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*FuturEnzyme:*

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

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MANUFACTURERS’ NEEDS AND

SPECIFICATIONS: PROTOCOL

DELIVERABLE NUMBER D2.1

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Document information sheet

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# Manufacturers’ needs and specifications: Protocol

## 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.

## Henkel’ needs and specifications

Table 1 summarizes the HENKEL’ needs and specifications.

**Table 1.** HENKEL’ needs and specifications.

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|  | **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. |

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|  | * 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 |

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|  | 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 |
| Benchmark enzymes | 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. |
| Substrates | 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**  **No. ID Soil components Textile Provider**   1. C-S-61 Beef lard2 CO CFT1 2. PC-09 Pigment/oil PES/CO CFT1 3. PC-S-132 Pigment/sebum3 PES/CO CFT1 4. CS-S-05s Mayonnaise with carbon black4 CO CFT1 5. C-S-10 Butterfat with colourant5 CO CFT1 6. PC-S-16 Lipstick, pink6 PES/CO CFT1 7. C-S-17 Make up7 CO CFT1 1CFT: CENTER FOR TESTMATERIALS (https://www.cftbv.nl)   2C-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).  3PC-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).  4CS-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.).  5C-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.) |

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|  | 6PC-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 C24 to C36 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)).  7C-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 No. Soil components**   1. Cuff and collar1 2. Natural skin fat1 3. Butterfat2 4. Olive oil 5. Frying fat3 6. Lard4 7. Tomato beef sauce   1Cuff and collar could contain natural skin fat/human sebum consisting of esters of glycerol (triglycerides), wax and cholesterol.  2Butter 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.  3Frying 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).  4Beef 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. |
| Remarks | 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. |

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## Evonik’ needs and specifications

Table 2 summarizes the EVONIK’ needs and specifications.

**Table 2.** EVONIK’ needs and specifications.

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|  | **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-](https://www.sigmaaldrich.com/ES/en/product/sigma/h3506?context=product) [1000 units/mg solid](https://www.sigmaaldrich.com/ES/en/product/sigma/h3506?context=product)). |

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|  | 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. |
| Substrates | 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**  **No. ID Provider**   1. High molecular weight hyaluronic acid produced after Evonik fermentation with *B. subtilis* 2. High molecular weight hyaluronic acid (ref. 53747) Sigma-Aldrich 3. Low molecular weight (50 kDa) hyaluronic acid HyaCare® 50 Evonik 4. Low molecular weight hyaluronic acid (<10 kDa), Hyalo-Oligo Kewpie Corp.   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 |

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|  | 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. |

## Schoeller’ needs and specifications

Table 3 summarizes the SCHOELLER’ needs and specifications.

**Table 3.** SCHOELLER’ needs and specifications.

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| **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\* |

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

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| **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. |

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| **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. |

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

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| 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. |

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| **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. |

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| **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 |

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| 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. |

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| **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. |

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| **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 |

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| 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. |

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| **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/m2 |
|  |  |  | 61488Z | Pre-treated |  |
| 2 | Woven | 61988 | 61988F1 | Raw | 92% PA, 8% EL 280 g/m2 |
|  |  |  | 61988F1 | Pre-treated |  |
| 3 | Woven | 67007 | 67007 | Raw | 88% PA,12% EL 135 g/m2 |
|  |  |  | 67007 | Pre-treated |  |
| 4 | Woven | 3X58 | 3X58 | Pre-treated | 100% PES 100 g/m2 |
| 5 | Woven | 66299 | 5237/00 | Raw | 92% CO, 8% EL 240 g/m2 |
| 6 | Warp-knitted | E03130 | E03130 | Raw | 80% PA, 20% EL 160 g/m2 |

1PA: 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

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## 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 |

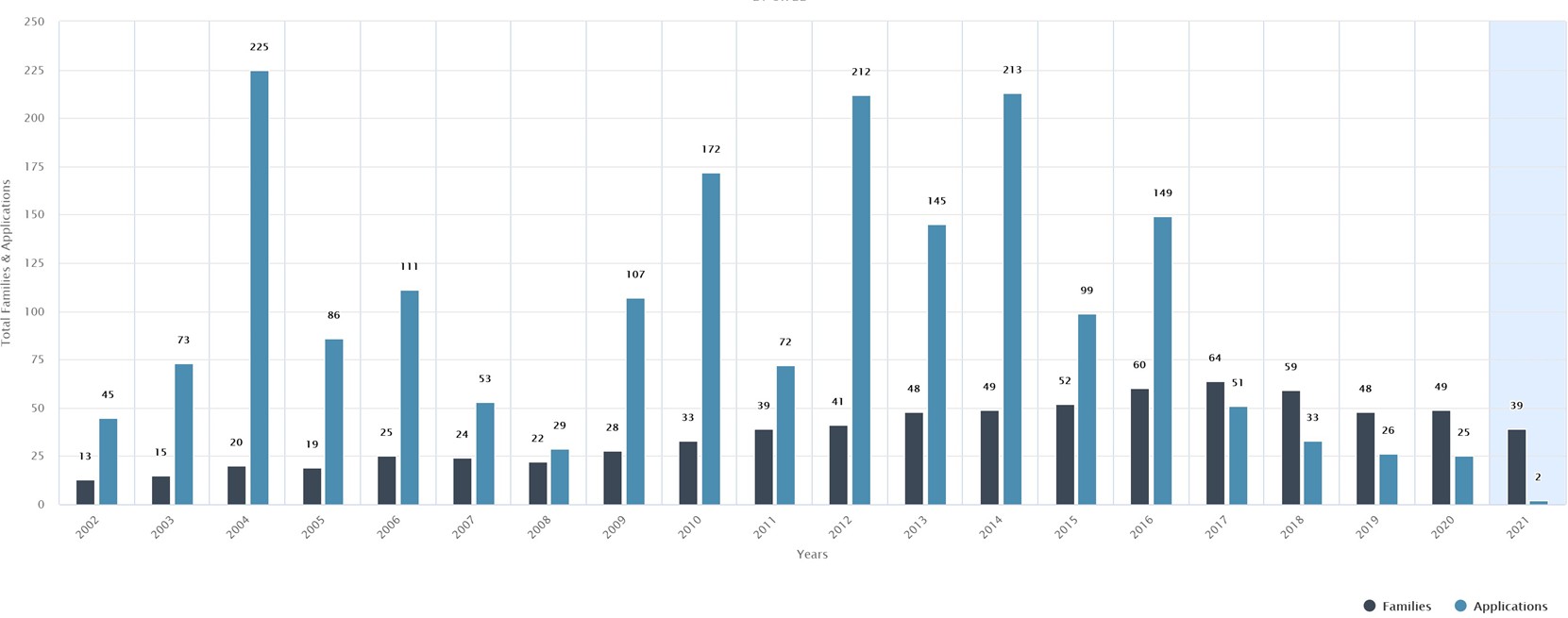
As a result of the background search, 169 results of patents were obtained, according to a number of keywords (Figure 1). About 67.1% of the patent applications are still active / alive, while the rest have expired or been abandoned. The analysis of how the presentation of new registries (families) has 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”

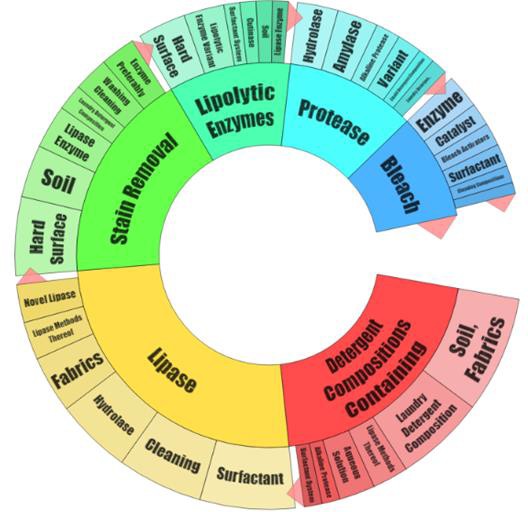
Based on the above needs and specifications we performed a background search regarding the use of enzymes in the production of detergents, with special interest in lipases, with the aim of making the patent and non-patent documents that are part of the state of the art related to this technology available, 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 |

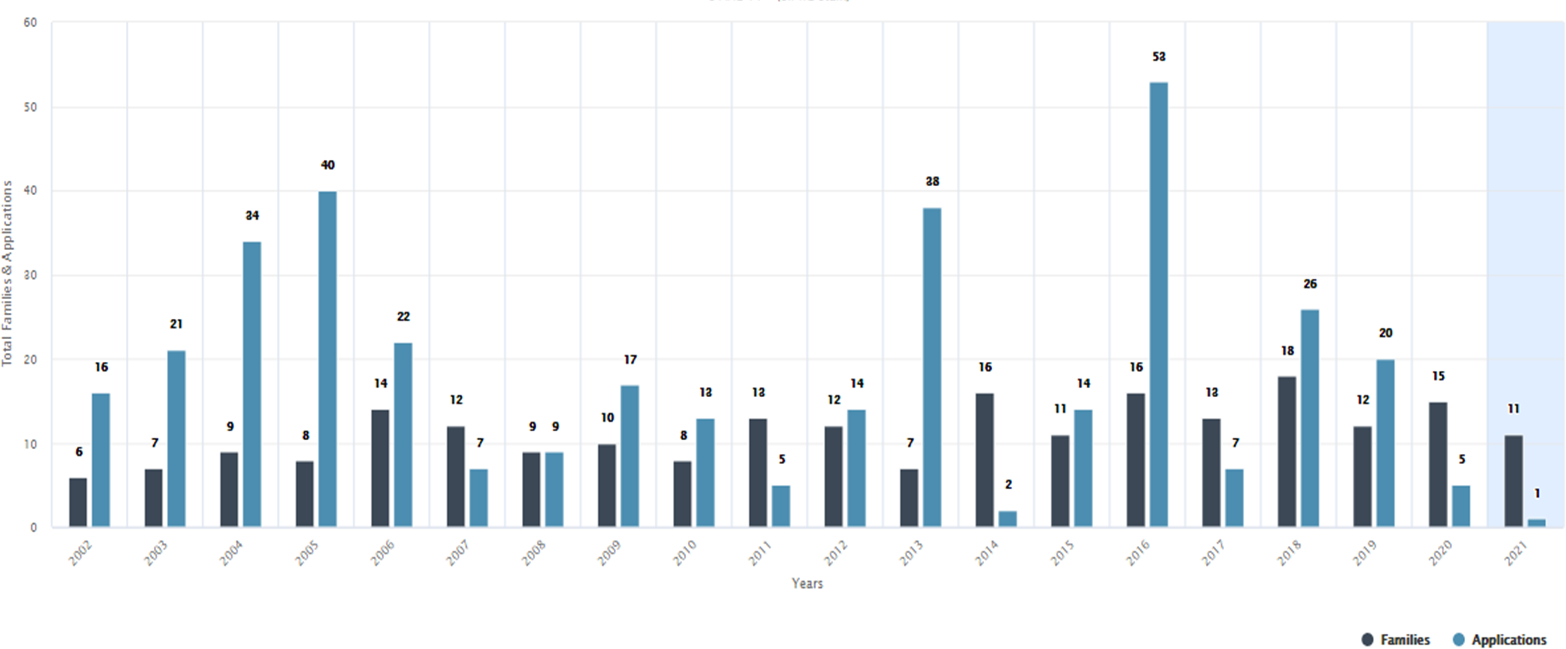
As a result of the background search, 93 results of patents were obtained, according to a number of keywords (Figure 3). About 33.7% of the patent applications are still active / alive, while the rest have expired or been abandoned. The analysis of how the presentation of new registries (families) has 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.

## 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:

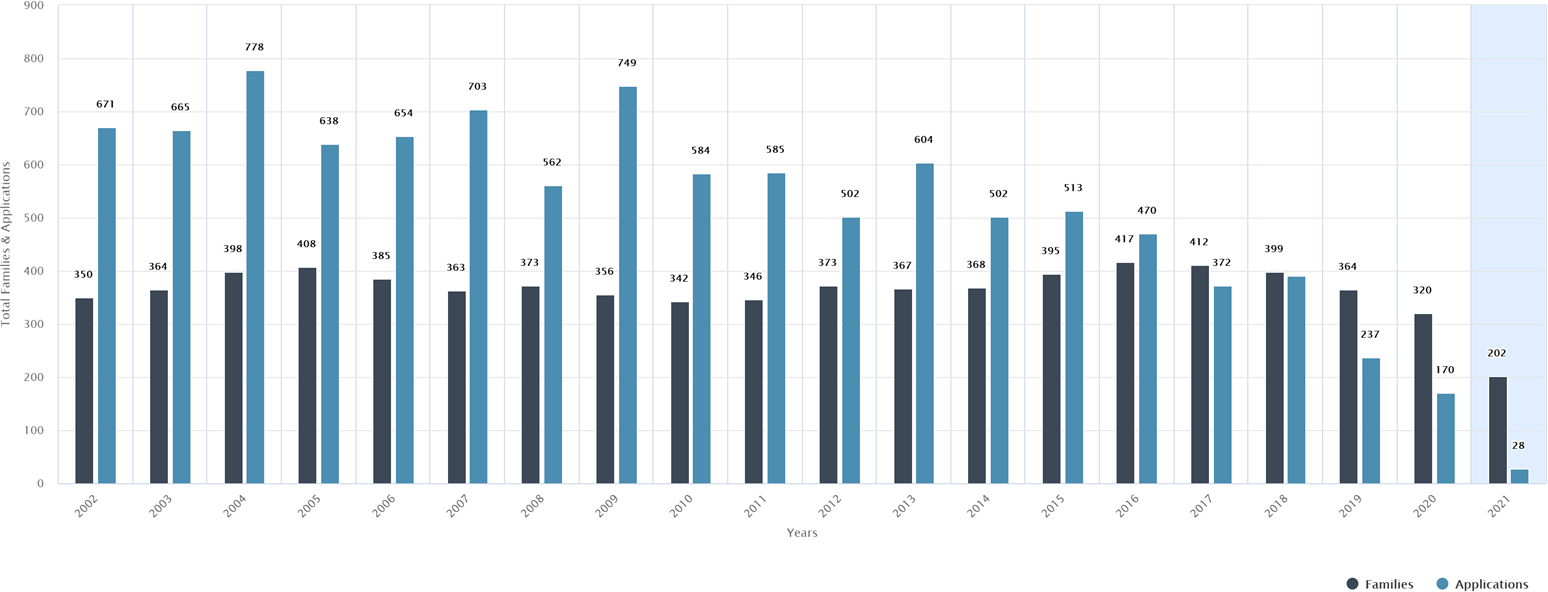
|  |  |  |
| --- | --- | --- |
| **Search Strategy** | **Key words** | **Result** |
| Search to find everything related to the use of  enzymes in textile industry | Textile, fiber, fibre, nylon,  polyester and enzyme | 22,823 |
| Search to find everything related to the use of enzymes in textile industry (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.



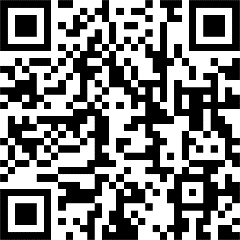
**Figure 6**. Most recent 20-years patent families and applications.

**Table 6**. Top 10 countries by patent families and applications

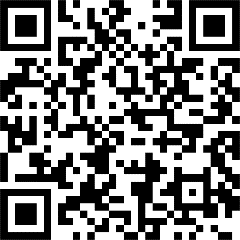
|  |  |  |  |
| --- | --- | --- | --- |
| **COUNTRY** | **FAMILIES** | **APPLICATIONS** | **GRANTS** |
| CHINA | 1522 | 1764 | 719 |
| UNITED STATES OF AMERICA | 938 | 2106 | 1357 |
| JAPAN | 765 | 1149 | 502 |
| GERMANY | 671 | 978 | 309 |
| CANADA | 520 | 733 | 313 |
| AUSTRALIA | 488 | 821 | 293 |
| 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”:



27