Confined device:
The Softer Shock Bioreactor

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Acknowledgments

Working on the design of a bioreactor is not an easy task and requires multidisciplinary competences from various domains.

We are grateful to **Dr. Souad Fehaili**, PhD in food processes and coordinator of the production major at Sup'Biotech, who shared her expertise on bioreactors. Without her, we would not have been able to push our very fledgling idea so far! You can find our complete interview annexed to this report.

Many thanks to **Dr. Estelle Mogensen,** PhD in plant pathology and laboratory manager at Sup'Biotech, who answered our different interrogations about filtration systems and allowed us to have a more precise insight of their bacterial retention capacities.

Summary

Introduction

We believe in the Softer Shock project, and are convinced that a thermo-inducible system could bring a significant help for the crop protection at the age of climate changes. However, we are aware that safety and regulations are the main challenges compromising the commercialization of such a project today. Indeed, regulations in our country as well as other parts in the world make it impossible to disseminate Genetically Modified Organisms (GMOs) in an open environment such as a field. Despite our different safety strategies to limit as much as possible the organism dissemination and DNA transfer, as well as its presence on the fruits at the time of harvest, we are not able to guarantee a 100% security under the law, which as for now compromises the implementation of our solution within the agricultural sector.

The potential dangers for the environment or human health lie in the micro-organisms themselves, and more precisely their modified genetic material. However, in the end, the core elements are the protective compounds produced by those organisms more than organisms themselves. That is why, to meet our French clients' and current consumers' needs, we followed our entrepreneurship approach to the end and conceived an alternative application of the Softer Shock project into a contained system. The aim is to use the same thermo-responsive biological entities as smart bioproduction agents to deliver the protectants (and the protectants only!) to the plants. Despite being more realistic for a possible commercialization today, designing a suitable thermo-dependent containment system is not an easy task and requires the collaboration of various interdisciplinary competences.

What might it look like?

In this report, we go through each compartment of our theoretical confined device and try to find the most appropriate technical elements. At the end, we discuss the added value of our final system compared to the direct application of micro-organisms on the plants, as well as the existing techniques for plant protection, to assess its real relevance for agricultural actors.

I. Bacterial culture: core element for the bioproduction of plant protectants

First, we must find a way to safely contain our modified micro-organisms. These are the core elements of the project, as they build up the thermo-reactive system.

A) Choice of the expression host

When cultivated inside a bioreactor, the microorganism choice is based on very different criteria compared to the initial Softer Shock project. We do not need to take the grapevine ecosystem into account anymore, nor the possible survival of the bacteria outside the plant. In the present system, the chosen microorganism will serve as an expression host for an industrial application, and will stay in a tightly controlled medium. We will therefore favor its culture and production capacities. We need an organism surviving and growing easily in temperatures ranging from 10°C to 40°C under a well-known growth cycle, receptive to genetic manipulations, and producing the plant protectants efficiently in those conditions.

Since our proof-of-concept was performed with *Escherichia Coli*, choosing the same strain will be interesting as we won't need to optimize the recombinant genetic material inserted for a different strain. This well-known intestinal bacterium has been widely used in various bioprocess technologies including the production of biofuels, biopharmaceuticals, food colorants or therapeutic molecules, the most famous example being the production of insulin for medical purposes (The MJ, 1989). Those numerous examples also confirm that this microorganism does not require high culturing costs, which is important as we want to help farmers at an economic cost that respects their budget constraints. Finally, their fast expression pattern coupled with a short doubling time will allow an optimal protectant production in response to brutal meteorological events.

B) A continuous feeding mode for an autonomous protecting system

The advantage of a thermo-reactive system is to bring a self-adjusting protection, without a need for the farmer to anticipate meteorological events and manually spray the solution. This is why bacteria should be cultivated in a continuous culture mode. A chemostat appears as an appropriate device for this kind of cultivation.

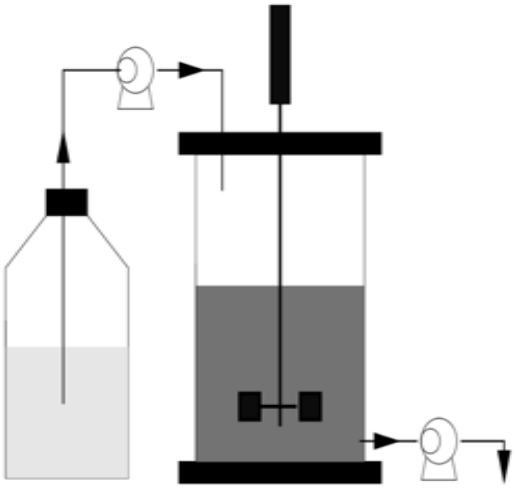


Figure 1: Basic schematic of a continuous cultivation in chemostat mode (Bernard O., 2004)

Cultivated species inside this system are fed with a solution containing a precise and limited amount of an essential nutrient, allowing a steady bacterial concentration. This fresh medium enters the bioreactor at the same rate as the spent medium is removed, so the volume inside the vessel remains constant. This system has been widely used by the scientific community to model *in vivo* events, especially for the study of specific human microbiota (David R. Drake and Kim A. Brogden, 2002).

A first tank containing fresh medium has to be settled upstream the bioreactor. This solution will contain all the essential components for bacteria survival: yeast extract, NaCl and tryptone. The medium will enter and leave the bioreactor under a precise and similar flow rate, imposed by a pump. Besides regulating the medium flow, the pump also has a role in preventing the phenomenon of pressure loss due to internal frictions between the liquid and the wall. We chose to work with volumetric pumps, as this type is particularly adapted to small volumes and rates as involved in this case. The internal medium will be kept under constant agitation thanks to a stirrer. The culture reservoir will have to be regularly replaced,

every few weeks approximately, to get rid of dead cells. This can be achieved by using a draining system, linked to a waste tank and a centrifuge system allowing the precipitation of dead bacteria.

How often should we drain?

Even in a continuous culture system, dead bacteria have to be regularly removed. The lifespan of *Escherichia Coli K-12* has been proved to be more than 8 hours. To determine how long a specific strain can live under cold or high temperatures, we should run our own experiments. The bacteria should be maintained at a regular temperature allowing their survival without triggering any protectant production (20°C for example), before inducing cold or heat shocks. For that we should grow the bacteria until the OD600 reaches 0,6. Then, we put them in a cold system below 15°C or in a heat system above 37°C, and calculate the OD600 at regular intervals until reaching the deceleration phase. That will be our referential for the determination of when to add nutrients and to remove waste. There are also specific bacteria strains for a better protein expression such as DL21. In that case we should study the lifespan of that strain using the same conditions as explained before. After that we should decide which strain is more useful to work with depending on the lifespan, the production capacities under certain temperatures and the waste material generated by the bacteria strain in question. The bacteria that possess the correct balance between all parameters, will be chosen for the bioreactor.

C) Thermo-regulation system

Generally, chemostats allow a very precise regulation of the different medium parameters (temperature, pH, oxygen levels…). In our case, the needs are particular: rather than being kept constant, the medium temperature should correspond to the outside air to keep the interesting capacity of our bacteria to respond directly to meteorological events.

We first thought about implementing our bioreactor outside, so micro-organisms could be directly exposed to current climatic conditions. As we did not find any cases of exterior confined bioreactors, we went towards several experts in order to get their opinions about this. We presented our idea to the Higher Council for Biotechnology (HCB) in Paris, and found out that the confined aspect would be questioned in this case. Indeed, the confined use of GMOs under the French legislation is based on the existence of a physical structure defined as a containment building. The bioreactor

alone would then not be sufficient to guarantee safety, as farmers will stay confronted to the risks of overflow and leaks, as well as the need to clean and maintain the installation. We also received the insights of an expert in food processing, who warned us about the difficulty of implementing a bioproduction system outdoor. In addition to the bioreactor itself, the filtration and recycling systems will require the installation of several tanks and pipes. For all those reasons, we decided to design an interior system.

“I quickly discussed with an expert about the contained uses of GMOs, current French legislation is based on the existence of a building defined as a containment; he did not know of any cases of "outdoor" confined bioreactors. According to him it would be rather complicated to implement an overlay that allows both containment, management of typical incidents overflows leaks etc and access to the bioreactor for maintenance and handling. And there is the question of waste management to think about, but regulation evolves, who knows!”- *Mr. Remondet Martin (scientific LEADER in charge of economics, ethicAL and social ISSUES at the HCB).*

The outside air temperature will be detected by a thermal sensor, placed externally, and coupled to a medium thermoregulation system. Therefore, the medium temperature will be modulated according to the outside conditions. This possibility could offer a significant improvement compared to the microorganism application on the leaves. Indeed, it allows a better control of the compound production and the bacteria survival throughout all temperature ranges. In case of extreme temperature events, the cultivated bacteria will only be submitted to a temperature drift that is sufficient to activate the production of protectants, without compromising its survival nor the speed of production. In addition, bacteria will be protected from other harsh conditions including rain, wind, or even hail.

We could define the medium temperatures as follows: as soon as the external temperature falls below 15°C, a cryostat is automatically activated and cold water is produced to cool down and keep the medium to a constant temperature of 15°C. In case of a temperature increase above 37°C, a boiler is automatically activated and hot water is produced to heat and keep the medium to a constant temperature of 37°C. Cold and hot water will circulate into a double envelope structure surrounding the principal culture tank.

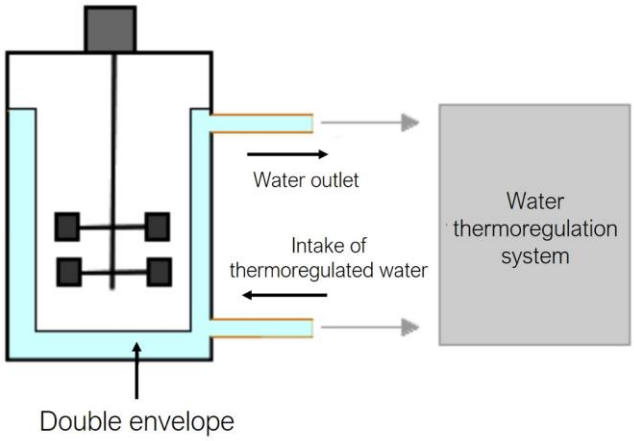


Figure 2: Basic schematic of the double envelope thermoregulation system (www.genie-bio.ac-versailles.fr)

II. Filtration system: the guarantee of microorganism containment

When leaving the culture tank, the medium must be filtered so that bacteria are retained while protective compounds join the aspersion system. Given the respective sizes of both elements, using a specific filter with pores substantially smaller than bacteria size could appear as an efficient solution. Several filter types exist and serve for bacterial retention, notably in the case of air or water purification systems, as well as sterilization processes. Depending on the strain considered, *E. Coli* measure between 0.5 to 3 µm whereas the compounds of interest should be at the nanometer scale. In our case, a microfiltration membrane with 0.2 µm pores can be used to remove cells from the medium. The filtration chamber will be composed of two pipes: one for the filtrate, composed of water, protecting compounds, other medium components and joining the aspersion system, and one for the retentate containing bacteria. To prevent components accumulation on the membrane and guarantee an optimal filtration, a "cross-flow" filtration system appears interesting.

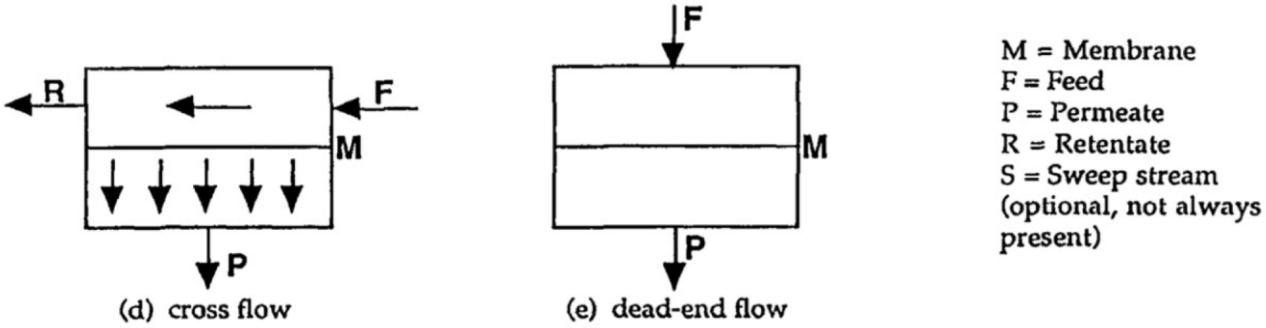


Figure 3: Schematic of cross-flow membrane-based separation principle compared to dead-end flow (Koros WJ et al, 1996)

In "cross-flow filtration", or "tangential flow filtration", the feed continuously flows tangentially against the filter surface. While bigger elements keep on flowing away in the same direction, smallest particles pass through the filter and join a perpendicular pathway (Koros WJ et al, 1996). This system is widely used to separate biomolecules in laboratories, but also at the industrial scale. The main advantages of such a system for

our application lie in increasing of the usage convenience and lowering the liquid consumption.

Firstly, the liquid dynamic allows the membrane to be washed at the same time, and prevents the formation of clogs. This will limit the frequency of maintenance operations, during which the filter must be replaced to avoid fouling. We could for example use a "backwashing technique" to regularly clean the entire system. The system also falls within an eco-responsible strategy: when using crossflow filtration, less liquid passes through the filter pores and results in a more concentrated filtrate. Moreover, instead of accumulating on the membrane surface, the bacteria contained in the retentate can be directly reinjected in the culture tank (Schwartz L).

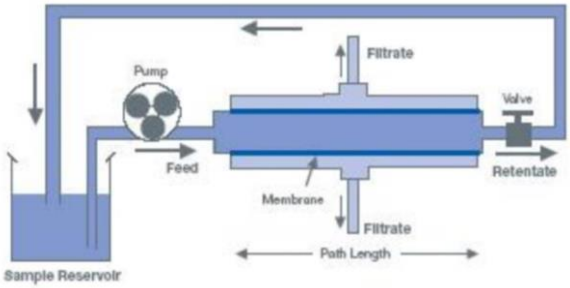


Figure 4: Complete flow path through a simple tangential flow filtration system (Schwartz L)

For our purpose, we want to limit as much as possible any eventual compound loss. Indeed, the challenge with this technique is to prevent the compounds contained in the medium from continuing with the main flow and being reinjected in the culture tank instead of being filtered. The crossflow rate is an essential parameter governing the filtration efficiency. By regulating it with a pump, we can precisely modulate the transmembrane pressure and consequently optimize the driving of liquid through the membrane (Schwartz L). We can also take the membrane properties into account to optimize the filtration process. For example, using a hydrophobic membrane could facilitate the passage of hydrophilic molecules, as it is the case for ice-binding proteins.

We send an email to Dr. Fehaili Souad, in which we asked her if it was possible to put a hydrophobic membrane in the filter tank, this was her answer:

""Yes, you can use hydrophobic filtration membranes. These gas membranes must be installed at the entrance of the filtration tank. They exist at Merck's

company, Millipore and other filter manufacturers." - *Dr. Souad Fehaili, PhD in food processes and Production major coordinator at Sup'Biotech*

Another strategy would be to install a 0.2 µm dead-end flow microfiltration membrane directly at the bioreactor outlet. In response to extreme temperatures, the protective compounds will be instantly injected into a containment tank while being synthesized, and then dispersed by the aspersion system once a certain concentration has been reached. Some advantages result from the fact that the filtration system is not separated from the tank: there is no need for a recycling loop anymore, and less water will be required. We could also install a second membrane to create a double security system.

The choice of the filtration system to use will depend on the filtration efficiency allowed by both strategies: some tests should be performed to evaluate the protectant concentration in the final filtrate obtained as well as the quantity of water required.

We considered the regulations towards GMOs, and found out that a filter alone was sufficient to consider the system as confined. This filter will have to be validated by an accreditation body and pass some tests as the ASTM F838 - 15a, or "Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration", commonly used to validate sterilization filters (www.astm.org).

We could also think of other separation techniques instead of using a filter. We could for example implement a decantation chamber to let the bacterial content precipitate and fall within a secondary pipe, while retrieving the supernatant containing the protectants for the aspersion. The choice of any technique is difficult to make at this stage. Ideally, we would have to perform small-scale tests, then at a larger scale, to assess the efficiency and rentability of each system.

III. Overhead aspersion: protecting vineyards smartly

Once produced, the protective compounds will be automatically spread on plants to carry out their protective action. We studied the existing watering techniques and tried to take our inspiration from them, to potentially link our device to existing machines already installed in the plantations. For the stake of water savings, and to reduce the risks of pathogen development on wet leaves, most farmers currently use micro-irrigation systems. In these systems, an underground pipe network delivers water drop by drop to the rhizosphere or at the soil surface level (Haman DZ & Izuno FT, 2003). However, this strategy is not appropriate for our application as our protectants will not have any role at the underground level, but only at the leaf surface. It will then be more convenient to separate our aspersion device from classical underground irrigation systems. We therefore considered systemic applications methods, as it is the case for phytosanitary products or winter sprinkling for example. We decided to choose a classical overhead sprinkler, which will allow an automatic spreading of our solution at the plant surface.

Figure 5: Sprinklers dispersing water on vineyards (www.aquaval.fr)

Numerous types of sprinkler heads exist, depending on the desired aspersion pattern and frequency. In our case, the sprinkler will be activated in response to a temperature change. We can turn towards rotary heads, designed to deliver a single stream of liquid and allowing an optimal distribution across the field. “Pop up” heads could also be of interest, as they automatically raise from the ground when activated and disappear the rest of the time (www.homedepot.com). Beyond vineyards, the choice of the sprinkler type will be specific to each crop benefitting from Softer Shock protection.

The choice of the protectant to be produced will directly depend on the aspersion method chosen. For the cold protection, we explored two different strategies (cf. Applied Design part). When aspersing vineyards, the proteins will be directly active inside the solution so mixing antifreeze proteins with water would not really be coherent. We will focus on the strategy of ice formation by the action of ice-nucleating proteins.

During harsh temperature falls, farmers currently use continuous water sprinkling to allow the constant formation of ice at the plant surface. This isolating ice layer creates a constant 0°C environment to prevent frost damages inside the plant. However, the technique requires a need to anticipate cold episodes, and above all, huge water quantities. This problematic comes from the fact that water needs to continuously solidify to liberate latent heat. Thanks to our method, the water applied will be enriched with ice-nucleating proteins, favoring ice formation compared to the classical method. This improvement could allow important water savings by optimizing the water solidification process. In addition of being more environmental-friendly, our solution could bring economic benefits to farmers.

In the context of heat protection, our system could be installed in addition of the classical underground irrigation methods. It will then consist in a complementary "smart watering" method, depositing a reflective layer in addition of water. Here again, by lowering the leaf temperature, evapotranspiration will be limited, and lower amounts of water will be required to prevent dryness.

Which quantity of water would be used inside the bioreactor?

"The water volume will depend on the needs of your strain (*E.Coli*) as well as the amount of active compounds to produce (area of the vineyard)."- *Dr. Souad Fehaili*

Once applied, a question can be raised towards the faith of the different medium components at the plant surface but also in the surrounding environment. In addition to water, the medium nutrients and amino acids could be absorbed by insects or microorganisms present at that time. In addition to our effort to concentrate the protectants in the filtrate as much as possible, we should also make some field tests to verify that our solution does not disrupt the microbiota, nor attract species potentially pathogenic for the plant.

What will be the medium composition?

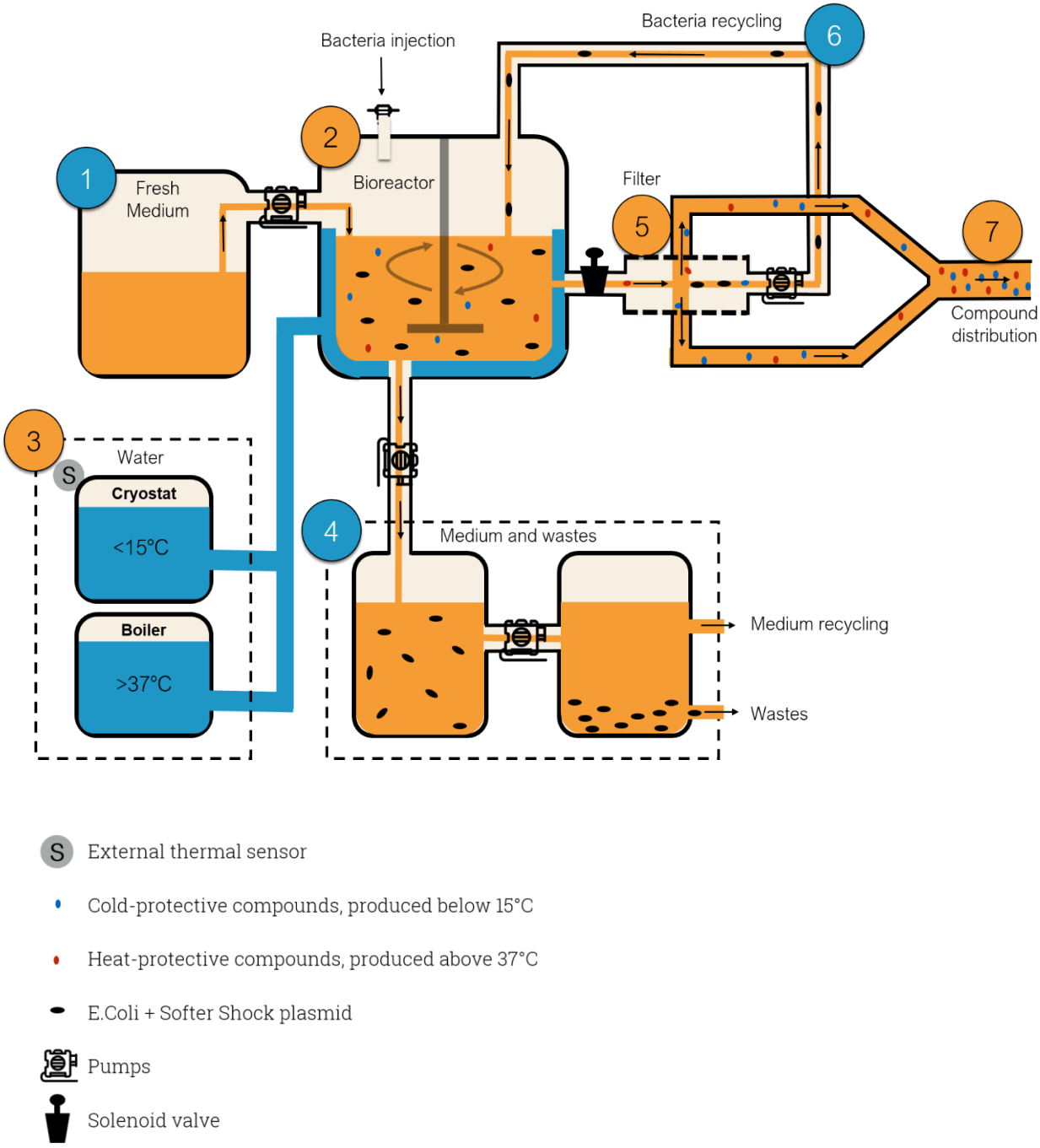
With a few exceptions, the medium contained inside the bioreactor will be globally similar in composition to the initial project one. We used adjuvants in our spray to reduce risks for the environment and the human health, but they will not be necessary in the bioreactor in which the bacterial solution will stay in a confined area. We can keep the idea of adding a synthetic amino acid into the medium. Formerly elaborated for killswitch purposes, this could also be interesting in our confined system to limit eventual alterations of the surrounding ecosystem. Indeed, once in the Nature, the synthetic amino acid will not be recognized.

Compared to automatic watering devices, often time-dependent, our system will respond to temperature. We therefore need a thermosensitive pumping system to conduct the microorganisms to the filtration and aspersion systems only when precise temperature thresholds are reached. This can be achieved using a solenoid valve, a control element able to activate in response to an electric signal. Linked to the thermal sensor, this system could allow a thermoregulated control of the liquid flow (www.relevantsolutions.com). During sudden spring cold episodes, the response needs to be relatively quick to avoid the most damages possible. As soon as the temperature falls below 15°C, the cultured bacteria will start producing ice-nucleating proteins. When reaching 10°C, the medium flowing out of the culture tank will be directed towards the filtration system to make the protective solution. Once activated, the sprinkler will start spreading the solution ready to do its job! In the same way, we could imagine a first temperature threshold of 30°C to start producing the white compounds, and a second threshold at 35°C to spread them on the crops.

Some tests are required to assess the bacteria production efficiency according to the different temperatures, and especially if we choose to synthesize ice-nucleation proteins. On the long term, and if produced in too large quantities, those proteins could alter the medium comportment by enhancing ice formation. Therefore, the protectant must be transposed to the filtration tank as soon as possible.

What is challenging is that each grape variety, and more extensively each crop type, has its own characteristics and its own sensitivity to changing temperatures. We could even go further, and imagine a connected system that would allow the farmer to manually set those thresholds depending of its crops and the climatic conditions in his geographical region

IV. Final device: added value and comparison with initial Softer Shock project and existing methods



Scheme 1: Schematic of the Softer Shock confined system and its components

1. Feed reservoir
2. Bioreactor culture tank
3. Water thermoregulation system
4. Waste containers comprising a draining and a centrifuge tanks
5. “Cross-flow” microfiltration system
6. Recycling loop
7. Distribution system joining sprinkler heads

The bioreactor alternative to the Softer Shock project emerged throughout the discussions we had with diverse experts, and especially the number of feedbacks towards the fear of GMOs dissemination in the environment. This novel theoretical application comes along with a whole list of new priorities and challenges compared to the initial project. We made a summary of the main aspects differing from one technique to another, and discussed their pros and cons.

Choice of the bacterial chassis

The role of the thermo-responsive micro-organism is completely different in a bioreactor than in an open environment. Retaining it into a confined system allows us to focus on its bio-production abilities and efficiency into a tightly controlled environment, rather than its survival among the whole phyllosphere species and the external climatic conditions. This choice was one of the principal challenges of the initial project. Indeed, using microorganisms to prevent temperature-linked damages on plants is quite pioneering, and finding a microorganism that filled the total list of criteria in terms of survival under extreme temperatures, biosafety and prevalence on the plant was almost impossible. Microorganisms used as bio-production agents are far more referenced and the list of criteria to meet is critically lowered.

But after all, aren’t reflexion and creativity two pillars of the iGEM competition?

Ethics, safety and regulations

These are inevitably the main obstacles preventing the development of the initial project right now. The problematic of GMOs in an open

environment is highly delicate: as we discussed the project with winemakers, we realized that the vision of GMOs highly differed from one person to another, and from one region to another. That is why we decided to go beyond this regulatory obstacle, and show that we thought our initial project in a responsible manner. However, the use of a confined system would probably be more widely accepted today. The farmers and companies we talked to globally showed a real interest for this solution. Nevertheless, the economic viability of such an installation remains to be demonstrated.

Automation

The bioreactor has the advantage of being automatically regulated. Instead of manually applying the protection solution on his crops, the farmer will let the bioreactor continuously maintain a stable bacterial population and self-react to meteorological events following the temperature thresholds previously specified. The bioreactor can be continuously activated during high-risk time periods, or even throughout the year. However, saying that this installation is "autonomous" would not be completely true. Indeed, the need for regular draining, the formation of biofilms, air bubbles, but also medium contamination in bioreactors are common issues that always require some monitoring and maintenance from the user at a certain point. Of course, farmers are confronted with some issues of this type when dealing with the classical aspersion methods, but the stake is critical in this case as GMOs are contained inside the device and should never come out. We can wonder, if in any case there will be an automated thermo-regulated system, what is the advantage of using biological thermo-reactive agents and not directly the protectants with a completely electronic device? The compounds will be produced continuously and only one type at a time in response to temperature events. Without this system, we would need two separate tanks containing proteins in substantial amounts that would need to be regularly replenished.

Economic statement

Everything that is going to be written for the economic statement is completely theoretical due to a lack of time and resources.

To establish an economic statement, we would like to contact an automation engineer, expert on bioreactors to discuss about the bioreactor complexity (pumps mechanisms, study the correct flow) to settle a global budget and to study the rentability of the bioreactor in the long run. We would have to do an important work of reflexion around the vine industry,

to settle the pros and the cons for the winemakers, and to prepare a business plan and Grant diagrams to study the feasibility of the bioreactor. Climate disorders are going to increase year after year, and the society has to find potential solutions to anticipate the risks.

We are aware that the production of this bioreactor will be expensive, especially the aspersion system. To build the bioreactor we can find ecofriendly solutions and produce zero waste, using recycled materials. We can contact companies that work on recycling materials to find partnerships and use their materials (zero cost) to build the bioreactors in exchange of visibility. In that case we would reduce expenditures and it may be more interesting for winemakers. We would have to do a huge "field work", enter in contact with winemakers, communicate with them, listen to their proposal and present the best options to solve their problems due to climate disorders. This part has already been studied during the iGEM competition to have society perception about the impact the initial Softer Shock project would have.

Environmental statement

Here, the difference will be specially in the way winemakers and the society would perceive the use of GMOs. With the bioreactor the level of security and safety is higher and so the society is more willing to use the solution Softer Shock, because no GMOs will be pulverized on the environment. The bioreactor itself could be also built with recycled materials to respect the environment and reduce waste production, and fall within a circular economy. In that case the Softer Shock project would be more willing to be accepted by the society, especially in France. The current French regulations prohibit the diffusion of GMOs in the environment. This in one of the reasons why we decided to propose to build a bioreactor, to adapt our project to society needs. Always keeping Softer Shock roots remains very important to us but we wanted to integrate the project into French perception of GMOs and synthetic biology.

Conclusion and Perspectives

After having presented this project to several winemakers and plant care professionals, we tried to get the most of this system in term of economic viability and practical use. We realized that implementing such a system implied reviewing some of the initial project priorities. In term of usage, a dual response would not necessarily be the most accurate for this final application. Using either a cold-responsive or a heat-responsive batch according to the corresponding season appears as a more profitable system. Therefore, the relevance of having a thermo-responsive biological system compared to a fully automated one can be questioned. In the last case, the choice of the protectants would be the only aspect to consider when designing the project, and the device will be comparable to any other bioproduction factory.

All this reflexion process lead us to the conclusion that transforming our thermo-responsive biological system into a feasible installation matching all regulatory, economic, and practical requirements was not as easy as it seemed. In some way, that comforted us in the feeling that directly applying our microorganisms on the leaves could really be of great benefit for farmers in the future. With this idea, we want to propose a “futuristic” biocontrol approach, in accordance with the vision of the plant as a whole ecosystem in which species interact with each other. Through all our searches towards general public and expert perception, as well as the Frostban project 30 years ago, we could see how fast mentalities towards the use of GMOs were evolving worldwide. Even if Softer Shock is purely at the theoretical stage, we really wanted to show that with a lot of knowledge and cautiousness, big things can be achieved!

We can conclude by saying that, even though our project aimed at helping grapevines endure temperature stresses, hence a “Softer Shock”, we tried to, with all our efforts and studies, give the project’s name a double meaning.

A Softer Shock for plants, certainly, but also a possible Softer Shock between synthetic biology and society. We believe in such perspectives and that is what we tried to reflect with this project.

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Annex: interview with Dr. Souad Fehaili

Position: PhD in Sciences and Food Processes, Coordinator of the Production Major at Sup'Biotech
Institution: INRA, Sup'Biotech Paris

Dr. Souad Fehaili is our process engineering teacher. Thanks to this relationship, we could easily contact her as soon as the bioreactor idea came to our minds. Elaborating a confined bioproduction system is not an easy task, and requires multidisciplinary competences in various domains. That is why we were really lucky to get her precious technical and strategic advices on the subject.

Do you know any examples of bioreactors implemented outdoors? Would this system be considered as confined?

→ Of course, some bioreactors can be implemented externally. It is notably the case of photobioreactors, which need light to ensure the growth of photosynthetic micro-organisms.

In order to preserve the interesting property of our micro-organisms to respond to external temperature changes, the medium temperature inside the bioreactor should correspond to the outside air. Can we achieve this with an absence of heating or cooling system? Or metabolic reactions will heat up the medium by themselves?

→ You don't necessarily need to install your bioreactor outdoors to reproduce the outside air temperature inside the medium. According to me, it will be in fact far more convenient to put it in a contained facility given all the additional filtration, waste, recycling, and thermo-regulating systems you will have to connect. You can easily regulate the medium temperature according to the outside thanks to a thermal sensor set up outside and directly linked to a thermo-regulation system.

How could the medium temperature be regulated in this situation?

→ You will need a double envelope bioreactor. The thermal sensor will be connected to a water thermoregulation system, and activate either a cryostat to produce cold water, either a heater to produce hot water. The thermoregulated water will then circulate into the bioreactor external envelope in order to either cool or heat the medium. The advantage of a confined bioreactor is that you will be able to control the temperature and keep it at an optimal production value. When applying your microorganisms on the plants, they have to resist high temperatures.

We would like to use a continuous culture system in order to keep the autonomy of our self-regulating system. We found chemostats, which appeared well adapted for this objective. What do you think of this option?

→ A chemostat would perfectly fit your requirements. Two tanks need to be installed: a feed tank containing fresh medium, and a waste tank. The limitation is that at some point, you need to get rid off dead bacteria. You will then need to regularly empty the tank (every few weeks, but the precise interval could be determined by some tests), centrifuge the medium and eliminate the precipitated microorganisms. The entering and leaving medium flow rates will be regulated by some pumps. Since you are working with low volumes, volumetric pumps should be more suitable. In addition, they are also important to prevent the pressure loss due to internal frictions between the liquid and the wall. They restore the fluid energy and impose a flow.

What would be the best strategy to retain the microorganisms while letting the protectants of interest pass through to be disseminated? Are filters considered as containment systems?

→ The filtration system is a good idea. You will end with you different fluids: the fluid having passed through the filter is called filtrate, and will contain water, protective compounds and medium elements. The retained portion, containing bacteria, is called retentate. Decantation can turn out to be another interesting separation technique for your project. It could be advantageous if you want to limit the need for cleaning, as bacteria will end clogging the filter. The assessment of the best separation technique would require some tests at small scale. What could be interesting is to find a way to concentrate the filtrate after having passed through the filter. This would allow your product to be competitive by lowering the water consumption.

Based on our culture and filtration systems, can we consider our entire device as confined?

→ Filters can certainly be considered as containment systems as long as they are certified by an accreditation body. You could try to contact experts in this domain to get more precise information about the required certifications. In the same way, the entire equipment materials will be hermetic. However, there will still be a need to maintain the continuous culture and to clean the different parts. For this, filters will need to be transiently and exceptionally removed and the culture tank opened.

As the medium temperature will not be constant, should we find a way to modulate the microorganism and medium entry in function, as the growth rate will probably change?

→ Ideally you should. But unfortunately, precisely regulating the flow rate of entering medium according to the growth rate resulting from changing temperatures would be difficult. The entering flow rate is usually constant.

What would be the best dispersion technique to use?

→ I would say you could mimic the classical aspersion method, but it depends on the liquid viscosity. If too viscous, this technique will not be adapted.

Do you have other remarks?

→ You should contact an automation specialist regarding the whole aspect of the bioreactor automated regulation. It is important that you highlight precisely what would be the added value compared to the initial project idea, but also to already existing plant protection techniques. What is important is also to determine if your microorganism will survive after the filtration step. If it is the case, you will be able to add a recycling loop and make the retained microorganisms re-enter the culture tank. If not, they will have to be dropped into the waste container.