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## Intrinsic degradation of volatile fatty acids in laboratory-compacted clayey soil

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### Abstract

Volatile fatty acids (VFAs) represent the major organic constituent of landfill leachate and provide the greatest potential for leachate induced organic contamination of groundwater (e.g. as represented by an increase in the concentration of dissolved organic carbon and chemical oxygen demand). Long-term diffusion tests were performed for laboratory-compacted clayey soil plugs exposed to continuous supply of synthetic leachate containing VFAs. Significant microbial activity developed upon exposure of the soil's indigenous microorganisms to these degradable contaminants. The growth of heterotrophic aerobic bacteria (HAB, which include facultative anaerobes), sulfate reducing bacteria (SRB) and methanogenic bacteria carrying out fermentation and mineralization of the VFAs became evident after 30–50 days of testing. The maximum microbial counts of  $(2-8) \times 10^8$  and  $(0.1-1) \times 10^8$  cfu/g for HAB and SRB were localized in the soil layer at the interface with the source of organic and inorganic nutrients. Regardless of this rapid growth in microbial population, the VFA consumption was small and measurable only after a lag of 140–180 days. It is considered that this lag of otherwise readily degradable organic compounds (such as VFAs) persisted due to a combination of the effects of a high initial concentration of these acids (2.4 g/l as dissolved organic carbon, DOC) applied to carbon starved soil microorganisms and the small pore size of the compacted clay. Once the significant amounts of gas were generated from fermentation, conditions developed for improved mass transport and exchange of the nutrients and bacteria and the outcome of the intrinsic degradation was more apparent. The breakdown of VFAs that followed after the lag was localized near the top of the soil

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and was characterized by a short half-life of 0.75–5 days for DOC (total VFAs as dissolved organic carbon).

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## 1. Introduction

Many modern municipal solid waste (MSW) landfills are constructed with a barrier system that includes a leachate collection system and a compacted clay liner which, together, control advective contaminant migration (Daniel and Koerner, 1995; Rowe, 2001; Rowe et al., 1995; Manassero et al., 1998). However, due to the concentration gradient between the leachate at the top of the clay liner and the unpolluted surroundings, diffusion of the contaminants inevitably proceeds. While slow, diffusion of contaminants represents a potential threat to groundwater resources, thus, it becomes important to consider the potential for contaminant degradation mechanisms in these liners. Generally, environmental rather than thermodynamic constraints have been recognized as controlling in the degradation metabolism of organic compounds found in landfill leachate. Diffusion, which governs the bioavailability of organic substrates, is considered a factor that severely limits microbial respiration and consequent biodegradation (McMahon and Chapelle, 1991; Verstraete and Top, 1999). Phelps et al. (1988) stressed that unsaturated conditions and soil texture with 20% clay impaired the size and activity of the indigenous microorganisms. In addition to low hydraulic conductivity and suboptimal pH, Chapelle and Lovley (1990) attributed small pore size ( $<0.05 \mu\text{m}$ ) and very low effective porosity (0.078) to low ( $<0.025 \text{ year}^{-1}$ , i.e.  $\sim 28$ -year half-life) rates of acetate turnover observed in clayey confining beds. In cases of fast acetate turnover and/or fatty acids consumption reported for marine sediments, the reaction was either drastically impeded or completely ceased below the few top centimeters of a bioreactive layer (Nedwell, 1984).

The focus of this paper is the intrinsic degradation of volatile fatty acids (VFAs) that are in direct contact with compacted clayey soil. These acids have been recognized as the major, readily degradable organic contaminants in landfill leachates (Harmsen, 1983; Robinson, 1995), and in the aquifers below landfills (Blakey and Towler, 1988). Biodegradation of these acids has been studied and metabolic pathways are well known (Dolfing, 1988; Widdel, 1988; Chapelle, 1993). Propionate, butyrate (and other short chain  $\text{C}_3$ – $\text{C}_6$  acids) and, most importantly acetate and hydrogen, released as reduced intermediates of oxidation/reduction steps in the breakdown of complex organic matter are utilized as growth substrates by mixed cultures. Acetate and hydrogen are well recognized as methane precursors, and competitive substrates for both methanogenic and sulfate reducing bacteria (Cord-Ruwisch et al., 1988; Hoehler et al., 1998). The metabolic processes and microorganisms instrumental in incomplete and complete oxidation of VFAs have been confirmed in sanitary landfills (Aragno, 1989; Sleat et al., 1989; Fielding et al., 1988; Barlaz et al., 1989; Bennett, 1998).

There is, however, very little information about VFA emissions from sanitary landfills in natural low hydraulic conductivity deposits or engineered clay liners. Early work of

Hoeks and Borst (1982) with buffered leachates made of various VFAs and sandy loam indicated potential for fermentation. This soil allowed percolation and, thus, was not a low hydraulic conductivity diffusion barrier, nevertheless, VFAs degradation was fast, reportedly with visible methane bubbling out of the columns. It was stated, although not shown, that at long retention times common to field conditions, VFAs would be completely mineralized from the leachate in the top first meter of soil beneath the waste pile. In the other document on VFAs degradation, McMahon and Chapelle (1991) reported a build-up of acetate and formate in an organic-rich sulfate reducing aquitard. These acids were driven by diffusion gradient into the underlying deep anaerobic aquifer, where the conditions were conducive to their fast mineralization.

Numerous studies of various natural subsurface environments have already confirmed that many indigenous microorganisms are not strictly autotrophic but, rather, are capable of utilizing organic substrates (and VFAs) and carrying out terminal oxidation/reduction reactions (Ghiorse and Wilson, 1988; Kölbel-Boelke et al., 1988; Jones et al., 1988; Phelps et al., 1988; Martino et al., 1998). Considering the decades (if not centuries) of contaminant–substrate retention associated with modern lined landfills, it is likely that the metabolic activity of soil bacteria would eventually start and continue. Consequently, biologically driven organic contaminant removal from the environment may be effective, even if it proceeds at rates that would, in other man-made industrial systems, be considered “impractically” slow.

The primary objective of this work is to examine whether degradation of a few representative organic pollutants, found in municipal solid waste landfill leachate, could be initiated and carried out by indigenous microorganisms present in the compacted clay. In this paper, emphasis is placed on the C<sub>2</sub>–C<sub>4</sub> volatile fatty acids, acetate, propionate and butyrate recognized as predominant products of organic waste hydrolysis and fermentation. The conditions that would eventually lead to their breakdown (i.e. mineralization in the soil) are examined. Experiments to simulate the adverse conditions imposed by diffusion controlled mass transfer limitations and the small pore size in compacted clay are described. Finally, the rates of degradation for the volatile fatty acids tested and conditions examined are inferred.

## 2. Materials and methods

The intrinsic degradation tests were performed in a glass–cell assembly connected to a continuous feed distribution network, intended to simulate degradation under the 1-D diffusion dominated mass transport for a long period of testing (Fig. 1). The fluid in the source reservoir was continuously replaced by a synthetic medium (Table 1) prepared to resemble both the organic, as well as inorganic composition of the typical (MSW) leachate. This synthetic leachate was prepared such that there was negligible bacterial content and although it contained a significant concentration of fermentable substrates and inorganic nutrients, it was neither designed for optimal growth of indigenous soil bacteria nor for optimal rate of removal of organic compounds. The flow rate (2.5–4.0 l/day) through the source reservoirs was controlled such that the concentrations of designated pollutants remained essentially constant with time.

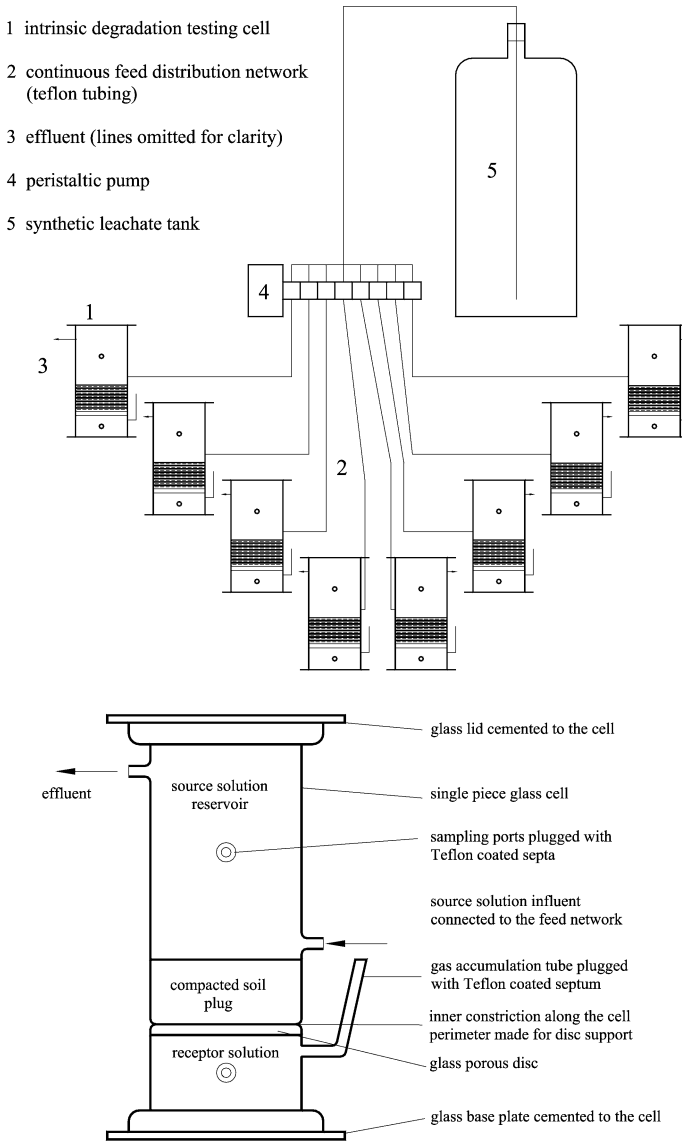


Fig. 1. Schematic of glass-cell testing assembly and intrinsic degradation cell.

Volatile (short chain) fatty acids (VFAs), in the form of acetate, propionate and butyrate represented a major source of dissolved organic carbon (DOC) and chemical (and biochemical) oxygen demand (COD, BOD), while volatile organic chemicals (VOCs) spiked to this synthetic leachate were representative of several micropollutants frequently found in domestic landfills. This synthetic mix was chemically reduced and designed to simulate “stabilized” landfill leachate at the beginning of methanogenic stage, except that,

Table 1  
Composition of the synthetic leachate used for intrinsic degradation experiments

General indicators	
COD (mg/l)	17,500
TSS/VSS	negligible
Temperature (°C)	24 ± 2
pH (–) NaOH (pH adjustment)	6.0
ORP (mV) 3% w/v Na <sub>2</sub> S × 9H <sub>2</sub> O (Eh adjustment)	– (80–120)
Volatile fatty acids (VFAs) <sup>a</sup> added as (ml/l):	
Acetic (ethanoic)	7
Propionic (propanoic)	5
Butyric (butanoic)	1
Volatile organic chemicals (VOCs) quantified as (mg/l):	
Dichloromethane (DCM)	4.5
1,2-Dichloroethane (1,2-DCA)	4.5
Trichloroethene (TCE)	2.5
Benzene	3.5
Toluene	3.0
Ethyl-benzene	2.4
Xylene isomers	2.5
Inorganic nutrients (mg/l)	
K <sub>2</sub> CO <sub>3</sub>	325
KHCO <sub>3</sub>	312
K <sub>2</sub> HPO <sub>4</sub>	30
NaHCO <sub>3</sub>	3015
NaNO <sub>3</sub>	50
NH <sub>4</sub> HCO <sub>3</sub>	2440
CO(NH <sub>2</sub> ) <sub>2</sub> , urea	695
CaCl <sub>2</sub> × 2H <sub>2</sub> O	3350
MgCl <sub>2</sub> × 6H <sub>2</sub> O	3115
MgSO <sub>4</sub> × 7H <sub>2</sub> O	320
Trace metal solution (ml/l)	2

Each of the VFA supplied in the synthetic leachate can be fermented (“dehydrogenated” or disproportionated, i.e. is partly electron donor and partly electron acceptor), acetate ~ 0.12 M, propionate ~ 0.067 M, butyrate ~ 0.011 M. Sulfates are relatively low (~ 0.002 M) and bicarbonates are high (~ 0.0668 M), which is sufficient to sustain methanogenesis upon generation of hydrogen from fermentation (H<sub>2</sub>/HCO<sub>3</sub><sup>–</sup> = 4:1).

<sup>a</sup> Incomplete oxidation of VFAs present in the synthetic leachate generally proceeds either as acetogenic dehydrogenation (of propionate: CH<sub>3</sub>CH<sub>2</sub>COO<sup>–</sup> + 3H<sub>2</sub>O → CH<sub>3</sub>COO<sup>–</sup> + H<sup>+</sup> + 3H<sub>2</sub> + HCO<sub>3</sub><sup>–</sup>, or butyrate: CH<sub>3</sub>CH<sub>2</sub>COO<sup>–</sup> + 2H<sub>2</sub>O → 2CH<sub>3</sub>COO<sup>–</sup> + H<sup>+</sup> + 2H<sub>2</sub>) or as acetogenic sulfate reduction (of propionate: 4CH<sub>3</sub>CH<sub>2</sub>COO<sup>–</sup> + 3SO<sub>4</sub><sup>2–</sup> → 4CH<sub>3</sub>COO<sup>–</sup> + 4HCO<sub>3</sub><sup>–</sup> + 3HS<sup>–</sup> + H<sup>+</sup>, or of butyrate: 2CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>COO<sup>–</sup> + SO<sub>4</sub><sup>2–</sup> → 4CH<sub>3</sub>COO<sup>–</sup> + H<sup>+</sup> + HS<sup>–</sup>). Complete oxidation of these acids and products of their incomplete oxidation proceeds in terminal reduction/oxidation steps as aceticlastic methanogenesis (CH<sub>3</sub>COO<sup>–</sup> + H<sub>2</sub>O → CH<sub>4</sub> + HCO<sub>3</sub><sup>–</sup>), as H<sub>2</sub>-utilizing (HCO<sub>3</sub><sup>–</sup>/CO<sub>2</sub>-reducing) methanogenesis (4H<sub>2</sub> + HCO<sub>3</sub><sup>–</sup> + H<sup>+</sup> → CH<sub>4</sub> + 3H<sub>2</sub>O)(Dolfing, 1988) or as sulfate reduction (CH<sub>3</sub>COO<sup>–</sup> + SO<sub>4</sub><sup>2–</sup> → 2HCO<sub>3</sub><sup>–</sup> + HS<sup>–</sup>) (concepts and equations from Dolfing, 1988; Widdel, 1988).

although not sterilized, it did not have an active microbial population characteristic of a MSW leachate. This chemically reduced synthetic leachate would not be conducive for bacteria characteristic of oligotrophic (oxidized) waters, had they been present in the medium or introduced by contamination. Thus, the high pumping rate resulted in frequent supply of nutrient medium essentially free of anaerobic bacteria.

The clayey soil tested (characterized in Table 2) was obtained from a location at the Halton Waste Management Site where there had been no contact between the soil and leachate, (Milton, Ontario, Canada; see Rowe et al., 2000; Hrapovic, 2001). The soil was dried under nitrogen atmosphere, pulverized and passed through US sieve no. 4, remolded in distilled water at about 14.5% moisture content (selected since it gives the lowest practical hydraulic conductivity), and compacted according to the standard Proctor procedure (ASTM). The compacted soil was then extruded from the mold and cut into 3-cm thick plugs, which were placed into glass cells (Rowe et al., 1997). Soil sterilization was not performed since the objective was to preserve indigenous microorganisms for this intrinsic degradation experiment.

Expecting the measurable degradation to take place at a slow rate after a considerable lag period, the test series started with eight replicate cells. At times when the information might be chronologically useful or when changes became obvious, one cell or a pair of cells was terminated (sacrificed) and analyzed for designated parameters.

The concentration of the 11 organic chemicals selected (three fatty acids and eight volatile organic compounds) was monitored over time in the cell solutions and soil pore water in order to assess the prospects of their intrinsic degradation in the laboratory-compacted clay liner. Only the results relating to the fate of the three volatile fatty acids are presented in this paper.

### 2.1. Analytical measurements

VFA concentrations were obtained by gas chromatography (GC), using a Shimadzu GC-9A and Varian 3400 instruments equipped with flame ionization detectors (FID) and 15 m × 0.53 mm (or 0.25 mm) ID, 0.5 μm film NUKOL™ (Supelco, Bellfonte, PA) capillary columns. Injections to the Varian 3400 were automated (Varian 8200 autosampler) and performed using solid phase micro extraction (SPME) with 10-min immersion/2-min desorption of a 75-μm Carboxen™/Polymethylsiloxane fiber (Supelco, Bellfonte, PA). Peak integration and quantification was done either by Varian Star Chromatography software or an in-line HP 3369A data processor. The detection limit for acetic acid, recognized as most difficult to separate, was 3–10 mg/l. All aqueous VFA samples were acidified in 1% H<sub>3</sub>PO<sub>4</sub> prior to analytical separation. Isovaleric acid was used as internal calibration standard. Samples from cell reservoirs were regularly withdrawn by use of a syringe, while soil pore water samples were obtained by squeezing the cut soil layers at 24 MP in a stainless steel mold.

The possible log populations (PLP) of colony forming units (cfu/ml or cfu/g) of heterotrophic aerobic bacteria (HAB) and sulfate reducing bacteria (SRB) were estimated by use of the Biological Activity Reaction Test (BART™), which employs a set of reactions to identify a consortium of microorganisms present in the system (Cullimore, 1993). HAB-BART, designed to monitor the activity of versatile consortium predom-

Table 2

Some characteristics of the Halton Till used for the intrinsic degradation experiments (after Rowe et al., 2000; Hrapovic, 2001)

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Halton till clayey soil: texture, mineralogy, pore water solutions and bacteria count

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Unweathered clayey silt till: upper till–lower till (6.2–7.5 m depth)

*Soil texture (USDA)*

Clay (<0.002 mm)	22–15%
Silt (0.002–0.05 mm)	48–39%
Sand (0.05–2 mm)	20–30%
Gravel (2–76.2 mm)	10–16%

*Mineralogy of clay fraction*

Clay minerals	~ 48%
Illite	~ 37%
Chlorite	~ 8%
Vermiculite/smectite	~ 3%
Quartz	~ 27%
Total carbonates	~ 17%
Feldspar	~ 7%
Organic matter	~ 1%
Organic carbon	0.14–0.49%
CEC (cmol/kg)	26%
Dry density (kg/m <sup>3</sup> )	1800%

*Original pore water content (mg/l)*

Bicarbonate <sup>a</sup>	840
Chloride	100–160
Nitrate <sup>a</sup>	~ 20
Sulfate <sup>a</sup>	~ 3800–5500
Calcium	450–520
Magnesium	450–640
Potassium	45–70
Sodium	220–300
pH	7.6

*Synthetic pore water content (used as initial receptor solutions) (mg/l)*

NaHCO <sub>3</sub>	700
CaCl <sub>2</sub> × 2H <sub>2</sub> O	500
MgSO <sub>4</sub> × 7H <sub>2</sub> O	3400
KHCO <sub>3</sub>	150
NH <sub>4</sub> HCO <sub>3</sub>	20
Ca(NO <sub>3</sub> ) <sub>2</sub>	30

*Bacteria count (cfu/ml) and activity (ATP) (ng/g)*

As compared plug: at 14.5% moisture content, dominant pore size 0.1 μm and initial porosity  $n = 0.34$

HAB <sup>b</sup>	$1.5 \times 10^3$ – $5 \times 10^5$ (average: $9.0 \times 10^4$ )
SRB <sup>b</sup>	$1.5 \times 10^4$ – $1.5 \times 10^5$ (average: $7.5 \times 10^4$ )
ATP	1.5–4.8

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Levels of naturally occurring sulfates are high (0.03–0.04 M) and in excess of the relatively to slowly diffusing donors (VFA). Initially, this soil is oxidized and oligotrophic conditions exist. HAB: heterotrophic aerobic bacteria. SRB: sulfate-reducing bacteria.

<sup>a</sup> Identified electron acceptors naturally present in the Halton till pore water.

<sup>b</sup> Indigenous microorganisms capable of fermentation and sulfate and sulfate reduction.

inating under aerobic conditions, can also indicate the metabolism of facultative and anaerobic bacteria capable of fermentation. Both HAB- and SRB-BART™ have been widely used and the “PLPs” are well established. The BIOGAS-BART™ was used only to confirm the presence of methanogenesis, since the quantification of possible colony forming units is not yet well defined. Activity of the viable soil bacteria was checked by measuring adenosine-5-triphosphate (ATP). A bioluminescence assay was performed with a Optocomp I luminometer (MGM Instruments) following the procedure developed by the reagent kit supplier Celsis (former Lumac, Landgraaf, the Netherlands). Soil organic carbon content was obtained using modified Walkley–Black method (Allison, 1965).

### 3. Results and discussion: laboratory intrinsic degradation tests

The soil parameters changed significantly with time due to intrinsic degradation driven by the indigenous bacteria in soil. The change of porosity for the approximately 3-cm thick soil plugs (Layer 1 being the portion of the plug adjacent to the synthetic leachate) is given in Fig. 2. The growth of selected genera and activity of soil indigenous microorganisms induced by degradation of VFAs is shown in Fig. 3. Finally, the data used to assess the rate of intrinsic degradation of the selected volatile fatty acids are given in Figs. 4 and 5. Fig. 4a shows the concentration of a particular acid in the receptor solution with time, while Fig. 4b, c and d show the variation in concentration in the pore water vs. depth for acetate, propionate and butyrate at the time of test termination for the different cells. Fig. 5a shows the variation in concentration of VFAs

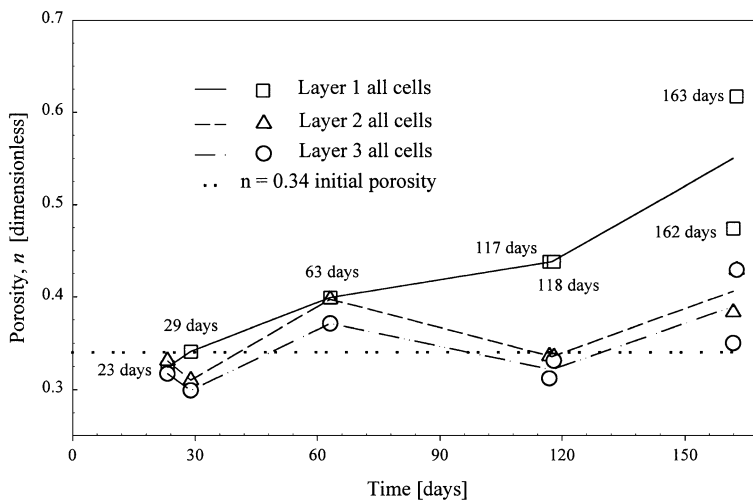


Fig. 2. Variation of porosity in 3-cm thick soil compacted plugs over time and position;  $n = \omega G_s / (1 + \omega G_s)$ , calculated for each layer at designated times based on water content,  $\omega$ .

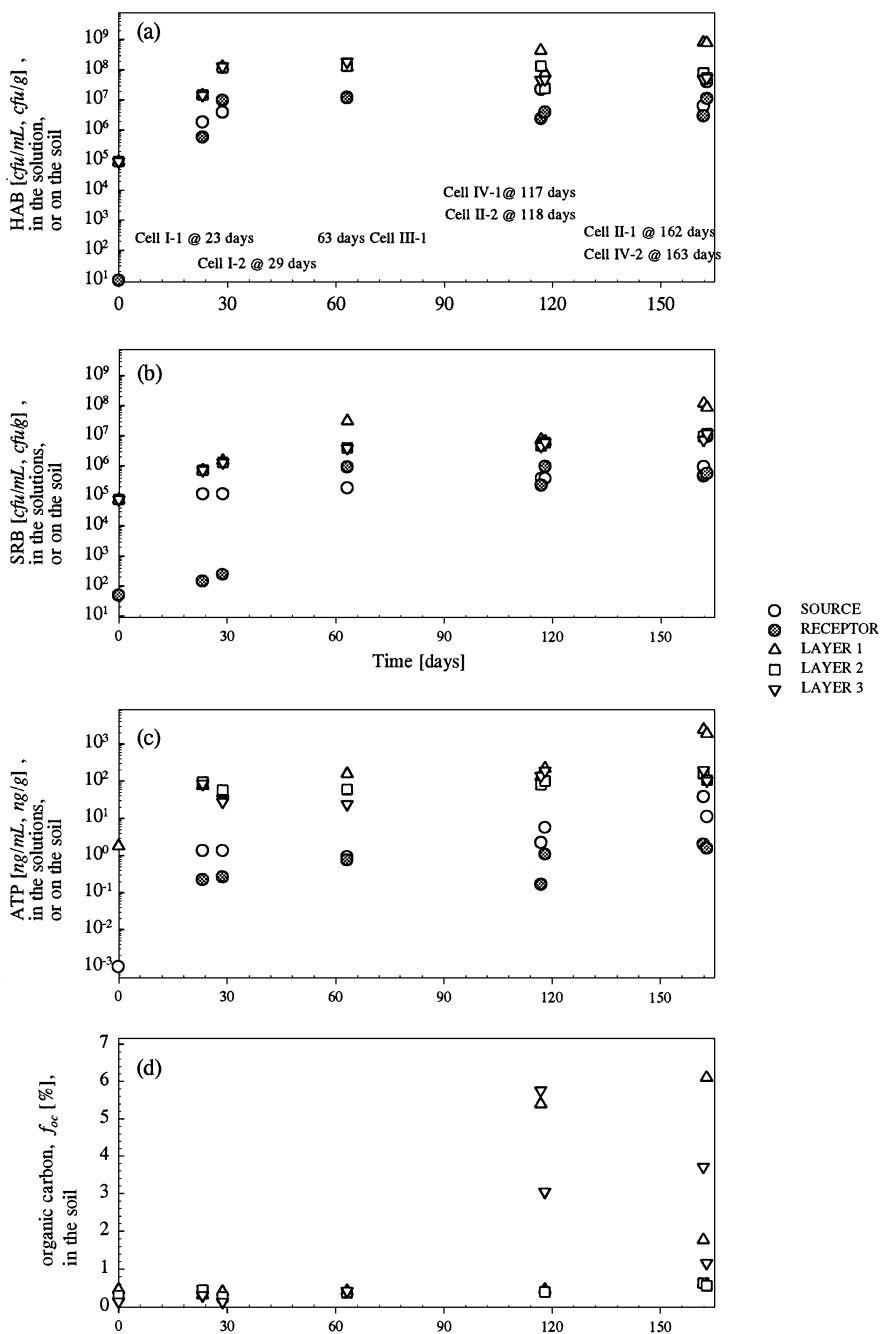


Fig. 3. Bacteria count and activity in 3-cm thick compacted soil plugs: (a) HAB, (b) SRB, (c) ATP, (d)  $f_{oc}$  organic carbon (values for soil [cfu/g] are expressed per dry mass).

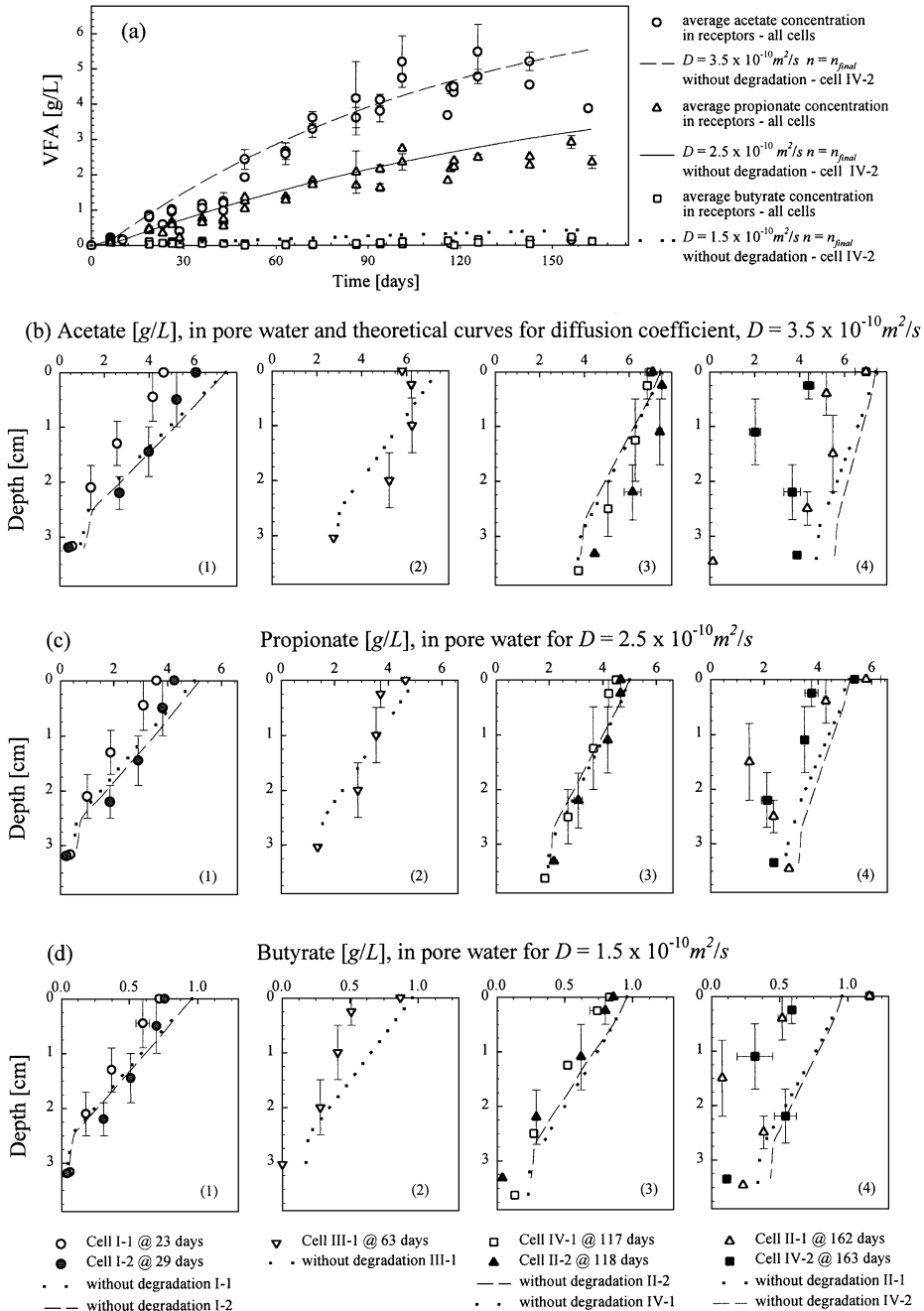


Fig. 4. Intrinsic degradation of VFAs in 3-cm compacted clayey soil: (a) receptor solution, (b) acetate depth profiles, (c) propionate depth profiles, (d) butyrate depth profiles.

in terms of dissolved organic carbon (DOC) in the receptor, while Fig. 5b shows the variation in DOC with depth at the termination of tests run for four different periods of time. The best estimates of the diffusion coefficients ( $D$ ) and half-lives for VFA decay were obtained using computer program POLLUTE v.6.5 (Rowe and Booker, 1999), which solves mass transport equation coupled with first order decay in the saturated pore space. A summary of the monitored and estimated parameters and their change in time is given in Table 3.

The soil plugs gradually started to change in appearance upon the exposure to the synthetic leachate. The initial rusty (tan, earth) color, characteristic of oxidized soil started subtly to fade. Dark gray or black tiny dots (<1-mm diameter) first appeared along the contact surface with the cell walls and soil, becoming visible on some of the plugs within less than a month of testing. Their sporadic appearance likely reflected nonuniform distribution of soil ferric and/or ferrous ions and sulfates, which turned into black iron sulfides upon microbially driven reduction.

The tests were terminated at different times to allow an evaluation of the migration of the VFAs through the clay plug with time.

### 3.1. The first termination (23–29 days)

As is evident from Fig. 4a, breakthrough of all of the VFAs was detected in the receptor of all cells, indicating that VFAs had diffused through the entire plug. Cells I-1 and I-2, terminated after 23 and 29 days, respectively, looked intact and unaffected by discoloration. The receptor solutions were clear but had a hint of  $H_2S$  odor.

The porosity of each layer cut from the soil plug(s) remained unaffected relative to the initial value of 0.34 throughout the entire thickness (see in Fig. 2 and Table 3). BART™ confirmed a uniform increase in number of heterotrophic aerobic bacteria (HAB) (including both heterotrophic aerobes and facultative anaerobes), as well as sulfate reducing bacteria (SRB) in the soil layers relative to the background levels, as shown in Fig. 3a and b, respectively (for layer designation, see Table 3). Relative to mid range initial count of  $9 \times 10^4$  cfu/g, on average the HAB population grew about 160-fold in 23 days in cell I-1 plug and about 1400-fold in 29 days in the Cell I-2 plug, as shown in Fig. 3a. The variation in count is within the expected bounds given the system heterogeneities. Relative to initial average population count of  $7 \times 10^4$  cfu/g, growth of SRB appeared uniform and less vigorous ( $\sim 10$ – $20$ -fold increase) for the two plugs as shown in the Fig. 3b. A high count of HAB in the source and receptor solutions in contact with the two tested soil plugs is indicative of growth of this ubiquitous group of bacteria. Measurements of ATP, shown in Fig. 3c confirmed a 10–30-fold increase in activity of the viable soil biomass in the soil plugs and detectable, although low, activity in the source and receptor solutions. Methanogens were not detected in any of these “early terminated” tests.

As expected, diffusion of VFAs did proceed without significant biodegradation at this early stage of testing. Measurements of the three VFA indicated very distinct diffusion profiles through the soil plugs, which could be reasonably modeled using diffusion coefficients given in Table 3. All of the acids would have been in their dissociated form at the pH tested and as such did not exhibit any measurable sorption onto the soil.

Table 3  
Change of soil porosity induced by intrinsic degradation of VFAs

Cell	"3-cm" thick compacted clay plugs													
	I-1		I-2		III-1		IV-1		II-2		II-1		IV-2	
	Terminated after (days)		29		63		117		118		162		163	
	$n^a$	$h^b$	$n$	$h$	$n$	$h$	$n$	$h$	$n$	$h$	$n$	$h$	$n$	$h$
Layer 1	0.32	0.90	0.34	1.00	0.40	0.50	0.44	0.50	0.44	0.50	0.47	0.80	0.62	0.50
Layer 2	0.33	0.80	0.31	0.90	0.40	1.00	0.34	1.50	0.34	1.20	0.38	1.40	0.43	1.20
Layer 3	0.32	0.80	0.30	0.6	0.31	1.00	0.31	1.00	0.33	1.00	0.35	0.60	0.43	1.00
Change of parameters modeled over the period (days)	0–55				55–140				140–163					
Porosity of Layer 1	0.34 (initial) or as calculated				0.44 or as calculated				0.55 or as calculated					
Diffusion coefficient ( $D$ ) ( $m^2/s$ )														
Acetate	$3.5 \times 10^{-10}$				$3.5 \times 10^{-10}$				$3.5 \times 10^{-10}$					
Propionate	$2.5 \times 10^{-10}$				$2.5 \times 10^{-10}$				$2.5 \times 10^{-10}$					
Butyrate	$1.5 \times 10^{-10}$				$1.5 \times 10^{-10}$				$1.5 \times 10^{-10}$					
VFA as DOC	$3.5 \times 10^{-10}$				$3.5 \times 10^{-10}$				$3.5 \times 10^{-10}$					

<sup>a</sup> Total porosity (–) assuming 100% saturation, calculated from measured gravimetric moisture content,  $w$ , and specific gravity,  $G_s$  (2.75 for Halton till) as  $n = wG_s / (1 + wG_s)$ .

<sup>b</sup> Measured thickness of the layer cut from the soil plug at the time of termination (cm).

Independent batch sorption tests confirmed such a lack of sorption, and consequently sorption was taken as “zero” in modeling (Hrapovic, 2001). The long-dash theoretical curve in Fig. 4a and b(1), generated with diffusion coefficient of  $D = 3.5 \times 10^{-10} \text{ m}^2/\text{s}$  for acetate, is in reasonable agreement with the data points collected for cell I-2. The datapoints collected for Cell I-1, although forming a similar diffusion profile, appeared to deviate from the calculated profile for  $D = 3.5 \times 10^{-10} \text{ m}^2/\text{s}$ . The propionate datapoints were reasonably consistent with the theoretical curve for  $D = 2.5 \times 10^{-10} \text{ m}^2/\text{s}$ , but with a somewhat better fit for the Cell I-1 data points than that generated for acetate (see Fig. 4a and c(1)). Pore water data are in good agreement with the theoretical curve for  $D = 1.5 \times 10^{-10} \text{ m}^2/\text{s}$  for butyrate (Fig. 4a and d(1)) for both Cells I-1 and I-2, which assumes no degradation.

In all cases, the greatest deviation from the theoretical curves is at the very top of the sample. Although this deviation could possibly be explained by biodegradation at or near the top of the plug given the increase in soil bacteria, this hypothesis is excluded based on the lack of other consistent evidence of consumption of substrates within 29 days of testing. As will be discussed in the following subsection, there was no evidence of biodegradation of propionate for at least the first 118 days of testing and better fits to the diffusion without degradation were obtained at 63 and 117–118 days than 23–29 days. Also, it will be noted that the discrepancy between the observed and the predicted diffusion profile is consistent for acetate, propionate and butyrate, which would not be expected if the explanation were degradation. A more likely explanation is that the source solution injected shortly before termination of these tests was lower than the target value, influencing the data near the top of the samples.

It appears that the rate of diffusive transport of the VFA substrates was unaffected by biodegradation over 23–29 day period of testing. The data presented in Fig. 4b(4b(1), c(1) and d(1) suggested that the diffusion coefficients inferred from the independent short term tests (Hrapovic, 2001) are applicable to these tests.

### 3.2. The second termination (63 days)

As testing progressed the changes in the soil plugs and solutions became more visible. The top surface of the plugs became dull gray, but the soil core in the middle remained rusty. After 40 days, the spotty formations of metallic sulfide on soil cell contact surface grew in size (2–5-mm diameter) and turned markedly darker, with tendency to localize at the bottom of the plug (i.e. at the point in contact with the receptor solutions). The source solution appeared increasingly cloudy with traces of unpleasant mix of “fishy” (possibly from *Pseudomonas*) and “septic” odor (possibly from soil *Enterobacteria*; BART™, User Manual, 1999), while the receptor remained transparent, but somewhat darkened and with unmistakable  $\text{H}_2\text{S}$  odor. The source reservoirs were constantly replenished with new, chemically reduced, synthetic leachate in order to keep constant concentration of the VFAs (and simulate anaerobic conditions that prevail in the waste fill). In contrast, the soil plugs were initially oxidized (remolded and compacted from the naturally oxidized soil), which might have been conducive to the growth of heterotrophic aerobic and facultative bacteria (HAB), which are more aggressive and more tolerant to variations in oxygen and pH than strict anaerobes. The generation of  $\text{H}_2\text{S}$  (rotten eggs) odor, a characteristic of the

activity of sulfate reducing bacteria (SRB), was qualitative evidence of the gradual development of reducing conditions in the receptor solutions, which started as oxidized ( $E_h = +80$  mV) synthetic pore water (see Table 2) in contact with initially oxidized soil.

During routine sampling at 36 days, Cell III-2 was damaged by an accumulation of gas in the receptor solution and it was not possible to recover data for the soil plug. The same incident almost reoccurred with Cell III-1 at  $\sim 63$  days, prompting test termination. The results plotted in the Fig. 2 indicate a small although noticeable increase in porosity for all three layers cut from this sample. HAB counts for Cell III-1 (see Fig. 3a) indicated a small 4-fold and marginal 1.5-fold increase for the source and receptor reservoirs respectively relative to the data at earlier times (23–29 days) and a uniform and apparently unchanged count of  $\sim 10^8$  cfu in the soil plug. SRB (Fig. 3b) exhibited noticeable  $\sim 30$ -fold increase in the top layer (i.e. Layer 1 in the contact with source solution) and only a slight ( $\sim 4$ -fold) increase in the rest of the plug, relative to earlier times of termination. It appears that within 63 days both HAB and SRB proliferated with VFAs as the predominately available substrate. This is particularly obvious from the SRB data plotted for receptor solutions (Fig. 3b). More importantly, the BIOGAS-BART™ reacted positive for methanogenesis in all three soil layers of the Cell III-1 plug after termination, indicating that reduced conditions conducive to methanogenesis had been established throughout the originally oxidized plug by 63 days. ATP measurements (Fig. 3c) demonstrated a significantly increased (100-fold) ATP content in the top layer. Organic carbon content of the soil (Fig. 3d) remained unaffected.

At 63 days, the breakthrough of the acids into the receptor solutions was characteristic of diffusion dominated transport evident from Fig. 4a. The comparison of the concentration data with the diffusion depth profile for acetate in Fig. 4b indicated a surplus of acetate with depth suggesting the possibility of acetate generation. Propionate gave a distinct diffusion profile (Fig. 4c) that was in reasonable agreement with theoretical curve suggesting that there was limited, if any, degradation of propionate. In contrast, the butyrate profiles for receptor concentration vs. time (Fig. 4a) and for concentration vs. depth (Fig. 4d) deviated significantly from the calculated diffusion profile assuming no degradation, suggesting that these losses could be attributable to degradation.

### 3.3. The third termination (118 days)

Increased activity of indigenous bacteria accompanied by a complete transformation of the appearance of the soil became fully evident by 118 days. A new “thinning” and/or “caviar”-like fluidized dark gray structure was formed at the top interfaces with the source solutions. At termination of Cells IV-1 and II-2 after 117 and 118 days, the top fluidized layer was  $\sim 5$ – $7$ -mm thick, blackened, swollen and cracked with tiny ( $\sim 1$  mm) gas bubbles sporadically popping up into the source solution. The middle of the soil plug was rusty and had visible although tiny (1–2 mm) cracks filled with liquid. The bottom of the plug had already recognizable 3–5 mm “black crust,” indicative of microbially driven sulfate reduction and consequent formation of metallic sulfides. Both solutions had strong odor, the source had a dominantly “septic” odor while the receptor had a more distinctly “rotten eggs” ( $H_2S$ ) odor.

Fig. 2 indicates an increase in porosity in Layer 1 while the porosity of the lower soil plugs remained unaffected relative to the initial reading. Although there was evidence of fermentation in the upper soil layer, HAB and SRB counts did not apparently increase (Fig. 3a and b). There was a moderate increase in soil ATP content (Fig. 3c). These data suggest that the activity of viable bacteria was virtually the same at the top and at the bottom and only slightly lower in the mid 1 cm of the particular plug. Methanogenesis was detected within all soil layers. Furthermore, after 118 days of testing both the source and receptor solutions tested positive for methanogenesis for the first time, suggesting that conditions for the syntrophic association of methanogens with more aggressive SRB were gradually being established. A significant increase in organic carbon content ( $f_{oc}$ ) was observed, particularly in the top and bottom soil layers, as indicated in Fig. 3d. This newly formed soil organic carbon was not examined in detail, however it is considered likely that it is linked to bacterial metabolism.

The pore water data shown in Fig. 4b(3) indicate a surplus of acetate, implying that it was being generated in the system. These extra amounts of acetate are considered to have originated from the breakdown of butyrate (Zinder, 1993). This “surplus” of acetate in Halton till plugs would suggest that rate of fermentation (breakdown of propionate and butyrate) at 118 days of testing may exceed the rate of anaerobic respiration (consumption of  $H_2$  and other fermentation products by terminal trophic bacteria), as observed by McMahan and Chapelle (1991). Propionate data for both IV-1 and II-2 plugs (Fig. 4c(3)), however, indicated consistent diffusion profiles which were still, (i.e. after 117 and 118 days) in excellent agreement with the theoretical diffusion profiles for  $D=2.5 \times 10^{-10} \text{ m}^2/\text{s}$ . Thus, for propionate, degradation could not yet be discerned. Butyrate data for both soil plugs (Fig. 4d(3)) were below those expected based on diffusion with no degradation. The receptor data for butyrate (Fig. 4a) clearly indicated losses, which given the circumstances, could only be associated with biodegradation. When the three acids were replotted as VFAs–DOC in Fig. 5a and b(3), the data suggested that DOC was conserved. The typical Fickian diffusion profile at 118 days further suggests that butyrate was converted to acetate without measurable breakdown of acetate. Data in the receptor solution seem to be encompassed in the range of  $D_{DOC}=(2.5-3.5) \times 10^{-10} \text{ m}^2/\text{s}$  assuming no mass (i.e. DOC) loss to degradation. Data for the pore water, expressed as DOC recovered from total measured VFAs concentration in the Fig. 5b(3) indicated excellent agreement with  $D=3.5 \times 10^{-10} \text{ m}^2/\text{s}$  diffusion (dotted) fit-line at  $\sim 118$  days. Thus, a satisfactory account could be obtained for the total mass, by considering the observed “surplus” of acetate but without taking into account loss of DOC due to degradation prior to 118 days. Nevertheless, the growth of SRB,  $H_2S$  odor, as well as methanogenesis, combined with the receptor profiles for butyrate provides qualitative evidence of mineralization. Bearing in mind that the amounts of acids (and consequently VFAs–DOC) applied to the indigenous microorganisms were substantial (g/l), this lack of measurable decline prior to day 117 possibly reflected a load-imposed lag to degradation. In addition, it is known (Chapelle and Lovley, 1990) that the rates of organic carbon metabolism (usually originating from  $\mu\text{M}$  or low mM amounts of VFAs) in deep aquifers and clayey confining beds are extremely slow (likely  $< \text{than } 10^{-4}-10^{-6} \text{ mM C/l/annum}$ ), yet these sediments remain metabolically active generating  $\text{CO}_2$  and/or methane. It appears that in this experiment, the

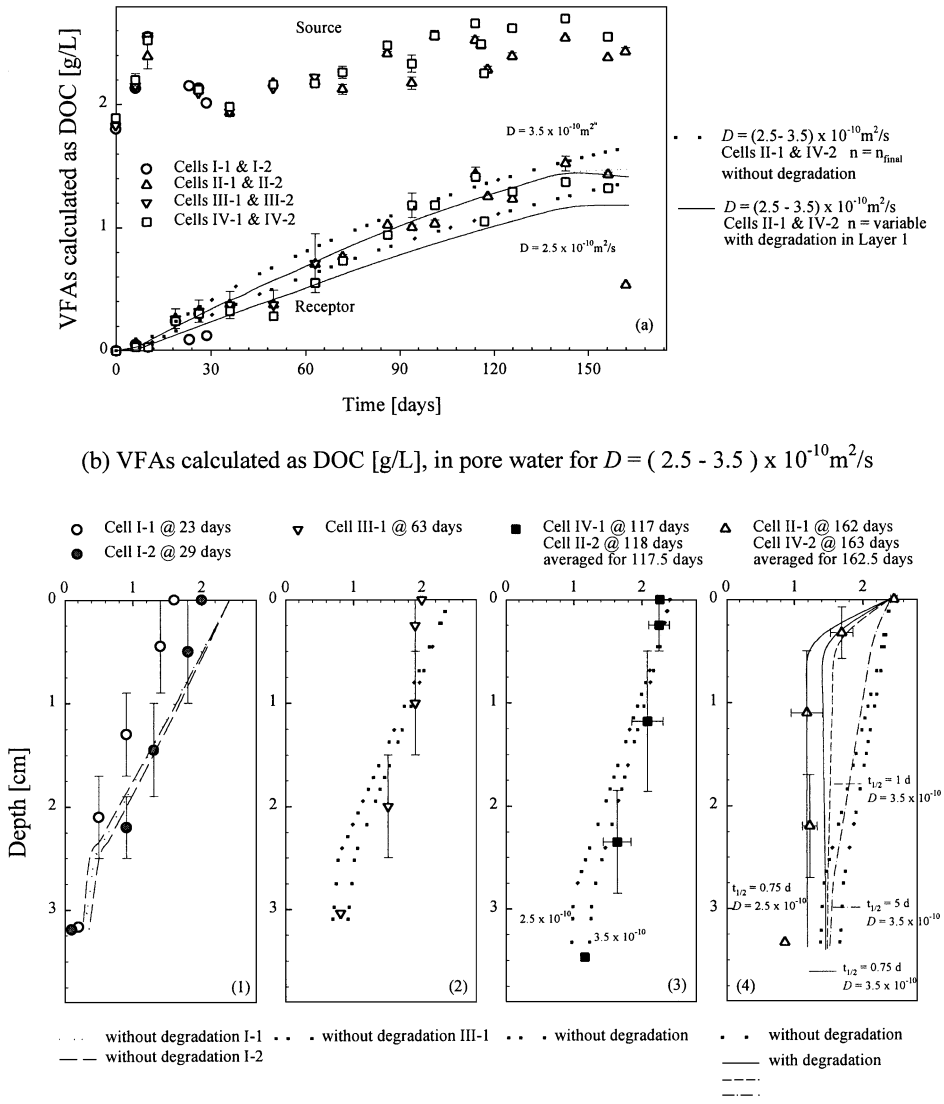


Fig. 5. Intrinsic degradation of VFAs (as DOC) in 3-cm thick compacted soil plugs: (a) source and receptor solution; (b) depth profiles.

VOCs-DOC degradation considered in Layer 1 only and modeled as:  
 $t_{1/2} = 0.75$  day in Layer 1 (0.65 top cm) after 140 day-lag;  
 porosity: initially 0.34 and as calculated at designated times (in Table 3):  
 $n = 0.34$  (0 to 55) days;  $n = 0.44$  (55 to 140) days;  $n = 0.55$  @ 140 days  
 (note: lines with the same symbol represent the range of diffusion coefficients where  $2.5 \times 10^{-10} \text{ m}^2/\text{s}$  gives lower DOC in solution or pore water than  $3.5 \times 10^{-10} \text{ m}^2/\text{s}$ )

mineralization of the VFAs tested could not be measured and discerned from diffusion even at such late stage, although the generation of fine gas bubbles which could only be released from the fermentation of these acids, could be seen by the naked eye.

### 3.4. The fourth termination (163 days)

The remaining two cells (II-1 and IV-2) were terminated after 162 and 163 days, respectively. At this time, the solutions in contact with soil had pungent decaying odor, “septic” prevailing in the source and “rotten eggs” ( $\text{H}_2\text{S}$ ) in the receptor, pointing to evident thriving of gas generating bacteria and development of anaerobic conditions. The top soil layers (Layer 1) were dark gray and expanded with thinner consistency than observed earlier, while the soil cores remained rusty in the middle.

There was (see Fig. 2) a significant increase in the porosity of Layer 1 (from  $\sim 0.34$  to  $0.62$ ) for Cell IV-2, while the increase for Cell II-1 was more moderate (from  $0.34$  to  $0.47$ , Table 3). Generally, the observed increase in porosity in both plugs was considered to be a direct consequence of VFA fermentation and the generation of gases associated with terminal degradation processes (Chapelle, 1993). With an increase in porosity, there was a slight increase in HAB and particularly SRB counts (10-fold) and ATP ( $\sim 10$ -fold) in the top layer of each cell, as can be seen in Fig. 3a, b and c. In other layers where there was no significant change in porosity, the saturation pattern observed with the count of indigenous microorganisms was maintained. For soil sampled from Layer 1, the BIOGAS-BART™ reacted after only two days of incubation compared to 20 days (or longer) at the earlier times of sampling, indicating that methanogenic activity had increased at this (final) stage of testing. Such fast response also indicated that the top interface became highly reduced, with hydrogen levels conducive for methanogens (possibly  $E_h < -0.3$  V,  $\text{H}_2 \geq (10\text{--}40$  ng/l or  $7\text{--}20$  nM)] (Chapelle, 1993). However, the environment at 163 days was also conducive for sulfate reducers, thus, becoming the locus of very intense activity of the two hydrogen utilizing bacterial groups.

Thriving of sulfate reducers and methanogens has been observed in natural anoxic sediments (Senior et al., 1982; Phelps et al., 1988) where the slow and steady decay of complex organic matter takes place. Since these “terminal” bacteria consume  $\text{H}_2$  (produced by propionate and butyrate degraders), as well as acetate to produce  $\text{H}_2\text{S}$ ,  $\text{CO}_2$ ,  $\text{CH}_4$  it was anticipated that such terminal and even visible gas generation would eventually result in decreasing amounts of VFAs in the receptor solutions and Halton till pore water.

The evident increase in porosity of the expanded top layer noted at this late time of testing coincided with the increased activity of the indigenous soil bacteria and consequently with the increased rate of VFAs (DOC) degradation. The small pore size ( $0.1$   $\mu\text{m}$  initially in compacted plugs tested) could be expected to restrict growth and transport of the soil bacteria (typically of similar,  $0.2\text{--}0.4$ - $\mu\text{m}$  size) and is often listed as imposing constraints to microbial activity (Chapelle and Lovely, 1990; Frederickson et al., 1997). Thus, the eventual beginning of VFA degradation within 3-cm thick Halton till plugs could be attributed to the relief of this “physical” constraint rather than to the high count/and or activity of fermenting and  $\text{H}_2$ -utilizing bacteria. Indeed, the most prominent increase in count of HAB, SRB and ATP occurred at early stage of incubation after only  $\sim 30\text{--}50$  days (Fig. 3a, b and c), at which point no increase in porosity in the top layers was detected (Figs. 2 and 3). It is hypothesized that during this incubation of about 2 months, soil microorganisms were growing at the expense of evidently very low levels of VFAs, and available carbon was used for biomass synthesis rather than conversion and mineralization (gas production) of VFAs. At the final termination, the active population appears stable

(at  $7.5 \times 10^7$ – $8 \times 10^8$  and at  $\sim 1.5 \times 10^7$ – $1 \times 10^8$  cfu/g for HAB and SRB, respectively), and capable of VFA fermentation and methanogenesis.

As a result of the microbial activity, the pore water concentrations at 163 days indicated loss of acetate, propionate and butyrate. Acetate degradation was manifest only in the pore water (see Fig. 4b(4)), while the receptor solution profiles (Fig. 4a) still indicated “generation” (i.e. an increasing trend) with only the final two points that suggested decline. For the first time in the test series, the propionate pore water profiles (Fig. 4c(4)) indicated significantly lower readings than predicted for “diffusion-only” without degradation. The propionate concentration (Fig. 4a) for receptor solutions showed a declining pattern, more obvious than for acetate, suggesting notable degradation from about 110 days onward. Eventual degradation of propionate is to be expected since both facultative heterotrophs and sulfate reducers (thriving in the “fluidized” Halton till; Fig. 3) could cause anaerobic oxidation of propionate and butyrate to acetate (Dolfing, 1988; Widdel, 1988). The same observation holds for butyrate concentrations for these final terminations at 163 days: both pore water and receptor solution readings were significantly lower than those predicted for diffusion with no degradation. The pore water data for the three acids displayed noticeable scatter and patterns which did not form neat diffusion profiles as was seen at earlier times (Fig. 4b(1–3), c(1–3) and d(1–3)). Since the most of the scatter appeared upon intensified degradation, analytical error was ruled out. Rather, the observed patterns were attributed to the uneven rates of acetate, butyrate and propionate breakdown, as well as the rates of gaseous product conversion, all mediated by a mixed culture existing in such a complex miniecosystem. At this late stage of testing, it seems that quite high VFA contamination was finally being removed by indigenous microorganisms from Halton till without culture enrichment and under adverse mass transfer conditions.

When the measured acid concentrations were plotted as DOC ( $C_0 = 2.4$  g/l as DOC or 17.4 g/l as COD) (Fig. 5), some of the scatter diminished and the degradation trend became more obvious. The depth profile in Fig. 5b(4), plotted from averaged readings for both II-1 and IV-2 Cells indicated somewhat uniform DOC distribution along the plug thickness with a tendency to equilibration with the DOC in receptor solution. Unlike the depth profiles collected for earlier times (Fig. 5b(1–3)) when degradation was not effective, this last (Fig. 5b(4)) profile at  $\sim 163$  days did not have distinct diffusion pattern. The profile collected for the receptor solution concentrations (Fig. 5a) was, however, quite characteristic of diffusion dominated transport and the data points remained within the proposed DOC diffusion bounds of  $(2.5\text{--}3.5) \times 10^{-10}$  m<sup>2</sup>/s almost until the very last sampling. Thus, the results of the final stage of the experimentation with this assembly revealed that intrinsic degradation of the VFAs tested proceeded very slowly becoming readily measurable only in the soil (pore water) after  $\sim 163$  days of testing, while the receptor solutions, with the exception of butyrate, remained only marginally affected by degradation until about 160 days. This finding is somewhat different from one reported by McMahan and Chapelle (1991), who showed that organic acids transported by diffusion from clayey (organic rich, thus, not oligotrophic) aquitard into the aquifer, mainly degraded in the aquifer. Although degradation in the receptor solution (hypothetical aquifer) could not be ruled out, it was clear from the experiments that VFAs could degrade in this compacted clayey soil (Halton till). The pore water profiles provided unequivocal evidence that intrinsic degradation dominantly took place in the soil. This

natural clayey soil with quite high sulfate ( $\sim 5$  g/l) and bicarbonate ( $\sim 1$  g/l, see Table 2) content in pore water was the source of active bacterial groups (Table 2) which had developed the obviously advantageous ability to ferment VFAs and to respire using electron acceptors other than oxygen (Chapelle, 1993; Kölbl-Boelke et al., 1988).

Modeling of degradation of VFAs, expressed as DOC (dissolved organic carbon), was conducted assuming that degradation would be localized and dominant at and near the two interfaces as suggested by the observed increased microbial activity at these locations. A simple approach assuming first order decay at the top source solution/soil interface (i.e. “Layer 1”) was adopted for coupled reaction under dominant diffusive transport. The use of first order decay is likely to be valid where there is no significant additional growth of biomass at the expense of growth substrate and growth (test) substrate concentration is low. The first of these conditions is likely justified, the second somewhat more questionable but as will be seen the first order approach did provide useful results. This simulation took into account a gradual change of porosity in time as given in Table 3 and diffusion coefficients inferred independently (Hrapovic, 2001). It was assumed that after 140-day lag, degradation prevailed in the top (expanded) layer at a fast rate, (i.e. half-life of 0.75–1 day). This resulted in a very good fit to the collected data, as shown by the solid lines plotted in Fig. 5a for receptor solution and in Fig. 5b(4) for the latest (and last) pore water profile recovered at 163 days. The details pertaining the changes of the parameters are given in the legends to Fig. 5 and Table 3.

It is recognized that the approach used to predict an impact of VFAs–DOC (concentrations) in the tested system, relies on certain simplifications, which might not hold in a natural large-scale system. Intrinsic degradation of VFAs–DOC is modeled as a first order reaction, but without identifying the underlying mechanism of biodegradation. At the same time, it was evident from the tests that indigenous microorganisms grew at the expense of the degraded VFA substrates. The rate of the microbial growth in these compacted plugs could not be assessed with certainty due to its complexity and dependence on the system itself. Growth apparently followed a logistic-like saturation pattern, with maximum growth at the early stage of incubation when there was no measurable removal of the growth substrate. No attempt was made to explain degradation using a model that could account for growth of indigenous microorganisms on a particular substrate (e.g. a Monod model) or to couple the growth to the substrate removal because such a simulation would require more parameters be known in advance than were available. In view of the limited number of data points that could be collected even in laborious experiments such as those presented herein, it was considered that such complex simulation was not justified.

#### 4. Conclusions

Results of this study demonstrate that acetate, propionate and butyrate are subjected to intrinsic degradation mediated by indigenous microorganisms in the soil. Each of the acids tested exhibited diffusive breakthrough, and was considered to be unaffected by degradation for at least the first 50 days of testing. As time elapsed, microbial activity became more evident, particularly in the upper centimeter of the soil plug in contact with the source of contaminants. This upper interface was gradually fluidized by gases generated

from VFA fermentation, its compacted structure loosened and favorable conditions arose for enhanced mass transfer and microbial growth. The biodegradation was largely localized in this reduced and fluidized soil layer. However, regardless of early observed changes in soil appearance, the significant increase in bacterial count and ATP content, as well as confirmed methanogenesis, the consumption of VFAs was small and delayed for 140–180 days. This was attributed to the high concentration of VFAs (2.4 g/l as DOC) introduced to this, initially oligotrophic microenvironment, as well as on severe limitation of exchange of incoming carbon and nutrients through compacted clayey soil. Once the microorganisms acclimated, the fermentation advanced and the acids, expressed as DOC, were removed from the soil at very fast rates, resulting in short half-lives of 0.75–5 days.

Based on the findings from the laboratory tests, which simulated very adverse conditions for degradation, it is hypothesized that an environment conducive to intrinsic degradation might develop in actual waste disposal facilities. It is recognized that field conditions in the real landfill and field compacted clay liner will be more adverse than simulated in these tests due to the heterogeneities of the large scale system and limited means of controlling the inputs. The presence of the waste fill and the confining stress it exerts to the compacted clay liner will impose additional limitations to the already nonoptimized degradation. Therefore, intrinsic degradation of VFAs in compacted clay liners and confining deposits is expected to be slower than in the tests reported herein. However, even the slow degradation rates after an acclimation period of many months, could potentially be very effective in the removal of significant contamination. While field validation is highly desirable, the data reported in this paper do suggest that it may be possible to more effectively design landfill barrier systems to take advantage of the intrinsic degradation processes due to bacteria present in soil.

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