



EXPERIMENTATION OF ANTIMILDEW ATX80

Carry out by leather tanning Institute Of Arzignano (VI)

A - Choosing the material to be tested

SAMPLE

Normal hide of tanning.

- 3% chrome sulphate
- 6-7% fat acids
- 15% water
- The rest is only hide

ANTIMILDEW ATX80

An antimildew is a substance that neutralizes the negative effect of oxygen. The oxidation of the products, that takes place in the presence of oxygen, takes to an undesired dark color and/or a bad smell and a taste known with the name of "rancidity". The antimildews are substances that react with oxygen and therefore neutralize its negative effect. Vitamin C is an example of antimildew.

6 OILS OF TANNING 1, 2, 3, 4, 5, 6

Names, characteristics and why they are used.

Oil: natural or artificial organic substance with vegetal, animal or mineral origin, based on hydrocarbons or glycerin esters and superior fat acids, liquid at room temperature, insoluble in water and in solvents. It is used in tannery field to lubricate the dermic layer, to facilitate the sliding of the fibres and avoid the superficial oxidation of the leather.

WORKING WITH DISHES

Dishes give more advantages because the mildews have everything they need (oxygen...) and the growth is certain; moreover it's possible to observe better the results.

LABORATORY INSTRUMENTS USED FOR THE TRIAL

- autoclave
- becher
- electronic precision balance
- bunsen
- biological safety hood
- graduated cylinder
- dishes
- sterile pipette
- pro-pipette
- spatulas
- slides and their cover

CULTURE MEDIUM

Sabouraud n°9 tryptone soya broth TSA n°10

Sabouraud dextrose agar:

to make this medium you have to weight 15 gr in 500 ml of water, put into the autoclave at 121°C for 15 minutes.

B - Execute the Trials

FIRST TRIAL: SOWING THROUGH INCLUSION AND SPATULA

January, 12th 2007

TARGET

To study mildew development in oils for tannery and evaluate the microbial concentration.

PROCESS

We used 5 different oils of tannery, chosen casually, and for each one we carried out a trial with:

- sabouraud medium and inclusion technique
- sabouraud medium and superficial use of spatula
- TSA medium and inclusion technique
- TSA medium and superficial use of spatula

Every operation has always been carried out under biological safety hood

Oils: 1, 2, 3, 4, 5

Total dishes: 20

That is 2 media for each oil for inclusion and the same for the use of spatula.



*Olio 3 - SPATOLAMENTO SUPERFICIALE
 Terreno Sabouraud*

RESULTS

After 1 week of incubation.

Technique	Medium	Oil	Growth	Microbial conc.
Inclusion	sabouraud	1	Yes	Irrelevant
		2	Yes	Irrelevant
		3	Yes	Irrelevant
		4	Yes	Irrelevant
		5	Yes	irrelevant
Spatula	sabouraud	1	Yes	3
		2	Yes	1
		3	Yes	>50
		4	Yes	3
		5	Yes	7
Spatula	TSA	1	Yes	1
		2	Yes	10
		3	Yes	6
		4	Yes	1 (no countable the rest)
		5	Yes	no countable
Inclusion	TSA	1	Yes	>30
		2	Yes	2
		3	Yes	no countable
		4	Yes	1
		5	Yes	5

SECOND TRIAL: DISH CONTACT

TARGET

To withdraw the mildews directly from hide and begin the first tests with antimildew ATX80.

PROCESS

To prepare 10 dish contact with sabouraud medium and withdraw the mildews with dish contact from hide in 5 different zones:

- don't apply the antimildew ATX80 to the first 5
- apply 0,1 ml of antimildew ATX80 to the last 5 with a sterile pipette
(withdrawal realized near 2 bunsen lamps)

RESULTS

DISH WITHOUT ANTIMILDEW ATX80	GROWTH	MICROBIAL CONC.
A	Si	No countable
B	Si	No countable
C	Si	No countable
D	Si	No countable
E	Si	No countable

DISH WITH ANTIMILDEW ATX80	GROWTH	MICROBIAL CONC.
A	Si	No countable
B	Si	No countable
C	Si	No countable
D	Si	No countable
E	Si	No countable



SABOURAUD MILDEW C
With antimildew ATX80



SABOURAUD MILDEW C
Without antimildew ATX80

THIRD TRIAL: DILUTIONS

February, 26th 2007

TARGET

- to establish the lowest quantity of antimildew ATX80 that allows mildew growth
- to establish the quantity of antimildew ATX80 that stops mildew growth
- to establish the temperature and the time necessary for mildew to grow
- to establish the most sensible oil, that is the oil where mildews grow less

PROCESS

A Use the pure antimildew ATX80 at 20%:

To prepare a diluted solution at 2% because the quotient with antimildew ATX80 is 1:10.

After preparing the diluted solution at 2%,

add 1 ml of: antimildew ATX80

9 ml of diluting.

The operation is carried out in condition of sterility, under biological safety hood and using 2 sterile pipette.

B To prepare 6 jars for each oil everyone has to contain:

- 40 ml deionized water
- 0,6 gr agar-agar
- 2,6 broth sabouraud, oil

C To establish how many ml of oil are to be used.

The oils 5, 1, 6 are too dense and with unknown density.

We have to calculate it, because density is an indispensable parameter.

Procedure to calculate oil density:

- to weigh an empty cylinder of 10 ml
- to add 5 ml deionized water
- to add 2 "sticks" of oil
- to close the cylinder
- to homogenize and weigh

Density = Weight/Volume

Density = (final weight-initial weight)/(final volume-initial volume)

Legend

Final weight = weight of cylinder+5 ml deionized water+2 "sticks" of oil

Initial volume = volume of cylinder+5 ml deionized water

Final volume = volume of cylinder+5 ml deionized water+2 "sticks" of oil+ deionized water

D To prepare 6 jars with agar sabouraud and deionized water.

olio 5	density: 1,1 gr/ml	For 2,8 ml we have to use:	
olio 6	density: 0,975 gr/ml	olio 5	3,08 mg
olio 1	density: 1 gr/ml	olio 6	2,73 mg
		olio 1	2,80 mg

To add the PURE solution into 3 jars		To add the DILUTED solution into 3 jars	
Dilution	ml	Dilution	ml
1%	0,5	1%	0,5
0,5%	0,25	0,05%	0,25
0,25%	0,125	0,025%	0,125

E To pour into dish the contents of every jar.

To inoculate the solution with mildew when the medium solidifies.

F To prepare the solution with mildew.

To withdraw mildew directly from hide with sterile handle: to pour the mildews into a test tube where you have put a physiologic solution first. Stirring.

To distribute 0,1 ml of solution with mildew using sterile pipette like a spatula and distribute with etaleur.

We have controlled the growth after 4 days at room temperature.



NOTE

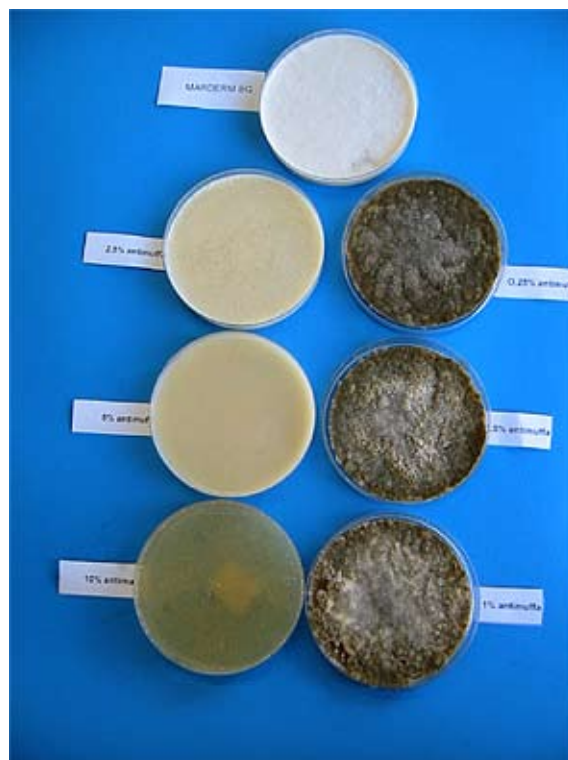
The growth occurs in all the dishes, but some oils have shown a better reaction with the antimildew ATX80; it's easy to recognize that the dishes with 1 and 5 oils have a less quantity of mildew.

DILUTION	ML OF ANTIMILDEW ATX80
10%	5ml
5%	2,5ml
2,5%	1,25ml

The following step is to increase of 10% the concentration of antimildew ATX80, repeating the experiment and using only the antimildew ATX80 and not the diluted solution.



Oil 1
Sabouraud medium
antimildew ATX80 1%



Oil 1
Sabouraud medium
antimildew ATX80 10%

NOTE

Confronting control:

- dish (medium, oil, mildew solution)
- first dish (medium, oil, mildew solution, antimildew ATX80 1%)
- second dish (medium, oil, mildew solution, antimildew ATX80 10%)

You can notice that:

- white mildew grew in the control dish
- green mildew grew better in the first dish with antimildew ATX80 1%
- both mildew disappear and the antimildew ATX80 react with the medium that changes its color in the second dish with antimildew ATX80 10%

CONCLUSIONS

It was demonstrated that an antimildew action is present: it can depend on the kind of oil, on the interaction oil- antimildew ATX80 or on the different oil concentration.

Two kind of mildews were found: white and green.

White mildews gets the better of green ones in normal condition.

When antimildew ATX80 1% is present, the white mildew is more sensible, disappears and takes the windward the green mildew.

We have obtained that:

- 1% is the minimum quantity of antimildew ATX80
- 10% is the maximum quantity of antimildew ATX80

FOURTH TRIAL: MILDEW IDENTIFICATION

MATERIALS

- Slides and their cover
- bunsen lamp
- sterile handle
- sabouraud medium

PROCESS

- To pour a little bit of medium of a slide, just to have 1 cm
- to withdraw the mildew from the dish with sterile handle and graze it on the slide,
- adding a drop of water
- cover the slide and wait

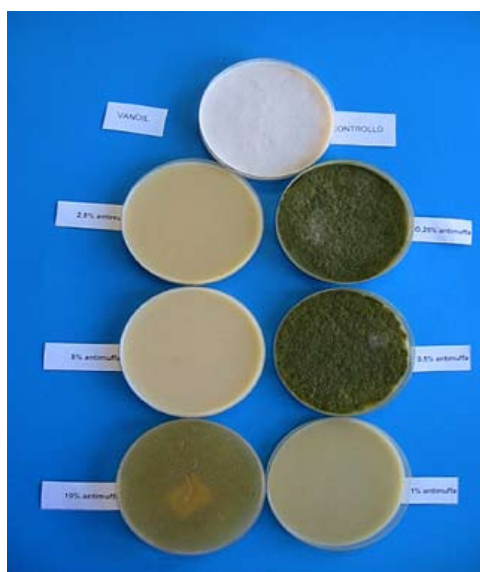
CONCLUSION

After 4 days at room temperature it's possible to observe the growth at the microscope. We discovered that the mildews belonged to the genus *Aspergillus* (in particular *Aspergillus terreus*).

Aspergillus belongs to a kind of 200 mildews, found in all the world. First, the aspergillus has been catalogued in 1729 by priest and by italian biologist Pietro Antonio Micheli. Observing the fungi to the microscope, Micheli has been attracted from the figure of an aspergillum (saint sprayer of the water) and has called the kind consequently.

Some characteristics of *Aspergillus terreus*:

- colourless smooth Conidioforo (100-250 micron)
- cupule sub-globular vesicle (10-16 micron)
- double-seriated cylindrical metule
- filiadi originating on the half or the two upper thirds party of the vescicola's surface
- head with a columnar disposition
- globular/sub-globular smooth hyaline-yellow pale conidi (diameter = 2-3 micron)
- Hyaline clamido spores in the vegetative part, that's submerged by hypha



The antimildew ATX80 has better reacted with the medium that contain oil 5 and 1.

It can be noticed that:

- **with the control the white mildew grows**
- **with minimal solution of antimildew ATX80 0,25% and 0,50% the green mildew prevails**
- **With solution 1% there's still the antimildew ATX80's effect: the medium appears turbid but the green mildews disappear completely**
- **After solution 2,5% the antimildew ATX80 reacts with the medium: all the mildews disappear, the medium remains turbid until 5% and to 10% changes colour**