LIFE PROJECT – ECODEFATTING
LIFE13 ENV/IT/00470

"Environmentally friendly natural products instead of chemical products in the degreasing phase of the tanning cycle"

DELIVERABLE - ACTION C.3
Report on environmental monitoring of semi-industrial defatting phase with natural products

1. Table of contents.

2. List of abbreviations, acronyms and symbols

°C	**C**elsius degree
pH	**hydrogenionic concentration**
µg/L	**micro g**ram **per l**itre
µm	**micro m**eter
µS	**micro S**iemens
Al	**al**uminum
As	**ar**senic
B	**b**oron
Ba	**ba**rium
Be	**be**ryllium
BOD	**b**iological **o**xygen **d**emand
$Ca(NO_3)_2$	**ca**lcium **nitrate**
Cd	**ca**dmium
centi-	$\mathbf{10^{-2}}$
cm	**c**enti**m**etre(s)
Co	**co**balt
COD	**c**hemical **o**xygen **d**emand
Cr	**ch**romium
Cu	***cypri*****um** (copper)
$CuSO_4$	**copper sulphate**
CVAA	**c**old **v**apor **a**tomic **a**bsorption
DMA	**d**irect **m**ercury **a**nalyzer
EN	**E**uropea**n** Standards
F.	***F****unalia*
Fe	***fe****rrum* (iron)
$FeSO_4$	**iron(II) sulphate**
g/l	**g**ram **per l**itre
Hg	***h****ydrar****g****yrum* (mercury)
HCl	**h**ydro**ch**loric **acid**
ICP-OES	**I**nductively **C**oupled **P**lasma **O**ptical **E**mission **S**pectroscopy
ICP-OES	**I**nductively **C**oupled **P**lasma **O**ptical **E**mission **S**pectroscopy
ICP-MS	**I**nductively **C**oupled **P**lasma **M**ass **S**pectroscopy
ISO	**I**nternational **O**rganization for **S**tandardization
K	**K**elvin (absolute temperature)
KCl	**potassium ch**l**oride**
KH_2PO_4	**potassium dihydrogenphosphate**
L (l)	**l**itre(s)
l/min.	**l**itre **per min**ute
mg	**mi**lli**g**ram(s)
mg/L(l)	**mi**lligram(s) **per l**itre
$MgSO_4 \cdot 7H_2O$	**mag**nesium **sulphate heptahydrate**
micro-	$\mathbf{10^{-6}}$
milli-	$\mathbf{10^{-3}}$
min.	**min**utes
mm	**millim**etre(s)
Mn	**man**ganese
$MnSO_4$	**man**ganese **sulphate**
$NaNO_3$	**sodium nitrate**
ms	**millis**econd(s)
Ni	**ni**ckel
nm	**nanom**eter(s)
O_2	**oxygen**
Pb	***plumbum*** (lead)
ppb	**p**arts **p**er **b**illion
ppm	**p**arts **p**er **m**illion
Sb	***stibium*** (antimony)
Se	**se**lenium
Tl	**th**a**l**lium
V	**V**olt
Zn	**zi**n**c**

3. Introduction

This report presents the results of the environmental impact of defatting wastewaters, coming from the demonstration action B.2 at semi-industrial level (**Figure. 3.1**).

Figure 3.1 Defatting operations at semi-industrial level

Samples of sheep, pig and cow skins were subjected to defatting, following the same experimental procedure described in the deliverable report of action B.1: but, lowering the skin/water ratio by weight from 1000 to 100 factor, since the scale of defatting in action B.2 was approximately 200 times higher. The defatting product used in action B.2 was EDF-20, which was selected from the laboratory demonstration B.1, according to the evaluations reported in the deliverable of the same action. EDF-20 was used in different percentages, leading to different effluents that were characterized in terms of their biodegradability and metal content. The data confirmed the observations made in the deliverable report of action C.2. One of the defatting effluents coming from action B.2 was subjected to a fungi based technology for the treatment of wastewaters downstream. The choice of fungi microorganisms in this monitoring action was based on the assumption, to integrate the data obtained at laboratory level described in the deliverable report C.1. The results would be indicative for the subsequent application of a more complex consortium of microorganisms such as activated sludge (typically used in water treatment plants) that will be consistent with the progression of the defatting job from the semi- to the pre-industrial level, which is representative of a typical production scale of a tannery.

4. Physico-chemical characterization of defatting effluents from action B.2

The defatting effluents were characterized by the physical-chemical properties and biodegradability reported in **Table 4.1**.

Table 4.1 Wastewaters parameters

Parameter	Method	
pH		
Conductivity at 25 °C (µS/cm)	UNE-EN 27888:1994	(ISO 7888:1985)
BOD (mg O_2/l)	UNE 77004:2002	(ISO 6060:1986)
COD (mg O_2/l)	UNE-EN 1899-1:1998	(ISO 5815:1983)
Biodegradability (BOD/COD)	BOD / COD	

The CODs and BODs were expressed in mg O_2/l, the conductivity in µS, whereas the biodegradability was a pure number as the result of the ratio between figures with the same unit of

measure. The COD and BOD parameters were determined, using the same equipment shown in the deliverable report for action C.1 and C.2 (**Figure 4.1**).

Figure 4.1 a) COD; b) BOD equipment

a)  b) 

The conductivity, COD and BOD values of the wastewaters were similar to those obtained at laboratory scale, despite the higher amount of fat in the effluents due to the greater effectiveness of the degreasing process on a bigger scale (**Table 4.2**).

Table 4.2 Characterization of effluents from action B.2

Skin	EDF20 (%)	Defatting (%)	pH	Conductivity (µS/cm)	COD (mg/l)	BOD (mg/l)	BOD/COD
Sheep	2	42,1	6,96	89,300	6,400	1,480	0,23
	3	44,7	6,86	118,000	9,100	1,280	0,14
	4	52,6	6,95	110,800	9,900	1,880	0,19
	5	57,9	6,5	129,800	9,800	1,800	0,18
Pig	2	52,7	7,1	123,400	8,900	1,760	0,20
	4	58,0	6,9	138,100	9,200	1,830	0,20
Cow	2	53,6	6,8	86,000	5,400	1,620	0,30
	4	60,7	7	91,000	5,850	1,450	0,25

The direct correlation between conductivity and the amount of defatting agent was consistent to the trend of the defatting percentage. The biodegradability, reported as the theoretical ratio between BOD and COD, was consistently across the low limit of the slow biodegradability window: but as mentioned in the deliverable report of action C.2, the effluents are usually mixed up inside the tannery and they are diluted at least by a factor of 5, which decreases the COD and thus tends to increase the estimated biodegradability. Therefore, the biodegradability at this level is an estimate to understand the real capacity of active sludge to specialize over time, when interacting with the contaminants of tanneries' wastewaters.

5. Determination of metal content in defatting effluents from action B.2

As mentioned in the deliverable report C.1 and C.2, the monitoring of metals is fundamental to check the quality of defatting products (*i.e.*, the final leather or even leather goods) and control any potential contamination within the processing sequence of raw hides to leather. The analysis of Al, As, B, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Be, Ba, Sb, Tl, Pb, Se and Cd metal was carried out in semi

quantitative fashion, using the Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) and Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (**Figure 5.1**).

Figure 5.1 a) ICP-OES; b) ICP-MS

a)  b) 

ICP-MS measurements were carried out with a quadrupole Agilent 7700 spectrometer, equipped with a collision cell system. Samples were introduced into the plasma by an auto sampler Agilent ASX-520. The instrument was fitted with a MicroMist Agilent standard nebulizer for 7700 and a Scott-type double-pass glass spray chamber cooled to 4°C. A solution of 10 µg/L of iridium in 2% HNO_3 was used as internal standard, according to the conditions of the instrument (**Table 5.1**).

Table 5.1 Instrumental parameters for the Agilent 7700 ICP MS

Operating conditions			**Acquisition parameters**	
Radiofrequency power	1550 W		**Dwell time**	300 ms
Plasma flow rate	15.0 L/min		**Number of sweeps**	300
Carrier flow rate	1.05 L/min			
Helium	4.5 mL/min			
Octapole bias	-18.0 V			
Octapole RF	200 V			
KED	3.5 V			
Sampling depth	8 mm			

ICP-OES measurements were carried out with an Optima 8000 ICP-OES Spectrometer. ICP-OES is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions, emitting electromagnetic radiation at wavelengths characteristic of a particular element. It is a flame technique with a flame temperature ranging from 6000 to 10000 K. The intensity of the emission is indicative of the concentration of the metal within the sample. Approximately 350 mg of unknown sample were placed in a Teflon container with 2 ml of 69% HNO_3, 1 ml of 30% H_2O_2 and 6 ml of conc. HCl at room temperature for 1 h. After that, the mixture was put in a microwave digester for 30 min at 180 °C. The liquid was then transferred in 50 ml flasks and analysed.
Mercury content was determined by Milestone Direct mercury analyser DMA-80 (**Figure 5.2**).

Figure 5.2 DMA-80

The technique associated to the DMA-80 instrument does not require any sample preparation and delivers comparable results to those obtained with Cold Vapor Atomic Absorption (CVAA) or ICP-MS instruments, that however do require sample preparation, often turning tedious, costly, and time-consuming. The DMA-80 combines the techniques of thermal decomposition, catalytic conversion, amalgamation, and atomic absorption spectrophotometry. Controlled heating stages are implemented to first dry and then thermally decompose the sample, which is confined inside a quartz tube. A continuous flow of oxygen carries the products of decomposition through a catalyst bed, where all mercury species are reduced to elemental Hg and driven to a gold amalgamator where the mercury is selectively trapped. The system is purged with argon gas and the amalgamator is subsequently heated, releasing all mercury vapors to the single beam, fixed wavelength atomic absorption spectrophotometer. The intensity of the absorption is measured at 253.7 nm and it is proportional to the content of mercury in the sample.
The effluents were analysed from the processing of sheep skins, obtained both as Crust and Wet Blue specimens. Usually the crust skin is achieved after the liming stage of hide processing, by first fleshing and subsequently splitting the limed hide, to separate the crust from the grain. The wet blue skin normally comes out at the end of a tanning procedure using chromium (III) salts, which are not carcinogenic (**Table 5.1**).

Table 5.1 Metal content[a] in defatting effluents from action B.2

Crust leather					Wet-Blue leather				
Al	3.65		Mn	-	Al	2.54		Mn	-
As	-		Ni	0.67	As	-		Ni	0.43
B	-		Pb	-	B	-		Pb	-
Ba	0.19		Sb	-	Ba	0.14		Sb	-
Be	-		Se	5.59	Be	-		Se	4.98
Cd	-		Tl	-	Cd	-		Tl	-
Co	-		V	4.98	Co	-		V	5.15
Cr	0.48		Zn	-	Cr	0.50		Zn	-
Cu	-		Hg[b]	0.05	Cu	-		Hg[b]	0.06
Fe	1.90				Fe	2.20			

a): values expressed in ppm; b) values expressed in ppb.

The total metal content found was quite low in both effluents and most of the metals analysed were below the detection limit. Even more important was the low content of mercury, just 5% of the threshold limit of 1 ppb for drinking water. Thus, it could be highlighted that the overall hide processing did not influence the quality of the wastewaters in terms of metal contamination. This finding is quite important, because any abnormal value of metals in the effluents can turn a spotlight on the hide processing sequence, the quality of defatting products used and even on the quality of the incoming leather. In this particular case, the metal analysis may be intended also as an indirect environmental indicator, since animals grown in a contaminated environment may lead to contaminated hides/skins.

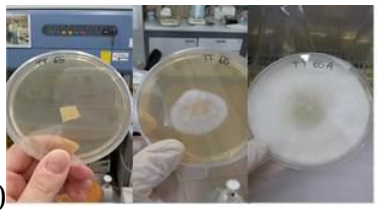
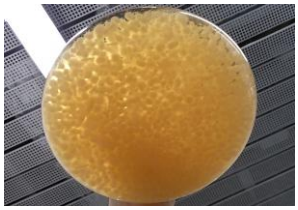
6. Treatment of defatting wastewaters from action B.2 with fungi

The purpose of the treatment of defatting wastewaters with bacteria was to demonstrate the potential reduction of their environmental impact. However, the results were general indications

about the potential biodegradability of those effluents, since the defatting process is widely dependent by the region of origin and type of hide/skin and the complexity of the processing. Every tannery effluent is sent to water treatment plants, where it is treated with activated sludge, that are microorganism consortia of high complexity. Therefore, it was interesting to demonstrate the treatment of tannery effluents from action B.2 with a selected fungus species, that is *Funalia trogii* normally found within rotten wood.

F. trogii was first grown on Petri dish in sterile conditions using malt extract agar (MEA) as solid medium, consisting of a mixture of glucose (20 g/L) triptone (2 g/L) malt extract (20 g/L) and agar (20 g/L). Subsequently, it was grown in liquid medium of Basidiomycetes reach medium (BRM) consisting of asparagine (0.6 g/L) KH_2PO_4 (1 g/L) KCl (0.5 g/L) yeast extract (0.5 g/L) glucose (10 g/L) triptone (10 g/L) $MnSO_4$ (3 mg/L) $FeSO_4$ (10 mg/L) $CuSO_4$ (3 mg/L) $ZnSO_4$ (2 mg/L) $MgSO_4{\cdot}7H_2O$ (1 g/L) $Ca(NO_3)_2$ (50 mg/L) to boost the growth and obtain a suitable amount of mass. Thereafter, the solid mass was homogenized mechanically and recovered by filtration. The fungus thus treated, was suspended in Czapek liquid medium, consisting of inorganic salts: $NaNO_3$ (6 g/L) KCl (0.52 g/L) $MgSO_4{\cdot}7H_2O$ (0.52 g/L) KH_2PO_4 (1.52 g/L) $FeSO_4$ (10 mg/L) $CuSO_4$ (3 mg/L) and $ZnSO_4$ (2 mg/L) (**Figure 6.1**).

Figure 6.1 *F. trogii* growth; a) on Petri dish; b) in BRM; c) in Czapek

a)  b)  c) 

Then, the culture was put in contact with the effluent collected from the processing of sheep skins from action B.2 in the presence of glucose as the source of living for the fungus (**Figure 6.2**).

Figure 6.2 Treatment of EDF20 effluents with *F. trogii*.

The activity of *F. trogii* was controlled by dosing the enzymes produced during its growth, using a standard kit of reagent for laccase and peroxidase enzymes, containing 1-hydroxybenzotriazole as enzyme mediator. In this case, an aliquot of the supernatant solution of the suspension containing the biomass, was treated with the reagent, developing a fast reaction to produce a benzotriazole coloured derivative, which could be quantified on a 0.5-1 min. time scale with a spectrophotometer. It was found that *F. trogii* was still alive after a week., but formation of a gel like material was

observed indicating that a potential interaction may have taken place between the fungus and the chemical waste of EDF20 (**Figure 6.3**).

Figure 6.3 Treatment of EDF20 effluents with *F. trogii*.

Thus, the supernatant was analysed for its COD and BOD content, observing an increase of biodegradability after the treatment with the fungus (**Table 6.1**).

Table 6.1 Analysis of defatting effluent from ovine skin processing

Parameter	Before	After
BOD (mg O_2/l)	480	5500
COD (mg O_2/l)	6080	12175
Biodegradability (BOD/COD)	0.08	0.45

It could be said that the increased biodegradability may have been the result of the presence of fungus biomass and glucose as fungus nutrient: but, it is undoubtedly clear that the BOD parameter had increased more than 11 times, whereas the COD had just doubled. This is a clear sign of fungus vitality and metabolic activity, which had helped in improving the overall profile of the defatting effluent towards depuration. In addition, the formation of the gel material may represent a good opportunity within a water treatment plant scheme, since effluents may undergo a simple mass setting in a dedicated tank, letting the supernatant flow over.

7. Conclusions

The defatting effluents from the semi-industrial scale demonstration showed a very low content of heavy metals, confirming the results of the lab scale monitoring action. This is quite important, since metal analysis demonstrated the reliability of the process, which does not add any extra pollutant. Moreover, metal analysis was confirmed as a good tool for even indirect environmental monitoring, since it may reveal trace elements accumulated within the animals' body: a sign of bad quality environment they live in. The overall biodegradability of those defatting effluents was in the low range window, indicating a potential difficulty for the depuration of such effluents. Actually, it was demonstrated that even the use of a fungi microorganism was capable to increase the biodegradability of the effluents, leading also to the formation of solid/semisolid mass, which may represent a plus during the purification process of tannery wastewaters in a water treatment plant.