BIOTECHNOLOGY AND MICROBIOLOGY IN TEXTILE SECTOR
Content

- LEITAT
- Introduction
- Processing
- Microbiology
- Testing
- Conclusions
LEITAT is a Technological Center, founded in 1906, that accounts for nearly a century of experience and expertise in several technologies applied to industrial sectors.
A BIOPROCESS is any process that uses complete living cells or their components (e.g., enzymes) to obtain desired products.
R&D (Drug Discovery)

Identification of new oncology targets and generation of NME (New Molecular Entities) and specially monoclonal antibodies (New Biological Entities - NBEs) to modulate them.

Complete characterization of its *in vitro* & *in vivo* profiles in order to deliver these innovative drugs as clinical development candidates.
We market a broad battery of oncologic *in vitro* & *in vivo* models to the pharmaceutical industries and others. We also provide the basis to identify unrevealed indications for known leads.

We also offer preclinical model design support and advising & consultancy for research projects.

**Monoclonal Antibody Generation**
- custom Mab (client needs)
- Immunization, screening, cloning and characterization

**Oncologic *in vitro* & *in vivo* models**

*In vitro assays*
- Proliferation, viability, apoptosis, target-related, migration, adhesion

*Ex vivo assays*
- Rat aorta

*In vivo assays*
- Subcutaneous, orthotopic, metastasis, angiogenesis, non-invasive techniques

**Basic preclinical histology**
- We support customer needs in the histology area.
  - paraffin embedding
  - sectioning
  - standard staining

BIOTEC 2008
Substitution of conventional processes by more sustainable ones, less harmful and less aggressive for humans beings and the environment, and that are done in mild conditions.

We use **enzymatic catalysis** as a substitution of chemical reagents.
ENZYMES are protein macromolecules present in any living organism.

They are natural catalysts with many advantages with respect to chemical catalysts:

- Smoother reaction conditions (low/moderate T and P)
- High specificity with respect of chemical reaction (avoiding side reactions)
- High specificity for the substrate
- Biological material (Ecofriendly)
Introduction

BIOTECHNOLOGY, in the textile context, mainly refers to textiles processed by enzymatic catalysis, biological management of its effluents, and to biological devices fitted in textile substrates.

BIOTECHNOLOGY is a key technological line in order to make possible the changing from conventional processes to those that are environmentally sustainable.
The use of enzymes allows the diminution or eradication of harmful chemical products.

- Reduction of water amounts needed for washing/rinsing
- Energy saving (diminution of working temperatures)
- Obtaining of textiles/goods well-finished, of high quality, and processed in a healthy and environmentally friendly way.

**Drawbacks**

- Enzyme recuperation and reuse
- Scale up in industrial environment
- Higher process time
- ¿Economically feasible?
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Objective: investigation, developing and implementation to substitute textile conventional processing.

Biotechnology in Textile:

- Natural fibers improvement
- Biobased fibers (biopolymers)
- Diagnostic tools for adulteration and quality control
- Chemical processes substitution
- New textiles with antimicrobial properties
- New washing powders
- Waste water treatment
- Finishing
BIOTECHNOLOGY: Fibers production

• Natural fibers improvement:

COTTON
- Transgenic cotton: caterpillar resistant
- Naturally colored cotton

• Synthetic fibers:

PROTEIN POLYMERS (seda de araña “Dragline”)
POLYMERIC POLYSACCHARIDE (chitosan, hyaluronic acid, PLA)
POLYEster (PHB) synthesized by microorganisms
BIOTECHNOLOGY: Other applications

• Quality control and adulteration control:
  - Animal fiber identification (Cachemira) by means immunologic assays.
  - New *versus* regenerated fibers

• Washing powder development:
  - Specific and efficient stain removal
  - Smoother washing conditions
### BIOTECHNOLOGY: Washing powders

<table>
<thead>
<tr>
<th>Spot origin</th>
<th>Examples</th>
<th>Main ingredient</th>
<th>Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food</td>
<td>Meat, chocolate, milk, potato, oil, fat</td>
<td>protein Starch Oil/fats</td>
<td>Proteases Amilases Lipases</td>
</tr>
<tr>
<td>Human Body</td>
<td>Blood, perspiration, grease</td>
<td>Protein Human grease</td>
<td>Proteases Lipases Cellulases</td>
</tr>
<tr>
<td>Others</td>
<td>Grass, Cosmetics</td>
<td>protein Fats/lubricants</td>
<td>Proteases Lipases</td>
</tr>
</tbody>
</table>

**Spot origin**
- BBQ sauce spot
- Washing powder without enzymes
- Washing powder WITH amilases
• Waste water treatment:
  - Cotton desizing and scouring
  - Bleaching
  - Dyeing

Biotechnology approximation:
  - Enzymatic treatment with catalases (Hydrogen peroxide) and laccase (dyes)
<table>
<thead>
<tr>
<th>ENZYME</th>
<th>SUBSTRATE</th>
<th>APPLICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulase</td>
<td>Cellulose</td>
<td>- Jeans finishing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Cotton smoothing</td>
</tr>
<tr>
<td>Amilase</td>
<td>Starch</td>
<td>- Desizing</td>
</tr>
<tr>
<td>Pectinase</td>
<td>Pectine</td>
<td>- Washing (alkaline products replacement)</td>
</tr>
<tr>
<td>Catalase</td>
<td>Peroxides</td>
<td>- Bleaching</td>
</tr>
<tr>
<td>Laccase</td>
<td>Pigments and dyes</td>
<td>- Wastewater treatment</td>
</tr>
<tr>
<td>Lipase</td>
<td>Oils and fats</td>
<td>- PET hidrofobicity improvement</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>Pigments and dyes</td>
<td>- Excess dye</td>
</tr>
</tbody>
</table>
COTTON
Biotechnology

α Amylase

Pectinase

Desizing

Catalase

Bleaching

Excess of bleaching agent

Excess of dye

Dyeing

Finishing

Cellulase

Grey fabric

Finished fabric

Desizing

Stone washed

Bleaching

Cellulase

Laccase

Finished garment

Better quality of end products (value added)

Reduction of pollution and residues

Reduction of cost (energy, water and raw materials)

DYEING
## Introduction

<table>
<thead>
<tr>
<th>Process</th>
<th>Conventional</th>
<th>Enzymatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desizing</td>
<td>NaOH pH=10, T=90°C</td>
<td>Amilase pH=7.2, T=60°C</td>
</tr>
<tr>
<td>Scouring</td>
<td>NaOH pH=10, T=90°C</td>
<td>Pectinase pH=7.2, T=60°C</td>
</tr>
<tr>
<td>Excess of bleach</td>
<td>Rinse with water excess</td>
<td>Catalase pH=7.2, T=40°C</td>
</tr>
<tr>
<td>Excess of dye</td>
<td>Rinse with water excess</td>
<td>Peroxidase</td>
</tr>
<tr>
<td>Polishing</td>
<td>-</td>
<td>Cellulase pH=7.2, T=60°C</td>
</tr>
</tbody>
</table>
BIOTECHNOLOGY: Scouring

Removal of natural or added impurities (waxes, powders...).

Pectinase acts only on the primary cell wall.
BIOTECHNOLOGY: Scouring

Methods

Traditional
- 2 – 5 g/l NaOH 100%
- 30 – 60 min at 100°C
- Water rinsing necessary
- Neutralization before dying

Alternative
- Enzymatic treatment (pectinase)
- Optimal temperature 55 - 60°C
- Temperature increase to 85°C to eliminate all waxes
- No neutralization needed
1. DESIZING + SCOURING

**Treatment 1: conventional**
1 h, 90 °C, pH=10
NaOH + Cotemoll + Amplex
Rinse at 90 °C for 15 min
3 Rinses at RT (3 x 10 min)

**Treatment 2: enzymatic (combined)**
180 min, 60 °C, pH=7.2
Cotemoll + Amplex + Aquazym + Scouzym
Rinse at 90 °C for 15 min
3 Rinses at RT (3 x 10 min)
1. DESIZING + SCOURING

Cotton Type

1
2
3
4

60 90 120 180 C t(min)

BLUE: starch presence
YELLOW: starch absence

Weight reduction

%

60 90 120 180

minutes
BIOTECHNOLOGY: Scouring

Bioscouring

Alkaline treatment

- Low fiber attack
- Lower weight loss
- Fiber surface smoothing
- Aspect improvement
- No alkaline wastes on fiber
Bleaching

- Hydrogen peroxide is the universal bleaching agent
- Alkaline conditions are needed
- Rinsing is necessary to avoid interferences with other processes

Catalase

$$\text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \text{O}_2$$

Decolorization

Laccases and peroxidases can oxidize a wide range of substrates (ex. azo dyes...)

Detoxified product + $\text{N}_2$
Samples of correctly desized denim

Samples of incorrectly desized denim with visible marks
**Biofinishing**

Most textiles based on natural fibers tend to fray. With use these threads tend to form small “balls”.

Biofinishing eliminates free threads and reduces this effect by partial hydrolysis with cellulase.
Treatment 3: global processing
180 min, 60 °C, pH=7.2
    Cotemoll + Amplex + Aquazym + Scouzym

Bleaching Reaction: 60 min, 90 °C, pH=10.4
    H$_2$O$_2$ + Na$_2$SiO$_3$ + Na$_5$P$_3$O$_{10}$ + MgSO$_4$

Catalase addition 20 min, 40 °C
Cellulase addition

Rinse at 90 °C for 15 min
3 Rinses at RT (3 x 10 min)

Further works will be done…
Cotton processing by enzymatic catalysis is possible

Although the required time for enzymatic desizing+scouring is longer, this lost is compensated by time recovered between processes

There is a reduction in the production costs (water and energy)

First trial for the combination of steps in cotton processing in a (semi) continuous way

Starting point for future research and developments in enzymatic catalysis combinations
WOOL
**Carbonization**

Vegetable impurities elimination with sulphuric acid can be substituted by enzymatic treatment (cellulases, pectinases, xylanases and proteases).

**Anti-shrinking**

Chemical treatments are performed to modify fiber surface and reduce or eliminate friction gradient root and end. Anti-shrinking treatments consist in a type of smoothing or surface cover. Some studies have been performed showing that proteases and lipases can perform this action.
POLYESTER
BIOTECHNOLOGY: PES modification

OBJECTIVE: Reduce hydrophobicity
Reduce electrostatic charge

Polyethylene tereftalate chemical structure (PET)

Lipase / Esterase / Cutinase

Hydroxyl groups formation on fiber surface

R COO R' + H2O → R COOH + HO R'

w/o treatment
Biotech treatment
BIOTECHNOLOGY: PES modification

Conventional (sulphuric acid)

Alternative (enzymes)
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BIOTECHNOLOGY: Microbiology

- Antimicrobial finishing:
  - Surface treatment
  - Covalent linking
  - Microencapsulation
  - Copolymerization or biopolymerization

Staphylococcus aureus

Aspergillus niger
1.- Antimicrobial finishing
2.- Antifungal finishing
3.- Anti mite finishing
4.- Sanitary textiles
6.- Lab tests
The use of antimicrobial products in the textile sector is an area of increasing interest.

The control of bacteria can be achieved using normal finishing processes of the textile industry, to create a value added of great attraction for the consumers and the businessmen.

The antimicrobial finishing can control smells, discoloration, spots and degradation result of the microbial attack to the fabrics.
Antimicrobial products are chemical substances synthesized partially or totally in the laboratory that inhibit the growth of microorganism. Nowadays, the use of essential oils and natural products are increasing.

Depending on its action we can distinguish:

- **Bacteriostatic**: avoid the growth and multiplication of microorganism, without destroying them completely, so they can growth again when the antimicrobial product disappears.

- **Bactericide**: their action is lethal for bacteria and irreversible.
Antimicrobial finishing according to the mechanism of their action:

- **Migrants**: Products that spread and act as a poison for microorganisms.

- **Non-migrants**: Products that destroy the microorganism when they are in contact with it (acting on the membrane). This type of products can be fixed chemically on the fibers using resins, etc.
Mechanisms of antimicrobial incorporation on textiles:

> Incorporating the product in the polymer before the extrusion. Only applicable to synthetic fibers.

✓ By means of surface finishing of the woven.
Main benefits of the application of anti-bacterial finishing are:

- **Control the bad odor**: skin own bacteria proliferate in the humid and warm atmosphere of our clothes. While bacteria reproduce produce gases (butyric acid), which are the familiar scents of the sweating. With this finishing bacteria from the skin or the environment cannot grow and therefore produced gases are reduced and bad odor does not appear.

- **Increase the life of textiles**: the smaller proliferation of bacteria diminishes the injurious action produced by its metabolism.
• **To prevent spots and changes in the aspect:** produced by the excessive proliferation of bacteria in a certain zone of the textile (for example zone of the armpits).

• **To avoid infections:** of great application in hospitals and health centers. They allow to reduce the risks of post-operative infections, disruption that suffer one of each ten people who are hospitalized. Its main application would be in mattresses, bed clothes (for example: absorbent covers for people who suffer of incontinence), dressings… The development of these textiles is really important for the use in “specially dangerous” areas that need an strict sterility control (immuno-depressed patient, ICU, infected by MRSA…).
Nevertheless, the use of the anti-bacterial finishings must follow the following norms:

➢ With respect to the natural barrier of the skin that acts avoiding the external microbial incursions and prevents the fixation of pathogenic bacteria. For that reason, the indiscriminate elimination of those microorganisms has non-desired consequences. The rupture of the ecological balance of the bacteria due to the excessive application of antiseptics increases the risks of infection. In order not to alter to the ecosystem of the skin, anti-bacterial fabric manufacturers must take care of this balance. They must obtain effective finishing with a high selective capacity.

➢ They do not have to kill the germs, but simply limit their development.
• Since it might be impossible to avoid direct contact of the anti-bacterial product with the skin and do not exist precise norms for the manufacturers of these fabrics, both they and consumers must be very prudent when selecting to use an antimicrobial textile product.

• Possible long term development of resistance to the substances used by the common bacteria.
Some of the anti-bacterial substances that are used in the textile industry are:

- Bamboo
- Chitosane
- Zinc chloride
- Phenolic compounds (thimol, carvacol)
- Silver compounds: with different mechanisms of action which makes it very effective:
  - Reaction with different groups of protein and enzymes.
  - Interference in the functions of DNA and RNA
  - Modification of the plasmatic membrane of the cell.

Due to these three mechanisms of independent action, it is very difficult for bacteria to develop resistance to this type of compounds.
The main applications of the anti-bacterial finishings are:

- **Industrial area:**
  - Work clothes
  - Air cleaners

- **Hospital area:**
  - Surgery clothes
  - Gauzes, bandages
  - Bed Clothes (sheets, protectors.)
  - Mouth protection

- **Final uses:**
  - Sport clothes
  - t-shirts, jackets
  - Tights
  - Underclothes
• Fungi contribute to textile accelerated degradation of cellulose origin and protein constitution through the enzyme secretion.

• The decomposition of textiles is considered as a harmful process directly implicated in loss of its specific properties.

• It is necessary to consider that besides visible fungi (*Aspergillus Niger*), the textiles also can be attacked by microfungi (*Gliocadium, Penicillum*...).
Some fungal effects on fabrics are:

- Changes in the color
- Diminution of the tensile strength
- Variation in the percentage of fibers extension
- Changes in the aspect
The main applications of the anti-fungals are:

• **Industrial area:**
  - Work Clothes
  - Air cleaners
  - Tools and clothes for the nautical sector

• **Hospital area:**
  - Gauzes, bandages
  - Respiratory tract protection

• **Final uses:**
  - Sport clothes
  - Underclothes
• Mites are arthropods, that can be found in different atmospheres; domestic dust, mattresses, sofas, carpets…

• Mites use human dead skin cells and other organic products to growth.

• They are recognized as responsible of the allergies produced by house dust since the end of years 60.

• The species that produce most frequently it are those of the *Dermatophagoides* family:
  
  • *Dermatophagoides pteronyssinus*
  • *Dermatophagoides farinae*
  • *Dermatophagoides microceras*
• Traditional ways to avoid them and to reduce their harmful effects are:
  • Avoid humid and closed places
  • Use of de-humidifiers apparatus that reduce the level of mites.
  • Ventilate rooms
  • Use of equipment that emits ultrasounds

• The most effective system is the use of covers for mattress and pillows with anti-mites substances.
• Some substances used to avoid the proliferation of the mites are:

  • piperonil Butoxide
  • 2 phenyl phenol
  • Piretrine: obtained natural compound of certain flowers of chrysanthemum.
  • Esbiol
  • Benzyl benzoate
  • permetrine: it is applied for example to the wool

• Butoxide and 2-phenyl phenol are enhancers of the anti mite activity of the other compounds.
The main applications of the anti mite products are:

- **Industrial area:**
  - Control the development of mites or insects in warehouses where wools or other weaves are accumulated.

- **Final uses:**
  - Bed clothes
  - Carpets
Most post-operation infections take place during the surgery because microrganims can reach the open wound.

Surgery clothes have to guarantee:

- sanitary personnel protection
- patient protection
- Prevention of post-operation infections

The operating room dressing gowns must be made of cotton with a density of 42 - 80 thread/cm and in addition must be waterproof.

After 75 cycles of washing they lose the barrier properties and must be replaced.

The alternative is the use of disposable dressing gowns (cellulose + plastic)
The European test EN 13795 establishes the requirements for cloth, dressing gowns and suits of clean air of surgical uses like sanitary products, for patients, clinical personnel and equipment. This test represents the technical link to fulfill the specifications of the Sanitary Product Directive 93/42/EEC.

They explain requisite of production and product, like the testing methods and the levels of fulfillment.
• Some properties that are evaluated are:

- Material resistance to the penetration of microorganisms in different conditions: in dry air (IN ISO 22612) and in humid environment (IN ISO 22610). In both cases samples are subjected to mechanical stress.

To test dry penetration *Bacillus subtilis* spores are used. This bacteria grows in the ground and can be easily transported by organic and inorganic particles.
Particle loss capacity, because these can transport microorganisms. The sample is subjected to compression and torsion movements that imitate the movements that they take place during the medical practice. The particles are moderate that oscillate between 3.25 is µm (ISO 9073-10).
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In the microbiology lab the effectiveness of the previous finishings can be tested following different norms:

- Tests related to anti mite products:
  - AATCC 194:2006
  - EN ISO 3998:1977

- Tests related to antifungal finishes:
  - AATCC 30:2004
  - UNE EN 14119:2004
  - UNE EN 846
LABORATORY TEST

- Tests related to anti microbial finishings:
  - UNE EN ISO 20645:2004
  - SN 195:920
  - JIS L 1902:2002
  - ISO 20743:2007
  - AATCC 100
  - ASTM E 2149-01
The referring norms to anti-bacterial specify the use of two types of bacteria (a representative of the group of the Gram - and Gram + bacteria) although, depending the final use of the sample or of the concrete specifications of the client they can use others.

- Staphylococcus aureus
- Klebsiella pneumoniae
- Psedomonas aeruginosa
- Escherichia coli
- MRSA
- Candida albicans
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¿IS BIOENGINEERING IN THE TEXTILE INDUSTRY USEFUL?
Yes, if new materials, products and processes can be developed to increase competitiveness and develop new market opportunities.

¿IS IT NECESSARY?
To improve processes and products and to reduce the impact of the industry on environment, health and safety.

¿IS IT POSSIBLE?
Yes in many applications. Necessary to make it sustainable and economically feasible.

¿WHAT IS NEEDED?
Know-how, state of the art and enterprising.