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CHARACTERIZATION OF EFFLUENTS FROM A TANNERY INDUSTRY: A CASE STUDY OF CÓRDOBA PROVINCE, ARGENTINA

Caracterización de efluentes obtenidos a partir de una curtiembre: un caso de estudio de la provincia de Córdoba, Argentina

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Key words: environmental pollution, effluent quality, physical, chemical and microbiological characteristics, operational parameters, toxicity.

ABSTRACT

Tannery effluents are considered to be severe pollutants around the world. The characterization of such effluents is a very important task in order to verify the compliance with legal requirements of the discharge and, if necessary, to optimize their treatment. In this study, we used the effluents from a local tannery (Córdoba, Argentina) that had implemented only a primary treatment. The effluents were periodically collected during one year to assess their physical, chemical, and microbiological characteristics, as well as their toxicity. The physical and chemical characteristics showed variations independently of the sampling time, although, in general, a high nutrient load was the most frequent finding. Moreover, parameters such as chemical oxygen demand, biochemical oxygen demand, total nitrogen, total phosphate, total phenols, Cr(VI) and settleable solids exceeded the limit set by the provincial legislation for the discharge of effluents into surface water bodies. The effluents were also characterized by a high count of bacteria, where pollutant-tolerant and some pathogenic bacteria were found. In sampling 9, *Bacteroidetes*, *Firmicutes* and *Proteobacteria* represented up to 93 % of the total number of sequences obtained, with *Paludibacter*, *Tissierella Soehngenia*, *Proteocatella* and *Proteiniclasticum* as the most abundant genera. In concordance with these results, all samples showed toxicity according to *L. sativa* and AMPHITOX bioassays. Altogether, data from this report indicate the need to optimize primary treatment of the effluents, as well as to include complementary treatments in order to reduce the negative environmental impacts of the release of this kind of runoffs.

Palabras clave: contaminación ambiental, calidad de los efluentes, características físicas, químicas y microbiológicas, parámetros operacionales, toxicidad.

RESUMEN

Los efluentes de las curtidurías se consideran contaminantes graves en todo el mundo. La caracterización de dichos efluentes es una tarea muy importante para verificar el cumplimiento de los requisitos legales de vertido y, en caso de ser necesario, optimizar el tratamiento de efluentes. En este estudio utilizamos los efluentes de una curtiduría local (Córdoba, Argentina) que había implementado sólo un tratamiento primario. Los efluentes fueron recolectados periódicamente durante un año para evaluar sus características físicas, químicas y microbiológicas, así como su toxicidad. Las características físicas y químicas mostraron variaciones independientemente del tiempo de muestreo, aunque, en general, el hallazgo más frecuente fue una alta carga de nutrientes. Además, parámetros como demanda química de oxígeno, demanda bioquímica de oxígeno, nitrógeno total, fosfato total, fenoles totales, Cr(VI) y sólidos sedimentables, superaron los límites establecidos por la legislación provincial para el vertido de efluentes en aguas superficiales. Los efluentes también se caracterizaron por un alto recuento de bacterias, encontrándose bacterias tolerantes a contaminantes y algunos patógenos. En la muestra 9, *Bacteroidetes*, *Firmicutes* y *Proteobacteria* representaron en conjunto hasta el 93 % del número total de secuencias obtenidas, siendo *Paludibacter*, *Tissierella Soehngenia*, *Proteocatella* y *Proteiniclasticum* los géneros más abundantes. De acuerdo con estos resultados, todas las muestras presentaron toxicidad según los bioensayos de *L. sativa* y AMPHITOX. En conjunto, los datos de este informe indican la necesidad de optimizar el tratamiento primario de los efluentes, así como de incluir tratamientos complementarios para reducir los impactos ambientales negativos de la liberación de este tipo de efluentes.

INTRODUCTION

Due to an increase in the number of industries water pollution is a serious problem faced by the modern world (Yadav et al. 2016, Liang et al. 2017). Among the industrial activities, the leather industry has gained a negative image in society attributable to the generated effluents, which leads to the decline of the receiving water bodies' quality (Akhand and Rao 2013, Amanial 2016).

In the past few decades, developing countries have witnessed a sharp increase in leather production as a consequence of a decline in this activity in developed countries due to more stringent control requirements regarding environmental pollution, as well as high labor costs (Haydar and Aziz 2009). Consequently, Argentina is among the leading producers of bovine leather in the world (FAO 2013). The Matanza-Riachuelo basin in the province of Buenos Aires groups around 170 tanneries, a sector dominated by a group of large companies with international projection (Greenpeace 2012, Martínez and Romero 2017). However, barriers to its commercialization may appear due to the identification of some critical points or processes related to the technical and quality specifications of the products, which is a quite relevant topic to continue towards the recovery of substances (Reyes et al. 2009).

In this industry, animal hides are transformed into leather in a sequence of many complex steps, consuming high volumes of water and using large quantities of chemicals, such as sodium sulfide, lime, sodium chloride, ammonium sulfate, bactericides, chromium salts and vegetable tannins (Cooman et al. 2003). Only about 20 % of the chemicals utilized in the tanning process are absorbed by the leather manufacturing process, while the rest of the compounds are released as liquid effluents (Akhand and Rao 2013). As result, tannery effluents are mainly characterized by several key parameters such as high concentrations of chromium salts, nitrogen, sulfonated oils and grease, suspended solids, chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5), as well as high levels of dyes, surfactants, solid waste fragments and waste skin trimmings, among others (Deghles and Kurt 2016, Sungur and Özkan 2017). These compounds can be removed from the effluents by appropriate treatments, including primary and secondary treatments such as physical and chemical methods and biological strategies, respectively (Sungur and Özkan 2017). Among the first, filtration, coagulation/flocculation, sedimentation, oxidation processes (Fenton based processes, ozone based methods, photocatalysis) and electrochemical treatment have been successfully applied (Lofrano et al. 2013). In relation to biological strategies, aerobic

and anaerobic processes, based in metabolic activity of the contaminant-tolerant bacteria, are frequently used (El-Sheikh et al. 2011, Lofrano et al. 2013). Additionally, tertiary treatments can also be applied in order to completely treat wastewaters. Constructed wetlands, using selected plant species, may be an interesting tertiary treatment option for leather tannery wastewater (Calheiros et al. 2012). In spite of all these possibilities, and others that were not mentioned, many industries do not usually treat their effluents. This may be due to the fact that effluent treatment plants, in general, are costly to construct and complicated to operate. In addition, the legal and social cost would be higher. As a consequence, many tanneries release their effluents without treatment or insufficiently treated, which causes many deleterious effects, including environmental degradation, alteration of water resources, as well as damage to animal and human health (Durai and Rajasimman 2011, Rangaraj et al. 2014, Yadav et al. 2016).

A complete characterization of the effluents generated from tanneries would be appropriate to understand the problem associated with them, including the design of effective treatment facilities, as well as to verify the compliance with legal requirements for the discharge (Haydar and Aziz 2009, Islam et al. 2014). In this regard, there are few studies in Argentina compared to the rest of the world (Villegas et al. 2008, Martínez and Romero 2017, González et al. 2018). On the other hand, the evaluation of the effluents toxicity is necessary since it allows us to estimate their toxic impact on living beings. Toxicity tests using higher plants (*Lactuca sativa* seeds test) and amphibian embryos (AMPHITOX test) have been recommended by many international organizations for the determination of ecological effects and also to study the standard toxicity of pure chemicals, phenolic compounds and effluents (Herkovits et al. 2002, Angelini et al. 2011, Aronzon et al. 2011, Charles et al. 2011, Park et al. 2016, Lyu et al. 2018, Paisio et al. 2019). Therefore, this study was aimed at performing an integral characterization of the effluents discharged by a local tannery during one year, in relation to several physical and chemical, microbiological and operational parameters. Additionally, we analyzed the acute toxicity of the samples by means of two bioassays, through *L. sativa* L. seeds and *Rhinella arenarum* embryos.

Taken together, data from this research reveals the existence of poor leather processing systems, the lack of achievement of legislation standards that assess the environmental safety of effluent discharge procedures, and it also provides an overview of the characteristics of effluents and their toxicity, which are the basis to design an effective treatment strategy.

MATERIALS AND METHODS

Sample collection

Effluent sample collection was made from a tannery located in a small village, situated in the south of Córdoba province, Argentina (32º 33' 20.4'' S, to 64º 23' 46.9'' W). This tannery operates with an average processing capacity of 5000 goat and 200 cow skins per week, using 50 000 liters of water daily, a relevant production volume to be considered. This industry has implemented an effluent treatment plant, in which a primary treatment of effluents from the pre-tanning process is performed. The effluents are consecutively exposed to the following continuous processes (**Fig. 1**): solids filtration (box 1), oxidization of sulfides (box 4), degreasing by flotation and decantation of fine solids (boxes 5 and 6). Finally, the effluents are discharged into a natural water receiving body through a channel from which the samples used in this work were collected (box 7). On the other hand, effluents deriving from the tanning process

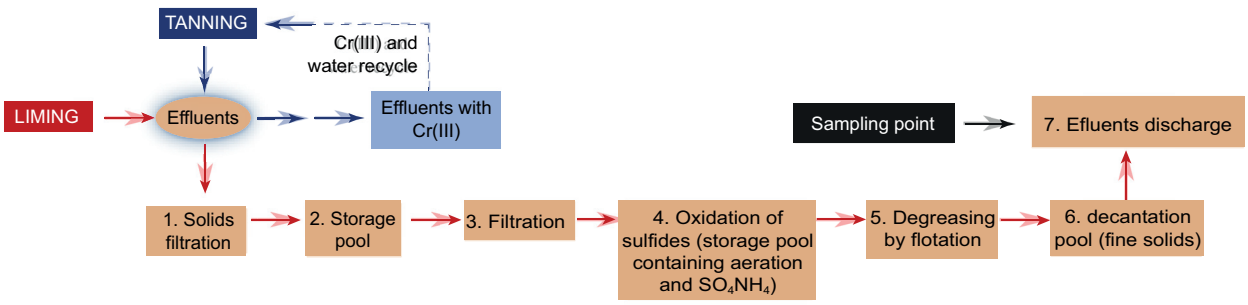

Fig. 1. Schematic representation of the primary treatment of effluents discharged by the studied tannery.

are treated in an independent route that allows the recycling of Cr(III) and water.

The study was conducted from 2017 to 2018. Effluent samples (by triplicate) were collected at 40 days intervals, during one year (number of samplings = 9). All effluent samples were taken in sterile polyethylene containers and transported to the laboratory for their processing. Separate containers were used (1) for physical, chemical and microbiological parameters and (2) for the 16S rRNA gene amplicon sequencing study.

Additional information register

The industry staff involved in the maintenance of the effluents was interviewed in order to obtain relevant information on the predominant processes (pre-tanning, tanning) carried out during the sampling period and the type of leather processed (cow, goat). Data from this register facilitated the correlation study between the physical, chemical, and microbiological parameters of the effluents with operational parameters. The prevailing average ambient temperature (AT) of the sampling period was registered and correlated with the above-mentioned parameters.

Determination of physical and chemical parameters of the effluent samples

The pH values were determined with a HANNA Waterproof Tester and settleable solids values (SS) were measured by a volumetric method (Ojeda-Suárez 2004). Organic matter was measured as BOD_5 and COD levels. BOD_5 levels were determined as the oxygen consumed by the microorganisms (Ojeda-Suárez 2004) and COD levels were quantified using a commercial HACH kit (HR2125915) and also a multiparametric spectrophotometer. Other commercial kits (HANNA: HI93767A-B and HI93758C) were also used to determine nutrients in the effluents such as total nitrogen (N) and total phosphates (P), according to HANNA specifications. Cr(VI) was quantified using *diphenylcarbazide* in acid solution at 540 nm and phenols were evaluated by the spectrophotometric method (Wagner and Nicell 2002). The data obtained was compared with the limits established by current provincial legislation for the discharge of liquid effluents into surface water bodies (MINSPCBA 2016).

Determination of microbiological parameters of the effluent samples and microbial community structure study by 16S rRNA gene amplicon sequencing

A total count of bacteria (TCB) for all tannery effluent samples was performed in rich agar (Beringer 1974) by the microdroplet protocol reported by Somasegaran and Hoben (1994).

For the 16S rRNA gene amplicon sequencing study, effluent samples (320 mL) were taken in duplicate from sampling 9, centrifuged at 4 ºC and 17 000 rpm for 10 min and from the obtained pellets, the DNA extraction was carried out (Wizard Genomic DNA Kit, Promega, USA). DNA samples were purified (Petric et al. 2011) and their purity and DNA concentration were estimated by the A260:A280 ratio and optical density, respectively.

The 16S rRNA gene (V4 region) was amplified from the extracted DNA samples, according to the amplification conditions from the sequencing company (Mr DNA, Texas, USA). PCR products were checked in agarose gels (2 % w/v) and purified using calibrated AMPure XP beads. The purified amplicons were paired-end sequenced (2 × 300) by Mr DNA (Texas, USA) using the MiSeq PE300 platform (Illumina, USA). The raw data associated with sample 9 was deposited in the NCBI (SRA database) under Biosamples SAMN10873103 and SAMN10873104 that belong to BioProject accession number PRJNA520999.

Data analysis was performed as described by Fernández et al. (2019) using the FastQC and MOTHUR v. 1.39.5 software (Schloss et al. 2009, Andrews 2010). Also, SILVA-database was used to align the filtered sequences (Quast et al. 2013), while Greengenes 16S rRNA database (gg_13_8_99) was used to carried out the sequence identification (DeSantis et al. 2006). Sequences were clustered into operational taxonomic units (OTUs) at 97 % relevant quantitative similarity. Alpha diversity from sampling 9 was estimated through Good's coverage and indices such as Heip, Chao1, and Shannon-Weaver, using the OTUs previously obtained and employing a subsample with 7347 sequences. The sequences processing and the rarefaction curves are shown in **Table SI** and **Fig. S1** in the supplementary material, respectively.

Acute toxicity bioassays: *L. sativa* and AMPHITOX tests

A toxicity test using *L. sativa* L. seeds was performed following the methodology described by Sobrero and Ronco (2004). For that, twenty seeds distributed on paper filters and into glass plates were impregnated with the different effluent samples or under tap water (control), in quintuplicate (n = 5). Plates were incubated in a dark chamber and under controlled conditions (average ambient temperature 25 ± 2 ºC, 80 % relative air humidity), for five days.

Later, the number of germinated seeds was registered.

On the other hand, acute toxicity bioassays were carried out with *R. arenarum* embryos as established by Herkovits and Pérez-Coll (1999). Ten embryos with a larval development of the stage 25 were placed per glass plates containing 10 mL of the different effluent samples or water as control (in triplicate). The plates were maintained at 20 ± 2 ºC, for five days. Daily, the survival (%) of the tadpoles was registered and all liquid samples were replaced.

Statistical analysis

All experiments were performed in duplicate, with the exception of toxicity assays which were done in quintuplicate. Results are shown as the means and standard deviation values. Principal component analysis (PCA) was carried out using Origin Pro (2017) software, which allowed us to visualize the dispersion patterns between the different tannery effluent samples and to check correlation with the set of physical, chemical, and microbiological characteristics, as well as the environmental (average ambient temperature) and operational parameters.

RESULTS AND DISCUSSION

Physical and chemical characterization of the tannery effluents and operational parameters

The physical and chemical characteristics of the tannery effluent samples, as well as some operational parameters of the industry analyzed during a whole year are summarized in **Table SII**. In general, the effluents were characterized by a brown/gray color, they were turbid, and had a putrid odor, which is characteristic of the tannery effluents and is probably due to decomposition of organic matter, as well as to the emission of sulfides and ammonia from these wastewaters.

The effluents presented high COD values that ranged between 1364 and 5328 mg/L, emphasizing the need of a secondary treatment to reduce their organic matter (**Fig. 2a**). Moreover, BOD_5 levels represented in average approximately between 40 and 60 % of each COD value detected in the samples (**Fig. 2** and **Table SII**). Since this parameter is a measure of the content of organic substances that are biologically degradable with consumption of oxygen (Islam et al. 2014), the results indicate that a large proportion of organic matter of these effluents is not feasible to degrade. This was confirmed through the COD/BOD_5 relation, which ranged between 1.54 and 4.08, indicating a very slow biodegradability, according to the classification criteria described by Wincler (2012). Similar findings were described for this type of effluents by Ates et al. (1997) and Haydar and Aziz (2009). On the other hand, nutrient levels such as total nitrogen (N) and total phosphate (P) reached values ranging from 93 to 665 mg/L and 14.0 to 59.7 mg/L, respectively. In general, nutrient levels followed the relationship DQO > BOD_5 > N > P (**Fig. 2a**).

The COD:N:P ratio found in the tannery effluents under study was in average 100:9.9:1.3 (**Table SII**). Various COD:N:P ratios have been recommended for

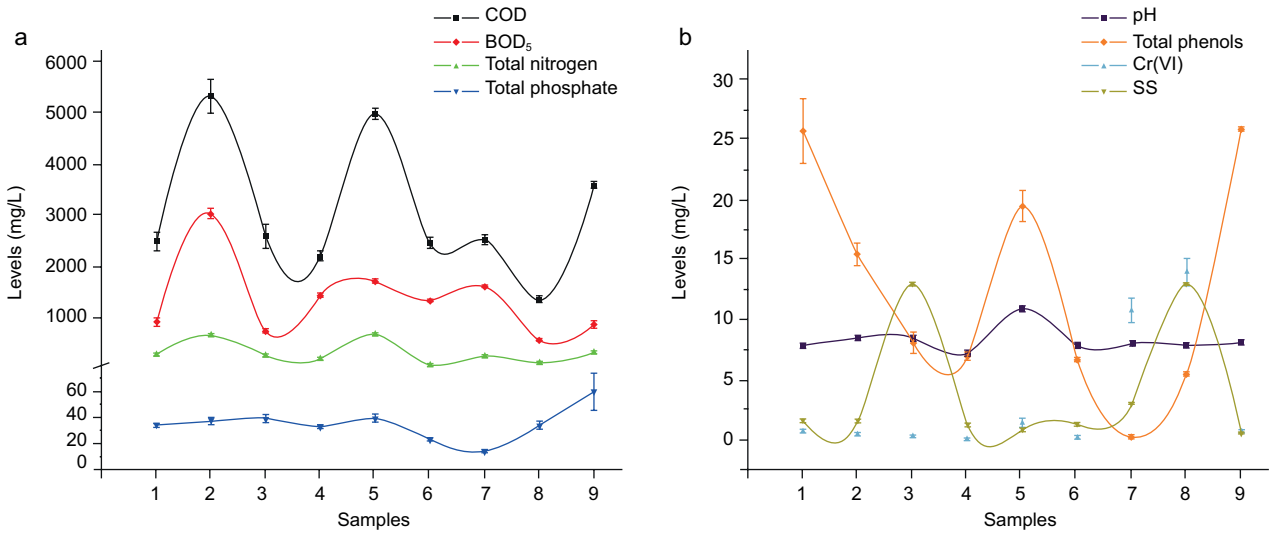

Fig. 2. Variation of the physical and chemical parameters in the tannery effluent samples. (a) Chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total nitrogen, and total phosphate; (b) pH, total phenols, Cr(VI), and settleable solids (SS).

aerobic wastewater treatment (Metcalf et al. 1991, Henze et al. 1997, Maier 1999). Conventionally, the COD:N:P ratio in aerobic systems was reported to be 100:5:1 to ensure the presence of sufficient nutrients for biomass growth (Ammary 2004, Metcalf et al. 2014). However, various studies reported different COD:N:P ratios to efficiently remove nutrients, depending on the initial characteristics of the effluents, as well as on the technologies to be applied to achieve it (Krishnan et al. 2008, Hao and Liao 2015, Hamza et al. 2018). For example, Hamza et al. (2019) indicated that the amount of nutrients needed for biomass growth did not follow the conventional organics to nutrients ratio (COD:N:P) of 100:5:1 when dealing with high-strength organics wastewater. The highest removal efficiency was achieved at COD:N:P ratio of 100:2.8:0.4, where COD, TN (*Kjeldahl nitrogen)* and P removal was 98.8 ± 0.3 %, 100.0 ± 0.0 %, and 99.3 ± 1.0 %, respectively. In addition, the COD:N:P ratio of 100:1.1:0.4 allowed the fastest rate of removal in batch optimization experiments. From the above results, we can suggest that the optimal COD:N:P ratio of the tannery effluents under study should be adjusted and/or modified in the future for *in situ* bioremediation.

Values of most of the analyzed nutrients exceeded the limits established by the provincial legislation, which means they are seriously dangerous for the environment (MINSPCBA 2016). The discharge of effluents rich in organic matter can produce eutrophication and may also affect the survival of gill breathing animals into the receiving water bodies, which could lead to important ecological imbalances (Islam et al. 2014, Noorjahan 2014, Amanial 2016).

The whole scenario gets worse if we consider that Cr and phenolic compounds were also present in the analyzed samples (**Fig. 2b**). Cr(VI) concentrations registered in the samples in general were less than 1.5 mg/L, although some samples (7, 8) had values up to 10-14 mg/L probably caused by an accidental discharge of effluents deriving from the tanning process or operational problems in the Cr(III) recycling. Cr(III) is used for tanning processes, and due to its oxidation during reactions, Cr(VI) is present in wastewaters (Homa et al. 2016). Taking into account that Cr(VI) concentration limit is < 1 mg/L, the found values were high and demonstrated that the metal recycling system adopted by the tannery is probably deficient and/or some operational problems were occurring. On the other hand, phenols are used in syntan production, a synthetic tanning agent; thus syntans contain phenolic hydroxyl groups and have the ability to react with collagen to produce leather. Syntans are not completely absorbed by the skin and remain present in the effluents (Rema et al. 2010). In the studied effluents, total phenols concentrations varied from 0.21 to 25.89 mg/L, greatly exceeding the maximum limits established by the legislation (≤ 0.1 mg/L). Phenolic compounds and heavy metals such as Cr(VI) are among the chemicals of major concern, as they tend to persist in the environment over a long period of time, exerting toxic effects on microorganisms, animals and humans (Bharagava and Mishra 2018, Bhattacharya et al. 2019, Wang et al. 2019). Most of these compounds can easily penetrate the skin through absorption, can readily be absorbed from the gastrointestinal tract and pose a high risk for human health and wildlife (Gami et al. 2014, Anku et al. 2016).

Settleable solids levels (0.5-13.0 mL/L) fluctuated between each sampling (**Fig. 2b**) and were also above the legislated values (≤ 1 mL/L, after 120 min). The high level of total solids present in the tannery effluents could be attributed to accumulation during the processing of the finished leather (Islam et al. 2014). These levels lead to high turbidity in the receiving water bodies, that could later result in poor photosynthetic activity of the aquatic systems and also in clogging of the fishes' gills and respiratory surfaces (Ates et al. 1997, Islam et al. 2014).

On the other hand, pH values were close to neutrality or alkaline (7.26-8.54). Sampling 5 presented pH values close to 11, probably due to inadequate neutralization after the pre-tanning process. In this sense, it is well known that large fluctuations in pH values of the released effluents are detrimental to some aquatic species (Rajeswari 2015).

Physical and chemical characteristics of this tannery effluent did not differ significantly form other effluents, as long as manufacturing procedures and effluent treatments used by other tanneries are similar (Islam et al. 2014, Chowdhury et al. 2015, Gutterres et al. 2015, Amanial 2016). Therefore, it is relevant to perform a correlation analysis between physical and chemical characteristics and operational parameters. In relation to main processes carried out in the tannery during the sampling time, a simultaneous prevalence of pre-tanning and tanning different sampling times was detected, with the exception of a single sampling period in which the tanning process was exclusively carried out. On the other hand, with respect to the type of leather processed during the sampling time, this parameter varied between cow/goat leathers and goat leather alone, which probably reflects in differences in the effluent characteristics, since the reagents and their concentrations used for

tanning each type of leather are different, as well as the chemical composition and quality of the hides and skins processed in the tannery. The potential correlation between chemical characteristics and these production features is shown below.

Microbiological characterization of the tannery effluent samples

The effluents were all characterized by a high count of bacteria (6-8 log CFU/mL; **Fig. 3a**). This result is consistent with the high nutrient values found (C expressed as COD, N and P) (**Fig. 2a**), which could explain the development and reproduction of such microorganisms. The amount of total bacteria considered as acceptable in effluents to be discharged into surface water bodies is not frequently legislated, in contrast with the total count of coliforms or thermo-tolerant bacteria, due to their implication in human health. A reduction in the total count of bacteria that is being discharged into the environment could be achieved by applying a simple microbial inactivation process using common bactericidal agents, such as sodium hypochlorite, which ensure reduction of the microbial flora, including potential pathogens.

Obtaining accurate information about the composition and status of the native bacterial communities within complex matrices such as effluents, gives us not only information about their quality but also allows us to have a potential biotechnological tool. The structure of bacterial communities is closely related to the performance and stabilization of biological wastewater treatment systems that can be applied in the future (Mielczarek et al. 2013, Chen et al. 2017). Analysis of the microbial communities was made in sampling 9 (in duplicate). The analysis showed a total of 439 608 high quality sequences, which were clustered to generate 5136 OTUs (**Table SI**). Good's coverage estimator and rarefaction curves indicated that both samples were sequenced in depth and that the size of the libraries was sufficient to cover almost 100 % of the bacterial communities (**Table SI** and **Fig. S1**).

A bacterial community in a certain niche is characterized by the bacterial diversity that involves the number of species present (richness) and their relative abundance (equitability) (Kim et al. 2017). Results of the Shannon-Weaver index showed an average value of 3.417, which could be interpreted as a normal to high diversity value in comparison with natural ecosystems (Pla 2006). On the other hand, the richness evaluated by the Chao1 index and the equitability assessed by the Heip index showed average values of 417.915 and 0.129, respectively (**Table SI**).

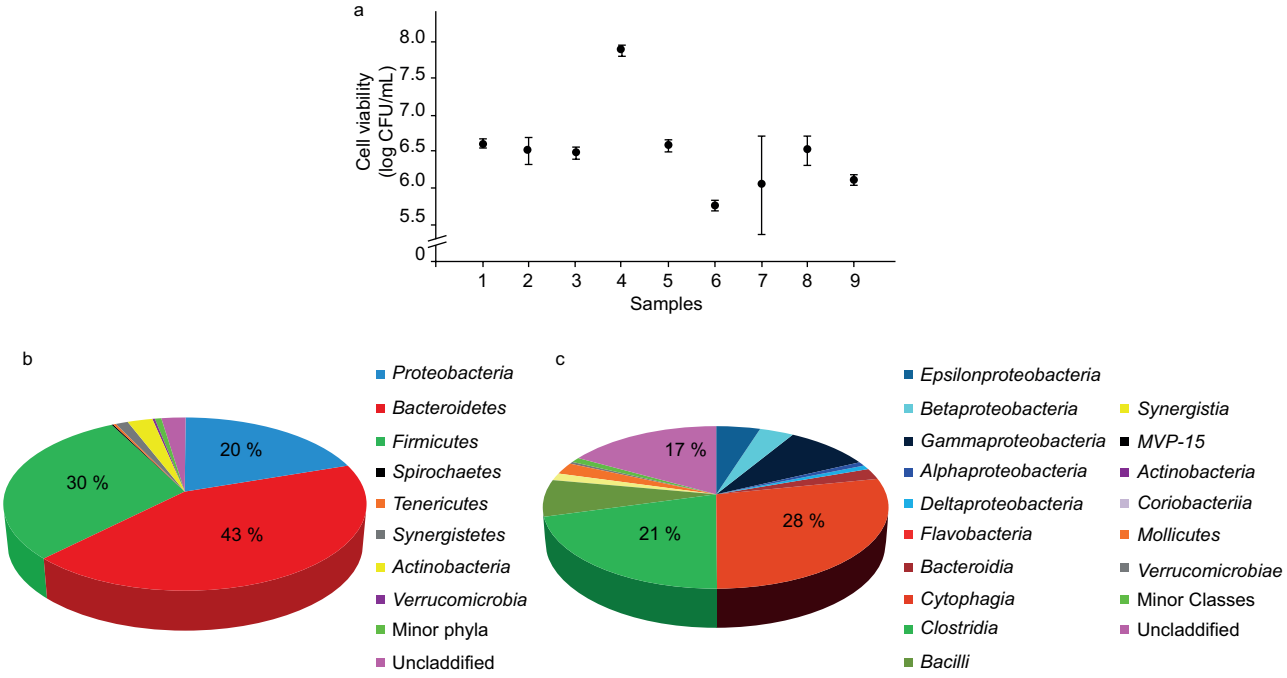

Fig. 3. (a) Total count of bacteria in the tannery effluent samples, (b) distribution pattern of bacterial phyla, and (c) classes obtained by a 16S rRNA gene amplicon sequencing study from sampling 9 (in duplicate). Bacterial taxa with relative abundances < 0.05 % were classified as minor taxa.

Analysis of the bacterial composition at phylum and class levels (**Fig. 3b, c** and **Tables SIII, SIV**) showed that Bacteroidia (28 %) and Clostridia (21 %) were the predominant classes within phyla Bacteroidetes and Firmicutes, respectively, in sampling 9. The Bacilli class, member of the phylum Firmicutes, has the second highest median detection frequency (7 %), after the class Clostridia. Within the third most abundant phylum (Proteobacterias, 20 %), the classes Gammaproteobacteria (9 %), Epsilonproteobacteria (5 %) and Betaproteobacteria (4 %) were homogeneously represented. Other phylum such as Synergistetes and Actinobacteria were also present in this tannery effluent sample, while relative abundance of sequences of phyla such as Spirochaetes, Tenericutes and Verrucomicrobia was no higher than 1 %. Bacterial profile at phylum level found in this work for tannery effluents is in good agreement with previous studies in relation to different industrial wastewaters' matrices and domestic effluents (Yang et al. 2011, Chen et al. 2012, Zhang et al. 2012, Giordano et al. 2016, Liang et al. 2016, Liang et al. 2017).

At lower taxonomic levels, the most represented genus within the Bacteroidia class was *Paludibacter* (6 %) (**Table SV**), which might play an important role in sulfate removal in wastewaters according to Liang et al. (2013). Other genera such as *Tissierella*, *Soehngenia*, *Proteocatella* and *Proteiniclasticum* within the Firmicutes class were also represented in the sample 9 (5-7 %). *Proteiniclasticum* and *Tissierella* have been reported as genera involved in the degradation of aromatic hydrocarbons (Dong et al. 2011).

Lysinibacillus and *Staphylococcus* within the Bacilli class and *Cloacibacterium* within the *Flavobacteria* class were also detected (1-2 %) in this work. *Lysinibacillus* spp. are bacterial pathogens, while *Cloacibacterium* are extremotolerant organisms and both have been also reported in this type of effluents (Chowdhary et al. 2017, Nascimento et al. 2018). Members of the Proteobacteria phylum, such as *Brachymonas*, *Campylobacter*, *Halomonas*, *Acinetobacter* and *Rickettsiella* showed similar sequence relative abundances (1-4 %) (**Table SV**). *Brachymonas* has been characterized by its high tolerance to toxic compounds such as sulfide and chromium, and also by its denitrifying capacity (Seyoum et al. 2004, Leta et al. 2005, Kim et al. 2014). Similarly, *Halomonas* and *Acinetobacter* are bacterial genera widely known by their capacity of removing high Cr(VI) and phenol concentrations (Bhattacharya and Gupta 2013, Paisio et al. 2013, Hora and Shetty 2014, Mabrouk et al. 2014, Ontañon et al. 2015, Albokari et al. 2018). On the other hand, sequences of pathogenic taxa were also frequently found in other tannery wastewaters. *Ricketsiella* and *Arcobacter* genera are pathogens of arthropods and humans, respectively (Chowdhary et al. 2017). In addition, *Campylobacter* is reported as ubiquitous in sewage and it was found that human and animal wastes from abattoirs and animal treatment plants are major sources (Stampi et al. 1999). In relation to other bacteria classified as pathogenic to humans, only two genera (*Escherichia* and *Klebsiella*) were found, and with a sequence relative abundance not higher than 0.0005 %.

In summary, the structure of the bacterial community within the effluent was very diverse and included several genera described as tolerant and removers of various pollutants and some pathogenic bacteria. In this sense, the fact that bacteria with the ability to remove different contaminants are present is advantageous in terms of the feasibility to obtain good results if secondary treatments are installed in the industrial effluent treatment plant. On the other hand, bacterial inactivation of the effluent should be considered, prior to its release into the environment, as an action to protect human health against pathogenic bacteria.

Distribution of the different samples according to different parameters

Principal component analysis (PCA) was used to visualize the dispersion patterns between the different samplings performed over one year, based on physical, chemical, and microbiological characteristics, operational parameters and average ambient temperature (**Fig. 4**).

Of the total observed variance, 77.39 % was explained by the first three components of the PCA. A group formed by the major number of samples (1, 2, 3, 6, 9) was characterized by higher values of COD, BOD_5, total nitrogen (N), total phosphate (P), and total phenols; all of them organic compounds feasible to degrade by microorganisms. The rest of the samples were separated from this group according to specific characteristics. In this sense, sampling 5, on the right side, showed a high pH value. Also, the processed leather type and the predominant process at this sampling time were goat leather and tanning, respectively. These operational characteristics were also present in samplings 7 and 8, located at the top of the PCA near sampling 5, and were also characterized by higher Cr(VI) concentrations and SS levels. Sampling 4, on the left side of the PCA, could be associated with its highest count of bacteria.

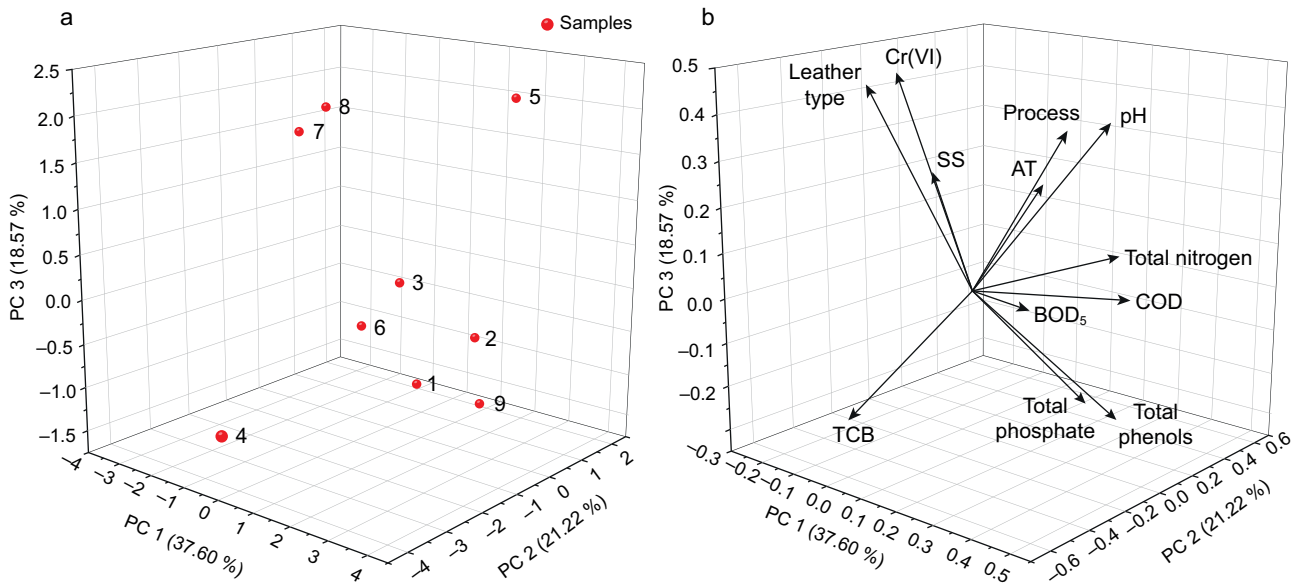

Fig. 4. Principal component analysis based on the physical, chemical, microbiological, and operational parameters (black lines) and the different tannery effluent samples (1-9, red dots). COD: chemical oxygen demand, BOD_5: biochemical oxygen demand, N: total nitrogen, P: total phosphate, SS: settleable solids, AT: average ambient temperature, TCB: total count of bacteria.

Similarly to our work, a positive correlation between chrome and solids was also observed by various researchers (Akhand and Rao 2013, Islam et al. 2014). This was expected since both are involved in the tanning process, either as a reagent or as a waste of the reactions.

Although occasionally certain samples showed a different dispersion pattern than usual, it is important to note that all samples were characterized by high nutrient concentrations, and this dispersion may be due to operational problems (for example a poor Cr(VI) recovery or the use of great concentrations of alkalinizing agents), as well as different industrial processes that were being carried out. Based on these results, the implementation of an adequate treatment to remove high organic loads from the effluents should be the main objective in an attempt to optimize the treatment of these wastes before their discharge.

Toxicity of the tannery effluents

Toxicity tests using standardized organisms that commonly inhabit water bodies impacted by the discharge of effluents, are useful indicators to estimate the effluents toxic effect on the receiving ecosystems (Charles et al. 2011).

In general, the different effluent samples showed toxicity to *L. sativa* seeds and *R. arenarum* embryos, since these effluents completely inhibited germination of the seeds (100 % inhibition) and promoted an immediate death of the amphibian embryos (0 % survival). These results could be directly correlated with the high values of most of the physical, chemical, and microbiological parameters described in this study. It could be stated that the direct discharge of the effluents into the environment would pose negative impacts on the development of plants and amphibian species and their survival. Cr and phenols are known to be toxic pollutants that can produce damage to plants and amphibians. In plants, these pollutants induced oxidative stress that causes alterations in the cell membranes permeability, seed germination inhibition, as well as degradation of photosynthetic pigments causing a reduction in growth and consequently in plant biomass (Panda and Choudhury, 2005, Michałowicz et al. 2009, Gami et al. 2014, Stambulska et al. 2018, Amin et al. 2019). In amphibians, these compounds have been found to produce various adverse effects ranging from acute toxicity (lethal and teratogenic effects) to sublethal and chronic effects on the endocrine system, neurotoxicity, liver and kidney damage and respiratory effects (Croteau et al. 2008, Paisio et al. 2009, Anku et al. 2016).

In addition, the oxidizable organic matter content of different effluents was responsible of the reduction in seeds germination and seedling growth and of the clogging of gills fishes within the receiving water bodies (Pandey et al. 2008, Doke et al. 2011, Narain

et al. 2012, Islam et al. 2014, Dashti et al. 2015).

Our results were in agreement with those described by other authors that reported high toxicity and mutagenicity of the non-treated tannery wastes for species from different trophic levels, such as aquatic ecosystems organisms, microorganisms and also on human cells when evaluated using bioassays (Alam et al. 2010, Masood and Malik 2013, Kumari et al. 2014, Nazir et al. 2019).

It is important to note that this work allowed integration between physical and chemical variables and toxicological characteristics of the samples, which are complementary analytical tools for monitoring the effluents quality. Therefore, reliable indices on the toxicity impact of the effluents released into aquatic environment were achieved.

CONCLUSIONS

This study revealed that the parameters of effluents released from a tannery industry located in the South of Córdoba province (Argentina) in nine samplings distributed during a 1-yr period showed variations that generally follow a same pattern, mainly related to a high nutrient organic load. Moreover, values obtained for several physical and chemical parameters exceeded the legislated ranges, thus implicating environmental risk (corroborated by its toxicity to *L. sativa* seeds and *R. arenarum* embryos), which allows us to emphasize the need to treat the effluents generated. In this regard, the effluent treatment plant of the tannery under study, that only includes primary treatment, would need adjustments, for example in the sedimentation/flocculation process to increase the removal of solids, a rigorous control of pH levels (and their neutralization) and Cr recovery. Important requirements in the case of implementing a secondary treatment are the high values of these parameters, which could could limit the biodegradability of effluents. The levels of phenols, COD and nutrients could also be reduced after its implementation, either by anaerobic (high levels) and/or by aerobic processes such as activated sludge systems, among the most frequent procedures. However, the subsequent chlorination method is another aspect that would require focus.

The implementation of an additional tertiary treatment such as the construction of a wetland could be an environmentally friendly strategy to increase the efficiency of removal in the case of persisting residual organic matter or other contaminants. In this way, the entry of pollutants into the environment could be reduced, and, therefore, their impact on the biota.

The processed leather types and predominant processes at the sampling time constituted factors affecting effluents' characteristics such as SS, Cr concentration, and pH, therefore the novelty of this study. In this sense, it would be interesting to take into consideration which industrial processes are being carried out for the operational adequacy of the effluent treatment plant in each case.

Finally, the occasional fluctuations detected in some parameters, either physical, chemical, or operational parameters represent a difficulty to implement the treatment of an effluent, because it will require facilities capable of facing fluctuations in the characteristics of the effluents without reducing the efficiency of the treatment. Therefore, more exhaustive studies would be essential to achieve the design of a treatment plant according to the generated effluents.

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SUPPLEMENTARY MATERIAL

TABLE SI. SEQUENCE READ DETAILS AND ALPHA DIVERSITY ANALYSIS OF THE SAMPLES BASED ON OPERATIONAL TAXONOMIC UNITS (OTUs).

Sample name	Number of sequences	OTUs	Good's coverage	Chao1 average	Heip average	Shannon-Weaver average
S9a	58 776	1020	0.99	285.555	0.141	3.213
S9b	380 832	4116	0.98	550.275	0.118	3.621

Richness was evaluated by Chao1 index, equitability was assessed by Heip index, while diversity was calculated by Shannon-Weaver index. For the analysis, a total number of 7347 sequences (average length of 253 bp) were used. Letters "a" and "b" indicate replica. OTUs were defined at 97 % sequence similarity.

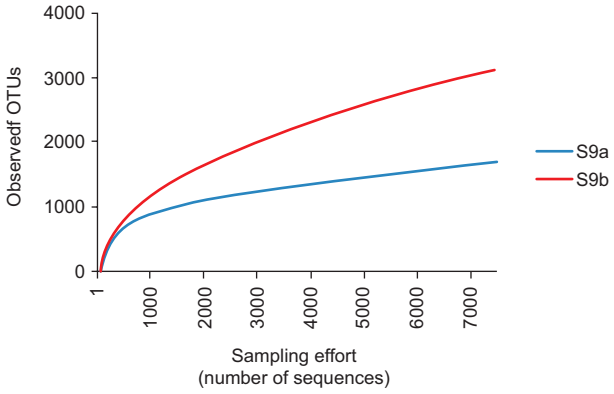

Fig. S1. Rarefaction curves for sequences clustered in operational taxonomic units (OTUs) defined at 97 % sequence similarity. Letters a and b indicate replica.

TABLE SII. PHYSICAL, CHEMICAL, AND MICROBIOLOGICAL CHARACTERISTICS OF THE TANNERY EFFLUENTS AND OPERATIONAL PARAMETERS REGISTERS.

Physical and chemical parameters/sampling	1	2	3	4	5	6	7	8	9	Guideline values*
pH	7.95 ± 0.15	8.54 ± 0.17	8.50 ± 0.09	7.26 ± 0.08	10.98 ± 0.10	7.88 ± 0.08	8.07 ± 0.15	7.94 ± 0.10	8.20 ± 0.01	6-9
COD (mg/L)	2492 ± 185	5328 ± 320	2590 ± 243	2215 ± 87	4976 ± 110	2468 ± 96	2527 ± 87	1364 ± 61	3589 ± 70	<250
BOD_5 (mg/L)	932 ± 77	3026 ± 103	742 ± 24	1436 ± 33	1721 ± 41	1339 ± 28	1604 ± 19	554 ± 12	878 ± 84	≤ 40
COD/BOD_5	2.67	1.76	3.49	1.54	2.89	1.84	1.57	2.46	4.08	
Total nitrogen (N; mg/L)	290 ± 25	664 ± 39	263 ± 18	217 ± 11	665 ± 56	93 ± 9	264 ± 22	118 ± 9	335 ± 18	≤ 10
Total phosphate (P; mg/L)	34.0 ± 1.7	37.2 ± 2.5	39.0 ± 3.0	33.0 ± 1.0	39.3 ± 3.5	22.6 ± 1.1	14.0 ± 0.8	33.7 ± 3.7	59.7 ± 14.1	≤ 0.5
COD:N:P	100:11.6:1.4	100:12.5:0.7	100:10.2:1.5	100:9.8:1.5	100:13.4:0.8	100:3.8:0.9	100:10.4:0.6	100:8.7:2.5	100:9.3:1.7	100:5:1(**)
COD/N	8.59	8.02	9.84	10.20	7.48	26.53	9.57	11.55	10.71	
COD/P	73.29	143.22	66.41	67.12	126.61	109.20	180.5	40.47	60.11	
Total phenols (mg/L)	25.80 ± 2.70	15.54 ± 0.90	8.12 ± 0.87	7.00 ± 0.05	19.52 ± 1.30	6.72 ± 0.14	0.21 ± 0.03	5.52 ± 0.15	25.89 ± 0.23	≤ 0.05
Cr(VI) (mg/L)	0.71 ± 0.05	0.48 ± 0.07	0.31 ± 0.02	0.07 ± 0.03	1.45 ± 0.40	0.14 ± 0.01	10.80 ± 1.05	14.04 ± 1.18	0.67 ± 0.01	≤ 0.1
Settleable solids (SS) (mL/L)120 min	1.6	1.6	13	1.2	0.8	1.3	3.0	13.0	0.5	≤ 1
Microbiological parameters										
Total count of bacteria (TCB) (CFU/mL)[1]	$4.0 \times 10^6 \pm 5.0 \times 10^5$	$3.3 \times 10^6 \pm 1.5 \times 10^6$	$3.0 \times 10^6 \pm 5.0 \times 10^5$	$7.5 \times 10^7 \pm 1.3 \times 10^7$	$3.8 \times 10^6 \pm 7.0 \times 10^5$	$5.8 \times 10^5 \pm 1.0 \times 10^5$	$1.1 \times 10^6 \pm 1.25 \times 10^6$	$3.3 \times 10^6 \pm 1.5 \times 10^6$	$1.3 \times 10^6 \pm 1.7 \times 10^5$	N.R.
Operational parameters										
Processed leather type	Cow-goat	Cow- goat	Cow- goat	Goat	Goat	Cow- goat	Goat	Goat	Cow- goat	
Processes carried out by the tannery	Pre-tanning-tanning	Pre-tanning-tanning	Pre-tanning-tanning	Pre-tanning-tanning	Tanning	Pre-tanning-tanning	Pre-tanning-tanning	Pre-tanning-tanning	Pre-tanning-tanning	
Average ambient temperature (AT) (°C)	19.5	19.0	21.0	7.0	17.5	21.5	19.5	25.5	22.5	N.R

Data correspond to mean values ± standard deviation (n = 2); numbers indicate samples taken at different time of the year.
[1]Total count of bacteria (TCB) was performed in TY agar (Beringer 1974); *guideline values established by the local legislation for the discharge of liquid effluents into surface water bodies (MINSPCBA 2016); **COD:N:P ratio in aerobic systems suggested by Ammary (2004) and Metcalf et al., (2014).
N.R.: non-regulated parameter.

TABLE SIII. RELATIVE ABUNDANCE (%) OF BACTERIAL PHYLA.

Phylum	Sample 9
Proteobacteria	20.01 ± 3.41
Bacteroidetes	42.72 ± 2.95
Firmicutes	30.05 ± 6.78
Spirochaetes	0.11 ± 0.07
Tenericutes	0.29 ± 0.28
Synergistetes	1.27 ± 1.20
Actinobacteria	2.58 ± 3.56
Verrucomicrobia	0.26 ± 2.52
Minor	0.64 ± 0.08
Unclassified	2.48 ± 0.51

Data correspond to mean values ± standard error (n = 2); bacterial phyla with relative abundance < 0.05 % were classified as minor taxa.

TABLE SIV. RELATIVE ABUNDANCE (%) OF BACTERIAL CLASSES.

Class	Sample 9
Epsilonproteobacteria	4.96 ± 0.26
Betaproteobacteria	3.81 ± 2.16
Gammaproteobacteria	9.41 ± 0.93
Alphaproteobacteria	0.89 ± 0.76
Deltaproteobacteria	0.90 ± 0.71
Flavobacteriia	1.95 ± 1.88
Bacteroidia	27.85 ± 10.35
Cytophagia	0.28 ± 0.28
Clostridia	20.63 ± 11.37
Bacilli	7.30 ± 6.15
Synergistia	1.27 ± 1.20
MVP-15	0.08 ± 0.04
Actinobacteria	2.36 ± 2.31
Coriobacteriia	0.21 ± 0.21
Mollicutes	0.26 ± 0.25
Verrucomicrobiae	0.21 ± 0.20
Minor	0.89 ± 0.02
Unclassified	17.09 ± 11.54

Data correspond to mean values ± standard error (n = 2). Bacterial classes with relative abundance < 0.05 % were classified as minor taxa.

TABLE SV. RELATIVE ABUNDANCE (%) OF BACTERIAL GENERA.

Genus/treatment	Sample 9
Sphingomonas	0.06 ± 0.05
Paracoccus	0.52 ± 0.52
Brachymonas	1.66 ± 0.47
*Comamonadaceae*_unclassified	0.91 ± 0.79
*Alcaligenaceae*_unclassified	0.15 ± 0.04
Alcaligenes	0.05 ± 0.01
Massilia	0.25 ± 0.25
Arcobacter	0.97 ± 0.27
Campylobacter	3.97 ± 0.00
Alishewanella	0.12 ± 0.12
Halomonas	2.24 ± 0.24
Marinobacterium	0.21 ± 0.04
Acinetobacter	4.10 ± 0.97
Pseudomonas	0.63 ± 0.54
Rickettsiella	1.40 ± 1.33
Desulfomicrobium	0.60 ± 0.41
Myroides	0.15 ± 0.08
Cloacibacterium	1.79 ± 1.80
Paludibacter	5.82 ± 4.14
Macellibacteroides	0.08 ± 0.03
*Porphyromonadaceae*_unclassified	0.22 ± 1.11
Prevotella	0.28 ± 0.28
Blvii28	0.14 ± 0.12
p-2534-18B5_unclassified	19.59 ± 15.36
*Bacteroidetes*_unclassified	12.59 ± 9.56
*Bacillaceae*_unclassified	0.59 ± 0.59
Lysinibacillus	1.80 ± 1.80
Staphylococcus	1.04 ± 1.04
*Bacillales*_unclassified	2.16 ± 2.14
Facklamia	0.58 ± 0.14
Atopostipes	0.30 ± 0.24
Tissierella Soehngenia	5.40 ± 2.68
Proteocatella	5.78 ± 4.36
Proteiniclasticum	7.31 ± 3.61
Syntrophomonas	0.27 ± 0.27
*Veillonellaceae*_unclassified	0.56 ± 0.56
*Firmicutes*_unclassified	2.00 ± 1.46
Other unclassified bacteria	2.49 ± 0.51
vadinCA02	0.73 ± 0.71
HA73	0.12 ± 0.12
Dethiosulfovibrio	0.40 ± 0.36
PL-11B10_unclassified	0.08 ± 0.04
*Microbacteriaceae*_unclassified	0.31 ± 0.31
Arthrobacter	0.40 ± 0.40
*Intrasporangiaceae*_unclassified	0.68 ± 0.68
Bifidobacterium	0.34 ± 0.33
Minor	6.99 ± 0.44

Data correspond to mean values ± standard error (n = 2); bacterial genera with relative abundance < 0.05 % were classified as "minor" taxa.