Merging marine sustainability and beauty

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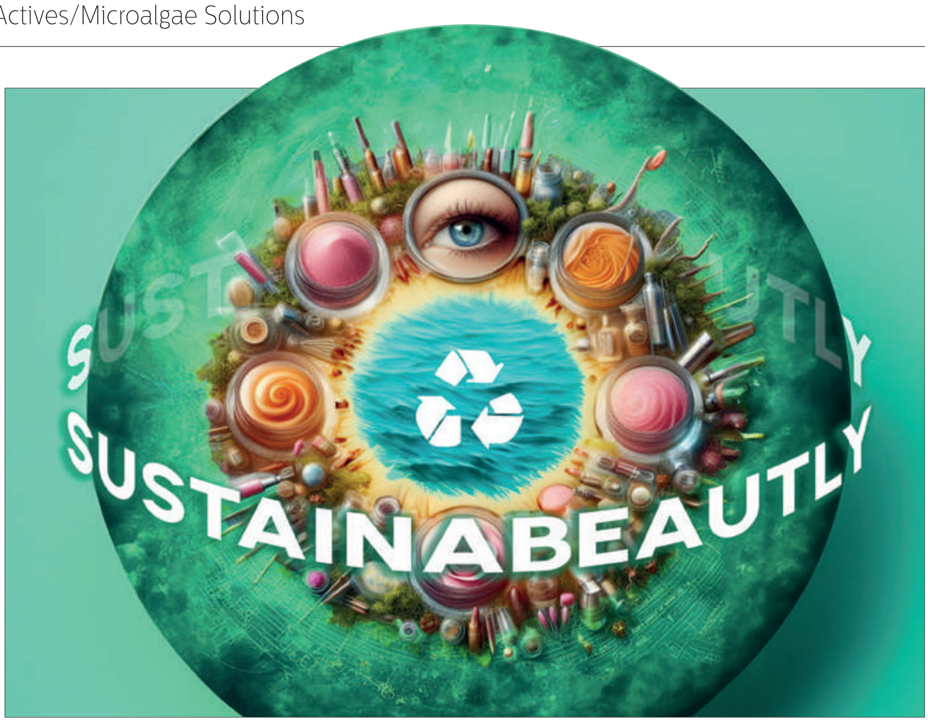

Ultraviolet rays enter through the atmosphere and heat the Earth's surface, which radiates the heat again. Greenhouse gases (GHGs) absorb the heat, leading to an increase in the temperature surface.

Those GHGs are also responsible for air pollution. Those factors produce a double effect. The first is anthropogenic climate change, with the consequences that we all know: water scarcity, melting polar ice, intense droughts and so on.

The second effect is the production of reactive oxygen species (ROS) that, when overproduced, can be harmful for all living organisms. ROS are molecules that contain at least one oxygen atom and one or more unpaired electrons.

Examples are oxygen free radicals or hydroxyl radicals. ROS are produced naturally because of our metabolism functioning, but in small quantities. The problem arises when they are accumulated in our bodies as they trigger numerous reactions leading to skin tissues damaging.[1]

ROS can lead to:

- Inflammation through prostaglandin E2 synthesis
- Damage the skin barrier oxidizing lipids and proteins.
- Increase sebum secretion (due to high levels of oxidized lipids)
- Produce acne lesions (produced by $^1O_2$).
- $^1O_2$ reduces the expression of matrix metalloproteinase (MMP-1) in dermal fibroblasts, leading a reduction of collagen production and, hence, an impairment in the dermal matrix

Unveiling the 'Sustainabeautly' concept

Luckily, this situation is reversible, as there are antioxidant molecules with the capacity of avoid ROS formation or scavenge them. Potential antioxidant molecules are ascorbic acid, vitamin E, carotenoids, or polyphenols.[1] Natural extracts, contain those antioxidant molecules and can accomplish multifunctional benefits for our skin, apart from the antioxidant action.

'Sustainabeautly' arises as a concept that merges sustainability and beauty. One cannot repair the damage caused by unsustainability without taking care of the planet, as it is a bidirectional action: repair, but also protect.

MC Actives/Microalgae Solutions produce natural, marine, and sustainable active ingredients with demonstrated efficacy. Sustainability is present along all the production line of the ingredient, specifically in two major steps: upstream processing: holobiont cultivation; and downstream processing: ingredient fabrication.

Upstream processing: holobiont cultivation

Phytoplankton cultivation itself for cosmetic ingredient production has various advantages over traditional crops. For instance, phytoplankton can be grown without fertile soil, prevent and reduce water pollution without the need for fertilizers. However, its cultivation at pilot or large scale is a limiting factor as high quantities of water and energy supply are needed.[2]

Systems to cultivate phytoplankton is divided into two main groups: open and closed reactors, although there exist also hybrid systems.

Closed photobioreactors are employed for cosmetic ingredient production as standardization and production reproducibility is possible compared to the other systems, while open reactors are used in other fields like agriculture.

Moreover, closed reactors produce biomass with more quality, and has lower contamination risks. Other advantages of closed reactors versus other systems of cultivation are that there are less water (evaporation) and CO_2 losses, it requires less space for its installation, the culture growth rate is higher, and it does not depend on the weather.[3]

However, closed photobioreactors has a series of drawbacks such as: high setup cost, it needs more energy inputs, and it is difficult to scale up.[3]

To overcome those disadvantages, we develop a cutting-edge technology to produce phytoplankton holobiont: Phycosphere Biodynamic Technology® (PBT). PBT consists

ABSTRACT

MC Actives and Microalgae Solutions have launched three marine ingredients with a 'blue biotech' patented technology (PBT®) under a sustainable approach, that includes use of green solvents, upcycling of solvents and sustainable pathways trough a zero-waste production, maximizing the use of all the fractions of the process (supernatant and pellet). The sustainable-marine ingredients (Phycoskin® One, Osmocean Phycoskin®, and OpenSee Phycoskin®), are focused on different actions on the skin: whitening, anti-ageing, and eye contour care

of a system that biomimics the conditions of the natural marine environment of the phytoplankton holobionts, a combination of phytoplankton cells and its microbiota.

Applying bioengineering, we cultivate holobionts in a green-lab grow, providing the necessary conditions to enhance the production of bioactive molecules. PBT saves energy (-70%) compared to traditional cultivation systems by using low-energy pumps.

PBT also saves water (-50%) compared to traditional cultivation systems by recirculating it. Furthermore, water use for cleaning is not necessary after cultivation while the traditional reactors need to be cleaned. PBT also negates the use of antibiotics and chemical products, leading to less pollution when compared with traditional technologies.

PBT technology allows to grow sustainable holobionts taking care of planet Earth while producing the raw material that is used in the following step: the biomass processing for active production. The above makes PBT a sustainable technology, with low environmental impact, in contrast to the old technologies used to grow phytoplankton.

Downstream processing: ingredient fabrication

After phytoplankton holobiont cultivation, it is collected and processed in our laboratories following environmental-friendly techniques. This process includes: i) bioactive molecules extraction, and ii) final product fabrication.

Sustainability can be understood as a rational way of improving processes to maximize production while minimizing the environmental impact.[4] Under this perspective, we process phytoplankton holobionts using safe and environmentally friendly solvents such as water and 96% ethanol; upcycling solvents for successive bioactive molecules extraction; and valorising residual biomass after extractions (pellets) to create new, upcycled ingredients.

In this way, our ingredients are focused on achieving the maximum sustainability concept, towards a zero-way approach.

An upcycling process?

Upcycling is a creative response to extract additional value from what would otherwise be wasted.[5] We pursued a zero-waste approach by designing extraction processes in which the waste is revalorised and converted into a new, valuable ingredient with cosmetic efficacies. We have developed two upcycling processes.

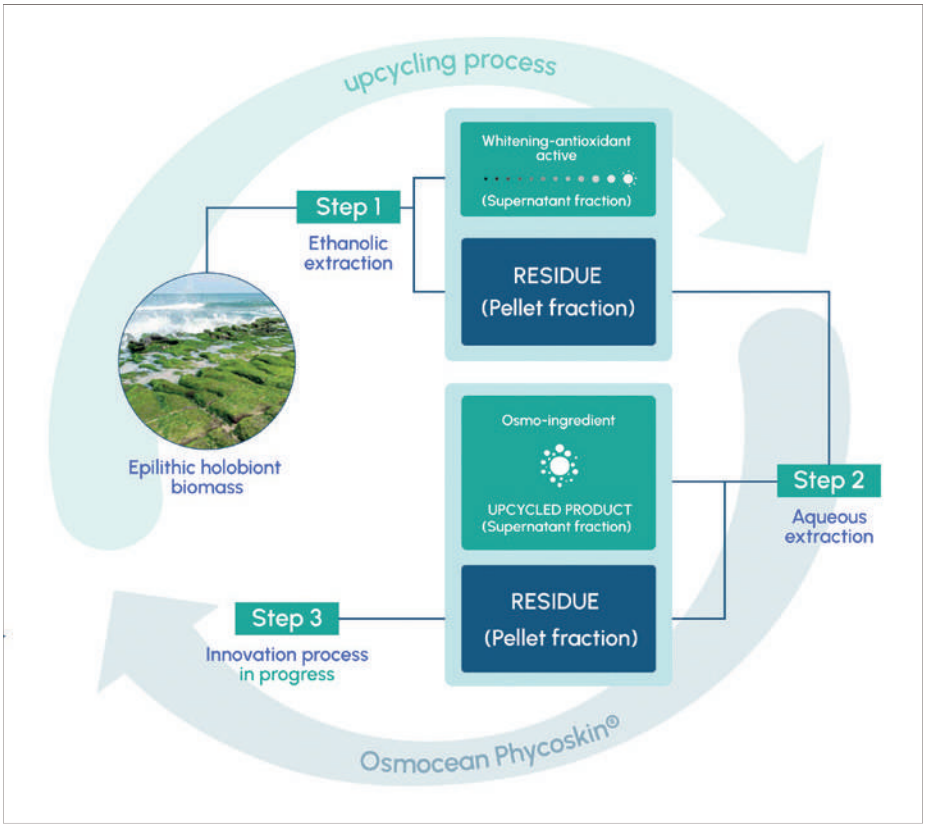

Figure 1: Cosmetic upcycling process 1

Upcycling process 1

Epilithic holobiont biomass is produced and processed in our laboratory. The first step is an ethanolic extraction from which we obtain the first active ingredient: the whitening-antioxidant active, and a residue (pellet fraction 1).

The pellet fraction 1 is then used for a second extraction (aqueous extraction), obtaining a second, anti-ageing active ingredient based on osmolytes, and another residue (pellet fraction 2), that is under research (Figure 1). After this process, ethanol is recovered for successive extractions.

Cosmetic efficacy of the ingredients coming from the upcycling process 1

The first ingredient produced in this process is the whitening-antioxidant active. As stated in the April 2022 issue of Personal Care Global,6 the ingredient reduces the pigmentation by 30% on spots in summer, and its mechanism of action was demonstrated by 51% of tyrosinase inhibition.

After producing this ingredient, a pellet (pellet fraction 1) is used for the second aqueous extraction producing the anti-ageing ingredient based on osmolytes (hereafter, the Osmo-ingredient). The cosmetic efficacy of this ingredient was described in the April 2023 issue of Personal Care Global.[7]

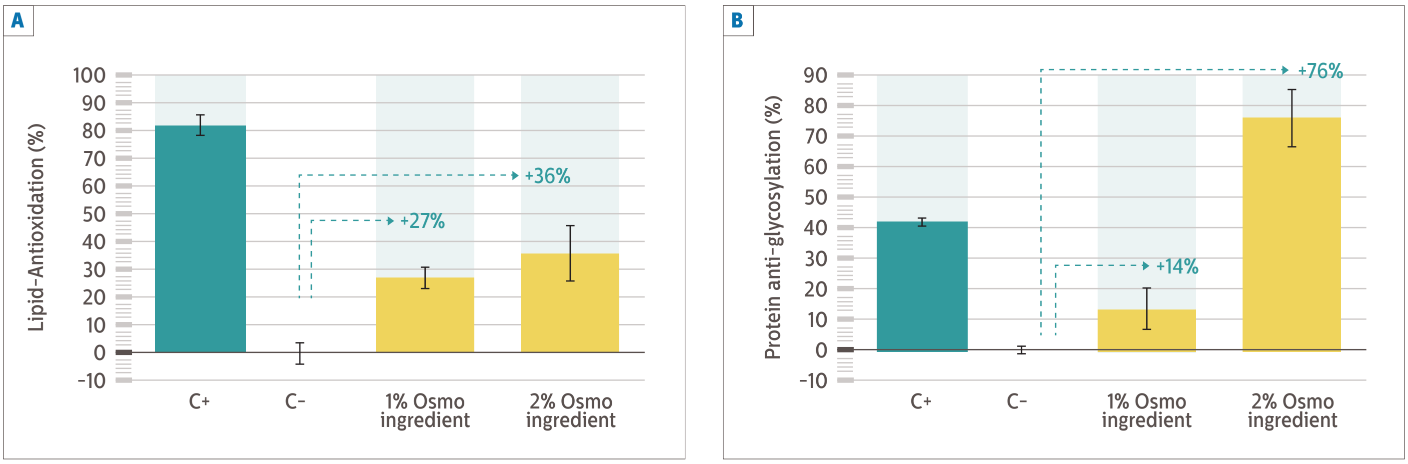

Figure 2. A: Capacity of protection against lipid alteration by Reactive Species. $p<0.05$ Osmo-ingredient vs C-; C-: distilled water; C+: 0.03% Vitamin E analogue. 2B: Capacity of protection of the cellular structure against protein alteration by glycosylation derived from oxidative and non-oxidative processes. $p<0.05$ Osmoingredient vs C-; C-distilled water; C+: 0.7% aminoguanidine

There are new results revealing the high antioxidant capacity of this ingredient as 2% of the Osmo-ingredient produces a protein anti-glycosylation of 76% compared to aminoguanidine, and 36% of lipid-antioxidation compared to vitamin E analogue (Figure 2A and 2B).

This antioxidant action prevents the break-down of collagen and elastin fibres, and structural lipids, leading to an antioxidant protection of the epidermis and dermis. Moreover, 2% of the ingredient has shown to scavenge peroxyl radicals produced in the human skin (ORAC assay) with a value of 83.87 mmol Trolox equivalent/g, values like those reached by bakuchiol.[8]

Upcycling process 2

Pelagic holobiont biomass is produced and processed in the laboratory. The first step is an ethanolic extraction from which we obtain a first active ingredient that is under development (ingredient 1), and the residue (pellet fraction 1).

The pellet fraction 1 is then used for a second extraction (aqueous extraction), obtaining a second active ingredient: an eye contour active (ingredient 2) and another residue (pellet fraction 2), that is under research (Figure 3). After this process, ethanol is recovered for successive extractions.

Cosmetic efficacy of the ingredients coming from the upcycling process 2

The first ingredient produced in this process is an ingredient that is currently being tested in *in*

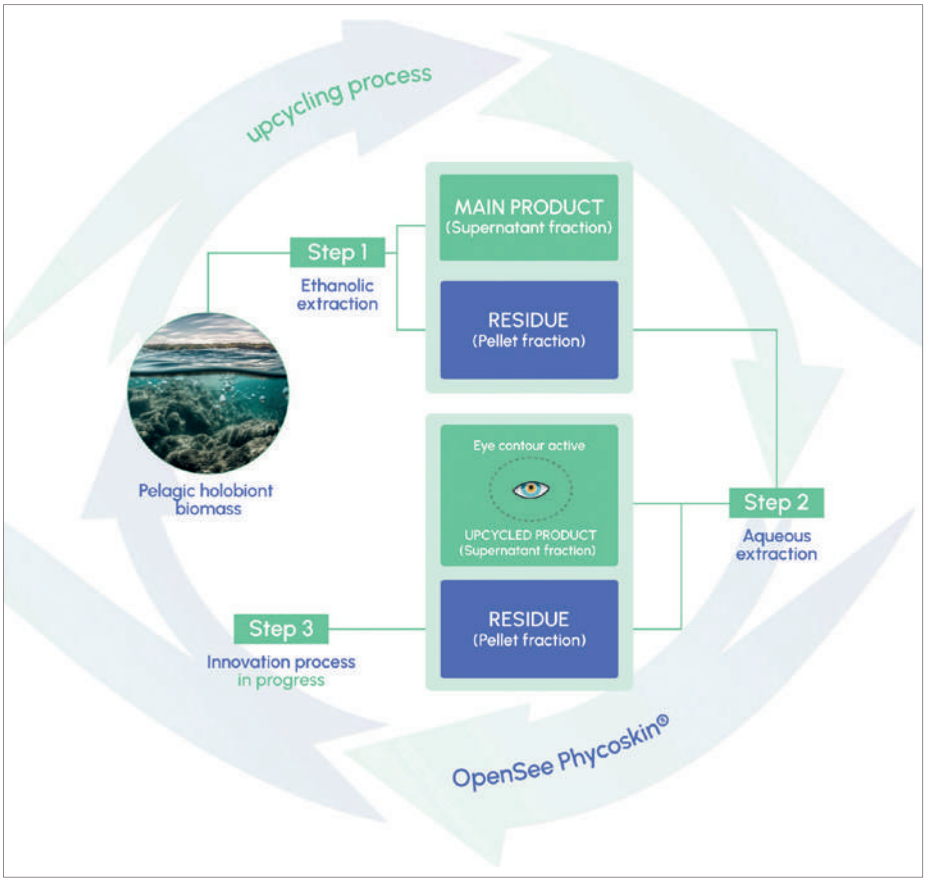

Figure 3: Cosmetic upcycling process 2

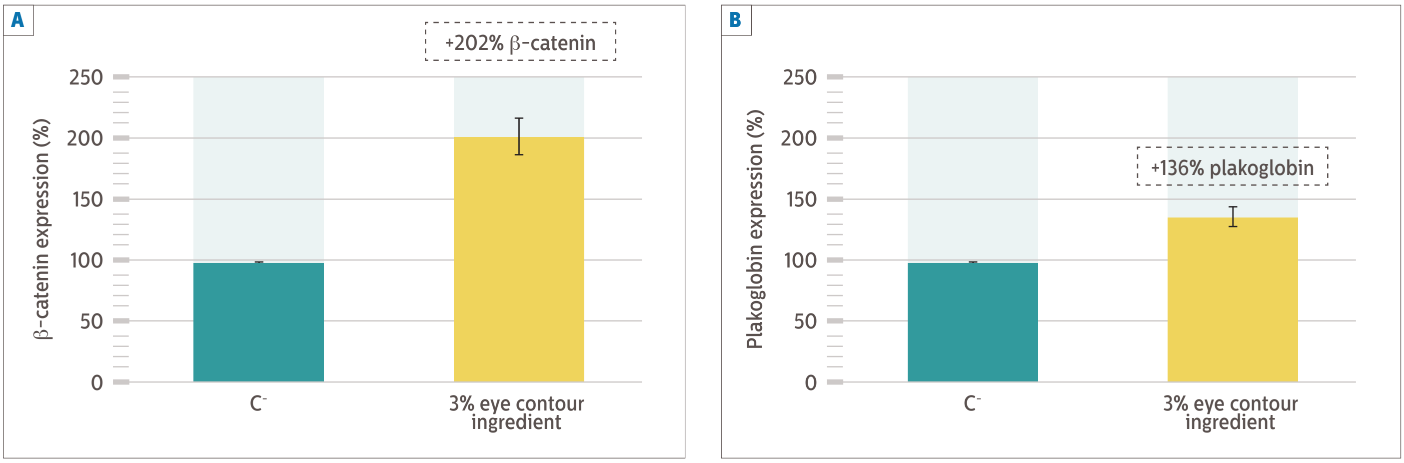

Figure 4: Epidermal cell adhesion proteins expression in HaCaT cells using the eye contour ingredient. A. β-catenin expression and B. Plakoglobin expression. Results of densitometry analysis. Replicates=2.C-: basal medium culture without the active ingredient

vitro tests. After producing this ingredient, the pellet (pellet fraction 1) is used for the second aqueous extraction producing the eye contour active. The cosmetic efficacy of this ingredient was published in the March 2024 issue of Personal Care Global.[9]

Two new clinical tests were performed for this eye contour ingredient. The first one demonstrated the capability of the ingredient to be a natural alternative of vitamin K1 oxide in attenuating the signs originated in the eye contour, specifically eyebags and dark circles pigmentation.

The second clinical study demonstrated the flash effect of the ingredient in just 40 minutes by increasing elasticity (+20%), and moisture (+15%). New *in vitro* tests revealed an increase in protein production implied in the cell adhesion of the epidermal cells (Figures 4A and 4B), and an increased production of dermal proteins: collagen (ten-fold increase) and elastin (1.57-fold increase) (Figures 5A & 5B).

New results reveal an antioxidant capacity in terms of peroxyl radicals scavenging of the eye contour ingredient at 3% of 81.4 mmol Trolox equivalent/g, values like those reached by bakuchiol.[8]

The two upcycling process leads to the production of four active ingredients, following the 'sustainabeautly' approach: restoring beauty with sustainable processes.

Those are only two examples of the upcycling process carried out to produce the marine ingredients, in which the residues 'pellet fraction' coming from the ethanolic extraction is used to obtain other high-value ingredients.

The active ingredients obtained from the 'sustainabeautly' approach showed amazing quantitative and qualitative (visible) cosmetic efficacy.

Conclusions

'Sustainabeautly' is the link between creating ingredients to repair the damage caused by climate change in the skin, under a sustainable point of view, and taking care of the environment.

Natural cosmetic actives can be produced in an environmentally sound way through a sustainable upstream (raw material production process) and downstream (transformation of raw materials into actives) production processing. **PC**

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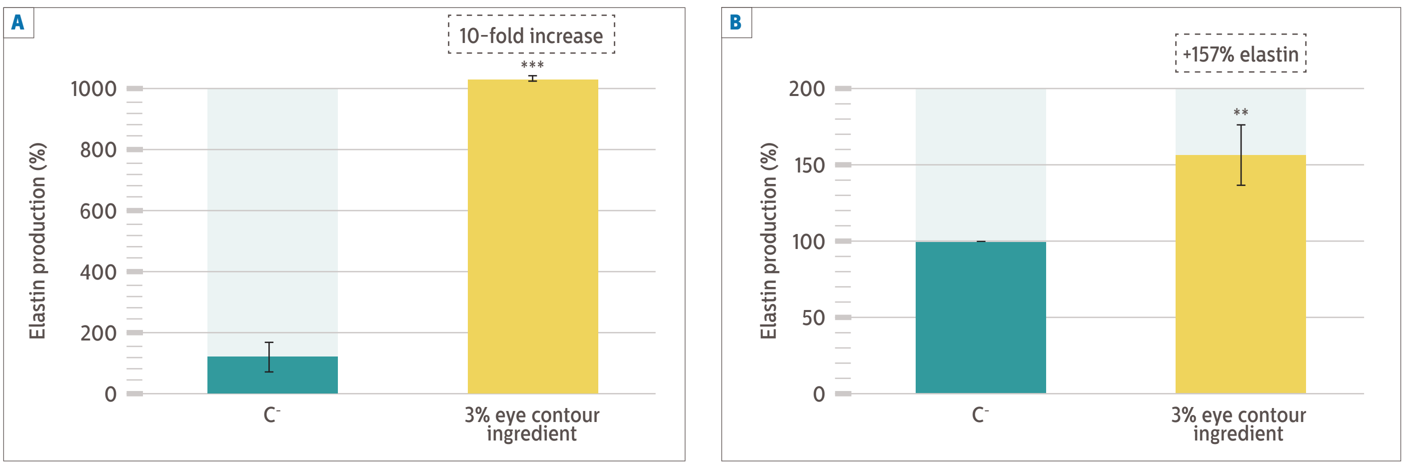

Figure 5: Dermal proteins expression in HDFc cells using the eye contour ingredient. A. Collagen type I and B. Elastin. C-: basal medium culture without the active ingredient. Replicates = 2. Student's test. *: 0.05 > p ≥ 0.01; **: 0.01 > p ≥ 0.001. Images represent Immunofluorescence assays. Green: collagen type I; blue: cell nuclei of HDFc. Red: elastin