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Title: Enzymes for consumer products to achieve climate neutrality

Running title: enzymes, consumer products and climate change

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LAY SUMMARY (200 words limit)
Accumulated greenhouse gas emissions are expected to increase from 36.2 Giga-tons (Gt) to 60 Gt over the next three decades. The global surface temperature has increased by +1.09˚C since 2001, and might increase by +2.2˚C in 2100, +3.6˚C in 2200 and +4.6˚C in 2500. These emissions and temperature rises cannot be reduced in their entirety, but they can be lowered by using enzymes. Enzymes are proteins that catalyze biochemical reactions that make life possible since 3.8 billion years ago. Scientists have been able to "domesticate" them in such a way that enzymes, and their engineered variants, are now key players of the circular economy. With a world production of 117 Kilo-tons and a trade of 14.5 Billion-dollars, they have the potential to annually decrease CO_2 emissions by 1 to 2.5 Billion-tons (Bt), the carbon demand to synthesise chemicals by 200 Million tons (Mt), the amount of chemicals by 90 Mt, and the economic losses derived from global warming by 0.5%, while promoting biodiversity and our planet's health. Our success to increase these benefits will depend on better integration of enzymatic solutions in different sectors.

ABSTRACT (250 words limit)
Today, the cheosphere's and biosphere's compositions of the planet are changing faster than experienced during the past thousand years. CO_2 emissions from fossil fuel combustion are rising dramatically, including those from processing, manufacturing and consuming everyday products; this rate of greenhouse gas emission (36.2 Giga-tons accumulated in 2022) is raising global temperatures and destabilizing the climate, which is one of the most influential forces on our planet. As our world warms up, our climate will enter a period of constant turbulence, affecting more than 85% of our ecosystems, including the delicate web of life on these systems and impacting socioeconomic networks. How do we deal with the green transition to minimize climate change and its impacts while we are facing these new realities? One of the solutions is to use renewable natural resources. Indeed, nature itself, through the working parts of its living systems, the enzymes, can significantly contribute to achieve climate neutrality and good ecological/biodiversity status. Annually they can help decreasing CO_2 emissions by 1 to 2.5 Billion-tons, carbon demand by about 200 Million-tons, and chemical demand by about 90 Million-tons. With current climate change goals, we review the consequences of climate change at multiple scales and how enzymes can counteract or mitigate them. We then focus on how they mobilize sustainable and greener innovations in consumer products that have a high contribution to global carbon emissions. Finally, key innovations and challenges to be solved at the enzyme and product levels are discussed.

KEYWORDS: bioeconomy, climate change, consumer products, cosmetics, detergent, enzymes, greenhouse gas emissions, textiles

CLIMATE CHANGE: A GLOBAL CHALLENGE

According to the Intergovernmental Panel on Climate Change in its AR6 report from 2021 to 2022, greenhouse gas (GHG) reached averages of 410 ppm of carbon dioxide (CO_2), or 0.410 g of CO_2/L of air, causing a constant warming uprising during the last four decades [1]. In 1800, the CO2 level was 285 ppm, a constant value since the year 0 of our era. Increasing GHG emissions are a direct consequence of a continuously growing consumption of fossil fuels that nowadays produce 84.3% of global energy, while only 11.4% comes from renewables [2, 3] (**Figure 1A**). The last time the atmospheric CO_2 concentration reached the current level occurred more than three million years ago [4]. A total of 36.2 Giga-tons (Gt; 1 Gt=10^9 tons) of CO_2 have been

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released into the atmosphere, which may increase to 60 Gt by 2050 if current trends continue [5]. If we compare this amount with distances travelled by car (an average European car emits 0.175 kgCO_2 equivalent (CO_2e)/km) and the amounts of carbon sequestered by trees (a mature tree sequesters approximately 0.917 kgCO_2e per month), this amount will be equivalent to approx. 342 trillion km (1.5 million times the distance to Mars) and 65 trillion tree-months (21 times the number of trees globally) [6]. CO_2 acts as a barrier trapping the sun's heat on Earth. As a consequence, the global surface temperature from 2001 to 2020 increased by +1.09˚C (compared to the period from 1850 to 1900) [7]. Scientists from the IPCC foresee at least a 50% likelihood that global warming will reach or exceed +1.5˚C during the period from 2021 to 2040 and an increase of up to 5.5°C over the next century [8,9]. However, other recent projections suggest that mean global warming will achieve +2.2˚C above present-day levels by 2100, and will continue to rise to +3.6˚C in 2200 and +4.6˚C in 2500. This warming is also projected to be unequally distributed [10]. As example, over the last 30 years, the temperature increase in Europe was +1.5˚C, at a rate of +0.5˚C every 10 years, more than double the global average; this being said, GHG emissions in Europe over the same period have been reduced by 31%, and the target is to reduce them to 55% by 2030 [11]. This means that the drastic actions required to fight climate change must go beyond a local scale.

CO_2 is not the only molecule directly affecting global warming. Indeed, humans have synthesized more than 140 000 artificial chemicals and mixtures of chemicals, and approximately 220 Billion-tons (Bt) of those are produced and disposed each year, thus contributing also to global warming like CO_2 [12]. The global carbon demand to synthesize those chemicals and derived materials, 450 Million-tons (Mt) per year in 2020 mostly sourced from fossil resources, is expected to increase at an annual rate of 2.7%, reaching 1000 Mt per year by 2050 (**Figure 1B**). This is why massive increase of carbon recycling by 2050 is necessary because *de novo* carbon mining either from fossil or renewables is just not possible in this amount using the technologies available (**Figure 1B**).

Different projections and scenarios may have to be reviewed because of the changes that countries are making in response to new realities, particularly to the SARS-CoV-2 pandemic and new political facts. Independently of these revisions, when environment changes, nature has the potential to stabilize itself. However, nature can only respond to slow changes. The only instance that self-equilibration did not occur was approximately 250 million years ago, when the planet warmed up, contributing to mass extinction [13, 14]. To gain some perspective, in the past 2 million years, several temperature changes have occurred on our planet. Before times of industrialization and globalization, a rise of +5˚C in global temperature took approximately 5000 years. The increase in GHG emissions and temperature, first acknowledged in 1856, is now happening 20 times faster [15-17]. This rate is too quick to allow nature to stabilize by its own, forcing us to take drastic steps to adapt to the acute extreme heat events that the world is facing [18].

Climate change is a global challenge whose effects must be considered beyond 2100 [10]. A number of solutions that can help mitigate climate change are currently available, including shift to renewable energy sources, electric. and low-carbon alternatives. These actions also include reduction of food loss, waste generation, deforestation and ecosystems damage, etc. [19]. Such actions, and others to be implemented in the future, are being and will be effective and sustainable in the long term only if socio-economic and policy reforms are considered, and if we all first know the consequences of climate change at multiple levels, from micro- to macro-scale, and also the possible solutions at different levels. Here, we break down some of such consequences and one of the solutions to mitigate or even reverse these deleterious

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effects. To this end, we need enzymes, which are not only the working parts of living systems, but also constitute one of the cornerstones of a circular (bio)economy.

CLIMATE CHANGE: SOCIAL AND ECONOMIC CONSEQUENCES

Climate change is provoking extreme weather phenomena such as large drought periods, more frequent torrential storms, and drastic changes with an overall increase in temperature that will result in the thawing of permafrost and melting of ice at the poles. These factors have a direct threatening effect on biodiversity and human life [20]. The socioeconomic consequences of climate change can also be substantial (**Table 1** [21-50]).

From an economic point of view, the total value of equities traded on the world's stock markets is approximately USD 70 trillion per year, and it has been suggested that climate change could cause a potential loss in traded equities of approximately USD 7 trillion per year [41]. The final effect on the global economy will depend on different climate change scenarios and mitigation actions [51]. For instance, agriculture will be the sector most affected by heat stress in the period 2050-2100. This is exemplified by projected total global gross domestic product losses of 2.6-4.0%, agricultural productivity losses of 2-15%, food price increases of 1.3-56%, and a food-demand gap of 1.26 Bt [10, 46-48, 52-54].

Reducing food and water security and transforming their distribution will affect human health and life expectancy [49, 55]. As an example, a recent study by Carlson *et al.* shows evidence of how climate change can increase cross-species viral transmission risk [56]. Indeed, climate and land-use change will produce novel opportunities for viral transmission among previously geographically isolated wildlife species, facilitating zoonotic spill over, thus increasing the risk of novel epidemic and pandemic outbreaks with already well-known consequences at the social and economic levels.

In addition, climate change is expected to affect civil and political rights, including rights to live, access to safe food and water, health, security, shelter, and culture, and contributes to humanitarian crises by creating new problems or exacerbating existing problems for vulnerable populations [57]. Finally, it should be highlighted that all projections unambiguously confirm that the actions we take in the coming years to respond to the climate crisis will determine the future of billions of human lives given that the global effects of climate change are not felt homogeneously across the planet [44]. Indeed, by 2070, approximately 3.5 billion people will live in conditions and surroundings with high vulnerability to climate change, and it has been estimated that for every degree of temperature increase, a billion people will be forced to live in uninhabitable places, exacerbating hostilities and giving rise to conflicts [44]. A recent study concluded that the present warming since 1980 elevated conflict risk in Africa by 11% [45].

VULNERABILITY OF THE ECOSYSTEMS AND THE ASSOCIATED WEBS OF LIFE

It is important to remark that the vulnerability of humans and ecosystems, including the life forms inhabiting them, are interdependent, and that safeguarding biodiversity and ecosystems is fundamental for climate-resilient development [1]. According to a recent study, more than 85% of the ecosystems will be affected by climate change by 2070-2090, and 16-30% of plant and animal species might go extinct [36-38].

It is worrisome that climate change effects on microorganisms are rarely considered, although scientists have warned that there is an urgent need to keep a close eye on this matter [58]. A 16% loss in microbial diversity by 2100 is projected by predictive models if the rate of GHG emissions continues [36, 39]. A redistribution of microbial diversity is also foreseen,

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causing the composition of bacteria to undergo a strong and generalized global homogenization process across locations [59]. Again, these changes will not occur equally globally or over different taxa [9, 10, 36, 58, 60, 61]. As example, typical desert bacteria, including phylotypes such as *Geodermatophilus* spp., *Mycobacterium* spp., *Venturia* spp., and *Devriesia* spp., and microbial producers of antibiotic resistance genes such as *Streptomyces* spp., will become increasingly common in the future. This will occur because the plasticity of thermal response originates from different strategies of adaptation [62]. Examples include differences in: i) the physiological plasticity, defined as the extent to which an organism can change its physiology in response to environmental cues; ii) the regulation of genes (e.g., temperature-dependent expression of isoenzymes and/or epigenetic regulation); and (iii) the genetic adaptation that drives the selection of new enzyme variants for which the reaction rate is adapted to changing environmental conditions (e.g. advantageous mutations or acquisition of new genes). The latter mechanism is particularly important in short generation time (and high turn-over) organisms, such as microorganisms, capable of timely adapting to new conditions.

The consequences of future microbial redistributions, which may be a direct or indirect consequence of climatic change, are currently not fully understood. As microscopic organisms are necessary for the planet with a crucial role and influence on carbon cycles and the storage of carbon, avoiding its release into the atmosphere, these changes should not be underestimated [63-66]. Indeed, it is assessed that, since the start of the industrial revolution, microorganisms through the enzymes they contain have absorbed almost half of all our CO_2 emissions, while also carrying out many essential functions, such as nutrient recycling, crop fertility, detoxification of pollutants, regulation of carbon storage, and even production and absorption of GHG such as methane and nitrogen oxides [67-70]. Therefore, the imbalance in the abundance and diversity of microorganisms expected by 2090 may also contribute to climate change [36,60]. Thus, recent studies have demonstrated that under a +4˚C warming scenario, microbial production of CO_2 will rise by 0.05-0.15% as a consequence of global warming and its effect on prokaryotic biomass [40]. At the same time, using a projected warming of +1.9˚C by 2100, the carbon sequestration by microbes could decrease by 17 ± 7% [71].

All the above, including GHG emissions, hazardous waste disposal, global carbon demand, socioeconomic impacts, and alterations in ecosystems and their delicate web of life (**Table 1**), are some of the issues that need to be addressed regarding climate challenges. It is essential to handle the so-called green transition by developing new technologies capable to help achieving climate neutrality. How do we do this in energy, food, raw materials, consumer products, etc.? These questions need to be solved due to climate change, new realities, including new political facts, and re-politicizization of adaptation decision-making [72]. The lessons we have learned from these realities are critical to allow strategic autonomy and building sustainable systems. In this context, it is now accepted that transforming the fossil-powered linear economy towards a circular (bio)economy is critical to our strategy to achieve climate neutrality (**Table 1**). Repowering the industry with enzymes can contribute to improve and accelerate this transformation. This is discussed hereinafter.

ENZYMES: KEY PLAYERS TO ACHIEVE CLIMATE NEUTRALITY

Enzymes, as a part of nature, are active proteins that catalyze biochemical reactions. They build and maintain all living organisms, increasing the reaction rates of both syntheses and break down reactions *in vivo*, but also *in vitro* [5, 73]. All living organisms on Earth, including

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plants, animals, microorganisms, and humans, would never have evolved the way they have without the help of enzymes. Since their initial appearance on Earth 3.8 billion years ago, these catalytic proteins have been allowing life to thrive through adaptations to multiple conditions, including extensive ice ages or global warming, and to new chemicals introduced into the environment, including plastics [74, 75]. This ability to adapt to a multitude of different conditions, i.e. their striking versatility, assigns to them a realistic and outstanding role also in reducing GHG emissions. **Table 2** summarizes the different products that are produced by enzymes. Natural products such as structural protein-based biomaterials or fibres must be extracted and downstream processed before they can be used in different applications. Additionally, a large number of important products for our daily life are manufactured using enzymes *in vitro* including commodity chemicals, bioplastics, and many others.

How much can enzymes contribute to fight climate change and global warming? Before quantifying their contribution, one should consider the multiple benefits that enzymes can introduce in industrial processes and products: (i) lower energy footprint; (ii) reduction of waste production and chemical consumption; (iii) reduction of environmental impacts across several categories, acidification, eutrophication, photochemical ozone, and energy use; (iv) making process conditions safer; and (v) using renewable feedstocks, to name a few [5, 64-66, 77, 78]. As such, the most comprehensive comparative environmental assessments conducted over 15 years have revealed that implementing enzymatic processes in place of conventional chemical ones generally leads to reduced contributions to global warming by saving up to 155 kg CO_2 per kg of product, depending on the product [29]. Recent estimations suggest that the full climate change mitigation potential of enzymes may range from between 1 and 2.5 Bt of CO_2 emissions per year by 2030 [27]. This reduction would be equivalent to the annual emissions of about 16-40% of all cars on the road worldwide (estimated to be 1.4 billion). Obviously, enzymes have a solid potential to transform our planet into a global powerhouse to drive the green transition (**Table 1**). In addition, carbon tax implementation (USD 40-80 per tonne CO_2) is expected to force industries not only to reduce their carbon footprint, but also to convert CO_2 into valuable chemicals and materials, which is key to reduce CO_2 emissions into the atmosphere [79]. Here, enzymatic processes may have a key role [5]. Enzymes also contribute to lowering the carbon footprint by supporting the production of about 90 Mt bio-based chemicals, which represents about 0.04% of the total chemical worldwide demand (**Table 1**) [12, 35].

It is worth mentioning that not all enzymes contribute equally to the fight against climate change, simply because their different performances and because the products or processes they assist may have a greater or lesser impact in terms of energy, water and chemical consumption and waste generation. For example, one of nature's fastest-working enzymes, carbonic anhydrase, reacts 1 million times per second to convert CO_2 into HCO_3^- (bicarbonate). As such, this enzyme together with other CO_2-converting enzymes has greater potential to help fight climate change, contributing to the capture of 14% of the GHG emissions that needs to be reduced by 2050 [80, 81].

THE GROWING CONCERN OF CLIMATE CHANGE REQUIRES NEW ENZYMES

It is estimated that our planet is home to 1 trillion (10^{12}) microbial species living and operating in a broad range of working conditions, although only approximately 420 000 have been formally described in GenBank. Additionally, the amount of DNA sequences representing different species deposited in databases is huge with the number of bases doubling

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approximately every 18 months [82, 83]. Every strain, representing each species, is expected to be a wide reservoir of enzymes [84]. As an example, the genome of a single bacterium, such as *Escherichia coli*, contains 4391 predicted genes, among which approximately 607 are enzymes catalysing more than 700 reactions. A single fungal strain contains more than 16 000 genes, among which at least 800 are enzymes that support at least 1069 reactions. However, the estimated diversity of some environmental samples reached 100 000 microbial species per gram, which theoretically overestimates the number of enzymes at our disposal [85]. Indeed, it is estimated that nearly 10^{10}-10^{15} proteins exist across all life forms inhabiting our planet, 40% of which may be catalytically active proteins, that is, enzymes [86].

This astronomical number is far from the number of enzymes we have been able to observe and to have in our hands. Thus, approximately 270 000 enzymes have been identified that all together support approximately 6500 different reactions; the protein structure of 170 000 of them has been characterized [84, 87, 88]. What is significant is that with these enzymes, which represent a tiny fraction of those at our disposal in ecosystems, significant global economic and environmental achievements have been made (**Table 1**). The following data serve as examples: the worldwide enzyme production reached 117 Kilo-tons per year [43]; nowadays the trade in enzymes represent 0.037% of total world trade (ca. USD 14.5 billion) with a projected annual growth rate from 2022-2028 up to 6.5%; and enzymes are expected to reduce economic losses derived from global warming by 0.5%, and if enzymes become more important, they should contribute to gain [42, 43]. Access to, or design of a higher number of enzymes will thus allow the industrial reconversion needed to complete the green transition and to achieve climate neutrality.

SUSTAINABLE CONSUMER PRODUCTS TO FIGHT CLIMATE CHANGE

The use of enzymes, whether they are new or naturally occurring enzymes, will contribute significantly to the protection of the environment. This occurs during production, use, or disposal through the conservation of resources, reducing global GHG emissions, promoting energy-efficient processes and the use of renewable energy, minimizing the use of toxic agents, reducing waste, and conserving water [5, 89, 90] (**Table 1**). Therefore, enzyme-derived products have the potential to benefit both the environment and our quality of life [91, 92]. Together with these benefits and stringent environmental regulations, the main driving force supporting the green trend in industry is related to the increased concern of consumers regarding climate change and the environment and the augmented awareness of the impact consumers can have on their everyday consumption choices. Indeed, according to Silva de Oliveira *et al.*, ca. 90% of consumers will buy a product with an environmental benefit and have a more positive image of a company that supports biotechnology [93]. Furthermore, 50% of consumers are willing to recognize a green premium for a more sustainable greener alternative. This consumer trend is important, as there is strong evidence that consumption habits are interlinked with awareness of climate and environmental change [94]. Thus, changes in consumption behaviour can significantly decrease environmental impacts [95]. For example, negative environmental impacts are expected to decrease if sustainable choices, instead of fashion choices, are prioritized. Indeed, in the 21st century, the fashion industry has been found to be responsible for 10% of GHG emissions [94]. Therefore, constant innovation is needed to pursue a 100% sustainable model of production and consumption that could help to effectively fight climate change while even improving the quality of goods.

Below, we review to what extent enzymes can mobilize sustainable and greener innovations in consumer products, to mitigate and even reverse the effect of climate change.

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In particular, we focus on textiles, detergents, and cosmetics that contribute globally to carbon emissions (**Table 1**), which can be reduced by the use of enzymes.

GREENING TEXTILES THROUGH ENZYMES

The contributions of the textile industry to climate change depend mainly on the type of textile. However, one of the main environmental issues in the process chain of textiles is that finished textiles commonly do not meet the desired requirements in the final inspection and return to production to achieve improvements. Such production and correction cycles, which are large chemical and energy-consuming processes, make the textile industry one of the largest contributors to climate change, with up to 10% of GHG emissions occurring worldwide [25]. Each kg of plastic-based fabric emits on average approximately 11.9 kg of CO_2, which accounts for a total of approximately 1291 Mt CO_2 (equivalent to 7.3 trillion km travelled by car), given the worldwide production of 119 Mt textiles [24,25] (**Figure 2A**). Therefore, being more aware of the impact of their purchasing decisions, textile sustainability is becoming an important new driver for industries and consumers [96].

To pursue a greener textile industry, different eco-responsible approaches are being investigated and developed [93, 97, 98]. They include: i) the utilization of alternative sources of fibrous raw materials that mitigate the negative impacts of traditional cotton culture, such as bamboo; ii) the utilization of natural dyes and pigments; iii) the use of supercritical CO_2 for reduction and cleaning operations, instead of water; iv) the production of durable and high-quality fabrics; and v) the implementation of heat recovery, so the energy used to warm water (especially in the dyeing and finishing processes) comes from that generated in other steps, such as the stentering frames or the steam boilers, the use of groundwater for the cooling process and returning it with the same quality, to mention a few. These approaches do not consider the application of enzymes so far.

Nonetheless, enzymes also have the ability to play a significant role in supporting the conversion of the textile industry into a zero-waste, zero-pollution, fully sustainable market. This potential stems from the fact that enzymes can be applied to all steps of the textile production chain. This may start with the production of biopolymers with the potential to replace common fabrics [99, 100]. Subsequently, the removal of chemicals used in all steps required to achieve the final fabric can be envisaged from the starting polymers, including fibre spinning, weaving and knitting, solvent cleaning, dyeing, washing, finishing, cutting and sewing, in this order [93, 101-105]. This requires highly time- and energy-intensive washing processes that are responsible for the highest amount of GHG emissions, approximately 9.6 kg of CO_2 per kg of fabric [24]. Indeed, dyeing of the textile materials requires a significant amount of water, and prior to the dyeing procedure, the removal of sizing products, such as silicones, paraffins, mineral oils, and waxes, is needed. These residual spinning oils added to yarns in order to allow for them to spin, will generate emissions during the drying and fixation steps and can have a negative impact on the subsequent dyeing/finishing processes themselves. Additionally, the processed water is circulated through the system again. The goal of using enzymes is to promote the reduction of the rinsing steps and their duration, optimize the dyeing process, and help discoloration and neutralization of the water resources used. Life cycle assessments demonstrated that enzymes could reduce the overall carbon footprint of fibre spinning, solvent cleaning, dyeing, washing, and finishing of fabrics: 1 g of enzyme can save 0.42-1.0 kg CO_2 per kg of dry weight yarn, which can be translated to a worldwide reduction of approximately 50-119 Mt $CO_{2,}$ giving the worldwide production of 119 Mt textiles [24, 25, 28, 29] (Filho et al., 2019; UNECE/FAO, 2019) (**Figure 2A**).

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For this reduction to affect the whole process, the impact associated with the enzyme production must be low. Here, the question which environmental impacts are associated to the enzyme production arises. The carbon footprint of the enzyme production can vary greatly, even for the same enzyme depending on the raw materials, the production method and the transportation. For the most advantageous method, a value as low as 8.9 grams CO_2 eq. per gram of enzyme from cradle-to-gate was found in recent life cycle assessments [106] (**Table 3**). These values clearly demonstrate the competitive advantages that enzymes bring to textile bio-processing, and that the contribution of the enzyme production to the entire carbon footprint of a textile bio-process is significantly low.

Enzymes will be also essential to avoid the accumulation of recalcitrant garments in landfills. In this context, enzymes can be applied in the biodegradation of the current textile materials in such a way that they can even be reused to produce new recycled textiles. Tackling this issue can prevent our planet from accumulating 3400 Mt of waste by 2030 [107-109]. Note that each second, a truckload of clothes is thrown away or incinerated. Adding enzymes to the recycling process can result in substantial savings of 5.5 kg of CO_2 per kg of textile material compared to chemical processes [30]. If all textiles (119 Mt) were recycled with enzymes, then an overall reduction of approximately 655 Mt CO_2 (equivalent to 3.7 trillion km travelled by car) could be achieved.

Whether sustainable clothing might be a marketable product rather than a real commitment to reduce environmental impact and climate change, will depend on our ability to offer new enzymes to transform procedures, since a very large amount of textile products is being generated. Such newly-developed enzymes may be directed at least in two key steps required to achieve the fabric on rolls. The first step consists of removing residual spinning oils / sizing products that, if not eliminated, will otherwise generate emissions during the drying and fixation steps. The second step consists in the dyeing process of the textile materials, that needs a lot of water, that further needs to be discolored and circulated in the system again. Currently, these additives/preparation materials and residual dyes are removed by a water/surfactant process and reducing the rinsing steps/duration is the expected goal when adding enzymes into the cleaning processes, and enzymes are needed to support water-based, low temperature, fewer water discharge, and fewer energy consumption processes.

GREENING DETERGENTS THROUGH ENZYMES

According to IndexBox estimates, by end 2020, approx. 26 Mt washing laundry detergents were consumed [34]. It is difficult to find reasonable and valid (public) numbers for potential CO_2 annual emissions of washing industry, because the high intra-country variability (average factor of 6.5) in the average GHG emissions related to the laundry washing process [110]. However, estimates for more than 840 million domestic washing machines in 2016 suggested more than 62 Mt CO_2, equivalent to 350 billion km travelled by car (**Figure 2B**) [23]. For countries with a mainly fossil-based electricity system, the dominant source of variability in GHG emissions results from consumer choices in the use of washing machines; in this context, predictive models foresee a potential reduction of 39 Mt of CO_2 worldwide per year if water and energy-efficient washing machines are employed [23]. For countries with a relatively low-carbon electricity mix, variability in emissions is mainly determined by laundry product-related parameters. It is at this latter point that enzymes play a major role, being one of the standard and commercially available key ingredients (added in amounts of 0.3-3%) in laundry detergent formulations since decades (early 1970s) to make the washing cycle effective and more sustainable [110]. In this case, we are talking about enzymes that efficiently break down

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different types of stains to enable the surfactants to better capture and keep these materials in the wash water. Adding these enzymes allows rebalancing the levels of surfactants and washing temperature, which in turn can contribute to lowering CO_2 emissions without compromising washing performance. Indeed, the potential lowering of the energy savings by facilitating reduced wash temperatures and the impact of the use phase of a detergent product, accounting for about 60% of CO_2 emissions, are among the major roles of enzymes in detergent products. As example, the average GHG emissions related to enzymatic-laundry washing processes were estimated to be 500 g CO_2 per wash cycle at 60°C, which can be reduced to 330 g CO_2 per wash cycle when the water temperature is lowered to 30°C, which means a drop of 35% [110].

The following three data from the "I Prefer 30°" campaign [31] confirm these arguments. First, the European average wash temperature in 2020 was 42.4°C. Second, 90% of the energy the washing machine uses goes towards heating the water. Third, data collected through the "I Prefer 30°" campaign, which promotes washing at 30°C, estimated a saving of 1307.9 GWh/year of current total laundry energy in the five campaign countries, based on a 3°C reduction of the average wash temperature. If a 3°C reduction was to be achieved across the 23 European countries, the reduction would be approximately 12% of 22.1 TWh/yr (2.49 TWh/yr, equivalent to about 1.4 Mt/year CO_2, and to about 122 000 cars not driven) [111]; this reduction can be 18% if the temperature is reduced by 5°C (instead of 3°C). In the USA, this reduction could achieve 2.3 Mt/year $CO_{2,}$ equivalent to 200 000 cars not driven [32].

Implementing better performing enzymes may significantly reduce the carbon footprint of the washing laundry sector further. These enzymes should have strong resistance to laundry ingredients (anionic and nonionic surfactants, chelators, bleach or oxidizing agents) and be efficient enough to eliminate stubborn stains at low temperatures without the extensive use of chemical additives; this is essential to decrease the percentage of chemical surfactants in the detergent formulations and to achieve washing programs with as low emissions as possible. Additionally, enzymes have to be stable at different temperatures to increase market opportunities, as for example in emerging markets, where enzymes and enzyme containing products can be exposed to higher temperatures, especially during transport. In theory, the optimal enzyme has a high robustness against chemical ingredients, is inexpensively producible, and has especially high washing performance at low wash temperatures. Hence, there is plenty of potential of such enzymes for laundry detergents to help achieving climate neutrality.

GREENING COSMETICS THROUGH ENZYMES

The cosmetics market is experiencing a fast boost worldwide, with an annual growth of 5.8%. This increase might be attributed to the fact that 34% of males showed more interest in cosmetics products and purchased these goods at higher rates than ever before in early 2020, while the current interest from women was maintained [112, 113]. In particular, the skin care industry is projected to increase by 24.3% from now to 2025 [114]. While cosmetic products are produced and used (approximately 5 g person/year) in less volume than detergents or textiles, their consumption also leads to a major environmental impact, reflected by the fact that more than 120 billion units of cosmetic products are released worldwide into the environment each year [115]. In this scenario, cosmetic companies are emphasizing the fight against climate change, as revealed by an analysis of sustainability report topics, therefore applying strategies to reduce their impact on the environment [116]. Accordingly, there is a growing attention directed to obtaining new sustainable bioingredients produced with the use

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of enzymatic technologies [117]. Indeed, in the manufacturing of personal care or cosmetic items, the production and extraction of active ingredients are the major sources of environmental impact, accounting for approximately 20% of the total impacts of cosmetic items. Recent estimates foresee from 0.78 to 2.33 kg CO_2 per 1 kg of final cosmetic product, which considering a global production of approximately 10 000 tons of cosmetics and personal care products, will account for a total of 8-23 Mt CO_2 (equivalent to 45-113 billion km travelled by car) (**Figure 2C**) [26]. These emissions are expected to be lowered by 23% if eco-ingredients are produced with enzymes [26]. As for the textile and detergent sectors, implementing novel and better performing enzymes, capable of supporting water-based and fewer energy consumption processes with which to produce cosmetic ingredients, may significantly reduce this carbon footprint while offering innovative consumer products.

SYNERGY TO BETTER BIOPROSPECT AND DESIGN NOVEL ENZYMES

As discussed before, the growing concern on climate change and the request for greener consumer products require the search for or design of new enzymes capable of maintaining high catalytic performance during a number of uses and catalytic cycles in an enzyme's lifetime, and whose production cost and carbon footprint is as low as possible [106, 118]. This last issue is currently feasible through platforms capable of managing and testing the high-throughput expression of more than 1500 enzymes per experiment [119]. The option to find such new enzymes, although being challenging, costly (€30k per enzyme) and time-consuming (15 months per enzyme), is realistic given the recent technological advances. Indeed, the use of bioinformatics, machine-learning, accurate protein structure prediction, and data-driven artificial intelligence techniques, are essential to fully exploit the potential of sequencing data as a source of new enzymes (**Figure 3**) [120, 124].

These developments must go hand in hand with experimental strategies to test computational predictions and platforms that speed up their incorporation in appropriated synthetic biology chassis and their repurposing through novel engineering techniques with ultrahigh-throughput methods [120, 125-129] (**Figure 4**). To highlight, for more than 98% of natural enzymes the average catalytic efficiency (k_{cat}/K_M) value is $\sim 10^5$ M^{-1} s^{-1}, and it is desired that k_{cat}/K_M values approach the physical limit of diffusion rate ($\geq 10^9$ M^{-1} s^{-1}), to ensure their industrial transfer [130]. Pending access to such natural enzymes, these levels can be achieved using engineering techniques. Indeed, artificial intelligence (AI) techniques are being used to create completely novel enzymes that open the possibilities to enrich industrial applications because of their increased properties [131, 132]. Thus, the list of potential enzymes, including those, is almost infinite!

But for the synergy between these techniques being effective, it is essential to link sequences encoding enzymes with the specifications (for example, enzyme's activity, stability, lifetime) and needs (for example, substrates to transform, working conditions, etc.) of industries [133]. In relation to this, it remains to be clarified whether current machine-learning, AI, and engineering techniques would be effective when applied to new enzymes and to approach future climate concerns, or whether new tools would need to be implemented, such as data-driven predictive tools (**Figure 3**).

Clearly, the potential of computing and AI for searching or repurposing enzymes, will depend on available computing capacities. As an example, if one uses a personal computer with a single core at 3.6 GHz, the search of enzymes in sequence databases may take approximately 142 minutes (or 1.86 g CO_2 emission) using Diamond (as the fastest search standard). The same analysis using a computing cluster takes approximately 18 minutes (or

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0.11 g CO_2 emission) using the minimum configuration with a single node composed of 40 cores at 2.5 GHz, from a hypothetical maximum of up to 134 nodes. Finally, if one has access to cloud resources (with up-to-date hardware), the search for a single genome usually takes 1-5 minutes (or 0.06 g CO_2 emission) [134]. A search for an enzyme encoded somewhere in the entire DNA from an environmental sample, the metagenome, that is sequenced, assembled, annotated, and classified *de novo* and further subjected to molecular simulations and virtual screening causes estimated carbon footprints of the average computational running times range from 113 to 5477 kgCO_2e [6]. This amount is equivalent to the amount of carbon sequestered by 103-5020 tree-months, or 19-958 km travelled by car. Thus, it is difficult to estimate what the carbon footprint associated with the screening of a single enzyme would be, given that these data refer to the analysis of samples whose enzyme content is *a priori* unknown, but values ranging from 0.008 to 0.38 kgCO_2e per enzyme may be suggested (**Table 3**).

Access to advanced supercomputers and AI would not only enable faster searches but also minimize the associated carbon footprint. As an example, it is worth noting that, considering all possible applications, AI, information and communication technology (ICT), supercomputers and quantum computing have the potential to reduce GHG emissions by between 2.6 and 5.3 Gt CO_2, equivalent to 14.8-30.3 trillion km travelled by car [135, 136]. However, supercomputers still consume a high amount of energy, especially for cooling: the world's supercomputers have an annual carbon footprint in the broad region of 3 million tonnes. Consequently, supercomputing facilities are urgently needed that do not produce any carbon emissions because they use 100% renewable power.

DESIGNING NOVEL BIO-PROCESSES FOR DECARBONIZATION

Many of the above-described products and processes will rely on the implementation of more and better performing enzymes. But there is also a need for enzymes that not only make these products and processes more sustainable and environmentally friendly, but also allow the design of novel pathways for CO_2 fixation and ultimately building up products by using atmospheric CO_2. Therefore, developing bio-based CO_2 capture technology at industrial scale will be a very urgent task to decarbonize their production processes [137]. Currently, seven different pathways involved in CO_2 fixation are known and they can be exploited for enzyme-driven decarbonization. The best-studied pathway is the Calvin-Benson-Bassham cycle with ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) as the main CO_2-fixing enzyme [138]. It is used by green plants, algae, cyanobacteria and many other microorganisms. Yet another pathway is the Wood-Ljungdahl pathway. It is well conserved within the acetogenic bacteria and the methanogenic archaea and is often associated with extreme habitats [139]. A key enzyme here is hydrogen-dependent CO_2 reductase (HDCR), the only known biocatalyst that can reduce CO_2 to formate using only H_2 as electron donor. As the reaction is fully reversible, HDCR can be used for H_2 production as well as carbon capture and production of formate as a starting material for a variety of high value products [140]. In addition, nature has evolved efficient few other pathways to fix CO_2 from the atmosphere among them the 3-hydroxypropionate bicycle, the 3-hydroxypropionate/4-hydroxybutyrate cycle, dicarboxylate/4-hydroxybutyrate cycle (DC/HB cycle), and the reverse tricarbonyl acid cycle [141, 142]. Altogether, these pathways and their respective enzymes will be important for the built up of enzyme-driven CO_2 fixing biotechnological processes.

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POSITIVE IMPACT OF ENZYMES ON BIODIVERSITY AND PLANET'S HEALTH

The benefits that enzymes bring in terms of reducing GHG emissions and supporting the bio-processes for decarbonization and recycling carbon from renewables (**Table 1**) have a direct influence on controlling or minimizing climate change and its effects. However, enzymes can also help to reduce the need and consumption of chemicals (**Table 1**), and to establish recycling and biodegradation processes that help to reduce pollution and remediate contaminated sites. This is an important issue as environmental pollution resulting from human activity is detrimental to ecosystems at different levels, such as biodiversity level which, as mentioned above, is crucial to maintain the planet's health status [36]. Note that recent estimates of bacterial and archaeal diversity suggested the existence of at least 2.2-4.3 million prokaryotic operational taxonomic units, that have inhabited on Earth over 3.8 billion years ago, and the diversity and distribution of up to 60% of the global ocean microbiome and 85% of terrestrial ecosystems are associated with temperature and contamination [36, 143, 144]. They co-exist with higher complex forms that includes plants, animals, fungi, and single-celled organisms with true nuclei (that is, all "eukaryotes"), of which about 1.8 million species are being described to date through the Earth Biogenome Project [145]. The grand aim is to minimize the influence on, or even rehabilitate or restore, the biodiversity of our ecosystems. Enzymes, as part of the nature-based solutions and circular bio-based systems, have the potential to substantially contribute to avoid the release of chemicals to, and remove pollutants from, environmental sites to improve the biodiversity status, thus extending the Natura 2000 network, that marked a significant step forward in environmental management [146]. Access to new enzymes that are not only capable of producing biobased chemicals but also help degrade pollutants in our ecosystems is critical to maintaining biodiversity and the health of our planet [147].

CONCLUSIONS

Climate change is here to stay unless humankind manages to knock it off. The only feasible approach encompasses the development of new methods, techniques and processes, mainly aiming at reducing GHG emissions. This is not an easy path, it won't be all fixed by tomorrow, but it is in our hands to gradually reduce the damage to our environment, which also means to ourselves and all living forms. It is important to remark that signs of the effectiveness of the measures tackled are already beginning to be visible. For instance, in the EU Member States and the UK, fossil emissions in 2019 decreased by nearly 3.8% [148]. This tendency needs to be extended to the whole planet and maintained or, even better, accentuated. However, monitoring this trend will be challenging in the following years because of the disturbances produced by the irruption of the worldwide SARS-CoV-2 pandemic, and the new realities and political facts we are facing [72]. The actions to fight climate change need to be engaged at local and global levels, and at personal, industrial and government levels. There is evidence that eco-consciousness is increasing across all regions and that at least 170 countries and many cities are including adaptation in their climate policies and planning processes [1]. Social media, publicizing, or initiatives like the Earth Hour, the International day of Climate Action, or Fridays for Future are also of great importance to create awareness of the consequences of inaction. The IPCC also calls out a warning: the effectiveness of adaptation to reduce climate risk will decrease with increasing warming, so we all need to grow in the same direction, and we need to do it now, so we can make the world green again. We have demonstrated here that, apart from new policies and actions, more prototypes of enzymes are needed to become techno-economic capable of implementing technologies that can contribute to the

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sustainable development for a circular (bio)economy, mitigate climate change, and contribute to blueprint roadmaps for avoiding the release of chemicals and for rehabilitation or restoration of ecosystems. At this point, with only several thousand commercially available enzymes, the environmental impacts have already been significantly reduced by 1 to 2.5 Bt of CO_2, including those emissions associated with everyday consumer products [5]. It remains to be quantified what benefits we will be able to achieve if we succeed to access and transfer new enzymes, either native, engineered or *de novo* designed, to industry, thereby offering to consumers innovative greener and sustainable products. The problems persist, but the signs are promising.

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CONFLICT OF INTEREST

None declared.

AUTHORS' CONTRIBUTIONS

M.F. and P.M-E. writing-original draft, review and editing and project administration; all other co-authors writing-review and editing.

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FIGURE LEGENDS

Figure 1. Distribution of primary energy supply worldwide (**A**) and global carbon demand (in Mt carbon, with indication of percentages depending on the source) to synthesize chemicals and derived materials (**B**). Adapted from [2, 3].

Figure 2. Schematic workflow of the key steps to produce or bioprocess textiles (**A**), washing laundry detergents (**B**), and ingredients to be incorporated into cosmetics (**C**), and the benefits enzymes could introduce in terms of the carbon footprint. Data for textiles according to [30]. Data for washing laundry according to [23, 31, 32]. Data for cosmetics according to [26]. Abbreviation: WW, worldwide; EU+USA, Europe and USA (as no reliable WW data are available).

Figure 3. Schematic workflow for the bioprospecting of enzymes for circular (bio)economy and climate change mitigation. Shown are the steps related to extraction, sequencing, assembling, annotation and virtual screening of new enzymes from the metagenomes of environmental samples, followed by their accurate protein structure prediction, high throughput characterization, and iterative improvement by engineering; finally, validation of computational predictions to design of predictive tools with which to artificially design *de novo* new enzymes.

Figure 4. Schematic workflow for the engineering of enzymes for circular (bio)economy and climate change mitigation. Shown are the steps for iterative improvement of enzyme performances by either rational design or directed evolution.

Graphical abstract legend. Repowering industry with naturally occurring or artificially repurposed enzymes, to boost consumer products innovations and to achieve climate neutrality.

TABLES

Table 1. Parameters representing the effect and the consequences of climate change and how they can be minimized with the help of enzymes.

	Quantification	
Parameter	Worldwide value (year in brackets)	Reductions achieved by using enzymes (year in brackets)
GHG (CO_2) emissions/year	36.2 Gt (accumulated in 2022)[1] 60 Gt (accumulated by 2050)[1] 49 700 Mt (only in 2022)[2,3] 700 Mt/year (increase rate from 1990-2019)[2]	1000-2500 Mt/year (by 2030)[4]
Global carbon demand for chemicals	450 Mt/year (in 2020)[5] 1000 Mt/year (by 2050)[5]	45 Mt (in 2022)[5] 200 Mt/year (by 2050)[5]
Total amount of chemicals	220 Bt/year[6,7]	90 Mt/year[8]
Ecosystem alterations	85% affected (by 2090)[9]	Not quantified

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Plant, animal species loss	16-30% (by 2070)[10]	Not quantified
Microbial diversity loss	16% (by 2100)[11]	Not quantified
Microbial CO_2 increase	0.05-0.15% (under a +4°C warming scenario)[12]	Not quantified
Economy loss	7 trillion USD/year[13]	0.5% reduction/year[14]
People vulnerability	3.5 billion (by 2070)[15]	Not quantified
Conflict risk increase	11% (in 2022)[16]	Not quantified
Food price increase	1.3-56% (by 2050)[17]	Not quantified
Global food demand	60% (by 2050)[18]	Not quantified
Food-demand gap	1260 Mt (by 2050)[18]	Not quantified
Agricultural productivity loss	2-15% (by 2100)[20]	Not quantified

[1] According to [4, 5, 21].

[2] According to [22].

[3] Approximately 62 Mt, 1291 and 8-23 Mt correspond to the washing laundry, textile, and cosmetic sectors, respectively [23-26].

[4] According to [27]. This includes: i) a Worldwide reduction of 50-119 Mt/year in the textile sector, according to [24, 25, 28-30]; ii) a Worldwide reduction of 1.9-5.3 Mt/year in the cosmetic sector, namely the bioprocessing of ingredients for cosmetics, according to [26]; and iii) a reduction in the EU of 1.4 Mt/year and in the USA of 2.3 Mt CO_2 in the washing laundry sector according to [23, 31, 32].

[5] According to [2, 3].

[6] According to [12].

[7] The production of 1 kg textile requires approximately 3 kg of chemicals, which according to a worldwide production of 119 Mt textiles [24, 25, 33], can be translated into approximately 357 Mt potential chemicals. In the case of the washing laundry sector according to IndexBox estimates, in 2019 approx. 24 Mt of washing laundry detergents were consumed worldwide, and according to an increase of 9.5% in 2020, the total amount by end 2020 reached about 26 Mt [34], which can be potentially flushed into the water system. For cosmetic sector no reliable data are available.

[8] According to [35].

[9] According to [36].

[10] According to [37, 38].

[11] According to [36, 39].

[12] According to [40].

[13] According to [41].

[14] According to [42,43].

[15] According to [44].

[16] According to [45].

[17] According to [46].

[18] According to [47].

[19] According to [48].

[20] According to [10, 49, 50].

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Table 2. Enzymes allow, both *in vivo* and *in vitro*, the development of unique and innovative functional products (including materials) or processes that are key in the circular (bio)economy. Adapted from [76].

Sector	Examples	Number of companies	Number of employees	Total sales in billion €
Automotive sector	Car body parts reinforced by natural fibres, car interior lining and seats based on bioplastics, tyres based on dandelion	17	756 000	36
Building industry	Wooden structures, composite materials reinforced by natural fibres, insulation materials, biobased screw anchors, biobased concrete mixtures	317 300	1 900 000	172
Chemical industry	Bioplastics, biobased platform chemicals	2121	434 313	186
Energy	Pellet stoves, biogas, biodiesel fuel, bioethanol, synthetic fuels, algae, kerosene, enzymes for better oil extraction	923	220 157	466
Agriculture & forestry	Precision agriculture, plant and animal breeding, short-rotation forestry, aquaculture	285 000	1 000 000	32
Mechanical engineering	Bioreactors, bioprocessing engineering, agricultural technology and equipment, greenhouse technology, biolubricants	6277	978 000	207
Pharmaceutical industry	Biopharmaceuticals, medicinal plants and herbs	923	135 773	36
Food & beverage industry	Enzymes, fragrances, amino acids, natural food additives, probiotics, food lupin protein	6000	555 000	41.4
Consumer goods	Biobased tensides, bioactive constituents in cosmetics, enzyme-based additives for cleaning agent	-	-	203
Textiles & clothing	Natural raw materials for synthetic fibres, high-tech fibres made of spider web, plant tannins	1300	111 313	11.33

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Table 3. Carbon footprint of key tasks associated to the screen and production of enzymes.

Task	Carbon footprint
Bioinformatic and computational screen	113 to 5477 kgCO_2e per analysis[1] 0.008 to 0.38 kgCO_2e per enzyme[2]
Production	8.9 g CO_2e per g enzyme[2]

[1] According to [6].

[2] Some of the tasks reported by Grealey *et al.*, 2022, include the analysis of up to approx. 15 000 genes in a computational run; while the equivalence may not be appropriate, the given carbon footprint per gene (or enzyme) refers to this number [6].

[3] According to [106].

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Figure 1

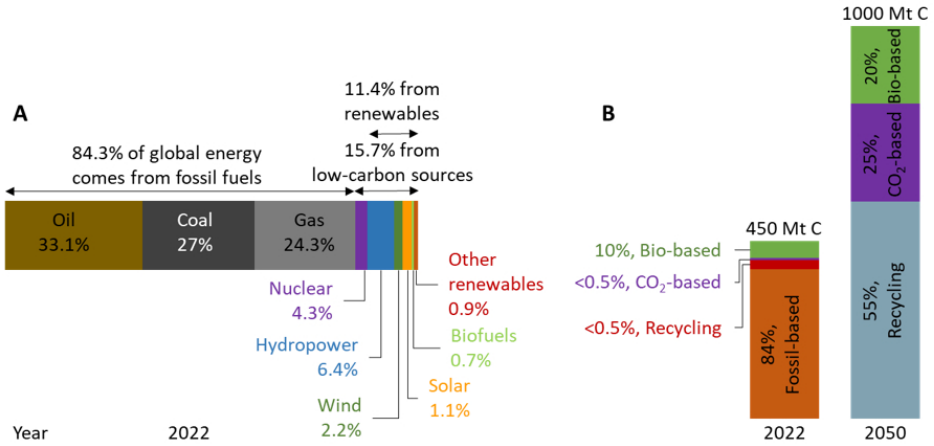

Figure 1. Distribution of primary energy supply worldwide (A) and global carbon demand (in Mt carbon, with indication of percentages depending on the source) to synthesize chemicals and derived materials (B). Adapted from [2, 3].

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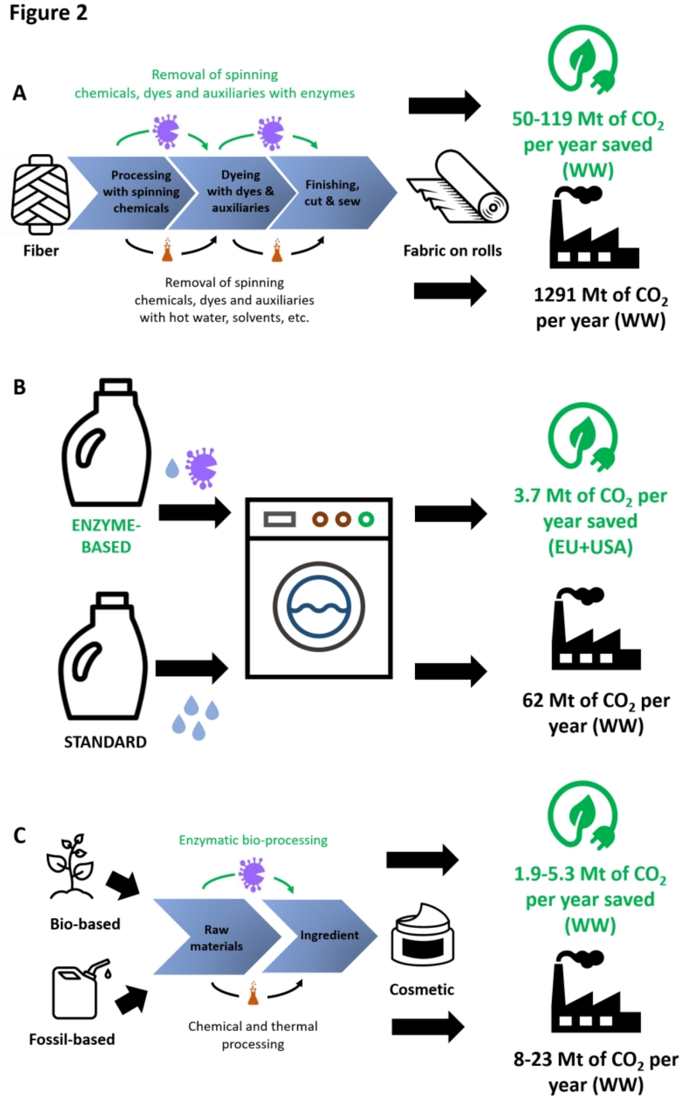
Figure 2



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294x493mm (150 x 150 DPI)

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Figure 3

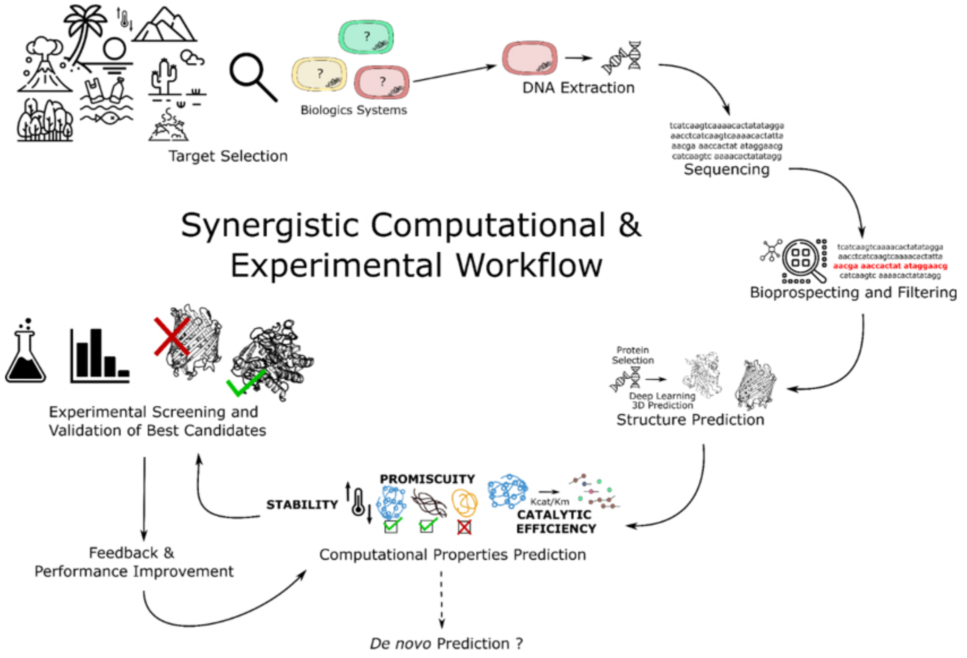

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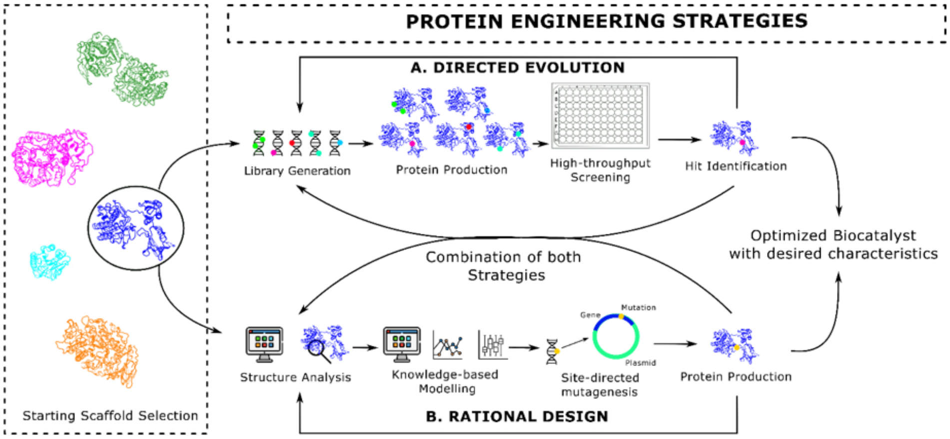
Figure 4



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164x90mm (150 x 150 DPI)

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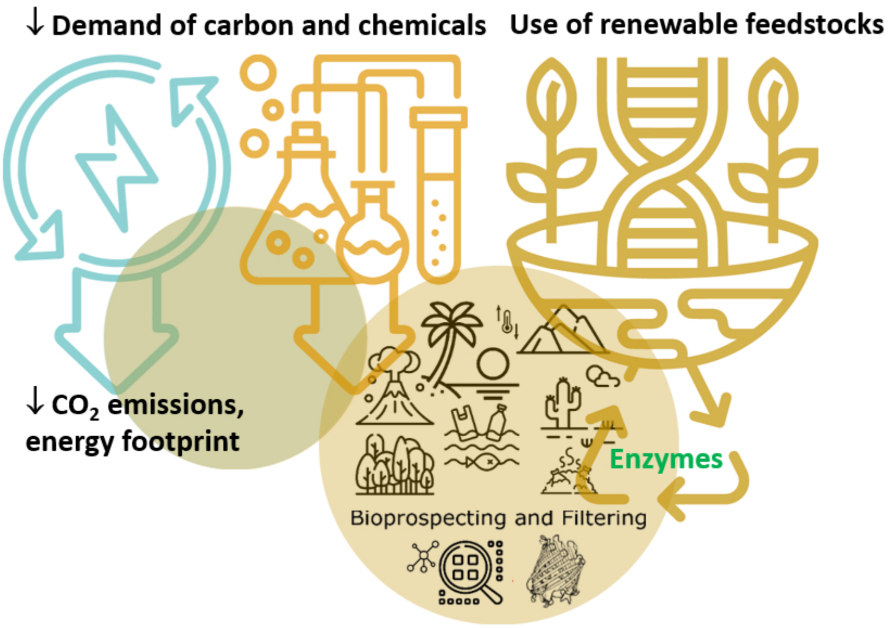
Graphical abstract



155x115mm (150 x 150 DPI)

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