Procédés d'obtention d'acides gras à partir de microalgues et leurs évolutions

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Abstract: *Microalgae have the potential to produce lipids with a high productivity such as two to three times what we may do with plants. We know also that the lipid quality can add value (EPA and DHA) to cosmetics and food complements. Even if the industrial scale is not reached yet, we know that we have to adapt to the lipid extraction process. In fact, the main problem is to remove water and to release lipid from cells in order to decrease the use of organic solvents. Others technologies are under process to prevent the use of such solvents. Also, the purpose of this article will be to expose the new extraction processes and their industrial potential for cosmetic applications. Then, some technologies of drying, supercritical CO_2 extraction, enzymatic extraction, oil extraction, and new solvents to be used for extraction will be described. These may bring some outlooks for the future, thanks to the multiple lipid activities on physiology and to the global sustainability of the system.*

Key words: *microalgae, blue biotechnology, lipids, extraction, technologies, sustainable development*

The cosmetic industry answers not only to expressed requests of the consumers, but also to the latent needs. Besides the efficacy, the products are expected to present a certain innovation and sensory properties. The consumers want transparency and sustainability too, and the industry is working on that. For instance, the cosmetic ingredient must be as much stable as possible, and without preservative. Cosmetic products are applied on healthy skin. Therefore, the zero risk strategy is leading all developments. Even if considered of second importance, hygiene and personal care have been proved to be essential in all cultures and civilizations to improve well-being and self-esteem. The cosmetic industry is subject to upstream and downstream pressure. For instance, regulation is getting harder, especially with REACH (all substances must be registered and their harmlessness proved). Besides, consumers are more demanding and more aware of risks, and the product efficacy should be proved by complementary tests (*in vitro*, *in vivo*, clinical). Transparency and traceability have become keywords, as well as environment-friendly.

Microalgae are at the origin of life on our planet. The story begins somewhat 3.8 billion

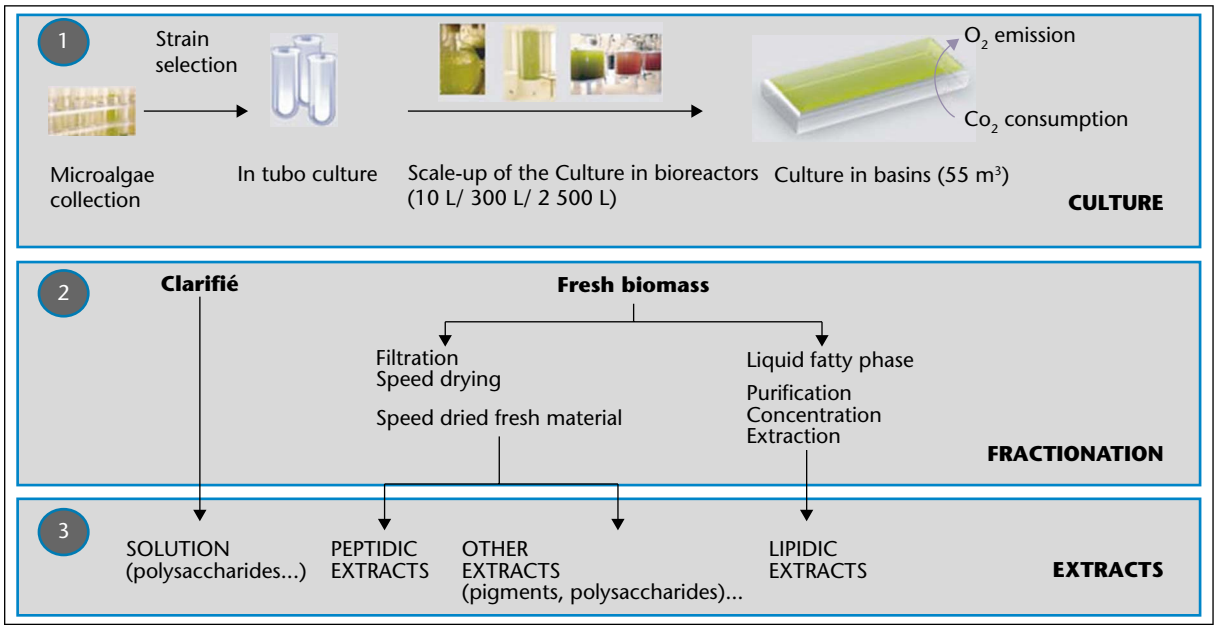

Figure 1. *General process developped in Soliance Blue biotechnology center.*

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years ago, with the apparition of the first cyanobacteriae (also called blue algae). They were the very first living organisms on Earth. By being autotrophic, those organisms produced oxygen via photosynthesis mechanisms. Then, under the action of UV rays, part of the oxygen (O_2) that they produced was transformed into ozone (O_3) that accumulated in the stratosphere; thus, a protective layer against UV was formed, allowing a noticeable reduction in their biocide effects.

An atmosphere being created, life could go on and develop, giving birth to other groups of plants and later on animals. As human beings, we have to remember that our existence is directly linked with the emergence of those marine microplants. They are the first link of the global food chain initiated in water. Being able to photosynthesize, they rely on solar energy and on simple elements such as CO_2, water, and minerals to produce their own organic material. Thus, they are necessary for the survival of all the other species. Animal plankton feeds from microalgae, before being eaten by small fishes, themselves eaten by fishes of greater sizes, themselves becoming food for humans. Microalgae contain important quantities of fatty acids, especially those called essential fatty acids that human can't synthesize. Consequently, they are of vital importance for the natural ecosystem and for our own survival.

Microalgae's lipids add value to the industry

Biodiversity is an inspiring innovation or the other way round? Similar to microscopic plants, microalgae constitute a pool of incredible molecules, in such a way that we can consider them as little organisms that have all the resources they need' inside them. They are characterized by an impressive ability to adapt to their environment that allowed them to survive for millions of years. We are benefiting from millennium of evolution and natural innovation to be inspired by and to innovate with. While studying the mechanisms they have developed to resist UV radiation, draught, extreme temperatures, and hypersalinity, we can come up with new industrial applications at the service of mankind and its environment. Biomimetism, *i.e.,* imitation of nature, represents a particularly interesting way to go for the development of active molecules. Considering the existing biodiversity (thousands of strains and many more till to be discovered) and the ability to adapt, we can only but imagine the potentials that microalgae can offer to us.

For instance, microalgae created biological protection strategies such as antioxidants (carotenoïdes, β-carotene and astaxanthine, tocopherols, or ascorbate) and enzyme systems (superoxide dismutase) against molecular oxygen and oxidation. Confronted to an aggressive environment, microalgae produce exopolysaccharides. In order to protect themselves from UV rays, they synthesize mycosporine (MAA), scytonemine, and long chain polyunsaturated fatty acids (AG-PUFA).

Soliance has been working on microalgae for years (to develop cosmetic ingredients of natural origin), especially long chain polyunsaturated fatty acids, which are our main concern here. These molecules are of great interest in terms of applications: cardiovascular, neurodegenerative, and inflammatory diseases, for example. They also present interesting properties when applied topically. Biological activities of AG-PUFAs on the skin have been proved through various mechanisms. For instance, they stimulate the activity of the proteasome, an enzymatic complex present in the cells, in order to ensure the degradation of oxidized proteins and the renewal of neo-synthesized proteins (Friguet *et al.*). Thus, cutaneous cells are detoxified.

Various processes to extract these molecules have been developed.

Various strategies for lipid extraction

Soliance has been working for years on these technologies. First, by maintaining a phyto-

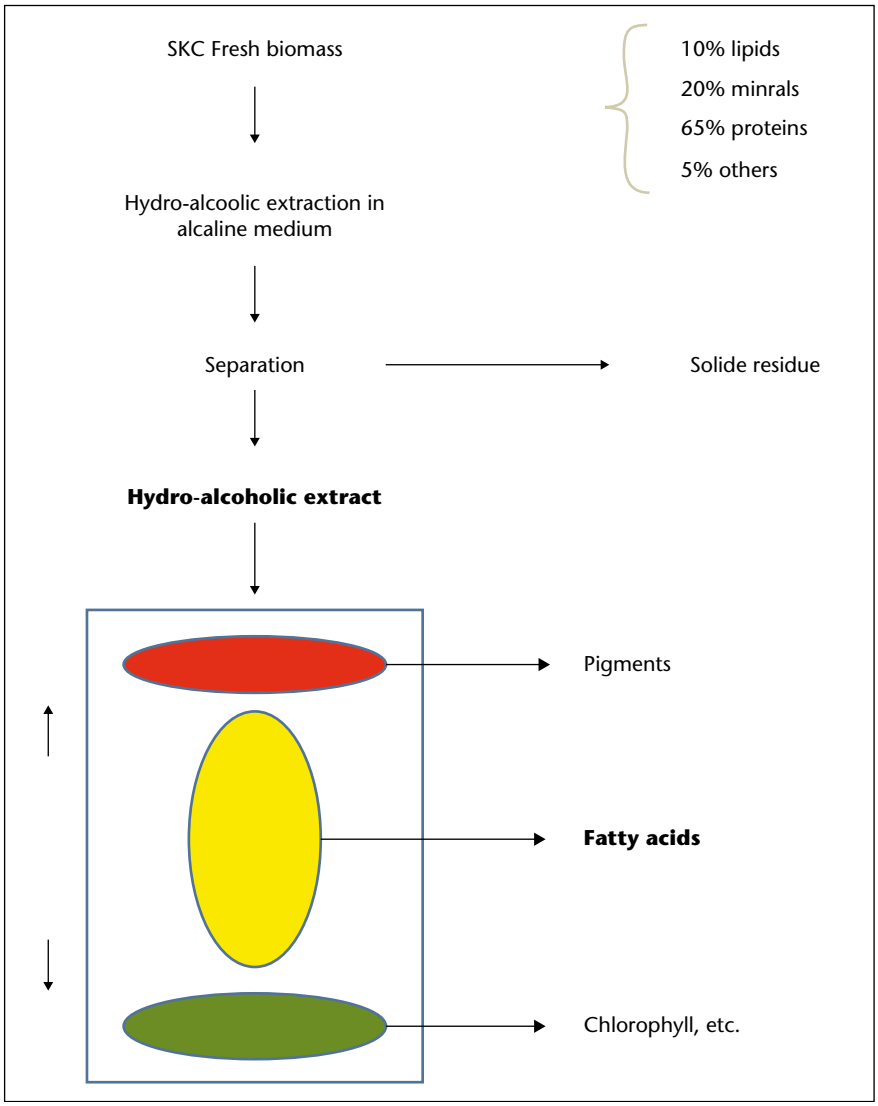


Figure 2. *Specific process developped by Soliance Blue on Skeletonema costatum micralgae.*

plankton collection, which is an untapped reservoir of biodiversity. Then, by optimizing microalgae culture and active molecules extraction from the biomass, to develop new cosmetic ingredients. The uniqueness of the Marine Spring Water that provides the culture medium basis, and exclusive developments are also Soliance expertise *(figure 1)*.

Soliance has based its extraction process on pharmacognosy strategy. Microalgae are cultivated from the small scale of the laboratory to 55 m^3 basins through several scale-up techniques. Then, the biomass is collected and frozen. A first hydroalcoholic extraction is realized to get the most polar and soluble fractions. Then, apolar solvents are used to extract the most apolar molecules such as pigments, and polar such as chlorophylls. The AG-PUFAs are at least subject to molecular distillation to eliminate any residue trace. The obtained lipidic extracts are particularly rich in AG-PUFAs (> 25%) and free fatty acids (> 40%) *(figure 2)*.

This process uses extraction solvents. It enables the obtention of lipidic fractions particularly rich in AG-PUFA that are not so much colored, but it is not very environment-friendly and therefore not subject to certifications (Ecocert, Natrue, etc.). It is still expensive and the solid residues have to be treated to recycle the solvents.

Other technologies have been developed to improve the ecological profile of the extraction. Enzymatic extraction, using proteases and cellulases to "open" the cells and release their content, is an option. Supercritical CO_2 extraction is even better, since it doesn't use any chemicals. Soliance uses the enzymatic extraction for one of the microalgae valorization *(figure 3)*.

This process is solvent-free and the extract is obtained directly with a lipidic excipient, but it is still expensive, and not selective enough (especially towards chlorophyll and needs a treatment of the solid residue).

Outlooks

Other technologies used to obtain AG-PUFAs are also promising. They are related to new challenges in process engineering concerning the culture, the separation, or the extraction. We may mention a few examples:

– **microalgae culture**, which implies optimization of the strain and improvement of the culture conditions;

– **separation**, with continuous technologies, or by floating technique, for instance;

– **drying**, which may be processed with a microwave or in more classical manners;

– **extraction**, various methods have been recently developed and used: sonication and microwave, supercritical CO_2, electroporation, enzymatic way, affinity techniques, and bio-based solvents.

In terms of outlooks and forecasts, it is important to note that functionalization of the AG-PUFAs is a good way to improve the bioavailability of the molecules, which is essential for any active ingredient, whether it is designed for food, pharma, or cosmetic industry. A way is glycosylation *(figure 4)*.

Another technique is through biological pathway, such as those used to produce sophorolipids *(figure 5)*.

Sustainability and environment

Microalgae are at the core of ecology. Our planet is threatened by the impacts of human activities. In terms of natural resources, our consumption of energetic substances (fuel, gas, coal) as well as minerals (calamine, precious metal) or plant- and animal-derived

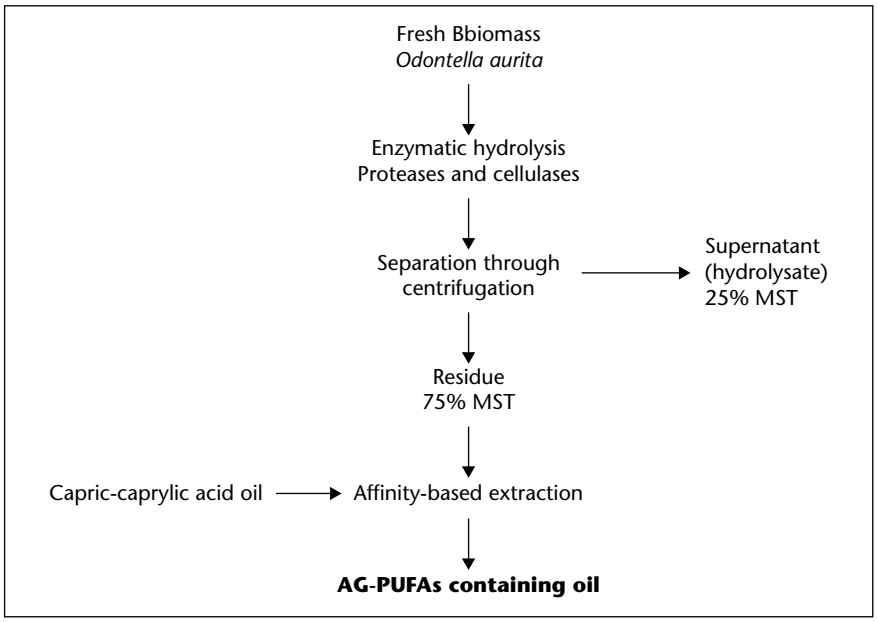


Figure 3. *Specific enzymatic process developped by Soliance Blue on Odontella aurita micralgae.*

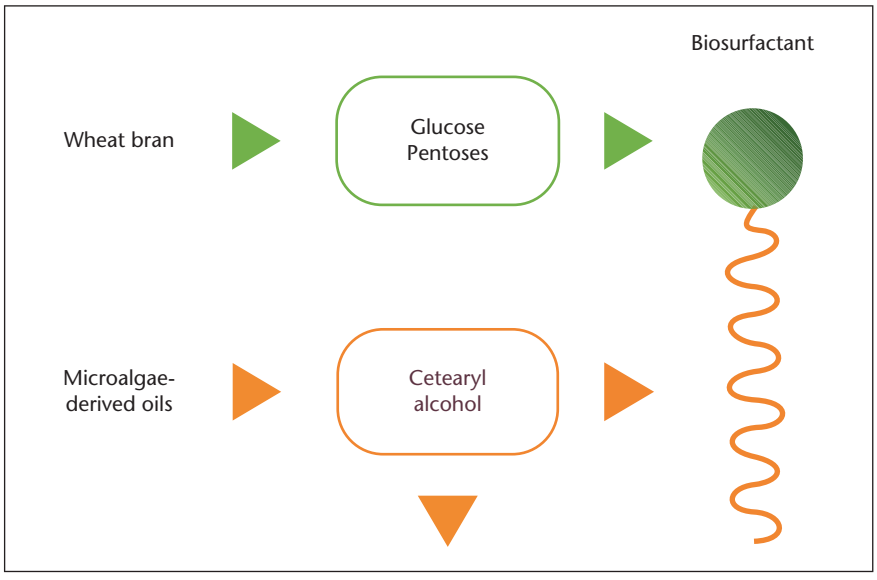
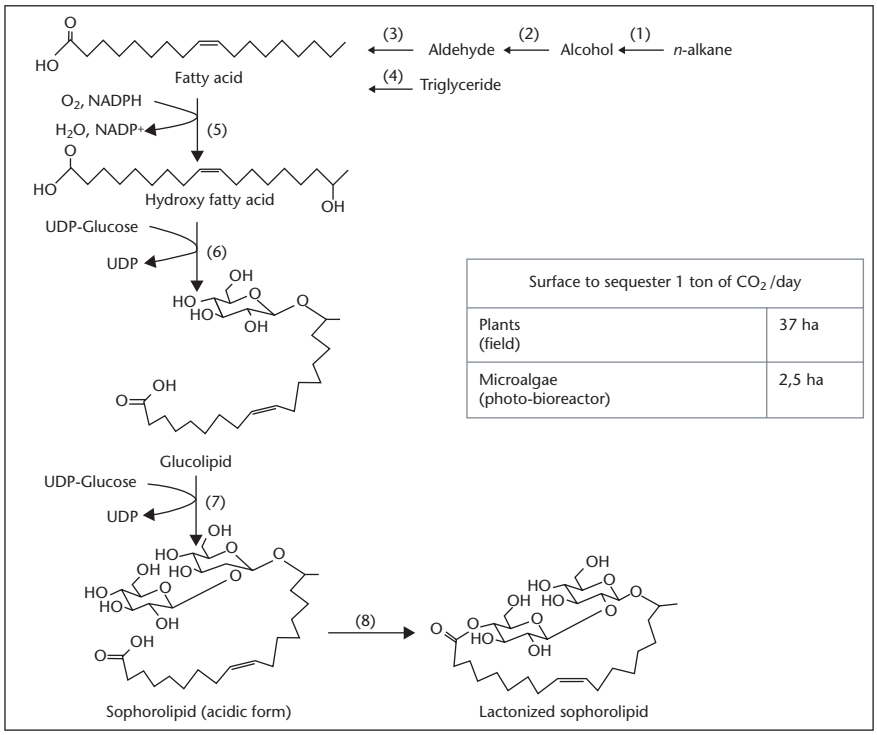


Figure 4. *Functionalization of AG-PUFAs through glycosylation.*



Surface to sequester 1 ton of CO_2 /day	
Plants (field)	37 ha
Microalgae (photo-bioreactor)	2,5 ha

Figure 5. *Functionalization of AG-PUFAs through biological pathway.*

substances is far more higher than what Earth can naturally recreate. It is therefore necessary to find today the innovative solutions of tomorrow. Microalgae consume more O_2 than they emit CO_2, and they represent 80% of the emitted O_2 on the planet! Moreover, they represent a renewable and natural raw material for the industry. By cultivating microalgae, we spare fish from being used for their oil content.

Pure water is becoming an increasingly scarce and precious resource. International development projects taking part in its savings and depollution are growing more numerous.

Progressively, the demographic explosion creates a competition between arable lands – dedicated to human nutrition – and building lands – dedicated to housing. It is a particularly important data as cultivations not dedicated to human nutrition will be less and less taken into consideration. Industries whose activities depend on those crops will have to find raw materials elsewhere. From now on, light is shred on natural renewable resources that do not enter in competition with human nutrition!

Carbon balance

Atmospheric pollution due to GEGs (greenhouse effect gases) and noxious substance emissions represent a real concern for the future of our planet. The CO_2 consequences on our climate are various and complex: global warming, ocean acidification, forest poisoning, etc. It is therefore urgent for us to find solutions to secure the future of our planet.

Indeed, we now know that microalgae can sequester CO_2, and even more interestingly, we now know how to push their metabolism to increase their CO_2 consumption. Thanks to photosynthesis, they constitute the first oxygen producer on Earth, far ahead all the forests together. They produce 80% of the oxygen of our planet, and can be considered as the "Earth lungs."

Conclusion

Microalgae are definitely of great interest for the human being. Many applications can be identified, such as in food, pharmaceuticals, and cosmetics. AG-PUFAs applications as active ingredients dedicated to cosmetic products are certain, but improvements have still to be made. Cleaner processes are to be developed, with always the economical constraints related to cost and yield in mind. Besides, production processes should always be worked at different scale, in order to enable industrial productions. Regarding the chemical evaluation of the obtained oils, the actual techniques have to be improved. One interesting outlook is nevertheless the functionalization of the microalgal oils.