Horizon 2020 Work programme
Food Security, Sustainable Agriculture and Forestry, Marine, Maritime and Inland Water Research and the Bioeconomy
Call
H2020-FNR-2020: Food and Natural Resources
Topic name
FNR-16-2020: ENZYMES FOR MORE ENVIRONMENT-FRIENDLY CONSUMER PRODUCTS

FuturEnzyme:
Technologies of the Future for Low-Cost Enzymes for Environment-Friendly Products
Final ID: 101000327

04/08/2021

MANUFACTURERS' NEEDS AND SPECIFICATIONS: PROTOCOL

DELIVERABLE NUMBER D2.1

MANUEL FERRER
CSIC
MARIE CURIE 2, 28049 MADRID, SPAIN

Document information sheet

Work package:	WP2, Machine learning enzyme bio-prospecting integrated into an industrial context
Authors:	CSIC (Manuel Ferrer, Patricia Molina) ITB (Sara Daniotti, Ilaria Re) CLIB (Tatjana Schwabe, Tobias Klement) Henkel (Susanne Wieland, Christian Degering) Evonik (Moniec van Logchem, Hans Henning Wenk) Schoeller (Roland Lottenbach, Rainer Roesch, Nazanin Ansari)
Document version:	1
Date:	04.08.2021
Starting date:	01.06.2021
Duration:	48 months
Lead beneficiary:	CSIC
Participant(s):	CSIC, ITB, CLIB, HENKEL, EVONIK, SCHOELLER
Dissemination Level:	Confidential, only for consortium's members (including the Commission Services)
Type	Report
Due date (months)	3
Contact details:	Manuel Ferrer, mferrer@icp.csic.es; Patricia Molina, patricia.molina@icp.csic.es

Summary

Manufacturers' needs and specifications: Protocol

1. Scope of Deliverable

This deliverable will consist in a report containing information about manufacturers' needs, and enzymes and products specifications (working/storage conditions and stabilities, compositions, etc.) for implementing 3 innovative, real-life, and environment-friendly products (detergents, textiles and consumer care products). Such draft information and the identities of benchmark enzymes and working parameters will be collected from manufacturers and through screening academic publications and patents. This report that will be delivered at month 3, will be continuously updated within the life-time of the project. In addition to that, the report will also contain information about the real-life substrates suggested and to be provided by industrial partners to partners involved in enzyme screening and characterisation. The report will be made available in the internal FuturEnzyme repository.

2. Henkel' needs and specifications

Table 1 summarizes the HENKEL' needs and specifications.

Table 1. HENKEL' needs and specifications.

	LIQUID/DOSE CAP DETERGENT
Products to be made	Laundry & Home Care (LHC)'s leading premium liquid detergent and/or unit dose caps products with enzymes.
Request	Enzymes for removing fatty oil stains.
Innovation	Innovation will come because the use of enzymes will improve removal of stubborn stains at low temperatures while decreasing chemical usage. A central point is to lower the amount of surfactant in the detergent formulation as much as possible by adding enzymes.
Priority enzymes to be targeted	Among all enzyme classes discussed in the proposal, priority target will be enzymes for removing specific fatty oil stains, that will include: • True lipases (EC 3.1.1.3) • Esterases (EC 3.1.1.1) • Cutinases (EC 3.1.1.74) and related fatty-oil degrading hydrolases
Non-priority enzymes to be targeted	Aside the priority classes, other enzyme classes relevant to detergents are also considered, that include: • Proteases/peptidases, suitable for protein-based stain removal (i.e. blood, milk, grass) at low temperature, e.g., type family S08 (alcalase), type papain (EC 3.4.22.2), type savinase-esperase (EC 3.4.21.14), type subtilisin-alcalase (EC 3.4.21.62), type trypsin and protease inhibitor. • Amylase (EC 3.2.1.1) and other glycoside hydrolases • Peroxidases and related enzymes (EC 1.1.3.-, EC 1.11.1.- or EC 1.10.3.2), very specific in the potential use case (to be discussed in more detail in case they become relevant).
Specifications that enzymes should meet	The enzymes should be active and stable under conditions relevant to the wash cycle and to storage. Below, the specifications are summarized: • The enzymes should be stable for at least 2 to 3 months at 30˚C in the liquid detergent formulation. Note: This stability refers to the stability of the enzymes in the detergent formulation.

	• The enzymes should be effective and stable at a washing temperature between 20 and 40˚C and at pH 7.0-8.5, at least during an operation time of a common wash cycle (120 min). Note: This stability and activity refer to that of the enzymes in a wash liquor mimicking the detergent-water mixture in a washing machine; this wash liquor consists in about 50 g liquid detergent per 20 liter of water. In general, Henkel strongly recommends to concentrate on the screening methods which can be performed in a wash liquor matrix (instead of standard buffers, etc.) as early as possible, since this affects the enzyme properties often quite strongly. Henkel will provide to partners involved in enzyme screening and characterization (CSIC, BANGOR, CNR, IST-ID, UDUS, UHAM) a sample of the LHC's leading premium liquid detergent without benchmark enzymes. Addresses of partners to receive from Henkel the detergent product without enzyme: Prof. Peter Golyshin Centre for Environmental Biotechnology (CEB) School of Natural Sciences Thoday bldg. 2nd floor, 313.2 Bangor University, Gwynedd, LL57 2DG Bangor, United Kingdom Phone: +44 (0)1248 383587, ext 3629 Prof. Michail M. Yakimov Marine Molecular Microbiology & Biotechnology CNR - Institute for Biological Resources and Marine Biotechnology Spianata San Raineri, 86 – 98122 Messina, Italy Phone: +39 090 6015437 Dr. Alexander Bollinger Institut für Molekulare Enzymtechnologie (IMET) Heinrich-Heine-Universität Düsseldorf Forschungszentrum Jülich Wilhelm Johnen Straße, Bldg 15.8, 01/303, 52428 Jülich, Germany Phone: 02461 616966 Prof. Carla de Carvalho iBB-Institute for Bioengineering and Biosciences Department of Bioengineering, Torre Sul, 7º piso Instituto Superior Técnico Av. Rovisco Pais 1049-001 Lisboa Portugal Phone: + 351 218 4195 94 Prof. Dr. Wolfgang Streit Universität Hamburg Department of Microbiology and Biotechnology

<table>
<tr><td></td><td>Ohnhorststrasse 18, 22609
Hamburg, Germany
Tel: +49 40 42816 463/461

Prof. Manuel Ferrer
Instituto de Catálisis y Petroleoquímica (ICP-CSIC)
C/Marie Curie nº2, 28049, Madrid, Spain
Phone: +34 91 585 4872</td></tr>
<tr><td>Benchmark enzymes</td><td>For comparisons, Henkel will provide to partners involved in enzyme screening and characterization (CSIC, BANGOR, CNR, IST-ID, UDUS, UHAM) a sample of the LHC's leading premium liquid detergent with benchmark enzymes.

This product will be provided, as information about specific benchmark enzymes integrated into LHC's products cannot be disclosed by Henkel. Instead, CSIC has started a large bibliographic and patent search so as to find benchmark enzymes, patented and of use in detergents, that we can use for comparisons (see Section 5). In addition, CSIC has contacted (27.07.2021) a representative of Novozymes in Spain, Gerard Santiago (GSG@novozymes.com), in order to get free samples of enzymatic preparations commonly sold or dispensed for preparing detergents; once received, the information and samples will be shared with partners, so that they can use for comparative purposes.</td></tr>
<tr><td>Substrates</td><td>Priority standard substrates will correspond to those relevant to the enzyme classes to prioritize, in particular fatty oils. Below, a list of (A) commercially available standard soils on textiles and (B) natural soils of interest with high consumer relevance for the detergent products to be developed are detailed.

A: Commercially available standard soil textiles
<table>
<tr><th>No.</th><th>ID</th><th>Soil components</th><th>Textile</th><th>Provider</th></tr>
<tr><td>1</td><td>C-S-61</td><td>Beef lard[2]</td><td>CO</td><td>CFT[1]</td></tr>
<tr><td>2</td><td>PC-09</td><td>Pigment/oil</td><td>PES/CO</td><td>CFT[1]</td></tr>
<tr><td>3</td><td>PC-S-132</td><td>Pigment/sebum[3]</td><td>PES/CO</td><td>CFT[1]</td></tr>
<tr><td>4</td><td>CS-S-05s</td><td>Mayonnaise with carbon black[4]</td><td>CO</td><td>CFT[1]</td></tr>
<tr><td>5</td><td>C-S-10</td><td>Butterfat with colourant[5]</td><td>CO</td><td>CFT[1]</td></tr>
<tr><td>6</td><td>PC-S-16</td><td>Lipstick, pink[6]</td><td>PES/CO</td><td>CFT[1]</td></tr>
<tr><td>7</td><td>C-S-17</td><td>Make up[7]</td><td>CO</td><td>CFT[1]</td></tr>
</table>
[1]CFT: CENTER FOR TESTMATERIALS (https://www.cftbv.nl)
[2]C-S-61 - Beef fat, coloured with Sudan red dye (based on bibliographic records beef lard is mainly constituted by triglycerides based on C16:0, C18:0 and C18:1, as well as C12:0, C14:0, C16:1, C17:0 and C18:2 in lower amount).
[3]PC-S-132 - Pigment/sebum (based on bibliographic records sebum is a complex lipid mixture composed of wax and sterol monoesters and cholesterol esters, such as cholesteryl oleate, oleyl oleate, palmityl palmitate, tristearin, and triolein).
[4]CS-S-05s - Mayonnaise with carbon black (based on bibliographic records mayonnaise is mainly constituted by emulsion of oil, egg yolk , as well as vegetable oil that included saturated, monounsaturated and polyunsaturated fatty acids, lipids, triglycerides, cholesterol and phospholipids, e.g. C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, etc.).
[5]C-S-10 - Butterfat with colourant (based on bibliographic records butter fat is mainly constituted by triglycerides such as C10:0, C12:0, C14:0, C16:0, C18:0, C18:1, C18:2, C18:3, etc.)</td></tr>
</table>

<table>
<tr>
<td></td>
<td>
[6]PC-S-16 - Lipstick, pink (based on bibliographic records lipstick is mainly constituted by wax (e.g. beeswax that consists of esters of straight-chain alcohols with carbon chains from C_{24} to C_{36} such as triacontyl palmitate, carnauba wax, candelilla wax, etc.), oil (such as petrolatum, lanolin, cocoa butter, shea butter, mango seed butter, shea butter, avocado butter, avocado oil, jojoba, castor, and mineral oil), and pigment (e.g. carmine red/pink or carminic acid, eosin)).
[7]C-S-17 - make up (based on bibliographic records make up is mainly constituted by paraben esters such as methyl, propyl, ethyl, butyl or isobutylparaben, isopropyl myristate, caprylic/capric triglyceride, tocopheryl acetate, etc.)

B: Natural soils of interest
<table>
<tr><th>No.</th><th>Soil components</th></tr>
<tr><td>1</td><td>Cuff and collar[1]</td></tr>
<tr><td>2</td><td>Natural skin fat[1]</td></tr>
<tr><td>3</td><td>Butterfat[2]</td></tr>
<tr><td>4</td><td>Olive oil</td></tr>
<tr><td>5</td><td>Frying fat[3]</td></tr>
<tr><td>6</td><td>Lard[4]</td></tr>
<tr><td>7</td><td>Tomato beef sauce</td></tr>
</table>
[1]Cuff and collar could contain natural skin fat/human sebum consisting of esters of glycerol (triglycerides), wax and cholesterol.
[2]Butter fat is mainly constituted by triglycerides such as C10:0, C12:0, C14:0, C16:0, C18:0, C18:1, C18:2, C18:3, etc.
[3]Frying fat may include coconut (triglycerides of C8:0, C10:0, C12:0, C14:0, C-16:0, C18:0, C18:1 and C18:2), palm (mainly C16:0, C18:0, C18:1, C18:2 and C18:3) , butter, lard (fat from pigs) or tallow (beef or sheep fat).
[4]Beef lard may include triglycerides based on C16:0, C18:0 and C18:1, as well as C12:0, C14:0, C16:1, C17:0 and C18:1.

CSIC has already ordered (22.07.2021) the above standard soil textiles, and once received they will be distributed among partners.
</td>
</tr>
<tr>
<td>Remarks</td>
<td>According to the above priority enzymes and soils on textiles and natural soils, the methods for screening and characterizing the enzymes (e.g. lipases) need to be adapted by partners, as detailed in Deliverable 3.2. In general Henkel strongly recommends to concentrate on the screening methods which can be performed in a wash liquor matrix (instead of standard buffers) as early as possible, since this affects the enzyme properties often quite strongly. It is similarly crucial to screen on textiles as soon as possible, too, as they are more challenging than stains alone.</td>
</tr>
</table>

3. Evonik' needs and specifications

Table 2 summarizes the EVONIK' needs and specifications.

Table 2. EVONIK' needs and specifications.

	COSMETIC FORMULATIONS
Products to be made	EVO's leading cosmetics integrating ingredients produced by enzymes.
Request	Enzymes for degrading hyaluronic acid to products of defined size to be integrated into cosmetics.
Innovation	Hyaluronic acid is widely used for cosmetic applications where it mainly acts as natural moisturizer and as anti-aging active. Specially, the biological anti-aging activity is limited by the enormous molecular size of hyaluronic acid that can reach up to 2,000 kDa and interferes with its penetration into the skin. Fragmentation of large hyaluronic acid polymers can markedly improve its penetration abilities. Nevertheless, pro-inflammatory responses have been reported for very small hyaluronic acid fragments (5-15 kDa) which are recognized by special receptors of the immune-system; therefore, size matters, and has to be above or below a specific threshold. In this case it should be below 5 kDA, prefered 1-2 kDa, so that the new molecule will better penetrate into the skin, making the cosmetic more effective, and the production process more sustainable.
Priority enzymes to be targeted	Priority targets will be enzymes degrading hyaluronic acid: • Heparanase (EC 3.2.1.166) • Hyaluronate lyase (cd01083 - EC 4.2.2.1) • Hyaluronidase (EC 3.2.1.35, EC3.2.1.36, pfam03662, pfam01630).
Specifications that enzymes should meet	Hyaluronic acid is actually produced by fermentation of *Bacillus subtilis* (non-pathogenic) and an environmentally friendly, solvent free recovery process. Existing technologies like thermal degradation are unsuitable for achieving the targeted molecular weight and polydispersity. We can envision two options for producing small hyaluronic acid with 1-2 kDA molecular weight: • An enzyme that can be added during the fermentation to prevent additional process steps to make the small hyaluronic acid. The possibility that the new enzyme can be integrated into the *Bacillus subtilis* that produce the hyaluronic acid may be also evaluated. • An enzyme that can be added after fermentation in the current solvent free process, which should improve the LCA. The fermentation conditions and the thermal denaturation conditions cannot be provided by Evonik. CSIC will start a large bibliographic and patent search to find most common conditions for such processes.
Benchmark enzymes	Based on current state of the art to reduce hyaluronic acid with MW 800-1000 kDA to smaller molecular weight products, the following enzymes are being tested and can be used as benchmark: • Hyaluronate lyase from *Streptococcus pyogenes* (Sigma-Aldrich Co. LLC, ref. 56177, 8.0 units/mg protein; 5.0-15.0 mg/mL). • Hyaluronidase from bovine testes (Sigma-Aldrich Co. LLC., ref. H3506; 400-1000 units/mg solid).

<table>
<tr>
<td></td>
<td>The use of these enzymes resulted in too less reduction of molecular weight and (too) long (>24 h) process time. For molecular weight determination Evonik uses GPC-MALDI, and CSIC high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), whose description is provided in Deliverable 3.2.</td>
</tr>
<tr>
<td>Substrates</td>
<td>Priority real-life substrates will correspond to that relevant to the enzyme classes to prioritize, in particular, hyaluronic acid. The following hyaluronic acid substrates are available:

A: Available hyaluronic acid substrates
<table>
<tr><th>No.</th><th>ID</th><th>Provider</th></tr>
<tr><td>1</td><td>High molecular weight hyaluronic acid produced after fermentation with B. subtilis</td><td>Evonik</td></tr>
<tr><td>2</td><td>High molecular weight hyaluronic acid (ref. 53747)</td><td>Sigma-Aldrich</td></tr>
<tr><td>3</td><td>Low molecular weight (50 kDa) hyaluronic acid HyaCare® 50</td><td>Evonik</td></tr>
<tr><td>4</td><td>Low molecular weight hyaluronic acid (<10 kDa), Hyalo-Oligo</td><td>Kewpie Corp.</td></tr>
</table>

Evonik and CSIC will provide partners involved in enzyme screening and characterization (CSIC, BANGOR, CNR, IST-ID, UDUS, UHAM) samples of the hyaluronic acid and the specification of the delivered material. In particular, Evonik has delivered (12.07.2021) a sample (5 grams) of the hyaluronic acid produced after fermentation with B. subtilis, and the lower molecular weight hyaluronic acid HyaCare® 50 average MW 50 kDa. CSIC will provide to partners hyaluronic acid from Sigma-Aldrich (ref. 53747) as well as Hyalo-Oligo (from Kewpie Corp.).

Addresses of partners to receive hyaluronic acid samples:

Prof. Peter Golyshin
Centre for Environmental Biotechnology (CEB)
School of Natural Sciences
Thoday bldg. 2nd floor, 313.2
Bangor University, Gwynedd, LL57 2DG
Bangor, United Kingdom
Phone: +44 (0)1248 383587, ext 3629

Prof. Michail M. Yakimov
Marine Molecular Microbiology & Biotechnology
CNR - Institute for Biological Resources and Marine Biotechnology
Spianata San Raineri, 86 – 98122
Messina, Italy
Phone: +39 090 6015437

Dr. Alexander Bollinger
Institut für Molekulare Enzymtechnologie (IMET)
Heinrich-Heine-Universität Düsseldorf
Forschungszentrum Jülich
Wilhelm Johnen Straße, Bldg 15.8, 01/303, 52428
Jülich, Germany
Phone: 02461 616966</td>
</tr>
</table>

	Prof. Carla de Carvalho iBB-Institute for Bioengineering and Biosciences Department of Bioengineering, Torre Sul, 7º piso Instituto Superior Técnico Av. Rovisco Pais 1049-001 Lisboa Portugal Phone: + 351 218 4195 94 Prof. Dr. Wolfgang Streit Universität Hamburg Department of Microbiology and Biotechnology Ohnhorststrasse 18, 22609 Hamburg, Germany Tel: +49 40 42816 463/461 Prof. Manuel Ferrer Instituto de Catálisis y Petroleoquímica (ICP-CSIC) C/Marie Curie nº2, 28049 Madrid, Spain Phone: +34 91 585 4872 According to the substrate to be used (hyaluronic acid), the methods for screening and characterizing the enzymes need to be adapted by partners, as detailed in Deliverable 3.2.

4. Schoeller' needs and specifications

Table 3 summarizes the SCHOELLER' needs and specifications.

Table 3. SCHOELLER' needs and specifications.

Priority	**1**
Possible applications/scope	Cleaning/pretreatment of synthetic fibres
Substrate	Polyester fibres (PES) / polyamide fibres (PA) containing elastane (polyether-polyurea copolymer)
Desired effect/change	Fully removal of spinning additives (see details below*)
State of the art	Solvent cleaning or insufficient washing, which creates problems in the subsequent processing
Impact to Schoeller	Huge
Impact to other textile producers	Huge
Priority High-Med-Low	High
Lab application possible?	Yes
Test method	Analytical extraction
Effect/result proof	Reducing dyeing, finishing problems and second quality products
How to quantify	1. Avoiding solvents 2. Bulk trial dyeing comparison
Reducing reworks and off-quality	Yes
Comments	-
Priority enzymes to be targeted	Lipases, cutinases, poliuretanases, amidases
Conditions for process/product	See details below*

Screening method for enzymes	The methods for screening and characterizing the enzymes need to be adapted by partners, as detailed in Deliverable 3.2.

Priority	**2**
Possible applications/scope	Chalk marks
Substrate	Cotton (CO), polyester fibres (PES), polyamide fibres (PA)
Desired effect/change	Solving the problem of writing on the finished textile
State of the art	F-based marks for hydrophobic materials
Impact to Schoeller	Huge
Impact to other textile producers	Huge
Priority High-Med-Low	High
Lab application possible?	Yes
Test method	Physical, observational
Effect/result proof	With less chemicals, similar effects
How to quantify	Calculating the sparing amounts of chalkmarks
Reducing reworks and off-quality	Yes, sparing quite a lot of money through the whole textile processing chain
Comments	-
Priority enzymes to be targeted	Lipases, esterases, poliuretanases, amidases, cellulases
Conditions for process/product	
Screening method for enzymes	The methods for screening and characterizing the enzymes need to be adapted by partners, as detailed in Deliverable 3.2.

Priority	**3**
Possible applications/scope	Replacement of the bleaching processes
Substrate	Cotton (CO)
Desired effect/change	Decoloring of natural fibres and cotton hasks
State of the art	Chemical bleaching (Chlorid or Peroxid)
Impact to Schoeller	Low
Impact to other textile producers	High
Priority High-Med-Low	High to Low
Lab application possible?	Yes
Test method	Chemical test tensile, degree of whiteness and DP (degree of average polimerization)
Effect/result proof	Achieving maximum whiteness and reducing dye stuff
How to quantify	Saving on chemicals
Reducing reworks and off-quality	To some extent
Comments	-
Priority enzymes to be targeted	Bleaching enzymes (oxidoreductases)
Conditions for process/product	See details below*
Screening method for enzymes	The methods for screening and characterizing the enzymes need to be adapted by partners, as detailed in Deliverable 3.2.

Priority	**4**
Possible applications/scope	Surface functionalization/modification
Substrate	Polyester fibres (PES), modification and plasma treatment
Desired effect/change	Generating functional groups/layers

State of the art	Heating (natriumhydroxide) and atmospheric plasma
Impact to Schoeller	Medium
Impact to other textile producers	Medium
Priority High-Med-Low	Low
Lab application possible?	Yes
Test method	Physical testing (permanent treatments)
Effect/result proof	Bonding strenghts and higher washability
How to quantify	Managable
Reducing reworks and off-quality	No
Comments	-
Priority enzymes to be targeted	Lipases, cutinases, esterases
Conditions for process/product	See details below*
Screening method for enzymes	The methods for screening and characterizing the enzymes need to be adapted by partners, as detailed in Deliverable 3.2.

Priority	**5**
Possible applications/scope	Improved hydrophilicity
Substrate	Polyester fibres (PES) / polyamide fibres (PA) containing elastane (polyether-polyurea copolymer)
Desired effect/change	Higher absorbency (by pre-processing) and better humidity management (finishing)
State of the art	Solvent cleaning
Impact to Schoeller	Huge
Impact to other textile producers	Huge
Priority High-Med-Low	High
Lab application possible?	Yes
Test method	Physical testing- absorbency
Effect/result proof	Improved dyeing process, moisture management
How to quantify	Hydrophil tests for uniform hydrophilicity
Reducing reworks and off-quality	Yes
Comments	-
Priority enzymes to be targeted	Lipases, cutinases, poliuretanases, amidases, proteases (subtilisin, bromelain type)
Conditions for process/product	See details below*
Screening method for enzymes	The methods for screening and characterizing the enzymes need to be adapted by partners, as detailed in Deliverable 3.2.

Priority	**6**
Possible applications/scope	Improved hydrophobicity
Substrate	Polyester fibres (PES) / polyamide fibres (PA) containing elastane (polyether-polyurea copolymer)
Desired effect/change	Better water /soil repellency with less chemicals, removal of residual substrates
State of the art	Higher amounts of chemicals
Impact to Schoeller	Huge
Impact to other textile producers	Huge
Priority High-Med-Low	High

Lab application possible?	Yes
Test method	Physical testing
Effect/result proof	Improved water and soil repellency with less chemicals
How to quantify	Reduction of used chemicals
Reducing reworks and off-quality	Yes
Comments	-
Priority enzymes to be targeted	Lipases, cutinases, poliuretanases, amidases, proteases (papain)
Conditions for process/product	See details below*
Screening method for enzymes	The methods for screening and characterizing the enzymes need to be adapted by partners, as detailed in Deliverable 3.2.

Priority	**7**
Possible applications/scope	Improved fixation of PA dyeing (amino multiplier?)
Substrate	Polyamide fibres (PA)
Desired effect/change	Better fixation with fewer color consumption
State of the art	Chemicals treatment
Impact to Schoeller	High
Impact to other textile producers	High
Priority High-Med-Low	Medium
Lab application possible?	Yes
Test method	Fastness, dye consumption tests
Effect/result proof	Less dye materials and improved fastness
How to quantify	Dye stuff consumption and fastness
Reducing reworks and off-quality	Yes, especially reducing chemicals
Comments	-
Priority enzymes to be targeted	Amidases, proteases (alcalase, subtilisin), lipases, esterases
Conditions for process/product	See details below*
Screening method for enzymes	The methods for screening and characterizing the enzymes need to be adapted by partners, as detailed in Deliverable 3.2.

Priority	**8**
Possible applications/scope	Fewer water consumption in the dyeing process
Substrate	Polyester fibres (PES), cotton (CO)
Desired effect/change	Still large amounts of water is consumed in dyeing process; yet to be defined whether reduction is possible by enzyme treatment
State of the art	Extensive rinsing process a high water and time consuming process
Impact to Schoeller	High, technical feasibility with enzymes hard to realise
Impact to other textile producers	High
Priority High-Med-Low	High - see comments
Lab application possible?	Yes
Test method	-
Effect/result proof	-
How to quantify	Water energy saving
Reducing reworks and off-quality	-
Comments	-
Priority enzymes to be targeted	Lipases, cutinases, cellulases

Conditions for process/product	See details below*
Screening method for enzymes	The methods for screening and characterizing the enzymes need to be adapted by partners, as detailed in Deliverable 3.2.

Priority	**9**
Possible applications/scope	Higher effectiveness of existing enzyme treatments on natural and synthetic fibres
Substrate	Cellulosic fibre
Desired effect/change	Desizing, bleaching, bio-polishing
State of the art	Chemicals
Impact to Schoeller	Too Low
Impact to other textile producers	Relevant
Priority High-Med-Low	Low
Lab application possible?	-
Test method	-
Effect/result proof	-
How to quantify	Quite time-consuming compared to the existing processes
Reducing reworks and off-quality	-
Comments	Schoeller is using amylases for desizing of cellulosic frequently
Priority enzymes to be targeted	Cellulases and amylases
Conditions for process/product	See details below*
Screening method for enzymes	The methods for screening and characterizing the enzymes need to be adapted by partners, as detailed in Deliverable 3.2.

*Conditions for bio-processing with enzymes in the applications above, are briefly summarised below.

The substrate generally used for bio-processing includes paraffin, mineral oil, silicon oil, acrylic acids, and ester oils, and those chemicals need to be eliminated at the end of the processing procedure by the action of enzymes to avoid extensive water consumption.

Chemistry used for polyamide (PA)/polyethylene terephthalate(PET)/polyester (PES) fibres, would be:

- Thermostable ester oils as lubricants.
- Various fatty alcohol, fatty acid or fatty acid amide derivatives, ethoxylated or ethoxylated / propoxylated as emulsifier / wetting agent / cohesion component.
- Phosphoric acid esters, phosphonic acid derivatives as antistatic agents.
- Small amounts of antioxidants, corrosion protection agents and in some cases in-can preservatives.

Chemistry used for polyurethane (PUE) filaments would be:

- Low-viscosity silicone oils (PDMS) as lubricants.
- Low-viscosity mineral oils as lubricants.
- Magnesium stearate as a release agent.

Regarding texturing preparation, as a rule, 2 preparations are applied.

1. First, spin preparation during the spinning of the partially orientated yarn (POY) filament (layer approx. 0.4 percent by weight): ethylene oxide (EO) / propylene oxide (PO) copolymers as lubricants, fatty alcohol alkoxylates as wetting / spreading agents. Possibly small amounts of fatty acid ethoxylate as wetting / spreading agent or cohesive component. Smallest amounts of phosphoric acid ester as an antistatic agent.
2. During texturing, before winding, a winding oil (application approx. 1.5 - 3 percent by weight): mineral oil as a lubricant, fatty alcohol / fatty acid ethoxylate as an emulsifier.

In Europe in particular, there are always discussions in connection with emissions on the stenter caused by spool oil, and mineral oil in particular is held responsible for this. That is why there are also more thermally stable winding oils, but they are correspondingly more expensive and therefore not very common. There the mineral oil gets through replaces thermostable ester oils or carbonic acid esters (Bozetto technology).

In order for the partners to start the screening and characterisation in WP2-WP4, Schoeller will provide partners the required raw textile materials. The same material will be kept by for intern measurements at Schoeller. About 1 meter of each material is needed for standard material testing and first evaluations. At least, the following materials will be sent to partners:

Variant	Type	Article No.	Based material	Available Status in stock	Comp.[1] / Weight
1	Woven	61488	61488Z	Raw	92% PA, 8% EL 180 g/m^2
			61488Z	Pre-treated	
2	Woven	61988	61988F1	Raw	92% PA, 8% EL 280 g/m^2
			61988F1	Pre-treated	
3	Woven	67007	67007	Raw	88% PA,12% EL 135 g/m^2
			67007	Pre-treated	
4	Woven	3X58	3X58	Pre-treated	100% PES 100 g/m^2
5	Woven	66299	5237/00	Raw	92% CO, 8% EL 240 g/m^2
6	Warp-knitted	E03130	E03130	Raw	80% PA, 20% EL 160 g/m^2

[1]PA: Polyamide; EL: Elastane; PES: Polyester.

Extra details about the materials:

- The main aim for sending cotton fabric is the bleaching degree and whiteness. The main aim for sending the synthetic fabrics is to evaluate cleaning effects and spinning additives.
- With this list, both PES and PA are available as main synthetic material bases used in Schoeller products.
- Similar composition and different weight of variants 1-3 can be a good baseline for evaluating the weight parameter.
- Variant 4 is only available in pre-treatment or dyed status (for now), ordering the raw material is under clarification and will be communicated soon.
- Any raw material on Schoeller stock potentially can get a desired pre-treatment, but it takes longer than the already available pre-treated variants on stock.

Addresses of partners to receive standard fabrics by EVO for testing and first evaluations:

Prof. Peter Golyshin
Centre for Environmental Biotechnology (CEB)

School of Natural Sciences
Thoday bldg. 2nd floor, 313.2
Bangor University, Gwynedd, LL57 2DG
Bangor, United Kingdom
Phone: +44 (0)1248 383587, ext 3629

Prof. Michail M. Yakimov
Marine Molecular Microbiology & Biotechnology
CNR - Institute for Biological Resources and Marine Biotechnology
Spianata San Raineri, 86 – 98122
Messina, Italy
Phone: +39 090 6015437

Dr. Alexander Bollinger
Institut für Molekulare Enzymtechnologie (IMET)
Heinrich-Heine-Universität Düsseldorf
Forschungszentrum Jülich
Wilhelm Johnen Straße, Bldg 15.8, 01/303, 52428
Jülich, Germany
Phone: 02461 616966

Prof. Carla de Carvalho
iBB-Institute for Bioengineering and Biosciences
Department of Bioengineering, Torre Sul, 7º piso
Instituto Superior Técnico
Av. Rovisco Pais
1049-001 Lisboa
Portugal
Phone: + 351 218 4195 94

Prof. Dr. Wolfgang Streit
Universität Hamburg
Department of Microbiology and Biotechnology
Ohnhorststrasse 18, 22609
Hamburg, Germany
Tel: +49 40 42816 463/461

Dr. Fabrizio Beltrametti
BioC-CheM Solutions Srl
Via R. Lepetit, 34
21040 Gerenzano (VA)
Italy
Phone: +39 02 96474404

Prof. Manuel Ferrer
Instituto de Catálisis y Petroleoquímica (ICP-CSIC)
C/Marie Curie nº2, 28049 Madrid, Spain
Phone: +34 91 585 4872

5. State of the technology

CSIC and ITB prepared reports related to the IDENTIFICATION OF THE STATE OF THE TECHNOLOGY in the three sectors mentioned above. The objective of these reports is to locate that bibliography (both patent documents and non-patent literature) referring to the use of enzymes in the following applications:

- Hyaluronic acid production (breaking) processes, mainly in the field of cosmetics;
- Use of enzymes, mainly lipases, in detergent compositions;
- Use of enzymes in the field of textile production/treatment.

In a potential second stage, as much information as possible will be extracted from the documents retrieved in the searches on the type and characteristics of the enzymes that have been described for these processes and products, the conditions applied (amount of enzymes used, temperature, times, etc.), and on the companies behind these publications and developments together with their contact details. These reports will allow, among others:

- To be at the forefront of new inventions and developments (enzymes, products and processes) in the three technological areas of interest, so that we will have the technical information regarding the processes that have been developed or are being developed in those areas of knowledge;
- To carry out a comparison with our own processes/products or the development of the same;
- To identify the main applicants/actors in the areas under study, which could be considered as potential companies of interest, licensees, partners interested in the technology or for disseminating project activities via social media;
- To know the positioning of the technology, new trends, versatility, etc.

The outcome of the above search will allow deciphering the specifications that enzymes commonly match for process and product development for consumer products similar to the ones to be developed in FuturEnzyme. Below, the summarised outcome of the bibliographic and patent search is provided. In a further phase (August-September 2021), the reading of the documents found during the search process will allow detailing such specifications, which can be compared to those that enzymes to be developed in the frame of FuturEnzyme will have. This information will be included in an updated version of this deliverable.

5.1. State of the technology “Production of hyaluronic acid for cosmetics”

Based on the above needs and specifications we performed a background search regarding the enzymatic production methods of hyaluronic acid for cosmetics, with the aim of making the patent and non-patent documents that are part of the state of the art related to this technology available, namely, regarding the enzymatic production methods of hyaluronic acid for cosmetics. These documents are those located in the background search strategy that will be detailed below. For the retrieval of the state of the art documents, the PatBase database was consulted. PatBase is one of the most reliable databases used daily by patent professionals around the world as their main search tool. Organized by patent families, PatBase offers extensive full-text coverage of more than 95 issuing authorities around the world. Starting from the needs and specifications data, a search was carried out in this database that provides bibliographic data on patent and non-patent documents. To retrieve the patents information, a search strategy was designed using the keywords: “hyaluronic acid” and “enzyme” along with their synonyms and variants. In addition, the search has been limited to the cosmetic application using words like “cosmetic” and classification codes: A61K8 - Cosmetics or similar preparations. The search for scientific literature was performed using keywords such as “hyaluronic acid” and “enzymes”.

The search strategy that has been followed is:

Search Strategy	**Key words**	**Result**
Search to find everything related to obtain hyaluronic acid and its derivatives	Hyaluronate, hyaluronidase, hyaluronic acid	8,671
Search to find everything related to obtain hyaluronic acid using enzymes	Hyaluronate, hyaluronidase, hyaluronic acid and enzyme	852
Search to find everything related to obtain hyaluronic acid using enzymes in the cosmetic industry (including the classification code)	Hyaluronate, hyaluronidase, hyaluronic acid, enzyme and cosmetic	99

As a result of the background search, 169 results of patents were obtained, according to a number of keywords (Figure 1). About 67.1% of the patent applications are still active / alive, while the rest have expired or been abandoned. The analysis of how the presentation of new registries (families) has evolved and their extensions to the different countries (applications) allows us to conclude that it is a developing technology that has experienced growth in recent years (See Figure 2). In fact, almost 80% of patents have been applied for in the last 10 years. The countries in which it has been extended the most and, therefore, may represent potential markets of interest, are the United States, Japan, Australia, China and Canada. Within the European content, Germany (113 families) and Spain (86 families) stand out (See Table 4).

Figure 1. Main concepts and keywords retrieved from the searches.

Table 4. Top 10 countries by patent families and applications.

COUNTRY	FAMILIES	APPLICATIONS	GRANTS
UNITED STATES OF AMERICA	151	735	514
JAPAN	144	335	168
AUSTRALIA	119	267	173
CHINA	118	228	117
CANADA	116	224	114
GERMANY	113	225	66
BRAZIL	86	132	32
SPAIN	86	158	157
SOUTH KOREA	82	146	64
MEXICO	64	107	38

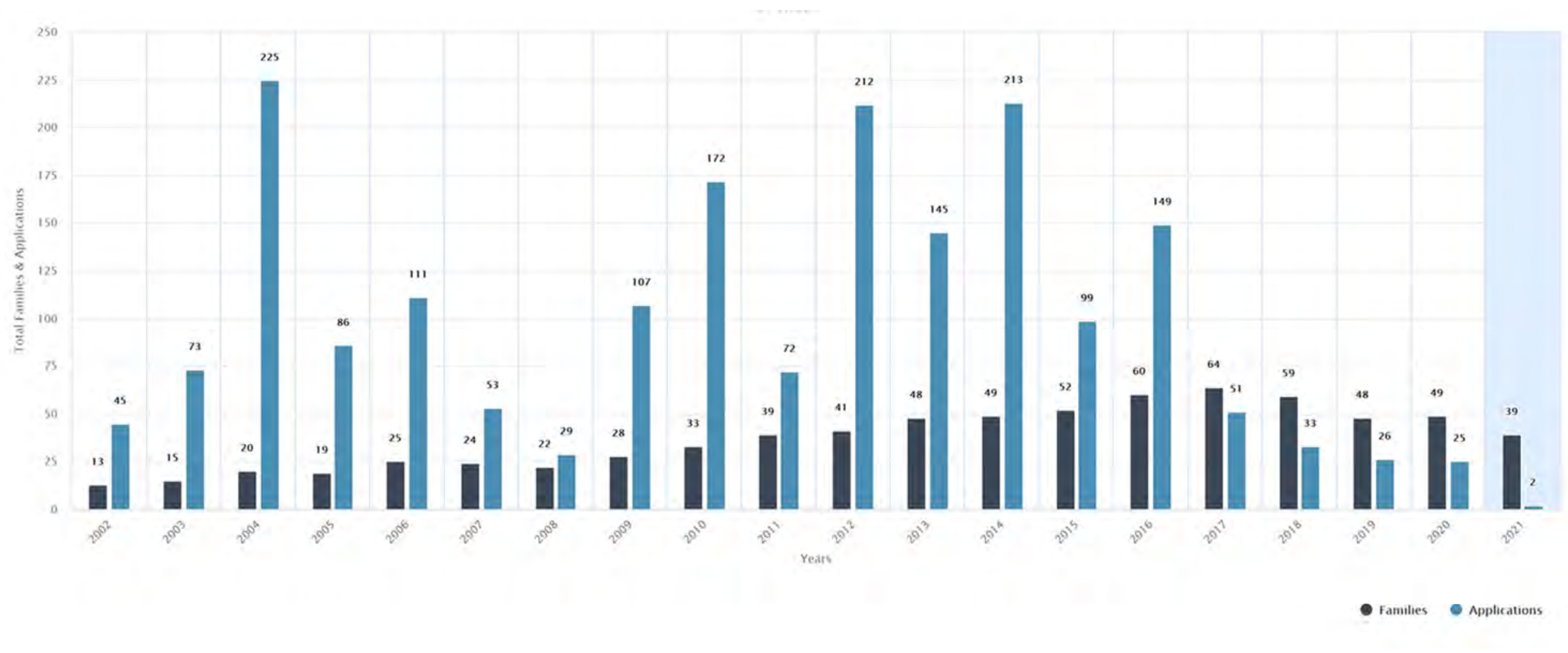

Figure 2. Most recent 20-year patent families and applications.

5.2 State of the technology “Use of enzymes in detergent compositions”

Based on the above needs and specifications we performed a background search regarding the use of enzymes in the production of detergents, with special interest in lipases, with the aim of making the patent and non-patent documents that are part of the state of the art related to this technology available, namely, regarding the use of enzymes in detergents. These documents are those located in the background search strategy that will be detailed below. For the retrieval of the state of the art documents, the PatBase database was consulted. PatBase is one of the most reliable databases used daily by patent professionals around the world as their main search tool. Organized by patent families, PatBase offers extensive full-text coverage of more than 95 issuing authorities around the world. Starting from the needs and specifications data, a search was carried out in this database that provides bibliographic data on patent and non-patent documents. To retrieve the patents information, a search strategy was designed using the keywords “lipase” and “detergent” along with their synonyms and variants. In addition, the codes of the international patent classification have been used to narrow the search:

- C11D: Detergent compositions; use of a single substance as a detergent; soap or its manufacturing; resin soap; glycerin recovery
- C12N9: Enzymes, e.g. ligases; proenzymes; compositions containing them (tooth cleaning preparations containing enzymes A61K 8/66, A61Q 11/00; medical preparations containing enzymes A61K 38/43; detergent compositions containing enzymes C11D).

The search strategy that has been followed is:

Search Strategy	Key words	Result
Search to find everything related to enzymes like lipase and its applications in detergents	Lipase, enzyme, detergent	11,958
Search to find everything related to enzymes like lipase and its applications in detergents (using the classification code C11D)	Lipase, enzyme, detergent	7,507
Search to find everything related to enzymes like lipase and its applications in detergents (using the classification code C11D) and limited to oil stains	Lipase, enzyme, detergent, oil stain	93

As a result of the background search, 93 results of patents were obtained, according to a number of keywords (Figure 3). About 33.7% of the patent applications are still active / alive, while the rest have expired or been abandoned. The analysis of how the presentation of new registries (families) has evolved and their extensions to the different countries (applications) allows us to conclude that it is a mature technology that in the last twenty years has maintained a constant growth (See Figure 4). The countries in which it has been extended the most and, therefore, may represent potential markets of interest, are Brazil, United States, Canada, Japan and China; within the European content, Germany (23 families) and Spain (15 families) stand out (See Table 5).

Figure 3. Main concepts and keywords retrieved from the searches.

Table 5. Top 10 countries by patent families and applications.

COUNTRY	FAMILIES	APPLICATIONS	GRANTS
BRAZIL	50	56	2
UNITED STATES OF AMERICA	48	95	50
CANADA	47	60	12
JAPAN	45	62	18
CHINA	45	62	21
AUSTRALIA	40	63	12
INDIA	29	29	4
ARGENTINA	25	31	0
MEXICO	23	31	6
GERMANY	23	31	12

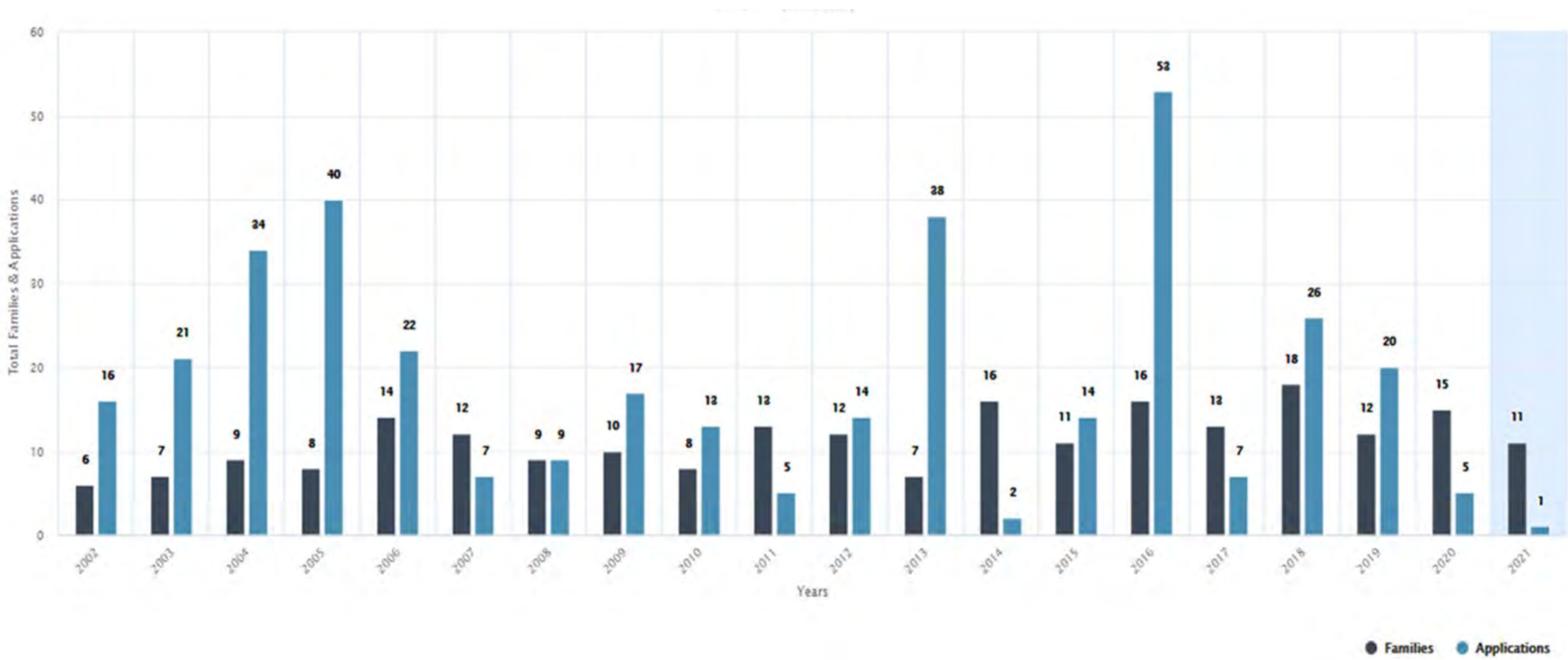

Figure 4. Most recent 20-years patent families and applications.

5.3. State of the technology “Use of enzymes in textile industry”

Based on the above needs and specifications we performed a background search regarding the use of enzymes in the textile industry, with the aim of making the patent and non-patent documents that are part of the state of the art related to this technology available, namely, regarding the use of enzymes in the textile industry. These documents are those located in the background search strategy that will be detailed below. For the retrieval of the state of the art documents, the PatBase database was consulted. PatBase is one of the most reliable databases used daily by patent professionals around the world as their main search tool. Organized by patent families, PatBase offers extensive full-text coverage of more than 95 issuing authorities around the world. Starting from the needs and specifications data, a search was carried out in this database that provides bibliographic data on patent and non-patent documents. To retrieve the patents information, a search strategy was designed using the keywords “textile”, “fiber”, “polyester”, “nylon” or “polyamide” and “enzyme” along with their synonyms and variants. In addition, the search has been limited to the textile application using classification codes.

- D06M: Treatment, not elsewhere provided for in class D06, of fibers, threads, yarns, fabrics, feathers, or fibrous articles made from these materials
- D06B: Textile treatment using liquids, gases or vapors
- D06P: Dying or printing of textiles; dying of leather, skin or solid macromolecular substances of any form

The search strategy has been narrowed based on the different applications:

Search Strategy	Key words	Result
Search to find everything related to the use of enzymes in textile industry	Textile, fiber, fibre, nylon, polyester and enzyme	22,823
Search to find everything related to the use of enzymes in textile industry (using classification codes D06M/D/P)	Textile, fiber, fibre, nylon, polyester and enzyme	2,755
Search to find everything related to the use of enzymes in textile industry (using classification codes D06M/D/P) in the last 20 years	Textile, fiber, fibre, nylon, polyester and enzyme	2,588
Search to find everything related to the use of enzymes in textile industry (cleaning/pretreatment of synthetic fibre)	Textile, fiber, fibre, nylon, polyester, enzyme, clean, pre-treatment and synthetic fiber	14
Search to find everything related to the use of enzymes in textile industry (chall marks)	Textile, fiber, fibre, nylon, polyester, enzyme, clean, pre-treatment and write	15
Search to find everything related to the use of enzymes in textile industry (replacement of the bleaching processes)	Cotton, decolour, enzyme	28
Search to find everything related to the use of enzymes in textile industry (surface functionalization/modification)	Textile, fiber, fibre, nylon, polyester and enzyme, functional modification	13
Search to find everything related to the use of enzymes in textile industry (improved hydrophilicity)	Textile, fiber, fibre, nylon, polyester and enzyme, hydrophilicity	14

Search to find everything related to the use of enzymes in textile industry (improved hydrophobicity)	Textile, fiber, fibre, nylon, polyester and enzyme, hydrophobicity	10
Search to find everything related to the use of enzymes in textile industry (dyeing process)	Textile, fiber, fibre, nylon, polyester and enzyme, fix, dye	71
Search to find everything related to the use of enzymes in textile industry (higher effectiveness of existing enzyme treatments on natural and synthetic fibres)	Cellulose, textile, fiber, fibre, enzyme, design, bleach	125

As a result of the background search, 2,588 results of patents were obtained, according to a number of keywords (Figure 5). About 43.2% of the patent applications are still active / alive, while the rest have expired or been abandoned. The analysis of how the presentation of new registries (families) has evolved and their extensions to the different countries (applications) allows us to conclude that it is a mature technology that in the last twenty years has maintained a constant growth (See Figure 6). The countries in which it has been extended the most and, therefore, may represent potential markets of interest, are China, United States, Japan, Canada and Australia. Within the European content, Germany (671 families), Spain (365 families) and Austria (343 families) stand out (See Table 6).

Figure 5. Main concepts and keywords retrieved from the searches.

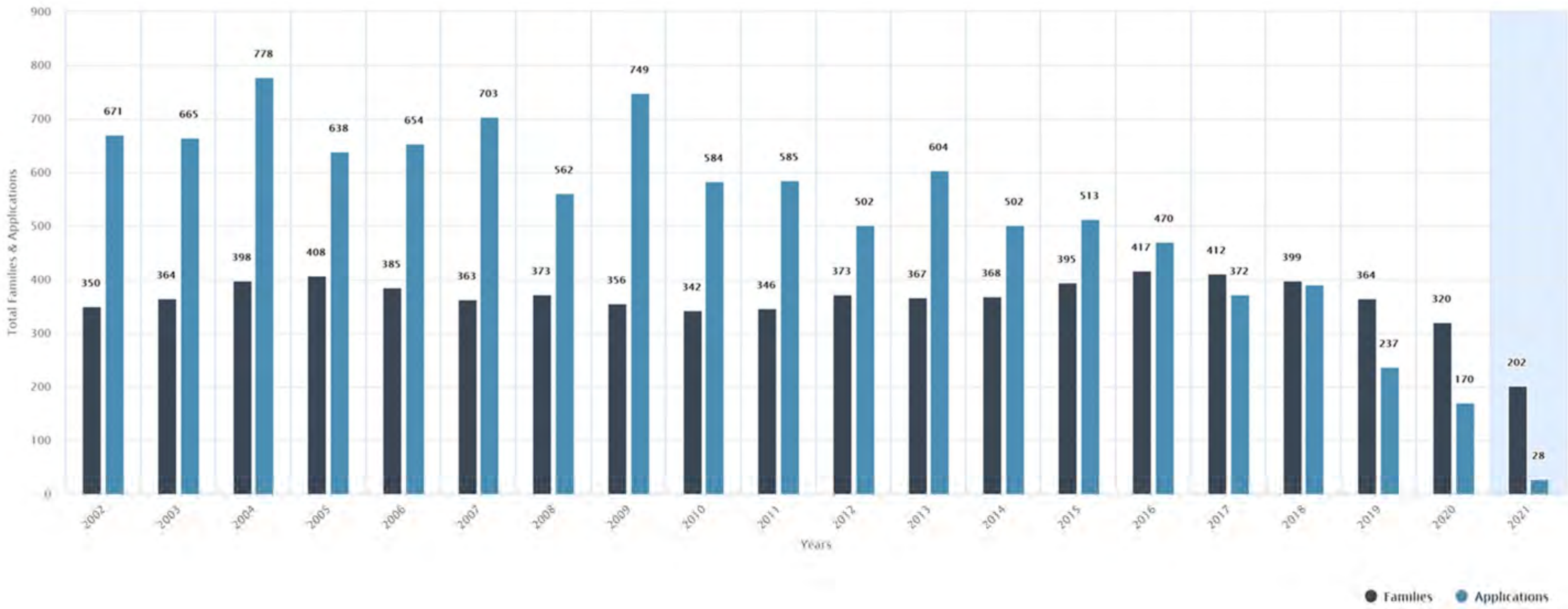

Figure 6. Most recent 20-years patent families and applications.

Table 6. Top 10 countries by patent families and applications

COUNTRY	FAMILIES	APPLICATIONS	GRANTS
CHINA	1522	1764	719
UNITED STATES OF AMERICA	938	2106	1357
JAPAN	765	1149	502
GERMANY	671	978	309
CANADA	520	733	313
AUSTRALIA	488	821	293
BRAZIL	470	611	112
SPAIN	365	465	465
AUSTRIA	343	428	427
MEXICO	314	416	74

The results showing bibliographic data of the most relevant patent and scientific documents located in searches can be accessed through the following QR codes (password: FuturEnzyme€01/06/2021).

QR code for State of the technology "Production of hyaluronic acid for cosmetics":

QR code for State of the technology "Use of enzymes in detergent compositions":

QR code for State of the technology "Use of enzymes in textile industry" divided in "Cleaning pretreatment", "Chalk marks", "Bleaching process", "Surface functionalization", "Hydrophilicity", "Hydrophobicity", "Dying process" and "Cellulose fibers":