From natural fermentation to an industrial and innovative biomimetic process

Today, more than half of all companies say they are committed to sustainable development[1], but it is becoming increasingly clear that reconciling environmental and economic performance involves many difficult choices. Only 21% of companies have a clear roadmap for implementing their sustainability strategies. At AFYREN, we believe that **sharing knowledge** and **experience** can contribute to a more sustainable future. In our blog posts, we seek to share the expertise we have developed on our own journey toward a **sustainable, circular business model**.

In 2021, the world used up the equivalent of 1.7 planets' worth of resources to satisfy production and consumption needs. This statistic, calculated every year by the NGO The Global Footprint Network, is one of the many stark environmental warnings that is pushing companies and ordinary citizens to be more careful in the use of our resources. At the same time, the appeal of so-called natural products has now surpassed that of conventional goods[2] . These two trends underscore some of the difficult choices manufacturers face. They are caught between an insatiable demand for natural products that respect the environment, the need to find solutions that reduce the use of fossil fuels and the temptation to promote agroforestry crops that compete with food chains.

Many have realized that a solution based on a sharp focus on circularity could solve both their dependence on oil and **reduce** their **impact on the environment**. This is why biotechnology, and specifically fermentation, is becoming increasingly attractive and visible in the industrial landscape.

Modern fermentation technology uses living organisms to obtain molecules used in multiple markets such as the chemical industry, cosmetics, human and animal nutrition and pharmaceuticals. The opportunities are very broad, and biotechnology **solutions** have been identified capable of meeting the challenges of a new kind of industrial production — one that combines **environmental protection** and **economic performance**.

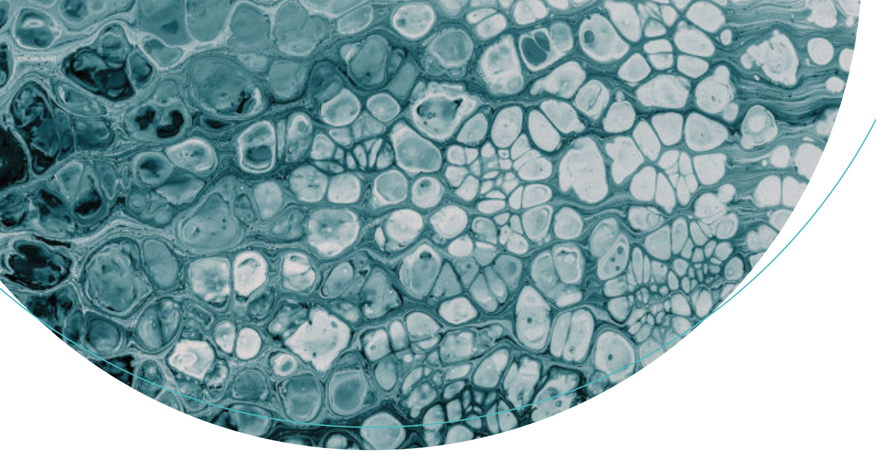
Industrial biotechnology, known as «white» biotechnology, has seen thousands of fermentative processes flourish at the pilot or laboratory development stage. However, scaling up to industrial production is such a **technical and economic challenge** that it can be difficult to distinguish which ones can be relied upon to sustainably replace conventional ingredients in very large volumes, without losing quality, performance and competitiveness.

What are the criteria for identifying fermentation processes capable of meeting industrial requirements and the challenges of scale-up?

Since its first uses in antiquity, what has biotechnology led us to gain mastery over?

In this post, we'll take a look at about how AFYREN, striving to reconcile performance, sustainability and competitiveness, has been able to overcome certain constraints during the development of its process.

[1]According to a survey conducted by Sphera
[2] https://www.bcg.com/publications/2019/pure-growth-natural-products



Fermentation, an ancient cycle of renewable resources for humanity

The very first **circular economy** was established long before humans. In order for Earth's biosphere to sustain itself, metabolic cycles were established, in which microorganisms feed on energy and matter to grow, and excrete compounds, which are put back into the natural cycle. In the case of fermentation, this process can take place in an «aerobic» or «anaerobic» environment, i.e. with or without oxygen. In either case, the microorganisms will draw these atoms from a substrate, such as carbohydrates, to obtain energy. The product of this transformation is a varied set of molecules (metabolites) which can in turn be used by other microorganisms, or enter into natural physiochemical processes (evaporation, crystallization, acid-base equilibrium, oxidation-reduction, etc.).

Industrial fermentation as a method to obtain viable biobased products

Fermentation bubbled up as man settled down. Our ancestors used the process of fermentation very early, with increasing craft and functionality depending on the level of mastery. It has been used since the Sumerian era, between 8000 and 4000 B.C., to preserve fresh products resulting mainly from agriculture. Beyond improving survival conditions, fermentation has been at the origin of a culinary culture specific to every geographical area: wine, beer, cheese, yoghurt, fermented vegetables (sauerkraut, gherkins, kimchi...), vinegar, soy sauce, bread, sausage, cured meats, and the list goes on.

The other great pivot in the use of this process occurred in the 19th century, with the beginnings of **enzymology**, when Louis Pasteur demonstrated that micro-organisms were responsible for the transformations observed during fermentation. In the 20th century, the birth of biotechnology and the industrial use of microorganisms led to the development of many products of interest: the first vaccines, the first antibiotics, and baker's yeast.

Over the last century, industries have developed that use fermentation processes in very large capacities to obtain bio-sourced industrial products for non-food applications. The best known and most widely produced is **bioethanol**, with more than 90 million tons produced per year worldwide. It has applications in «green» fuels, chemistry, food, perfumery and pharmaceuticals. There are other fermentation products, such as lactic acids, glutamic acids, etc., that are less well known but just as essential in our daily lives.

The development of production technologies, the sequencing of micro-organism genomes, and the development of new strains, have allowed the emergence of brand new fermentation processes. New molecules with high added value, until now obtained from conventional resources, could be produced thanks to fermentation, including high-value molecules such as vanillin.

The challenges of industrializing a fermentation process

While fermentation presents immense **potential for industrial use**, physical constraints must be put into perspective to determine the optimal conditions for profitable exploitation. The traditional approach in white biotechnology is to maximize a finished product (FP) and minimize its co-products. The development of an industrial process is based on three key indicators: **productivity** (g/l/h of finished product), **maximum product titer** (in g/l), and **production yield** (in g of FP/ g of substrate). A fermentation process must be developed that achieves the maximum of these three elements for the target product, with the aim of minimizing capital investment (CAPEX) and production costs (OPEX).

Getting past the development phase

While this approach, which follows the triptych: strain + substrate + product, has long been proven on an industrial scale for simple components, it can be very limited when it comes to scaling up industrial units for the production of ingredients using more complex strains, such as yeast.

Developing a suitable strain for a specific product sometimes requires implementing a whole **process of metabolic engineering** of microorganisms upstream — to maximize microbial characteristics like **productivity** or **resistance** to environmental stresses. The long development times and costs of a high-performance strain can quickly impact the profitability of the finished product and jeopardize its viability from the start.

Substrate sourcing and abundance

Once a successful strain is developed, an abundant supply of resources suitable for the development of these particular microorganisms during fermentation must be **secured**, while complying with the **regulatory parameters** governing the use of these genetically modified microorganisms (GMO/GM). Sugars such as sucrose or glucose, from sugar beet, sugar cane, potatoes or cassava, are the most common substrates. Sustainable development issues, including competition with the food chain, and transport costs, must be taken into account. The choice of substrate is critical because the supply must be sourced as close as possible to the production site, and other considerations should be taken into account: the possible evolution of the market price, potential geopolitical constraints, and interchangeability in case of supply difficulties. There are some well known efforts to overcome these sourcing constraints. For example, so-called «2G» ethanol tends to be produced from various non-food resources (like wood, straw, miscanthus, or short rotation coppice).

Implementation of the fermentation process

The other element that affects the key parameters of industrial fermentation are the **stress conditions** (physical, chemical or biological) to which the micro-organisms must be subjected, since they can only multiply under specific temperature and pressure conditions. Moreover, in order to guarantee the homogeneity of the medium and allow the microorganisms to efficiently access the substrate, which are sometimes in various forms and phases, the medium must be agitated while guaranteeing the integrity of the microorganisms. The size of the reactors could therefore be limited or the agitation process could become complex, energy consuming and therefore costly.

There are other so-called tipping points to watch out for. When a microorganism metabolizes a substrate, it generates «**metabolites**» that concentrate in the medium and can, at a certain level, become harmful to the microorganisms or the medium. At this point, the productivity reaches a maximum and the growth of the population can reach a limit. For some microorganisms, productivity and titer are low and therefore require very large fermentation volumes, which can affect the overall yield. Under these conditions, costs become difficult to reduce, and only products with high added value are of economic interest. At the biological level, if the environment in which the microorganism evolves is contaminated by other microorganisms, the productivity and/or the quality of the finished product will be affected, in particular because of the recourse to complex and expensive purification processes and the need for sterilization (and thus energy or antimicrobial products). The bill can be multiplied tenfold and greatly affect the economic viability of a project.

If conventional fermentation can provide access to complex metabolic processes, yielding molecules that would be difficult or impossible to obtain simply and directly in nature, **industrial scale-up** and **competitiveness** with petro-sourced products are the criteria that determine the viability of the project.

AFYNERIE® : a biomimetic process for innovative and competitive fermentation

To overcome these constraints, the original approach we chose at AFYREN is **biomimicry**, which aims to mimic nature as closely as possible while maintaining industrial performance objectives. Our process is based on metabolic chains found in natural ecosystems (like the stomachs of ruminants, or lakes). And we produce a set of molecules (not just one target), using raw substrates without pretreatments, such as beet pulp. We also use a mix of microorganisms rather than a single one, to transform all the material available with a rich and varied natural enzymatic load.

In this industrial approach, which places moderation at the heart of its economic model, the aim is to find the best outlet for each of the molecules produced naturally by these micro-organisms, instead of seeking maximum optimization for a single valuable product.

This approach allows us to overcome technical and economic constraints since the fermentation is not very constrained itself and therefore offers several advantages. All the natural micro-organisms live in symbiosis, so we don't need to develop one or more efficient strains through genetic modification, to sterilize the environment or to use very specific inputs to ensure their survival and productivity.

Finally, this population of microorganisms transforms various substrates with high yields and can work with a **wide range** of agricultural co-products distributed all over the world that don't compete with human food. Since the AFYREN process can be applied to a wide range of organic residues, the resources are not a limiting factor to production and the process is local and circular. Using local resources as close as possible to producers and downstream users makes sense and encourages the development of an integrated circular economy.

The process is ultimately **transposable** wherever there are fermentable resources that allow high fermentation volumes, while maximizing the transformation to obtain valuable products, and little unexploited residue left. In the end, AFYNERIE's technology makes it possible to manufacture **biosourced molecules** with a very **small carbon footprint** (one-fifth that of petro-sourced equivalents) while preserving natural resources. More broadly, the technology proves that it is possible to marry Ecology and Economy and to contribute to the **transformation of industrial value chains**.