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Microbial gum formation from the decomposition of cotton gin trash

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Abstract

This investigation evaluated production of extracellular polysaccharides (EPS) from the decomposition of cotton gin trash (CGT) in aerobic, anaerobic and continuous percolating environments. A further variation of pre-rinsing CGT was investigated in order to determine if the pollution potential from CGT could be minimized. Since previous investigations have used a colorimetric method for quantifying EPS that yields varying results when the constituent sugars are at different molar concentrations, a gravimetric method was used to quantify EPS production. The accumulation of uronides in the bound EPS suggests that the bound portion is critical in seal formation. The anaerobic environment showed an initial increase of EPS to 3 mg/g CGT and then maintained a constant level. The aerobic environment produced 9 mg of EPS/g CGT after 26 days with a continuing upward trend. The continuous percolating environment had concentrations similar to the aerobic environment and showed decreased hydraulic conductivity with increased EPS.

Keywords: Biological sealing; Microbial gums; Polysaccharides; Cotton gin trash

1. Introduction

CGT is a major by-product of the cotton ginning industry with more than 90 000 Mg (100 000 tons) produced in Georgia alone (USDA, 1996) much of which is currently placed in landfills. In the past the most popular disposal method of CGT was incineration. Due to Federal clean air emission requirements, incineration is no longer economically achievable (Mayfield, 1991). With land application, the nutrient benefit and organic matter of CGT are utilized; however, incorporating CGT directly into the soil also introduces plant diseases and weed seeds. Even composting does not eliminate viruses such as verticillium wilt (Thomasson, 1990). Thus, new uses of CGT are desirable.

Tollner et al. (1983) have shown that CGT may have potential as a soil seal. Lynch et al. (1957) linked C/N environments greater than 15 to enhanced production of soil sealing EPS. This relationship suggests the use of a readily decomposable material such as CGT, with a C/N of 28, as a substrate for generation of EPS and lagoon sealing. Past work has shown that plugging from EPS is a key mechanism in the sealing of waste lagoons (Ritter et al., 1984; Chang et al., 1974; Avinemelch and Nevo, 1964; Hills, 1976). Ritter et al. (1984) observed a gradual formation of a biological seal in swine waste lagoons, but still suggested liners for loamy sand and sandy loam to reduce groundwater impact. Waste water treatment lagoons and constructed wetlands require low soil conductivity to minimize pollution potential from leaching. Because of the number of waste water treatment ponds and the sizes of constructed wetlands, an economical method of sealing is required. Tollner et al. (1983) measured concentrations of EPS in the soil layer applied with plant residue and its impact on hydraulic conductivity. The possibility exists to accelerate the sealing mechanism by applying a layer of CGT, simultaneously finding a useful disposal method for CGT and eliminating groundwater contamination from waste lagoons.

Plants produce polysaccharides in the form of cellulose (50–70% of plant dry weight), hemicellulose, gums and mucilages. Microbial produced polysaccharides are classified into four groups: cell-wall materials, capsular, intracellular and extracellular (Greenland and Oades, 1975). EPS are long chain carbohydrates of varying molecular weight and structure. Of the extracellular polysaccharides, two subdivisions are of interest: free and bound. Free EPS have been excreted into the surrounding environment and are soluble. Bound EPS are linked to cell walls or inorganic particulates and are not readily soluble. Tollner et al. (1983) concluded uronides

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were important in the formation of a biological seal. Uronides are polysaccharides consisting of the simple sugars D-glucuronic acid and D-galacturonic acid, both of which have C6 as part of a carboxyl group. Martens and Frankenberger (1990a) incorporated biological materials in soil and found no free uronides were detectable after decomposition and that they existed only in the bound state. The bound polysaccharides of the biological materials contained as much as 25–50% uronides, yet the free EPS in the biological material/soil mixture contained no uronides. The low levels of uronides outside plant materials suggest that they are rapidly utilized as a carbon energy source for microbial activity. During the decomposition of CGT, EPS are produced as by-products from microbes. The majority of polysaccharides in soil are believed to be by-products of microbial metabolism (Greenland and Oades, 1975).

The case for bioconversion of cotton gin trash (CGT) into EPS is sparked by stringent disposal requirements and possible beneficial use from bioconverted CGT. Tollner et al. (1983) also noted that the soluble portion associated with CGT caused high pollution loading of the initial water passing through CGT before seal formation. Rinsing and/or off-site productions of EPS are potential methods for minimizing the pollution associated with the use of CGT as a biological sealant. The mechanism which allows EPS to form a hydraulic seal is not clearly understood. The purpose of this study was to further investigate the sealing mechanism, by (1) varying the decomposition environment as a preliminary investigation to off-site bioconversion of CGT to EPS, (2) considering the effect of prior rinsing and (3) determining both bound and free production of EPS instead of composite EPS because of the difference between uronide concentrations in the bound and free states.

2. Methods

2.1. Sample preparation and percolation test

CGT was gathered from a local gin (The Bostwick Gin, Bostwick, GA) in January 1997 and represented the 1996 harvest. CGT was stored at 2.5°C up to 12 months before use. It was ground with a 0.37 kW Wiley Mill fitted with a 6 mm screen. Due to the clumps of cotton fiber, homogeneity of the substrate was difficult to achieve while measuring 10 g samples and should be noted as a possible source of variability.

Anaerobic, aerobic, and continuously percolating environments for decomposition were created. For anaerobic and aerobic environments, 10 g of CGT were placed in 500 ml flasks with 250 ml of water. Erlenmeyer flasks were used for the anaerobic environments and were left undisturbed for the desired time of the trial. For the aerobic environment, shaker flasks were placed on a shaker at 200 rpm for the desired time. All samples had three replicates. Aerobic trials were measured at 4, 10, 18 and 26 days. Anaerobic trials were measured at 4, 8, 14 and 28 days. A zero time sample was generated by adding the water and then immediately performing the measurements. To test the effects of rinsing CGT, the sample was created as before, but then was placed on the shaker for 30 s and the contents poured out over the nylon mesh. CGT was replaced in the flask and water was added again until the original weight was achieved.

The continuous percolating environments were created in 500 ml Nalgene sample bottles. A 3.18 mm (1/8 in.) hose connector was placed on the bottom container, and a 18.8 mm (3/4 in. sch. 80) nipple was threaded into the side of the bottle at 100 mm from the bottom. One hundred grams of autoclaved sand, forming a 20 mm layer, was placed on top of a coarse nylon mesh to prevent channeling of flow. A 25 g layer of CGT was then compacted to a density of 0.22 g/cm^3, creating a 30 mm layer over the sand. Another 100 g of sand was placed over CGT to hold CGT in place. The 18.8 mm (3/4 in. sch. 80) nipple was connected to a carbon-filtered water reservoir maintained at a constant level 30 cm above the top layer of sand. Percolation rates and selected effluent constituents for three pre-rinsed and three non-rinsed samples were assessed at 0.5, 1, 2, 4, 8 and 24 h. Orthophosphate, nitrate and ammonia levels were measured in percolant samples. Other flow rates were measured periodically as was the free chlorine residual of the incoming water. Samples were destroyed and EPS measured between 4 and 24 days in replications of three. Three pre-rinsed samples were obtained by placing the 25 g sample in 250 ml of water and shaking for 30 s by hand. The rinse water was poured off and the moist CGT was collected.

2.2. Quantification of EPS

EPS determination began with a separation of plant material with a Spectra/Mesh® nylon filter with a mesh opening of 70 μm, allowing water, dissolved solids, and suspended solids to freely pass through. The liquid was centrifuged (16 000 × g for 30 min). The supernatant was pipetted into two samples: one for TSS measurements and the other was precipitated with 2 v/v of 2-propanol for two days (Stevenson, 1994).

The solids retained by the nylon mesh were soaked in an equivalent volume of 1N HCL for an hour at room temperature (19–23°C). The short hydrolysis time was designed to cleave only the readily accessible bonds of bound EPS. After the acid hydrolysis, the sample was filtered again and the procedure for the free EPS was repeated. Samples were filtered with Whatman GF/C paper and oven dried at 105°C until a constant weight was achieved. A schematic diagram of the procedure is shown in Fig. 1.

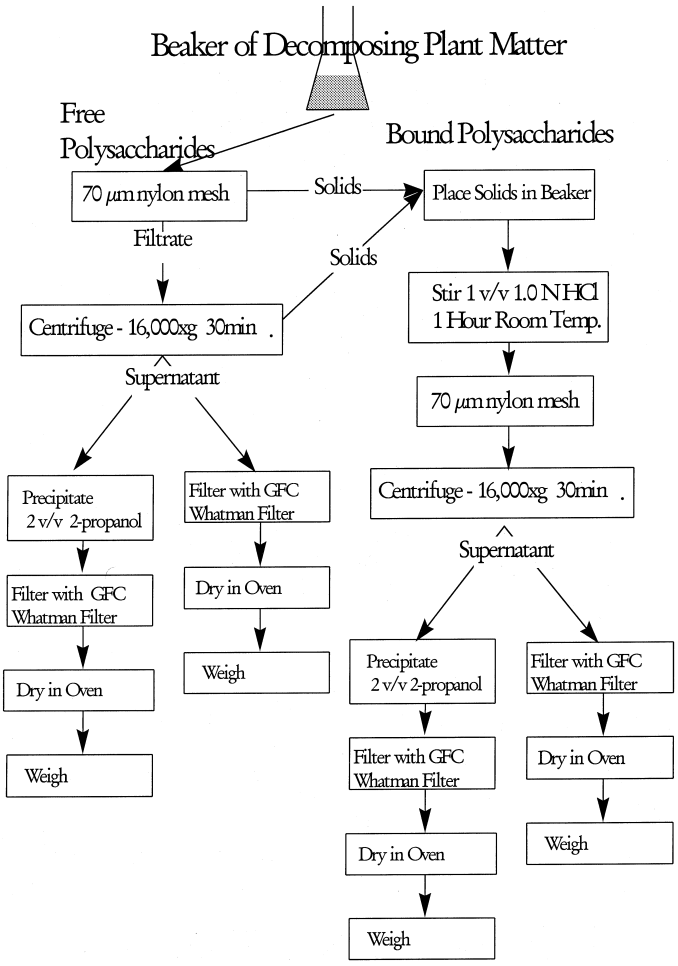

Fig. 1. Schematic diagram for EPS determination.

In order to account for the different environment used by Norberg and Enfors (1982) and to characterize the samples, gas chromatography-mass spectrometry (GC-MS) was performed on the samples collected on the filter paper. The samples were hydrolyzed using freshly prepared 1 M methanolic-HCl for 16 h at 80°C. N-acetylation was performed by addition of methanol (0.5 ml), acetic anhydride (50 μl) and pyridine (10 μl). The sample was left at 80°C for 15 min and dried under nitrogen. The released sugars were derivitized with Tri-Sil and samples were run on GC-MS using a Supelco column. Myo-inositol was also added (40 μl) as an internal standard.

2.3. Hydraulic conductivity

The orifice equation was used to calculate the relationship between resistance and flow for the empty container. The flow through the container and sand was used to calculate the hydraulic conductivity of the sand. With this information, the hydraulic conductivity through the CGT layer could be determined. As the flow decreased, the resistance of the container became negligible.

2.4. Statistical analyses

The statistical analyses consisted of performing several regression analyses and general linear model (GLM) evaluation coupled with standard error value analyses (computed via spreadsheet) for bound and free EPS in the aerobic and anaerobic environments on a daily basis. The NCSS statistical package (Hintz, 1997) was used to make the GLM-ANOVA computations. The aerobic and anaerobic treatments were first analyzed as a randomized complete block, three replicates. Main effects were a bound – free effect, rinsed–non-rinsed effect and an anaerobic–aerobic effect. The time effect was nested in the main effects. The continuous percolation treatment series was analyzed separately due to some apparent effects of residual chlorine in the incoming percolant. In the event of significant interactions, the GLM or one-way ANOVA procedures were run at each level of one of the interacting variables. An alpha error of 10% was selected as the cutoff point for assessing statistical significance. In cases where the time effect was not significant the one-way ANOVA model was used.

Post hoc tests having a lower alpha are indicated using $p \leqslant$ xx notation.

3. Results and discussion

3.1. Measurement of the EPS

The gravimetric method for determining the EPS appears to quantify the amount of EPS produced. However, the organic and non-organic constituents plus other contamination will overestimate actual results. The GC analysis showed that the samples averaged 65% (± 5%) carbohydrate. Ten percent of the remaining mass was accounted for in the TSS. The remainder of the weight included organic and inorganic constituents of the polysaccharides and possibly other compounds which precipitated in the alcohol. This remaining component likely adds to the mass of the remaining polysaccharides and it is appropriate to include it in the context of soil sealing. The gravimetric method is independent of the molar concentrations of sugars, which is important because the molar concentrations vary considerably during the decomposition process. Due to the limited accuracy of gravimetric methods, this method would be handicapped by low concentrations.

3.2. The EPS Production

Considering all three environments and all times, a significant three way interaction ($p < 0.01$) existed between the type of EPS (bound or free), environment (aerobic, anaerobic and continuous percolating), and time. Not considering the measurements at day 0, bound EPS and free EPS production was related to time ($p < 0.10$).

The anaerobic runs appeared to reach a maximum production of free EPS of approximately 10 mg/g of CGT after the first reading at 4 days and then remained constant (Fig. 2). The bound samples reached a maximum of 4 mg/g CGT with no significant difference with respect to time or aeration status after the 4 day reading. Pre-rinsing seemed to consistently reduce the free EPS though the effect was not statistically significant.The bound EPS was significantly lower than the free EPS as one would expect (Fig. 3). The relatively constant levels of both the bound and free EPS suggest that EPS are stable in anaerobic environments.

The aerobic runs showed a peak in the free EPS at the 10 day samples with an average of 9 mg/g CGT and then a drop to 0 at 26 days (Fig. 4). The rinsing treatment was not significant although the second order effect in time was significant. The concentration of bound EPS remained similar in the aerobic environment until the 18 day sample where they showed an increase, which was observed again at the 26 day sample (Fig. 5). The low

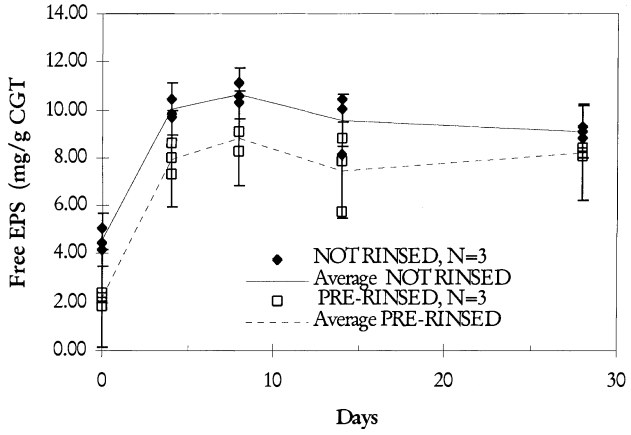

Fig. 2. Free EPS production from the anaerobic decomposition of CGT.

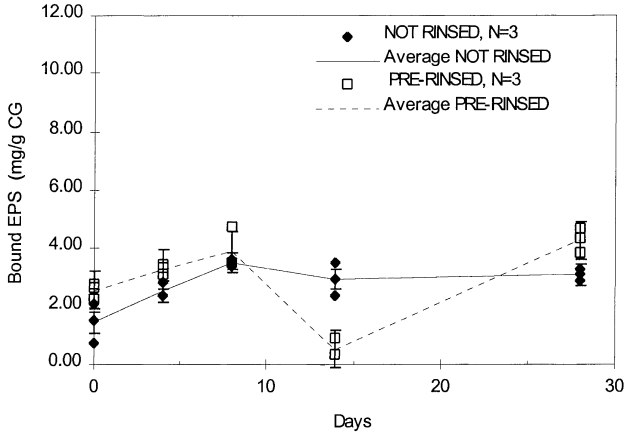

Fig. 3. Bound EPS production from the anaerobic decomposition of CGT.

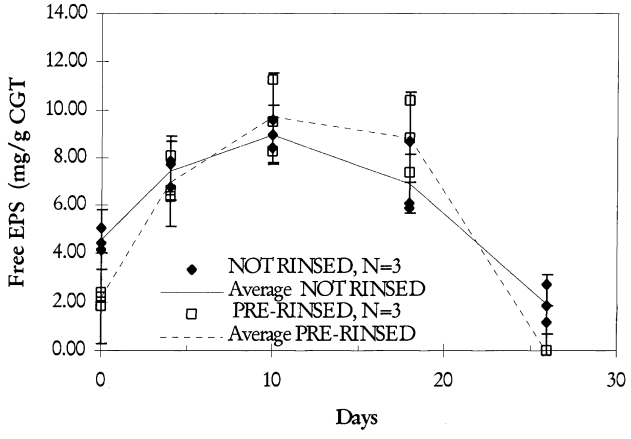

Fig. 4. Free EPS production from the aerobic decomposition of CGT.

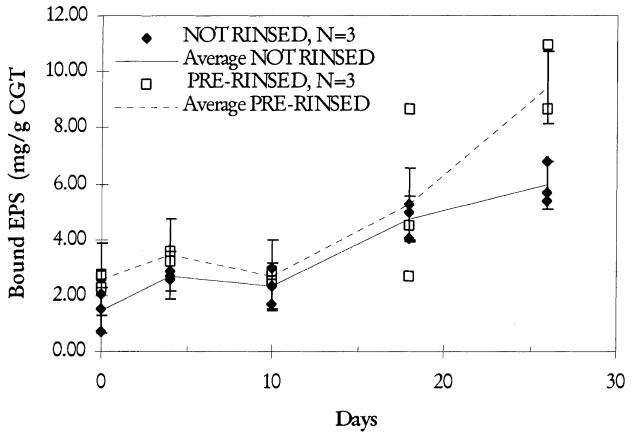

Fig. 5. Bound EPS production from the aerobic decomposition of CGT.

free EPS at the 26 day sample could be explained by an increased microbial population consuming any free EPS. The corresponding increase of bound EPS can be explained by the microbes not releasing EPS and therefore increasing the amount bound to the microbes in the nutrient deficient environment of the nearly decomposed CGT. Failure to release EPS in a nutrient deficient environment was noted by Greenland and Oades (1975).

No relation between production of EPS and rinsing was observed. However, at day 0, the pre-rinsed free EPS were significantly lower than the non-rinsed ($p < 0.01$). This difference in free EPS can be attributed to washing away of the free EPS during the rinsing process. The bound pre-rinsed samples had slightly higher levels of EPS.

Difficulties in supplying a continuous chlorine-free water supply did not allow for the development of a relationship of EPS production and time for the continuous percolating environment. A failed filter allowed free chlorine levels to reach 35 mg/l which resulted in an average of 350% increase in hydraulic conductivity. Once free chlorine levels were reduced average flows returned to the pre-chlorine levels within 9 days (Fig. 6). The apparent correlation between increased free chlorine and hydraulic conductivity suggests that the biological sealing mechanism is adversely affected by chlorine.

Chang et al. (1974) presented data which can be plotted to show decreased hydraulic conductivity with increase in EPS concentration. Fig. 7 shows their data plotted with data from the continuous percolation run of the present study. The point with the lowest EPS and highest hydraulic conductivity is the measurement at day 0. The soil hydraulic conductivities are generally 100 times greater than CGT and increased EPS affected hydraulic conductivity greater in the soil than in CGT. The greater effect of EPS on hydraulic conductivity and higher levels of EPS in CGT suggest a soil-CGT interaction which would lead to greater sealing.

3.3. Rinsing effects

Pre-rinsing in the aerobic and anaerobic environments did not have a significant effect on production of EPS. In the continuous percolating environment the non-rinsed CGT had lower average hydraulic conductivities, which could be attributed to edge effects of the container, because the dry CGT swells and makes a better seal against the side of the container, but the difference becomes negligible after 16 days. Total mass of pollutants passed in the first 24 h was calculated with numerical integration of the product of flow and pollutant concentration with time. Total normalized pollutant mass for orthophosphate and nitrate (averages shown in Fig. 8) were significantly higher ($p < 0.05$) for the pre-rinsed compared to the non-rinsed, even though pollutant concentrations were lower. The ammonia average was not significantly different. Due to lower initial

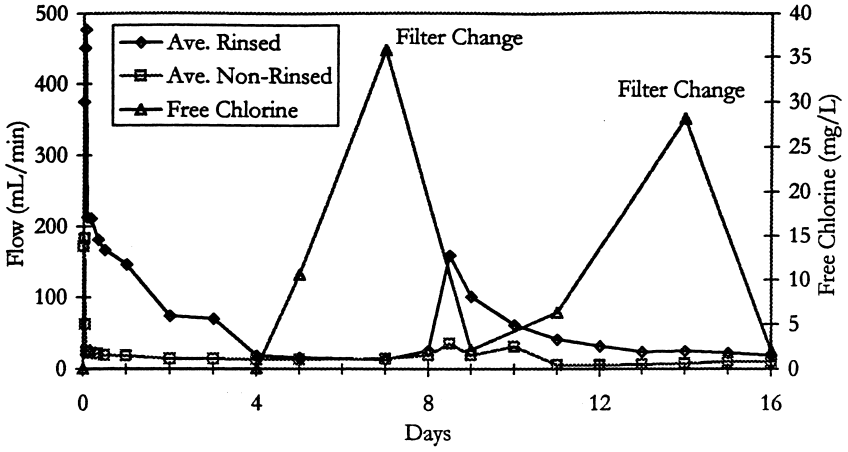

Fig. 6. Flow through continuous percolating system and free chlorine. Each point represents one measurement value for purposes of documenting free chlorine in the flow to the rinsed and non-rinsed runs.

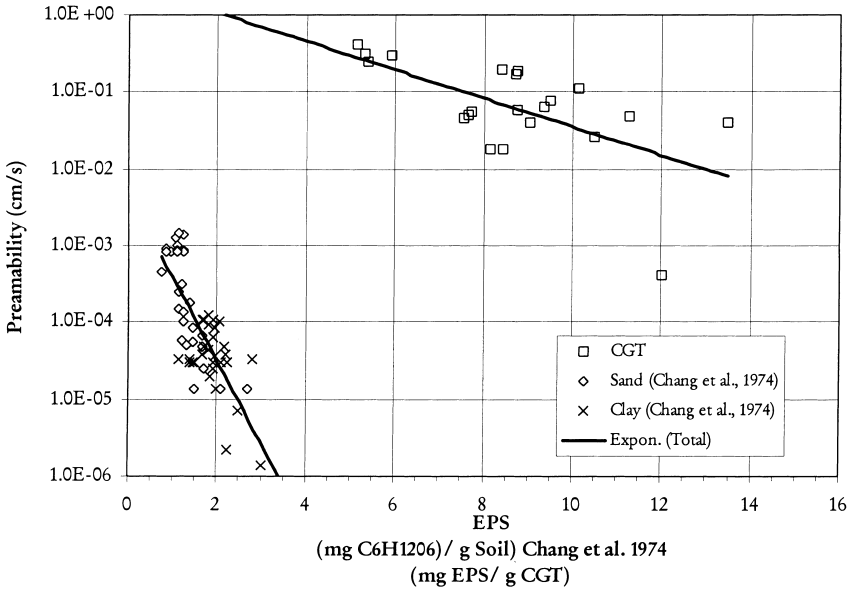

Fig. 7. Relationship between EPS concentration and hydraulic conductivity.

pollutant concentrations, pre-rinsing the CGT can reduce total pollutant loading, only if lower initial hydraulic conductivity could be developed.

3.4. EPS Constituents

GC-MS was used to verify the carbohydrate constituents of a composite of the 28 day anaerobic, 18 day aerobic, 16 day continuously percolating (pre-rinsed and non-rinsed), and 0 day control. Free and bound samples were analyzed for all cases except in continuous percolating samples because concentrations were too low for sample recovery. Not all the constituent percentages add to 100%, because only a base search of 12 expected sugars was performed (Table 1).

The anaerobic and aerobic free samples had similar totals within the hexose, pentose and uronide portions. Within the hexose portions the rhamnose and galactose fraction varied. The free uronide portion is drastically reduced after decomposition occurred both aerobically and anaerobically.

The 28 day anaerobic bound samples almost matches directly the 0 day bound samples suggesting that after 28 days, no accumulation of microbial bound polysaccharides occurred. The aerobic bound sample more than doubled the amount of uronides to 72.6% while hexoses decreased. The continuous percolating bound sample increased uronides to 81.2% and 79.1% for the pre-rinsed. These samples support the findings of Martens and Frankenberger (1990a) of

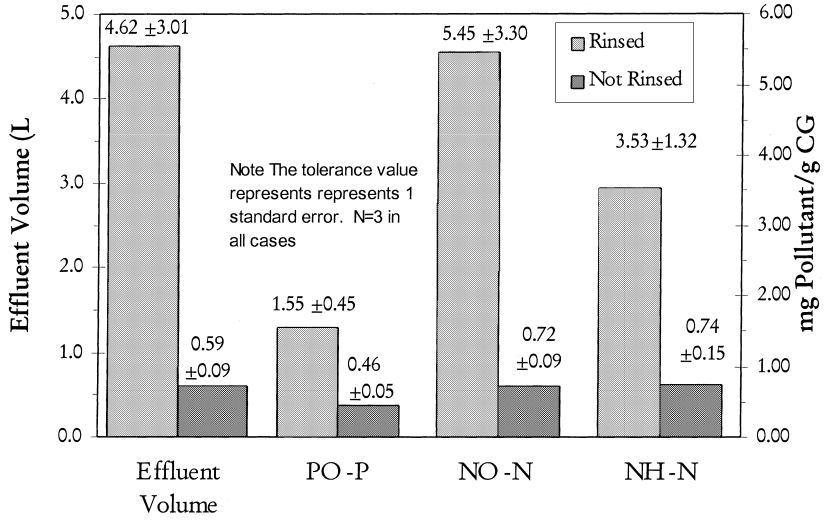

Fig. 8. Average normalized pollutant mass and effluent volume from the first 24 h.

Table 1
Sugar fractions of recovered EPS

	0 day free	28 day anaerobic free	14 day aerobic free	0 day bound	28 day anaerobic bound	14 day aerobic bound	16 day continuous percolation	16 day continuous percolation (rinsed)
Rhamonose	8.4	11.4	41.5	5.8	6	3.6	3.8	3.1
Fucose	0.8	5.3	2.9	0	0	1.8	0	0
Glucose	17.7	26	12.2	38.3	35.4	9	4.8	8.5
Galactose	21.7	24.8	8.6	9.7	7.5	4.9	4.4	4.3
Mannose	6.7	11.3	5	4.5	6.4	2.4	1.8	1.9
N-acteyl galactosamine		1.6	9					
N-acteyl glucosamine		3.7	3.6					
Total hexose	55.3	84.1	82.8	58.3	55.3	21.7	14.8	17.8
Arabinose		5.4	9			3.4	2.4	1.6
Xylose		8.1	6.6			2.3	1.6	1.5
Total pentoses	0	13.5	15.6	0	0	5.7	4.0	3.1
Galacturonic	26.4	2.4	0	22.4	33.2	72.6	81.2	79.1
Glucuronic Acid	8	0	1.5	10	0		0	0
Total uronides	34.4	2.4	1.5	32.4	33.2	72.6	81.2	79.1

low-level free uronides in plant soil mixtures, even with large percentages in plants before introduction to the decomposing environment. However, in the soil matrix higher glucuronic acid content was observed, while Martens and Frankenberger (1990b) indicated all galacturonic acid.

4. Conclusion

No free EPS were recovered in the continuous percolating environment or in the aerobic environment after 26 days, suggesting they were rapidly utilized as a carbon and energy source. No uronide accumulation occurred in the free EPS recovered from the anaerobic environment. The high concentration of uronides in the bound EPS suggests bound EPS are the key to seal formation. Quality of the water used in continuous percolation is an issue which requires additional study.

Both aerobic and continuous percolating environments produced substantial quantities of bound EPS with over 70% uronides. Since the continuous percolating environment produced the highest percentage of uronides, production of EPS for lagoon sealing in a batch aerobic or anaerobic process is not preferred. Increases in bound EPS in the continuous percolating environment resulted in decreased percolation. Previous data show a greater effect of EPS on hydraulic conductivity in soil than the similar increases of EPS in CGT. A soil–CGT mixture may utilize the advantages of greater production of the EPS and lower hydraulic conductivities. Rinsing CGT resulted in lower concentration of pollutants, but higher total amounts due to greater flows. Advantages of rinsing can be realized if lower initial hydraulic conductivities can be achieved.

Acknowledgements

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