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# Characteristics and Biological Treatment of Leachates from a Domestic Landfill

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## Abstract

Waste material from urban areas is a major environmental concern and landfill application is a frequent method for waste disposal. The leachate from landfills can, however, negatively affect the surrounding environment. A bioreactor cascade containing submerged biofilms was used to treat newly formed (< 1 year old) leachates of the Jbel Chekir landfill located southwest of Tunis, Tunisia. A preliminary analysis indicated a highly biodegradable portion of the leachate substances ($BOD_5$/ COD = 0.4). The treatment system and performance was investigated at different organic loading rates. Results obtained during this study indicated a significant reduction in organic matter between 60 to 90% of Total Organic Carbon. The main bacterial genera responsible for removal of organic carbon in the leachate consisted of the genera *Bacillus*, *Actinomyces*, *Pseudomonas* and *Burkholderia*. The bacterial isolates inoculated into raw leachates further reduced TOC concentrations. TOC was reduced by *Pseudomonas* isolates by 70%. *Actinomyces* and *Bacillus* isolates reduced TOC by 69% and *Burkholderia* isolates resulted in the greatest TOC reduction of 77%. Consortia of the bacterial isolates reached TOC yield of about 84%.

**Keywords:** Biological treatment; Leachate; *Actinomyces; Bacillus*; *Pseudomonas*; *Burkholderia*; TOC; Consortia

**Abbreviations:** $BOD_5$: Biochemical Oxygen Demand In Five Days; CFU: Colony Forming Unit; COD: Chemical Oxygen Demand; D: Day; G DW: Gram Dry Weight; H: Hour; Ha: Hectare; L: Litre; Mg: Milligram; Min: Minutes; MSW: Municipal Solid Waste; N: Number Of Sample; TKN: Total Kjeldahl Nitrogen; TOC: Total Organic Carbon; UDS: Uncontrolled Dumping Sites; V: Volume

## Introduction

In Tunisia, the success of solid waste management is critical to approaching or achieving sustainability and is a national priority regarding environmental protection. To achieve this goal, a National Management Program for Solid Waste (PRONAGDES) was launched in 1993, to address the negative effects of solid waste and to improve management practices. The controlled landfill of Jebel Chekir near Tunis Tunisia was first developed in 1999, receiving the municipal solid waste (MSW) of the greater Tunis area. Controlled landfills solved some of the problems encountered from previous years of uncontrolled dumping. However, the leachates generated in discharges from the landfill still constituted a considerable risk to the environment. The Jebel Chekir landfill produces 200 to 250 $m^3$ leachate $d^{-1}$. Tunisian MSW contains a high percentage of wet organic matter and the leachate also contains high levels of organic material. For example Total organic carbon in undiluted leachate sampled from landfill of Jebel Chekir is abouy 6 Kg TOC/$m^3$/d. Because of these issues, a study of options in biological treatments seemed appropriate. The objectives of this study were (i) to monitor the changes in the microbial community during the biological treatment of Jebel Chekir leachate; and (ii) to isolate and identify the most prevalent bacterial groups involved in degrading landfill waste.

## Methods

### The Jbel Chekir Landfill

The Jbel Chekir landfill is the oldest and largest controlled-landfill site in Tunisia. It is used for the disposal of domestic solid wastes from the greater Tunis area. The landfill presently encompasses 46 ha of the 124 ha site and receives 2000 tons of municipal solid waste (MSW) per $d^{-1}$, which is made up of 85% wet household waste [1]. The high moisture levels in MSW contribute to the production of a large quantity of leachate. Currently the leachate is piped in and stored, as is, in 13 collection pools with a total capacity of 300 000 $m^3$. Newly formed leachate samples were less than 1 year old and taken from an operating Jebel Chekir landfill. The intermediate leachate samples were (six-year age) [2] and taken from a closed-landfill area. The aerobic bioreactor used in this study consisted of three bio reactors placed in succession (Figure 1). All reactors were cylindrical in shape with interior diameter of 30 cm and height of 42 cm. The three reactors were made from transparent PET to allow viewing of the process. The working volume of leachate was fixed at 18 L per reactor. Oxygen was continuously supplied to the reactors by an air compressor at 100 L $min^{-1}$.

### Leachate treatments

**Different organic loading rates:** Young leachate had a high organic content and was added to the pilot reactor at progressively increasing organic loading rates ranging from 0.6 to 6 Kg TOC $m^{-3}d^{-3}$ achieved by dilution with tap water (leachate/tap water (v/v)). Each

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reactor was seeded (1/10, v/v) of activated sludge originating from the Soliman municipal wastewater treatment (MWW). During treatment, temperature varied between 26 and 30°C. The MWW was replaced daily with an equivalent volume of landfill leachate. In general, a half liter of leachate replaced a half liter of removed MWW. Additional leachate was added the next increase in level once the carbon (TOC) was reduced by at least 90%. Also, pH values were maintained in range 6-7 by addition of acetic acid (1N) when pH rises of 7.2 values. The pH range of 6-7 was attributed to prevent free ammonia formation and to optimize the production of biomass since most of bacteria enzymes were optimized at pH of 6-7 [3,4]. The reactors were monitored for (i) microbial counts from the attached biofilms naturally developed by the microorganisms growing in the inner walls of the reactors. (ii) Treatment of young leachate at fixed organic loading rate 3.2.1. (ii) Treatment of young leachate at different organic loading rate and (iii) Identification of the bacterial isolates responsible for organic degradation.

**Fixed organic loading rate:** An additional study was conducted to test biofilm development on the reactor supports using a known leachate composition. For 45 d, the pilot station was supplemented with a glucose/peptone leachate composed mainly of glucose and peptone to possibly hasten biofilm production.

## Microbial Assays

**Screening protocol:** Isolated colony forming units (CFU) were used to predict the effect of known bacteria inoculation to the reactor. Young leachate yielded 80 strains from biofilms of the three reactors and thirty isolates [5] from various other environments (soil, compost and non-treated wastewater) were used in these studies. These isolates were purified and stored at -20°C. Isolates were screened to characterize their ability in degrading organic matter. This test consisted of inoculating 1 ml of an activated culture of each strain into 9 ml of young leachate. This mixture was left for 48h at 30°C and shaken (225 t/min$^{-1}$) to oxygenate the medium. The bottles used had empty volume to ensure a good oxygenation by agitation. Agitation was carried out by the agitator translation (Bioblock Scientific). Each sample was tested for TOC concentrations of the seeded and incubated leachate. Leachate without inoculation was used as a control. The results were expressed in percentage and calculated as:

Degradation efficiency (%) = 100 * ($TOCi–TOC_f$ ) / TOCi concentration.

Where: TOCi was the initial concentration of TOC; $TOC_f$ was the final concentration of TOC. Isolates that had TOC degradation rates greater than 40% were retained for the following tests. In addition, the removal of TOC by bacteria consortia was examined. These consortia consisted of various mixtures of the bacterial isolates.

**Microscopic observations:** Motic Digital Microscope was used for microscopic bacteria observation. Microfilms on the assembled support were observed using scanning electron microscopy (SEM, Quanta 200).

**Microbial counts:** Bacteria enumerated on nutrient agar (NA, Biorad) medium were incubated for 24 to 48 hours at 30°C. Cocci and rods were counted and identified by Gram staining and microscopic observation of the CFU isolated in NA medium broth. (3) *Actinomyces* were enumerated on a Glycerol-Arginine-Agar medium [6] [(g 1000/ml): Glycerol, 20; asparagine (used instead of the arginine), 2.5; NaCl, 1.0; $CaCO_3$, 0.1; $FeSO_4 7H_2O$, 0.1; $MgSO_4 7H_2O$, 0.1; agar, 20; and pH adjusted to 7.0]. Adequate dilutions of each sample were plated, poured, and incubated for 5 to 15 or 35 days at 28°C. The colonies having the specific characteristics of *Actinomyces* were counted. The morphological characteristics were followed by microscopic observations following Gram staining. (4) *Bacillus* groups were isolated after sporulation treatment at 80°C during 10 min. Pure culture isolation

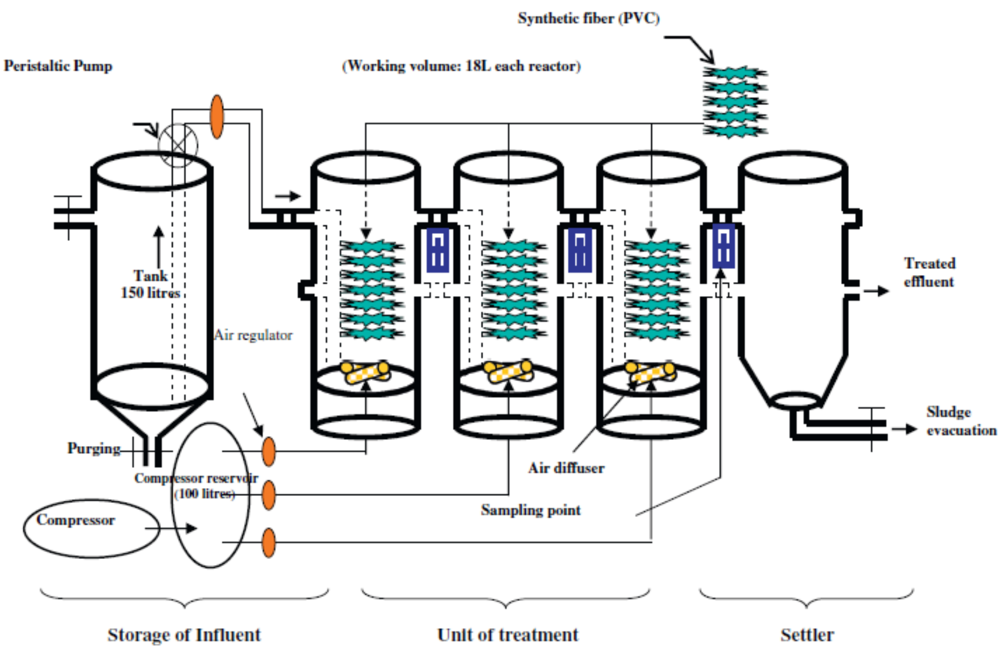


**Figure 1:** Schematic illustration of bioreactor prototype used to investigate landfill leachate from the Jbel Chekir landfill site south west of Tunis, Tunisia

| | | | Young Leachate | |
|---|---|---|---|---|
| Parameters | Units | N | min-max | Average |
| Age | year | 8 | - | <1 |
| Colour | - | 8 | Green black | - |
| pH | - | 8 | 7-7.9 | 7.5 |
| TKN | mg $L^{-1}$ | 8 | 2240-6048 | 3740 |
| N-$NH_4$ | mg $L^{-1}$ | 8 | 1728-4998 | 2570 |
| TOC | mg $L^{-1}$ | 7 | 6890-15960 | 10920 |
| COD | mg $L^{-1}$ | 7 | 4740-28120 | 13610 |
| $DBO_5$ | mg $L^{-1}$ | 1 | - | 2840 |
| $DBO_5$/COD | - | 1 | - | 0.4 |

$BOD_5$ = Biochemical Oxygen Demand in Five Days
CFU = Colony Forming Unit
COD = Chemical Oxygen Demand
N: Number of sample
TKN = Total Kjeldahl Nitrogen
TOC = Total Organic Carbon

**Table 1:** Characteristics of young leachate I from the Jbel Chekir landfill site south west of Tunis, Tunisia.

was obtained by plating consecutively the same clone several times on Trypticase-Soy-Agar (TSA, Biorad). *Bacillus* isolates were confirmed by Gram stain, oxidase and catalase assays [7]. (5) the most probable number method (MPN) was used for the enumeration of *Pseudomonas* using asparagine medium [(g $L^{-1}$): asparagine, 3.0; $K_2HPO_4$, 1.0; $MgSO_4$ $7H_2O$, 0.5] and acetamide medium [(g $L^{-1}$): acetamide, 10.0; NaCl, 5.0; $K_2HPO_4$, 1.39; $KH_2PO_4$, 0.73; $MgSO_4$, 0.5; phenol red, 0.012].

**Bacterial identification:** Bacteria were identified through classical methods based on the use of selective media and certain enzymatic reactions and the API system (bioMérieux). The API 20 NE tests were used for *Pseudomonas* and *Burkholderia* identification. However API 50 CHB combined with the API 20 E was used for *Bacillus* identification.

## Results and Discussion

### Characterization of Landfill Leachate

The characteristics of leachate samples were determined (Table 1). A $BOD_5$/COD ratio of 0.4 suggests that the material is a good candidate for biodegradation possibly due to the presence of volatile fatty acids [8].

### Treatment of young leachate

**Treatment of young leachate at different organic loading rate:** Young leachate having an increasing organic loading rate from 0.6 to 6 Kg TOC $m^{-3}$ $d^{-1}$ was added to the pilot reactor. The retention time was 36 hours. The mean TOC removal efficiency of each reactor was grouped in Table 2. The system performance showed a steady TOC reduction ranging from 80 to 90%, when the pilot reactor was supplemented with the lower organic loads (0.6 and 1 Kg TOC $m^{-3}$ $d^{-1}$). However, when the pilot reactor was fed by higher organic loads (2. 3 and 6 Kg TOC $m^{-3}$ $d^{-1}$), the rate decreased by 88, 70 and 60 %, respectively.

The bacteria present in the different bioreactor during this treatment consisted of two types of bacteria: rods and cocci (Figure 2). Although the three reactors had relatively the similar bacteriological compositions, only the biomass colonizing reactor1 success in adapting to the various imposed conditions and maintained some stability. This stability was reflected in an increasing rate of leachate, primarily through the effect of *Actinomyces* and *Bacillus* activity. The prevalence and the maintenance of the number of these two bacterial groups for almost all the period of the treatment suppose the good adaptation and the involvement of these two groups in the leachate treatment. *Bacillus* spp are generally known by their prevalence stressing media, because of their spore formation [7] and their production of broad range of enzymes [9].

*Actinomyces* are widespread heterotrophic microorganisms in nature and are known to produce antibiotics and enzymes and have resistance to certain stresses, such as, heavy metals [10]. *Actinomyces* and *Bacillus* are also known for their similarly producing enzymes of amylases, xylanases, proteases, lipases and cellulases which might contribute to enzymes are involved in organic matter degradation [11,12].

**Treatment of young leachate at fixed organic loading rate:** The

| | Leachate diluted | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 10 times | | 7.5 times | | 5 times | | 2.5 times | | Undiluted leachate | |
| Treatment | Organic loads* | yield | Organic loads* | yield | Organic loads* | yield | Organic loads* | yield | Organic loads* | yield |
| Pilot unit | 0.60 | 81.03% | 1.00 | 88.85% | 2.00 | 67.34% | 3.00 | 68.81% | 6.00 | 63.68% |
| Reactor 1 | 1.82 | 54.42% | 2.97 | 80.00% | 5.70 | 31.98% | 9.50 | 53.00% | 17.34 | 44.90% |
| Reactor 2 | 0.83 | 55.00% | 0.58 | 18.00% | 3.91 | 7.00% | 4.41 | 16.00% | 9.55 | 12.00% |
| Reactor 3 | 0.37 | 7.31% | 0.47 | 30.00% | 3.61 | 48.00% | 3.69 | 19.00% | 8.35 | 24.00% |

* Kg TOC/$m^3$/d
TOC=Total Organic Carbon

**Table 2:** The yield of the reactors and the pilot reactor in TOC reduction of leachate from the Jbel Chekir landfill south west of Tunis, Tunisia.

| | Waste water | | Synthetic leachate* | | Young leachate | |
|---|---|---|---|---|---|---|
| Treatment | Organic loads** | yield | Organic loads** | yield | Organic loads** | yield |
| Pilot unit | 0.16 | 91% | 1.33 | 99% | 2.5 | 91.61% |
| Reactor 1 | 0.5 | 84% | 4 | 85% | 8 | 26% |
| Reactor 2 | 0.09 | 4% | 0.6 | 12% | 6.69 | 36% |
| Reactor 3 | 0.07 | 3% | 0.5 | 3% | 4.25 | 82% |

*The synthetic leachate is composed mainly of glucose and peptone (composition is adapted from Trabelsi [15])
**Kg TOC/$m^3$/d
TOC=Total Organic Carbon

**Table 3:** The yield of the reactors and the pilot reactor landfill leachate from the Jbel Chekir landfill south west of Tunis, Tunisia.

treatment of undiluted young leachate was carried out with an applied load of approximately 2.5 kg TOC $m^{-3}d^{-3}$, with a retention time of 108 hours and neutral pH [13]. Under these conditions, reactors 1 and 2 showed a comparable TOC removal rate varying between 26 and 36% respectively. Reactor 3 showed a higher rate of TOC removal at 82.1%. Thus, the total TOC removal rate of the pilot reactor was about 91% (Table 3).

During the adaptation phase, the application of wastewater did not provide sufficiently developed biofilms in the three reactors. However, the application of the assembled leachate, had provided dense biofilms (the number of total bacteria increased by two logarithmic units (maximum number = 1.6 $10^{11}$ CFU $g^{-1}$ DW). This increase occurred when the pilot reactor was supplemented with the assembled leachate. Thus, it is an interesting favorable medium for bacteria growth.

When reactor 1 was fed by young leachate, the total bacterial number decreased in the initial stage (lag phase), and then increased thereafter to reach a maximum value of 4.3 $10^{11}$ CFU $g^{-1}$ DW. This rapid growth in microbial population showed that the total flora was well adapted to the treatment process. The formed microfilms could rebound from the shock caused by the addition of the young leachate.

In the same way for reactors 2 and 3, the adaptation phase did not lead to the formation of thick biofilms and the feeding of the pilot reactor by the assembled leachate caused a rapid and varied bacteria proliferation in reactors 2 and 3 (increase of three logarithmic units).

The microbial community of the reactor consisted of *Bacillus, Actinomyces* and *Pseudomonas* genera (Figure 3). These microbial populations significantly increased during the adaptation stage. For example the number of *Actinomyces* increases at the level of reactor 1, 2 and 3. The number of *Actinomyces* at the beginning of the treatment was $10^7$, $10^7$ and $10^8$ CFU $g^{-1}$ DW, respectively. At the end of the adaptation stage on reactor 1, 2 and 3, the number of *Actinomyces* was $10^{10}$, 4. $10^{10}$, 6.$10^9$ CFU $g^{-1}$ DW, respectively.

These results compared with the first phase of treatment considered

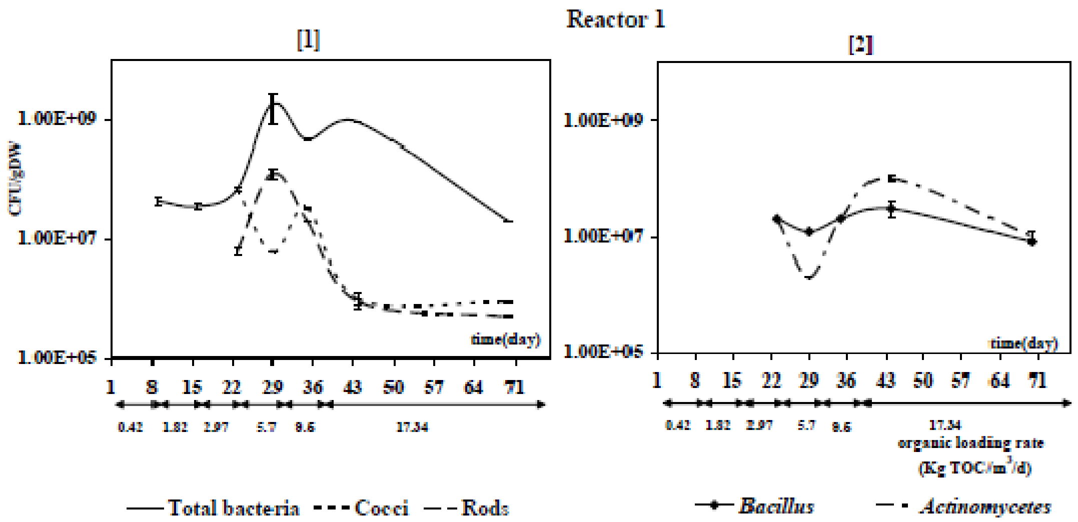


**Figure 2:** Main groups on the three reactors fed by young leachate at different organic loading rate landfill leachate from the Jbel Chekir landfill site south west of Tunis, Tunisia (CFU/g DW = Colony Forming Unit per gram Dry Weight of each sample).

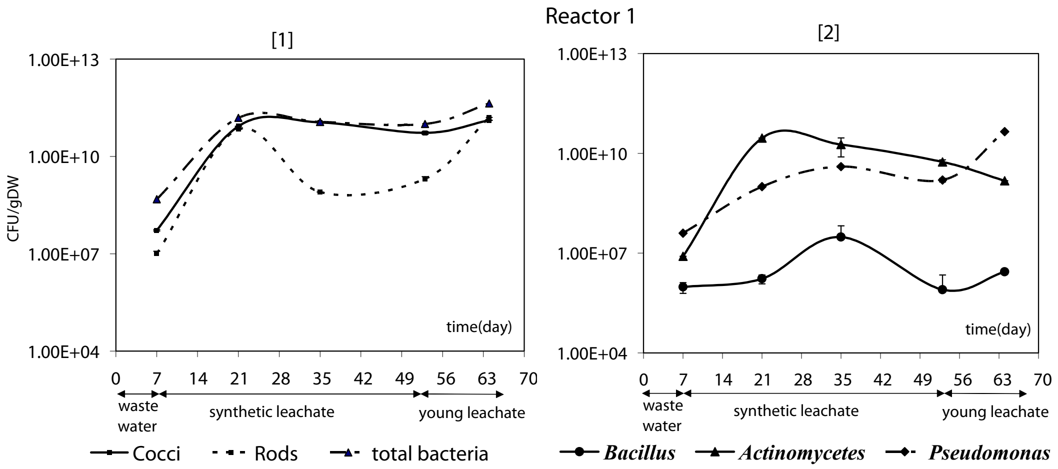


**Figure 3:** Main groups on the three rectors fed by young leachate at a fixed organic loading rate.

at different organic loading charge, indicates that this treatment phase was characterized by an important increasing of the number of all the bacterial groups in the three reactors. This observation reveals the important role of adaptation stage by assembled leachate as well as the role of pH maintenance to values close to neutrality (pH = 7). Indeed, a dense biofilm was able to resist to various treatment conditions. In addition, the pH value near the neutrality (pH = 7) is more favorable for the bacterial growth, which leads to higher populations and greater diversity [14]. Thus, under such conditions, the pilot reactor had a high output of 91%. Additionally, Reactor 3 had the lowest load (4.25 kg TOC $m^{-3}d^{-1}$) most suitable for degradation of the organic matter by the microflora. Thus, the reactor 3 presented the highest TOC removal rate (82%).

## Biological TOC removal by the various bacterial isolates

**Young leachate:** The study of biological TOC removal by the various bacterial isolates (endogenous and exogenous) showed that the degradation of the organic matter was inversely proportional to TOC concentrations. The TOC removal rate of the various isolates is represented in Figure 4. The degradation rate of TOC concentration of 11 $gL^{-1}$ is higher than those obtained at a concentration of 25 $gL^{-1}$. This result is similar for all tested isolates. The degradation effectiveness of the leachate by the individual cultures during 48 hours showed that all the bacterial isolates had an important role in TOC removal compared to the natural flora in the leachate, or the control (Figure 4). For TOC concentration of 11 $gL^{-1}$, *Bacillus* spp were the most efficient of the bacterial groups. However, for a higher concentrations (TOC = 25 g/L), *Pseudomonas*, *Actinomycetes* and *Burkholderia* degraded TOC at a higher efficiency than by *Bacillus* inoculation.

An antagonism test was performed between the bacterial isolates to define the ability to be combined in one consortium. This test showed that they were not antagonistic between each other. Thus several types of consortia were prepared by mixing individual strains: Consortium 1 was composed of *Bacillus* isolates, however consortium 2 contained *Actinomycetes* isolates. The consortia 3, 4 and 5 were grouped as *Pseudomonas* isolates, *Burkholderia* isolates and a mixture of all the bacterial isolates, respectively. The study of the biological removal of the TOC by the various consortia is presented in Figure 5.

Organic matter degradation was found to be inversely proportional to TOC leachate concentration. Organic matter degradation rates were higher for a TOC concentration of 11 $gL^{-1}$ where values were ranging between 69 and 85%. While the degradation rate of organic matter at a TOC concentration of 25 $gL^{-1}$ did not exceed 45%. Consortium 5 (composed by 43 strains), had a higher yield of TOC removal compared with the other consortia (85.5 and 45.6% for TOC concentrations of 11 $gL^{-1}$ and 25 $gL^{-1}$, respectively). In addition, the other consortia present a maximum of 77 and 42% for the same TOC concentrations (Figure 5). This result may be to the diversity of the microorganisms in the considered consortium, as presented in Figure 5. This same result was confirmed by Saraswathy et al. [15], who showed that the use of a heterogeneous consortium containing a high number of strains [16] was more advantageous than homogeneous one. The high diversity resulted in greater stability and increased the metabolic capacities due to greater physiological variation.

**Intermediate leachate:** The study of TOC reduction of intermediate leachate by the individual isolates showed the highest degradation rate for an initial TOC concentration of 3 $gL^{-1}$ with a maximum value varying between 77 and 87%. However, TOC reduction varied between 71 and 80%, for an initial concentration of 1 $gL^{-1}$ (Figure 6). This may be due to the lowest concentration of the leachate having the least biodegradable portion. Which may be especially composed of remnant

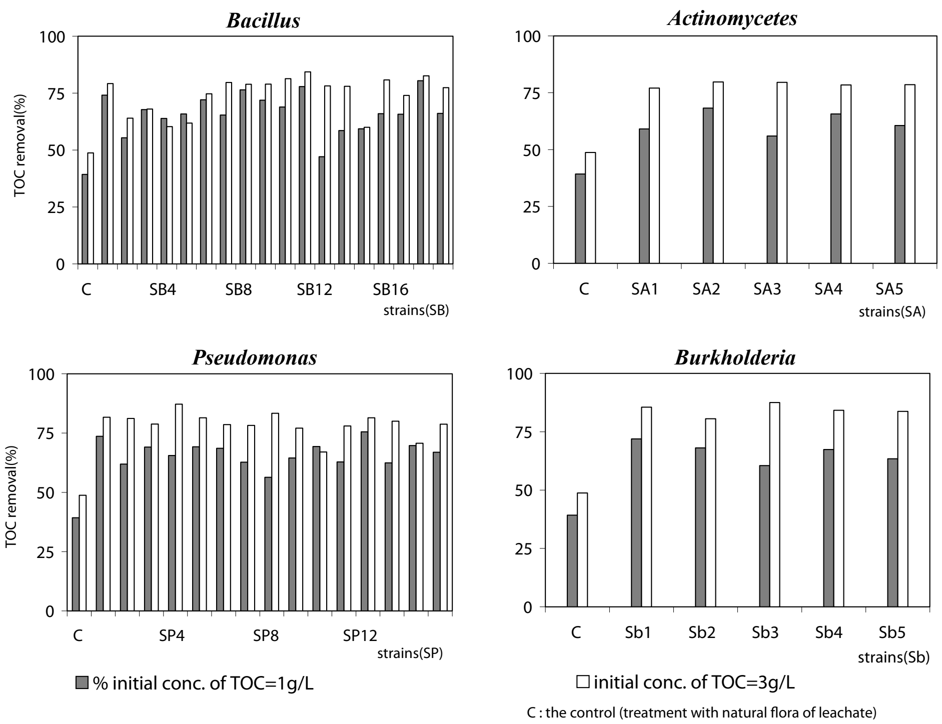


**Figure 4:** TOC Removal (young leachate) by the bacterial isolates (TOC removal % = 100*(initial concentration – final concentration) / initial concentration) landfill leachate from the Jbel Chekir landfill site south west of Tunis, Tunisia.

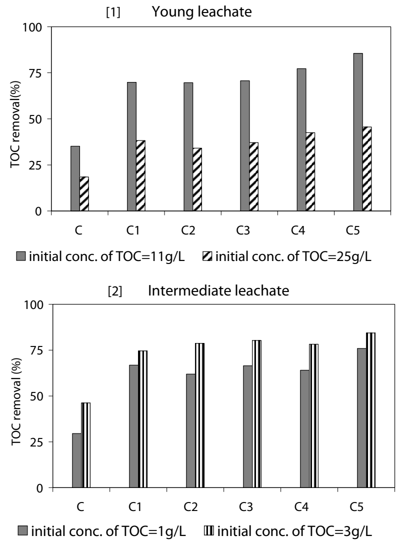


**Figure 5:** TOC Removal efficiency of the consortia (young and intermediate leachate) landfill leachate from the Jbel Chekir landfill site south west of Tunis, Tunisia. (C: the control (treatment with natural flora of leachate; C1: consortium1 (*Bacillus* isolates); C2: consortium2 (*Actinomycetes* isolates); C3: consortium3 (*Pseudomonas* isolates); C4: consortium4 (*Burkholderia* isolates); C5: consortium5 (mixture of all the bacterial isolates).

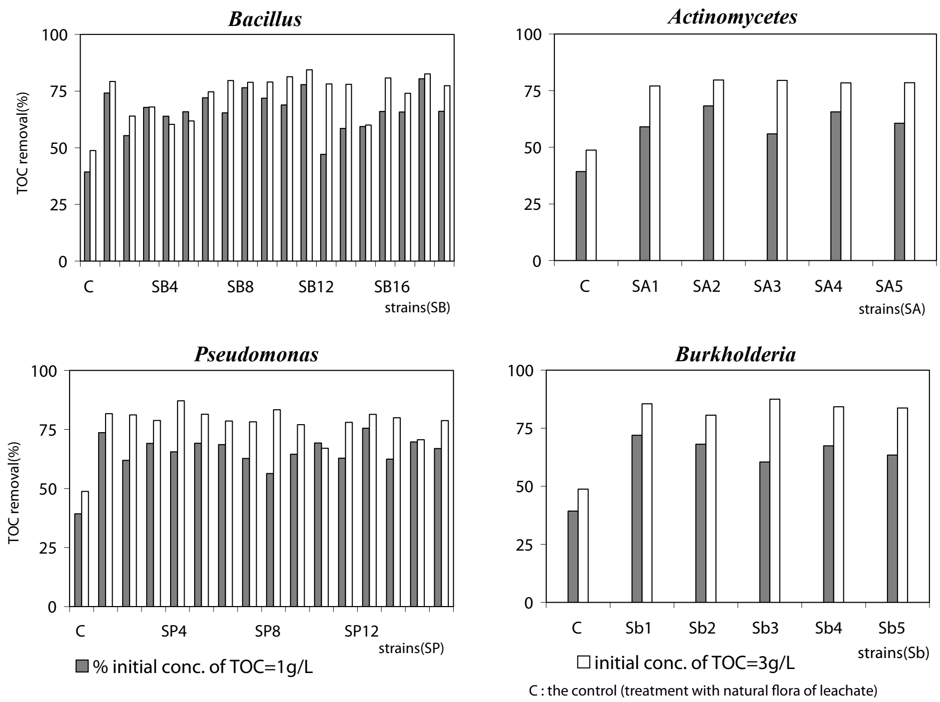


**Figure 6:** TOC Removal (intermediate leachate) by the bacterial isolates from the landfill leachate from the Jbel Chekir landfill site south west of Tunis, Tunisia.

or recalcitrant compounds like the humic acids [17] and presented a relatively weak TOC concentration?

**Identification of the bacterial isolates:** Aerobic microorganisms in young leachate were identified to understand the biology of the reactor and to detect possible pathogens. Using the microgalleries (API 20E, 20NE and API 50 CHB) and conventional tests (oxidase, Gram and catalase),bacteria were identified in young leachate. Three dominate genera were found including 21 bacteria belonging to *Pseudomonas* (n = 4), *Bacillus* (n = 12) and *Burkholderia* (n = 5) together with four *actinomyces* strains. Seven species were identified as *Pseudomonas aeruginosa* (n = 3), *Pseudomonas puptida* (n = 1), *Bacillus subtilis* (n = 2), *Bacillus circulans* (n = 1), *Bacillus pumilus* (n = 1), *Burkholderia cepacia* (n = 4), *Burkholderia gladioli* (n = 1). The *Pseudomonas* genera dominated the microbial flora associated with Jebel Chekir leachate due to its aerobic character and its common inhabitation of the natural environment. These strains were of great interest in the bioremediation process of a variety of aromatic hydrocarbons and dyes because they possess appropriate degradation pathways [18]. The Gram positive bacteria identified in young leachate are dominated by *Bacillus* strains. Species of this genus were frequently isolated from landfill materials [19,20]. None of these Gram positive bacteria are considered primarily as pathogens although some may be found in the environment.

## Conclusions

An aerobic treatment of Jebel Chekir landfill leachate was investigated in an aerobic pilot unit composed of three bioreactors. During the assay, the microbial biodiversity of this prototype was studied. The pilot unit succeeded to reach important TOC removal rates ranging between 60 and 90%. These selected isolates had a high capacity for degradation of organic matter at low or high TOC leachate concentrations (TOC = 1, 3, 11, 25 $gL^{-1}$). The differences in organic matter degradation at various leachate concentrations indicates that long-term studies are needed to investigate the changes in bacteria genera with time at these higher concentrations.

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