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(Article begins on next page)

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

3

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10

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

13

14 **Abstract**

15 The wastewater from the dumping site usually contains high pollutant levels. Biological process and physic-
16 chemical treatments are among several technologies for wastewater treatment. Using microorganisms in the
17 treatment of landfill leachate is an emerging research issue. Furthermore, bioremediation is a feasible approach
18 for pollutants removal from landfill leachate which would provide an efficient way to resolve the issue of landfill
19 leachate. In this study, the performance of yeast and bacteria isolated from kefir grains was assessed for landfill
20 leachate treatment. Kefir grains microbial composition was evaluated by molecular approaches (Rep-PCR and
21 16S rRNA gene sequencing). The obtained outcomes denoted that high concentrations of lactic acid bacteria and
22 yeast populations (over 10⁷ CFU/ml) were found in the kefir grains and were essentially composed of *Lactococcus*
23 *lactis*, *Lactobacillus kefirien*, *Bacillus* sp., *L. lactis*, and *Kluyveromyces marxianus*. The co-culture with 1% of
24 inoculum size was demonstrated as the most efficient in the degradation of different contaminants. The overall
25 abatement rate of chemical oxygen demand (COD), ammonium nitrogen (NH₄⁺ -N), and salinity were 75.8%,

26 85.9%, and 75.13%, respectively. The bioremediation process resulted in up of 75% removal efficiency of Ni and
27 Cd, and a 73.45%, 68.53%, and a 58.17% removal rates of Cu, Pb, and Fe, respectively. The research findings
28 indicate the performance of *L. lactis* and *K. marxianus* co-culture isolated from kefir grains for the bioremediation
29 of LFL.

30

31 **Practitioner Points**

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

35 -Biological treatment using co-culture strain is an effective approach to remove or- ganic matter, NH_4^+
36 $-\text{N}$ and heavy metals.

37

38 **Introduction**

39 Industrialization, urbanization increase, and technological advancements have induced a rapid growth in the
40 municipal solid waste (MSW) production. Throughout the world, appropriate management of MSW is becoming
41 one of the most challenging environmental problems. For decades, landfilling has been commonly applied as an
42 ultimate disposal practice for MSW (Klauson et al., 2015; Oulego, Collado, Laca, & Díaz, 2016). However, the
43 percolation and filtration of rainwater into the waste layers can produce important amounts of landfill leachate
44 (LFL) (He et al., 2016; Zhang et al., 2013). This waste- water is a complex mixture of several pollutants like organic
45 compounds, ammonia–nitrogen, inorganic salts (e.g., chlo- ride, sulfate, sodium, etc) and heavy metals (e.g.,
46 copper, iron, lead, manganese, etc) (Vaverková et al., 2018; Xie et al., 2012). Various factors influence the quality
47 of leachate such as the waste age, the climatic conditions, the waste composition as well as the depth of the
48 landfill site (Ghani, Yusoff, Zaman, Zamri, & Andas, 2017; Mandal, Dubey, & Gupta, 2017). Due to its com- plex
49 composition, landfill leachate must be properly treated to remove organic materials and ammonium–nitrogen
50 ($\text{NH}_4^+ -\text{N}$) before its discharge into the environment. Therefore, it is necessary to select sustainable processes

51 to manage and treat this particular effluent. Accordingly, several studies have been focused on LFL treatment
52 using different approaches such as electro-coagulation; nanofiltration; oxidation and photocatalysis
53 (Kamaruddin, Yusoff, Aziz, & Hung, 2015). However, the high costs and the results of secondary pollutants in
54 some cases are the major disadvantages of these processes. So far, biological processes have gained an interest
55 for the LFL treatment since they have been considered as the most environmentally friendly processes (Klauck
56 et al., 2017). The presence of microorganisms with important biodegradation potentials and resistance to
57 different contaminants could be a potential problem-solving of LFL treatment (Wang et al., 2018). As reported in
58 the literature, several investigations have been demonstrated that some microorganisms are able to degrade
59 dissolved organic matter contained in the wastewater (Sosa et al., 2017; Wang et al., 2018; Westlund & Yargeau,
60 2017). However, it is worthy to highlight that a few researches have focused on $\text{NH}_4^+ - \text{N}$ abatement rate of LFL
61 using bioremediation process (Cherni et al., 2020; Elleuch et al., 2020). In fact, several microorganisms have been
62 tested for the assimilation of different heavy metals (Abbas & Badr, 2015; Mohd et al., 2017). Others have
63 described the efficiency of polycultures (consortium) in wastewater treatments, including biomass production
64 and pollutants removal (Ayed, Abid, & Hamdi, 2019; Gonçalves, Pires, & Simões, 2016). The use of microbial
65 consortium for contaminants removal can be very beneficial since combining microorganisms was found to lead
66 to the improvement of a robust biological system that can operate under different stress conditions which can
67 enhance pollutants uptake loads (Ayed, Asses, Chammem, & Hamdi, 2016; El ouaer, 2020). In the same vein,
68 Kumari, Ghosh, and Thakur (2016) demonstrated the efficiency of LFL treatment using a consortium of
69 microalgae and bacteria. Bacto-algal mixed culture proved efficiency in organic matters degradation and heavy
70 metals biosorption. Furthermore, a study achieved by Zhang, Vahala, Wang, and Smets (2016) describes
71 communities and their biological activity in LFL treatment. It has been reported that the major factor that
72 affecting the bioremediation performance is the capacity of the added culture to display its activities and survive
73 in different physiological conditions (Song, Wang, Yue, & Li, 2013; Westlund & Yargeau, 2017). Highly adaptive
74 bacteria exceeded an important removal rate of pollutant substances. Consequently, a complex
75 symbiotic microbial consortium of several yeasts and bacteria would be interesting mixture to overcome

76 stressing LFL culture conditions. In this context, the present work supposes that kefir grains (KGs) as a microbial
77 consortium constituted mainly of some bacterial species such as lactobaccili, lactococci, and leuco- nostoc and
78 yeast species such as Kluyveromyces, Candida, and Saccharomyces growing in ecological niche (Bengoa,
79 Iraporda, Garrote, & Abraham, 2019; Richard, 2016) could be promote for the removal of pollutants. Thus, the
80 aim of this research is to assess the performance of Lactococcus lactis and K. marxianus monoculture and co-
81 culture in the biodegradation of landfill leachate.

82

83 **Materials and methods**

84

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

99

100 **LFL characterization**

101

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

107

108 **LFL physicochemical analysis.** The performance of biological LFL treatment is evaluated by measuring the
109 decrease of the organic matter, ammonium, and heavy metals. The analyses were assessed on raw and treated
110 leachate. The initial pH of the leachate was modified to the desired value using 1 M hydrochloric acid. PH, TDS,
111 and EC were measured by a multi parameter type «Consort C 860». COD, BOD5, and NH₄⁺ -N were
112 determined according to Rodier and Legube (2009). Total Kjeldahl nitrogen was measured according to Rodier
113 and Legube (2009). The concentrations of heavy metals were determined using flame atomic absorption
114 method (Analytic Jena AG Spectrometer AAS vario 6). The bacterial cell biomass was detected by optical
115 density of samples at 600 nm. The LFL characteristics are showed in Table 1.

116

117 **Main physicochemical characteristics of Jebel Chakir LFL.**

118 Physicochemical proprieties of Jebel Chakir LFL were determined according to the following parameters: COD,
119 pH, salinity, electrical conductivity (EC), and heavy metals (Table 1). The raw LFL showed a dark brown color,
120 physical, and chemical parameters presented an alkaline pH of 7.73 and high conductivity (20.6 ms/cm) as well
121 as considerable levels of COD (26.200 mg O₂/L) that can be attributed to the high initial organic matter in the
122 leachate. The relatively high levels of salinity (3.62 g/L) demonstrated the presence of inorganic contents in the
123 studied leachate. It was noticed that leachate samples contain significant amounts of toxic heavy metals such
124 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
125 reported by Ellouze, Aloui, and Sayadi (2008), the leachate presented an important quantities of organic

126 matter, nitrogen, and toxic heavy metals especially Fe (20.6 mg/L). The relatively high levels of contaminants
127 including organics, ammonia, inorganic substances, and toxic metals confirmed the high organic load of the
128 dumped garbage in Jebel Chakir LFL.

129 130 **Bioremediation process for landfill leachate treatment Inoculum preparation.**

131 The isolation and identification of bacteria and yeast strains were done as described previously in Section
132 Isolation and identification of microorganisms. The yeast strain was inoculated to 50 ml of synthetic nutrient
133 broth medium YPD (dextrose [2%], bacteriological peptone [1%], yeast extract [1%]) and incubated at 30°C for
134 48 hr with 150 rpm agitation speed. For the preparation of bacteria inoculum, the strain was inoculated to 50
135 ml MRS broth medium and incubated at 37°C for 24 hr with 150 rpm agitation speed. Then, the microbial
136 inoculums were used for bioremediation process.

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

147 148 **Results and discussion**

149 150 **Strain selection**

151 Kefir grains were analyzed microbiologically to identify the predominant microorganisms. A total of 54 bacteria
152 and 27 yeasts were subjected to Rep-PCR fingerprinting technique to estimate bacteria and yeast diversity of
153 kefir grains. Then, 11 bacteria and 4 yeasts were chosen as representative of each sub-cluster obtained (70% of
154 similarity) using the Pearson correlation. The sequences were aligned to the query sequences of the GenBank
155 16S rRNA and ITS-5.8S rDNA sequences database, resulting in identities of known sequences of 99%–98%, as
156 shown in Table S1 (Supporting Information). *Lactococcus lactis* and *K. marxianus* are among predominant
157 microbial populations from the kefir grains. Figure 1 presented the obtained strains after visualization under
158 ultraviolet light and the resulting profiles were determined by digital image capturing using a CCD UVI pro
159 Platinum 1.1. Recently, Elleuch et al. (2020) reported the cost effectiveness of kefir grains as a biological
160 pretreatment for landfill leachate. Overall, TOC, COD, NH₄⁺-N, and PO₃⁻ 4 decreased, respectively, by 93%,
161 83.33 %, 70%, and 88.25% with respect to the raw effluent (24,000 mg/L), thus reflect- ing the resistance of these
162 grains to the toxicity of leachate (Elleuch et al., 2020). Same conclusions were reached by Mohd et al. (2017) and
163 Wang et al. (2018) showing the efficiency of these genera of lactic acid bacteria and the yeast *Kluyveromyces*
164 *marxianus* for the removal of organic matter and toxic substances from wastewaters. In this context, lactic acid
165 bacteria and yeast strains isolated from KGs could be potential for the removal of organic matter and toxic
166 pollutants from LFL. In addition, Milanowski et al. (2017) worked on the biosorption of silver using *L. lactis* strains.
167 It was found that the lactic acid bacteria was able to grow and absorbed about 70%–96% of silver from 1 ppm
168 solution. Yadav et al. (2014) proved the performance of *K. marxianus* in the biodegradation of cheese whey (78%
169 of COD removal after 30 hr of incubation).

170

171 **Bioremediation treatment of LFL using the selected bacterial strain and yeast**

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

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

180

181 **Change in organic matter and NH₄⁺ -N during**

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

189 It is clearly seen that the consortium response seems to be better compared to both monoculture of *L. lactis* and
190 *K. marxianus*. The co-culture exhibited an appreciable COD reduction in a shorter degradation time only after 3
191 days. The COD reduction was comparatively higher with the samples at 1% of inoculum size. Furthermore, the
192 findings showed that the maximum COD removal rate increased greatly (75.8%) using the co-culture compared
193 with those of *L. lactis* (52.3%) and *K. marxianus* (56.2%), which suggested no competition or inhibition between
194 the two selected strains. It was possible due to the co-culture synergy effect on increasing the growth abil-
195 ity, biomass production, and enzyme activity. This outcome is promising because it proved not only the compatibility
196 of yeast and bacteria populations but their complementarity. In fact, an important COD concentration might
197 accelerate the growth of heterotrophic bacteria, which would consume oxygen and nutrients rapidly (Patureau
198 et al., 2001). In addition, some researchers have noted the benefits of applying mixed cul-
199 tures (Alcántara et al., 2015; Wilkie & Mulbry, 2002). The findings of our work seem to be more interesting
200 than those described in Razarinah, Zalina, and Abdullah (2014). As results of experiments, maximum simul-

201 taneous COD and BOD5 removal were achieved 89.14% and 2.11%, respectively, after 28 days of incubation using
202 immo- bilized *Trametes menziesii*. Recently, Er, Seow, Lim, Ibrahim, and Sarip (2018) tested *Brevibacillus*
203 *panacihumi* strain ZB1 for the removal of toxic compounds from LFL. As a result, COD and ammonia–nitrogen
204 degradation were attained ~40% and ~50%, respectively, after 42 days of incubation. On the other hand, the
205 variation trends of COD removal rate using *L. lactis* and *K. marxianus* were similar with 3% and 5% of inoculum
206 size, as shown in Figure 2. The most important COD removal rate using *L. lactis* and *K. marxianus* with 3%
207 inoculum size were 30.1% and 36.3%, respectively. The abatement rate of COD using *L. lactis* and *K. marxianus*
208 with 5% of inoculum size were 31.2% and 29.6%, respectively. The mixture of *L. lactis* and *K. marxianus* with 3%
209 of inoculum size increased the maximum COD removal rate to 38.2% and slightly decreased to 28.1% with 5% of
210 inoculum size after 10 days of incubation for LFL treatment. As far as the control samples, no important COD
211 degradation was observed. According to the literature, several studies reported that the inoculum size is a
212 relevant factor to improve the biodegradation of wastewater (Bohutskyi et al., 2016; Elleuch et al., 2020). Thus,
213 the inoculation of 1% of consortium had almost a positive effect on the degradation rate during the biological
214 process. However, the addition of 3% and 5% of consortium was unfavorable for the degradation and pollutants
215 removal. In the same vein, previous studies revealed the benefits of nutrients to accelerate and/ or facilitate the
216 biodegradation of contaminants. This is principally explained by the biostimulation of microorganisms through
217 the supply of nutrients such as carbon, nitrogen, and phosphorus (Dadrasnia, Azirun, & Ismail, 2017). For that
218 the possible interpretation was that the inefficiency of 3% and 5% could be attributed to the low nutrients
219 availability for their stimulation, which could have decreased the biodegradation process. Therefore, future
220 studies should focus on improving the proprieties of substrates, inoculums, and the environmental conditions.
221 In this research, the second result was $\text{NH}_4^+ -\text{N}$ reduc- tion in leachate. As shown in Figure 3. $\text{NH}_4^+ -\text{N}$ level in
222 the studied leachate was 780 mg/L. According to the literature, the high amount of $\text{NH}_4^+ -\text{N}$ was probably due
223 to the fermentation and hydrolysis of the nitrogenous fragments of biodegrad- able refuse. The obtained results
224 proved the strains co-culture capacity to survive under a considerable $\text{NH}_4^+ -\text{N}$ amount. Bioremediation
225 presented a great effectiveness in $\text{NH}_4^+ -\text{N}$ reduction for all tested treatments. The reduction rate of $\text{NH}_4^+ -\text{N}$

226 remarkably increased after one week and improved until the last day of the study. The maximum $\text{NH}_4^+ - \text{N}$
227 reduction of leachate with 1% of co-culture addition was approximately 85.9%. The highest $\text{NH}_4^+ - \text{N}$ removal
228 rate using co-culture with 3% and 5% of inoculum size was 23.06% and 33.29%, respectively. The same yields
229 were obtained for the samples treated by *L. lactis* and *K. marxianus*. Compared with the control, amending the
230 leachate with 1% of *L. lactis* and *K. marxianus* pure cultures increased the $\text{NH}_4^+ - \text{N}$ removal rate about 48.6%
231 and 37%, respectively. Although 21.05% and 26.47% $\text{NH}_4^+ - \text{N}$ removal rates have been recorded using *K.*
232 *marxianus* at inoculation sizes of 3% and 5%, respectively. The maximum $\text{NH}_4^+ - \text{N}$ reduction of LFL without
233 culture addition was approximately 15.4%. This result could be attributed to the effect of enzyme production
234 and biomass activity of the microorganism (Mohd et al., 2017). In view of that, our outcomes showed a signifi-
235 cant degradation of organic matter compared to other studies which highlighted the performance of the
236 consortium used in this work. For example, Raposo, Oliveira, Castro, Bandarra, and Morais (2010) reported 13%–
237 15% of COD removal using a consortium of *Chlorella vulgaris* and brewery wastewater native microalgal-bacterial
238 consortia after 20 days of treatment. Our findings indicated that the co-culture possessed pollutions removal
239 abilities for LFL. Moreover, the above results suggested that the inoculum size has a great effect on the organic
240 matter and $\text{NH}_4^+ - \text{N}$ degradation. 1% of inoculum size was found to be more efficient in the removal of
241 pollutants. For this reason, in the subsequent analyses using *L. lactis*, *K. marxianus*, and the consortium, only the
242 inoculation size of 1% will be considered.

243

244 **Change in salinity during bioremediation.**

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

251 marxianus, and consortium are resistant strains to high salts contents in LFL. This property was also reported by
252 Huang, Liu, Liang, and Mao (2014) and Tekarslan-Sahin, Alkim, and Sezgin (2018) who proved the ability and
253 tolerance of lactic acid bacteria and yeasts to survive at higher salinity value. In fact, the salinity was increased
254 from 3.62 to 0.8 g/L in leachate inoculated with consortium. Also, when *L. lactis* and *K. marxianus* were
255 inoculated, the salinity was reduced to 1.5 and 1.2 g/L, respectively. Thus, the salinity increase is attributed to
256 the growth and the accumulation in cell walls as well. Numerous research studies have proved that yeast cells
257 exposed to high salt contents show dehydration, physiological and biochemical variations, and gene modification
258 (Mage & Siderius, 2002). Applying detoxification mechanisms and ion transport, the cells demonstrate an
259 important tolerance to Na⁺ stress through osmotic regulation by adsorbing Na⁺ salts ability and tolerance of
260 lactic acid bacteria and yeasts to survive at higher salinity value. In fact, the salinity was increased from 3.62 to
261 0.8 g/L in leachate inoculated with consortium. Also, when *L. lactis* and *K. marxianus* were inoculated, the salinity
262 was reduced to 1.5 and 1.2 g/L, respectively. Thus, the salinity increase is attributed to the growth and the
263 accumulation in cell walls as well. Numerous research studies have proved that yeast cells exposed to high salt
264 contents show dehydration, physiological and biochemical variations, and gene modification (Mage & Siderius,
265 2002). Applying detoxification mechanisms and ion transport, the cells demonstrate an important tolerance to
266 Na⁺ stress through osmotic regulation by adsorbing Na⁺ salts inside the cell (Dhar, Sägesser, Weikert, Yuan, &
267 Wagner, 2011; François, Walther, & Parrou, 2012).

268

269 **Change in heavy metals during bioremediation.**

270 Recently, the biosorption of heavy metals by a variety of biomasses including bacteria, fungi and algae has been
271 demonstrated as important economical and effective alternatives (Mehta & Gaur, 2005; Romera, Gonzalez,
272 Ballester, Blazquez, & Munoz, 2006). Since the area of biosorption is huge, our study was restricted to the toxic
273 metals such as cadmium, nickel, copper, zinc, and iron biosorption using 1% of *L. lactis*, *K. marxianus*, and the co-
274 culture. As reported in Table 2, the maximum recorded removal rates of Ni, Cr, Cd, Pb, and Fe were 81.53%,
275 73.45%, 79.48%, 68.53%, and 58.17%, respectively, with the co-culture inoculation size of 1% (v/v). By comparing

276 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*
277 showed higher ability in the removal of Ni (39.77%) and Cd (62.63%). According to the literature, several studies
278 have been performed to explore LAB and yeasts in the wastewaters treatment (Reis et al., 2017; Zhang et al.,
279 2016). These outcomes were in agreement with Han et al. (2006) findings using beer yeast for the removal of
280 Cu^{2+} and Pb^{2+} from wastewater. The experimental results exhibited the capacity of yeast to consume Cu^{2+} and
281 Pb^{2+} and therefore, to reduce these toxic metals level in the culture medium. Otherwise, Bhakta, Ohnishi,
282 Munekage, Iwasaki, and Wei (2012) tested the performance of eleven LAB strains isolated from mud and
283 sludge in heavy metals removal rates from wastewater. They reported that *Lactobacillus reuteri* showed the
284 highest Cd^{2+} (25%) and Pb^{2+} (59%) removal capacities. In another research carried by Schut, Zauner, Hampel,
285 König, and Claus (2011), it was indicated that *Lactobacillus* species may have a great application in the reduction
286 of Cu^{2+} .

287

288 **Conclusion**

289 In the present research, the bacteria and yeast isolates from kefir product were applied for landfill leachate
290 treatment. The identification of the isolated microorganisms is presented to be *L. lactis* and *K. marxianus*. A
291 consortium was constructed from bacteria and yeast mixed culture. As far as COD, $\text{NH}_4^+ -\text{N}$, and heavy metals
292 removals are concerned; the results demonstrated that isolated bacteria and yeast strains have the ability to
293 reduce the COD value up to 50% and $\text{NH}_4^+ -\text{N}$ value up to 35%. Furthermore, the Addition of 1% (v/v) of inoculum
294 showed the best biodegradation rate compared to 3% and 5% (v/v). However, a co-culture would prove to be
295 more effective and beneficial compared to single strain. Significant results were obtained in co-culture (1% (v/v)
296 of inoculum size) which reduces the COD (75.8%), $\text{NH}_4^+ -\text{N}$ (85.9%), and salinity (75.13%). Also, the results
297 proved that applying a bacto-yeast co-culture to Jebel Chakir leachate is a suitable treatment to remove high
298 quantities of heavy metals like Ni (81.53%), Cu (73.45%), Cd (79.48%), Pb (68.53%), and Fe (58.17%). The LFL
299 bioremediation process could be promising and considered as an effective green technology in the removal of
300 organic compounds from LFL.

301

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306

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489 **Legend of figures:**

490

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

493

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

496

497 **Fig. 3** $\text{NH}_4^+ - \text{N}$ removal during the bioremediation treatment of LFL using single and co-cultures with different
498 inoculum size: 1% (a), 3% (b), and 5% (c).

499

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

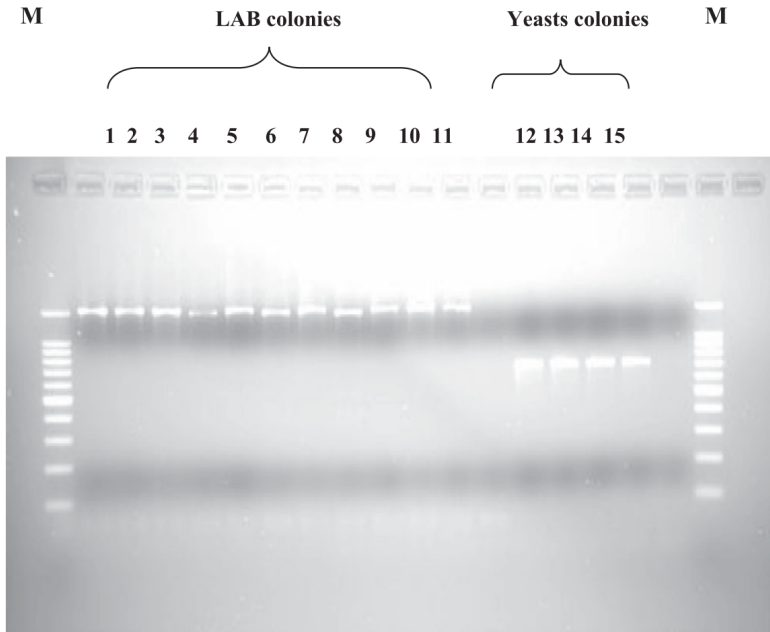
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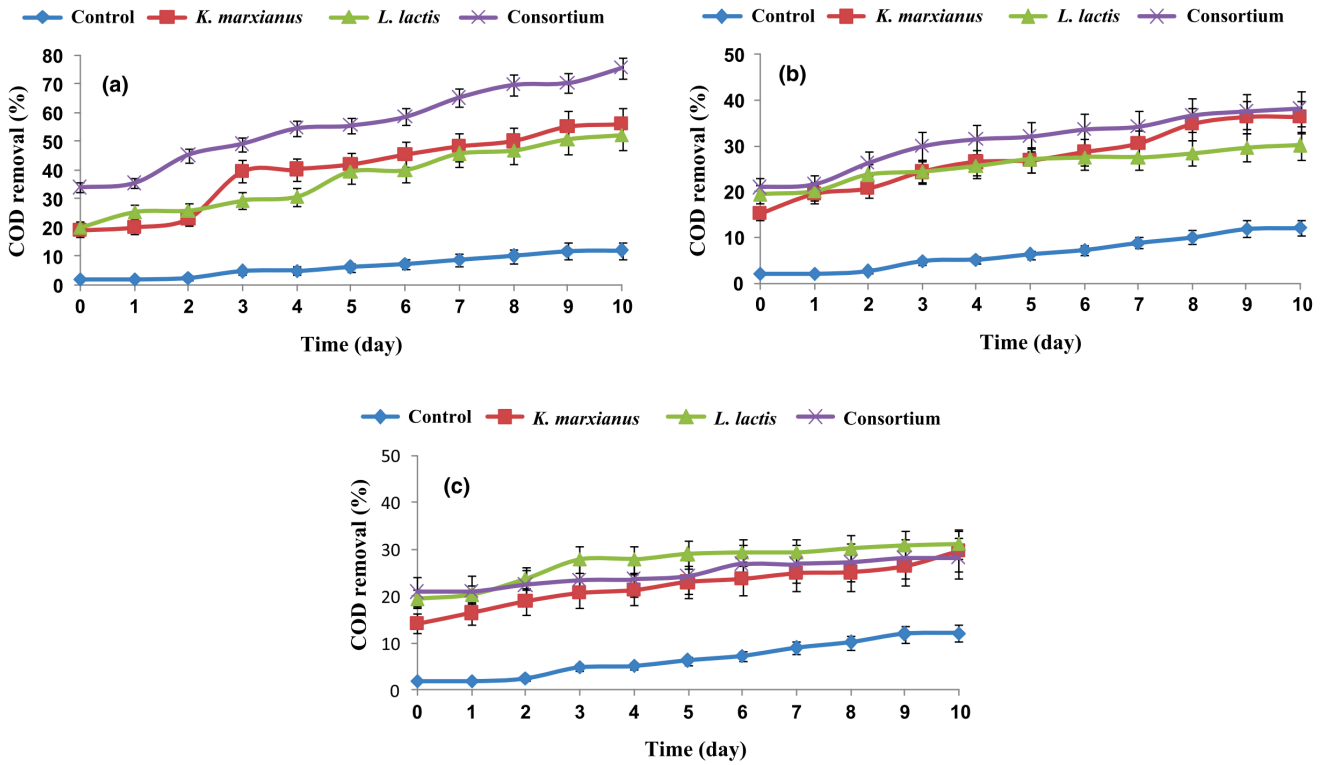
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Figure 1



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Figure 2



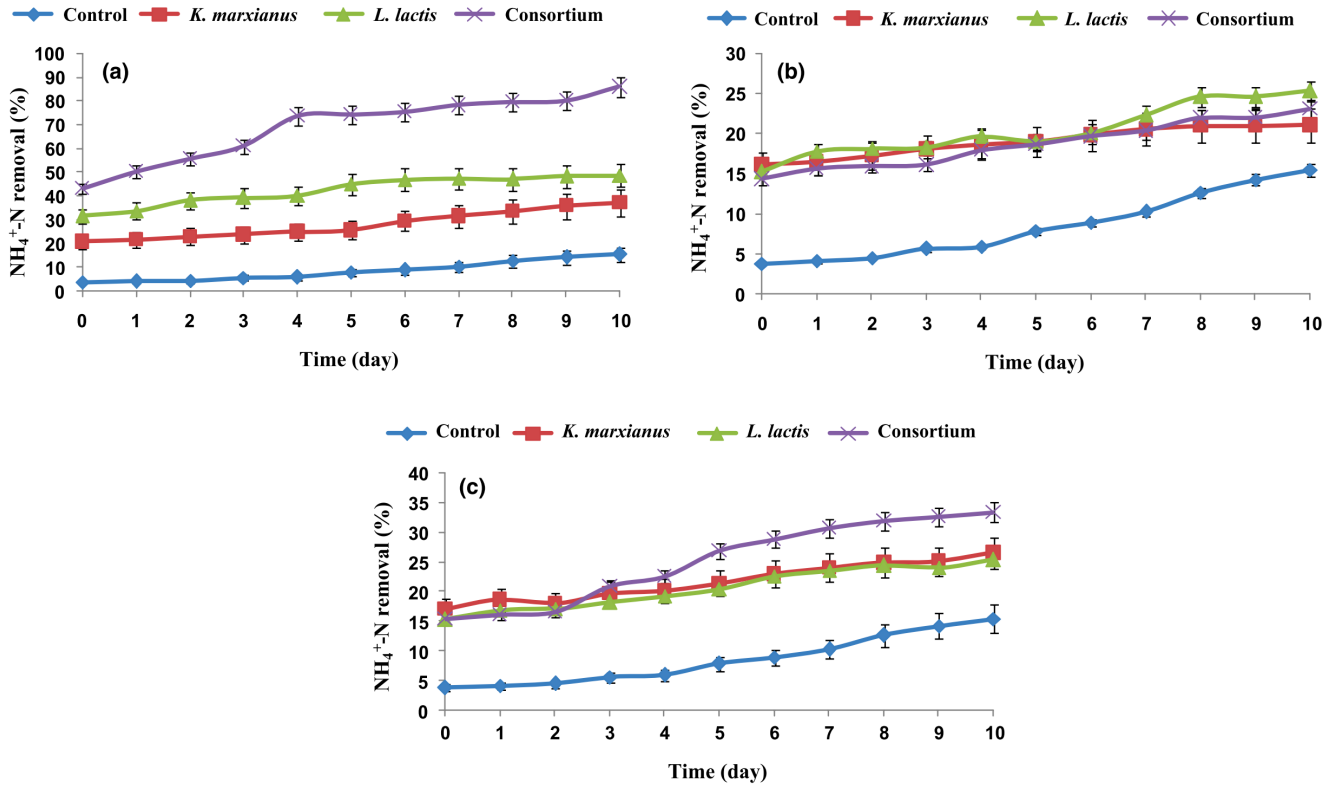
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Figure 3



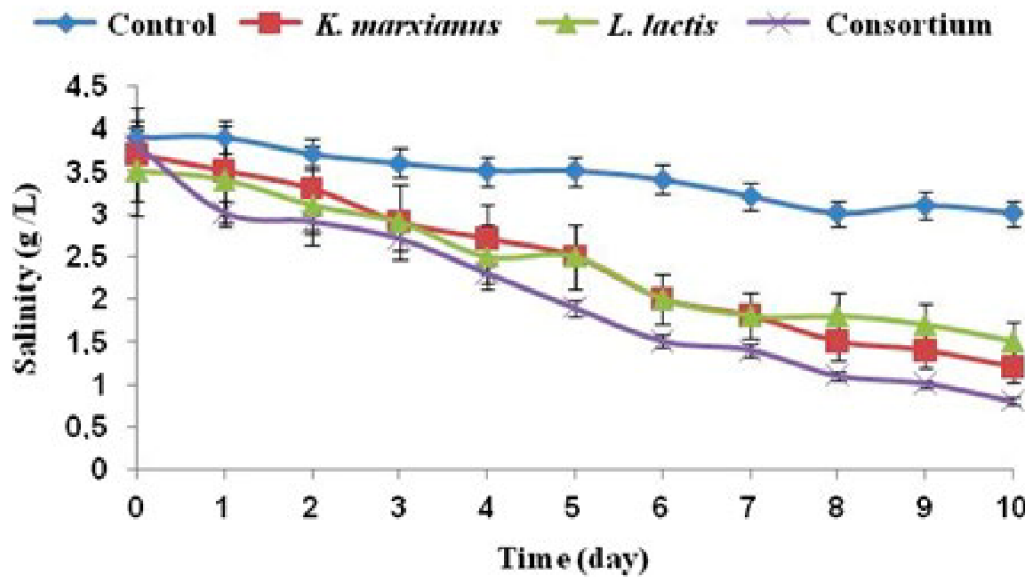
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Figure 4



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518 **Tables**
519

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

522

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

524

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

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

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