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Unhairing effluents treated by an activated sludge system

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Abstract

Leather tannery effluents are a source of severe environmental impacts. In particular, the unhairing stage, belonging to beamhouse processes, generates a significantly toxic, alkaline wastewater with high concentrations of organic matter, sulphides, suspended solids and salts. The objective of this work was to evaluate the biodegradability and toxicity of diluted unhairing wastewater after being treated by an activated sludge (AS) system. The biomass activity of the AS was also evaluated.

The AS system was fed for 180 days with diluted unhairing effluent. The operation strategy increased the organic load rate (OLR) from 0.23 to 2.98 g COD/l per day while the HRT was variable until operation day 113, when the HRT was near 1.1 days. Results show that when the organic load rate was lower than 2 g COD/l per day, the biological oxygen demand (BOD_5) efficiency was 99%, whereas the chemical oxygen demand (COD) was around 80%. The reactor operation was stable until 2 g COD/l per day. For higher values, the system was less efficient (COD and BOD_5 removal rate lower than 40%) and the relation of food/micro-organisms (F/M) was higher than 0.15.

Biomass evaluations through oxygen utilisation coefficients show that the specific oxygen uptake rate (SOUR) decreased from 1.11 to 0.083 g O_2/g MLVSS per day, in the same way the endogenous oxygen coefficient decreased from 0.77 to 0.058 per day. The reduction of biomass activity (measured as oxygen respiration) could be attributable to the inorganic compound content (ammonia and chloride) in the unhairing effluent. Also, the bioassays with *Daphnia magna* and *Daphnia pulex* showed that with these compounds, only between 24 and 31% of the toxicity of the aerobic-treated effluent can be removed. On the other hand, ultrafiltration (UF) analysis indicated that a COD fraction is recalcitrant to the aerobic treatment, principally those above 10,000 Da (around 55% of total unhairing influent COD).

Keywords: Unhairing effluents; Biological treatment; Ultrafiltration; Toxicity; Daphnidios

1. Introduction

Leather tanning generates many complex and high-loaded effluents that require treatment before being discharged into receiving waters [1]. The main characteristics of tannery effluents are high organic loading, high ammonia and organic nitrogen content and the presence of specific inorganic compounds (sulphide, chromium, sodium chloride, etc.) [2]. In particular, the unhairing stage associated with beamhouse processes generates a highly toxic, alkaline wastewater containing high concentrations of proteins, sulphide, suspended solids and salts (e.g. sodium chlorine) [3,4].

The application of biological processes to industrial effluents remains complicated principally due to the presence of inhibiting and/or bio-recalcitrant compounds [5]. In fact, it is only after a physical–chemical treatment that the activated sludge (AS) system has been used efficiently to remove organic matter and nitrogen [6,7]. Table 1 summarises data from aerobic treatment systems for tannery wastewater obtained by other authors.

The data show that chemical oxygen demand (COD) removal efficiencies vary from 12 to 87%, depending principally on the biodegradability of the particular effluent. Biological oxygen demand (BOD_5) removal efficiencies (67–98%) indicate that aerobic treatment (especially AS) is particularly useful for the elimination of readily biodegrad-

Abbreviations: AS, activated sludge; b', endogenous oxygen coefficient; BOD_5, biological oxygen demand; COD, chemical oxygen demand; D, dark; F, food; L, light; HRT, hydraulic retention time; LC_{50}, mean lethal concentration; M, micro-organism; MLVSS, mixed liquor volatile suspended solid; MW, molecular weight; MWCO, molecular weight cut-off; OLR, organic loading rate; OUR, oxygen uptake rate; SBR, sequencing batch reactor; SOUR, specific oxygen uptake rate; SVI, sludge volume index; T, temperature; TKN, total Kjeldahl nitrogen; TS, total solids; TSS, total suspended solids; UF, ultrafiltration; VS, volatile solids; VSS, volatile suspended solids

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Table 1
Comparison of various aerobic treatment process for different tannery wastewaters

Reactor[a]	Wastewater characteristics				Control parameters			Removal			Reference
	Type	BOD_5 (mg/l)	COD (mg/l)	TKN (mg/l)	HRT (days)	Sludge age (days)	MLVSS (g/l)	SVI (ml/g)	COD (%)	BOD_5 (%)	
SBR	Total	n.d.[b]	300–1400	50–200	6/cycle	–	2.5 3.9	–	12	n.d.	[5]
AS	Unhairing	7081	24089	18	18	4–10	1.1–2.5	–	n.d.	67	[2]
AS	Total	855–965	1412–1454	311–369	10–38	–		–	79–81	97–98	[7]
AS	Total	510	880–1213	65.8	12	–	2.9–3.8	80–100	72–87	n.d.	[8]
AS	Total	n.d.	1000	n.d.	8–48	–	1.0–3.6	–	80.5	n.d.	[9]

[a] Reactors: SBR, sequencing batch reactor; AS, activated sludge.
[b] n.d.: not determined.

able organic matter. Other studies on AS systems have indicated that the pre-treatment step can affect the COD removal efficiency [10]. Indeed, control of the specific bacteria in the AS process can improve the organic material removal, such as shown in the study of Ahn et al. [8], which demonstrated that the *Zoogloea ramigera* and its extracellular polymer can improve COD removal up to 15%.

However, compounds with high molecular weight, like proteins, can be recalcitrant in the aerobic process. Indeed, during the anoxic/aerobic treatment, high effluent (refractory) COD was often present at the end of the process. In this case, organic substrate removal occurred mainly during the anoxic period [5].

The objective of this work was to evaluate biodegradability and toxicity of the diluted unhairing wastewater after being treated by an activated sludge (AS) system. The biomass activity of the AS was also evaluated.

2. Materials and methods

2.1. Raw wastewater

Effluent samples (three samplings over a 1-year period) were obtained from a local tannery's beamhouse unhairing process effluent. Similar types of animal hides and reactive had been used in all the processes. Effluents had been previously treated by a physical system (filtration) to remove hair, pieces of skin, and fats. Samples were transported on ice in insulated coolers to the laboratory and stored in the dark at $4 \pm 1\,^{\circ}C$. This raw wastewater was diluted between 5 and 13 times, depending on the organic loading rate (OLR), to be later fed into the AS system.

2.2. Inoculum

The AS reactor was inoculated with sludge from an aerobic reactor that treated Kraft mill effluent.

2.3. Activated sludge system

The AS system, including an aerobic reactor (1.8 l) and a settling unit (0.6 l), was operated continuously for 180 days (Fig. 1). The sludge was periodically recycled and the excess was withdrawn from the settling unit to obtain a 30-day aged sludge. Oxygen concentration inside the reactor was above 6 mg/l due to a pump blowing air through a diffuser system that also provided mixing.

The AS operation consisted in two phases. In the start-up of the AS system (phase I), a solution of glucose (10 g/l) and nutrient (1 g NH_4Cl/l and 0.2 g H_2KPO_4/l) with a relation C:N:P of 100:5:1 was fed to increase the biomass to 3 g TS/l. During phase II (treatment), the AS reactor was fed with unhairing process wastewater without added nutrients via a peristaltic pump. During phase II, the organic load rate and hydraulic retention time (HRT) varied from 0.23 to 6.34 g COD/l per day and from 16.44 to 1.10 days, respectively.

2.4. Bioassays

Influent and effluent acute toxicity were determined by exposing *Daphnia pulex* and *Daphnia magna* juveniles (<24 h) during 24–48 h. Mortality was recorded at the end of exposure, where mortality was defined as a lack of organism mobility when the vessel was shaken. Toxicity tests were performed within 48 h after the sample obtainment. Organisms were obtained from in-house cultures that were fed three times weekly with a suspension of baker's yeast,

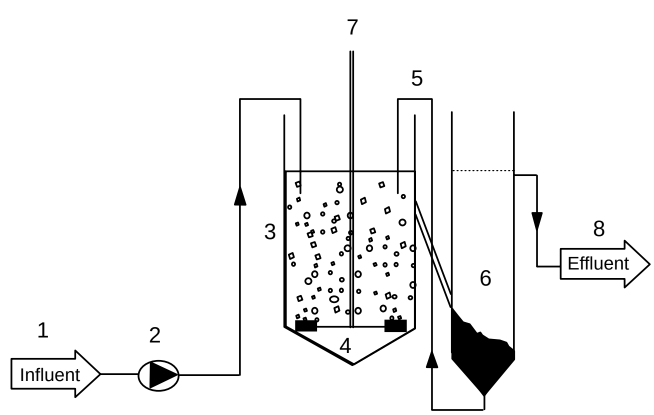

Fig. 1. A schematic presentation of the AS system: (1) influent; (2) pump; (3) aerobic reactor; (4) biomass; (5) biomass recirculation; (6) settler; (7) aerator; (8) effluent.

trout chow and alfalfa with an equivalent carbon content of 7.2 mg C/l on Monday and Wednesday, and 10.8 mg C/l on Friday. The culture medium was changed before feeding and neonates were removed within 24 h [11]. The solutions were not renewed and the organisms were not fed during the experiments. Oxygen concentration, pH and conductivity were measured at the beginning and end of each test. Cultures and animal exposures were conducted at $20 \pm 2\,^{\circ}C$ and were maintained on a photoperiod 16 h L:8 h D, and were characterised by a hardness of 125–250 mg/l as $CaCO_3$ for *D. pulex* and *D. magna*, respectively, and a pH ranging between 7.52 and 8.60 at 20 °C.

Four replicates, each containing five organisms, were assigned to the treatments and control. Borosilicate cups (30 ml) filled to 25 ml served as test chambers. The 24 and 48 h mean lethal concentration (LC_{50}) were calculated using the Probit and the Spearman–Karber methods as appropriate [12].

2.5. Oxygen uptake rate determination

Oxygen uptake rate (OUR) was measured using a closed respirometric unit of a BOD_5 bottle in which the dissolved oxygen concentration was measured using a WTW Oxycal 323B oxygen electrode connected with a Corning 450 pH-meter. Appropriate quantities of sludge were placed into the bottle. The electrode was dipped into the sample, allowing the displaced liquid to overflow preventing the air bubble accumulation inside the bottle. The sample was continuously stirred during measuring, and dissolved oxygen values were obtained every 30 s during 2 h. The OUR was determined by lineal regression from the slope obtained from the plotting of dissolved oxygen concentration versus time. Specific oxygen uptake rate (SOUR) and the endogenous oxygen coefficient (b') were determined according to the Eckenfelder and Musterman procedure [13].

2.6. Analytical methods

The measurement of COD, BOD_5, colour, total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), nitrate ($N\text{-}NO_3^-$), nitrite ($N\text{-}NO_2^-$), sulphide (S^{2-}), sulphate (SO_4^{2-}), chloride (Cl^-), total Kjeldahl nitrogen (TKN) and ammonia ($N\text{-}NH_3$) were performed according to Standard Methods [14]. Samples for COD, BOD_5, colour, $N\text{-}NO_3^-$, $N\text{-}NO_2^-$, TKN, S^{2-}, SO_4^{2-}, Cl^- and $N\text{-}NH_3$ were previously membrane filtered (0.45 μm).

Sludge volume index (SVI) was measured as the volume occupied (ml) by 1 g of mixed liquor suspended solids (MLVSS), dry weight, after settling for 30 min in a 1000 ml graduated cylinder.

Ultrafiltration was performed according to Vidal et al. [15] at 20 °C using a 450 ml stirred cell (Micro-Prodicon Model UHP 75) with an exposed membrane surface area of $38.5\,cm^2$. Two cellulose membranes were used of which the nominal molecular weight cut-off (MWCO) was 10,000 and 1000 Da. Nitrogen was applied over the liquid in the stirred cell. Prior to ultrafiltration, the pH of the wastewater was adjusted to 8.0 and the sample was then filtered through a 0.2 μm membrane filter in order to fractionate the lignin. Subsequently, 80 ml of the wastewater sample was filtered through a 10,000 Da MW membrane to provide approximately 75 ml of permeate and 3 ml of retentate.

3. Results and discussion

3.1. Raw wastewater characterisation

Table 2 presents the mean values and the physicochemical parameter ranges for the unhairing effluent. For all the samples, high COD and BOD_5 were found with mean values of 34.9 and 8.8 g/l, respectively. The relationship between COD/BOD_5 is 3.97, that shows the effluent biodegradation. Additionally, unhairing effluent samples presented a high contents of TKN (2040–4275 mg/l), chloride (18.4–27.1 g Cl^-/l) and sulphide (1.05–1.3 g S^{2-}/l).

3.2. Activated sludge operation

After start-up of the aerobic system (phase I), AS was fed with raw wastewater at a low organic load rate (0.23 g COD/l per day) that was progressively increased up to 6.34 g COD/l per day. Fig. 2 and Table 3 illustrate the operation and performance of the AS reactor. Results show that the removal efficiency for both BOD_5 and COD decreased as the OLR increased. In the best case (below 128 days of operation), BOD_5 and COD removal in the AS was above 99 and 60%, respectively. The effluent pH in the AS remained stable near 8.0 (see Fig. 2b). Table 3 shows the food/micro-organism (F/M) relationships, sludge volume in-

Table 2
Raw unhairing effluents characteristics

Parameter	Abbreviation	Unit	Average	Range
pH	pH		12.34	12.31–12.38
Chemical oxygen demand	COD	mg/l	34900	29600–40600
Biological oxygen demand	BOD_5	mg/l	8794.5	7005–10584
Colour		UPtCo	26095	21540–30650
Total solids	TS	mg/l	45540	39230–52670
Volatile solids	VS	mg/l	21377	15520–32510
Total Kjeldahl nitrogen	TKN	mg/l	3085	2040–4275
Ammonia nitrogen	$N\text{-}NH_3$	mg/l	220	189–275
N-nitrite	$N\text{-}NO_2^-$	mg/l	0.25	0.14–0.50
N-nitrate	$N\text{-}NO_3^-$	mg/l	0.66	0.2–1.6
Chloride	Cl^-	mg/l	21867	18400–27100
Sulphide	S^{2-}	mg/l	1149	1048–1284
Sulphate	SO_4^{2-}	mg/l	247	100–330

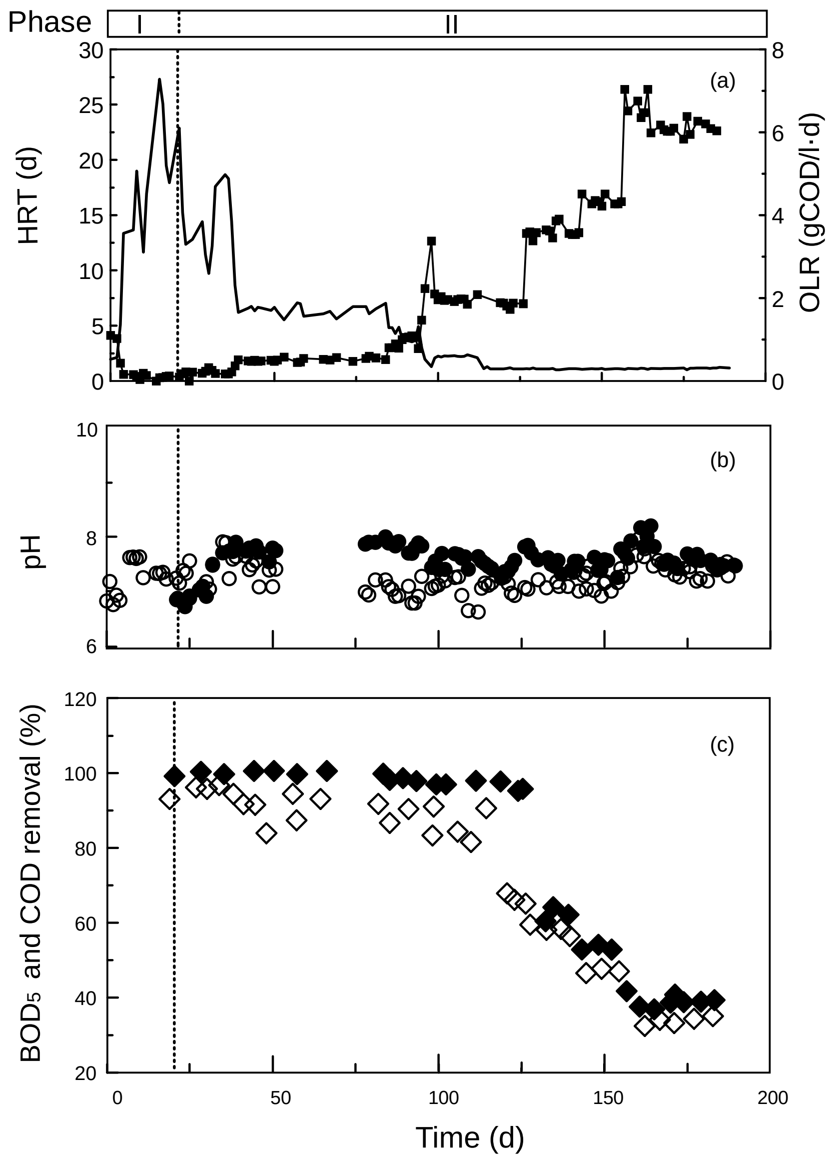

Fig. 2. Performance of AS reactor. (a) OLR (■) and HRT (—) values. (b) pH of the influent (○) and effluent (●). (c) Percentage of COD (◇) and BOD_5 (◆) removal.

dex, and the BOD_5/COD ratio for the influent and effluent values. AS reactor operation was very stable, the SVI range varied between 14.5 and 94.5 ml/g MLVSS, and no bulking phenomena were observed. The BOD_5/COD influent relationship was always higher than 0.37 indicating the possibility of biological degradation. However, residual organic matter content in the effluent was not removed by biological treatment as indicated by the AS operation optimum period (BOD_5/COD < 0.06, OLR < 2 g COD/l per day). Due to this, the recalcitrant COD, ranging between 0.1 and 1.3 g COD/l, might only be removed by a tertiary treatment [16] or combined oxidative–biological treatment [17]. In previous works, Jochimsen et al. [6] showed high values for COD removal from tan-yard wastewater (up to 95%) in a combined ozone and biological system. Additionally, Vidal et al. [16] concluded that a photocatalysis process after the biological treatment can improve the COD unhairing effluents up to 60%.

As shown in Fig. 3 and Table 3, the F/M ratio varied from 0.08 to 0.39 g BOD_5/g MLVSS per day during the overall reactor operation. It can be observed that the greatest BOD_5 removal efficiency (99%) was obtained when the F/M ratio varied between 0.08 and 0.15 g BOD_5/g MLVSS per day. According to the literature for activated sludge systems with conventional aeration, the optimal F/M ratio for tannery effluent treatment is 0.1 g BOD_5/g MLVSS per day with an HRT of 2.5 days [13]. Both values were determined as optimal in the present study. The F/M ratio is frequently used for treatment plant control, and it is related to SVI and sludge settling characteristics.

Table 3
Average removal parameters in the AS reactors during the operation

Period	OLR (g COD/l per day)		HRT (days)		F/M		SVI (ml/g MLVSS)	BOD_5/COD	
	Average	Range	Average	Range	(g BOD_5/g MLVSS per day)	(g COD/g MLVSS per day)		Influent	Effluent
Phase I									
0–21	0.11	0.09–0.13	16.80	13.36–19.47	–	–	–	0.6	0.01
Phase II									
22–37	0.23	0.21–0.26	16.44	14.41–18.67	0.08	0.14	94.5	0.63	0.06
38–84	0.51	0.48–0.56	6.55	6.20–6.75	0.09	0.16	72.0	0.57	0.04
85–95	0.93	0.78–1.09	4.21	3.54–4.93	0.07	0.19	67.1	0.43	0.04
96–112	2.00	1.94–2.09	2.18	1.98–2.27	0.15	0.35	34.5	0.43	0.06
113–128	1.88	1.86–1.89	1.11	1.10–1.14	0.15	0.43	14.5	0.52	0.03
129–143	3.63	3.53–3.90	1.10	1.05–1.17	0.19	1.0	–	0.37	0.29
144–156	4.34	4.22–4.50	1.10	1.05–1.13	0.39	1.09	24.7	0.39	0.32
157–178	6.34	5.95–7.09	1.10	1.03–1.18	0.35	0.9	76.1	0.39	0.39
179–189	2.98	2.84–3.06	1.18	1.16–1.19	0.31	0.7	16.9	0.39	0.48

F: food, M: micro-organisms, SVI: sludge volume index.

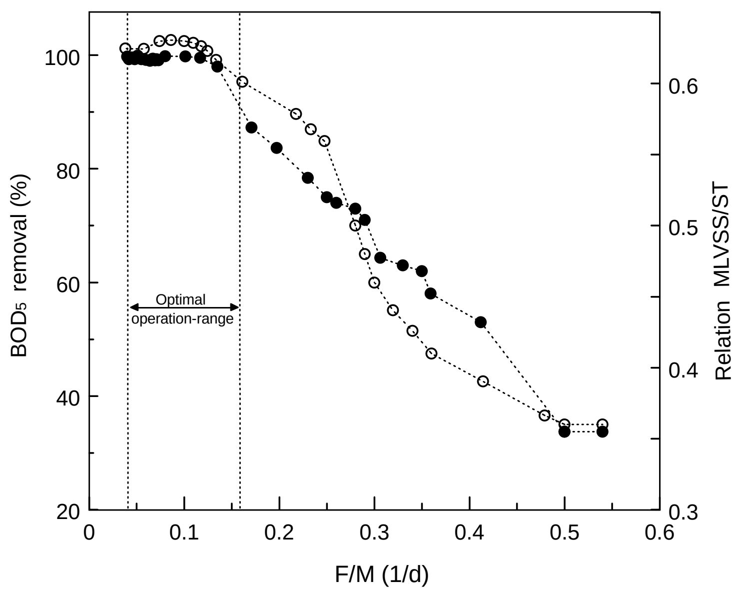

Fig. 3. Relationship of BOD_5 removal (●) and MLVSS/ST (○) vs. F/M ratio during activates sludge treatment.

At low F/M ratios, the amount of food present in the system is insufficient to maintain micro-organism growth, and they are driven to endogenous respiration. The residue left from endogenous metabolism consists mainly of cell capsules that are very light, generating sludge with poor settling characteristics. On the other hand, at high F/M ratios, filamentous micro-organisms predominate. This type of growth does not settle well either and remains almost indefinitely in suspension. Sludge under these conditions is referred to as a bulking sludge [18].

Table 4 shows the oxygen utilisation coefficients for the biomass of the AS system from operation day 113 to 189. It can be seen that the biomass activity, measured as the oxygen uptake rate (OUR), seems to increase from 0.456 g O_2/l per day (OLR 1.88 g COD/l per day) to 0.648 g O_2/l per day (OLR 2.98 g COD/l per day). However, the real oxygen utilisation per biomass unit in this experiment is measured by SOUR. The SOUR values for the last 79 days of AS operations decreased, from 1.11 to 0.083 g O_2/g MLVSS per day.

Table 4
Behaviour of the oxygen utilisation coefficients for the biomass of the SA system

SVI (ml/g MLVSS)	OUR (g O_2/l per day)	SOUR (g O_2/g MLVSS per day)	b' (day^{-1})
14.5	0.456	1.110	0.770
24.7	0.576	0.234	0.165
76.1	0.646	0.133	0.094
16.9	0.648	0.083	0.058

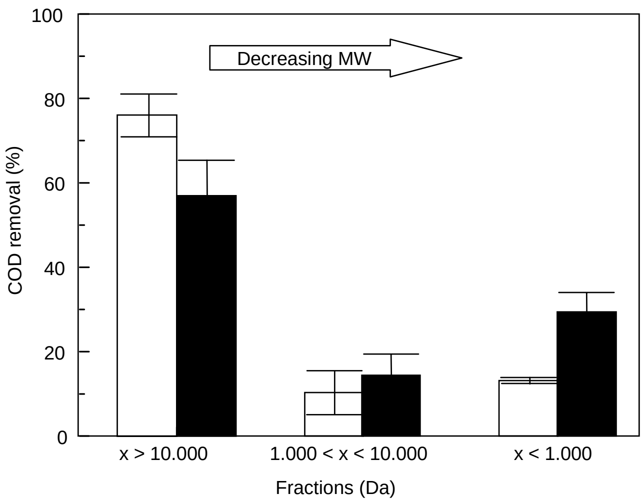

Fig. 4. Ultrafiltration of the influent (☐) and aerobic effluent (■).

On the other hand, Eckenfelder and Musterman [13] found for an AS plant treating organic chemical wastewater SOUR values from 0.07 to 0.42 g O_2/g MLVSS per day and an endogenous oxygen coefficient (b') is in the range between 0.18 and 0.22 per day. In our study, the b' coefficient ranged between 0.77 and 0.058 per day. The different oxygen utilisation coefficients show that the biomass activity in the system decreases, probably due to the presence of toxic compounds in the unhairing effluent. Indeed, studies on Daphnidios explain the presence of the specific inorganic compounds (like ammonia and chloride) content in the unhairing effluent [4].

In order to study the biodegradation of the different fractions of the unhairing effluent, UF analyses were done. Fig. 4 shows the UF of raw unhairing wastewater and aerobic-treated effluents. Since membrane filters of 10,000 and 1000 Da were used, raw wastewater and aerobic effluent were divided into three fractions. The COD concentration of each fraction was determined and compared. The COD content of the fraction up to 10,000 Da of the treated effluent was reduced in comparison with the corresponding fraction of the raw wastewater. The CODs of the 1000 Da $<$ MW $<$ 10,000 Da and the MW $<$ 1000 Da fractions of the treated effluent increased in 3 and 14%, respectively. However, the UF analysis indicated that aerobic treatment does not have the capacity to significantly biodegrade compounds with a molecular weight greater than 10,000 Da, when the majority are proteins. These types of compounds do not penetrate into the cell walls; rather, extracellular enzymes hydrolyse the large molecules into smaller ones. For this reason, no toxicant contribution can be considered, as explained by Klinkow et al. [19].

Bioassays with *D. magna* and *D. pulex* indicate that aerobic treatment of the unhairing effluent can only partially remove the toxic compounds (toxicity reduction range between 24 and 31%). The effluent toxicity reduction is partially due to the organic matter oxidation contained in the influent. These was explained by Cooman et al. [4]. As shown in Fig. 5, both *D. magna* and *D. pulex* responded in the same manner: the 24 h LC_{50} values of the aerobic effluent for both species ranged between 60 and 73%, and similar results were obtained after a 48 h exposure (60–75%). Previous studies performed by Cooman et al. [4] with the effluent of this study in our laboratory found, using an specific evaluation test (toxicity identification evaluation), that inorganic compounds like ammonia (29.8 mg/l) and chloride (1.9 g/l) caused the remaining toxicity in the effluent treated by activated sludge. Similar results were reported by van Sprang and Janssen [20] who indicated in their studies that ammonia can be responsible for toxicity at pHs higher than 8.0. In the same way, Kaiser and Palabrica [21] found that the 2 mg/l of free ammonia could reduce the 50% of the *Photobacterium phosphoreum* activity.

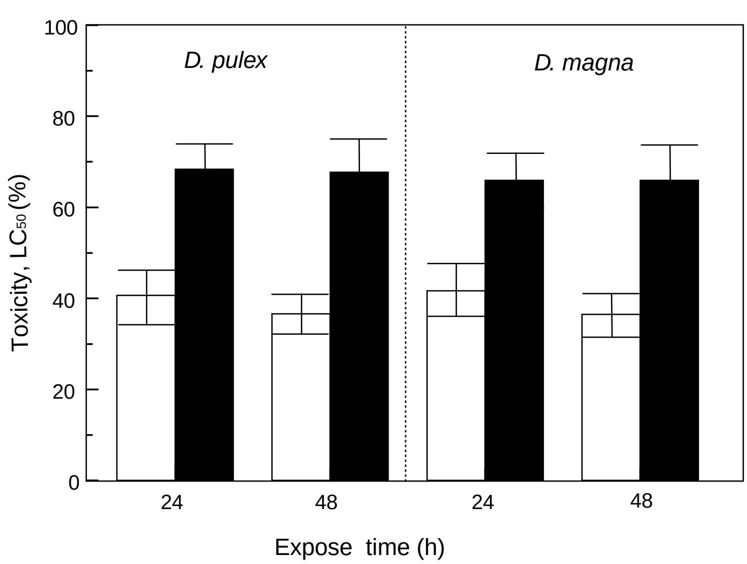

Fig. 5. Mean lethal concentration (LC_{50}) at 24 h and 48 h of the influent (☐) and aerobic effluent (■) against *D. pulex* and *D. magna*.

On the other hand, Klinkow et al. [19] found that the toxicity by *Vibrio fischeri* decreased with the type of effluent treatment. Consequently, tannery effluent after an aerobic treatment can decrease 40% of the toxicity in the high MW fraction, whereas tannery effluents after anaerobic–aerobic treatment can be non-toxic to *V. fischeri*. The amount of sulphide (0.01–0.21 g S^{2-}/l) in the aerobic-treated unhairing effluent was far below the 48 h LC_{50} value of 6.5 mg/l for *D. magna* found by Tišler and Zagorc-Konçan [22].

4. Conclusions

AS systems have the ability to remove more than 99% of the BOD_5 content in an diluted unhairing effluents at low OLR (<2 g/l per day and the F/M between 0.08 and 0.15). Whereas the COD removal was around 80%, the HRT for these conditions was near 1 day.

Biomass evaluations through oxygen utilisation coefficients show that the specific oxygen uptake rate decreased from 1.11 to 0.083 g O_2/g MLVSS per day, while the endogenous oxygen coefficient decreased from 0.77 to 0.058 per day. The reduction of biomass activity could be attributable to the inorganic compound content (ammonia and chloride) in the unhairing effluent, however, it does not affect the process efficiency.

Bioassays with *D. magna* and *D. pulex* showed that the toxicity of the aerobic-treated unhairing effluent could only be partially removed (toxicity reduction range between 24 and 31%). The remaining toxicity to both Daphnids is probably due to ammonia and chloride.

On the other hand, a COD fraction is recalcitrant to the aerobic treatment, where the principal recalcitrant fractions are those over 10,000 Da. The UF analysis indicates that fractions of unhairing effluent below 10,000 Da are partially degraded by AS.

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