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Simultaneous removal of organic compounds and heavy metals from Tunisian landfill leachate using dairy processing wastes

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Abstract

Landfill leachate production and management are identified as one of the greatest problems of sanitary landfill. In this research paper, the effect of the use of bactofugate (B) and the expired fermented milk 'Lben' (L) in the biological treatment of Jebel Chakir leachate was studied. Leachate samples were separately inoculated with both raw and reactivated (B) and (L) samples and incubated at 37 °C over 15 days. Both raw (L) and (B) inoculums ensure better results in terms of organic matter removal than the reactivated samples. However, the best removal performances were obtained with (L) inoculum. The chemical oxygen demand (COD) removal reached 50% with an initial COD concentration of 20.8 g L^{-1}, whereas 80% of ammonia nitrogen (NH_3-N) removal was recorded. Furthermore, heavy metal concentrations, especially of Cr^{3+} and Fe^{2+}, were reduced during the treatment with average removal rates of about 90%. Then, further kinetic investigations were performed using the (L) inoculum with an equipped bioreactor with air incorporation. Important COD removal efficiency (46%) was recorded within only 24 h. Heavy metal concentrations were also reduced during this process. The findings indicate that expired fermented milk could be a promising alternative for the biological pre-treatment of landfill leachate.

Key words: biological pre-treatment, COD removal, dairy releases, landfill leachate

INTRODUCTION

With industrial expansion and the growth of population, the amount of municipal solid waste (MSW) has increased all over the world. Landfill waste disposal represents a key management strategy in most developed countries, principally due to its economic advantages. However, landfill management may pose serious environmental impacts through the discharge of highly polluted wastewater, also known as leachate. This effluent is usually characterized by great concentrations of organic matters, ammonia and heavy metals (Modin *et al.* 2011). Moreover, several factors influence the characteristics of landfill leachate, such as the MSW composition, the seasonal weather variations, the landfill age and the decomposition stage in the landfill (Kurniawan *et al.* 2006). Thus, landfill leachate needs to be adequately treated to meet the reject standards.

Due to its composition, characteristics and the nature of the organic matters, the treatment of landfill leachates (LFL) is very complicated, expensive and requires various treatment processes. Thereby, several biological, physicochemical or combinations of both, processes have been applied, aiming to promote the degradation of the complex organic load of this effluent (Nogueira *et al.* 2015).

Due to their reliability, simplicity and high cost-effectiveness, biological processes have usually been considered as the most environmentally-friendly of treatment processes. Furthermore, biological processes involving microorganisms have been proven to be very effective in organic and nitrogenous matter removal, especially from immature leachates (Renou *et al.* 2008). For instance, Kumari

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et al. (2016) tested the treatment feasibility of leachate using a synergistic approach with microalgae and bacteria. Bacto-algal co-culture was found to be the most efficient in terms of toxic organic contaminants and heavy metals removal. In the last decade, lactic acid bacteria (LAB) have gained increasing importance in the detoxification of wastewaters and a wide range of pollutants (Kinoshita *et al.* 2013). As reported by Ghodhbane *et al.* (2016), several LAB have been isolated and identified from bactofugate including: *Lactococcus lactis subsp.lactis, Lactococcus lactis subsp. cremoris, Lactobacillus pentosus, Lactobacillus plantarum* and *Lactobacillus paracasei.* These bacteria are currently used as starters in dairy products such as yoghurts and fermented milks. In preceding studies, the pre-treatment and the reuse of waste dairy products with combined physical-chemical and biological processes were studied for promoting the treatment and reuse of several effluents (Kasmi *et al.* 2016; Kasmi *et al.* 2017). This research is performed with the aim of using dairy waste in the pre-treatment of Jebel Chakir landfill leachate. Bactofugate (B) and the expired fermented milk 'Lben' (L) are evaluated for their degrading potential. The effect of these products on the removal of organic matters, turbidity and heavy metals is investigated.

MATERIALS AND METHODS

Description of the study site and leachate sampling

The site of Jebel Chakir is a controlled landfill area. It is mainly used for the disposal of domestic solid wastes from the greater Tunis area and it is located 10 km south-west of Tunis. The site occupies 47 ha over a reserve total area of 124 ha (Figure 1). Jebel Chakir landfill receives 2,000 tons/day of MSW, of which 68% is organic materials. The volume of leachate generated from this site is estimated to be around 270 m^3/day.

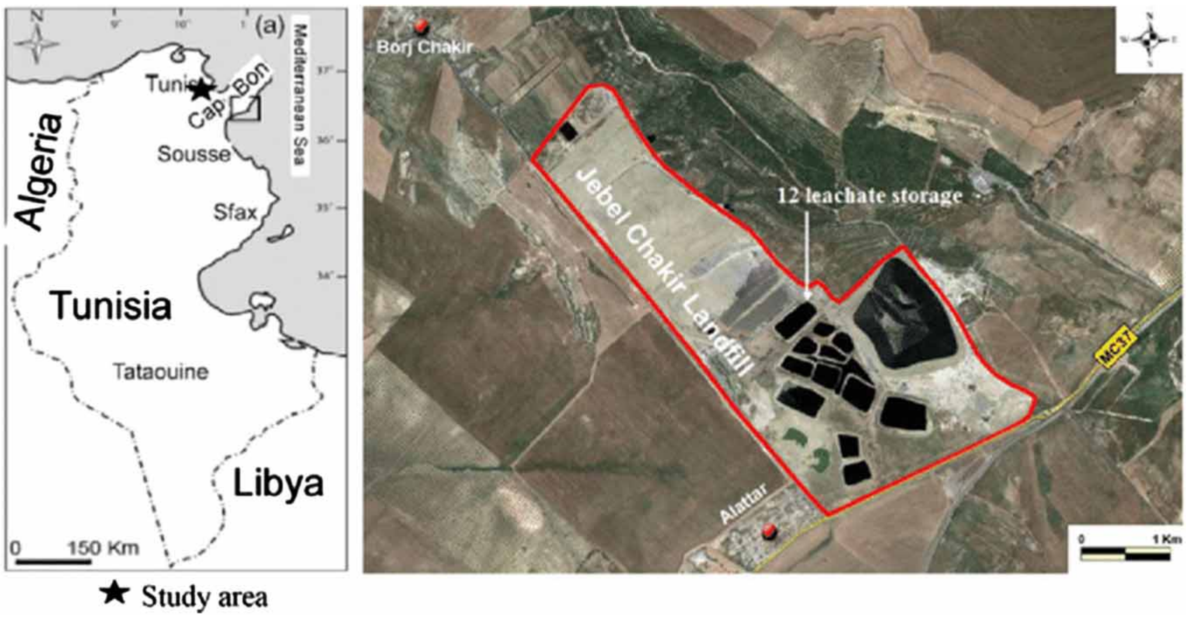

Figure 1 | View of Jebel Chakir landfill site with13 basins of leachate storage (Google Earth image).

Up-to date the leachate is piped and stocked without any treatment in 13 collecting pools with a total capacity of 300,000 m^3 (Tizaoui *et al.* 2007). Leachate samples were collected in 20 L tanks and transported to the laboratory. The leachate was stored in the dark at 4 °C in order to avoid its acidification and its chemical composition variation; and notably to limit the microbial activity.

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Dairy rejects sampling

The two dairy wastes used in this study, bactofugate and expired fermented milk 'Lben', were collected from a regional dairy industry (Centrale Laitière du Cap Bon) which is a part of Delice Group, leader of the food industry in Tunisia in the dairy sector.

According to the industry statistics, an average of 1,200 L is generated daily from bactofugation as separated milk (Belwarda 2012). The bactofugate is a dairy waste product, generated following the bactofugation of milk consisting of a special form of separation in which specific types of microorganisms are removed from milk and inactivated (Kolhe *et al.* 2009). The difference in density between spores and milk allows their sedimentation under the effect of a centrifugal force and the bactofugate is ejected outside (Hanno *et al.*1991).

The fermented milk 'Lben' is one of the most popular products in North Africa and the Middle East. In Tunisia, industrial 'Lben' is produced by spontaneous fermentation of cows' milk with mesophilic industrial starter cultures such as *Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. diacetylactis and Lactococcus lactis subsp. cremoris* (Samet-Bali *et al.* 2012). The withdrawal of damaged products or those over their sell-by date from the market creates a significant amount of dairy wastes unsuitable for sale. The Tunisian industrial fermented milk products 'Lben' used in this study had expired a month previously.

Batch conditions

Inoculation with dairy waste

The bactofugate (B) and the expired Lben (L) were assessed for their chemical oxygen demand (COD) removal potential. The reactivation of (B) and (L) products was carried out by incubation at 37 °C for 48 hours. 90 ml of leachate samples were separately inoculated with both raw (B) and (L) and reactivated (RB) and (RL) samples at 1% (v/v) in Erlenmeyer flasks (250 ml) and incubated at 37 °C for 15 days.

Inoculation with *Lactobacillus plantarum*

The used *Lb. plantarum* strain was previously isolated from dairy wastes and identified by Kasmi *et al.* (2018); where dairy wastes including bactofugate and decreaming water raw samples, in addition to the wasted fermented dairy products, were used for LAB isolation. Aseptic inoculation was realized with *Lb. plantarum* seed cultures prepared previously. Fermentations were performed in batch into Erlenmeyer flasks (250 mL) containing 100 mL of working volume. The basal culture conditions are: fermentation time, 24 h; temperature, 37 °C and inoculum 1% (v/v). The rotary speed of the shaker incubator was fixed at 150 rpm (Pescuma *et al.* 2008).

Bioreactor configuration

The biological treatment was carried out in a glass vessel reactor. The bioreactor was inoculated with 1% (v/v) of dairy product (L). The temperature was maintained at 37 °C using a thermally controlled water bath circulating through the fermentation vessel double wall. Agitation (100 rpm) was performed using a magnetic stirrer. Air incorporation was ensured by an air pump (Figure 2).

Analytical methods

Leachate as well as dairy rejects (B) and (L) were characterized in terms of physical and chemical parameters: the pH, electrical conductivity (EC) (mS/cm) and total dissolved solids (TDS) (g/L) of each sample were determined using multi-parameters device Consort C860. COD values were

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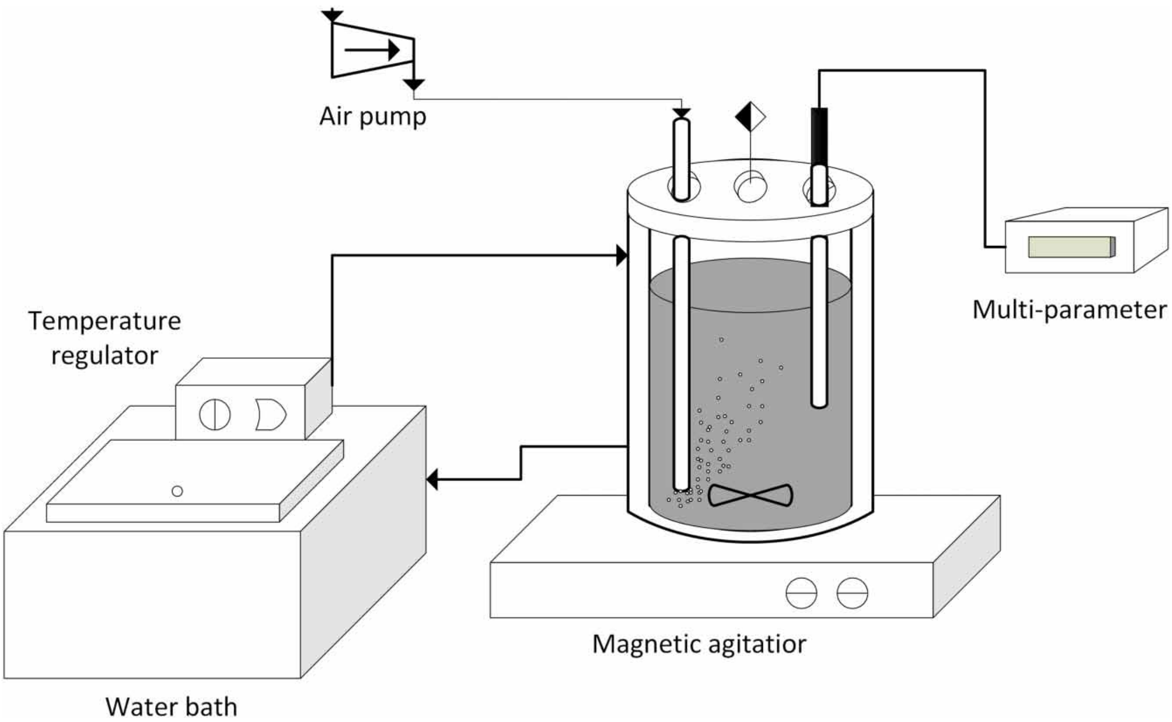

Figure 2 | Schematic representation of the bioreactor with air incorporation for landfill leachate treatment.

measured by the potassium dichromate colorimetric method using an open reflex system (Rodier *et al.* 2009).

As for the leachate, analyses were performed before and after the biological treatment. Treated leachate was decanted for one hour and the recuperated supernatants were filtrated using a 0.45 μm membrane filter. Filtrates' ammoniacal nitrogen was determined according to the NFT90-15 method (AFNOR 1999). The concentrations of heavy metals (Fe, Cd, Pb and Cr) of leachate filtrates were determined using the flame atomic absorption method (Analytic Jena AG Spectrometer AAS vario6).

Dry matter (DM), volatile suspended solids (VSS) and mineral matter (Mm) were determined according to the NFT12880 methods (AFNOR 2000). The turbidity was measured using the hand turbid meter Hach 2100p.

RESULTS AND DISCUSSION

Physical and chemical characterization of leachate

Table 1 provides a general characterization of the landfill leachate of Jebel Chakir. The leachate exhibited an alkaline pH of 8 with a dark-brown color, which was associated with a high organic pollutant charge (COD = 20 g O_2/L), high ammonia nitrogen content (2.25 g/L), high conductivity and high total suspended solids concentration (EC = 37 mS/cm, TDS = 24 g/L). Similar results were reported by Im *et al.* (2001); where the studied LFL organics concentration ranged from 21 to 26 g O_2/L. The presented results reveal that Jebel Chakir leachate contains considerable amounts of heavy metals such as Cu (1.19 mg/L) and Pb (0.87 mg/L) which contents are close to the Tunisian regulations for the discharge in a public sewer (1.5 and 0.5 mg/L, respectively). Unlike Fe (19.8 mg /L) and Cr (1.90 mg/L) that exceed the limits (5 and 0.5 mg/L, respectively). Similar results were obtained by Ellouze *et al.* (2008) indicating that leachate presented important concentrations of

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Table 1 | Characteristics of Jebel Chakir landfill leachate

Parameters	Unit	Raw leachate	Discharge standards of rejects
pH	25 °C	8.25 ± 0.2	6.5 < pH < 8.5
Conductivity	mS/cm	36.20 ± 1.1	–
Salinity	g/L	25.23 ± 1.5	–
COD	g O_2/L	20.80 ± 1	90
TDS	g/L	24 ± 2	–
Turbidity	NTU	979 ± 40	–
NH_3-N	g/L	2.25 ± 5	30
Cu	mg/L	1.19	1.5
Pb	mg/L	0.87	0.5
Fe	mg/L	19.8	5
Cr	mg/L	1.90	0.5
DM	%	3.58	–
MM	%	2.83	–
MVS	%	0.75	–

Fe (20.6 mg/L). The relatively high concentration of the analyzed heavy metals and the high COD and nitrogen concentration confirmed the high organics content of the dumped garbage in the landfill of Jebel Chakir.

Dairy products characterization

The dairy industry is usually considered to be one of the most polluting food industry sectors. This investigation proposes the reuse of bactofugate and industrial fermented milk (Lben) in leachate pre-treatment. Dairy products analysis revealed that the pH value of bactofugate was around 5 and its recorded COD value was 60 g O_2/L. Bactofugate presents a high mineral content (37 g/L) and its conductivity was assessed at 36 mS/cm. The pH value of the fermented milk was close to 4. The COD, the conductivity and TDS measured values were 112 g O_2/L, 37 mS/cm and 39.4 g/L, respectively.

Biological pre-treatment of leachate

Organic removal using bactofugate

Treatment of LFL was carried out using raw (B) and reactivated bactofugate (RB) samples.

Figure 3 presents the changes in COD concentrations of landfill leachate after treatment. pH values showed a moderate shift from 8.38 to 8.20. Low COD variation was recorded in the effluent during the first three days of treatment. However, from the fourth day a minor increase was noticed to 20.2 g O_2/L by the end of the experiment. Based on the complex composition of bactofugate (spores, LAB, yeasts …), the pH and COD evolutions may be related to the microbial growth and the secretion of several families of enzymes (Ledenbach & Marshall 2009).

As for the *Lb. plantarum* strain's performance on leachate, a slight variation of pH values was observed during the treatment (Figure 4). After three days of incubation with the LAB strain, only 11.5% of COD reduction was observed. At the end of the treatment, the percentage of COD reduction did not exceed 23%, compared to the bactofugate inoculation where the maximum abatement rate (4%) was obtained during the three first days. *L. plantarum* seems to be more efficient than bactofugate in the degradation of leachate organic matters. However, those findings remain far from the

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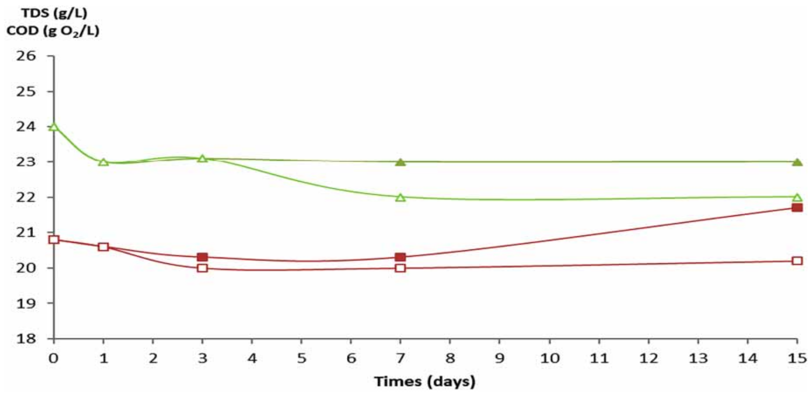

Figure 3 | Evolution of TDS (g/L) and COD (g O_2/L) during the biological treatment of leachate by inoculation using raw (B) and reactivated (RB) bactofugate (B: TDS (▲); COD (■) and RB: TDS (△); COD (□)).

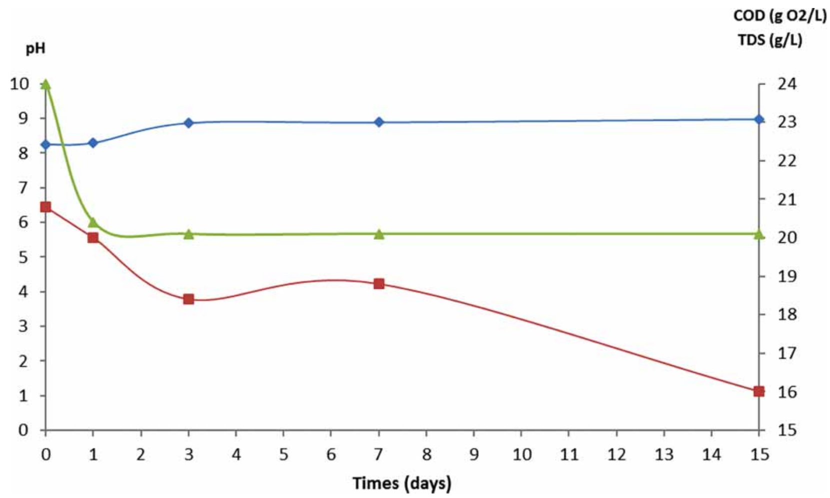

Figure 4 | Evolution of pH (◆), TDS (g/L) (▲) and COD (g O_2/L) (■) of leachate treated by *L. plantarum*.

reported results of Kasmi *et al.* (2016); where the *L. plantarum* strain exhibited significant COD removal exceeding 80% on dairy waste treatment.

Organic removal using fermented milk (Lben)

The landfill leachate was inoculated using the raw (L) and the reactivated expired Lben (RL) samples. As shown in Figure 5, the treated leachates by (L) and (RL) samples have similar profile evolutions. A slight variation in pH values was noticed. Yet, at the end of the treatment experiments (15 days), a drop of 47.1% in COD values was recorded for leachate treated by (L) samples to reach 11 g O_2/L. It is noteworthy that a COD removal rate of 17.3% was recorded only during the first day. Identically, the leachate treatment using (RL) samples resulted in COD removal rate of 42.3%, where 13.5% was noticed during the first day. Though, the obtained results confirm that there is no interest to use the reactivated samples for the leachate treatment. Both raw (L) and (B) samples ensure better results in terms of organic matter removal than the reactivated samples.

Besides, leachate inoculation using expired Lben has been demonstrated to be more effective for COD removal than its inoculation using bactofugate. This could be attributed to the indigenous microorganism of Lben samples as reported by Benkerroum & Tamime (2004) where yeasts

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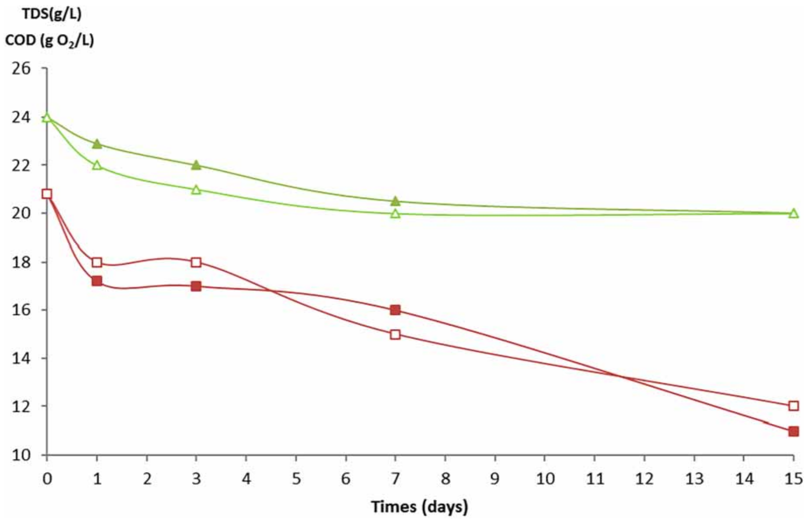

Figure 5 | Evolution of TDS (g/L) and COD (g O_2/L) of leachate treated by raw (L) and reactivated (RL) Lben (L: TDS (▲); COD (■) and RL: TDS (△);COD (□)).

and moulds (such as *Saccharomyces cerevisiae* and *Kluyveromyces marxianus*) are predominating. Several works have reported that yeast showed better removal efficiency than bacteria (Wichitsathian *et al.* 2004). Furthermore, several studies confirmed that some white rot fungus could be an effective biological actor for the treatment of LFL (Abdullah *et al.* 2013; Tigini *et al.* 2013).

Further investigations were performed in this work to focus on leachate pollutants removal using the expired fermented milk. Ammoniacal nitrogen content in leachate was assessed during the biological treatment using (L) samples. Results show an NH_3-N decrease over 15 days of treatment to reach a removal rate of 80%. Thereby, a reduction of 47% was recorded in COD values. Leachate ammoniacal nitrogen was metabolized by the expired Lben microorganisms for the benefit of their microbial growth (Fudala-Ksiazek *et al.* 2014). In addition, landfill leachate is highly loaded with heavy metals, whereas the recorded increase of pH values indicates a certain decrease in metal solubility (Harmsen 1983). Thus, levels of Cr^{3+} and Fe^{2+} were notably reduced, where reduction rates reached 90%. The Dm, VSS and Mm were also evaluated for the treated leachate using expired Lben. The results showed an increase of DM (from 3.72 to 4.15%), Mm (from 2.75 to 3%) and MVS (from 0.74 to 1.56%) concentrations. After the biological treatment, leachate turbidity was reduced considerably from 900 NTU to 300 NTU. All the determined parameters confirm the biodegradability of leachate using the expired Lben. Nevertheless, the obtained results remain less interesting compared to Kalčíková *et al.* (2014) findings using the white rot fungus *Dichomitus squalens* which was able to grow in the leachate, resulting in a COD removal rate of 60%. In another work, Wichitsathian *et al.* (2004) investigated the efficiency of yeasts and bacteria-based membrane bioreactors in leachate treatment. Both microorganism species showed significant COD removal performance (~70% in 24 h) in the membrane bioreactors equipped with air compressor. It is therefore supposed that air incorporation would have a beneficial effect on the microbial growth in leachate and on its organic matter biodegradation thereafter.

Bioreactor configuration with air incorporation

In this section, biological leachate treatment using the expired fermented milk (L) in a bioreactor equipped with air incorporation is proposed (Figure 2).

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Performance of bioreactor in the COD removal of landfill leachate

As shown in Figure 6, pH values of landfill leachate samples of Jebel Chakir discharge showed a nearly unchanged value between 7.6 and 8.1 during 24 h. This pH range is appropriate for the growth of LAB, which are probably best characterized as neutrophils (Hutkins & Nannen 1993). In terms of organic load removal, the COD concentration exhibited a linear decrease during 16 hours to reach 8 g O_2/L. Thereafter, COD values were stabilized to the end of the experiment. Those results confirm that air incorporation in the biological treatment of leachate-enhanced dairy indigenous microorganism performances for the leachate organic matter biodegradation. In fact, the biological treatment duration of leachate using expired L sample was reduced to 16 hours with a removal rate of 46%. Whereas, similar results (47.1%) were obtained without air incorporation after 15 days. Compared to Welander & Henrysson (1998) findings using a moving-bed biofilm reactor for leachate treatment, where the COD removal rate was around 20% during 40 days, the proposed treatment process for Jebel Chakir leachate in this study exhibited interesting results after only 16 hours of fermentation.

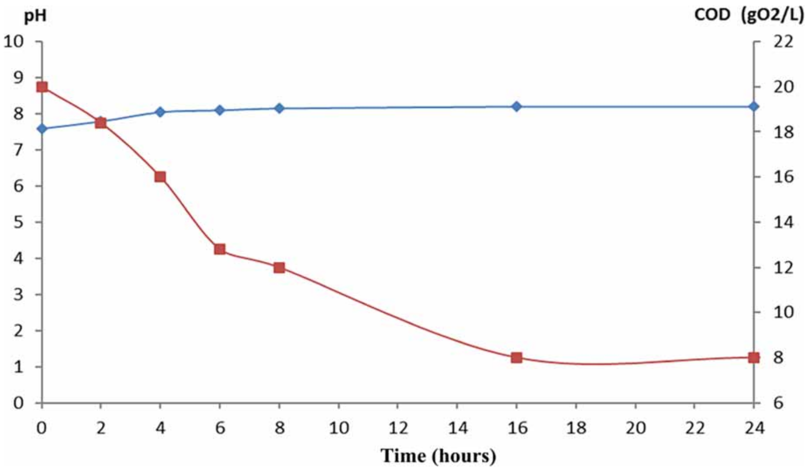

Figure 6 | Evolution of pH (◆) and COD (g O_2/L) (■) of leachate treated by (L) in bioreactor with air incorporation.

Performance of bioreactor in the heavy metals removal of landfill leachate

The results presented in Table 2 show that Fe was the predominant heavy metal in the leachate (19.1 mg/l) while Pb was the lowest (0.87 mg/l).The high level of Fe indicates the dumping of steel scrap in the landfill. This explains the brown dark color of the leachate, which is an oxidation product of ferrous to ferric form and the formation of ferric hydroxide colloids and complexes with humic acid (Kanmani & Gandhimathi 2013). After the treatment in bioreactor, the removal rate of

Table 2 | Heavy metals removal in leachate treated using expired Leben in the bioreactor with air incorporation

Parameters	Fe (mg/L)	Cd (mg/L)	Cu (mg/L)	Pb (mg/L)
Bioreactor input	19.80	1.13	1.19	0.87
Bioreactor output	11.18	0.95	0.08	0.17
Removal rate (%)	43.53	92.00	92.00	80.45
Tunisian limits into natural recipient	1.00	0.005	1.50	0.50
Tunisian limits into hydraulic recipient	1.00	0.005	0.50	0.10

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Fe, Cu, Cd and Pb were 43, 92, 92, 80%, respectively. According to Kanmani & Gandhimathi (2013), the high pH (from 7 to 9) in the leachate sample indicates low metal solubility. This latter decrease generally due to the precipitation of metal ions as insoluble hydroxides at high pH values.

Moreover, a number of factors influence the efficiency of biomass removal in biological processes such as the chemistry of the metal ions, the specific surface properties of the microorganisms, the cell physiology and the physical chemical influences from the environment. In the last decade, the application of bacterial biomass (Shakoori & Qureshi 2000) and fungi (Rehman *et al.* 2010) for the removal of heavy metals has gained increasing importance in the remediation of wastewaters. Rehman *et al.* (2010) proved that the treatment of LFL using C. *tropicalis* CBL-1 strains may have potential as a biosorbent of heavy metals-containing wastewaters. They reported that *C. tropicalis* was able to remove 73% of Cd after 12 days of incubation. LAB was also described as a potential biosorbent of heavy metals. However, studies investigating heavy metal biosorption by LAB are barely available (Kinoshita *et al.* 2013). Recently, Bhakta *et al.* (2012) tested 11 LAB strains isolated from mud and sludge for their efficiency in removing heavy metals from wastewater. They reported that *Lactobacillus reuteri* showed the highest Cd^{2+} (25%) and Pb^{2+} (59%) removal capacities. On the other hand, Schut *et al.* (2011) investigated the biosorption of Cu^{2+} by different *Lactobacillus* species from must and wine. *Lactobacillus buchneri* showed the highest biosorption at a maximum of 46.17 mg Cu^{2+} bound per mg cell in deionized water. In other research carried out by Ibrahim *et al.* (2006), it was indicated that *Lactobacillus rhamnosus* may have potential application in bioremediation of Cu^{2+} and Pb^{2+} in wastewaters.

Consequently, some studies have confirmed LAB potential for heavy metals removal. The applied approach using a bioreactor with air incorporation for LFL treatment and using fermented milk as a mixture of LAB and yeasts, not only promoted COD removal efficiency but also heavy metals reduction.

CONCLUSIONS

The release of Jebel Chakir leachate to the environment is one of the major environmental impacts related to disposal of waste. High values of EC, TDS and various toxic heavy metals were exceeding the permissible limit. Thus, a biological treatment process using bactofugate (B), expired Lben (L) and *Lactobacillus plantarum* were proposed. The obtained results proved that the expired fermented milk was the most effective in terms of COD and NH_3-N removal, with removal percentages of 47.1% and 80%, respectively, during only 15 days. Air incorporation for the treatment of leachate in a bioreactor exhibited similar COD removal efficiency during only 24 h. Moreover, the treatment in the bioreactor showed important heavy metals removal, especially for Cu, Cd and Pb, with removal rates exceeding 80%. These results highlight the positive effect of the expired fermented milk 'Lben' in the biological pre-treatment of leachate. The organic matter content, NH_3-N and heavy metals were efficiently degraded. Thus, the proposed approach could be a promising alternative for the recycling of dairy waste as well as for landfill leachate pre-treatment.

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