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ORIGINAL RESEARCH

Biodegradation of organic compounds and decrease in electrical conductivity by native consortium in effluents from the olive industry

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Abstract

Purpose Effluents from machined olive waters are highly polluting. These have high organic load values such as the biological demand of oxygen and the chemical demand of oxygen, salinity, and others, which far exceed current regulations. The objective of this work was to achieve, through bioremediation by native microorganisms, the reduction of effluent contamination.

Method Bioremediation was achieved by supplementing the effluent with a source of carbon, nitrogen, and phosphorus in the approximate ratio 100: 5: 1, under aerobic conditions at room temperature (25 ± 1 °C) for a period of 7 to 14 days.

Results The consortium of microorganisms (bacteria and yeasts) was identified as: *Pseudomonas aeruginosa strain Kasamber 11*, *Pseudomonas aeruginosa strain 1816*, *Klebsiella sp. strain DE004*, *Enterobacter sp. DKU NT 01*, *Pseudomonas sp. KC31*, *Bacillus sp. MG06*, Candida thaimueangensis NWP2-1, *Klebsiella sp. SI-AL-1B*, *Bacillus pumilus strain LX11*, *Bacillus sp. B9 (2015b)*, *Bacillus pumilus strain Y7*, *Planomicrobium sp. strain MSSA-10 16S*, *Candida thaimueangensis strain S04-2.2* and one microorganism without identification. A reduction of approximately 40-80% of specific parameters and contamination indicators such as biological oxygen demand (BOD_5), chemical oxygen demand (COD) and electrical conductivity was achieved.

Conclusion The microbial consortium achieved the reduction of the original contamination of the effluent from "mechanized olives" by biostimulation, transforming it into a less contaminated liquid that could be used for other uses or destinations.

Keywords Machined olive waters, Bioremediation, Native microorganisms, Effluent introduction

Introduction

Nowadays, two thirds of the world's population live in regions where they suffer from water shortages at least one month a year. Five hundred millions of people who live in areas where water consumption exceeds renewable water resources locally by a rate of two to one. Highly vulnerable areas where non-renewable resources continue depleting, such as fossil groundwater, have become heavily dependent on transfers from areas with abundant water resources and are constantly seeking alternative economic sources (UNESCO 2017).

The olive industry produces numerous effluents that contaminate the available water and soils reached by them. According to the International Olive Council (COI 2019) in its 2017-2018 campaign, world production was 3,284 thousand tons, where the European Union came out on top with a production of 912.5 thousand tons, followed by Egypt, Turkey, and Algeria with 750, 450 and 303.5 thousand tons, respectively. Argentina was the sixth one with a production of 106 thousand tons. Depending on the process, seasonality, and technology used in each company, the volume of effluent generated by this industry is very high. Approximately an estimated 10–30 million of litters of effluents

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are generated every year from the production of olive oil (McNamara et al. 2008) and the same is produced by the table olive canning industry (Borgo 2013).

In Argentina, these waters are mostly dumped into the environment untreated. In other places, the commonest treatment is to retain them in evaporation ponds. This procedure, however, causes bad smells and the possibility of polluting surface and ground waters (Beltran-Heredia et al. 2000) due to the toxicity for waterways, soil waterproofing and contamination of plants. Legislation bans these effluents from being discharged without treatment. Since the setting up of more severe regulations concerning public waste disposal, there is a growing interest in the development of new technologies and procedures for the purification of this waste. Nevertheless, not all the treatment used is economical and easy to develop for factories.

The "machined olives" are conformed by sliced olives, rolled and / or spitless used for to stuffed olives and olive paste. They represent approximately 60% of table olives. The whole process of machined olives is handled with water because olives have a soft and delicate texture due to the previous process.

This effluent is a characteristic emulsion due to the amount of fat and solids in suspension that are washed away by the water. The general analysis can be described as an acid effluent (pH 4.8 to 5.49) of high electrical conductivity (25,000 uS). It shows a BOD_5 greater than 25000 mg O_2 / L, and a COD of approximately 20000 O_2 / L.

Society's growing demand for decontamination of industrial effluents (liquid or gaseous), materialized in increasingly stringent restrictions, has driven, in the last decade, the search for alternatives that contribute to solving these environmental problems (Garzón et al. 2017).

Bioremediation is a process that uses the catalytic abilities of living microorganisms to degrade and transform pollutants in both terrestrial and aquatic ecosystems. It has enormous potential in mitigating environmental pollution. Bioremediation has focused on the exploitation of the genetic diversity and metabolic versatility. Both factors characterize the microorganisms that transform the contaminants into innocuous or less toxic products that can be integrated into natural biogeochemical cycles (Garbisu et al. 2000). Bioremediation has proven to be an alternative to establish new wastewater treatment systems and optimize conventional systems (Chen et al. 2015).

Among the main technologies that have been registered since the decade of 1970, bioremediation has proven to be profitable and efficient in the removal of certain pollutants (Garzón et al. 2017). Bioremediation of wastewater can be divided into three main technologies (Salinas et al. 2008): (i) natural purification, where contaminants are reduced by the action of native microorganisms without any external help; (ii) biostimulation, in which nutrients are incorporated into the system to accelerate biodegradation, and (iii) bioaugmentation, where specialized microorganisms are added to the treatment system to increase its efficiency. (Barrera and Zafra Mejía 2018). Biostimulation involves enhancing the capacities of native bacteria, requiring the identification of an optimal nutrient ratio (Hassanshahian et al. 2013).

Numerous investigations have been carried out using microorganisms capable of growing aerobically in different effluents of olives and oils industries. Some of them, reduced the initial organic load and the phenolic content in OMW (Olive Mill Wastewaters) as Hamdi (1993), Borja et al. (1995) and others. In particular, the pretreatments of OMW with *Aspergillus niger* (Hamdi et al. 1991), *Aspergillus terreus*, *Geotrichum candidum*, *Azotobacter chroococcum* and *Phanerochaete chrysosporium* (Gharsallah 1994), reduce considerably the COD and the polyphenols content.

Tabatabaei et al. (2020) studied the reuse of urban wastewaters for irrigation and concluded that several times treated wastewater disposal in the soil, in addition to improving the soil properties, causes the plants to benefit from the nutrients which exist in the wastewater. These authors also concluded that monitoring and treatment of wastewater prior to reuse is very important to ensure environmental protection. That is why other authors studied the bioremediation of OMW and they checked that it does not generally contain sufficient N and P for an adequate aerobic purification process. An experiment was, therefore, conducted to screen the most essential nutrients necessary for aerobic microorganisms such as nitrogen, phosphorous (Duke et al. 2000; García et al. 2007; Sanscartier et al. 2009), and sulphate for an effective aerobic biodegradation of OMW (Fadil et al. 2003; Ranalli 1994). In aerobic bioremediation processes, in general, a ratio of carbon and nutrients (BOD_5 / N / P), between 100/1/0.5 and 100/5/1 was used to guarantee microbial growth and desorption of contaminates (Nannipieri et al. 2003; Tabatabaei et al. 2012).

Due to the importance of bioremediation in the sustainability of the environment and its relatively economic cost, the main objective of this work was to reduce the fundamental parameters of pollution as indicators of COD and BOD_5 and conductivity by bioremediation. The second objective was to demonstrate that by adding nutrients such as reducing sugars and salts, it is possible to biostimulate or activate the native microorganisms of the effluent responsible for reducing the previously mentioned polluting parameters.

Materials and methods

Physicochemical analysis

In order to characterize the effluent used in the assay, a sample of three litres after agitation on the following analysis were performed according to APHA (1992) which were: pH, temperature, electric conductivity, dissolved oxygen, DQO, DBO_5, nitrates ammoniacal, nitrogen, phosphates, settling solids at 10 min and 2 hours, total soluble solids, fixed suspended solids, volatile soluble solids, chlorides, sulphates, sodium, potassium, total alkalinity, total carbonates and total polyphenols"

Bioremediation assay

Half a litre of effluent from machining waters was poured to five one-litre capacity Erlenmeyer flask, previously shaken. They were supplemented with a source of carbon, nitrogen, and phosphorus in a rate of about 100:5:1. To each Erlenmeyer flask, the following compounds were added: 10 g / L of glucose and 2 g / L in the form of the following salts: $(NH_4)_2 SO_4$, $K_2 HPO_4$, $K H_2PO_4$, TRIPLE 15®. The effluent was also supplemented with Mg by adding 1 g / L of $Mg CL_2$ and $MgSO_4$. Then, each Erlenmeyer flask was placed in a shaker Dragon Lab at 220 rpm for the incorporation of air during the experiment generating, aerobiosis conditions at room temperature (25 ± 1 °C) for a period of 7 to 14 days.

The evolution of different pollution indicators and other measurements over time were monitored, which are detailed below: BOD_5 and COD according to the standard method (APHA 1992); electrical conductivity (APHA 1992), pH per electrode (APHA 1992), total reductive sugars by DNS method (Miller 1959), and total polyphenols according to the Folin - Ciocalteau technique, by spectrophotometry (APHA 1992).

Microbiological analyses

The total number of bacteria was determined on plate count agar (Britania), the total numbers of fungi on glucose potato agar (Britania) and the enteric gram-negative bacilli on eosin methylene blue agar (Britania) diluted with effluent in a proportion of 35%, 50% and 100%. Then, the most representative microorganisms were isolated until the axenic cultures were achieved by means of grooves on the surface, preparing the culture medium with 35, 50 and 100% effluent to select the viable and effluent-resistant microorganisms.

Molecular microorganism identification

The viable native microorganisms were cultivated in specific culture media (eosin methylene blue, plate count agar and glucose potato agar) diluted with effluent in a proportion of 35%, 50% and 100%. Both phenotypic and molecular characterization of the strains were performed. For bacteria, the strains were identified by amplification of the 16S rDNA ribosomal gene from genomic DNA using the universal primer set for bacteria 27F (5 'AGAGTTTGATCCTGGCTCAG 3') and 1492R (5 'TACGGTTACCTTGTTACGACTT 3'). These primers give an amplification product of ~ 1,500 bp. DNA extraction was carried out from 24-hour cultures using two extraction techniques. Amplification was carried out in a final volume of 50 µl containing buffer STR (10x) (Promega) 5 µl (supplied with the enzyme), 0.1 µM of primers, 2 U of Taq DNA polymerase (Promega) and 50 ng of DNA. Amplification conditions consisted of an initial denaturation of 5 min at 94 ºC, followed by 30 cycles of denaturation (94 ºC, 1 min), annealing (55 ºC, 2 min) and extension (72 ºC, 2 min), and a final extension at 72 ºC for 7 minutes.

For Yeast, the strains were identified by amplification of the of the 26S subunit of rDNA. Amplification of the D1 / D2 domain of the 26S subunit of the rDNA was performed using PCR (Polymerase Chain Reaction). In the amplification, reactions of this work were used as 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) foward: NL-4 (5'- GGTCCGTGTTTCAAGACGG-3'), (ii) reverse: NL-1 (5'- GCATATCAATAAGCGGAGGAAAAG-3' Amplification reactions were performed in an automatic thermocycler (Perkin-Elmer, model 9700, Applied

Biosystems). The amplification products (4 µl) were separated by electrophoresis on agarose gels 1.5% (w/v) using TAE 1X (Tris, 24.2%; acetic acid, 5.71%; EDTA, 0.5 M pH8 10 ml) and 1 µl of Gel Green as running buffer and DNA stain, respectively. A 100 bp PB-L molecular weight marker (Bio-Logical Products) was included. The samples were mixed with 6X seeding buffer (Orange – Blue, Promega), run at 75 V for approximately 30 minutes 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 Analyzer.

DNA sequencing was carried out by Macrogen Services. Sequences were compared and aligned with sequences from the GenBank database by using the BLAST program of the Nacional Center for Biotechnology Information.

Results and discussion

Water pollution and the issues related to it are regulated by Resolution No. 778/96 of the Province of Mendoza, issued on December 23, 1996, and its amendments (Resolution No. 627/00), which establishes the parameters that they must have industrial and sewage discharges for agricultural reuse, in addition to modifying the "Polluter-Payer" principle of Art. 59, by the principle "He who pollutes pays".

In accordance, all establishments that discharge effluents into the public hydraulic domain must obtain the corresponding administrative authorization and must have an adequate effluent treatment system to comply with the technical requirements provided in current legislation. The authorization mentioned is the "Dumping Permit" and is granted by the General Directorate of Irrigation (DGI) Superintendent. If the establishments do not comply with the necessary requirements to obtain said permit, they must sign a "Discharge Permit Management Agreement", through which they are granted a period of time to adjust the quality of their effluents and improve their treatment systems.

Table 1 shows the characteristic results of the most important parameters contemplated by local and international legislation for irrigation water in agriculture according to FAO (1994) and their classification as tolerated, allowed, prohibited, and not complied with as the parameter has been quantified.

The consortium of native microorganism was composed of fourteen strains. Table 2 shows the identification and gram coloration.

The consortia were mainly composed of bacteria and to a lesser extent yeast. Among the bacteria, the best-known genres are *Pseudomonas sp., Bacillus sp., Klebsiella sp. Planomicrobium sp. and Enterobacter sp,* and among the yeasts, the genus *Candida sp* was the main one. These genres have been found in the treatment of other effluents as bioremediators.

Several studies have utilized bacterial consortia for bioremediation (Darvishi 2012; Ayed et al. 2016, 2019). The effectiveness of aerobic bacteria in reducing the phytotoxicity of olive oil mill wastewater (OMWW) varies greatly. Aerobic bacteria appear to be very effective against some phenolic compounds and relatively ineffective against others. For example, Ramos-Cormenzana et al. (1996) reported a 50% reduction in the phenolic content of OMWW by *B. pumilis*. This bacterium was able to completely degrade protocatechuic acid and caffeic acid but had much less effect on tyrosol (Ramos-Cormenzana et al. 1996).

Pseudomonas aeruginosa has been used in bioremediation of soils contaminated with crude (Ojewumi et al. 2018), also in arsenious waters (Pellizzari et al. 2015) and it is able to produce biosurfactants from olives suproducts (Mèrcade and Manresa 1994).

While *Candida thaimueangensis* has been found among the typical biodiversity of olive production processes (Lucena-Padrós et al. 2014) and *Planomicrobium sp.* was found in the maturation composting stages of the two-phase olive mill waste (Tortosa et al. 2017).
On the other hand, *Enterobacter sp* was found able to remove of heavy metals from contaminated domestic-industrial effluent (Bestawy et al. 2013). So, all these viable species of the consortium are related in one way or another to the bioremediation of effluents, but they had not been found associated in this way in other effluents.

As Darvishi (2012) comments, the biodegradable capacity of microorganisms is associated with their ability to produce enzymes. In recent years, many researchers have utilized OMWs as growth substrates for microorganisms, obtaining a reduction of the COD level, together with enzyme production. Lipases are among the most important classes of industrial enzymes. Many microorganisms are known as potential producers of lipases including bacteria, yeast, and fungi. Additionally, there are other microorganisms that produce glycosidases, lignocellulosic, and other enzymes that may have aided in biodegradation.

As can be seen in Fig. 1, the COD presented a contamination reduction rate (COD in time n / COD in ini-

Table 1 Initial composition of the effluents

Variable	Average	Unit of measurement	Resolution No. 778	FAO Water quality for irrigation
pH	6.2	-	Tolerated	Tolerated
Temperature	25	ºC	Permitted	Does not apply
Electric conductivity	0.37	dS/cm	Forbidden	Permitted
Dissolved oxygen	0.29	mg/l	Does not Comply	Does not Apply
DQO	17,410	mg/l	Forbidden	Does not Apply
DBO_5	15,500	mg/l	Forbidden	Does not apply
Nitrates	0.38	mg/l	Permitted	Permitted
Ammoniacal nitrogen	6.19	mg/l	Forbidden	Does not apply
Phosphates	7.74	mg/l	Forbidden	Forbidden
Settling solids 10 min	2,875	mg/l	Forbidden	Does not apply
Settling solids 2 hours	4,375	mg/l	Forbidden	Does not apply
Total soluble solids	9,878.86	mg/l	Forbidden	Does not apply
Fixed suspended solids	162.14	mg/l	Forbidden	Does not apply
Volatile soluble solids	9,716.72	mg/l	Forbidden	Does not apply
Chlorides	5,575	mg/l	Forbidden	Forbidden
Sulphates	137	mg/l	Permitted	Permitted
Sodium	3,098.2	mg/l	Forbidden	Forbidden
Potassium	1,271.8	mg/l	Does not comply	Does not apply
Total alkalinity	510.6	mg/l	Does not comply	Does not apply
Total carbonates	22.34	meq/l $CaCO_3$	Does not comply	Does not comply
Total polyphenols	1.55	mg ácido gálico/l	Forbidden	Does not apply

Table 2 Consortium of microorganisms

Strain Number	Identification/Access number	Gram	Feature
1	*Pseudomonas aeruginosa strain Kasamber 11*/Srain10 MM	-	Bacillus
2	*Pseudomonas aeruginosa strain 1816/* Strain 21MM	-	Bacillus
3	*Klebsiella sp. strain DE004/* Strain 22 MM	-	Bacillus
4	*Enterobacter sp. DKU_NT_01/* Strain 25 MM	-	Bacillus
5	*Pseudomonas sp. KC31 /* Strain 27 MM	-	Bacillus
6	*Bacillus sp. MG06 /* Strain 29MM	+	Bacillus
8	*Without idetification /* Srain 46 MM	-	Bacillus
9	*Klebsiella sp. SI-AL-1B* / Strain 88 MM	-	Bacillus
10	*Bacillus pumilus strain LX11* / Strain 91 MM	+	Bacillus
11	*Bacillus sp*. B9(2015b) / Strain *94* MM	+	Bacillus
12	*Bacillus pumilus strain Y7 /* Strain 100 MM	+	Bacillus
13	*Planomicrobium sp*. strain MSSA-10 *16S /* Strain 102 MM	+	Bacillus
14	*Candida thaimueangensis strain* S04-2.2 / Strain 18282 MM		Yeast

tial time) that was from 100 at the initial time to 10 on the seventh day. This is demonstrated in the practically exponential decrease of the COD curve until the seventh day and then it continues to vary very slightly and finally becoming almost asymptotic with the x-axis, with which the rate of decrease in pollutant was from 100 to 0.1 on day 14. This parameter started with an average of 13,575 mg O / L and the rate of decrease in contamination fell almost two orders of magnitude after 14 days of treatment, reaching a value of 172 mg

O / L. For what is appropriate for the legislation of Resolution 778, to allow public cause, it must be less than 250 mg O / L for COD. On the other hand, the decrease in COD was consistent with a 90% reduction in BOD_5 registered by the samples, which decreased from an average of 15,500 mg O / L to 1,500 mg / L. This value is greater than that indicated in the legislation: Resolution 778, to allow the public cause, it must be 120 mg O / L. Biodegradation could be complemented with Fenton reaction to achieve the indicated value as indicated by Lucas and Peres (2009). However, this process was more effective than the aerobic biodegradation and detoxification of wastewaters used by Fadil et al. (2003). They achieve COD removals to be 55.0%, 52.5% and 62.8% in wastewaters fermented with *Geotrichum sp*., *Aspergillus sp*. and *C. tropicalis*, respectively. The efficiency in terms of biodegradation is also multicausal if other examples are taken. To compare, Fadil et al. (2003) studied an effluent with 124 g / L of COD, pH 5,2, total solids (g=l) 92.4, volatile solids (g=l) 86.2, mineral solids (g=l) 6.2 ammonia (g=l) 0.15 reducing sugars (g=l) and 12.8 and total phenolic compounds (g tannic acid=l) 8.2 but these is an OMW (Oil Mill Wastewaters), its different in composition than the studied effluent. On the one hand, the effluent under study is similar only in total solids but most of other parameters are different. Furthermore, the consortia of microorganism is also different, whereas Fadil et al. (2003) used some acclimatized microorganism. In addition, they used different values of COD to establish a kinetics behave. Instead, this work used biostimulation of the viable native microorganisms. In general, these last conditions are more efficient than acclimatized microorganisms. But other studies have reported better ratios of degradation as García García et al. (2000). They achieved a reduction of 73% COD and 76% of phenol reduction using *Aspergillus Niger*, 75% COD reduction, 92% phenol reduction in OMWW, using *Phanerochaete chrysosporium*. In summary, the efficiency of using native microorganisms is that they generally have the necessary enzymatic pull to degrade the environment in which they live. This is a free solution that nature offers.

This result is important because it uses only the native microorganism, compared with Kyriacou et al. (2005) who studied the combined bioremediation and advanced oxidation of green table olive processing wastewater. They obtained promising results with *Aspergillus niger* achieving a reduction of COD about 66-86%. In addition, with electric coagulation, it finally achieved 98% of reduction. It was also consistent with the reduction of reducing sugars that was used as an indirect indicator of the microbial activity shown in Fig. 2, since it is the first carbon source apparently consumed by microorganisms in its exponential growth phase.

Fig. 2, shows the decrease in reducing sugars. It was very fast until they become indictable in quantity. On the fourth day, there are no more reducing sugars. This was consistent with the decrease in COD (see Fig. 1), which on day four is in full exponential decline to become practically asymptotic by day seven. This behaviour could probably be since microorganisms,

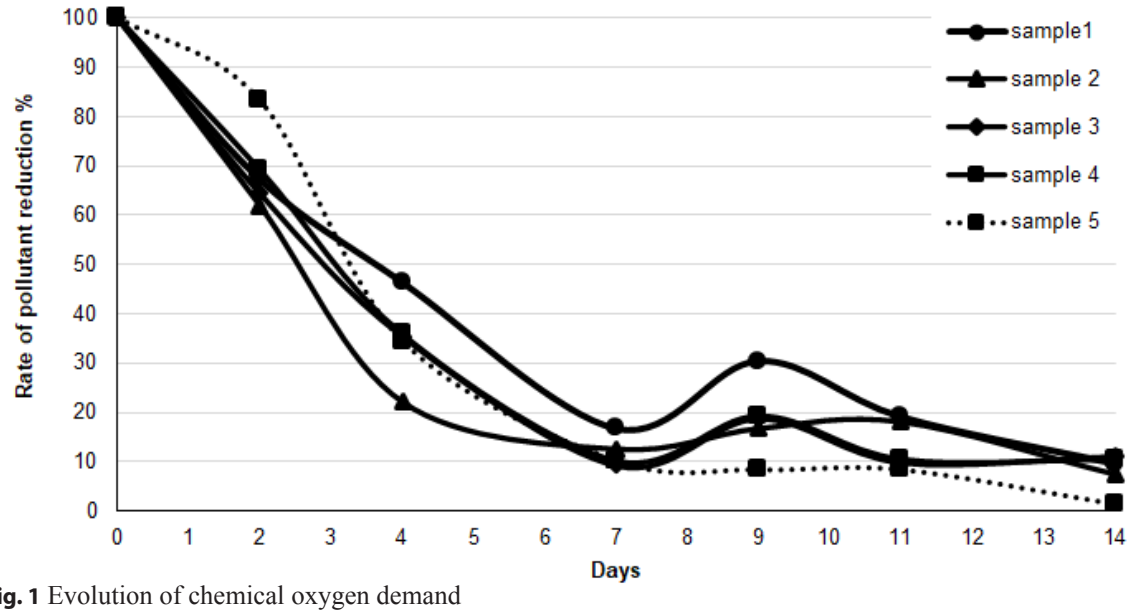

Fig. 1 Evolution of chemical oxygen demand

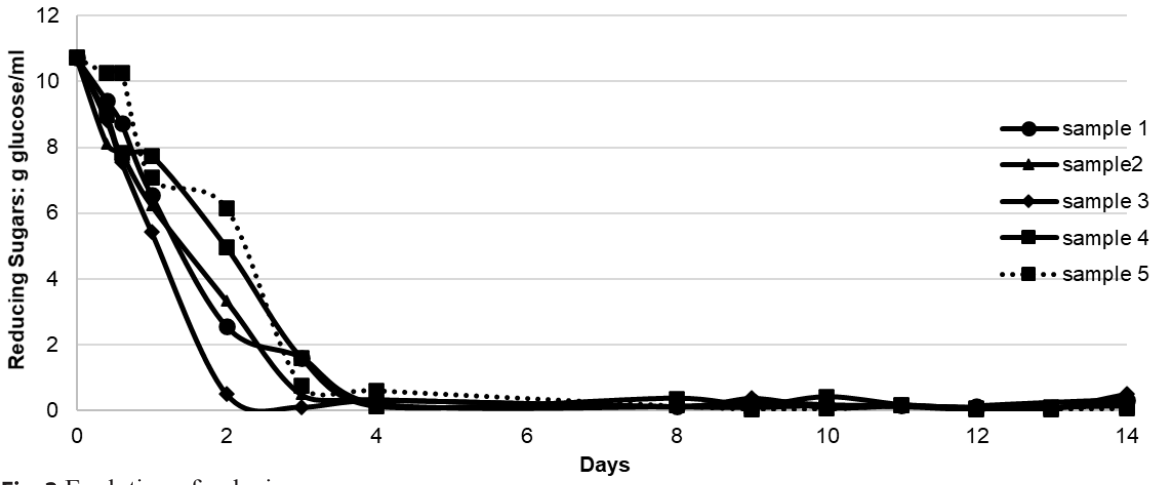

Fig. 2 Evolution of reducing sugars

which grow between the first 24 hours and 72 hours, would do so at the expense of the consumption of reducing sugars, which are the easiest to degrade and then continue to grow at the expense of other sources of complex carbon.

Similar behaviour was found by Laconi et al. (2007) in olive oil mill wastewater in aerobic conditions too.

Table 3 demonstrates the growth of microorganisms. It was followed by total counting of them. The growth of three important population groups were verified: aerobic mesophilic, coliforms and yeasts. The coliforms and aerobic mesophilic grew up during the first phase and continued growing up to the end. The yeasts were present in the last phase of the fermentation process.

As mentioned above, the growth of microorganisms was greater while there was a greater amount of organic matter in the system and specifically accompanied by the presence of reducing sugars. However, there was always growth of viable native microorganisms. This indicates that they were present throughout the process, but possibly their metabolic rate was reduced as the carbon source was lacking, which is why it reached the end of the process showing an asymptotic behaviour in the different parameters followed to visualize its behaviour.

Table 3 Total counting of Microorganisms

	UFC/ml		
Time (hours)	**Coliforms**	**Aerobic mesophilic**	**Yeast**
0	9.9889 E+12	1.41778 E+14	0.00 E+00
2	1.80008 E+15	5.26475E+17	0.00 E+00
4	7.82501 E+12	3.36572 E+16	0.00 E+00
6	1.6856 E+13	1.538 E+17	3.27 E+07
8	1.1732 E+11	1.9767 E+15	5.52E+07

The evolution of pH can be observed in Fig. 3. The evolution of pH was different for each Erlenmeyer flask with effluent, although the same effluent was used in all of them. Each one behaved as an individual bioreactor although there were some similarities to consider. At the beginning, on the first day, there seems to be a slight change in the pH of the repetitions. At the second day, the pH shows a small increase in three of the Erlenmeyer flasks (2, 3 and 4). This behaviour occurred mainly during the first 24 hours. This change could possibly be interpreted due to the microbial growth that transformed the medium (quantified data not shown). Probably, the inorganic nitrogen is used by them to generate basic proteins and cell membrane constitution (Nelson et al. 2005). In biostimulation and bioaugmentation techniques, commercial fertilizers with N and P content were used to guarantee the nutritional optimum of the bacterial metabolic process (Duke et al. 2000; García et al. 2007; Sanscartier et al. 2009). In this work, the amount of glucose nitrogen and phosphorous was

added by adding fertilizers to reach the 100: 5: 1 ratio as suggested by Nannipieri et al. (2003) in order to promote microbial growth.

On the other hand, samples 1 and 5 presented a decrease in pH between day 2 and 3, possibly due to the production of acids released by the aerobic respiration of sugars by citrate acids cycle (Nelson et al. 2005). They were almost completely breathed at 100 hours as shown in Fig. 2. In fact, if the Table 3 is observed between days 2nd and 4th , there is growth of microorganisms (coliforms and mesophilic aerobes). On the other hand, the yeasts grew from day 6th where the pH was near to pH 6. The decrease in pH has also been reported by other authors in similar aerobic respiration (Flamarique et al. 2016). This is also consistent with what was found by Barrera and Zafra Mejias (2018), who showed that the bioremediation processes for the purification of wastewater kept pH between 4 and 9.1, with a median of 7.1 as the optimal pH for the microorganism development.

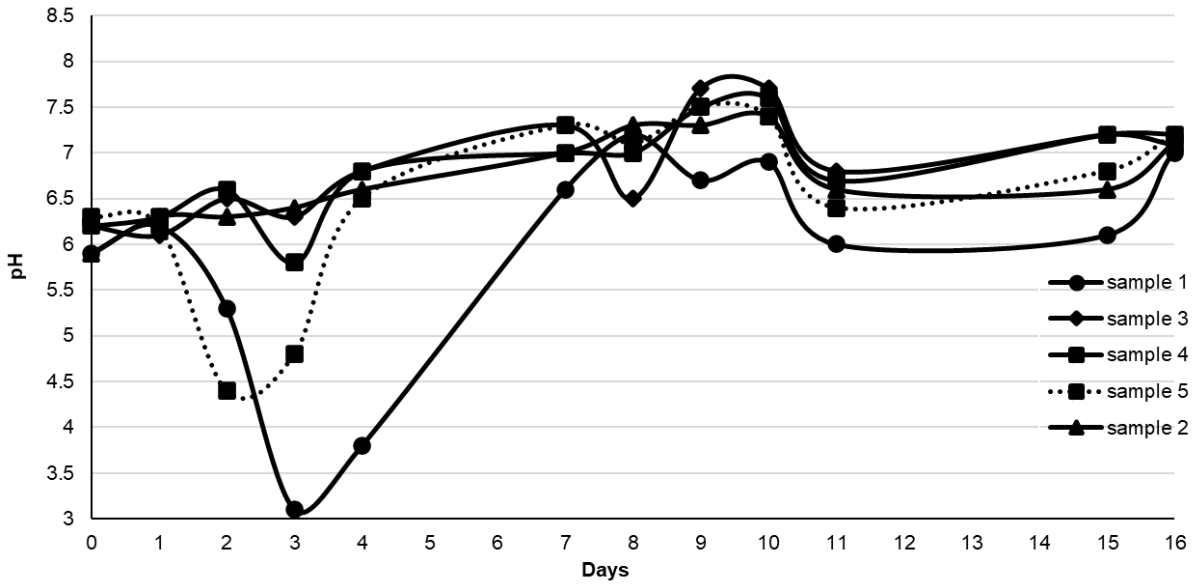

Fig. 3 Evolution of pH

This decrease in pH is consistent, also, with the organoleptic changes that were registered. The changes of colour seen in the opalescent effluent show how the microorganisms developed and multiplied. The microbial activity was consistent with the variations of pH that were seen in each sample, verifying that the increase of microbial mass and products of aerobic treatment raised almost two pH points towards the tenth day. The microbial activity and its respiration were consistent with the decrease in the chemical demand for oxygen. Probably, it could indicate the consumption of other more complex organic products than the glucose added in treated samples.

Fig. 4 shows the evolution of electric conductivity. The initial conductivity of samples 1, 2 and 3 was initially higher than that of samples 4 and 5, possibly due to the complex matrix treated. However, during the treatment, a decrease in conductivity of 35% was observed. The causes of which will be studied in detail in a future work. It is interesting to analyse this, since the mechanism by which the decrease in electrical conductivity occurred is not known and understood. This could be caused by any of the selected microorganisms or by their synergistic work.

Conclusion

It has been possible to reduce the fundamental parameters of pollution indicators of an effluent because of the action of bioremediation microorganisms obtained from effluent, viable natives. These parameters such as COD and BOD_5 decreased by approximately 90%, and the conductivity by 35%. It verified the presence of viable native microorganisms that consumed the reducing sugars until leaving traces of sugars. It is possible that they consume more complex compounds later. This was consistent with the decrease in COD. Further studies should be carried out to understand and relate the causes of decreased electrical conductivity. The laboratory results are promising as a first approximation to the

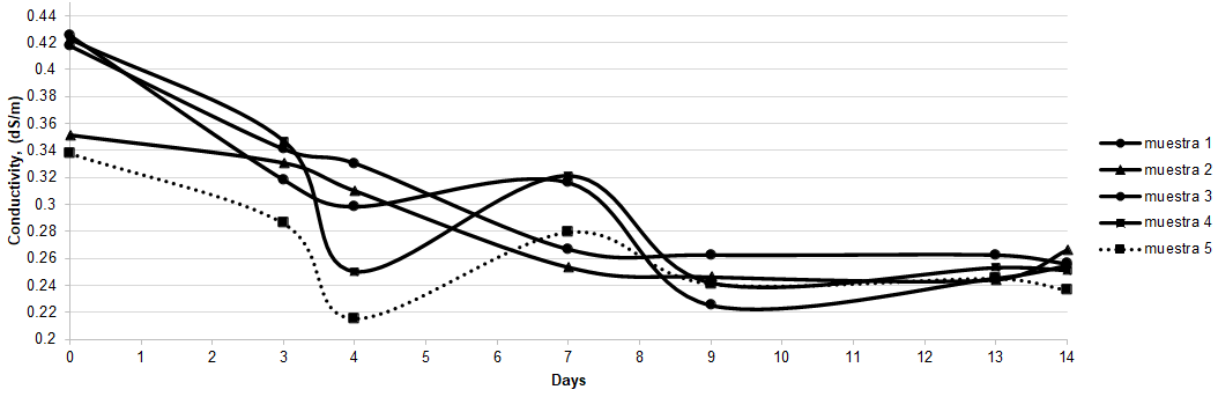

Fig. 4 Evolution of electric conductivity (EC)

bioremediation of this complex and polluting effluent for the local industry.

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UTN FRM: National Technological University: Mendoza Regional School.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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