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Mixed culture of Lactococcus lactis and *Kluyveromyces marxianus* isolated from kefir grains for pollutants load removal from Jebel Chakir leachate

Yasmin Cherni [1,*], Cristian Botta[2], Mariam Kasmi[1], Irene Franciosa[2], Luca Cocolin[2], Abdelwaheb Chatti[1], Ismail Trabelsi[1], Lobna Elleuch[1]

[1] Laboratory of Treatment and Valorization of Water Rejects, Water Researches and Technologies Center, Borj-Cedria Technopark, University of Carthage, 8020 Soliman, Tunisia

[2] Department of Agriculture, Forest and Food Sciences, University of Torino, Turin, Italy

Keywords: ammonium nitrogen removal, bioremediation, landfill leachate, microorganisms, organic materials removal

Abstract

The wastewater from the dumping site usually contains high pollutant levels. Biological process and physic-chemical treatments are among several technologies for wastewater treatment. Using microorganisms in the treatment of landfill leachate is an emerging research issue. Furthermore, bioremediation is a feasible approach for pollutants removal from landfill leachate which would provide an efficient way to resolve the issue of landfill leachate. In this study, the performance of yeast and bacteria isolated from kefir grains was assessed for landfill leachate treatment. Kefir grains microbial composition was evaluated by molecular approaches (Rep-PCR and 16S rRNA gene sequencing). The obtained outcomes denoted that high concentrations of lactic acid bacteria and yeast populations (over 107 CFU/ml) were found in the kefir grains and were essentially composed of *Lactococcus lactis*, *Lactobaccillus kefirien*, *Bacillus* sp., *L. lactis*, and *Kluyveromyces marxianus*. The co-culture with 1% of inoculum size was demonstrated as the most efficient in the degradation of different contaminants. The overall abatement rate of chemical oxygen demand (COD), ammonium nitrogen (NH+4 −N), and salinity were 75.8%,

85.9%, and 75.13%, respectively. The bioremediation process resulted in up of 75% removal efficiency of Ni and Cd, and a 73.45%, 68.53%, and a 58.17% removal rates of Cu, Pb, and Fe, respectively. The research findings indicate the performance of *L. lactis* and *K. marxia*nus co-culture isolated from kefir grains for the bioremediation of LFL.

Practitioner Points

-Isolation and identification of microorganisms from kefir grains was carried out. • Biological treatment of LFL using monoculture of (Lactococcus lactis; Kluyveromyces marxianus) and co-culture (5% of L. lactis and 5% K. marxianus) has been performed.

-Biological treatment using co-culture strain is an effective approach to remove or- ganic matter, NH+

4 −N and heavy metals.

Introduction

Industrialization, urbanization increase, and technological advancements have induced a rapid growth in the municipal solid waste (MSW) production. Throughout the world, appropriate management of MSW is becoming one of the most challenging environmental problems. For decades, landfilling has been commonly applied as an ultimate disposal practice for MSW (Klauson et al., 2015; Oulego, Collado, Laca, & Díaz, 2016). However, the percolation and filtration of rainwater into the waste layers can produce important amounts of landfill leachate (LFL) (He et al., 2016; Zhang et al., 2013). This waste- water is a complex mixture of several pollutants like organic compounds, ammonia–nitrogen, inorganic salts (e.g., chlo- ride, sulfate, sodium, etc) and heavy metals (e.g., copper, iron, lead, manganese, etc) (Vaverková et al., 2018; Xie et al., 2012). Various factors influence the quality of leachate such as the waste age, the climatic conditions, the waste composition as well as the depth of the landfill site (Ghani, Yusoff, Zaman, Zamri, & Andas, 2017; Mandal, Dubey, & Gupta, 2017). Due to its com- plex composition, landfill leachate must be properly treated to remove organic materials and ammonium–nitrogen (NH+4 −N) before its discharge into the environment. Therefore, it is necessary to select sustainable processes to manage and treat this particular effluent. Accordingly, several studies have been focused on LFL treatment using different approaches such as electro-coagulation; nanofiltration; oxidation and photocatalysis (Kamaruddin, Yusoff, Aziz, & Hung, 2015). However, the high costs and the results of secondary pollutants in some cases are le major disadvantages of these processes. So far, biological processes have gained an interest for the LFL treatment since they have been considered as the most environmentally friendly processes (Klauck et al., 2017). The presence of microorganisms with important biodegradation potentials and resistance to different contaminants could be a potential problem-solving of LFL treatment (Wang et al., 2018). As reported in the literature, several investigations have been demonstrated that some microorganisms are able to degrade dissolved organic matter contained in the wastewater (Sosa et al., 2017; Wang et al., 2018; Westlund & Yargeau, 2017). However, it is worthy to highlight that a few researches have focused on NH+4 −N abatement rate of LFL using bioremediation process (Cherni et al., 2020; Elleuch et al., 2020). In fact, several microorganisms have been tested for the assimilation of different heavy metals (Abbas & Badr, 2015; Mohd et al., 2017). Others have described the effi- ciency of polycultures (consortium) in wastewater treatments, including biomass production and pollutants removal (Ayed, Abid, & Hamdi, 2019; Gonçalves, Pires, & Simões, 2016). The use of microbial consortium for contaminants removal can be very beneficial since combining microorganisms was found to lead to the improvement of a robust biological system that can operate under different stress conditions which can enhance pollutants uptake loads (Ayed, Asses, Chammem, & Hamdi, 2016; El ouaer, 2020). In the same vein, Kumari, Ghosh, and Thakur (2016) demonstrated the efficiency of LFL treatment using a consortium of microalgae and bacteria. Bacto-algal mixed culture proved efficiency in organic matters degradation and heavy metals biosorption. Furthermore, a study achieved by Zhang, Vahala, Wang, and Smets (2016) describes communities and their biological activity in LFL treatment. It has been reported that the major factor that affecting the bioremediation performance is the capacity of the added culture to display its activities and survive in different physiological conditions (Song, Wang, Yue, & Li, 2013; Westlund & Yargeau, 2017). Highly adaptive bacteria exceeded an important removal rate of pollutant substances. Consequently, a complex symbiotic microbial consortium of several yeasts and bacteria would be interesting mixture to overcome stressing LFL culture conditions. In this context, the present work supposes that kefir grains (KGs) as a microbial consortium constituted mainly of some bacterial species such as lactobaccili, lactococci, and leuco- nostoc and yeast species such as Kluyveromyces, Candida, and Saccharomyces growing in ecological niche (Bengoa, Iraporda, Garrote, & Abraham, 2019; Richard, 2016) could be promote for the removal of pollutants. Thus, the aim of this research is to assess the performance of Lactococcus lactis and K. marxianus monoculture and co-culture in the biodegradation of landfill leachate.

Materials and methods

Isolation and identification of microorganisms Microorganisms were isolated from Tunisian kefir product. Seventeen gram kefir samples were aseptically taken and homogenized with sterilized Ringer's solution. The samples were homogenized for 3 min in a stomacher. The serial decimal dilutions were prepared in Ringer water and plated for bacterial and yeast counts. Bacteria strains were grown on MRS agar plate's counts (Man, Rogosa, Sharpe, Heywood, Lancashire, UK) agar supplemented with 0.025 g/ml of Delvocid (Sigma) and incubated at 30°C for 24 hr, whereas yeasts and molds were grown from W.L agar plate's counts (Wallerstein Laboratory Nutrient Agar) supplemented with 0.05 g/ml of Tetracycline (Sigma) at 25°C for 48 hr. Yeast and bacteria strains were randomly picked, subjected to Gram staining (for bacteria strains), purified and growth in YPD (dextrose [2%], bacteriological peptone [1%], yeast extract [1%]), and MRS broth, respectively. Purified strains were maintained at −20°C with 30% (v/v) of glycerol until the use in the bioremediation experiments. All purified isolates were subjected to DNA extraction (Cocolin et al., 2004). Afterward, amplification of the FD1-RD1 region of 16s rRNA (Weisburg, Barns, Pelletier, & Lane, 1991) and ITS-5.8S rDNA region (Korabečná, Liška, & Fajfrlik, 2003) was carried out for bacteria and yeast isolates, respectively. Bacteria and yeasts were identified by alignment of the sequenced amplicon with Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi).

LFL characterization

LFL sampling. Leachate samples were collected from Jebel Chakir landfill. It is located in the southwest side of Tunis City and has started operating in 1999. The site occupies 47 ha over a reserved total area of 124 ha (ANGed & GIZ, 2014; Ismail et al., 2011). In this study, raw leachate samples were collected from the collection systems at the Jebel Chakir landfill site in 20 L plastic barrels, transported to the laboratory and stored at the refrigerator before being used and analyzed.

LFL physicochemical analysis. The performance of biological LFL treatment is evaluated by measuring the decrease of the organic matter, ammonium, and heavy metals. The analyses were assessed on raw and treated leachate. The initial pH of the leachate was modified to the desired value using 1 M hydrochloric acid. PH, TDS, and EC were measured by a multi parameter type «Consort C 860». COD, BOD5, and NH+ 4 −N were determined according to Rodier and Legube (2009). Total Kjeldahl nitrogen was measured according to Rodier and Legube (2009). The concentrations of heavy metals were determined using flame atomic absorption method (Analytic Jena AG Spectrometer AAS vario 6). The bacterial cell biomass was detected by optical density of samples at 600 nm. The LFL characteristics are showed in Table 1.

Main physicochemical characteristics of Jebel Chakir LFL.

Physicochemical proprieties of Jebel Chakir LFL were determined according to the following parameters: COD, pH, salinity, electrical conductivity (EC), and heavy metals (Table 1). The raw LFL showed a dark brown color, physical, and chemical parameters presented an alkaline pH of 7.73 and high conductivity (20.6 ms/cm) as well as considerable levels of COD (26.200 mg O2/L) that can be attributed to the high initial organic matter in the leachate. The relatively high levels of salinity (3.62 g/L) demonstrated the presence of inorganic contents in the studied leachate. It was noticed that leachate samples contain significant amounts of toxic heavy metals such as Ni (3.52 mg/L), Cu (1.62 mg/L), Cd (2.73 mg/L), Pb (1.78 mg/L), and Fe (9.23 mg/L). Similar results were reported by Ellouze, Aloui, and Sayadi (2008), the leachate presented an important quantities of organic matter, nitrogen, and toxic heavy metals especially Fe (20.6 mg/L). The relatively high levels of contaminants including organics, ammonia, inorganic substances, and toxic metals confirmed the high organic load of the dumped garbage in Jebel Chakir LFL.

Bioremediation process for landfill leachate treatment Inoculum preparation.

The isolation and identification of bacteria and yeast strains were done as described previously in Section Isolation and identification of microorganisms. The yeast strain was inoculated to 50 ml of synthetic nutrient broth medium YPD (dextrose [2%], bacteriological peptone [1%], yeast extract [1%]) and incubated at 30°C for 48 hr with 150 rpm agitation speed. For the preparation of bacteria inoculum, the strain was inoculated to 50 ml MRS broth medium and incubated at 37°C for 24 hr with 150 rpm agitation speed. Then, the microbial inoculums were used for bioremediation process.

Experimental set-up. Bioremediation was performed at initial pH of 5. The process was achieved in batch with Erlenmeyer flask (50 ml) containing 20 ml of the wastewater. A set of experiments were carried out in duplicate. For the first set of experiments, the selected monoculture of (L. lactis; K. marxianus) was added separately in the test samples at different inoculum sizes (1%, 3%, and 5% [v/v]) and incubated in the orbital shaker with a rotation speed of 150 rpm at room temperature for 10 days. For the second set of experiments, three inoculum sizes (1%, 3%, and 5% [v/v]) were tested. A bacteria and yeast co-culture was prepared by taking 5% of L. lactis and 5% K. marxianus, respectively. Bacteria and yeast inoculums were added separately in the leachate samples and incubated in the orbital shaker with a rotation speed of 150 rpm at room temperature for 10 days. A blank experiment which consisted of raw leachate was run in the same condition of the test samples.

Results and discussion

Strain selection

Kefir grains were analyzed microbiologically to identify the predominant microorganisms. A total of 54 bacteria and 27 yeasts were subjected to Rep-PCR fingerprinting technique to estimate bacteria and yeast diversity of kefir grains. Then, 11 bacteria and 4 yeasts were chosen as representative of each sub-cluster obtained (70% of similarity) using the Pearson correlation. The sequences were aligned to the query sequences of the GenBank 16S rRNA and ITS-5.8S rDNA sequences database, resulting in identities of known sequences of 99%–98%, as shown in Table S1 (Supporting Information). Lactoccocus lactis and K. marxianus are among predominant microbial populations from the kefir grains. Figure 1 presented the obtained strains after visualization under ultraviolet light and the resulting profiles were determined by digital image capturing using a CCD UVI pro Platinum 1.1. Recently, Elleuch et al. (2020) reported the cost effectiveness of kefir grains as a biological pretreatment for landfill leachate. Overall, TOC, COD, NH+4 −N, and PO3− 4 decreased, respectively, by 93%, 83.33 %, 70%, and 88.25% with respect to the raw effluent (24,000 mg/L), thus reflect- ing the resistance of these grains to the toxicity of leachate (Elleuch et al., 2020). Same conclusions were reached by Mohd et al. (2017) and Wang et al. (2018) showing the efficiency of these genera of lactic acid bacteria and the yeast Kluyveromyces marxianus for the removal of organic matter and toxic substances from wastewaters. In this context, lactic acid bacteria and yeast strains isolated from KGs could be potential for the removal of organic matter and toxic pollutants from LFL. In addition, Milanowski et al. (2017) worked on the biosorption of silver using L. lactis strains. It was found that the lactic acid bacteria was able to grow and absorbed about 70%–96% of silver from 1 ppm solution. Yadav et al. (2014) proved the performance of K. marxianus in the biodegradation of cheese whey (78% of COD removal after 30 hr of incubation).

Bioremediation treatment of LFL using the selected bacterial strain and yeast

The progress in bioremediation rates depends, to a great extent, on the ability of the introduced microorganisms to survive and display their activities in difficult conditions (Bardi et al., 2017; Tigini, Prigione, & Varese, 2014). In recent years, various research studies on the detoxification and treatment of wastewaters using lactic acid bacteria (LAB) and yeasts have been carried out world- wide (Reis et al., 2017; Yi et al., 2017; Zhang et al., 2016).

In this work, the bioremediation was monitored to provide an insight into its efficiency in reducing the contaminant load in the leachate using the selected strains. During the bioremediation process, the effects of the treatment duration (each day) and the inoculum size (1%, 3%, 5% [v/v]) of L. lactis and K. marxianus were studied.

Change in organic matter and NH_4^+ −N during

bioremediation. Despite the modification of the samples, the LFL was too toxic to allow the growth and the metabolic activity of the introduced microorganisms L. lactis and K. marxianus were used to assess the performance of bioremediation treatment of LFL. Furthermore, pollutants removal efficiency of LFL using the mixture of L. lactis and K. marxianus was studied to evaluate if there was any synergy or inhibition effects of these two microorganisms on the pollution removal in the bioremediation treatment process. To our knowledge, the present work is the first investigating the ability of L. lactis and K. marxianus to degrade several pollutants from LFL. The variation of COD using the two microorganisms and consortium is reported in Figure 2.

It is clearly seen that the consortium response seems to be better compared to both monoculture of L. lactis and K. marxianus. The co-culture exhibited an appreciable COD reduction in a shorter degradation time only after 3 days. The COD reduction was comparatively higher with the samples at 1% of inoculum size. Furthermore, the findings showed that the maximum COD removal rate increased greatly (75.8%) using the co-culture compared with those of L. lactis (52.3%) and K. marxianus (56.2%), which suggested no competition or inhibition between the two selected strains. It was possible due to the co-culture synergy effect on increasing the growth abil- ity, biomass production, and enzyme activity. This outcome is promising because it proved not only the compatibility of yeast and bacteria populations but their complementarity. In fact, an important COD concentration might accelerate the growth of heterotrophic bacteria, which would consume oxygen and nutrients rapidly (Patureau et al., 2001). In addition, some researchers have noted the benefits of applying mixed cul- ture over single strains cultures (Alcántara et al., 2015; Wilkie & Mulbry, 2002). The findings of our work seem to be more interesting than those described in Razarinah, Zalina, and Abdullah (2014). As results of experiments, maximum simultaneous COD and BOD_5 removal were achieved 89.14% and 2.11%, respectively, after 28 days of incubation using immo- bilized Trametes menziesii. Recently, Er, Seow, Lim, Ibrahim, and Sarip (2018) tested Brevibacillus panacihumi strain ZB1 for the removal of toxic compounds from LFL. As a result, COD and ammonia–nitrogen degradation were attained ~40% and ~50%, respectively, after 42 days of incubation. On the other hand, the variation trends of COD removal rate using L. lactis and K. marxianus were similar with 3% and 5% of inoculum size, as shown in Figure 2. The most important COD removal rate using L. lactis and K. marxianus with 3% inoculum size were 30.1% and 36.3%, respectively. The abatement rate of COD using L. lactis and K. marxianus with 5% of inoculum size were 31.2% and 29.6%, respectively. The mixture of L. lactis and K. marxianus with 3% of inoculum size increased the maximum COD removal rate to 38.2% and slightly decreased to 28.1% with 5% of inoculum size after 10 days of incubation for LFL treatment. As far as the control samples, no important COD degradation was observed. According to the literature, several studies reported that the inoculum size is a relevant factor to improve the biodegradation of wastewater (Bohutskyi et al., 2016; Elleuch et al., 2020). Thus, the inoculation of 1% of consortium had almost a positive effect on the degradation rate during the biological process. However, the addition of 3% and 5% of consortium was unfavorable for the degradation and pollutants removal. In the same vein, previous studies revealed the benefits of nutrients to accelerate and/ or facilitate the biodegradation of contaminants. This is principally explained by the biostimulation of microorganisms through the supply of nutrients such as carbon, nitrogen, and phosphorus (Dadrasnia, Azirun, & Ismail, 2017). For that the possible interpretation was that the inefficiency of 3% and 5% could be attributed to the low nutrients availability for their stimulation, which could have decreased the biodegradation process. Therefore, future studies should focus on improving the proprieties of substrates, inoculums, and the environmental conditions.

In this research, the second result was NH_4^+ −N reduc- tion in leachate. As shown in Figure 3. NH_4^+ −N level in the studied leachate was 780 mg/L. According to the literature, the high amount of NH_4^+ −N was probably due to the fermentation and hydrolysis of the nitrogenous fragments of biodegrad- able refuse. The obtained results proved the strains co-culture capacity to survive under a considerable NH_4^+ −N amount. Bioremediation presented a great effectiveness in NH_4^+ −N reduction for all tested treatments. The reduction rate of NH_4^+ −N remarkably increased after one week and improved until the last day of the study. The maximum NH_4^+-N reduction of leachate with 1% of co-culture addition was approximately 85.9%. The highest NH_4^+-N removal rate using co-culture with 3% and 5% of inoculum size was 23.06% and 33.29%, respec- tively. The same yields were obtained for the samples treated by L. lactis and K. marxianus. Compared with the control, amending the leachate with 1% of L. lactis and K. marxianus pure cultures increased the NH_4^+-N removal rate about 48.6% and 37%, respectively. Although 21.05% and 26.47% NH_4^+-N removal rates have been recorded using K. marxianus at inocu- lation sizes of 3% and 5%, respectively. The maximum NH_4^+-N reduction of LFL without culture addition was approximately 15.4%. This result could be attributed to the effect of enzyme production and biomass activity of the microorganism (Mohd et al., 2017). In view of that, our outcomes showed a significant degradation of organic matter compared to other studies which highlighted the performance of the consortium used in this work. For example, Raposo, Oliveira, Castro, Bandarra, and Morais (2010) reported 13%–15% of COD removal using a consortium of Chlorella vulgaris and brewery wastewater native microalgal-bacterial consortia after 20 days of treatment. Our findings indicated that the co-culture possessed pollutions removal abilities for LFL. Moreover, the above results suggested that the inoculum size has a great effect on the organic matter and NH_4^+-N degradation. 1% of inoculum size was found to be more efficient in the removal of pollutants. For this reason, in the subsequent analyses using L. lactis, K. marxianus, and the consortium, only the inoculation size of 1% will be considered.

Change in salinity during bioremediation.

Salinity assessment values in the LFL ranged from 3 to 5 g/L. Such variation could probably be due to the possible precipitation of salts with other LFL compounds while it was kept under refrigeration. As illustrated in Figure 4, the salinity of different leachate samples increased rapidly at the beginning, then, it stabilized gradually over the experimentation time. The evolution trends of leachate salinity inoculated by 1% L. lactis, K. marxianus, and consortium were similar. The first observation could show the ability of different strains not only to survive but also to grow in a stress environment with a high concentration of salts. This finding confirms that L. lactis, K.

marxianus, and consortium are resistant strains to high salts contents in LFL. This property was also reported by Huang, Liu, Liang, and Mao (2014) and Tekarslan-Sahin, Alkim, and Sezgin (2018) who proved the ability and tolerance of lactic acid bacteria and yeasts to survive at higher salinity value. In fact, the salinity was increased from 3.62 to 0.8 g/L in leachate inoculated with consortium. Also, when L. lactis and K. marxianus were inoculated, the salinity was reduced to 1.5 and 1.2 g/L, respectively. Thus, the salinity increase is attributed to the growth and the accumulation in cell walls as well. Numerous research studies have proved that yeast cells exposed to high salt contents show dehydration, physiological and biochemical variations, and gene modification (Mage & Siderius, 2002). Applying detoxification mechanisms and ion transport, the cells demonstrate an important tolerance to Na+ stress through osmotic regulation by adsorbing Na+ salts ability and tolerance of lactic acid bacteria and yeasts to survive at higher salinity value. In fact, the salinity was increased from 3.62 to 0.8 g/L in leachate inoculated with consortium. Also, when L. lactis and K. marxianus were inoculated, the salinity was reduced to 1.5 and 1.2 g/L, respectively. Thus, the salinity increase is attributed to the growth and the accumulation in cell walls as well. Numerous research studies have proved that yeast cells exposed to high salt contents show dehydration, physiological and biochemical variations, and gene modification (Mage & Siderius, 2002). Applying detoxification mechanisms and ion transport, the cells demonstrate an important tolerance to Na+ stress through osmotic regulation by adsorbing Na+ salts inside the cell (Dhar, Sägesser, Weikert, Yuan, & Wagner, 2011; François, Walther, & Parrou, 2012).

Change in heavy metals during bioremediation.

Recently, the biosorption of heavy metals by a variety of biomasses including bacteria, fungi and algae has been demonstrated as important economical and effective alternatives (Mehta & Gaur, 2005; Romera, Gonzalez, Ballester, Blazquez, & Munoz, 2006). Since the area of biosorption is huge, our study was restricted to the toxic metals such as cadmium, nickel, copper, zinc, and iron biosorption using 1% of L. lactis, K. marxianus, and the co-culture. As reported in Table 2, the maximum recorded removal rates of Ni, Cr, Cd, Pb, and Fe were 81.53%, 73.45%, 79.48%, 68.53%, and 58.17%, respectively, with the co-culture inoculation size of 1% (v/v). By comparing the samples treated with 1% (v/v) of L. lactis and with 1% (v/v) of K. marxianus, it can be found that L. lactis showed higher ability in the removal of Ni (39.77%) and Cd (62.63%). According to the literature, several studies have been performed to explore LAB and yeasts in the wastewaters treatment (Reis et al., 2017; Zhang et al., 2016). These outcomes were in agreement with Han et al. (2006) findings using beer yeast for the removal of Cu^{2+} and Pb^{2+} from wastewater. The experimental results exhibited the capacity of yeast to consume Cu^{2+} and Pb^{2+} and therefore, to reduce these toxic metals level in the culture medium. Otherwise, Bhakta, Ohnishi, Munekage, Iwasaki, and Wei (2012) tested the performance of eleven LAB strains isolated from mud and sludge in heavy metals removal rates from wastewater. They reported that Lactobacillus reuteri showed the highest Cd^{2+} (25%) and Pb^{2+} (59%) removal capacities. In another research carried by Schut, Zauner, Hampel, König, and Claus (2011), it was indi- cated that Lactobacillus species may have a great application in the reduction of Cu^{2+}.

Conclusion

In the present research, the bacteria and yeast isolates from kefir product were applied for landfill leachate treatment. The identification of the isolated microorganisms is presented to be L. lactis and K. marxianus. A consortium was constructed from bacteria and yeast mixed culture. As far as COD, NH_4^+ −N, and heavy metals removals are concerned; the results demonstrated that isolated bacteria and yeast strains have the ability to reduce the COD value up to 50% and NH_4^+ −N value up to 35%. Furthermore, the Addition of 1% (v/v) of inoculum showed the best biodegradation rate compared to 3% and 5% (v/v). However, a co-culture would prove to be more effective and beneficial compared to single strain. Significant results were obtained in co-culture (1% (v/v) of inoculum size) which reduces the COD (75.8%), NH_4^+ −N (85.9%), and salinity (75.13%). Also, the results proved that applying a bacto-yeast co-culture to Jebel Chakir leachate is a suitable treatment to remove high quantities of heavy metals like Ni (81.53%), Cu (73.45%), Cd (79.48%), Pb (68.53%), and Fe (58.17%). The LFL bioremediation process could be promising and considered as an effective green technology in the removal of organic com- pounds from LFL.

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Legend of figures:

Fig. 1 Representative polymorphic profiles of LAB and yeast colonies isolated from the Kefir grains: M, molecular marker-1,500 bp.

Fig. 2 COD removal during the bioremediation treatment of LFL using single and co-cultures with different inoculum size: 1% (a), 3% (b), and 5% (c).

Fig. 3 NH+ 4 −N removal during the bioremediation treatment of LFL using single and co-cultures with different inoculum size: 1% (a), 3% (b), and 5% (c).

Fig. 4 Salinity removal during the bioremediation treatment of LFL using 1% of single and co-cultures.

ability

Figure 1

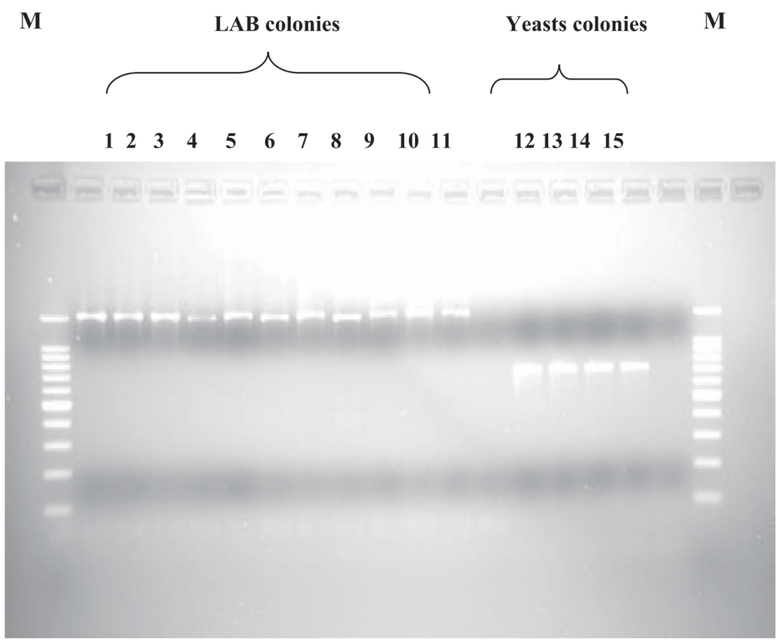


Figure 2

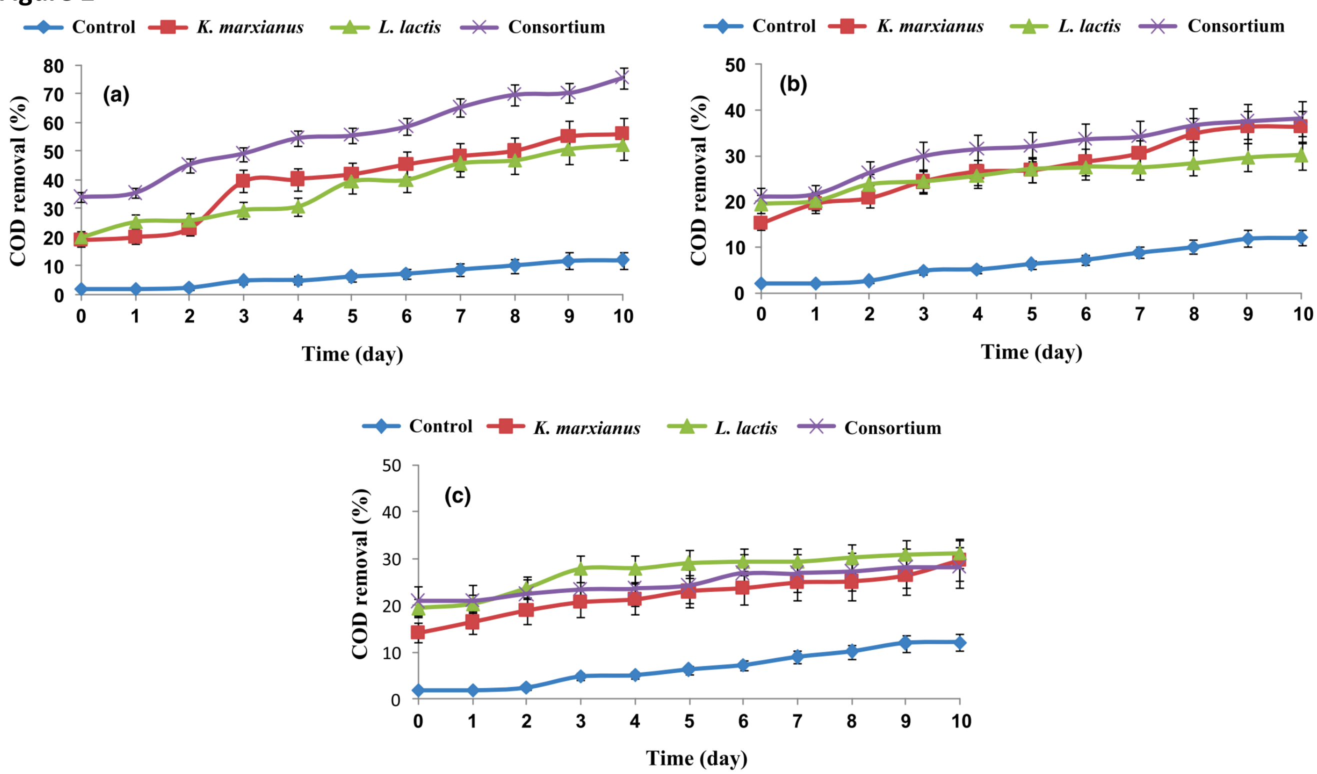


Figure 3

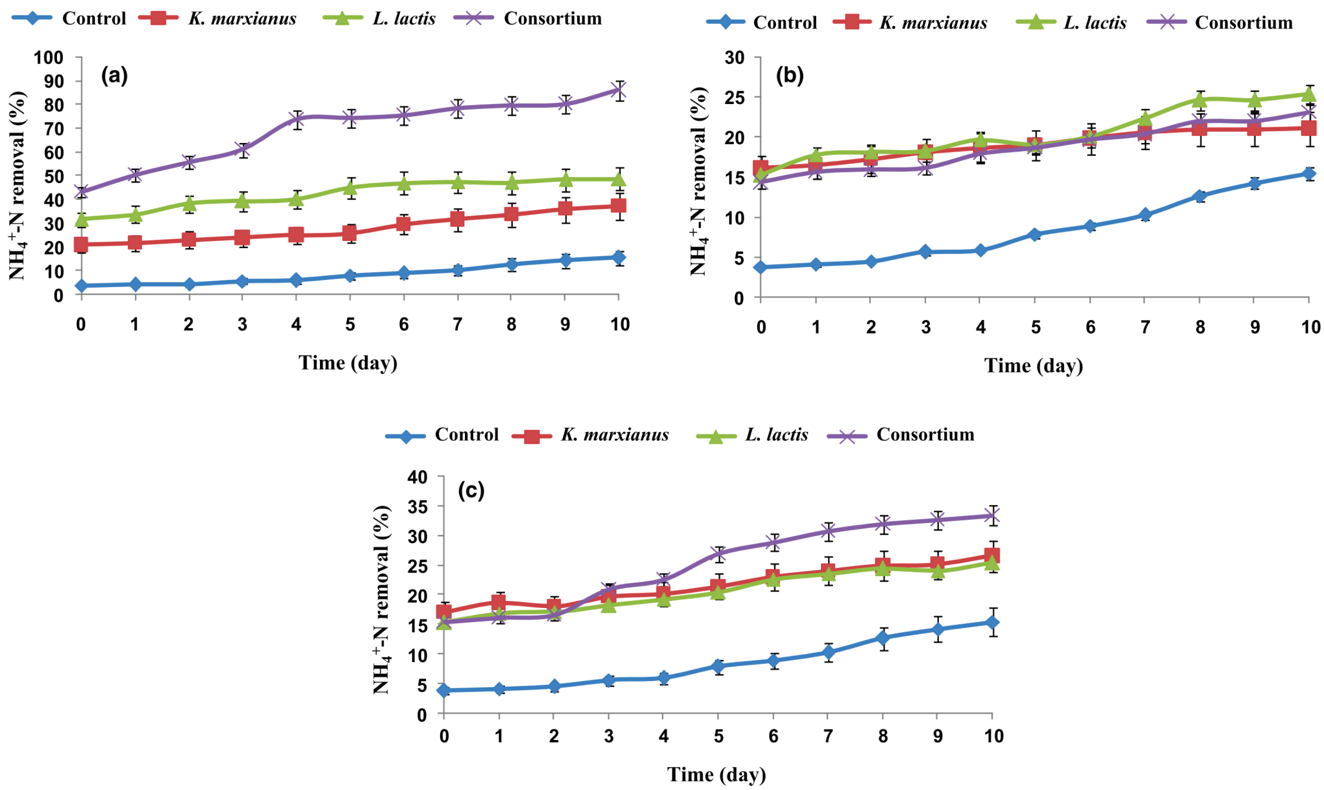
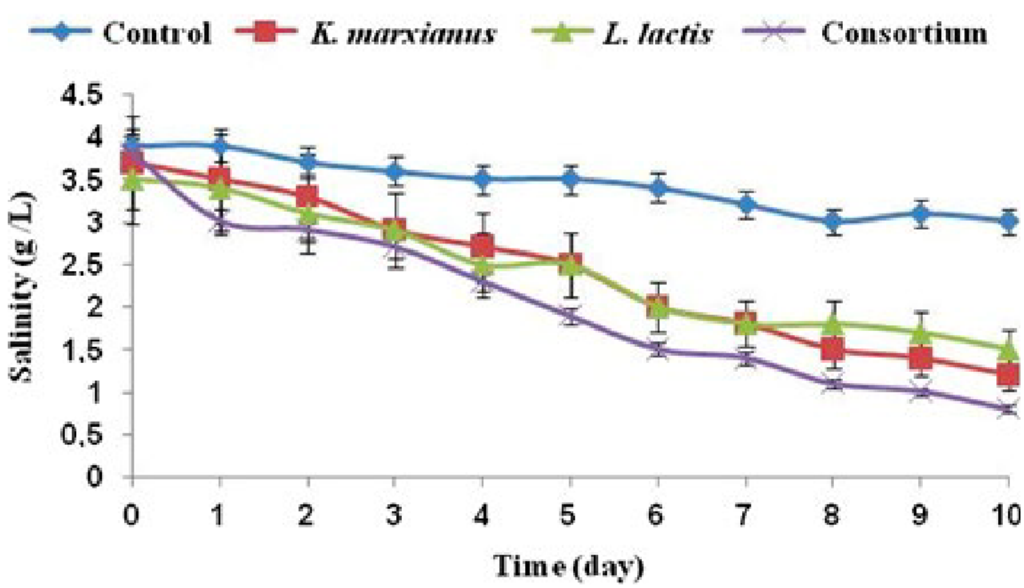


Figure 4



Tables

Table 1 Representative polymorphic profiles of LAB and yeast colonies isolated from the Kefir grains: M, molecular marker-1,500 bp.

Table 2 Heavy metals removal performance using single and co- culture after 10 days of treatment

PARAMETER	UNIT	VALUE
pH	–	7.73
COD	mg O_2/L	26,200
conductivity	mS/cm	20.6
Salinity	g/L	3.62
TKN	mg/L	1,640
TDS	g/L	3.4
$NH_4^+ - N$	mg/L	780
NO_3^-	mg/L	7.326
NO_2^-	mg/L	3.178
PO_4^{2-}	mg/L	28.292
Mg^{2+}	mg/L	15.6
Ca^{2+}	mg/L	12.3
Ni^{2+}	mg/L	3.52
Cu^{2+}	mg/L	1.62
Cd^{2+}	mg/L	2.73
Pb^{2+}	mg/L	1.78
Fe	mg/L	9.23

	REMOVAL RATE AFTER 10 DAYS OF TREATMENT (%)				
STRAINS	NI	CU	CD	PB	FE
1% of *K. marxianus*	14.20	42.59	54.94	56.17	38.46
1% of *L. lactis*	39.77	37.03	62.63	14.04	32.50
1% of co-culture	81.53	73.45	79.48	68.53	58.17