with methane. However, hydrogen is an important fermentation product in the presence of sulphate, since the SRB use hydrogen as electron donor for the reduction of sulphate, to such extent that the SRB will out-compete the MB for the available H_2 (9, 3). Considering substrate affinity and growth rates, SRB have a preference for hydrogen,

5

propionate, butyrate and acetate in that respective order. Growth and sulphate reduction on hydrogen, propionate and butyrate goes fairly well, while growth on acetate is in general slow (9). Hydrogen, produced by the fermentative bacteria is immediately used by SRB, and in that sense one can speak of syntrophic growth. The SRB keep the dissolved hydrogen concentration low, consequently the fermenting bacteria are not inhibited by the production of hydrogen.
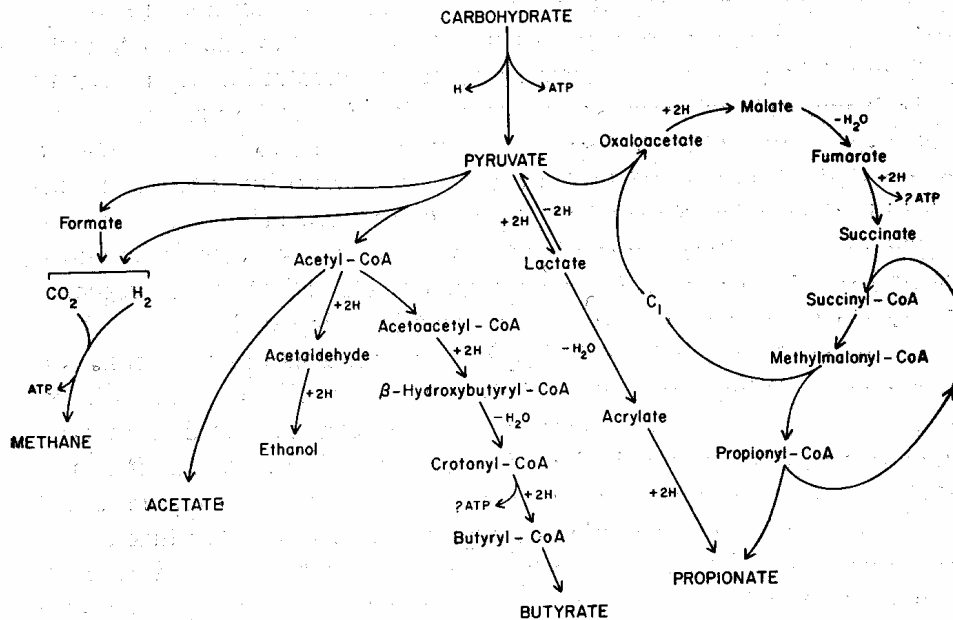
Figure 5. Degradation of cellulose by fermentation bacteria (6). The produced hydrogen will be used for SO$_4$ removal rather than for methane production in the studies reported here.

COD concentration

The COD concentration in R1-R4 is presented in Figure 6, showing that the COD concentration in the four reactors is similar. This result was not expected as it was anticipated that the COD concentration in R1 and R2 would be lower than in R3 and R4 due to COD utilization for sulphate reduction. The COD utilization in R3 and R4 can possibly be ascribed to the activity of MB, producing gas. COD utilisation can be seen in R2 after day 5, when more COD was used than produced and in R4 from day 8 to day 12, which coincided with the SO$_4$ addition and removal and with the VFA utilisation. Fresh grass cuttings were added to all reactors on day 21, which can be noted from Figure 6: a slight increase in the COD concentration in R1, R2 and R4 and a slight decrease in R3, the control reactor. This is an unexpected result, although it may indicate that not only the compost bacteria are responsible for the grass fermentation, but also the SRB (in R1, R2 and R4) in order to produce the VFA needed for biological sulphate reduction. It has been shown in the study of Greben & Baloyi (4) that SRB can ferment grass cuttings to VFA in absence of other fermentation bacteria.

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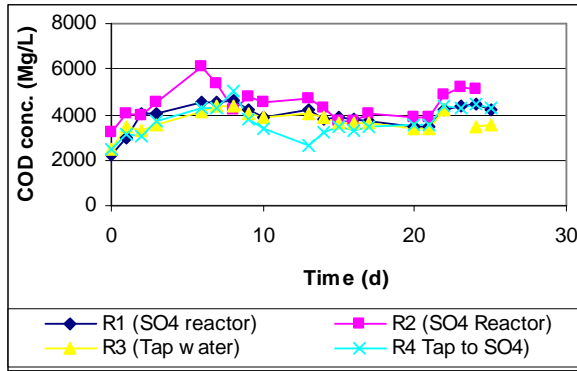
Figure 6. The COD concentration in R1-R4.

Study 2

Sulphate reduction

The sulphate removal in L1 is presented in Figure 7, which showed that the sulphate was removed from 1250 to 800 mg/ℓ from days 0 to11 and that on day 14, the sulphate was reduced to 40 mg/ℓ. On days 14 to18 (inclusive) 5.5 g Na_2SO_4 was added to L1, which was removed 16 to18 h after addition. These results indicate good sulphate removal.
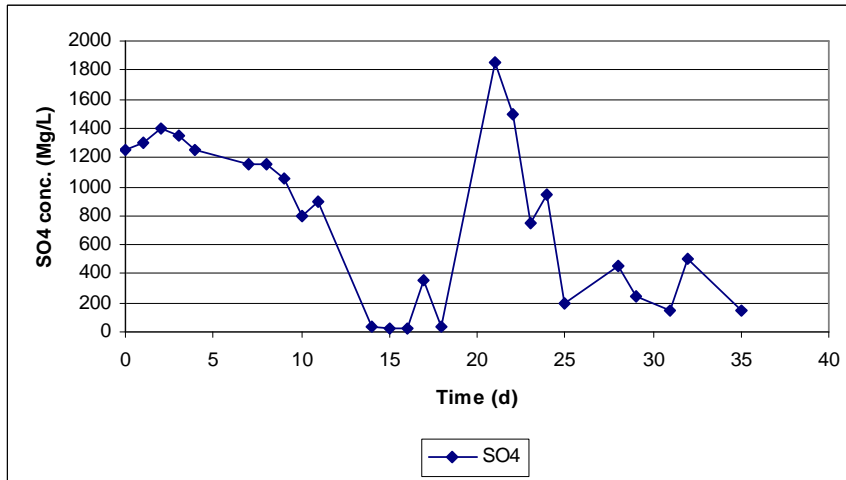

Figure 7.   The biological sulphate reduction in L1.

VFA production and utilisation

Propionate
The propionic acid concentration in L1 was lower than in L2 (Figure 8), which can be ascribed to the C3 utilization for the sulphate removal in L1 (Figure 7). Whenever the sulphate concentration was < 100 mg/ℓ, a fresh sulphate solution was added to the reactor, which was removed, resulting in propionic acid utilization in L1.
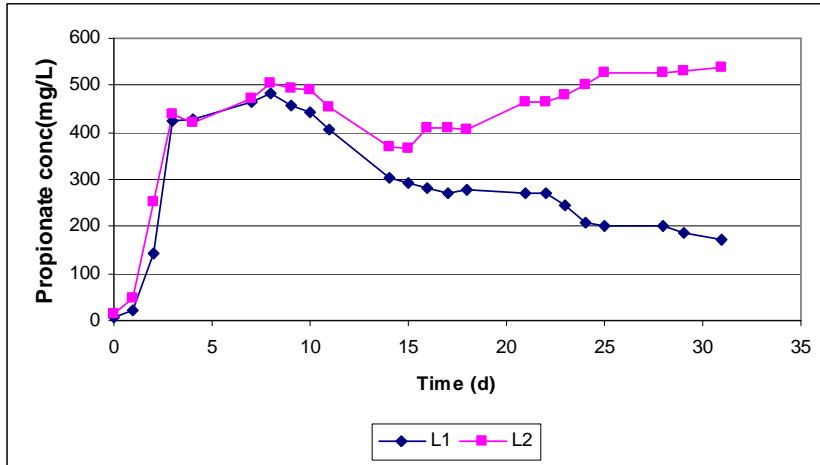
Figure 8. The propionate concentration in reactors L1 and L2

Acetate

Sulphate reduction and propionic acid utilisation in L1 resulted in the production of acetic acid (Figure 9). A higher acetate concentration can therefore be observed in reactor L1 compared to L2 (control reactor).
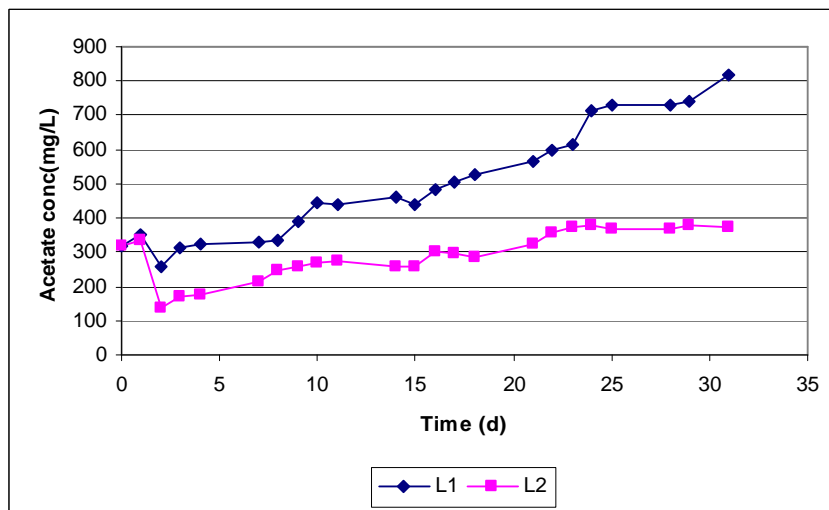
Figure 9. The acetate concentration in reactors L1 and L2

The acetate concentration increased with time to a concentration of about 800 mg/ℓ, which according to Hill (10) may result in process failure. The highest acetic acid concentration obtained in the control reactor which contained the Rumen fluid microorganisms and tap water was almost 400 mg/ℓ. When SRB utilise propionate and butyrate as energy sources to reduce sulphate to sulphide, reactions [1] and [2] can be applied, respectively. The total acetate, propionate and butyrate concentrations, measured in L1 and L2 over the experimental period, are given in Table 3.

The data in Table 3 show that the propionate and butyrate concentrations in L1 are considerably lower than in L2, which can be ascribed to the biological sulphate reduction in L1. As can be seen from reactions [1] and [2], for every

8

molecule of sulphate removed 3 molecules propionate were used, producing 3 moles acetate, while in the case of butyrate, 1 mol sulphate is reduced when 2 moles butyrate were used, producing 4 moles acetate. The VFA concentrations in L2 can be considered as the control VFA production, which shows that the C4 concentration differed 2639 mg/ℓ with that in L1, while for C3 this was: 3118 mg/ℓ, resulting in a total increase of 4720 mg/ℓ acetate (C2) due to the utilization of butyrate and propionate for the biological sulphate reduction.

Table 3.   The VFA (mg/ℓ) concentration in Reactors L1, L2 and L3

Reactor	Acetate	Propionate	Butyrate
L1	11436	6465	500
L2	6716	9583	3139

Butyrate

The butyrate concentration in L1 and L2 is given in Figure 10, which shows no butyrate concentration in the reactors during the first 14 days of operation. This lag in butyrate production might be due to an increased C3 production. The rumen organisms produce more propionic acid, while the compost bacteria generate a higher butyric acid concentration. Thereafter, the butyrate concentration in L1 was low, because after it was produced it was utilised for the sulphate reduction, while in L2 the butyrate concentration increased due to further cellulose degradation.
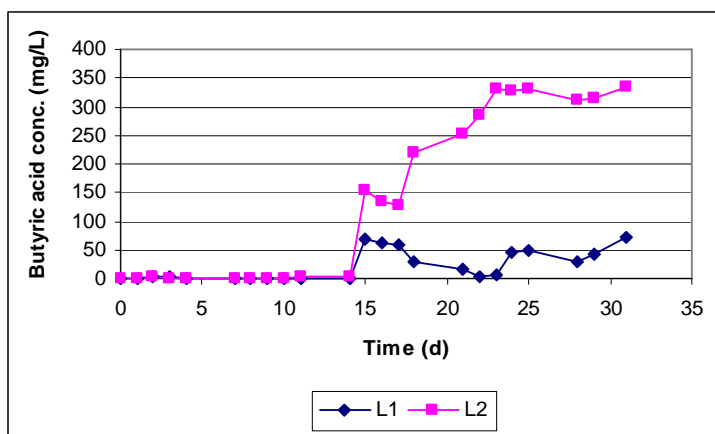
Figure 10. The butyrate concentration in reactors L1 and L2

Sulphate removed/VFA utilised

The high peaks in the graph in Figure 7 indicated the sulphate concentration after a fresh sodium sulphate solution was added to the reactor. The sulphate removal as shown in Figure 7 is due to the presence and utilisation of the C3 and C4 acids and other degradation products of cellulose. The total sulphate removal was calculated from day 0 to 21, during which period no fresh GC was added to the reactor. During that period 9 g SO_4 was removed, while 75 gram GC was added to the reactor. From this information, it can be deduced that in order to reduce 1 gram sulphate, 8 g GC was needed.

Utilisation of a potential waste product, such as grass cuttings, is beneficial in two ways, as it produces energy due to the formation of VFA, followed by SO_4 reduction or by methane production and it serves as an alternative in solid waste management. Grass waste is a major component of solid waste, comprising about 50% of the organic fraction of Municipal Solid Waste (11). The composition of fresh grass is given in Table 4. This data shows that 88% of the dry matter consist of cellulose and hemicellulose (both consisting mainly of coupled hexoses) which can be degraded by cellulose degrading bacteria, such as rumen bacteria and bacteria isolated from compost. Lignin is degradable as well, but this requires very long retention times (12).

Table 4. Composition of fresh grass (8)

Compound	Percentage w/w
Water	51.84
Celulose	14.00
Hemicelluse	28.30
Lignin	5.40
Ash	0.46

Rumen fluid bacteria can be used for the degradation of cellulose in grass, however these microorganisms require a temperature of 36-39 ˚C, while the compost bacteria can operate at ambient temperatures. Additional energy costs amount to R 0.50 in order to heat up 1 m^3 AMD from 22 to 37 ˚C to accommodate the rumen fluid bacteria in a sulphidogenic reactor treating 2000 m3/d AMD using GC as the energy source.

The composition of the produced VFA from cellulose and hemicellulose by rumen bacteria is presented in Table 5. The composition of the produced VFA changes in the presence of sulphate and SRB, as these bacteria will use the produced VFA and hydrogen in the presence of sulphate as the energy source and therefore almost no methane will be produced. Zoetemeyer (13) indicated that the reactor pH also influences the fermentative bacteria and thus the outcome of VFA formation.

Table 5. Composition of the VFA's produced by rumen bacteria when degrading cellulose (14)

VFA	Weight percentage
Acetate	60
Propionate	19
Butyrate	17
Rest	4

4   CONCLUSIONS

The results of this study showed that
- The microorganisms isolated from compost can degrade grass cuttings to high concentrations of butyric acid and small concentrations of propionic acid

- The rumen microorganisms produced more propionic than butyric acid
- The produced VFA can be utilised for the biological sulphate reduction
- Acetate was formed due to the utilisation of the C4 and C3 acids for the SO_4 removal
- When sulphate was added to the control reactor, the sulphate was reduced overnight, due to the presence of high concentrations of VFA, produced from grass cuttings
- The produced butyric and propionic acids were utilised by the SRB for biological sulphate removal.
- A clear relationship exist between the sulphate removal and the utilisation of VFA
- The compost bacteria can operate at ambient temperatures
- The rumen fluid microbes operated at 36-39 ˚C.

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