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Effect of biostimulation in the bioremediation of waters coming from debittering olives process

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ABSTRACT

The potential bioremediation of native microorganisms presents in lye waters, produced during the industrial debittering of green table olives was studied in this work. Two treatments, T1 and T2, as well as the corresponding control were carried out, whilst the biostimulation method was evaluated under aerobic conditions. The evolution of parameters such as BOD_5, COD, electrical conductivity and reducing sugars were measured, whereas the growth of native microorganisms was monitored by total plate count. COD parameter showed a decrease of 100% for both treatments, although electrical conductivity decreased around 35-30% in treatment T1 and 17–30 % in the treatment T2. The initial BOD_5 decreased consistently with the reduction in COD. The controls only had aeration, however in absence of biostimulation, similar results were observed in all treatments, due to the growth of microorganisms stimulated by the native source of organic matter. However, biostimulation accelerated the process, especially in the reduction of pH, transforming the alkaline effluent into a more neutral effluent.

1. Introduction

Currently, the availability of water has become one of the most important concerns. According to UNESCO, approximately 55 countries are under the minimum water stress threshold and 65% of the world's population suffers it (UNESCO, 2019). Al least one month a year, about 4 billion people experience severe water scarcity (UNICEF, 2021). UNESCO emphasized that the number of people affected by this issue is unnerving: many lives are being lost due to incidents caused by inadequate water, drinking water and sanitation services, outnumbering deaths caused by floods, droughts, and other disasters (UNESCO, 2019).

On the one hand, 80% of wastewater is dumped directly into riverbeds without any type of treatment, generating an elevated level of contamination, environmental deterioration and subsequently influencing the health and well-being of communities (UNESCO World Water Assessment, 2019). Looking ahead to 2030, it is a recommended and urgent aim to improve water quality by reducing pollution. The purpose is to eliminate discharges and minimize the emission of chemicals and hazardous materials, halving the percentage of untreated wastewater, increasing recycling and safe reuse globally (UNESCO, 2019). Several food industries, produce a large volume of wastewater and contribute to increased pollution.

On the other hand, during the biennium 2020/2021, three million tons of table olives, were produced worldwide. This represented an increase of around 77,000 tons over the previous year. Although the majority production was in Europe, it was followed by countries such as Egypt, Morocco, Turkey, and Algeria. Argentina was one of the emerging countries with 78,000 tons (International Olive Council, 2021). Regarding the national context, Mendoza is one of the producing provinces of table olives. A diagnostic study of the status of effluents from olive industry carried out in 2013 in Mendoza, concluded that most olives industries kept effluents untreated and diluted with drinking water or well water, increasing the water footprint. According to Valsecchi Punzi (2020) in Mendoza, food industries water consumption is estimated at 19.65 hm^3, nevertheless could be reduced 63% with an adequate reutilization. The effluent generated is approximately 180,000 m^3, additionally, since its sodium content and electrical conductivity characteristics, its agricultural reuse is prohibited, so a dilution is necessary to reach the accepted parameters (Duek, 2016). Additionally,

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Nomenclature

BOD_5	biological oxygen demand
COD	chemical oxygen demand
EC	electrical conductivity
TAE buffer	(Tris, 24.2%; acetic acid, 5.71%; EDTA, 0.5 M pH8 10 mL)
EDTA	ethylene diamine tetra acetic acid
T1	treatment one: 50% lye dilute with distilled water
T2	treatment one 25% lye dilute with distilled water
IDG	irrigation general directorate

it is estimated that in efficient factories the volume of drinking water consumed is 1.5 to 2.5 L/kg of olives, compared to 4 L/kg in less efficient factories.

Annually, the production of olives varies considerably and mills operate for a reduced period, however, a significant volume of effluents is generated (Gunay and Karadag, 2015), in addition, an important fraction of water consumed in olives production is discarded as wastewater, untreated to the environment. Green table olives undergo a debittering process, where the bitter taste conferred by oleuropein is eliminated and the olive flesh softens and by diffusion of the sodium content (Maldonado et al., 2022). This procedure consists of immersing olives in a NaOH solution that varies between 1 and 2.5%, influenced by the temperature, variety, industry, among others, whereas residual lye is generally reused up to six or more occasions depending on the factory (Arvanitoyannis and Kassaveti, 2008). The effluent is alkaline (pH 12), with an elevated Biological Oxygen Demand (BOD_5) content: 15,000 mg O_2/L, Chemical Oxygen Demand (COD): 23,000 mg O_2/L, total dissolved solids: 48,000 mg/L and a free NaOH concentration of 11 g/L approximately (Fernández Llano et al., 2001). The debittering stages and the subsequent washings constitute the most important polluting fraction of the entire process (Dermeche et al., 2013).

The discharge of untreated effluents is prohibited by global and local legislation, so there is a growing interest concerning the development of new technologies and the aforementioned effluents purification procedures, based the implementation of stricter relations on public disposal waste. However, most treatments are expensive, therefore, since olive industries produces a large amount of pollution, there is a need to solve the problem by developing optimized systems for its treatment. Among the various processes currently used, bioremediation is an economical, sustainable, and environmentally friendly process (Castro Viteri, 2022). It is based on the use of microorganisms such as yeast, fungi or bacteria, through the natural degradation, degrading hazardous substances into less or non-toxic substance (Arvanitoyannis and Kassaveti, 2008), using its catalytic characteristics transforming contaminants present in different ecosystems. Bioremediation can be executed both *in-situ* or *ex-situ* (Bala et al., 2022) and, so the importance of this technique lies in its wide application, and the bacteria genetic diversity and metabolic versatility.

Several authors have used bioremediation to degrade effluents, proving to be efficient the use of pre-treatments with aerobic, advanced oxidation and heat methods to remove toxic materials and improve the anaerobic treatment efficiency (Gunay and Karadag, 2015). González-González and Cuadros (2015) aerated the wastewaters form oils mills using native microorganisms, as a result, the polyphenol content was reduced the 56% in 24 h and 90% on the seventh day. Furthermore, Koutsos et al. (2018) used *Aspergillus niger* in combination with Fenton reaction to biodegrade olive industry effluents, reducing COD and phenols concentration by 66–86% and 65%.

Regarding the debittering olives wastewater, Aggelis et al. (2001) evaluated the performance of an anaerobic, aerobic and combined anaerobic-aerobic process separately, obtaining a removal of organic matter of 49% with a reduction of polyphenols of 12% considering the anaerobic treatment, whereas the aerobic treatment, a degradation efficiency of 71.6–75.9% was obtained regarding the reduction of organic matter. However, the pH of the study treatment was corrected as polyphenols were not efficiently reduced. The combined treatment was the most efficient, attaining an 83.5 and 28% reduction of organic matter and polyphenols respectively. The aforementioned is one of the few works that handles lye water, so it is necessary to detect a solution concerning this sort of wastewater.

Moreover, bioremediation can be combined with biostimulation (Adams et al., 2020), adding limiting nutrients and electron acceptors, namely, phosphorus, nitrogen, oxygen, or carbon, that would otherwise be available in low enough amounts to restrict microbial activity, therefore, the environment is modified and bacteria capable of bioremediation stimulated. Additionally, biostimulation has been successfully used in several olive effluents (Maldonado et al., 2022) contributing to the bioremediation by reducing COD, BOD_5 and electrical conductivity (EC), although the green olive debittering wastewater has not been tested.

Based on the above-mentioned, and given the importance of finding an effective and efficient solution, to mitigate the contamination of the debittering wastewater, it has been proposed the present work proposes to study the addition of nutrients such as oxygen, glucose and salts as biostimulants, hence, evaluate the performance of native microorganism in the green olive debittering wastewater at local level.

2. Materials and methods

2.1. Physicochemical analysis

For the characterization of the tested effluent, a sample of five litter of lye waters was taken, previously stirred shaken, to carry out the following analyses according to Standard Methods (Rice et al., 2017): COD by HACH DR2000 Spectrophotometer, HACH COD Digester, BOD_5 by HACH Sension TM6 Oximeter, pH by pH-meter, temperature with a digital thermometer with 0.01°C, EC with a conductivity-meter, solid sediments at 10 min and 2 h in Inhoff cone, Total soluble solids, fixed suspended solids, volatile soluble solids obtained by gravimetric method, chlorides by Mohr's method, sulphates by turbidimetric method, nitrates by ultraviolet absorption spectrophotometry, carbonates and bicarbonates by volumetry with ethylene diamine tetra acetic acid (EDTA). The elements: sodium, potassium, calcium, and magnesium were measured by spectrophotometry.

In order to avoid the degradation of sample, the analyses and trial were performed on the raw sample immediately after being taken from the processing plant.

2.2. Biostimulation test

The biostimulation bioremediation test consisted of carrying out two treatments in triplicate and their corresponding Controls. Treatment one: T1 (50% lye) consisted of placing half a litter of lye water diluted with 50% of distilled water and treatment two: T2 (25% lye) used lye water diluted with 75% distilled water, subjecting both to aerobic conditions and biostimulation. In both treatments, biostimulation was carried out by adding 2 g/Lof PO_4HK_2, 0.5 g/L of NH_4Cl, 1 g/L of sodium citrate, 1 g/L of KCl, 1 g/L of $MgSO_4$ and 1 g/L of triple 15®. A 15 g/L of anhydrous glucose was also added as a carbon source. The Controls (T1) and Control (T2) consisted of lye diluted to 50% and 25% with distilled water, respectively, but without the addition of salts. Aerobic condition was maintained by shaking the Erlenmeyer flaks on a shaker at 200 rpm for 14 days. The evolution of parameters was measured by taking samples at regular time intervals. The samples were analysed immediately to avoid degradation and errors. BOD_5, COD, EC, reducing sugars were evaluated using the Miller technique and polyphenols using the Foling Cicalteu method. In addition, the growth of native

microorganisms was monitored by total plate count.

2.3. Microbiological analyses

Microorganisms were then isolated by culture on plates, using the surface streak technique, until axenic culture of viable native microorganisms was achieved, on EMB Britania® agar for coliform growth, on Plate count Britania® agar for aerobic mesophylls growth and on Britania® potato dextrose agar for the growth of fungi and yeasts. All these media were diluted with distilled water and effluent in a proportion of 85 % and 80% distilled water and 15 % and 20 % effluent, in order to grow viable microorganisms adapted to lye water. Both, the phenotypic and molecular characterization of the strains were performed.

2.4. Characterization of microorganisms

2.4.1. Molecular identification of microorganisms (bacteria)

The strains were identified by amplification of the 16S rDNA ribosomal gene from genomic DNA using 27F (5′ AGAGTTTGATCCTGGCTCAG 3′) and 1492R (5′ TACGGTTACCTTGTTACGACTT 3′) as universal primers for bacteria. These primers give an amplification product of ~1500 bp. From the 24-h pure cultures, DNA extraction was performed using two extraction techniques.

Amplification was carried out in a final volume of 50 μL containing 5 μL Promega STR Buffer (10x) (provided with enzyme), 0.1 μM primers, 2 U Promega Taq DNA polymerase and 50 ng DNA. Amplification conditions consisted of an initial 5 min denaturation at 94°C, followed by 30 cycles of denaturation (94°C, 1 min), annealing (55°C, 2 min) and extension (72°C, 2 min), and a final extension at 72°C. for 7 min. The amplified product (4 μL) was analysed by electrophoresis in 1.5% (w/v) agarose gels in TAE buffer (Tris, 24.2%; acetic acid, 5.71%; EDTA, 0.5 M pH8 10 mL) with the addition of 1 μL of Gel Green, for DNA development. A 100 bp PB-L (Bio-Logical Products) molecular weight marker was included. Samples were mixed with 6X seed buffer (Orange-Blue, Promega), run at 75 V for approximately 30 min, and analyzed with Bio-Rad's Quantity One program.

The PCR products were sent to MACROGEN KOREA for sequencing. The partial 16S rDNA sequences obtained were compared with the sequences deposited in the GenBank database using the Basic BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) and aligned with each other by use of Jalview (www.jalview.org). From the alignments, a phylogenetic tree was built using the MEGA7 software (www.megasoftware.net). The obtained sequences were deposited in the GenBank database receiving their corresponding access numbers.

2.4.2. Yeasts: amplification of the 26S subunit of rDNA

For yeast, strains were identified by amplification of the 26S rDNA subunit. The amplification of the D1/D2 domain of the 26S rDNA subunit was performed by PCR (Polymerase Chain Reaction). In the amplification reactions of this work, we use as a template the genomic DNA extracted from the selected strain. The final volume of the reaction was 50 μL and universal primers were used (O'Donnell, 1993). The following primers were used: (i) forward: NL-4 (5′-GGTCCGTGTTTCAAGACGG-3′), (ii) reverse: NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) Amplification reactions were performed in a thermocycler automatic (Perkin-Elmer, model 9700, Applied Biosystems) The amplification products (4 μL) were separated by electrophoresis on 1.5% (w/v) agarose gels using 1X TAE buffer and 1 μL of Gel Green as running buffer and DNA staining, respectively. A 100 bp PB-L molecular weight marker (Bio-Logical Products) was included. Samples were mixed with 6X seeding buffer (Orange-Blue, Promega), run at 75V for approximate and analysed with Bio-Rad's Quantity One program. The bands were visualized by fluorescence in ultraviolet light and on the Doc BIORAD gel image analyser.

DNA sequencing was performed by Macrogen Services. The sequences were compared and aligned with sequences from the GenBank database using the BLAST program of the National Center for Biotechnology Information.

2.5. Statistical analysis

Tukey test was used in ANOVA analysis, with a significance level of 0.05, using Infostat 2020 software. The results are presented as mean ± standard deviation (SD) for n = 3.

3. Results and discussion

Water pollution and issues related to it are regulated by Resolution No. 52/2020 of the Province of Mendoza, which establishes the parameters that industrial and wastewater discharges must have for agricultural reuse. The resolution is based on the principle that "The polluter pays" (art. 59). Consequently, all industries that discharge effluents into the public hydraulic domain must obtain the corresponding administrative authorization and must have a treatment system of effluents adequate to meet the technical requirements set forth in the current legislation. The authorization mentioned is the "Discharge Permit" and is granted by the Superintendence of the General Directorate of Irrigation (GDI). If the establishments do not meet the necessary requirements to obtain said permit, they must sign a "Discharge Permit Management Contract", through which they are granted a term to adjust the quality of their effluents and improve their treatment systems. Based on this resolution and FAO (2002) recommendations, the lye water was analysed. Its characteristics are shown in Table 1.

Table 1 presents the results of the most important parameters of the effluents contemplated by local water legislation and their classification as: permitted, prohibited, and not complying, according to the quantification of the parameters to allow its discharge to the public hydraulic domain and possible agricultural reuse.

The untreated effluent cannot be discharged into treatment plants or public hydraulic system because it does not comply with the regulations of resolution 55/20, especially with regard to pH, EC, COD, BOD_5, sodium, total and suspended solids. Regarding the possibility of being used for agricultural irrigation, it could not be used according to local or international regulations due to its high pH, EC, and sodium.

Table 2 shows that the initial COD value for Treatment T1 was 41,900 mgO_2/L and decreased to 37,770 mgO_2/L seven days after the process started, which means a reduction of 9%. Then, at the end of the process (14 days), it was reduced by 98.5%. Its Control (T1) started with 41,900 mgO2/L and decreased 99% at the end of process to a value of 446 mgO_2/L. Treatment T2, started with an initial average value of 31,200 mgO_2/L and decreased 39% to a value of 12,050 mgO_2/L, after seven days. Finally, it reduced to 210 mgO_2/L in 14 days, representing a reduction of 99.3%. While its Control (T2) decreased 98%. The two Controls showed a similar behaviour: both decreased 98-99%, although more slowly. The analysis of variance and Tukey's test showed significant differences between T1 and T2, probably due the dilution of the lye, and between T1 and its Control (T1). The same happened between T2 and Control (T2) at the end of the process. (See Supplementary Material Tables 1 and 2). This could indicate that the Controls were degraded only with oxygen as biostimulant, without the need of salts. It was possibly due to the organic matter content of the reused lyes. These results are consistent with those reported in the aerobic treatment practiced by Aggelis et al. (2001) in debittering waters. They found an 83.5% reduction in COD. Taking into account that the COD value represents the organic matter of an effluent and indicates the degree of contamination, it could be said that the bioremediation carried out was totally effective and reduced the pollution load both, when the effluent was used with 50% or 25% of lye under aerobics conditions, independently of the salts as biostimulants.

The almost complete biodegradation is consistent with the initial BOD_5 value of the effluent which was 50,568 mg O_2/L with which the so-called Biodegradability Index (BOD_5/COD) was 0.73. This represents

Table 1
Characterization of the effluent prior treatment.

Variable	Average	standard error	Unit of Measurement	Resolution 52/20		FAO
				Transfer to treatment plants	Agricultural Reuse	Agricultural Reuse
pH	13.09	±0.2	-	Fail to comply	Fail to comply	Fail to comply
Electric conductivity	57,533.33	±2	uS/cm	Fail to comply	Fail to comply	Forbidden
COD	68,150	±500	mg/l	Fail to comply	Fail to comply	Does not apply
BOD_5	50,568	±500	mg/l	Fail to comply	Does not apply	Does not apply
Nitrates	0.0	±2	mg/l	Permitted	Permitted	Permitted
Settling solids 10 min	0.2	±0,1	mg/l	Fail to comply	Does not apply	Does not apply
Settling solids 2 horas	51	±0,1	mg/l	Fail to comply	Fail to comply	Does not apply
Total soluble solids	3 8.020	±10	mg/l	Fail to comply	Does not apply	Does not apply
Fixed suspended solids	36.384	±10	mg/l	Does not apply	Does not apply	Does not apply
Volatile soluble solids	1.636	±10	mg/l	Does not apply	Does not apply	Does not apply
Sulphates	0	±5	mg/l	Permitted	Permitted	Does not apply
Carbonates	6794.23	±10	mg/l	Does not apply	Does not apply	Does not apply
Bicarbonates	0	±10	mg/l	Does not apply	Does not apply	Permitted
Sodium	13,500	±0.5	mg/l	Fail to comply	Fail to comply	Forbidden
Potassium	2594	±0.1	mg/l	Does not apply	Does not apply	Does not apply
Calcium	40	±2	mg/L	Does not apply	Does not apply	Does not apply
Magnesium	120	±2	mg/l	Does not apply	Does not apply	Does not apply

Table 2
COD evolution.

Testing days	T1	Control (T1)	T 2	Control (T2)
0	41,900±170	41,900±535	31,200±462	31,200±356
7	37,770±362	25,000±481	12,050±500	10,361±438
14	615±46	446±26	210±55	650±45

Note: Results are stated as means ± SD (n = 3).

a quantitative approximation of the biodegradability of an effluent and can be used to control and operate a treatment plant (Hernández Muñoz, 1992) ensuring that it is a biodegradable effluent because the ratio is >0.4

The pH values evolved as shown in Figs. 1 and 2, they had similar behaviour in both T1 and T2. The pH decrease was faster than their respective Controls in the first 106 h, showing a statistically significant difference in that point for T1 and its Control (T1) but not for T2 and its Control (T2) (See Supplementary Material Table 3). Then it continued to decrease up to pH 8.2 and 8.3 at 265 h for T1 and T2 and their Controls, showing not statistically significant difference between them (See Supplementary Material Table 4). The decrease in pH was compatible with the possible degradation of organic matter that native microorganisms consumed in the presence of oxygen, generating acids by the Krebs cycle. These acids may be the cause of the transformation of a totally alkaline effluent into one closer to neutrality, reaching a final value 8,2-8,3 in both treatments. With these values, the treated effluent would meet the requirements to be discharged into the public hydraulic domain, according to ordinance 52/20 of Irrigation General Directorate.

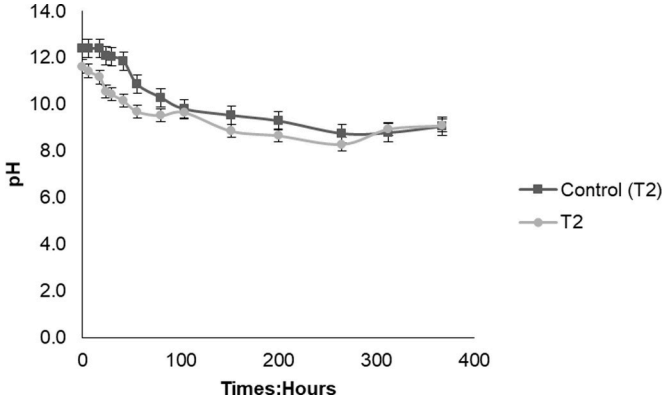

Fig. 2. Evolution of pH treatment T 2 (25% lye).

At the final of the process both treatments and their Controls did not show significative differences between them.

Regarding electrical conductivity (EC), the initial effluent had a very high value of 57.53 mS/cm corresponding to 100% NaOH, which means that it cannot be discharged into the public hydraulic domain without prior treatment. Resolution 52/20 of the General Directorate of Irrigation of Mendoza requires a maximum EC of 5.5 mS/cm to be discharged into the public hydraulic domain. Fig. 3 shows that the initial average value of EC was 36,64 mS/cm for the Control (T1) and 33.94 mS/cm for T1. T1 showed an average decrease in EC during the aerobic process to 23,94 mS/cm (29.5% of reduction). However, the Control (T1), that did

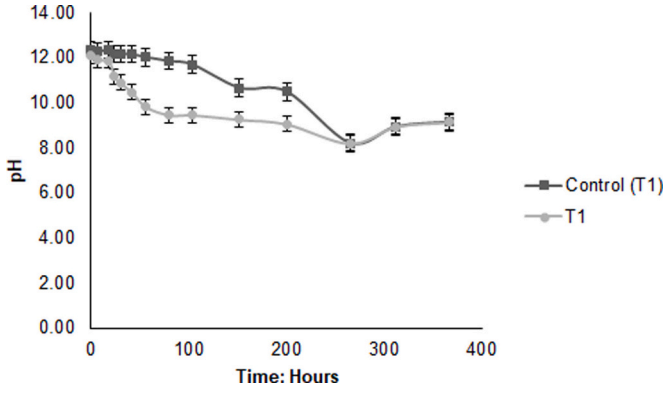

Fig. 1. Evolution of pH treatment T1 (50% lye).

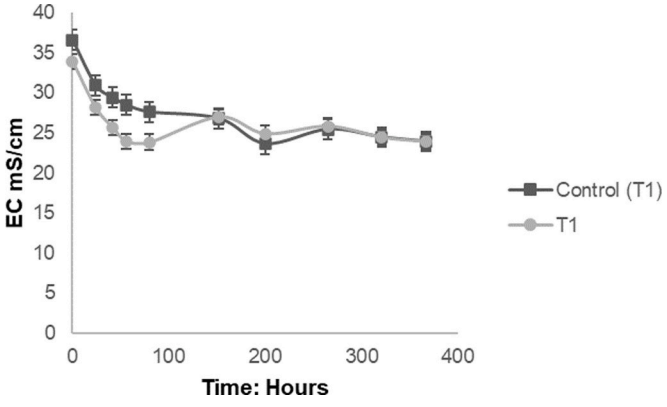

Fig. 3. Evolution of electrical conductivity for treatment T1 (50% lye).

not have biostimulation salts, decreased to 23,94 mS/cm also, showing a decrease of 35%. At the end of the process there was not significative difference between T1 and its Control (T1) (See Supplementary Material Table 5).

Fig. 4 shows the evolution of electrical conductivity (EC) for treatment 2 (25% lye) and its Control.

For Control (T2), the EC started with 22,15 mS/cm and it decreased to 14.53 mS/cm at the end of the test showing a reduction of 34% (statistically not-significant difference). For T2, the EC started with an average value of 18.00 mS/cm and then decreased to 15,02 mS/cm showing an average decrease of 17%.

In both cases, the electrical conductivity decreased, showing statistically significant difference at the end of the process between T1 and T2 (See Supplementary Material Table 5), possibly due the dilution of the lye. However, they did not reach the appropriate values to allow discharge into a public hydraulic domain or for agricultural use. Nevertheless, these decreases in EC is promising and also consistent with that found by Maldonado et al. (2022) for olive machining water, where the aerobic treatment showed a decrease in EC of 35%. In this case, more comprehensive studies are needed to understand the cause or the mechanism by which the decrease in electrical conductivity occurred.

Fig. 5 shows the monitoring of microorganisms in treatment T1, as an example to show the presence of native microorganisms. It can be seen that the latency period was reduced to zero due to the presence of biostimulation salts that made viable the growth of microorganisms. The mesophilic aerobic count, as well as coliforms, yeasts, and fungi showed a clear logarithmic increase. This is consistent with the degradation of the parameters COD and BOD_5 that indicated the presence of organic matter. In both treatments the growth was verified in a similar way along the time. There was also growth in the corresponding Control of each treatment (data not shown).

Table 3 shows the viable microorganisms responsible for the bioremediation of the effluent.

The consortium of viable microorganism included *Klebsiella* var, *Bacillus* var, *Planomicrobium* var and two varieties of yeast *Candida* var, *Rhodotorula* var. In the case of *Klebsiella, Bacillus, Planomicrobium* were also found in effluent from olive processing as part of the bioremediation consortium reported in previous investigations (Maldonado et al., 2022).

Candida tropicalis has been found to have bioremediation capacity for aromatic hydrocarbons (Badr and Abbas, 2015) and other petroleum-derived contaminants. In this type of effluent, it is characteristic to find phenolic compounds which have also been reported by other authors as degradable substances by the genus *Candida* (Zhou et al., 2011).

Rodotorula mucilaginosa is a species that has been found as a potential bioremediator of nanoparticles (NPs) in addition to being found as a bioremediator of contaminated water (Ruas et al., 2020).

The reducing sugars as shown in Figs. 6 and 7 were rapidly consumed

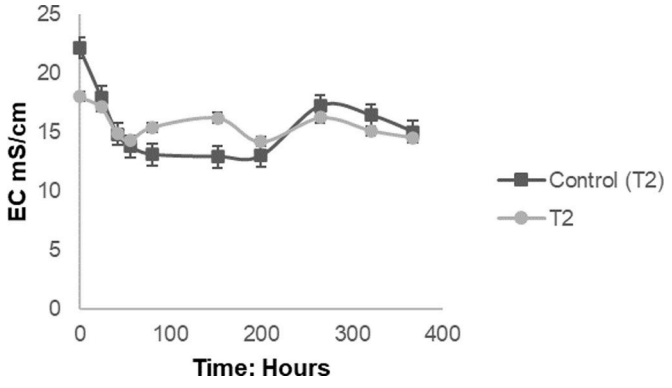

Fig. 4. Evolution of electrical conductivity for treatment T2 (25% lye).

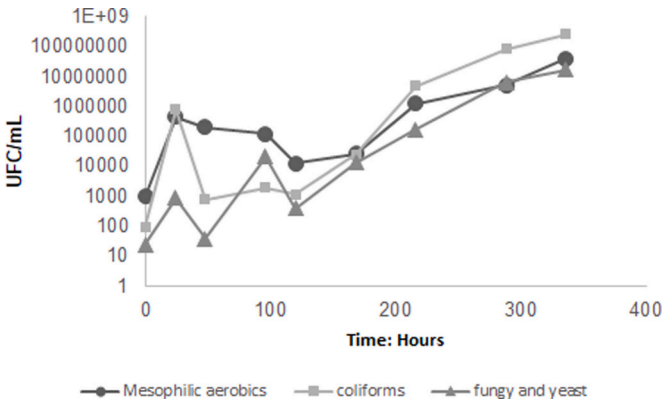

Fig. 5. Monitoring of microorganisms.

Table 3
Consortium of microorganisms.

axenic culture	Shapes	Gram	Identification
1	Bacilli	Gram -	*Klebsiella* var.
2	Yeasts		*Candida tropicalis* var
3	Bacilli	Gram +	*Bacillus* var.
4	Bacilli	Gram +	*Planomicrobium* var.
5	yeasts		*Rhodotorula mucilaginosa*
6	Yeasts		*Candida tropicalis* var

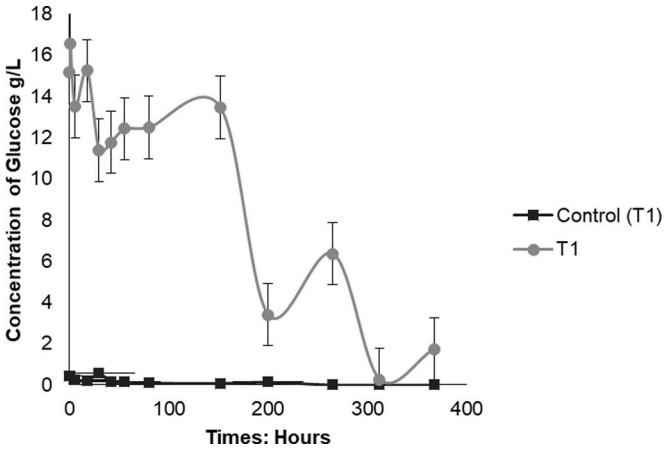

Fig. 6. Evolution of reducing sugars for treatment 1 (50% lye).

by the microorganisms in the treatments that had the addition of 15g/L of glucose as a carbon source, which possibly allowed the multiplication of the viable microorganisms. Nevertheless, the decrease was higher in treatment T1 since the final values were in a range between 4 and 0.64 g/L of glucose and in treatment T2 between 7.38 and 3.9 g/L of glucose. This consumption of the carbon source is consistent with the higher number of microorganisms after 72 h of testing for both treatments. Similar behaviour was found and reported by Maldonado et al. (2022). However, in both treatments it can be seen that their respective Controls had very low values of reducing sugars, behaving asymptotically with the x-axis. It could be thought that the degradation of organic matter as a consequence of microbial growth was possibly due to the fact that the initial contents of reducing sugars of the Control (T1) and Control (T2) were 0.24 g/L and 0.17 g/L, respectively. Although these values are low compared to glucose biostimulation (15g/L), it was a threshold content that allowed microbial development or, possibly, there could have been other unmeasured carbon sources that could promote it to sustain the degradation of their Controls. The degradation of both Controls

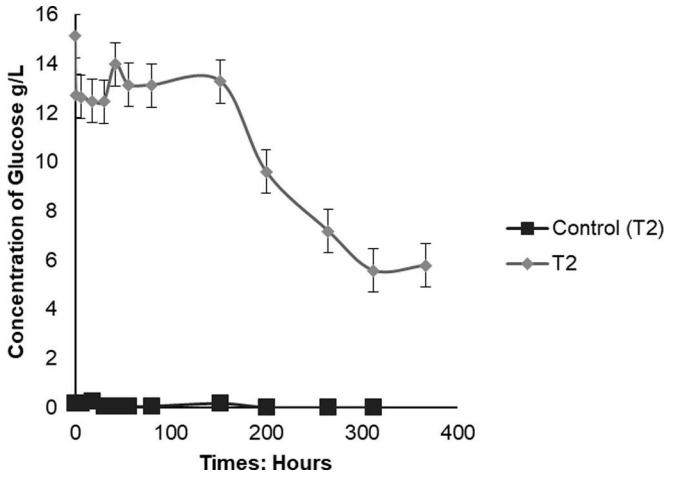

Fig. 7. Evolution of reducing sugars for treatment 2 (25% lye).

indicates the need to do more studies in the future, due the capacity of native microorganisms to live with very little quantities of sugars in presence of an oxidant atmosphere. This environmental condition was only obtained by oxygen biostimulation.

Table 4 describes some critical parameters before and after treatment to compare the results with local regulations (Resolution 52/20 of the Irrigation General Directorate). The pH value was one of the most affected by the treatment, making it now possible to discharge the wastewater into the public hydraulic domain, since it fell from 13 to 8.2 for treatment T1 and to 8.3 for treatment T2. Even though the COD decreased considerably in both treatments up to 99% (see Table 2), these final values were higher than the Maximum Allowed value for discharge into the public hydraulic domain. However, for treatment T2 the value was less than the Maximum Tolerable value for discharge.

The initial BOD_5 for the T1 treatment and its Control (T1) was 31,250 mgO_2/L and 24,210 mgO_2/L for T2 and its Control (T2) (data not shown in the table). After 14 days of treatment, these BOD_5 values presented a considerable reduction of almost 99% for T1 and T2 (see Table 4). Similar behaviour showed their Controls presenting a decreasing of 99% too (output BOD_5: Control (T1) =150 mg O_2/L and for Control (T2) = 16.2 mg O_2/L). However, the final value for T1 was higher than both the Maximum Allowed and the Maximum Tolerable values for discharge into the public hydraulic domain. While for treatment T2 the value was less than the Maximum Allowed value for discharge.

Sodium decreased considerably considering that the initial sodium value was 6750 mg Na/L in T1 and with the aerobic treatment it dropped to 5710 mg Na/L. In the case of T2, sodium was initially 3375 mg Na/L, and after aerobic treatment decreased to 871 mg Na/L. These decreases were also consistent with the reduction in conductivity. The decrease of Na is possible due to the bioaccumulation mechanism that some microorganisms use as a survival strategy in toxic environments (González-Hernández and Peña, 2002). Finally, the values of total polyphenols remained with little variation, since they decreased from 2.98 and 1.64 mg/L of gallic acid for T1 and T2 respectively to an average value of 2.92 for T1 and 1.53 mg/L of gallic acid for T2. Similar values were measured for the Controls: Control (T1) started with 2,79 mg/L of gallic acid and decreased up 1,48mg/L of gallic acid and Control (T2) started with 2,37 mg/L of gallic and decreased up 1,25mg/L of gallic acid. In all treatments, the values were higher than the Maximum Allowed and the Maximum Tolerable values. The treatment was not very effective for the reduction of polyphenols, possibly this could be enhanced with the Fenton reaction as applied by other authors. Lucas and Peres (2009), widely used in combination with other bioremediation strategies.

All these findings are very encouraging and although this is a pilot scale study, it could be carried out on a larger scale to study the real possibility of bioremediation of debittering wastewaters in the industry

4. Conclusion

Biostimulation under aerobic conditions turned out to be an effective treatment for the reduction of parameters such as COD, BOD_5, EC and pH in the green olive debittering wastewater when the effluent was used with 50% or 25% of lye, independently of the salts as biostimulants.

The COD decreased considerably in both treatments and in its Controls, presenting a decrease of 99%-98%.

The BOD_5 decreased considerably in both treatments and in its Controls as well, presenting a decrease of 99%.

There was an important reduction in EC in T1 (29.5%) respect T2 (17%). While its Controls decreased the EC in 34-35 % respectively, due to the lack of salts.

The pH values evolved similarly in T1 and T2. The pH decrease was faster than their respective Controls. They decrease to pH 8.2 and 8.3 at 265 h for T1 and T2 and their Controls. The decrease in pH was compatible with the possible degradation of organic matter that native microorganisms consumed in the presence of oxygen.

The Controls of both treatments, were degraded only with the action of oxygen and the organic matter presented in the original effluent, so more studies are necessary to do to understand deeply the aerobic conditions generated by the aeration that could modified the environmental conditions of process.

Native microorganisms were effective in reducing pollution, being an economical treatment available in the ecosystem.

CRediT authorship contribution statement

Mariela Maldonado: Conceptualization, Visualization, Project administration, Investigation, Writing – original draft, Writing – review & editing. **Lesik Dimitri:** Conceptualization, Validation, Visualization, Data curation. **Paula Giorlando:** Data curation. **Lisanti Leonel:** Methodology, Data curation. **Boscariol Adrian:** Data curation. **Contreras Simón:** Data curation, Validation. **Vanina Enriquez Tellez:** Data curation. **Carla Zaragoza:** Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial

Table 4
Effluent parameters before and after treatment.

Parameter	14 days treatment value		Resolution 52/20		Condition	
	T1 (50%)	T2 (25%)	Maximum Allowed	Maximum Tolerable	T1 (50%)	T2 (25%)
pH	8,2±0.2	8,3±0.2	6,5 a 8,5	5.5 a 9	Permitted	Permitted
COD (mg/L)	610±50	215±50	75mg/L	250 mg/L	Does not comply	Tolerable
BOD_5 (mg/L)	164±50	15,7±50	30mg/L	120 mg/L	Does not comply	Permitted
Sodium (mg/L)	5710±0.2	871±0,2	250mg/L	400 mg/L	Does not comply	Does not comply
Polyphenols (mg/L)	2,92±0.1	1,53±0,1	0,05 mg/L	1 mg/L	Does not comply	Does not comply

Note: experimental data are reported as mean ± se.

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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CONICET: National Council of Scientific and Technological Research
UTN FRM: National Technological University: Mendoza Regional

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.envadv.2022.100321.

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