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ORIGINAL ARTICLE

Aerobic microbial activity and solid waste biodegradation in a landfill located in a semi-arid region of Argentina

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Abstract The sanitary landfill of Bahía Blanca city, Argentina, located in a semiarid region, receives 270 tons/day of municipal solid waste. The objectives of the study were: (1) to determine aerobic microbial activity (MA) and waste biodegradation taking into account the age of the waste, and (2) to evaluate the degree of waste stabilization of the oldest cell. MA was determined by CO_2 release and chemical characterization in four cells of different ages of landfill. Furthermore, enzymatic methods were used to determine the effect on MA of leachate addition. Low annual rainfall, the sandy characteristics of the soil, and the use of landfill techniques where rigorous compaction is not performed, contribute to maintaining microenvironments with a sufficient quantity of O_2 to support aerobic degradation processes. Under these conditions, degrading processes are faster, thus explaining why MA in the landfill cell closed in 1992 is stabilized and behaves as an anthrosol. In areas where MA is limited by lack of water (arid and semiarid zones), refuse degradation is limited to favorable humid periods. For that reason, operations favoring fast and efficient aerobic activity should be implemented.

Keywords Landfill · Microbial activity · Leachate recirculation · Semi-arid region

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Introduction

Waste generation is inherent to human beings and results from their activities. This problem affects the entire world, particularly cities with a high level of urbanization.

Municipal solid waste (MSW) landfills are generally operated by conventional landfilling techniques, where anaerobic conditions are created within the landfill waste. Under anaerobic conditions, waste stabilization processes are slow and may increase the potential risks to human health and the environment (Erses et al. 2008). Waste stabilization generally occurs after periods of longer than 30 years and may extend to two or more centuries (Komilis et al. 1999; Warith 2003). Increasing attention is being given to the enhancement of waste stabilization to reduce the time required for waste degradation, and leachate recirculation and aeration were shown to be successful in accelerating the process of bioreduction within a landfill cell (Chugh et al. 1998; Reinhart and Townsend 1998; Komilis et al. 1999; Warith 2003). The main advantages of aerobic landfill technology are: rapid degradation, detoxification and stabilization of organic wastes, and reduction of emissions of methane gas, volatile organic compounds and odor. Such advantages both minimize environmental risks and allow for greater free space availability, thus extending the life of the landfill site and reducing costs (Reinhart and Al-Yousfi 1996; Triantafillopoulos et al. 2001; Hudgins and Read 2001; Erses et al. 2008).

Microbial activity (MA) in a sanitary landfill can be affected by moisture, pH, temperature, nutrients, the presence or absence of toxins, particle size and oxidation reduction potential (Reinhart and Al-Yousfi 1996). One of the main factors required for microbial biodegradation is moisture since lack of water is generally responsible for retarding degradation of MSW in conventional landfills. Furthermore, the potential moisture present is seldom distributed uniformly (Chugh et al. 1998; Dollar 2005).

According to estimations by the Municipal Office of the Under-Secretary for the Environment of the Argentinian government, 270 tons MSW are generated per day in Bahía Blanca city, which is located in the semi-arid pampean region of Argentina, characterized by 600 mm annual rainfall and alternating dry (no more than 100 mm) and humid periods (no more than 700 mm) (INTA 2011).

Bahía Blanca landfill has been operated by conventional landfilling techniques since 1988. Since there is no information on the degree of mass degradation in residues in this sanitary landfill, our study focused on its chemical and microbiological characterization. On the other hand, the oldest cell (closed in 1992) was chosen to evaluate the degree of stability because it was the only one old enough to show signs of such stability.

The objectives of this study were: (1) to determine aerobic MA and waste biodegradation taking into account waste age, and (2) to evaluate the degree of waste stabilization of the oldest cell examined.

Materials and methods

Bahía Blanca is located in the southwest of Buenos Aires province (Argentina), on the Atlantic Ocean coast, at 38° 44′ S and 62° 16′ W.

Microbial activity and chemical characterization of cells of different age

Sample collection

Samples from four cells of the landfill (closed in 1992, 1995, 1997 and 2001, respectively) were dug with an excavator (caterpillar). Material was extracted at a depth of 1, 2, 4 and 6 m in 3×3×6 m digs. Containers with 15-kg capacity were used in the sampling process to obtain representative samples. Samples were transported to the laboratory, where large objects (>30 cm) such as paper (9%), glass (6%), metal (2%), plastic objects (10%) and others were removed. A pool was made with the remaining organic material (58%) so as to perform microbiological and chemical determinations.

Determination of C mineralization rate

Microcosms in airtight containers with a capacity of 1 l were prepared. The technique of C mineralization by capture of CO_2 in NaOH and titration with HCl (Zibilske 1994) was used. From each sample, 100 g waste was processed and incubated for 15 days at 28°C. Determinations were made in duplicate. Mineralized organic carbon was calculated from these values (C–CO_2), considering the efficiency of microbiota assimilation of 40% (Coyne 2000).

Chemical determinations

Samples were mineralized by acid digestion at 440°C with a Hach Digesdahl®, using sulfuric acid (H_2SO_4,95–98%) and hydrogen peroxide (H_2O_2, 30%) following Hach's technique (Barbarino and Laurenço 2009). The remaining mineral fraction was separated from the solution by filtration with 0.8-μm pore size fibreglass, and dried at 105°C overnight before being weighed. The weight difference between the original sample and the solid remains was considered total organic matter (TOM). The mineralized and filtered solution was used for determining total P and N. P concentration was estimated following Murphy and Riley (1962), and N was quantified spectrophotometrically as ammonium based on Berthelot's reaction as modified by Verdouw and colleagues (1978). Oxidable organic matter (OxOM) was determined by oxidation with potassium dichromate ($K_2Cr_2O_7$) 1 *N* and H_2SO_4, according to the technique described by Walkley and Black (1934). Soil pH was electrometrically measured in a 1:5 soil: distilled water suspension. At the same time, one of the samples was dried at 105°C in order to measure moisture content by gravimetry. The following metals were analyzed according to Koirtyohann and Wen (1973): Cd, Pb, Cr, Mn, Ni, Cu and Zn.

Statistical analyses

Results from chemical analysis and biological activity (CO_2 release) were subjected to principal components analysis (PCA) using standardized data, and a correlation matrix was prepared using the program InfoStat (Di Rienzo et al. 2008).

Effects of leachate injection in cell 1992

During the final state of landfill stabilization, nutrients and available substrates become limiting and the biological activity shifts to relative permanent dormancy. If the 1992 cell was stabilized, the nutrients provided by the leachate injection would be able to maintain high MA only during the period for which carbon substrates were available.

Determination of the C mineralization rate

Microcosm experiments similar to those mentioned above were performed with waste from the oldest cell (1992) so as to determine the degree of cell stabilization. Leachate incorporation in a 5% v/v solution (Bouchez et al. 2001) was compared with a control.

Determination of the C mineralization rate was carried out in the same way, but in this case the NaOH used for titration was renewed every 24 h to detect when the highest level of activity is reached after the leachate addition. Values were expressed as the rate of daily released CO_2.

Since leachate addition may affect microbial activity owing to different contributions such as nutrients, moisture and microbiota in a second stage, addition of leachate was compared with that of sterile distilled water (5% v/v) with the purpose of evaluating exclusively the effect of moisture. In this case, MA was determined by hydrolysis of fluorescein diacetate (FDA) and quantification of dehydrogenase activity (DHA) (Zabaloy et al. 2008). The technique was changed because there was little available material and it was not possible to perform a new sampling (enzymatic techniques require smaller sample amounts).

Hydrolysis of FDA This is a simple, quick and widely accepted method for measuring total MA in different environments. FDA is a colorless compound that can be hydrolyzed by different microbial enzymes. The final product is fluorescein ($C_2OH_{12}O_5$), a compound which can be quantified by spectophotometry (Schnürer and Rosswall 1982). MA was measured by hydrolysis of FDA following the technique described by Zabaloy et al. (2008).

Determination of DHA activity Dehydrogenases play an important role in oxidizing organic matter and, therefore, measuring their activity yields information about the metabolic activity of microorganisms in a particular environment (Friedel et al. 1994). 2,3,5-Triphenyltetrazolium chloride (TTC) is a colorless compound that is reduced to triphenyl formazan (TPF) by DHA, becoming colored and able to be quantified by spectrophotometer (Tabatabai 1994). The technique described by Zabaloy et al. (2008) was used. For both enzymes, determinations were performed on days 1 and 8 after leachate addition.

Statistical analyses

MA results and the standard error of the mean (SE) were expressed based on a total of three replicates. The same data were subject to ANOVA of one factor to compare differences among the treatments using InfoStat tools (Di Rienzo et al. 2008). Probability values were established at a level of 0.05 for all statistical tests.

Results and discussion

Microbial activity and chemical characterization of cells of different age

Climate (semi-arid region), edaphological characteristics (sandy soils) and not totally compacted waste suggest the persistence of microenvironments supporting aerobic metabolism; therefore, aerobic microbial communities were studied. In future research, it would be interesting to complete the measurement of MA quantifying ATP production, which may enable us to ascertain if the predominant metabolic activity is aerobic or anaerobic (Madigan et al. 2004; Nannipieri et al. 2003).

Under the experimental conditions applied, respiration (CO_2 release) is related directly to the aerobic metabolic activity of microbial populations. Microorganisms respire at higher rates in the presence of large amounts of easily bioavailable organic matter, while respiration rate is slower if this type of material is scarce or toxic compounds are present (Barrena et al. 2005).

During the sampling periods, the Town council of Bahia Blanca performed methane measurements that were neither systematic nor in all cells. The results obtained were: 0.023% (cell 1992), 0.002% (cell 1995) and 0% (cell 2001) (personal communication). These results agree with those obtained in bioreactors submitted to aeration, where methane production is 0%, whereas rsamples not receiving aeration give a result of 30% (Leikam et al. 1999).

MA and the evaluated chemical parameters values were very variable, reflecting the heterogeneity of the studied material and the complexity of the system (Table 1). The oldest cell (1992) iswasthe only one that presented a distribution similar to a soil profile, where there is a larger quantity of organic matter and nutrients (C, N, P) on the surface than at depth. Figure 1 (samples 1, 2, 3 and 4) illustrates that the samples are grouped in relation to C, pH and MA variables. This may indicate a stabilizing tendency of waste, showing evolution into an anthrosol (soil generated by human activity) (Soil Survey Staff 1999).

As cell 2001 was not appropriately managed, the current depth is 4 m, rather than 6 m as in the others. Correlations could be established between the recorded MA and TOM in data from cell 1992 (Fig. 1). The pH was mostly alkaline, possibly due to the type of soil and the marine influence. Samples from cells 1995 and 1997, except those from the surface, showed an inverse relationship between pH and MA (mg of CO_2) (Fig. 1), which is particularly evident in samples 6, 8, 10 and 11. Samples from cell 2001 show

Table 1 Microbial activity (CO_2 release) and chemical variables of solid waste for each cell and depth. *TOM* Total organic matter, *Min OC* mineralized organic carbon, *OxOM* oxidable organic matter

Sample		CO_2 (mg g^{-1} waste)	TOM (%)	Min OC (mg g^{-1} waste)	OxOM (%)	C (%)	C/N	C/P	pH	Moisture (%)
Cell 1992										
1	1 m	15.7	11.8	7.2	4.4	6.8	17	23	7.4	15.6
2	2 m	11.5	9.1	7.0	2.5	5.3	15	35	8.4	19.6
3	4 m	10.3	7.5	4.7	1.6	4.3	20	47	8.1	14.1
4	6 m	7.5	2.9	5.2	1.1	1.7	10	21	8.3	15.3
Cell 1995										
5	1 m	6.7	15.3	3.0	6.1	8.9	15	90	8.0	39.2
6	2 m	2.2	25.1	1.0	0.7	14.6	91	256	9.2	16.8
7	4 m	9.4	21.1	4.3	3.0	12.2	40	149	8.7	20.6
8	6 m	3.6	22.0	1.6	1.8	12.8	52	169	9.1	20.8
Cell 1997										
9	1 m	14.6	31.6	6.6	7.0	18.3	28	109	6.9	32.9
10	2 m	5.8	28.7	2.6	1.7	16.6	88	153	8.6	24.9
11	4 m	6.8	19.2	3.1	0.8	11.1	39	117	9.3	18.7
12	6 m	11.7	27.1	5.3	2.7	15.7	57	169	9.1	18.7
Cell 2001										
13	1 m	16.3	29.7	7.4	5.5	17.2	47	212	7.9	29.2
14	2 m	12.2	45.0	5.6	5.6	26.1	26	770	8.5	28.1
15	4 m	6.2	38.7	2.8	7.3	22.5	29	109	8.9	35.8

greater TOM and moisture content. The PCA reveals that MA is influenced by different abiotic parameters and their interaction (Table 2).

The ratio of C/N and C/P reflects the balance of nutrients and is critical to ensuring good biodegradation; recommended ranges for C/N are 30–40, and for C/P less than 120 (US Composting Council 1994). In samples 6, 8 and 10, the ratio of C/N is high and MA is negligible (Table 1). These values could indicate the presence of recalcitrant organic matter.

N is considered an important regulator of decomposition rate. As a component of the C/N ratio, it is used as a measurement of the quality of the carbonaceous substrate and a predictor of the decomposition rate. Yet, this ratio is not always useful because the amount of total N is not the same as its availability. Samples 4, 5 and 15 show C/N ratios favorable for degradation, but MA is less than in other samples with greater C/N rate, thus indicating poor N availability.

On the contrary, samples 12 and 13 show non favorable rate C/N (57 and 47, respectively) and high MA (11.7 and 16.3 mg CO_2 g^{-1} waste). It is probable that, in these samples, the microbiota has assimilated an exogenous contribution of N, for example biological fixation of N, since a wide diversity of bacteria possess this capacity (Martínez Molina and Velásquez 1992). Nitrogen fixing

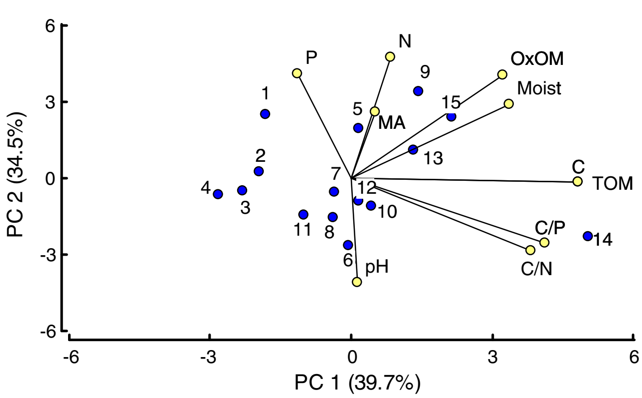

Fig. 1 Principal components (PC) analysis applied to microbial activity (MA) values and abiotic variables: pH, moisture (moist), oxidable organic matter (OxOM), C, N, P, C/N, C/P, total organic matter (TOM)

Table 2 Correlations of principal components (PC) with variables

Variables	PC 1	PC 2
TOM	0.95	−0.03
OxOM	0.64	0.74
C	0.95	−0.03
C/N	0.76	−0.53
C/P	0.81	−0.46
N	0.17	0.87
P	−0.22	0.75
pH	0.03	−0.75
Moisture	0.66	0.54
MA	0.1	0.48

bacteria such as *Bacillus megaterium*, *Bacillus brevis*, *Bacillus azotoformans*, *Alcaligenes* sp., *Klebsiella pneumoniae*, and *Pseudomonas pseudoalcaligenes* (Xie et al. 2006) were isolated from the refuse and leachate at an Atlanta landfill (Hale Boothe et al. 2001). This ability is also found among methanogenic (Magingo and Stumm 1991) and methanotrophic (Wilshusen et al. 2004) bacteria. This may explain why MA can occur with very unfavourable ratios of C/N. The role of N in regulating organic matter decomposition is too complex to be generalized by measurement of total N concentration (Sinsabaugh et al. 1993).

Mineralized organic carbon in the different samples incubated at constant temperature for 15 days varied between 1 and 7.4 mg C g^{-1} refuse (Table 1). Erses et al. (2008) reported that an aerobic bioreactor under laboratory conditions consumed 1.2 mg C ml^{-1} leachate after 374 days of operation. Although the conditions and methodologies in that study differed from those used here, the higher mineralization values in our work are worth stressing, considering that we conducted a short-term incubation (15 days vs ~1 year in Erses et al. 2008).

Metals that could have a critical toxic effect towards microbial populations (e.g., Pb, Cr) were not detected, and the concentrations of other metals seemed not to interfere with microbial processes according to our results (Table 3).

Effects on solid waste of leachate addition

OC mineralization in waste containing leachate

Cell 1992 was selected to assess whether organic waste was really stabilized or whether leachate contributed significantly to increased degradation. This new assay was performed to investigate the degree stabilization since it was not expected that a 12 year-old cell would be stabilized with conventional landfilling techniques.

CO_2 release values of samples collected at different depths followed the same trend. Stimulation ($P<0.05$) of MA due to leachate addition was observed within 24 h (Fig. 2).

The positive effects of leachate recirculation on the stabilization and reduction of waste volume, and the production of gases have also been established in sanitary landfills in the United States (Hudgins and Harper 1999), Austria (Reinhart and Al-Yousfi 1996) and Greece (Triantafillopoulos et al. 2001).

Results from microcosms without added leachate indicate very low MA values, which remain stable for 7 days, indicating basal respiration—dormancy—(Fig. 2). Since respiration values lower than 7 mg CO_2 g^{-1} waste day^{-1}

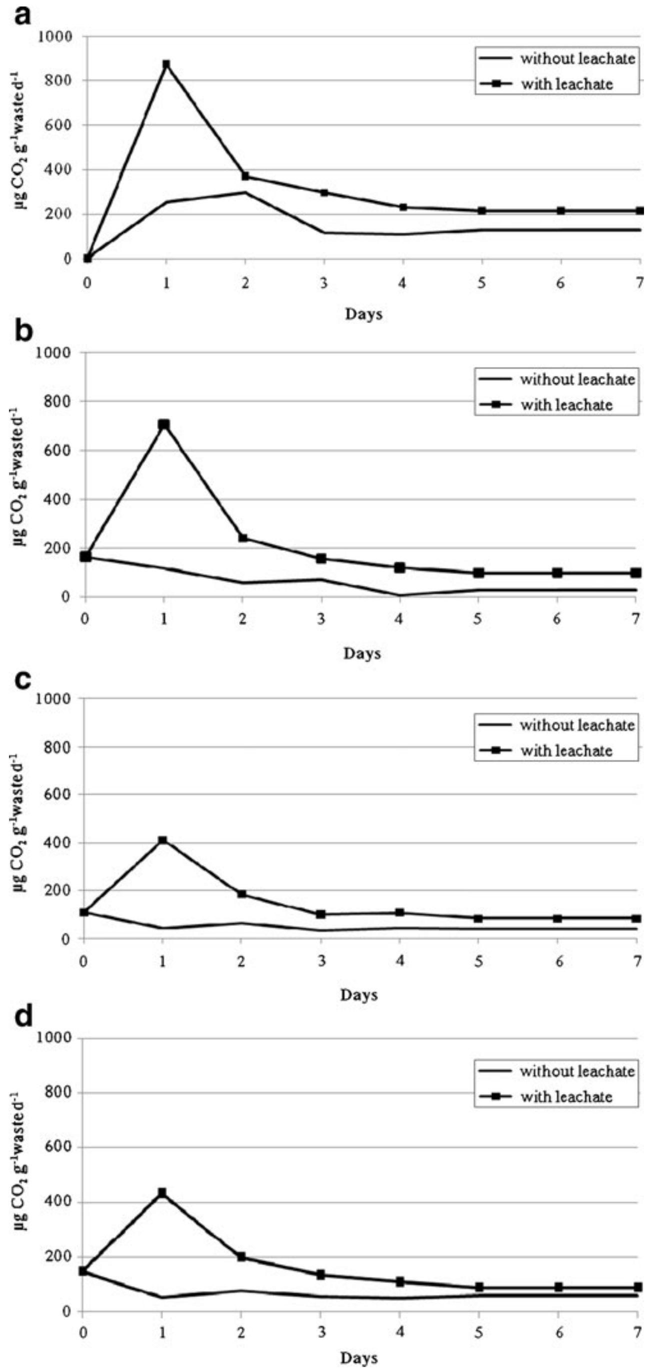

Fig. 2 Rates of CO_2 release from municipal solid waste (MSW) from different depths after incorporation of leachates. **a** 1 m **b** 2 m, **c** 4 m, **d** 6 m

Table 3 Metal contents expressed in μg g^{-1} dry matter determined using integrated samples (each of the four samples is formed by aliquots of different depths)

Cell	Cd	Pb	Cr	Mn	Ni	Cu	Zn
1992	nd[a]	nd	nd	220.9	nd	nd	nd
1995	nd	nd	nd	223.9	nd	46.0	123.3
1997	nd	nd	nd	195.4	181.5	61.4	226.7
2001	nd	nd	nd	139.1	155.1	nd	121.3

[a] Values below the detection limit of the method used

correspond to mature and well stabilized compost (Brewer and Sullivan 2003; García-Gil et al. 2003), the data obtained (0.06 μg CO_2 g^{-1} waste Fig. 2b) indicate good stability of some of the refuse in this cell. Visual and olfactory inspection of the waste analyzed revealed a much-degraded material in which it was not possible to distinguish the composing waste and which did not have the typical smell of putrefaction.

Despite the great difference in handling, age and composition of samples, the results of studies on compost were used for comparison purposes because no information on aerobic MA parameters in solid waste of sanitary landfills as measured by CO_2 release could be found.

This results again shows that CO_2 values decrease as depth increases (Fig. 2).

FDA and DHA measurement in waste containing leachate or water

Enzymatic measurements were made using samples of cell 1992 (2, 4 and 6 m depths). It was not possible to run these tests in surface waste (1 m) due to shortage of material and the impossibility of further sampling.

When the C mineralization technique was performed, it was observed that leachate incorporation into waste stimulated MA over a 24-h period, but it was not possible to establish the cause of this effect. Reasons may include an increase in waste moisture, nutrient contribution, or the contribution of a greater quantity and/or diversity of microorganisms. Previous studies (Yunes and Gómez 2009) have characterized leachate from the sanitary landfill of Bahía Blanca, finding a significant number of microorganisms related to organic matter mineralization. Enzymatic tests were performed to discriminate whether the increase in MA was due to the presence of either moisture or nutrients and microorganisms (Fig. 2). Thus, leachate incorporation was compared with that of sterile distilled water in equal volume. Thus, the moisture content of the samples increased from 16% to 20.3% (2 m), from 12% to 16.5% (4 m) and from 13.5% to 18% (6 m). These values do not coincide with those in Table 1 because samples lost 2% moisture in the storage month despite being kept at 4°C.

Enzymatic activities determined after 24 and 192 h (8 days) for both treatments are listed in Table 4. No significant difference between leachate and water ($P>0.05$) addition was detected, which indicates that moisture contribution may be the main cause by which leachate stimulates MA.

FDA hydrolysis values were lower than those obtained by Ryckeboer et al. (2003) when analyzing biowaste compost (vegetables, fruit and garden waste), and are also much lower than those reported by Smith and Hughes (2004) when studying the same material. These differences may be due to waste age since Ryckeboer et al. (2003) worked with 12-week-old compost samples, and Smith and Hughes followed the process with 1-year-old samples. On the other hand, data on DHA activity were lower than those presented by Tiquia et al. (2002) in a study intended to evaluate the dynamics of vegetable waste compost in Hong Kong. Again, samples analyzed by Tiquia et al. (2002) were much younger than those in the present study.

Among the techniques used for detecting MA, CO_2 release gives information on the true organic matter mineralization by microorganisms, even though sample volumes larger than 50 g are convenient in that they are representative. Regarding enzymatic determinations, they have the advantage of allowing the use of smaller sample volumes and, in particular, DHA is very sensitive as it registers the presence of enzymes associated with the bacteria cell membrane; i.e., if the enzyme is detected, the active bacterial cell is detected as well. Hydrolysis of FDA has been proposed as a means of measuring the global hydrolytic capacity of soils and as an indicator of MA. As hydrolytic enzymes may be present inside the bacterial cell or free in the soil, the total content of these compounds

Table 4 Microbial activity (MA) according to enzymatic hydrolysis of fluorescein diacetate (FDA) and dehydrogenase (DHA) measurement for samples taken at 2, 4 and 6 m depth. Values shown in brackets are standard error (SE); $n=3$. *TPF* Tryphenyl formazan

	FDA (μg fluorescein g^{-1} waste)		DHA (μg TPF g^{-1} waste)	
	With water	With leachate	With water	With leachate
2 m				
Day 1	81.1 (3.8)	79.4 (2.1)	9.8 (0.7)	10.1 (0.4)
Day 8	47.4 (6.0)	40.8 (3.4)	7.4 (0.3)	7.5 (0.5)
4 m				
Day 1	48.7 (0.3)	51.1 (2.9)	8.4 (1.6)	10.2 (2.0)
Day 8	40.5 (1.6)	42.8 (1.8)	8.4 (0.1)	9.2 (0.3)
6 m				
Day 1	57.7 (4.1)	52.9 (1.7)	5.8 (0.1)	6.2 (0.0)
Day 8	35.1 (3.0)	33.3 (2.2)	4.7 (0.5)	3.4 (0.0)

does not correspond directly to the density and/or activity of microbial populations (Shaw and Burns 2006).

Conclusions

The results of the present research allowed us to establish that the aerobic degradation processes in Bahía Blanca's sanitary landfill are very important. The low annual rainfall, the sandy soil, and the landfill techniques used (i.e., rigorous compactation is not performed), most likely contribute to maintaining microenvironments with a sufficient quantity of O_2. Under these conditions, degrading processes are faster, which explains why cell 1992 is stabilized and behaves as an anthrosol.

From the results obtained, it is concluded that, due to the environmental conditions of the Bahia Blanca landfill, it should be operated so that aerobic conditions, which prevail during dry periods, are favored.

The heterogeneity and complexity of waste composition with respect to pH, moisture and nutrient content and availability determines the different levels of MA observed. In this regard, the techniques used in our study (FDA, DHA and C mineralization) proved to be sensitive to such variations.

On the other hand, the main factor limiting MA in this region is the availability of water; leachate recirculation can accelerate waste degradation, mainly due to the increase in moisture content.

The results of this study may be helpful for landfill operation and engineers when choosing aerobic and anaerobic systems. In areas where MA is limited by lack of water (arid and semiarid zones), refuse degradation is limited to humid favorable periods. For that reason, an operation favoring fast and efficient aerobic activity should be implemented. At the same time, methods reflecting aerobic MA should be implemented and standardized.

Unfortunately, conventional landfill techniques where anaerobic conditions are created are performed similarly in geographic locations with completely different environmental characteristics and without previous knowledge of the biodiversity and microbial population density in each environment. Consequently, we highly recommend that landfills be managed according to the microbial ecology specific to each region, and MA—both aerobic and anaerobic—should be encouraged by observing the conditions and microbiota type predominant in the area.

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