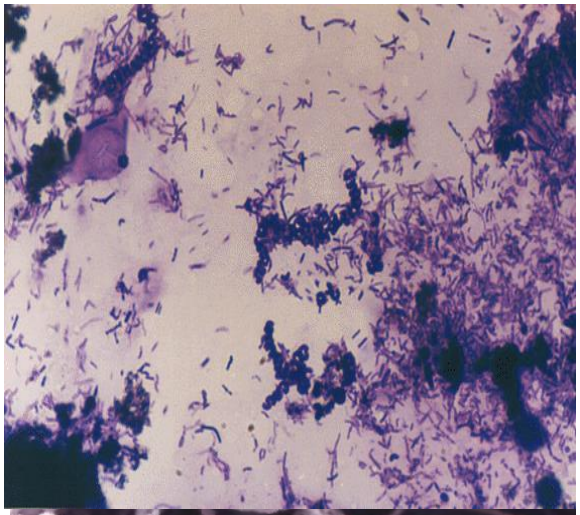
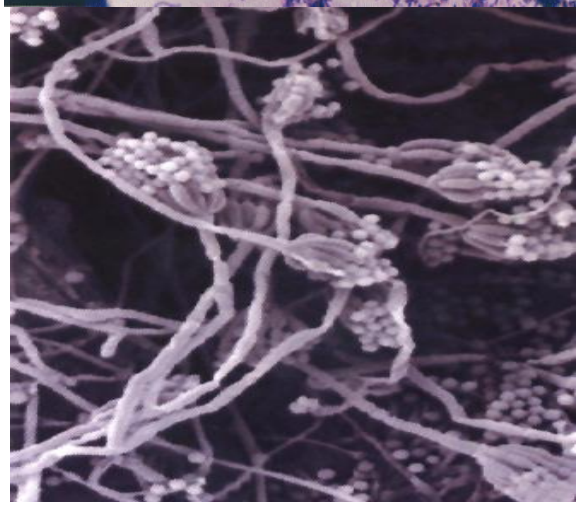
Buckman

Seminar on Microorganism Control





PRESENTATION PREPARED BY

Raf Leyman – EMEA Regulatory Affairs Manager
Luis Zugno – Global Technical Director Leather
Elton Hurlow – EMEA Leather Division Manager
Luca Ramadori – EMEA Area Manager

Topics Covered

SECTION A:

Microorganisms and their Control in Leather Industry

1. *Microorganism problems in leather production*
2. *Best practices in control of microorganisms*
3. *Importance of monitoring*

SECTION B:

Government Regulations & Market Requirements

1. *Government regulations on biocides*
2. *Risk assessment*
3. *Market restrictions on biocides*

SECTION C:

Questions and General Discussion

Buckman

SECTION A:

Microorganisms and their Control in Leather Industry

1. MICROORGANISM PROBLEMS IN LEATHER PRODUCTION

Buckman

Classification of Living Things

- Early scientific classifications included the 5 “Kingdoms of life”:

  Animalia, Plantae, Fungi, Protista, Bacteria
- Individual organisms were classified and categorized according to strict hierarchical naming conventions, e.g.:

  Fungi, Basidiomycota, Basidiomycetes, Agaricales, Agaricacea, Agaricus bisporus
  Animalia, Chordata, Mammalia, Primates, Hominidae, Homo Sapiens

Hierarchical Classification

Binomial Nomenclature

- Kingdom
- Phylum
- Class
- Order
- Family
- Genus
- Species

Carl Linnaeus
(1707- 1778)

Common Mushroom

- Improved understanding of cellular structure, brought 2 domains:
  - Prokaryotes (no organized nucleus or membrane bound organelles)
  - Eukaryotes (those with cells containing a nucleus)

Buckman

Classification of Living Things

Improved understanding at the genetic level, brought 3 Domains we use today.

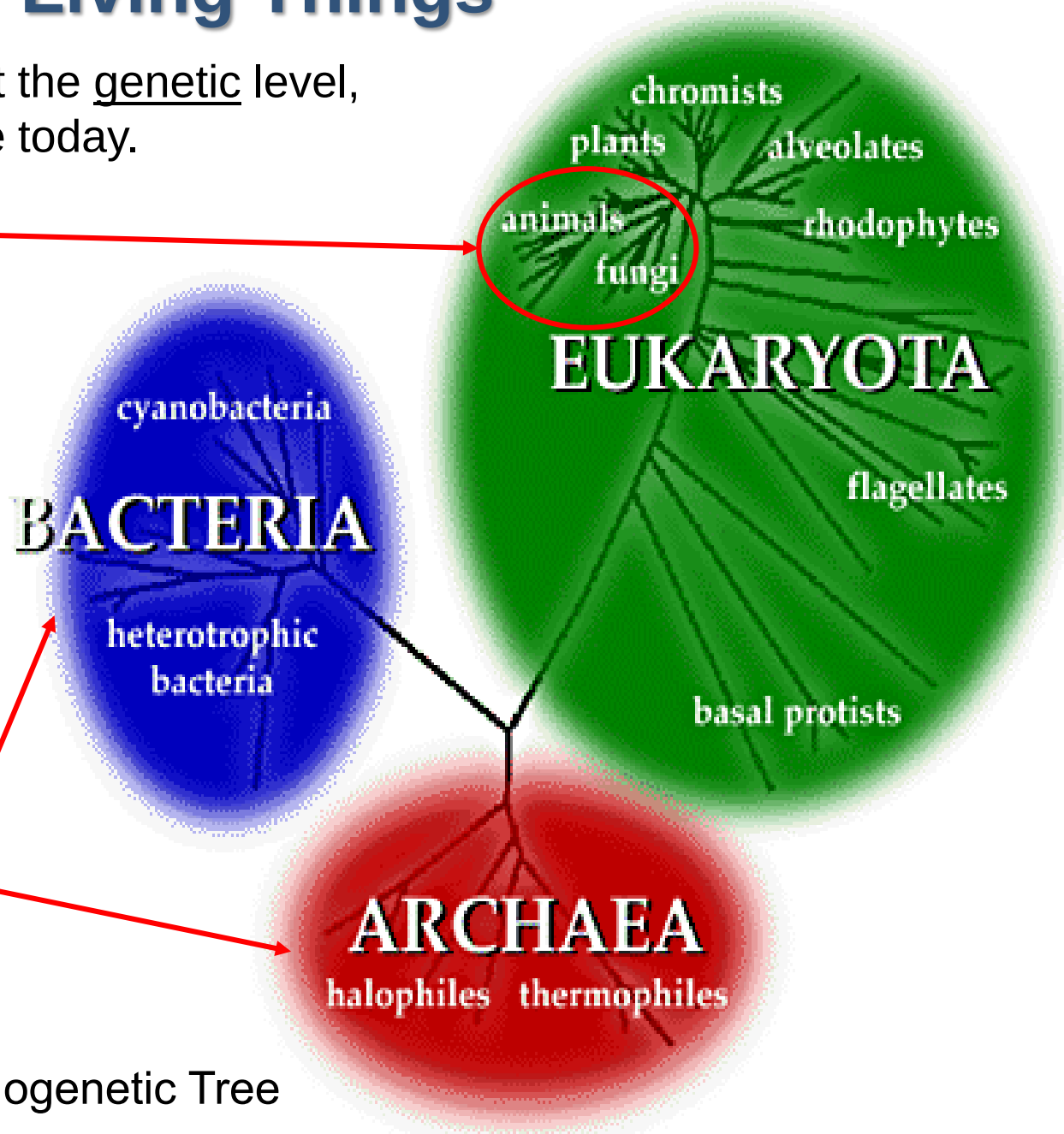
There is a close relationship between fungi & animals.

Fungi are not plants

Prokaryotes

Archaea and bacteria are genetically quite distinct

Archaea have much in common with Eukaryotes



Carl Woese 1990's Phylogenetic Tree

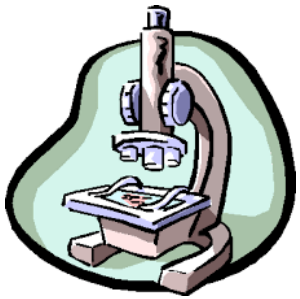
Most living things are Microorganisms

Size Relationships: Important first line of distinction

atoms	0.1 nm	10^{-9} m
molecules	1 - 10 nm	
viruses	8 - 200 nm	
bacteria	**0.5 - 2 μm**	10^{-6} m
archaea	**0.5 - 2 μm**	
fungi / yeast	**10 μm***	
protozoa	**10 - 1000 μm**	
algae	**>100 μm**	
nematodes	1 mm	10^{-3} m
flea	10 mm	
chicken egg	100 mm	
human	1.5 – 2 m	10^{0} m

Microorganisms

Macro-organisms



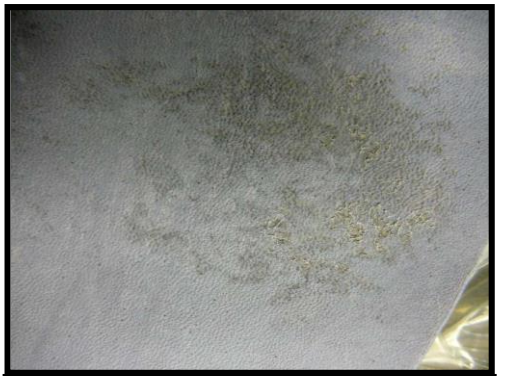
* Diameter of mycelium - fungi range widely in size from μm to m

Buckman

Microorganism Damage in the Leather Industry

Main problematic microorganisms are: ***Archaea, bacteria, fungi****
They cause many $millions damage in the leather industry worldwide each year

- Damage to hide and leather quality can occur
  - On the live animal
  - After slaughter and before arrival in the tannery
  - During leather processing
  - Storage and transport of leather
  - Storage and transport of leather articles
- Worker health
  - Mycotoxins, lost time & productivity, nuisance issues



€ £ $

* ***Yeasts*** are mono-cellular fungi

SECTION A:

Microorganisms and their Control in Leather Industry

2. BEST PRACTICES IN CONTROL OF MICROORGANISMS

Buckman

Microorganisms of concern

Organisms that cause damage

- Archaea
- Bacteria
- Fungi

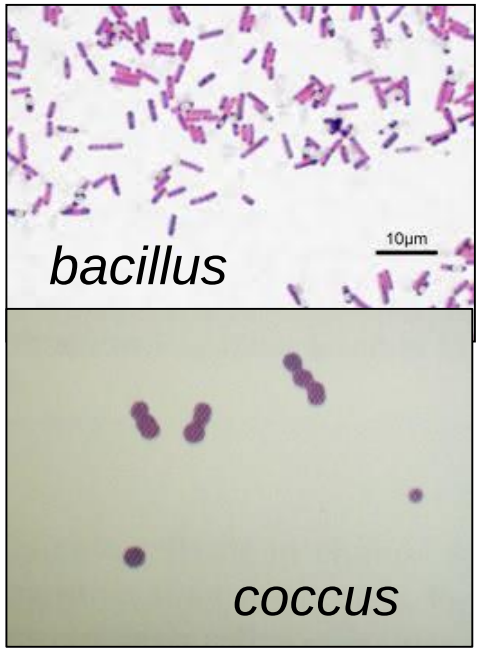
Chemical Substances used for Control

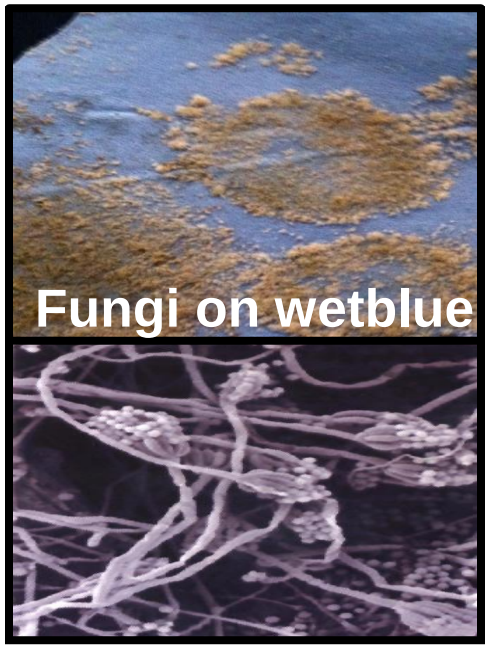
Longer Term Control

- Bactericides
- Fungicides

Short Term / Cleaning

- Sanitizers or Disinfectants





Buckman

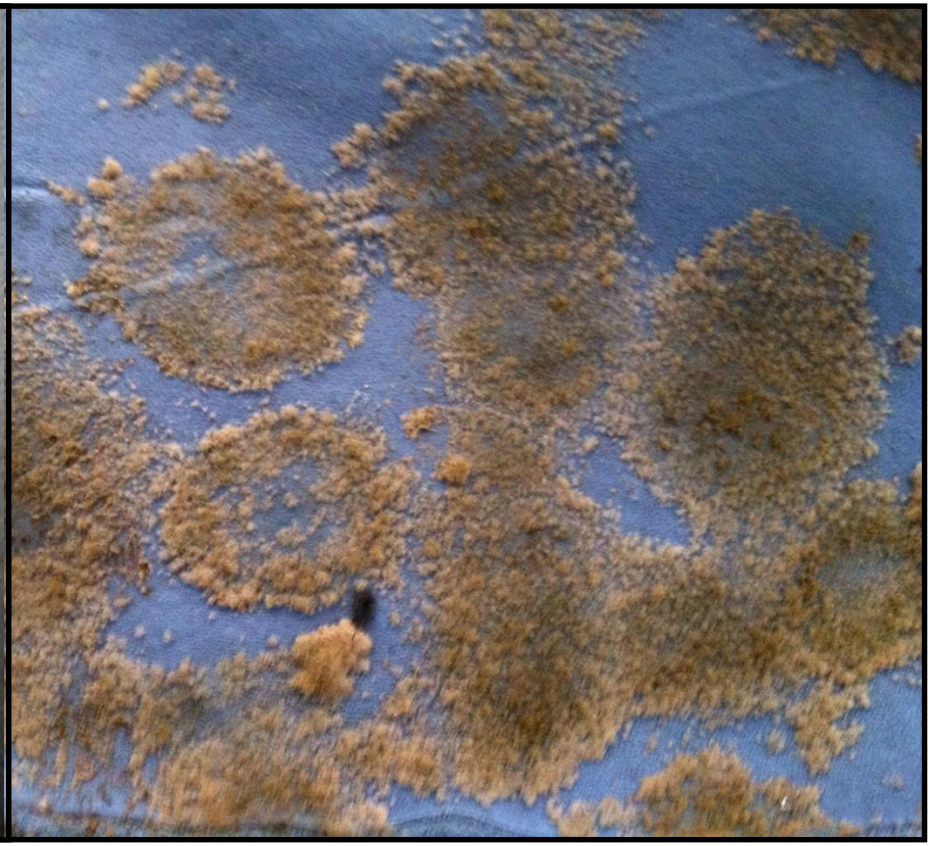
Two Main Tannery Problems within our Control

Attack during soaking

Bacterial damage to the grain due to a lack of adequate controls

Attack on wet leathers

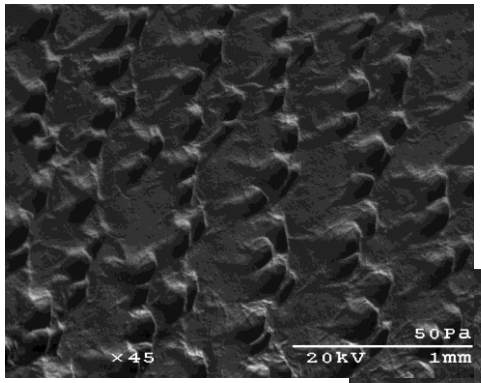
Fungal growth on wetblue due to insufficient preservation



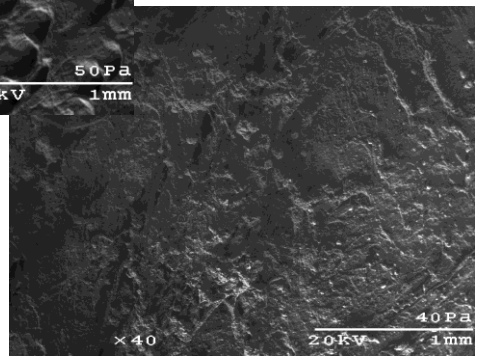
Bacterial Damage in Soak

Damage is due to bacterial **exo-enzyme*** attack and can result in:

- Exacerbation of existing damage
- Pin prick - follicular enlargement
- Loss of Grain or suede effect
- Loss of Enamel layer / sheen
- Increase in veininess
- Loss of hide substance
- Increased looseness
- Loss of physical strength properties
- Uneven chemical uptake
- Downgrading of hides or skins



Intact Grain



Damaged Grain

Enzyme activity is a function of:

- Type and amount of enzyme
- Temperature & pH
- Time available for function
- Level of nutrients
- Presence or absence of inhibitors

*It is not the number of bacteria that are a problem in soaking, but the amount of bacterial enzymes.

Buckman

Control during Soaking

General Considerations:

- Large numbers of bacteria are introduced from the hides or skins – dirt, manure, etc.
- "Fresh hides" are typically more contaminated than salt cured hides.
- It is NOT realistic to eliminate all bacteria during the soak
- For uniform results, we need to minimize the "exo-enzymes" released
- We do this by adding a suitable bactericide.
- Bactericides may be compared:
  - Chemistry
  - Mode of action
  - Speed of kill
  - Dosage or efficacy
  - Cost

*If bacteria are not controlled you are adding **variability** to the process*

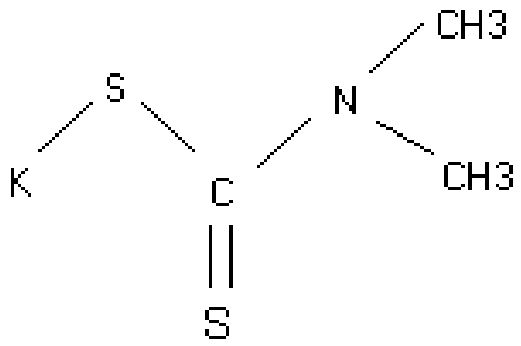
Most common bactericides in soaking are based on **dithiocarbamate** chemistry

Buckman

Bactericide Selection

Dithiocarbamates

- The most widely used bactericide for soaking worldwide
  - Economical application cost
  - Very effective at alkaline pH
  - Long lasting, slow kill – long $T_{1/2}$
  - Possible unhairing issues at higher concentration (>0.2%)
  - Possible lachrymation issues with some types of carbamates
  - Listed by IPPC as “Best Available Technique” for the leather industry
  - Available as K or Na salts



Potassium Dimethyl-dithiocarbamate

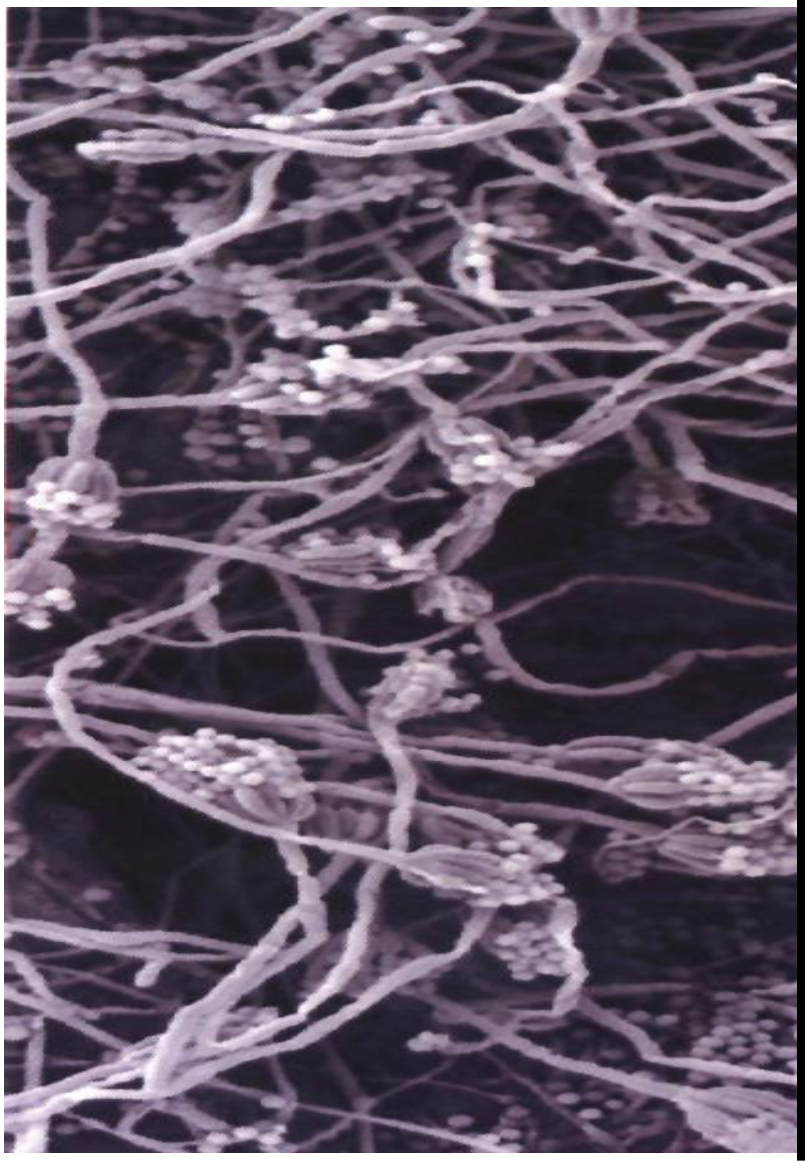
Bactericide Selection

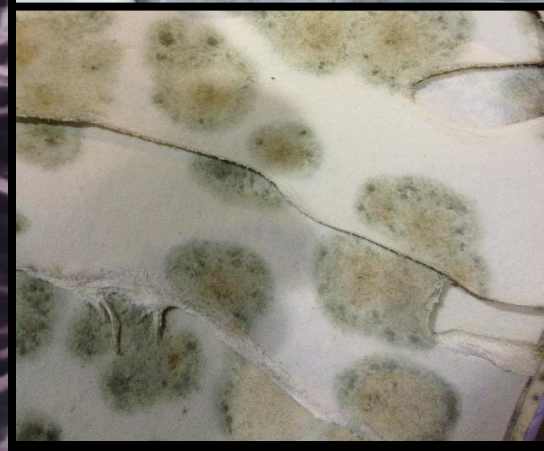
Other:

- Commodity Oxidizers: Chlorite, Hypochlorite; Bromine; Ozone; Peracetic acid
  - Competing action as they react with all organics
  - Excess dosage can cause problems with hair removal
  - Peracetic has strong smell
- Isothiazolinones (mix)
  - Good bactericides, Effective over a broad pH range
  - Broad spectrum, rapid kill
  - Moderate half life ($T_{1/2}$)
  - Cost effective for shorter soak
- Quaternary Ammonium Compounds:
  - These are good bactericides, but mainly used as surface sanitizers - not very effective in soak

Preservation of wet leather

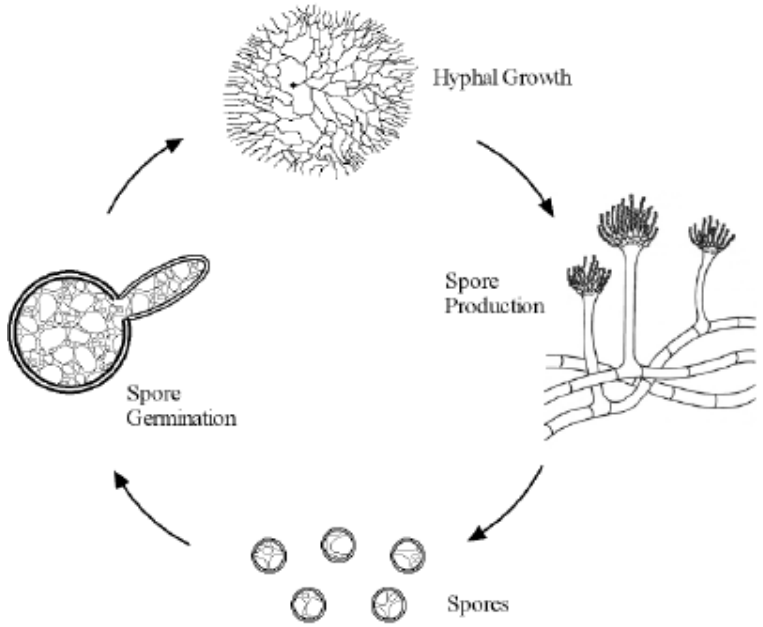
Fungi

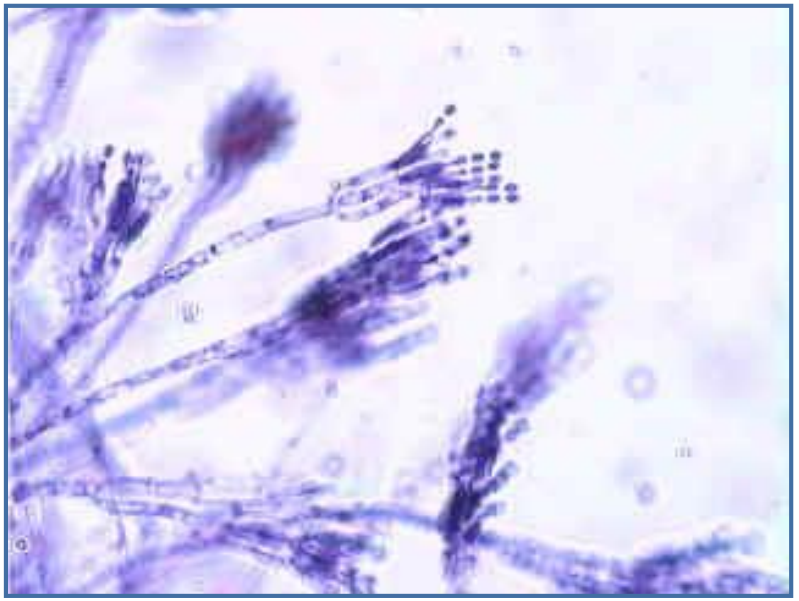




Fungi Growth Cycle

- Mature fungi produce spores which are dispersed in the air.
- Spores can remain dormant for years.
- Germination is triggered if sufficient moisture and nutrients are available.
- Growth structures are in the form of thread-like cells called hypha.
- Hyphae release enzymes that degrade surrounding nutrients which are absorbed
- The mass of intertwining hyphae network is called mycelium, which when visible is sometimes called mould
- To reproduce, fungi form fruiting bodies that release spores

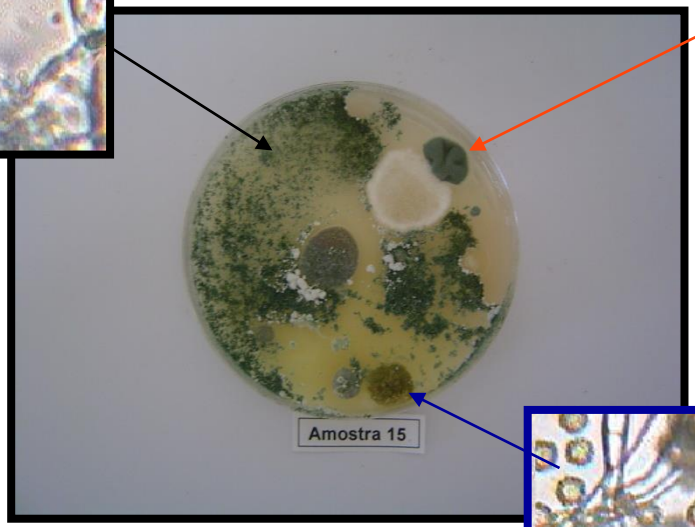


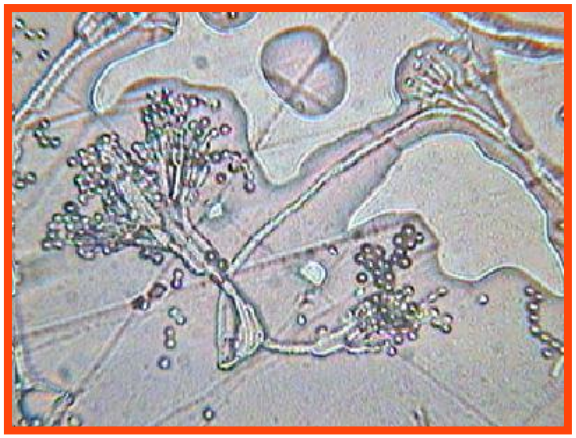


By the time we see mould growth on leather, the original spore has multiplied to represent thousands of individual fungal organisms.

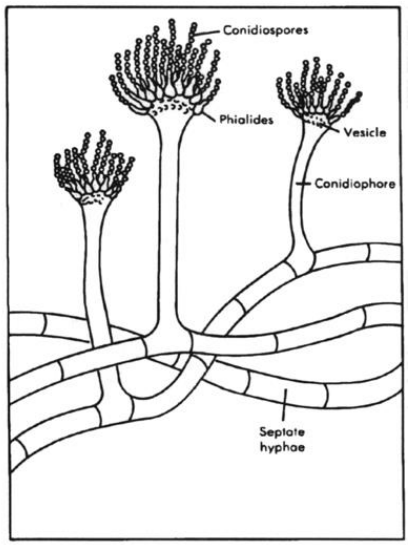
Typical Leather molds

***Trichoderma* sp.**





***Penicillium* sp.**

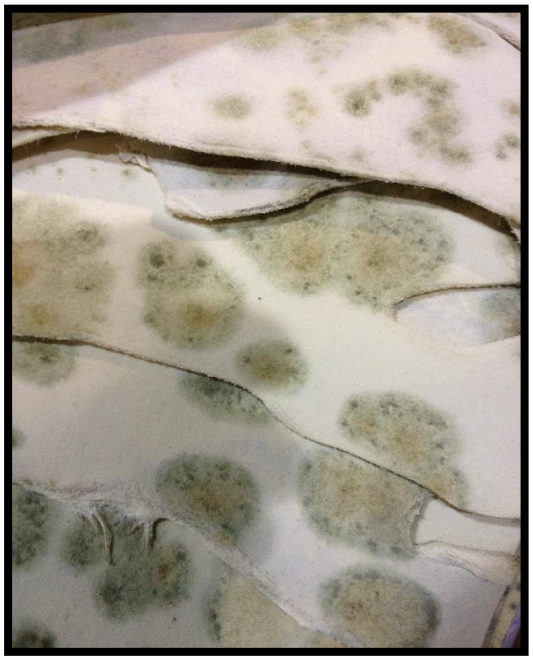


***Aspergillus* sp.**

Buckman

Problems caused by fungi

- Staining of the grain - can be from pigment in fungal spores but usually from physico-chemical changes in area of fungal growth
- Uneven dyeing or levelness problems
- Downgrading
- Time lost - Rework
- Opportunity lost - utilize molded stock in darker colours or different grades.
- Upset customers
- Worker health problems – some spores are toxic (mycotoxins)



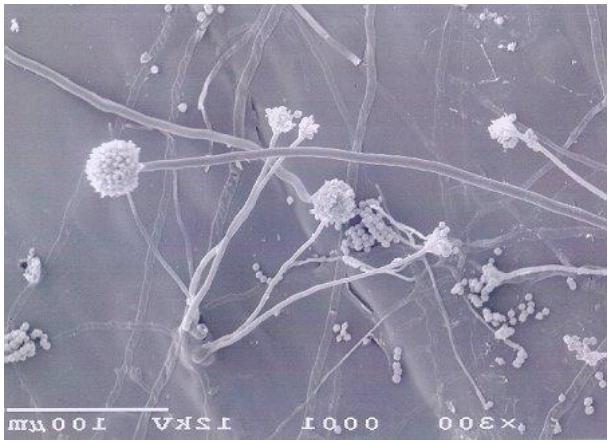
A definition of tanning:
"To prevent microbial enzyme attack".
Q: So why do we get fungal growth on tanned leather?
A: The main nutrients for fungal growth are fatty materials & sugars

Fungicide chemistry

There are not many active substances that are of significant commercial importance in the leather industry:

- **TCMTB -** 2-(Thiocyanomethylthio)benzothiazole
- **OIT** - 2-n-octyl isothiazolin-3-one (ITZ / OITZ)
- **CHED -** S-Hexyl-S'-Chloromethyl-cyanodithiocarbimate
- **PCMC** - *p*-Chloro-metacresol (CMK)
- **OPP -** ortho-Phenylphenol

> 98% of industry



Other actives encountered include:

- **MCABIA -** Carbendazim
- **DIMTS -** Diiodomethyl-*p*-Tolylsulfone
- **IPBC –** Iodo-propenyl butyl carbamate
- Sulfones, pyrithiones, etc.

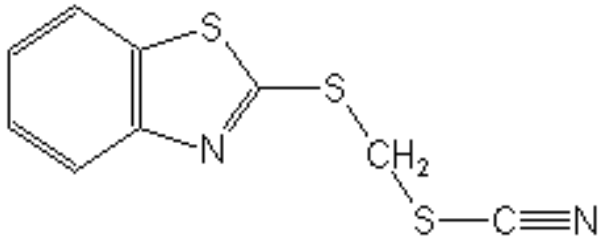
- **Multiple active blends:** TCMTB + OIT; TCMTB + OIT + CHED; OPP + PCMC, etc.

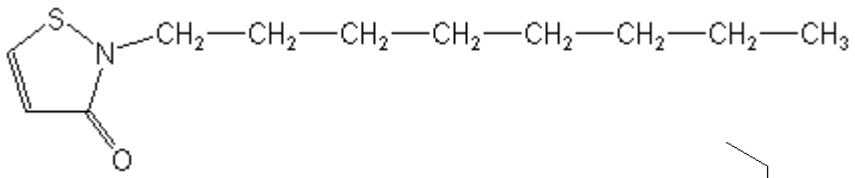
NOTE: Most of these active substances, except for CHED, have been around for a long time (>30 years).

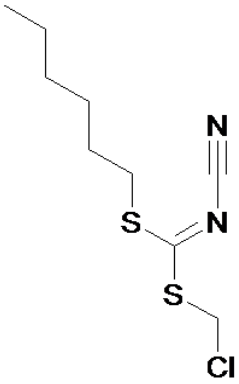
Buckman

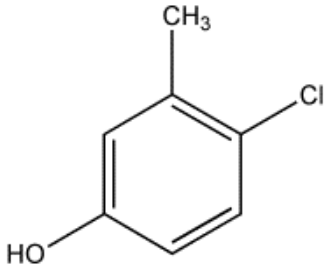
Fungicide Active Substances for Leather

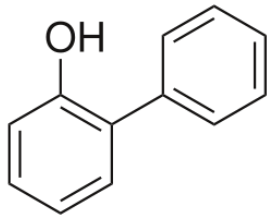
- TCMTB
- OIT (ITZ)
- PCMC (CMK)
- OPP
- **CHED**









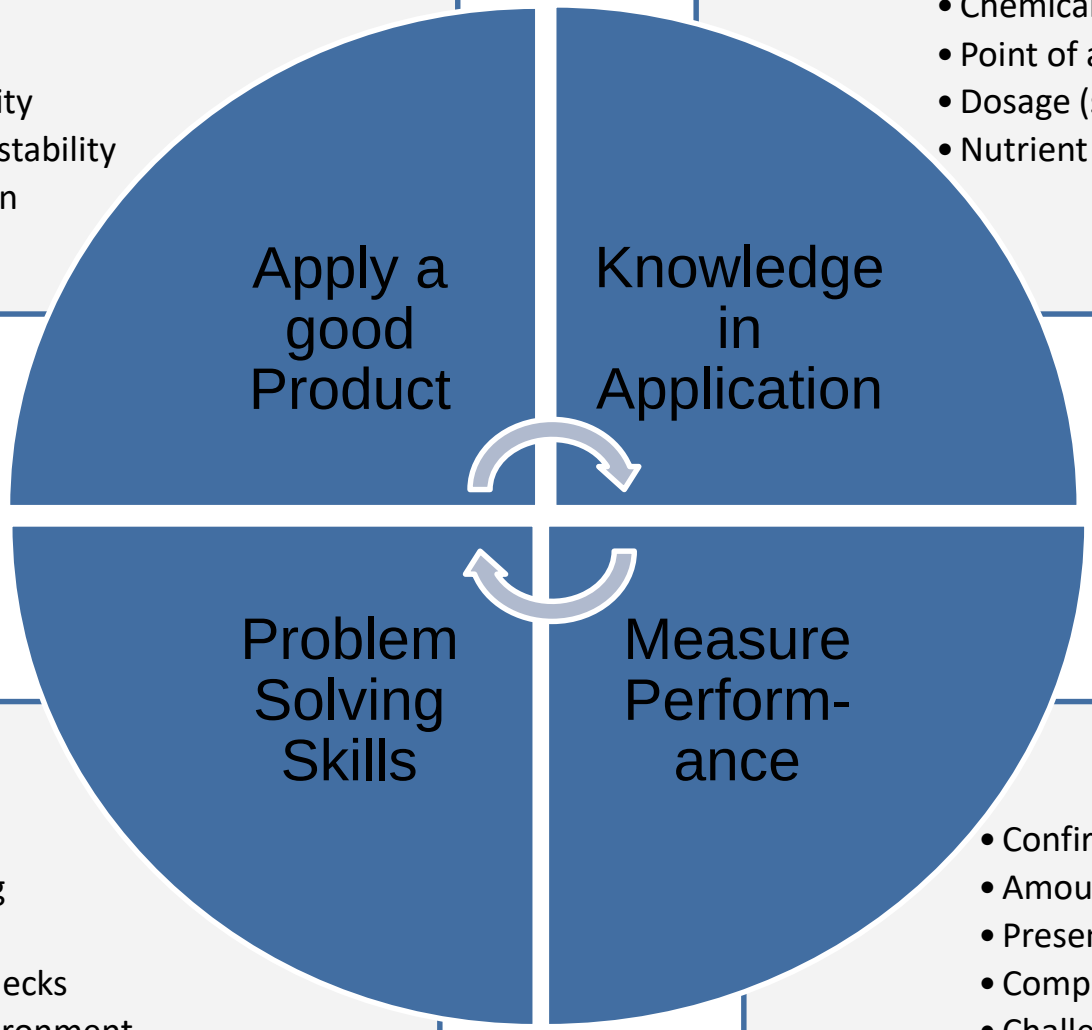


Buckman

Critical Success Factors

- Active Substance
- Concentration
- Blends & Synergy
- Formulation stability
- Emulsion size and stability
- Aqueous dispersion

- Review of process / recipe
- Chemical Compatibility
- Point of application
- Dosage (s)
- Nutrient levels / fats



- Troubleshooting
- Storage, Handling
- Spectrum, MIC
- Environmental checks
- In a dynamic environment problems can and will arise

- Confirmed uptake
- Amount and uniformity
- Present in active form
- Compatible process
- Challenge test - Tropical chamber, Petri dish

Buckman

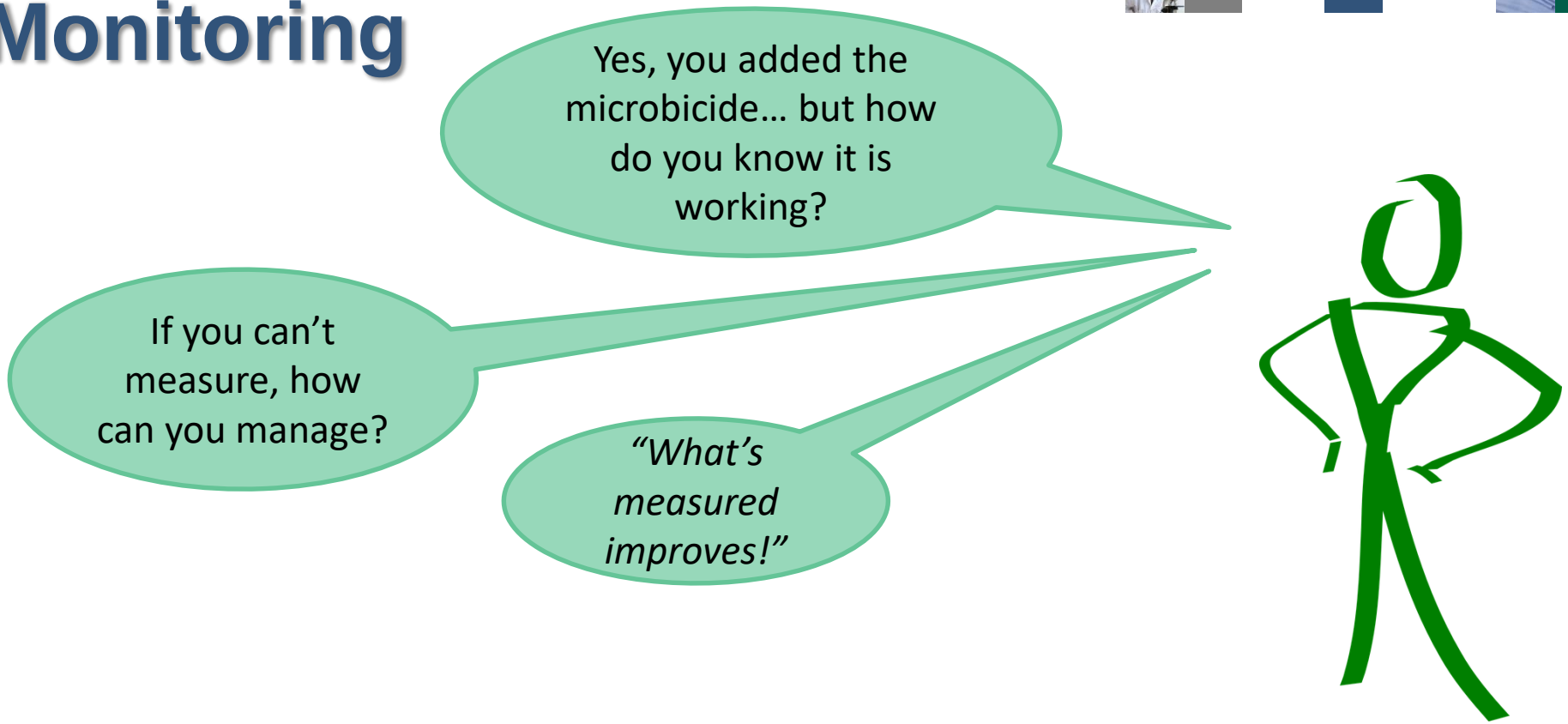
SECTION A:

Microorganisms and their Control in Leather Industry

3. IMPORTANCE OF MONITORING

Buckman

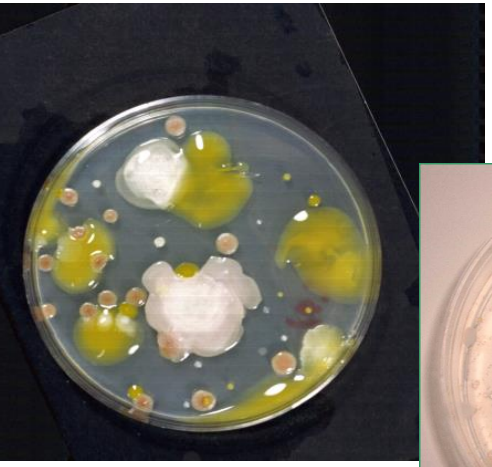
Monitoring



- Both the tannery and the microorganism world are dynamic environments
- Every tannery is different, and raw materials, process recipes, environmental conditions, etc. are constantly changing
- Monitoring is necessary to ensure performance

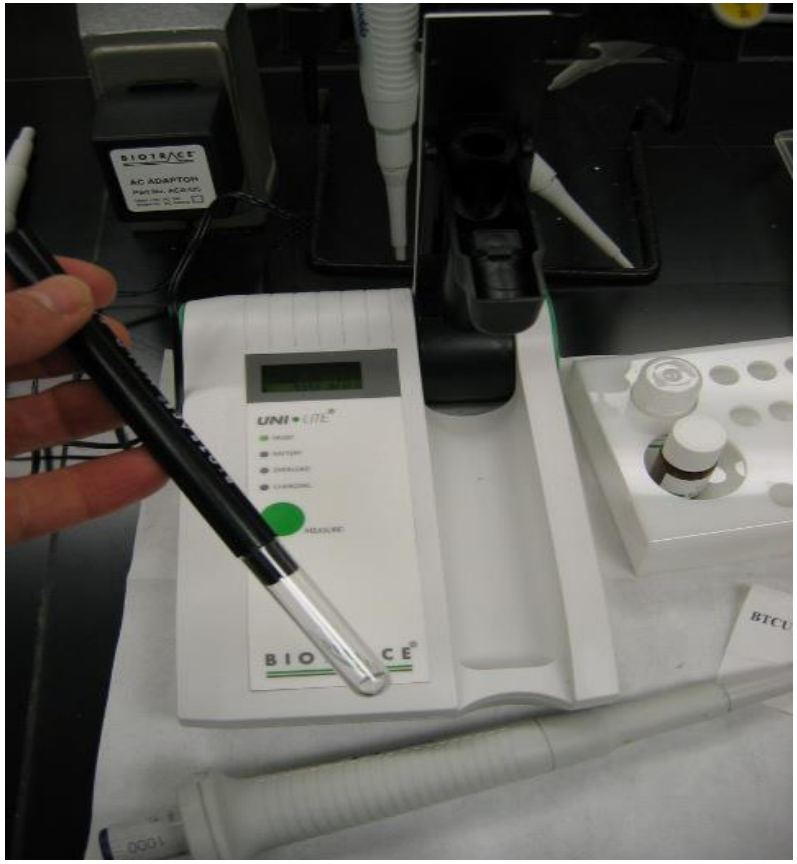
Buckman

Monitoring Bacteria in Soak

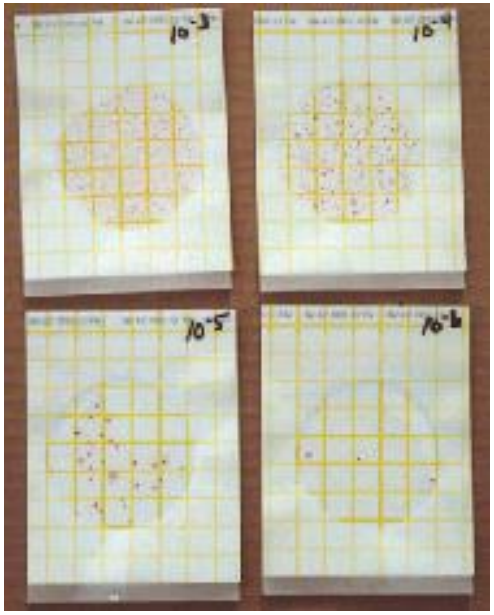


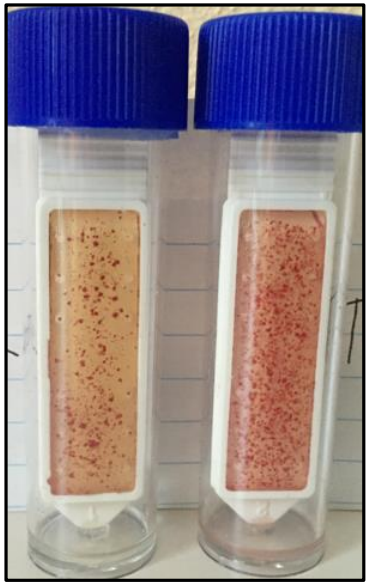
Plating Techniques

ATP Metabolic Activity



Petrifilm®



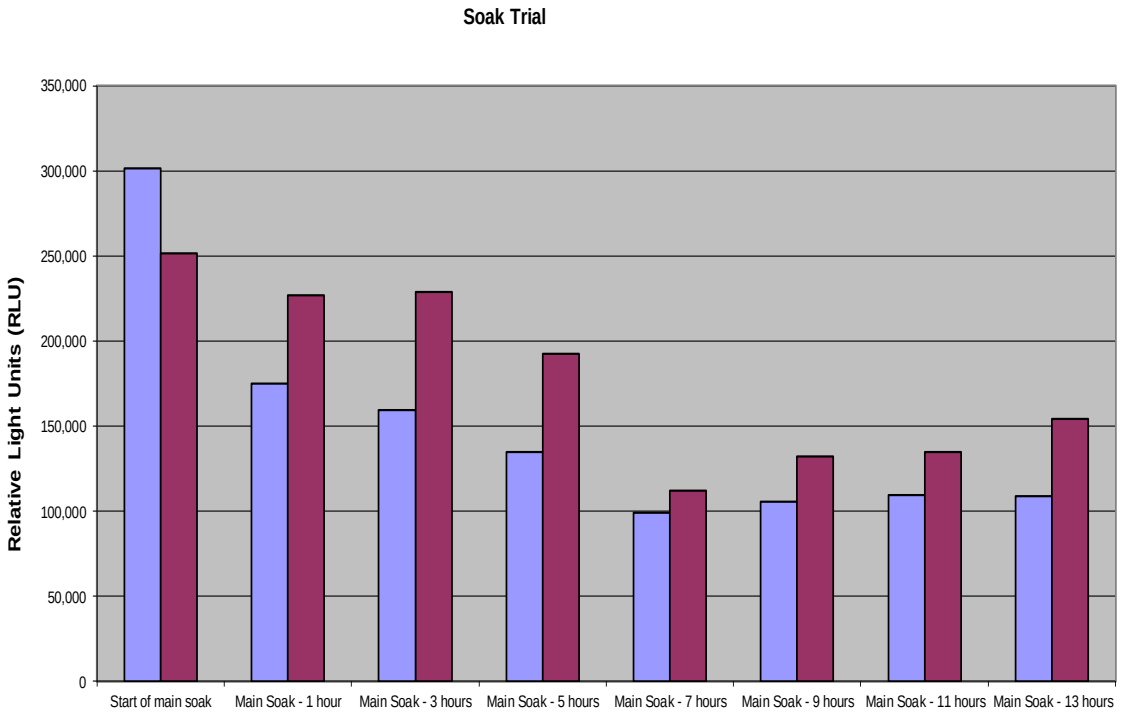


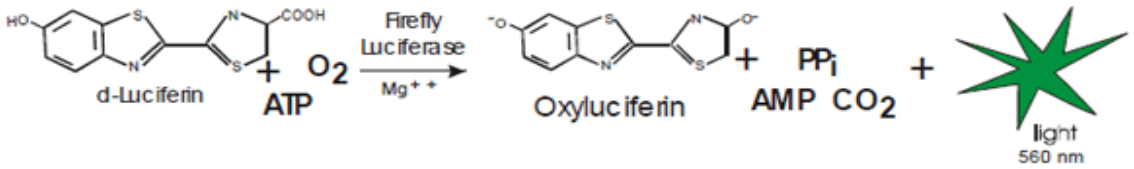
Bucheck / Dipslides

Buckman

ATP* Bioluminescence Assay

- Measurement of metabolic activity
- Directly correlated to all living microorganisms in a given system
- Monitor trends in real time
- Results are immediate
- Results are “actionable”





*ATP = Adenosine triphosphate

Buckman

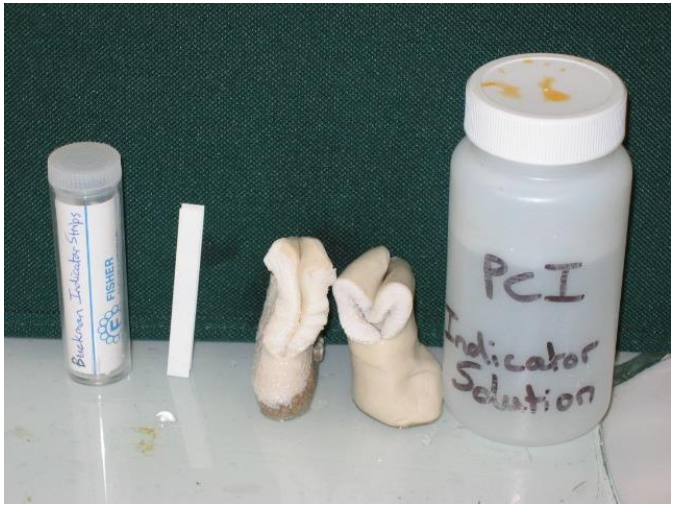
Setting up a Fungicide Program

Process & Environment Review:

- Understanding of raw materials, process recipes, and environmental conditions.
- Check to ensure compatible chemistries
  - Strong oxidizing agents
  - Reducing agents
  - Other potential interferences

Performance Requirements:

- Define post tanning operations and preservation conditions
- Ensure that dosage and uptake are aligned with preservation requirements



Buckman

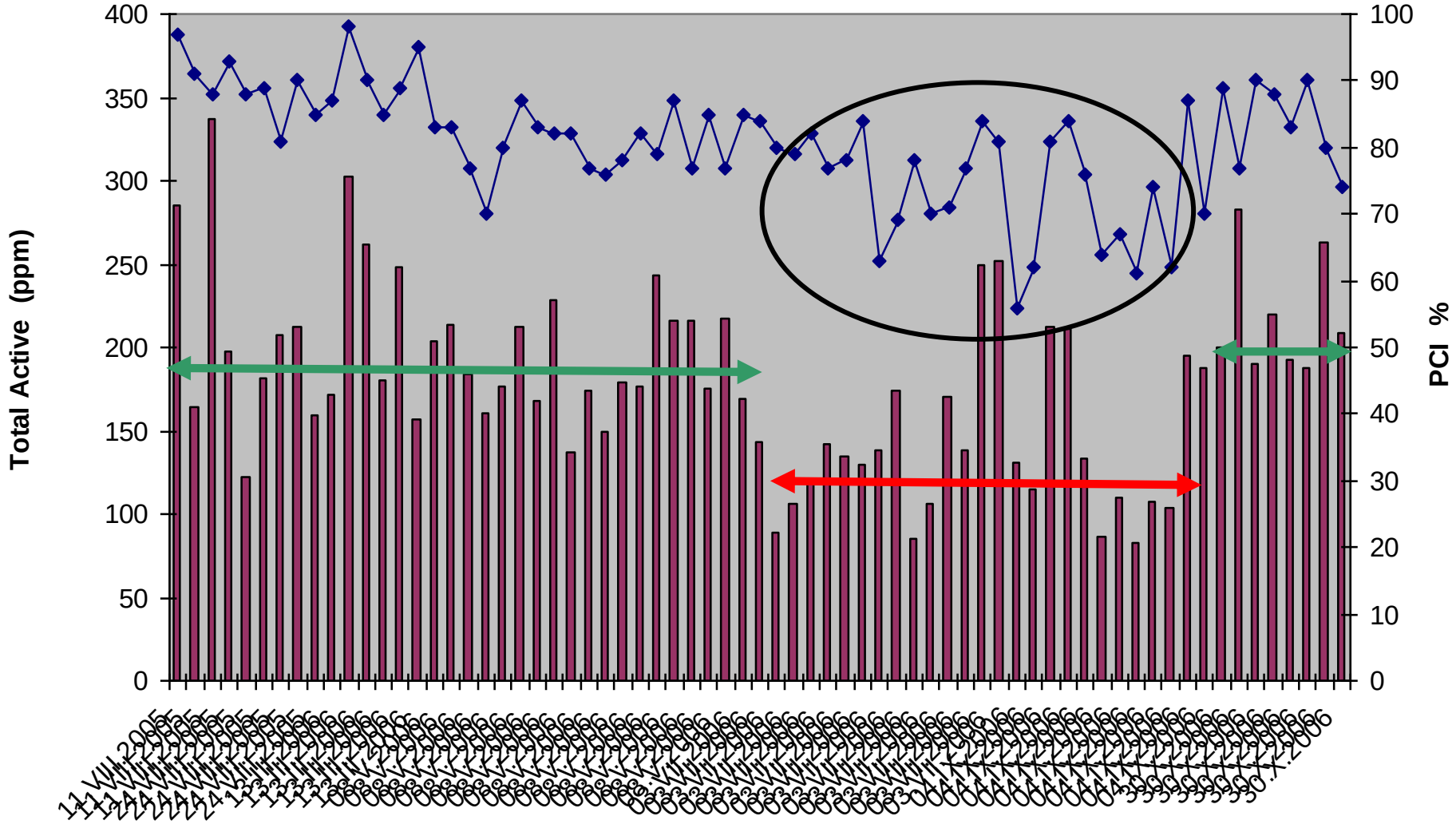
Uptake of Fungicide

Analytical Measurement of Active Substance:

- Solvent extraction → detection using HPLC or TLC
  PCI = Process Compatibility Index (TCMTB)
- **Quantity**: Critical minimum amount is required for performance
- **Uniformity:** Uptake and distribution

Reference: IUC 29 / EN ISO 13365

Total Active Substance
Corrected for Thickness, Moisture & PCI



Buckman

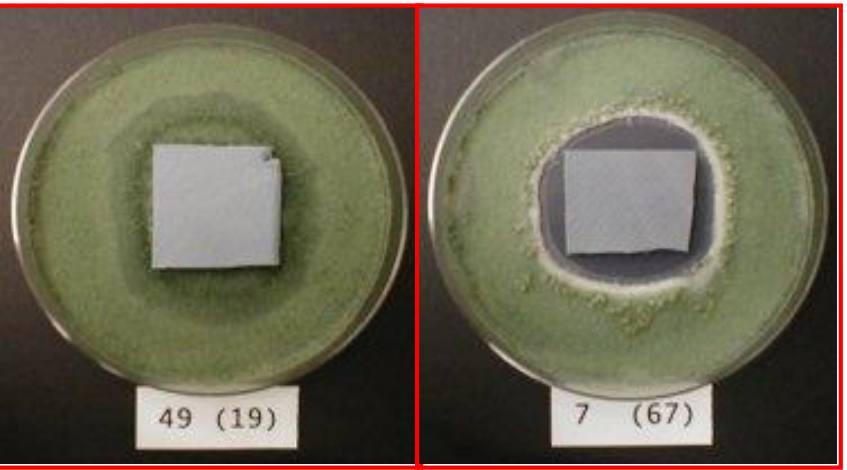
Challenge Testing

Environmental Chamber Test: (ASTM D7584-10)

- Controlled temperature and humidity
- Populated with various fungal species
- Exposure period – e.g. 4 to 8 weeks
- Monitor regularly for mould growth

Agar Plate Challenge Test:

- Controlled temperature and humidity
- Inoculated with various fungal species
- Monitor for growth



Buckman

Comment on Resistance

Do I need to periodically change my fungicide to prevent resistance?

NO!

- There are significant technical differences between industrial biocides and antibiotics.
- Forty years of leather industry experience has not provided one confirmed case of genetic resistance.
- Failures of fungicide programs are often blamed on resistance, but scientific evaluation indicates root cause problems are either:
  1. Insufficient fungicide addition
  2. Poor uptake and distribution
  3. Incompatibilities in processing