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Hydrolysis of newspaper polysaccharides under sulfate reducing and methane producing conditions

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Abstract

The initial decomposition rates of cellulose and hemicellulose were measured using toluene to specifically inhibit the microbial uptake of hydrolysis products during the degradation of newspaper under sulfate reducing and methane producing conditions. The amount of glucose and xylose accumulation in the first 2 weeks of incubation period was higher in the sulfate reducing condition compared to the methane producing condition. It was estimated that 28 and 6% of initially loaded cellulose in the sulfate reducing condition and the methane producing condition was hydrolyzed, respectively. Accordingly, the newspaper-cellulose hydrolysis rate constant was estimated to be 6.7 times higher in sulfate reducing condition than in methane producing condition. Based on the glucose accumulation patterns, when sulfate reducing bacteria (SRB) were inhibited by anthraquinone and molybdate (Na_2MoO_4), it may be suggested that SRB might have contributed to the hydrolysis of cellulose, while their effect on the hydrolysis of hemicellulose could not be elucidated.

Introduction

Almost half of the biomass synthesized by photosynthetic fixation of carbon dioxide is made up of cellulose and 15–36% of the synthesized biomass is lignin (Higuchi 1985). Biomass like cellulose, hemicellulose and lignin represent a major part of the organic materials in aquatic environments (DeBlois & Wiegel 1990; Ljungdahl & Eriksson 1985), where anaerobic conditions are often found to prevail. Under anaerobic conditions, the first, and rate-limiting step in the degradation of cellulose and hemicellulose is catalyzed by extracellular enzymes (Benner et al. 1984; Billen 1982; King 1986) during which these natural polymers are decomposed to smaller intermediates to facilitate transport through the cell membrane. What makes cellulose so resistant to bacterial hydrolysis can be traced to the crystalline structure of cellulose co-existing together with two other biomass constituents (i.e., hemicellulose and lignin). Cellulose and hemicellulose have been reported to be anaerobically biodegraded in their pure forms (Magee & Kosaric 1985). The presence of lignin in the lignocellulosic complex renders cellulose and hemicellulose less biodegradable or even completely refractory. It has been reported that lignin in lignocellulosic materials forms a three-dimensional complex structure with cellulose and hemicellulose and thus renders cellulose and hemicellulose less accessible to enzymatic attack (Bisaria & Ghosh 1981). Of the steric hindrance factors, the presence of lignin is therefore thought to be the most important factor affecting the degradation rate of cellulose.

In anaerobic environments, in addition to fermentative bacteria and methanogens, sulfate reducers have been reported to co-exist. Sulfate reducing bacteria (SRB) and methanogens compete for the utilization of volatile fatty acids, the key intermediate in the anaerobic degradation of organic material. Thermodynamically and kinetically, SRB are more favored than

methanogens, which has been confirmed experimentally in natural environments such as sediments, as well as in completely mixed anaerobic reactors (Lovely et al. 1982). SRB are of great ecological importance in the degradation of organic material in anaerobic environments. For example, in marine sediments up to 50% of the organic material may be oxidized by sulfate reduction (Jørgensen 1982). In some low-sulfate environments such as freshwater lakes, bacterial sulfate reduction may still be important in the degradation process (Bak & Pfennig 1991; Hordjik et al. 1985; Ingvorsen & Brock 1982; Ingvorsen et al. 1984).

Although large amounts of data exist on the processes and factors that govern the decomposition of bulk organic matter in anaerobic environments, information on the decomposition and hydrolysis in particular of specific natural polymers such as cellulose and hemicellulose is still lacking (Henrichs 1992; Webster & Benfield 1986). In addition, information on the hydrolysis step of cellulose and hemicellulose in sulfate reducing conditions is not available.

In a previous study, we investigated the degradation of four lignocellulosic substrates with varying lignin content and found that the degradation of the lignocellulosic substrate was faster under sulfate reducing condition than in methane producing condition (Pareek et al. 1998). To further investigate the degradation process under the two different anaerobic environments, hydrolysis of cellulose and hemicellulose (collectively referred to as polysaccharides) was examined. In the study presented here, an approach to measure the initial decomposition rate (hydrolysis rate) of cellulose and hemicellulose in lignocellulosic complex was used to compare the initial decomposition rates of these natural polymers under sulfate reducing and methane producing conditions. The method used for measuring the initial decomposition rates is based on selective inhibition of microbial carbohydrate uptake by toluene, without affecting the extracellular hydrolysis of cellulose and hemicellulose. The accumulation of hydrolysis products (i.e., reducing sugars) was followed over time, after the addition of toluene. In addition, the role of SRB in the hydrolysis of cellulose is also presented.

Materials and Methods

Source of inoculum

The microbial cultures used in this study were obtained from two simulated landfill column reactors, namely R1 and R3. R1 was operated as a methane producing reactor and R3 as a sulfate reducing reactor. Both reactors were initially loaded with newspaper and sawdust as the model solid waste. The simulated landfill column reactors were operated continuously for a period of more than 3 years. The volatile suspended solids from both of the landfill reactors were found to be low and similar, ranging between 40–50 mg/L. The objective of the experiments with the simulated landfill column reactor was to control the methane emission described in greater detail elsewhere (Pareek et al. 1998).

Lignocellulose sample and incubation

The model lignocellulose substrate used in this study was newspaper and the chemical composition of the lignocellulose was determined according to the methods describe by Hyodo et al. (1999). Accordingly, it was found that newspaper samples contained 50% (wt/wt) glucose, 7% xylose, 7% mannose, 23% lignin and minor amounts of arabinose and galactose. The newspaper used was unprinted to avoid ink, which is known to form a physical barrier restricting the hydrolyzable site to enzymal attack (Cummings & Stewart 1994).

Serum bottle reactor test outlined by Gupta et al. (1996), and biochemical methane potential test outlined by Owen et al. (1979), were used with a few modifications to study the initial degradation of newspaper under sulfate reducing and methane producing conditions. The vials seeding and incubation procedures have been previously described (Pareek et al. 1998) and are briefly described here. A precisely weighed amount of dry sample (newspaper, 1–1.5 g) was added to 100 mL serum vials along with 80 mL of nutrient and bacterial seed solution mixture. The mixture contained 90% by volume bacterial culture obtained as the leachate from either sulfate reducing or methane producing simulated landfill column reactors and 10% nutrient medium having a 10-fold concentration. All media preparation and organism transfer were conducted under oxygen-free nitrogen gas. The nutrient medium used for the sulfate reducing vial set was modified Postgate medium C with the following composition (g/L): KH_2PO_4, 0.5; NH_4Cl, 10; $CaCl_2$,

0.06; $MgSO_4 \cdot 7H_2O$, 0.06; $FeSO_4 \cdot 7H_2O$, 0.007 and trace elements B, Co, Cu, Mn and Zn 0.05 mg/L (each) (Reis et al. 1992), while the composition of the nutrient medium used for the methane producing vial set was (g/L): KH_2PO_4, 0.45; K_2HPO_4, 0.85; NH_4HCO_3, 2.0; $CaCl_2$, 0.016; $MgSO_4 \cdot 7H_2O$, 0.15; $FeCl_3 \cdot 6H_2O$, 0.003 and trace elements (mg/L) $CoCl_2$, 0.15; $ZnCl_2$, 0.03; $NiCl_2 \cdot 6H_2O$, 0.03; H_3BO_3, 0.03; Na_2MoO_4, 0.03. Newspaper cut into 1×5 cm strips was added to each vial along with a mixture of bacterial inoculum and nutrient solution. Control vials containing newspaper and sterile distilled water were used to measure any non-microbial degradation of lignocellulosic material. The initial sulfate concentration in the sulfate reducing microcosm was adjusted to 1 g/L (approximately), the sulfate concentration was monitored over the 6-week incubation period and sulfate as Na_2SO_4 was added as required to maintain a sulfate concentration of 500 mg/L (approximately). All the microcosms were sealed with butyl rubber stoppers and aluminum crimps and incubated anaerobically at 37 ± 1 °C in a reciprocating shaker.

Measurement of initial decomposition rates

Four sequential steps were carried out in measuring the initial degradation of cellulose and hemicellulose. In the first step, a precisely weighed amount of substrate (1–1.5 g) was introduced into 100-mL vials and 80 mL of a mixture of leachate and nutrient solution was introduced into these vials. In total, six groups of microcosms, each consisting of three microcosms, were prepared for the two anaerobic conditions. In the second step, the microcosms were sealed and incubated in a water bath. In the third step, a group of microcosms from each set (i.e., three microcosms) was removed, at a regular interval of 7–10 days, and toluene 3% (v/v) was injected into the microcosms. Toluene is known to selectively inhibit the microbial uptake of hydrolysis products without affecting the extracellular enzymes (Boschker et al. 1995). In step four, the microcosms were further incubated for 6 hours after the injection of toluene. During the incubation period after the addition of toluene, an accumulation of the hydrolysis products was expected to occur for the reason mentioned above. Finally, one of the microcosm was opened after 0, 3 and 6 hour intervals following the injection of toluene, and the accumulation of sugars was measured.

Role of SRB in the hydrolysis of polysaccharides

To understand and evaluate the role of SRB in the hydrolysis of polysaccharides, batch experiments consisting of four microcosms were carried out. The first microcosm consisted of normal sulfate reducing microcosm as described above. This vial set was referred to as the control microcosm and the initial sulfate concentration was adjusted to 1 g/L. The second and third microcosms were prepared in a similar manner to the control vial set but were inhibited by anthraquinone (10 mg/L) and molybdate (50 mg/L, Na_2MoO_4), respectively. The initial sulfate concentration in the second and third microcosms was also adjusted to 1g/L. The last microcosm was similar to the control microcosm except that no sulfate was added. All four microcosms were sealed after the addition of lignocellulosic substrate (newspaper, 1.5 g) and incubated for 7 days. Toluene 3% (v/v) was injected into all the microcosms at the end of incubation period and the accumulation of sugars was followed over a 6-hour incubation period.

Sugar analysis

For sugar analysis, samples were transferred from vials to disposable centrifuge tubes and then centrifuged at 10,000 rpm for 10 minutes. The supernatants were filtered (0.45 μm, Cellulose nitrate membrane filter, Advantec, Japan) under pressure. Internal standard 2-deoxy-D-glucose (5 μL, 10–100 μmol) was added to the filtered samples. The internal standard was added to the samples to calculate the concentration of the reducing sugars using the internal standard calibration method. The sugar analysis was carried out on a Dionex DX-500 chromatography system and the detector was an electrochemical detector (Dionex ED-40) in the pulsed amperometric mode. The column was from Dionex (CarboPac PA1 anion exchange column) with 1.0 mM NaOH as eluent at a flow rate of 1.0 mL/min. The column was preconditioned for 10 minutes after each injection by elution with 0.7 M NaOH (1.0 min), 0.2 M KOH (0.1 min), 0.1 M Na_2CO_3 (0.5 min) and 1.0 mM NaOH (8.4 min). The samples were manually injected into the chromatography which had a loop size of 20 μL. The reducing sugars were identified based on the retention time of the corresponding purchased standards as arabinose, galactose, glucose, xylose and mannose.

Sulfate analysis

Sulfate was analyzed using an ion chromatography Dionex QIC Analyzer (Column: Dionex Ionpac, AS4A 4 mm; Eluent: 0.025 N H_2SO_4; 1.8 mM Na_2CO_3; 1.8 mM $NaHCO_3$; Flow rate: 1 mL/min; Detector: Electro-cunductivity detector).

Results and discussion

Cellulose and hemicellulose hydrolysis product accumulation

The accumulation of sugars and the hydrolysis products of newspaper polysaccharides was followed over time after the injection of toluene during the degradation of lignocellulosic substrate (newspaper) in sulfate reducing and methane producing microcosms. The production of methane and carbon dioxide in methane producing microcosm, in addition to gas production and sulfate reduction in the sulfate reducing microcosms, was analyzed throughout the incubation period and duplicated as previously described by the authors (Pareek et al. 1998).

The increase in the amount of glucose and xylose accumulation in sulfate reducing and methane producing microcosms, incubated for 7 days before the addition of toluene, is presented in Figure 1(a) and (b), respectively. The unit used to express the increase in the amount of reducing sugar accumulation was micro-mole of reducing sugar accumulated per gram (dry weight) of newspaper introduced in the microcosm. With respect to the sampling time chosen (0, 3 and 6 hours), no lag phase was observed in the accumulation of glucose and xylose (hydrolysis product of cellulose and xylose, respectively) after the addition of toluene in sulfate reducing microcosm, indicating that toluene 3% (v/v) was very effective in inhibiting the uptake of hydrolysis products of polysaccharides by sulfate reducing microcosm. In other words, the extracellular hydrolysis process continued to function while the microorganisms were incapable of consuming the hydrolysis products. Toluene probably disturbs the proper functioning of the cytoplasmic membrane by increasing its permeability (Felix 1982). The uptake of hydrolysis product depends on a concentration gradient over the semipermeable cytoplasmic membrane, which probably collapses in the presence of toluene. After the addition of toluene the increase in the amount of glucose and xylose accumulation in the sulfate reducing microcosm was almost linear with time, which is evident from the high coefficient of linear regression coefficient (R^2 = 0.93–0.99). A slight deviation from linearity for glucose and xylose accumulation in methane producing microcosms was observed (R^2 = 0.86–0.98). This deviation from linearity for glucose and xylose accumulation in the methane producing microcosms may be due to a lag phase in the early period of incubation with toluene.

The amount of glucose accumulation after 6 hours of incubation with toluene was 10.57 μmol glucose/g (dry weight) in sulfate reducing microcosm, while the amount of glucose accumulation in methane producing microcosm was 0.19 μmol glucose/g (dry weight). The amount of glucose accumulation on day 7 in sulfate reducing microcosm was about 55 times greater than in methane producing microcosm after 6 hours of incubation with toluene. The amount of xylose accumulation after 6 hours of incubation with toluene was 3.07 μmol xylose/g (dry weight) in sulfate reducing microcosm, while it was 0.23 μmol xylose/g (dry weight) in methane producing microcosm. The xylose accumulation on day 7 after 6 hours of incubation with toluene was one order of magnitude greater in sulfate reducing microcosm compared to methane producing microcosm. On day 7, the amount of glucose accumulation in sulfate reducing microcosm was 3.5 times greater than the amount of xylose accumulation, whereas the xylose accumulation was greater than the glucose accumulation in the methane producing microcosm.

After 2 weeks of incubation, the accumulation of glucose and xylose after 6 hours of incubation with toluene is presented in Figure 1(c) and (d), respectively. The increase in the amount of glucose accumulation patterns were found to be similar to the glucose accumulation on day 7 in both sulfate reducing and methane producing microcosms, respectively, with almost equal amounts of glucose accumulation in sulfate reducing and methane producing microcosms. On the other hand, the amount of xylose accumulation in the methane producing microcosm was found to be greater than in sulfate reducing microcosm. As can be observed from Figure 1(c) and (d), the amount of glucose accumulation on day 17 was 5 times greater than xylose accumulation in the sulfate reducing microcosm, while on day 14 the amount of glucose accumulation was found to be greater in the methane producing microcosm than xylose accumulation, after 6 hours of incubation with toluene.

The amount of glucose accumulation decreased dramatically in the sulfate reducing microcosm after 2

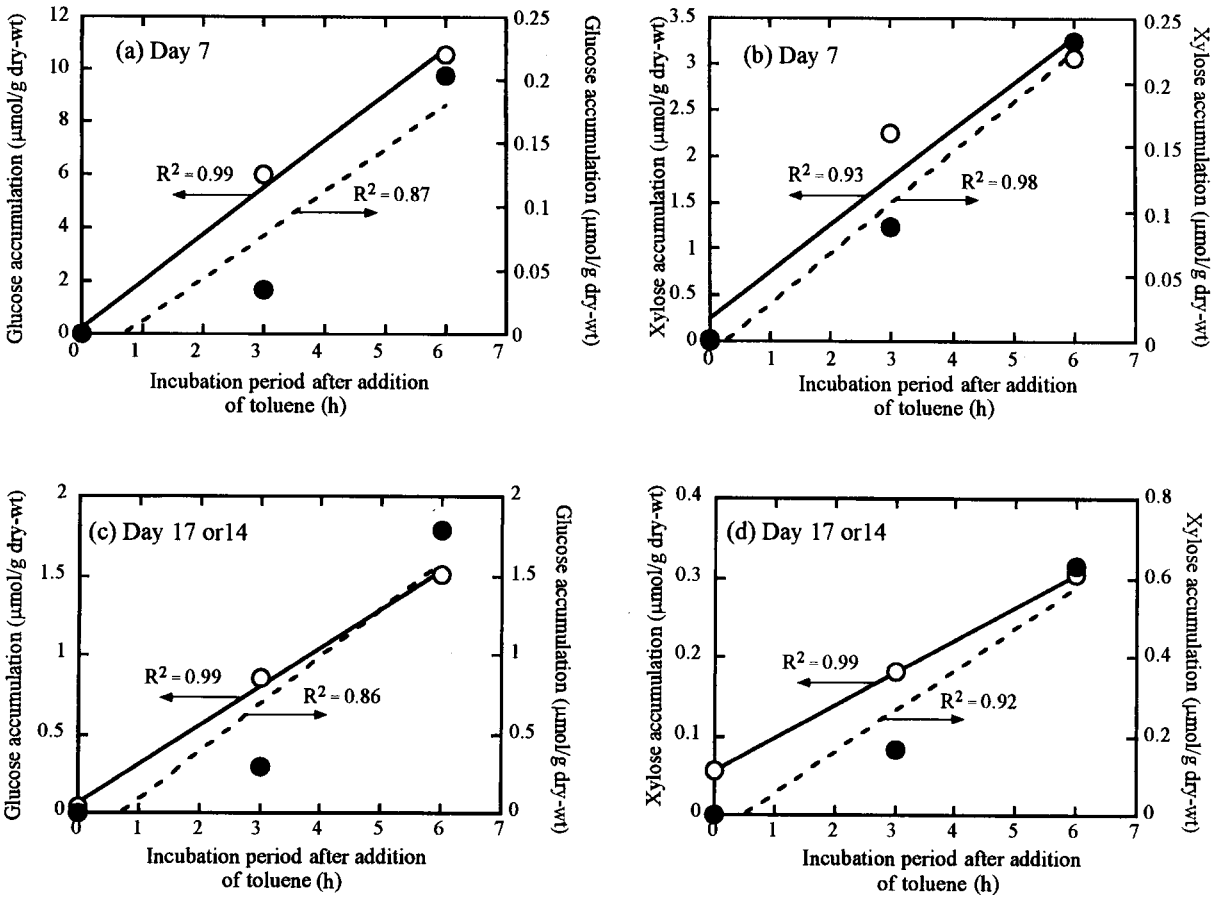

Figure 1. Accumulation of glucose (a) and (c) and xylose (b) and (d) on days 7 and 17 or 14, respectively in (○) sulfate reducing and (●) methane producing microcosms.

weeks (day 17) of incubation period, while an increase in the amount of glucose accumulation was observed in the methane producing microcosm. The higher amount of glucose accumulation in the first 2 weeks of incubation is indicative of the fact that cellulose hydrolysis was faster in the sulfate reducing microcosm during this period. The decrease in the amount of glucose accumulation, i.e., hydrolysis of cellulose in the sulfate reducing microcosm, may be due to the decrease in the active hydrolyzable site of cellulose. In the first 2 weeks of incubation, readily hydrolyzable cellulose may have been hydrolyzed leading to a high glucose accumulation rates in the sulfate reducing microcosm. After 2 weeks of incubation, the only cellulose available was presumably embedded in lignin. Thus, the reduction in the active cellulose surface on which cellulose hydrolytic enzymes could attack, could be responsible for the decrease in the amount of glucose accumulation after 2 weeks of incubation in the sulfate reducing microcosm. The lower amounts of glucose and xylose accumulation on day 7, and the relative increase in the amounts of glucose and xylose on day 14 in the methane producing microcosm, indicated that on day 7 hydrolysis of polysaccharides was slower in the methane producing microcosm compared to the sulfate reducing microcosm.

In addition to glucose and xylose, arabinose, galactose and mannose (hydrolysis product of hemicellulose), accumulation was also detected on day 7 and 14 in both sulfate reducing and methane producing microcosms (data not shown). The amount of arabinose and galactose accumulation was relatively lower than the amount of glucose and xylose accumulation mainly due to the lower quantities of these reducing sugars in the lignocellulosic complex used as the substrate. However, the relatively lower amount of mannose accumulation compared to xylose (al-

though their initial amounts were similar) cannot be explained.

Change in the hydrolysis product accumulation rate

The hydrolysis product accumulation rates, expressed as micro-moles of reducing sugar accumulated per gram (dry-weight) of newspaper per hour, were calculated on day 7, 10, 17, 25, 35 and 43 for sulfate reducing, and on day 7, 14, 25, 34 and 44 for methane producing microcosms, respectively (see Figure 2(a) and (b)). In the sulfate reducing microcosm, the highest glucose and xylose accumulation rates were observed on day 10 and these were one order of magnitude greater compared to those in the methane producing microcosm. On day 7, the glucose and xylose accumulation rates in sulfate reducing microcosm were 1.76 μmol glucose/g dry-wt/h and 0.51 μmol xylose/g dry-wt/h, respectively. On day 10, the glucose and xylose accumulation rates increased and reached the highest observed accumulation rates of 5.11 μmol glucose/g dry-wt/h and 1.21 μmol xylose/g dry-wt/h before decreasing to 0.25 μmol glucose/g dry-wt/h and 0.05 μmol xylose/g dry-wt/h on day 17. After day 17, the glucose and xylose accumulation rates in the sulfate reducing microcosm decreased gradually or remained constant until the end of the experimental period. The glucose accumulation rates in the sulfate reducing microcosm were always observed to be greater (3.5–5 times) than the xylose accumulation rates.

The glucose accumulation rate in the methane producing microcosm was 0.03 μmol glucose/g dry-wt/h on day 7, which increased to 0.29 μmol glucose/g dry-wt/h on day 14. It further increased to 0.35 μmol glucose/g dry-wt/h on day 23 and this was the highest glucose accumulation rate observed in the methane producing microcosm during the experimental period. After day 23, the glucose accumulation rate decreased to 0.01 μmol glucose/g dry-wt/h on day 34 and remained almost constant until day 44. The xylose accumulation rate increased from 0.03 μmol xylose/g dry-wt/h on day 7 to 0.11 μmol xylose/g dry-wt/h on day 14 after which it decreased on day 23 to 0.07 μmol xylose/g dry-wt/h. Thus, the highest glucose accumulation rate in the methane producing microcosm was observed on day 23, while the highest xylose accumulation rate was observed on day 14. In the methane producing microcosm, a higher xylose accumulation rate was observed compared to the glucose accumulation rate on day 7, after which the glucose

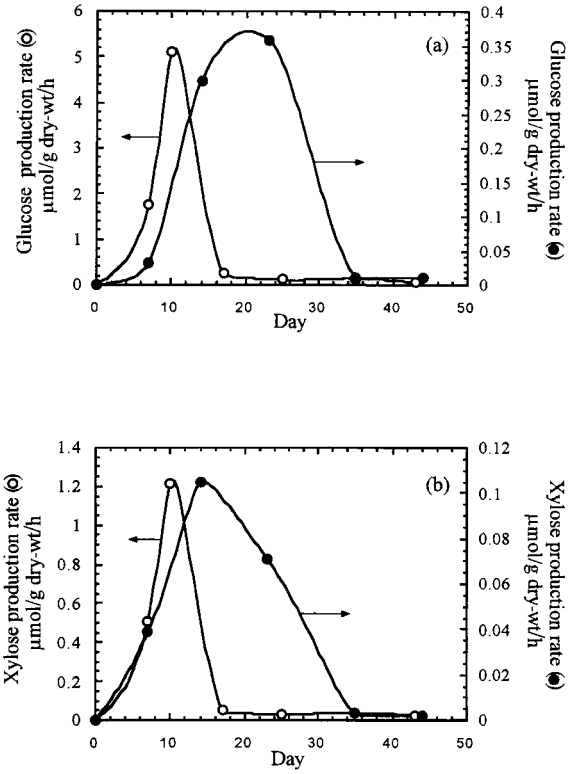

Figure 2. (a) Glucose and (b) xylose accumulation rates in sulfate reducing (○) and methane producing (●) microcosms.

accumulation rates were always higher than the xylose accumulation rates.

The data presented in Figure 2(a) and (b) indicate that higher rates of polysaccharide hydrolysis product accumulation were observed in the sulfate reducing microcosm than in the methane producing microcosm. The highest rate of glucose accumulation was attained in a shorter period of time in sulfate reducing microcosm (10 days) as compared to methane producing microcosm (23 days). The decrease in the hydrolysis product accumulation rates in the sulfate reducing microcosm (after day 10) and in the methane producing microcosm (after day 23) can be attributed to the hindrance caused by lignin, as it is known that lignin forms a three dimensional matrix in which polysaccharides are embedded.

The data points in the Figure 2(a) and (b) were interpolated and the area under the interpolated curve was numerically integrated (Al-Khafaji & Tooley 1986). The following assumptions were made while numerically integrating the area under the interpolate curve in Figures 2(a) and (b): (1) The interpolated curve represents the actual rates of hydrolysis product

accumulation; (2) The hydrolysis product accumulation rate is zero at time zero.

The area represents the total amount of hydrolysis product released during the experimental period which is presented in Table 1. After 43 days of incubation under the sulfate reducing condition, it was estimated that 1,195 μmol of initially introduced 4,174 μmol of glucose was released as hydrolysis product. Under the methane producing condition 176 μmol of initially introduced 2,782 μmol of glucose was released as hydrolysis product. In other words, 28% of cellulose was estimated to be hydrolyzed in sulfate reducing vials while only 6% of cellulose was hydrolyzed under methane producing condition i.e., approximately 4.5 times more newspaper cellulose was hydrolyzed in sulfate reducing condition compared to methane producing condition.

As the accumulation of the hydrolysis products was linear with time the hydrolysis of cellulose can be expressed as a zero order reaction as

$$(C - C_0) = -k_h t \quad (1)$$

where C is the amount of cellulose at time = t (mg); C_0 is the amount of cellulose at time = 0 (mg); k_h is the cellulose hydrolysis rate constant (day^{-1}); t is time (day).

The amount of cellulose initially loaded in microcosms can be calculated as

$$\text{Cellulose (mg)} = \text{Glucose (mg)} \times 0.9 \quad (2)$$

Thus, the initial amount of cellulose loaded in the sulfate reducing microcosm was 676.2 mg, while 450.8 mg of cellulose was loaded in methane producing microcosm. Using Equation (1), the following equations for sulfate reducing microcosm (3) and methane producing microcosm (4) can be derived

$$(C - C_0) = -27t \quad \text{Sulfate reducing microcosm} \quad (3)$$

$$(C - C_0) = -4t \quad \text{Methane producing microcosm} \quad (4)$$

The estimated zero order newspaper–cellulose hydrolysis rate constant under batch condition for the sulfate reducing microcosm (27 d^{-1}), and the methane producing microcosm (4 d^{-1}) was found to be about 6.7 times higher for sulfate reducing condition.

Role of SRB in hydrolysis of newspaper polysaccharides

As both the sulfate reducing microcosm and the methane producing microcosm consisted of anaerobic cultures of a complex mixed community of microorganism, different species of bacteria may have contributed to the hydrolysis of newspaper polysaccharides. For effective hydrolysis of cellulose and hemicellulose, extracellular or membrane bound enzymes must be produced by bacteria. The results presented in the previous sections do not provide any evidence for the difference in microbial community attributing to the hydrolysis of polysaccharides in the sulfate reducing and methane producing microcosms. Although an obvious difference is the presence of a large SRB community in the sulfate reducing microcosm as a sulfate rich medium was used for the sulfate reducing culture, the role of SRB has not been investigated in the hydrolysis of polysaccharides. In this section, the role of SRB in the hydrolysis was investigated.

The relative concentration of glucose and xylose accumulation in the four different microcosms is shown in Figure 3. The glucose production was the highest in the control (normal sulfate reducing) microcosm followed by the sulfate reducing microcosm inhibited by anthraquinone, the sulfate reducing microcosm without sulfate addition, and the least in microcosm inhibited by molybdate. On the other hand, xylose accumulation in 6 hours of incubation with toluene was the highest in the sulfate reducing microcosm inhibited by anthraquinone, followed by the control microcosm, the microcosm without sulfate addition, and the least in sulfate reducing microcosm inhibited by molybdate. It is now well established that molybdate at 50–100 mg/L specifically inhibits SRB (Speece 1996). Recently 9,10-anthraquinone has also been used to inhibit the sulfate reduction by SRB (Cooling et al. 1996). In the presence of sulfate, SRB use sulfate as the terminal electron acceptor to oxidize the organic substrate. In the absence of sulfate, most SRB are able to metabolize lactate or pyruvate, i.e., oxidation of organic substrate is replaced by fermentative reaction. Anthraquinone is known to inhibit the sulfate respiration or dissimilatory sulfate reduction pathway. In the presence of anthraquinone, sulfate respiration is inhibited, but the complete inhibition of SRB is not achieved as the SRB can thrive on the fermentation pathway. Thus, molybdate and anthraquinone can completely or partially inhibit the growth of SRB. The inhibition of SRB by anthraquinone and

Table 1. Numerically integrated area under the interpolated curves

Microcosm identity	Reducing sugars area[a]		Initial amount of cellulose[b] added	Cellulose hydrolyzed (%)
	Glucose	Xylose		
Sulfate reducing	1195	291	4174	28
Methane producing	176	50	2782	6

[a] Units: μmol.
[b] μmol glucose equivalent.

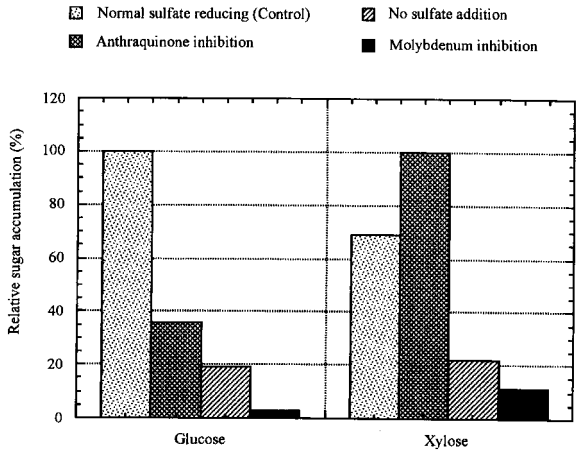

Figure 3. Relative sugar production under normal sulfate reducing (control), inhibition of SRB by anthraquinone, molybdenum and no sulfate addition conditions.

molybdate had a pronounced effect on the hydrolysis of cellulose which was manifested by lower amounts of glucose accumulation in the respective microcosms. A similar observation was made in the sulfate reducing microcosm without sulfate addition. Accordingly, it may be suggested that SRB might have contributed to the hydrolysis of cellulose. It can also be said that fermenting bacteria also contributed to the hydrolysis of cellulose in these microcosms. This was deduced from the fact that significant amounts of glucose accumulated as hydrolysis product in microcosm inhibited by anthraquinone and in microcosm without sulfate addition.

Relatively higher xylose accumulation in the microcosm inhibited by anthraquinone compared to the control microcosm cannot be adequately explained. Further investigations are necessary to explain this phenomenon. Another interesting observation with respect to the amount of xylose accumulation, similar to that of the methane producing microcosm (Figures 1), was that the amount of xylose accumulation in the microcosm inhibited with anthraquinone was higher than glucose accumulation. The amount of xylose accumulation in the sulfate reducing microcosm inhibited with anthraquinone was almost equal to the xylose accumulation in the methane producing microcosm on day 7. In addition, the pattern of hydrolysis product accumulation in the sulfate reducing microcosm inhibited by anthraquinone was similar to the methane producing microcosm on day 7.

Conclusions

Toluene 3% (v/v), used to selectively inhibit the uptake of hydrolysis product, led to an accumulation of hydrolysis products of newspaper polysaccharides. Under sulfate reducing conditions, the accumulation of glucose was linear with time indicating that no lag phase was observed in the hydrolysis product accumulation process. The amount of glucose and xylose accumulation until day 14 was higher in the sulfate reducing microcosm, leading to a higher accumulation rate of these reducing sugars in the sulfate reducing microcosms compared to the methane producing microcosm. In the sulfate reducing microcosm, the highest glucose and xylose accumulation rates were observed on day 10, while in the methane producing microcosm this was observed on day 23 and 14, respectively.

The area under the interpolated curve between the datapoints was numerically integrated and it was found that 28 and 6% of initially loaded cellulose in the sulfate reducing microcosm and the methane producing microcosm was hydrolyzed, respectively. Accordingly, the newspaper–cellulose hydrolysis rate constant was estimated to be 6.7 times higher in sulfate reducing condition that in methane producing condition. As hydrolysis is the rate-limiting step in the degradation of cellulose, higher hydrolysis rate will enhance its degradation. The faster degradation of lignocellulosic material under sulfate reducing condition compared to methane producing condition, as previously reported by the authors (Pareek et al. 1998),

may be due to the higher hydrolysis rate of cellulose under sulfate reducing condition. Although the experimental conditions used in this study and in natural environments may differ, it may be suggested that in sulfate reducing natural habitats, cellulose degradation will be faster than in methane producing environments. The same will also be true for landfills with high sulfate waste.

The role of SRB in hydrolyzing newspaper polysaccharides was investigated indirectly by inhibiting SRB by anthraquinone and molybdate. Accordingly, it was found that SRB may have contributed to the hydrolysis of cellulose, as higher amounts of glucose accumulated in control microcosm compared to microcosms inhibited by anathraquinone and molybdate. On the other hand, SRB did not seem to contribute to the hydrolysis of hemicellulose. Further investigations by isolating and identifying the microorganisms responsible for the hydrolysis of polysaccharides in the sulfate reducing microcosm are required to reconfirm the above findings.

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