Grass cellulose as cost-effective energy source for biological sulphate removal

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Abstract

Acid mine drainage (AMD) needs to be treated before it is discharged to water courses. The biological sulphate removal technology can be applied for the removal of salinity (sulphate), acidity and metals, the main pollutants in AMD. The aim of this study was to demonstrate that sulphate removal can be achieved using the fermentation products of grass-cellulose as cost- effective carbon and energy sources. Two studies were conducted. In the first study (an experimental period of 32 d) two stirred anaerobic batch reactors with a volume of 2.5 ℓ each were operated at 37 to 39 ˚C and at a pH of 6.7 to 6.9. Both reactors contained grass cuttings, sulphate-reducing bacteria and rumen fluid. The test reactor contained sulphate-rich water and the control reactor tap water. The results from this study indicated that grass cellulose could serve as an energy source for biological sulphate removal. In the second experiment a 20 ℓ continuously fed one-stage reactor containing grass cuttings, rumen fluid and immobilised sulphate-reducing bacteria, was fed synthetic sulphate-rich feed water. The results showed that sustained sulphate removal could be achieved when operating this reactor. The butyric and propionic acids formed were mainly utilised as the electron donors for the sulphate reduction, which resulted in increased levels of acetic acid. A clear relationship existed between the rate of sulphate reduction and the COD/VFA concentration in the reactors. It was concluded that sustained sulphate removal was achieved operating the continuously fed reactor using grass-cellulose as the carbon and energy sources.

Keywords: cellulose, fermentation, grass cuttings, rumen microbes, sulphate, VFA

Introduction

Acid mine drainage (AMD) originates from mining operations. It is formed when pyrite comes into contact with oxygen and water, producing elevated sulphate, metals, (especially iron) and acidity concentrations, the main characteristics of AMD. These effluents require treatment, either by chemical or biological means or through a combination of these methods (Maree et al., 2004), before discharge to receiving water bodies. At present, two biological treatment systems are in operation in South Africa: a 3 Mℓ/d pilot scale plant using waste ethanol as the carbon and energy source at Navigation Colliery and the Biosure Plant (10 Mℓ/d) at Grootvlei Mine using sewage sludge as the carbon and energy source. The price of ethanol is related to the oil price of which the costs have escalated, while large volumes of sewage sludge are not always available in most of the mining regions. In this study the focus was on evaluating the potential of grass-cellulose as a cost-effective carbon source for the biological sulphate removing technology.

Plant biomass is a sustainable source of energy when cellulose is utilised in anaerobic fermentation to produce volatile fatty acids (VFA) (Lynd et al., 2002). This process not only involves many species of degrading bacteria (Coughlan and Mayer, 1992; Schwarz, 2001), but sulphate-reducing bacteria (SRB) can also participate in the degradation of cellulose polymers and monomers to produce VFA (Oude Elferink, 1998). Greben and Baloyi (2004) showed that the anaerobic degradation of grass cuttings (GC) to VFA was enhanced when an SRB mixture was added to the fermentation process, even when no sulphate was present. The degradation of plant cellulose is a complex process, in which various natural occurring microbial communities are known to participate, e.g. the rumen micro-organisms. The rumen is a highly effective cellulosic ecosystem with a complex microbial population of bacteria, archaea, protozoa and fungi (Hungate, 1966). The degradative process is driven by bacteria and protozoa that efficiently mediate the anaerobic degradation of plant material by producing fibre-degrading enzymes (Lee et al., 2000; Schwarz, 2001). Sonakya et al. (2003) demonstrated the use of digested cattle feed as an inoculum for the production of VFA from GC during anaerobic digestion resulting in enhanced methane production.

The aim of this study was to demonstrate that sustainable sulphate removal can be achieved using grass-cellulose as the carbon and energy source through the fermentation of cellulous material to VFA by cellulose-degrading microbes originating from rumen fluid.

Materials and methods

Two studies were conducted. During the first study two stirred batch-operated reactors were used, to investigate whether biological sulphate removal could be achieved using grass-cellulose fermentation products (VFA) as the electron donors. During the second study a continuously fed single-stage reactor was operated, with the aim to investigate whether the above-mentioned process could be maintained continuously for an extended test period.

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Study 1: Batch-operated reactors

Reactors

Two stirred anaerobic reactors: L1 and L2 (Vol.:2.5 ℓ) were operated at 37 to 39°C and at a controlled pH of 6.7 to 6.9 to create an ideal environment for the rumen fluid micro-organisms (RB). The contents of the reactor were stirred by overhead stirrers.

Grass cuttings

Kikuyu grass cuttings (GC) were obtained from the CSIR, Garden Services, Pretoria. The GC used for Studies 1 and 2 were stored at 4°C. The length of the GC was 1 to 2 cm. The weight of the grass in these studies refers to air-dried grass. The moisture content of the GC was 7.6%, while 1 g GC/ℓ corresponded with a COD concentration of ≈ 1 g/ℓ.

Inoculum

Rumen fluid was obtained from fistulated cattle (Agricultural Department, University of Pretoria) and transported to the CSIR, where the inoculum was stored at 37°C. Rumen fluid typically contains 10^{10}-10^{11} bacteria and 10^6 protozoa per mℓ of fluid (Hungate, 1966). Some members of the original microbial consortia present in the rumen population were able to adapt to the reactor-environment and were responsible for degrading grass-cellulose to VFA (and other intermediates) in the reactors.

Experimental conditions

The experimental data for the operation of reactors L1 and L2 are presented in Table 1. The duration of Study 1 was 32 d. Daily samples (25 mℓ) were taken on weekdays. The volume loss due to sampling was replaced in Reactor L1 by a SO_4 solution of 2 500 mg/ℓ, which was responsible for an additional daily SO_4 concentration of 25 mg/ℓ to the reactor. This daily addition represented 1/100 of the original sulphate concentration. Reactor L2 received tap water to replace the loss in volume.

TABLE 1
The experimental conditions for Study 1

Reactor	Contents
L1	1 500 mg/ℓ SO_4 + 30 g/ℓ GC + 250 mℓ RB + nutrients
L2	Tap water + 30 g/ℓ GC + 250 mℓ RB + nutrients

RB: Rumen micro-organisms obtained from rumen fluid; GC: Grass cuttings

Study 2: Continuously operated reactor

Feed water

Sulphate-rich synthetic water was used as feed water for the single-stage reactor system (FR) containing an SO_4 concentration of ≈ 2 500 mg/ℓ, (Na_2SO_4, Crest Chemicals, Johannesburg) as well as a macro-nutrient solution (6.5% N, 2.7% P, 13.0% K, 7.0% Ca, 2.2% Mg and 7.5 % S) and micro-nutrient solution (0.15% Fe, 0.024% Mn, 0.024% B, 0.005% Zn, 0.002% Cu and 0.001% Mo) of which 1 mℓ/ℓ feed water was used respectively.

Reactor system and biomass

A one-stage anaerobic hybrid reactor system (FR) was operated, consisting of a fermentation section and a sulphate removal section (Fig. 1). A 20 ℓ Perspex reactor was used, which was operated at 37 to 39°C. The temperature was maintained by circulating heated water (water bath) through a water jacket surrounding the reactor. The bottom part of the reactor contained ceramic rings as packing material. Anaerobic sulphate-removing biomass (250 mℓ, volatile suspended solids (VSS) concentration of 9.6 g/ℓ), obtained from the biological sulphate-removing demonstration plant (Witbank, South Africa), was added to allow for SRB biofilm formation on the ceramic rings, to prevent washout of the biomass. The top part of the reactor received 1 000 g GC (from the same stockpile as used in Study 1) at the start of the study, and was supplemented with 150 g GC on Days 13, 32, 46 and 62 resulting in 4 experimental periods of 19, 15, 15 and 14 d, respectively. Rumen fluid (250 mℓ, VSS of 10.6 g/ℓ) obtained from fistulated ruminants (University of Pretoria, South Africa) was added to the GC. The dissolved oxygen (DO) concentration in the reactor was 0 mg/ℓ, indicating anaerobic conditions in the reactor. The feed water entered FR at the top of the reactor at a feed rate of 5 ℓ/d, resulting in a hydraulic retention time (HRT) of 4 d. A recycle stream (360 ℓ/d) was installed for improved mixing within the top section of the reactor. The effluent was discharged at the bottom of the reactor.

Sampling

The monitoring of the reactor system started 14 d after initiation of the study. Daily samples were taken from FR from the effluent (sample point: Fig. 1) during the different experimental periods, except during weekends.

Analytical methods

Determinations of sulphate, COD, pH, mixed liquor suspended solids (MLSS) and VSS were carried out according to standard analytical procedures as described in *Standard Methods*, 1985. With the exception of the MLSS, VSS and sulphide, all analyses were carried out on filtered samples (Whatman #1). The COD samples were pretreated 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 (FID). The column used was a HP-FFAP, 15 m x 0.53 nm, 1 µm. The GC/FID programme can be summarised as follows: initial oven temperature 30°C, for 2 min, temperature programmed to increase thereafter from 80°C to 200°C at 25°C/min, with temperature hold for 1 min at 200°C, FID temperature 240°C. The carrier gas (N_2) flow rate was set at 1 mℓ/min.

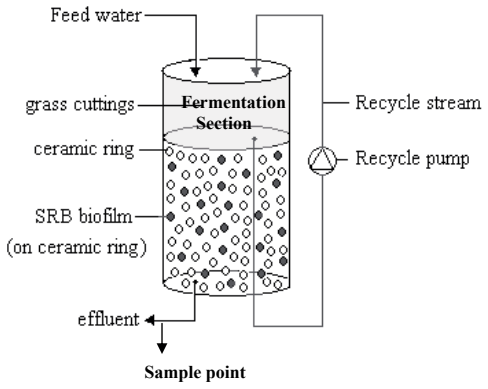

Figure 1
Schematic overview of one-stage reactor system

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Results and discussion

Study 1

Sulphate reduction

The sulphate removal profile of Reactor L1 is depicted in Fig. 2. The sulphate concentration decreased from 1 250 mg/ℓ to 800 mg/ℓ during the first 11 d, whereafter it was reduced to as low as 40 mg/ℓ within the next 3 d (Day 14). When the sulphate concentration dropped to < 100 mg/ℓ in Reactor L1, Na_2SO_4 was added to the reactor, such that the final reactor SO_4 concentration was ± 2 500 mg/ℓ (5.5 g Na_2SO_4). Sulphate was added on Days 14 to 18 (inclusive), as indicated in Fig. 2. The results showed that the increased sulphate concentration was typically removed within 16 to 18 h after each Na_2SO_4 addition. This rapid sulphate removal was ascribed to the available VFA and other intermediates of cellulose degradation, such as hydrogen, present in the reactor. After the last addition of sulphate, the reduction process was much slower as can be seen from Fig. 2, which was likely due to the lower levels of readily available energy sources, as no new GC were added to Reactor L1.

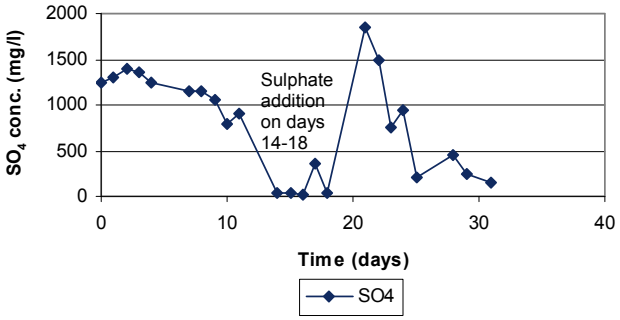

Figure 2
Biological sulphate reduction in Reactor L1

Propionic acid concentration utilisation

Initially the propionic acid concentrations in Reactors L1 and L2 (Fig. 3) were similar, but when the rate of sulphate removal increased in Reactor L1 (after day 14), the propionic acid concentration decreased to values of 200 mg/ℓ, while the C3 acid concentration in Reactor L2 continued to increase to concentrations > 500 mg/ℓ. Thus the rapid sulphate reduction in Reactor L1 (Fig. 2) resulted in a decrease in the propionic acid concentration. This result showed the relationship between the sulphate reduction and propionic acid utilisation.

Acetic acid concentration

Cellulose degradation results in the production of VFA, e.g. acetic, propionic and butyric acids. Sulphate reduction and the accompanied propionic acid utilisation in L1 resulted in the production of additional acetic acid as can be seen by the higher C2 acid concentration in Reactor L1 compared to Reactor L2 (Fig. 4). The final acetic acid concentration in Reactor L1 was ca. 800 mg/ℓ, while it was almost 400 mg/ℓ in the control reactor. When SRB utilise propionic and butyric acids as energy sources to reduce sulphate to sulphide, acetate is produced (Eqs. (1) and (2))

$$\text{Propionate}^- + \tfrac{3}{4}\,SO_4^{2-} \rightarrow \text{Acetate}^- + HCO_3^- + \tfrac{3}{4}\,HS^- + \tfrac{1}{4}\,H^+ \quad (1)$$

$$\text{Butyrate}^- + \tfrac{1}{2}\,SO_4^{2-} \rightarrow 2\ \text{Acetate}^- + \tfrac{1}{2}\,HS^- + \tfrac{1}{2}\,H^+ \quad (2)$$

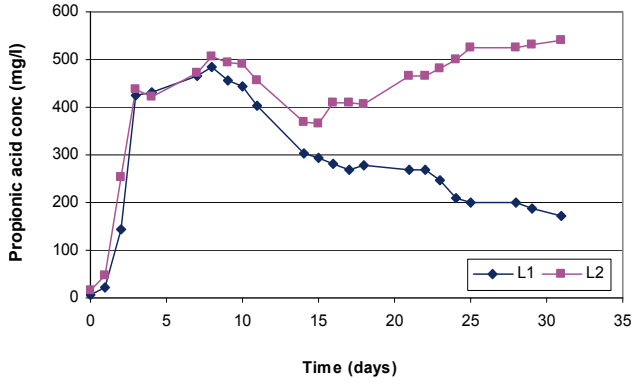

Figure 3
The propionic acid concentration in Reactors L1 and L2

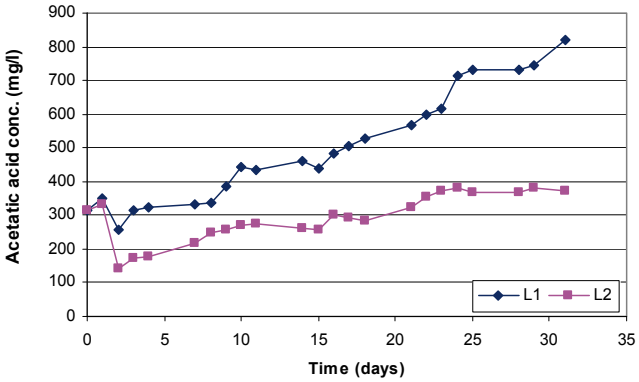

Figure 4
The acetic acid concentration in Reactors L1 and L2

Generally, when grass-cellulose is degraded by fermenting bacteria, short-chain VFA as well as methane are produced. Hydrogen produced in the presence of sulphate and SRB will typically be used as the preferred energy source by the SRB to such an extent that the SRB will out-compete the methanogenic bacteria (MB) for the available H_2 (Visser, 1995; Oude Elferink, 1998). Considering substrate affinity and growth rates, SRB have a preference for hydrogen, propionate, butyrate and acetate in that order. Growth and sulphate reduction on hydrogen, propionate and butyrate proceeds fairly well, while growth on acetate is in general slow for the SRB (Visser, 1995). When sufficient hydrogen, propionic and butyric acids are available for the SRB, acetic acid will not be utilised for the biological sulphate reduction, which can explain the steady increase in acetic acid concentration in Reactor L1.

Sulphate removed/VFA utilised

The sulphate removal, as shown in Fig. 2, was due to the production and utilisation of VFA and other degradation products of grass-cellulose. The total sulphate removal over the period from day 0 to 21 was 9 g SO_4, during which period 75 g GC was added to the reactor. This relates to the reduction of 0.13 g SO_4 for 1 g GC.

The results of Study 1 indicated that sulphate removal was achieved using rumen fluid bacteria for the degradation of grass-cellulose to short chain VFA and other intermediates. Thus it has been shown that GC, a potential waste product, can be used beneficially as the energy source for biological sulphate reduction, resulting in bio-waste utilisation rather than disposal in landfills (Yu et al., 2002).

Study 2

Sulphate removal when feeding synthetic feed water

The relationship between the available COD concentration and the sulphate reduction in FR is shown in Fig. 5. The feed SO_4 concentration showed an erratic pattern, which was due to the poorly dissolved sulphate solution. During the periods that the reactor COD concentration was < 1 000 mg/ℓ, the sulphate reduction was less efficient (≈ day 50); however when the COD concentration was > 1 000 mg/ℓ, the SO_4 concentration in the treated water was < 500 mg/ℓ. Fresh grass (150 g) was added to the reactor on Days 13, 32, 46 and 62, which resulted in 4 experimental periods (1 to 4, Fig. 5). It can be observed from Fig. 5 that after each grass addition (arrows), the COD concentration sharply increased, followed by a steady decrease during the periods of sulphate reduction. The COD concentration during Period 1 deviated from this pattern and was irregular. This observation can be ascribed to the addition of 1 000 g GC on Day 1 and possibly due to the poor mixing of the reactor content. The sulphate removal data obtained for FR, during the 4 experimental periods, are presented in Table 3. During each experimental period, 150 g grass was added to the reactor. The results indicated that the sulphate removal in FR was very stable and that the sulphate removed (based on average data) during Periods 2, 3 and 4 was similar at 176, 175 and 172 g over a period of 14 and 15 d, respectively.

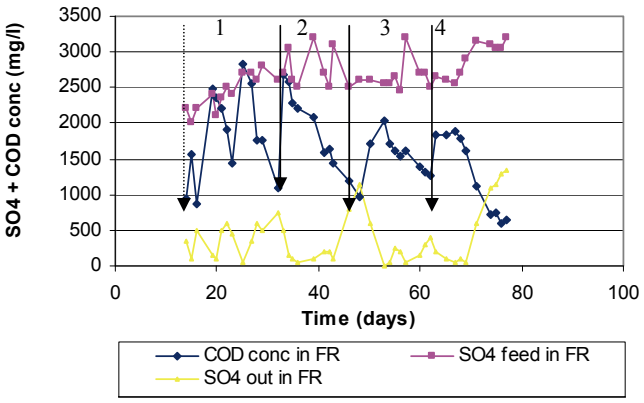

Figure 5
Sulphate removal and COD concentration in FR over 78 d

The chemical composition of the feed and treated water in FR (Table 2) showed that sulphate removal was followed by sulphide production. The $S^2_{produced}/SO_{4removed}$ ratios are 0.19, 0.21, 0.19 and 0.20 during Periods 1 to 4 in FR. Although these ratios are lower than the theoretical value of 0.33, it can be noted that the ratio throughout the 4 experimental periods was similar. The lower experimental ratios can be explained by sulphide to sulphur oxidation, by SO_4^{2-} to SO_3^{2-} reduction (SO_3^{2-} is not analysed in the daily routine) as well as by the fact that part of the sulphide produced escaped in the gaseous form, due to the lower reactor pH. Weast (1981) described that the pK_a value of the dissociation equilibrium of H_2S is about 7.04 at 18 °C. Above pH 8.0 to 9.0 virtually all dissolved sulphide is present in its ionised form, while at neutral pH values 20 to 50% of the dissolved sulphide is present as H_2S, depending on the reactor temperature (O'Flaherty & Colleran, 2000). The increase in reactor pH after sulphate reduction (due to alkalinity production) is thus beneficial for lower reactor sulphide toxicity. It is therefore advised to maintain the pH of the sulphidogenic reactor at between 7.5 and 8.5. In this study, however, the higher reactor pH may be harmful to the rumen fluid bacteria, which co-exist in the same one stage reactor and which require a pH between 6.6 to 6.9 (Hungate, 1966).

The higher sulphate removal of 194 g in the 1st period can be ascribed to a longer period of 19 d and to the supplementation of 1 000 g GC added on Day 1 (Table 3). The percentage SO_4 removal efficiency in FR during the 4 periods was 84, 91, 88 and 80%, respectively. The results in FR compared well with the 78% sulphate removal efficiency using manufactured propionic acid as the carbon and energy sources for the biological sulphate removal in a previous study (Greben et al., 2004).

TABLE 2
Chemical composition of the feed and treated water during the 4 periods in FR

Parameter	Feed-water FR	Treated water FR
Period 1		
COD (mg/ℓ)		1724
pH (value)	7.15	7.23
SO_4 (mg/ℓ)	2367	383
S^{2-} (mg/ℓ)		386
S^{2-}/SO_4 ratio		0.19
Redox (mV)		-173
Period 2		
COD (mg/ℓ)		1965
pH (value)	7.20	7.26
SO_4 (mg/ℓ)	2761	244
S^{2-} (mg/ℓ)		522
S^{2-}/SO_4 ratio		0.21
Redox (mV)		-174
Period 3		
COD (mg/ℓ)		1519
pH (value)	7.30	7.45
SO_4 (mg/ℓ)	2650	315
S^{2-} (mg/ℓ)		446
S^{2-}/SO_4 ratio		0.19
Redox (mV)		-171
Period 4		
COD (mg/ℓ)		1276
pH (value)	7.33	7.46
SO_4 (mg/ℓ)	2895	600
S^{2-} (mg/ℓ)		467
S^{2-}/SO_4 ratio		0.20
Redox (mV)		-154

TABLE 3
The sulphate removing data in the reactor system

Sulphate removal	Period			
	1	2	3	4
Av SO4 removal g/ℓ	2.04	2.52	2.33	2.29
Av SO4 removal g/d	10.21	12.58	11.67	11.49
Av g SO_4 removed during period 1	194	176	175	172
% SO_4 removal	84	91	88	80
Total SO_4 (g) removed over each period	435	245	223	190

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The results in Table 3 showed that during each period 435, 245, 223 and 190 g SO_4 was removed, respectively, during the 4 periods, when 150 g GC was added to FR. It was calculated from the total sulphate removal that from 1 g grass 1.6, 1.5 and 1.3 g SO_4 was removed for the 2nd, 3rd and 4th period, respectively, showing reproducible values during the latter 3 (comparable) periods. These results compared favourably with those obtained from Study 1, where it was calculated that 1 g GC removed 0.13 g sulphate.

VFA utilisation when feeding synthetic feed water

The data in Table 4 (based on average concentrations) indicated that most C3 and C4 acids were utilised for the biological sulphate reduction in FR, producing acetate according to Eqs. (1) and (2). The acetate concentration varied from 649 mg/ℓ to 449 mg/ℓ to 88 mg/ℓ and to 27 mg/ℓ from Periods 1 to 4, respectively. These results seem to indicate that less butyric and propionic acids were utilised, therefore less acetate was produced or alternatively that due to the lower concentration of butyric and propionic acids, the acetic acid was utilised for sulphate reduction in FR. Omil et al. (1997) observed that when no suitable energy source is available, SRB can utilise acetate for the reduction of sulphate. The lower VFA concentrations in FR (Table 4) during the consecutive periods agreed with the lower residual COD concentration during the 4 periods (Table 2).

TABLE 4
VFA profile in FR during the 4 experimental periods

VFA	Period			
	1	2	3	4
Acetate	649	449	88	27
Propionate	16	3	0	2
Butyrate	3	1	0	0

COD concentration

The graphs in Figure 5 and the data in Table 2 showed that the reactor COD concentration varied from 1 724 to 1965 to 1 519 and to 1 276 mg/ℓ, during periods 1 to 4, respectively. The highest reactor COD concentration corresponded with the highest sulphate removal. The residual COD concentration most likely comprised un-degradable COD (e.g. lignin), since most VFA (Table 4) were utilised, except for small concentrations of acetate. The final COD concentration in the effluent can be removed operating an aerobic system, in sequence to the anaerobic hybrid reactor.

Conclusions

The results of both Studies 1 and 2 showed that sulphate removal could be achieved when using the fermentation products of grass cellulose. It was furthermore evident from the results that a clear relationship existed between the sulphate removal, the COD concentration and the utilisation of VFA. When the VFA concentrations in FR decreased, acetate, as a product from the degradation of grass-cellulose and as a product from the utilisation of butyric and propionic acids, was seemingly used for sulphate reduction. Operating the batch test reactor indicated that the fermentation of 1 g GC removed 0.13 g SO4, while when operating the continuously fed reactor the degradation of 1 g GC resulted in the reduction of an average of 1.5 g of sulphate. The results obtained from the presented studies show promise for sustained sulphate removal using grass-cellulose as the carbon and energy sources.

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