Comparative study of the environmental impact of antibiotics and bacteriophages strategies. Qualitative approach based on life cycle analysis methods.

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Abstract

This study presents a comparative analysis of the environmental impact of antibiotics and bacteriophages using a life cycle assessment (LCA) methodology. The aim of the research is to evaluate and compare the potential environmental consequences associated with the production, use, and disposal of these two types of antimicrobial agents.

Preliminary findings suggest that antibiotics and bacteriophages differ significantly in their environmental profiles. While antibiotics are administered in multiple doses, phages only require one dose to treat a similar bacterial infection. This fundamental distinction is expected to contribute to variations in resource consumption, emissions, and waste generation between the two groups.

The production of bacteriophages is not made at an industrial scale, which gives us much more freedom in our choices, whereas for bacteria, we had to rely on models dating back to the 1970s. Indeed, antibiotic manufacturers do not share manufacturing data.

We acknowledge that this comparative study was conducted under certain limitations. The availability of comprehensive and specific data pertaining to the life cycles of both antibiotics and bacteriophages was scarce, which posed challenges in accurately quantifying their environmental impacts. Despite these limitations, this study aims to provide initial insights into the potential differences in environmental performance between antibiotics and bacteriophages, highlighting the need for further research and data collection to strengthen the validity of the findings, especially at an industrial scale in the case of bacteriophages.

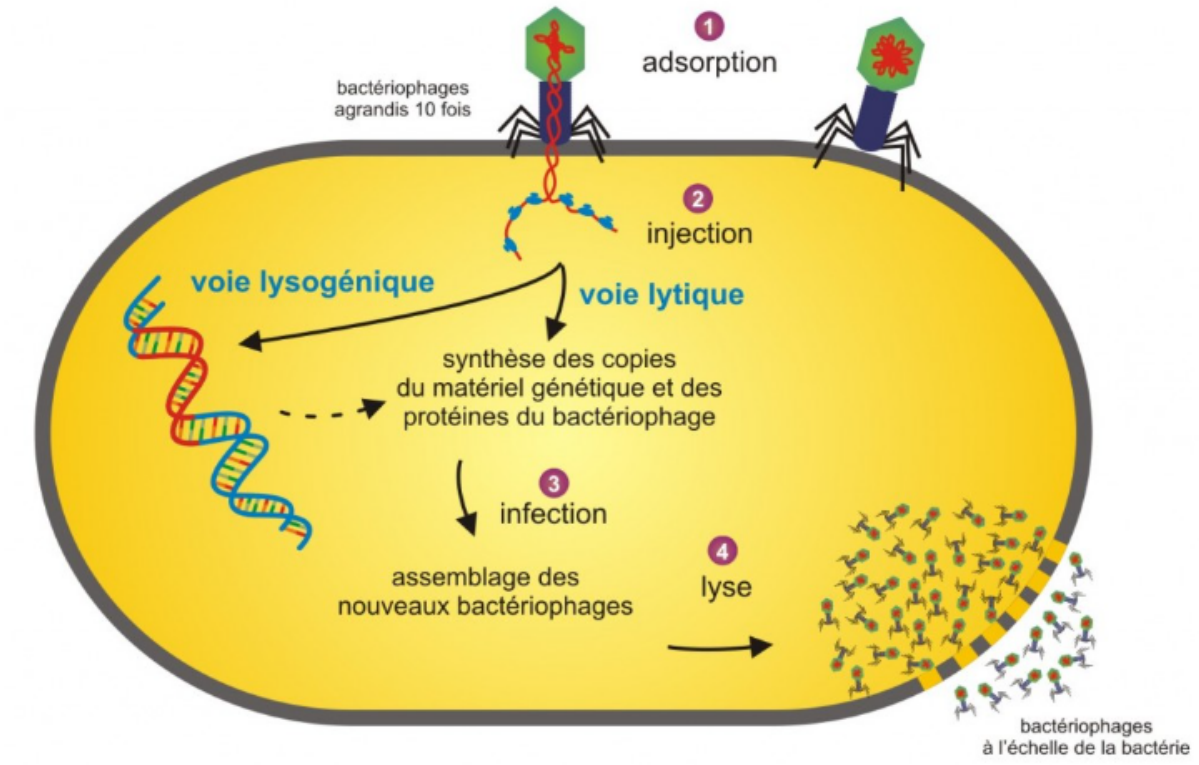
The outcomes of this comparative study could constitute a first step towards providing valuable insights for decision-makers in the healthcare and pharmaceutical industries, as well as environmental policymakers. By understanding the environmental implications of antibiotics and bacteriophages, stakeholders can make informed decisions regarding the selection and promotion of antimicrobial strategies that align with sustainable and environmentally responsible practices.

Introduction

Since their discovery, antibiotics have been the most widely used medication for treating bacterial infections. In France, in 2022, pharmacies sold 63 million boxes of amoxicillin, the most common antibiotic. However, there are other means of combating bacteria; lytic bacteriophages for example are highly effective and selective.

What is a bacteriophage ?

Briefly since it is not the object of this study, a bacteriophage is a virus, commonly found in nature, that specifically targets bacteria for reproduction. Like all viruses, it attaches itself to the target bacterium, injects its genetic material for replication, and the bacterium then produces thousands of copies of the bacteriophage until its death. These copies are subsequently released into the environment to continue the infection.



Goals

In recent years, the widespread use of antibiotics has led to bacterial populations becoming resistant to these drugs. This has prompted the pharmaceutical industry to finance the research for new antibiotic molecules, but this race comes with an economic, health and environmental cost. Therefore, some laboratories are now exploring the possibility of using bacteriophages on a larger scale. Bacteriophages have already been employed in several countries of the former USSR in the form of cocktails to treat bacterial infections. Although resistance to phages can also develop in the targeted bacteria, it appears at a slower rate. Additionally, alternative approaches involving non-lytic phages are being explored, including at institutions such as INRAE, which could help address the problem of bacterial resistance. Undoubtedly, bacteriophages represent a significant public health concern.

Beyond the public health dimension, in the context of a climate crisis, it is important to consider whether switching treatment methods would ultimately have a beneficial impact on the environment. This is the question that our study seeks to address by providing a preliminary comparison between the production of a selected antibiotic (amoxicillin) and the production of phages targeting the elimination of *Escherichia Coli* bacteria throughout their life cycle.

The intended audience of our study consists of Researchers at INRAE in the MICALIS unit working on innovative treatments with bacteriophages.
On a potentially larger scale, this could be extended to industrials who want to estimate the benefits of producing bacteriophages long term or governments who might want to arbitrate between the two solutions.

The results will be partially the property of K. Gloux and INRAE and, as such, will not be released publicly without their consent. If released it will be of INRAE's doing with our team's consent.

Methodology, Study Framework, and Hypothesis.

We considered two modes of treating bacterial infections:

- Antibiotics, which are proven medications with an established production and distribution chain.
- Bacteriophages, which are currently only produced at the laboratory scale in France.

To compare the two treatment methods, we chose to use an equivalent functional unit of a single dose for the treatment of a bacterial infection in a patient. On average, this corresponds to a box for amoxicillin and one dose of 10^{11} PFU for the bacteriophages.

The goal is to compare the life cycle impacts of each medication, including:

- Material extraction
- Product manufacturing
- Packaging
- Distribution
- Use
- Recycling and waste management

We have chosen to focus on production and distribution within France for a duration of one year. As there are numerous types of bacteriophages and antibiotics, we have limited this study to the treatment of an *E. Coli* bacterial infection, specifically the production of the corresponding phage and treatment with amoxicillin. This choice is based on the fact that *E. Coli* is one of the most common bacteria and a priority research area for the development of bacteriophage therapy.

Any co-product produced during the manufacturing process should be taken into account.
We will not consider the manufacturing of reusable machines and tools (especially lab equipment) nor the emissions linked to the buildings in our assessment, since the durability of those is assumed to be too large to take into consideration considering our functional unit. We will also not take into consideration the commuting of employees and consider that it would be equivalent between phages and bacteriophages.
We will not consider the upkeep of shops, drugstores and other places of distribution other than the cost of transportation for our material.

We decided to consider a French production (of material, importation, energy). This leads to assuming that the transport from the factory to the market will only be done inside the country. The energy mix is assumed to be the one mentioned for 2021 on the IEA website.
We will assume that the manufacturing and transport is done in a market that allows for equipment to be used to their maximum potential (full factories and trucks).
The equipment will be assumed to be of the best quality available on the market for an industrial scale, and that has already been tested and implemented, if possible. Otherwise, it will be based on lab studies and most widely used methods in this context.

As our study is a general study on existing academic and industrial materials and complemented by some experimental research (marginal), our data quality depends on the data quality of the research screened during our study, as well as the difficulty of theoretically scaling up lab studies. Our goal being to compare the two methods, as long as this uncertainty is taken into consideration during the analysis of the results, they are not an obstacle.

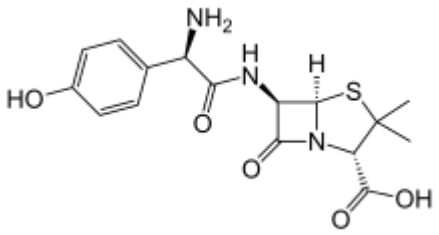
We will try to consider the impact on water, grounds, human health and air pollution, as well as interpret the consequences on climate change, at least in a qualitative and comparative way.

The impact on climate change will be evaluated by calculating an estimation of the emissions in equivalents of CO_2 ($kgCO_{2,\,eq}$), focused on the consumption of energy during the process.
The impact on human health will be evaluated using an estimated number of deaths and/or long term disabilities caused by the use of antibiotics and bacteriophages compared to the practical efficiency of the treatments.

Antibiotics

Elements of context

In our study, we aim at presenting results which are representative of the context of the report (France, in 2023). As the most consumed antibiotic in France is amoxicillin, we decided to choose it to be compared to phages. Yearly, about 63 million boxes of amoxicillin are sold in France. This antibiotic is used to treat bacterial infections like otitis, angina or digestive system infections.



Molecule formula of Amoxicillin

Amoxicillin, as opposed to bacteriophages, in a molecule. Its chemical formula is C16H19N3O5S. This derivative of ampicillin and member of the penicillin family was discovered in 1958 but used after 1972. The molecule attaches to the bacterium's cell wall and provokes its death by preventing the cell wall from being built. It covers moderate-spectrum, but some bacteria already have developed a resistance against amoxicillin, such as Citrobacter and Pseudomonas aeruginosa. Some E.coli have become resistant as well.

Manufacturing

Fermentation

The source microorganism is grown in large containers (100-150 m^3 or more) containing a liquid growth medium. Oxygen concentration, temperature, pH and nutrient levels must be optimum. As antibiotics are secondary metabolites, the population size must be controlled very carefully to ensure that the maximum yield is obtained before the cell dies.

A fermentation process requires the following :

1/ A pure culture of the chosen organism, in sufficient quantity

2/ Sterilised, carefully composed medium for growth of the organism

3/ A seed fermenter, a mini-model of production
4/ A production fermenter, the functional large model
5/ Equipment for drawing the culture medium in steady state, cell separation, collection of free supernatant, product purification and effluent treatment.

To evaluate the carbon emissions due to the fermentation, we use the database from ADEME : we choose the closest data available which were 1.06 kgCO2e/kg for the impact of plant-produced bacterial enzymes. Based on bacterial enzymes produced industrially, for a box containing 6 pills of 1g of amoxicillin each requiring the same amount of untreated bacterias, fermentation corresponds to carbon emissions of **400 tCO2e per year**.

Semi-synthesis

When extracted from the fungi, the 6-APA has a chemical structure which is very close to amoxicillin but still requires chemical reactions. This is what we call the semi-synthesis. We consider the reaction material as written off, given the period of study (one year). Besides, due to the lack of data available and time, we will essentially consider the emissions due to energy consumption in a quantitative way. The other consumptions will be studied qualitatively.

Reaction

Amoxicillin requires a semi synthesis from the precursor (+)-6-aminopenicillanic acid, or "6-APA". The industrial production of Amoxicillin was a process based on the Dane salt route used for another antibiotic named Ampicillin. The potassium ethyl salt is presently the preferred derivative. Yields can go above 90 %. The reaction is the following :

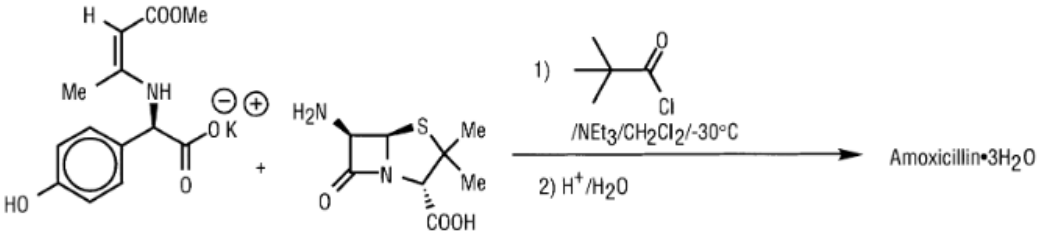


Fig. I.14 Industrial process for Amoxicillin

Comments on the mechanism

As the mechanism of the reaction was not as easy to understand as we expected, we did not have the possibility to determine without a doubt the co-products of the reaction. However, thanks to the advice of professor Nuel, we managed to understand that the hydrolysis (the second step) is a deprotection step. This is important because it means that both steps must be done one after

another rather than simultaneously. Also, the reactants can not be in contact with water until they have reacted.

Protocol

The protocol, as well as the reaction itself, were found in *Synthesis Of [-L- Lactam Antibiotics - Chemistry, Biocatalysis And Process Integration (Bruggink & Roy, 2021)*. We decided to include it in the report as it was in the book.

A. Preparation of Mixed Anhydride
A solution of the Dane salt in dichloromethane is treated with an equivalent of pivaloyl chloride. Addition is at such a rate so as to maintain a suitably low temperature (-30°C). The mixture is stirred for 1 hour to give the mixed anhydride of the Dane salt.
B. Preparation of 6-APA solution
A suspension of 6-APA in dichloromethane is solubilised with the help of an excess of triethylamine at 0/10 deg. C.
C. Preparation of Ampicillin Trihydrate
The 6-APA solution is slowly added to the mixed anhydride with good stirring. After an additional few hours of stirring, the mixture is hydrolyzed with water and HCl at around 0°C. The layers are separated and the aqueous phase is treated with base to give a final pH of 5. The crystallised Ampicillin trihydrate is collected, washed with water and acetone, and dried.

Moreover, one may assume that purification steps can be necessary although they are not mentioned in the protocol.

Mass balance, inputs and outputs

The complete tables are available in appendix n°5.

Yearly, 63 million boxes of amoxicillin are sold in France, with approximately 6 pills per box, containing 1000 mg of active agent. Therefore we intend to produce 378000 kg of amoxicillin each year, or 1036 kg daily. For the orders of magnitude to be easier to handle, we used the daily amount. The emissions will be multiplied by 365 at the end.

The introduced materials are : 6-APA, Dane salt, pivaloyl chloride, triethylamine, dichloromethane, chlorhydric acid, water and acetone. The waste produced is pivalic acid, potassium chloride and the rest of reactants since the yield is not 100 %. The solubility of amoxicillin allowed us to approximate that 31 m^3 of reacting medium was necessary every day. This figure gives the possibility to evaluate the other quantities of the table.

We were not able to evaluate the quantity of chlorhydric acid necessary nor the quantity of acetone used in the rinsing step. Unfortunately, the ADEME database did not provide any

information on the management of chemical waste. These elements would be relevant to consider in a deeper study.

Stirring and temperature control

During the whole reaction, the temperatures are maintained very low. The common refrigerant liquid that is used in the industry is a glycol-water mix, however it would not have satisfying properties at -30°C (mainly a high viscosity). Therefore, we searched for more relevant alternatives. We discovered that organic brines, such as potassium acetate or potassium formate allow low functioning temperatures with satisfying thermodynamic properties. They are non-toxic, even compatible with alimentary contact and do not contain any harmful component to the environment. Besides, these two products are used as eco-friendly deicer on roads or in airports.
Given the lack of precision in the resources we disposed of, we approximated that the first step (under -30°C) was about one hour long while the second step (0°C) was two hours and a half long. This allows us to evaluate the quantity of energy which is needed to lower the temperature of the reactants.

In order to keep dichloromethane under -30°C for 1 hour, we need 302.5 kWh. As for water at 0°C for 2.5h, we need 263.75 kWh. According to the ADEME database, using the French mix of electric energy, this corresponds to emissions of **10,78 tCO2eq per year**.

Unfortunately, we were not able to evaluate the impact of stirring. In a more advanced study, this factor should be taken into account.

Conditioning (Encapsulation)

The impact of an antibiotic depends on how it is conditioned. It will also influence the way it is inoculated to the patient : a liquid solution with a syringe, a pill to be swallowed with water, a powder to be dissolved in water, etc. In the case of amoxicillin, many options are available. Moreover, phages can also be under many forms of administration, which means that we have the choice of the format we want to study. For our work, we decided to consider pills. Indeed, the compressed tablet is the most commonly seen dosage form in use today. About two-thirds of all prescriptions are dispensed as solid dosage forms, and half of these are compressed tablets.
A treatment of amoxicillin usually requires about 6 inoculations of the product whereas in the case of phages, only one would be necessary.

First of all, it is important to remember that a pill does not only consist in the active component, but also many others called excipients : industrials use their properties to give the medicine its colour, taste, coagulation, antivomitive action and so on. These excipients are indicated on the

manual of the medicine as mandatory information, however their precise concentration is often not disclosed. This is why it was not obvious for us to determine the quantity and number of excipients we needed for our amoxicillin dose.

When the mixture of active compound and excipients is obtained, the formation of a pill requires four steps:

- Filling a compression room
- Eliminate (to level) the superficial quantity of solid in the chamber
- Generate the compression to obtain the pill
- Eject the pill

This is made with the help of a tablet press. Unfortunately, we did not have the opportunity to deepen our evaluation of this step's impact.

Packaging

A medicine is usually sold in a cardboard box, with a printed paper manual and the pills themselves wrapped in plastic and aluminium. Although we may suppose that phages would be packed in a similar way, it is important to take into account the fact that the difference of administration doses will impact the packaging that is necessary. Indeed, only one dose of phage is necessary for a treatment while it would require about 7 pills.
An article published in the *Algerian Journal of Environmental Science and Technology* deals with the life cycle analysis of the industrial production of an antibiotic. More specifically, an interesting figure shows the contribution of industrial wrapping to eutrophisation for 1 kg of spiramycin while decomposing the treatment into different components.

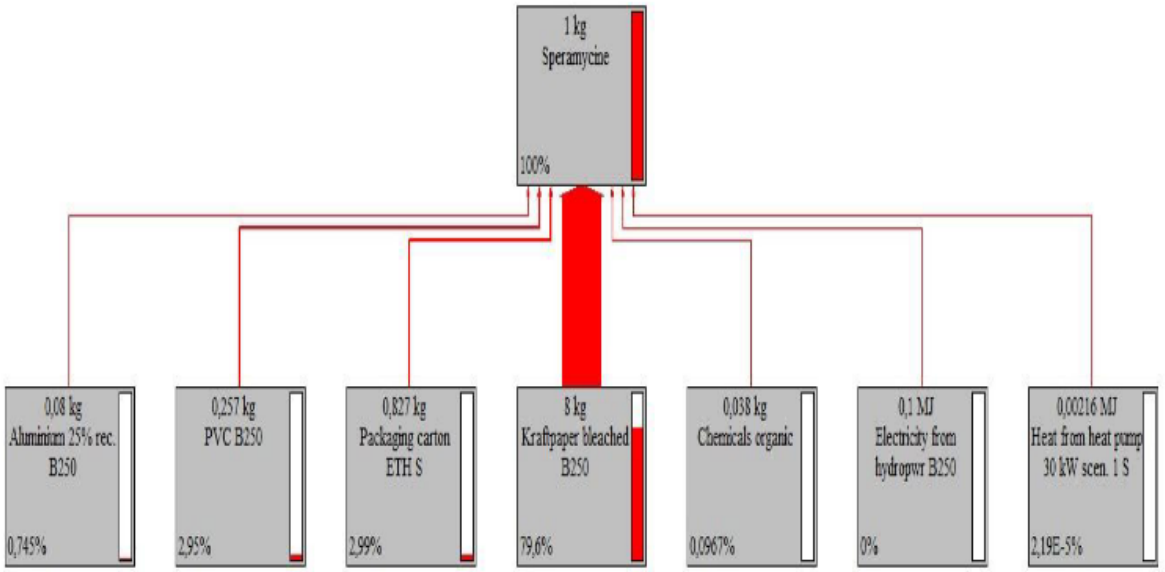


Figure 5. *Contribution d'enrobage industriel d'un antibiotique sur l'autrophisation par rapport à un kilogramme de spiramycine.*

This reference gives us the following components for a box containing 7 pills of 1g of amoxicillin each:

- 0,3 g of chemicals (solvents, glue, inks,etc.)
- 0.6 g of aluminium
- 1.8 g of Polyvinyl chloride (PVC)
- 5.8 g of cardboard (box)
- 56 g of bleached kraft paper (printed notice)

For a total of 71g approximately.
In order to evaluate the carbon emissions due to each material, we use the ADEME database.

It makes an annual consumption of:

- **16.8 t of chemicals (53 tCO2e)**
- **35 t of aluminium (275 tCO2e)**
- **113 t of PVC (341 tCO2e)**
- **364 t of cardboard (142 tCO2e)**
- **3.5 kt of bleached kraft paper (3 242 tCO2e)**

So a total of **4 054 tCO2e** due to the packaging.

Distribution

When it comes to distribution, it is likely that amoxicillin boxes and phage boxes would be managed the same way, the difference being the size of boxes. Hence, the calculations were made for bacteriophages and then a proportionality factor was added. **This approximation gives annual emissions of 391 tCO2e per year due to the distribution of amoxicillin.**

Use

Patients are supposed to ingest their pill with the help of a glass of water, or juice. Therefore, for each tablet, about 150 mL of tap water is consumed. **It makes an annual consumption of 66 150 m^3 of water.** According to the database of ADEME, the consumption of water corresponds to carbon emissions of **8.7 tCO2e**.

Disposal

Unused medicines

In France, pharmacies are required to accept the unused medicines that are brought by customers. This collection is financed by the eco-organism Cyclamed. However, in the context of our study, we shall consider that all the pills of a box are used for treatment. In a more precise approach, it would be relevant to take into account the unused tablets and their end-of-life.

Packaging

The printed instructions and packaging are not to be brought back to the pharmacist, but sorted like the rest of domestic waste. So they are counted as domestic packaging by another eco-organism named CITEO.
As we saw earlier, several materials are used in a box of tablets: aluminium, plastic (PVC), cardboard and paper. Earlier, we managed to evaluate the composition of a box of antibiotics.
According to CITEO, 73 % of paper and cardboard, 58 % of aluminium, 11 % of plastics and 58 % of aluminium are recycled.

These figures would give us that:

- 20.5 t of aluminium are recycled, leaving 14.8 t
- 12,5t of PVC is recycled, leaving 100,5 t
- 266 t of cardboard is recycled, leaving 98 t
- 2.5 kt of paper is recycled, leaving 1 kt.

To evaluate the carbon emissions due to the end-of-life of the amoxicillin, we use the database from ADEME.

Material	Carbon emissions due to the end-of-life (tCO2e)
Aluminium	11
PVC	363
Cardboard	269
Paper	168
TOTAL	**811**

We obtain that the disposal of amoxicillin boxes accounts for **811 tCO2e**.

Unfortunately, it is very unlikely that the plastic and the metal can be easily separated during the sorting process. In that case, recycling is not really an option as opposed to landfilling or incineration, despite the high recyclability of aluminium. Besides, the material risks being contaminated by grease or liquids remaining on other packages in the same bin (eg. from a pizza box, a bottle of juice).

Bacteriophages

Prescription and definitions

63 million of antibiotics packs are sold yearly in France. Since bacteriophages treatment is effective with a single inoculation, we consider that one box of antibiotics corresponds to one dose of phages.

Bacteriophages have the ability to attach themselves to bacterial cells, inject their genetic material into the cell, and hijack the cellular machinery to replicate themselves. In the case of lytic bacteriophages, this replication process eventually leads to the death and destruction of the targeted bacteria. To use bacteriophages for the treatment of bacterial infections, a specific phage or a combination of phages that are effective against the target bacteria is selected. These phages can be obtained from natural sources, isolated from environmental samples, or obtained from phage banks.

Considering phages prescription, we found that, on average, single doses of around 10 mL at a concentration of 10^{11} PFU/mL were inoculated. [8]
PFU stands for "Plaque-Forming Unit." It is a unit of measurement used in virology to quantify the number of viable virus particles in a sample. A plaque-forming unit refers to the ability of a single virus particle to form a visible plaque or localised area of cell death in a culture of susceptible cells.
Using the amount of antibiotics prescribed, this corresponds to $630 * 10^3$ L produced in a year.

Manufacturing

From raw materials to packaging

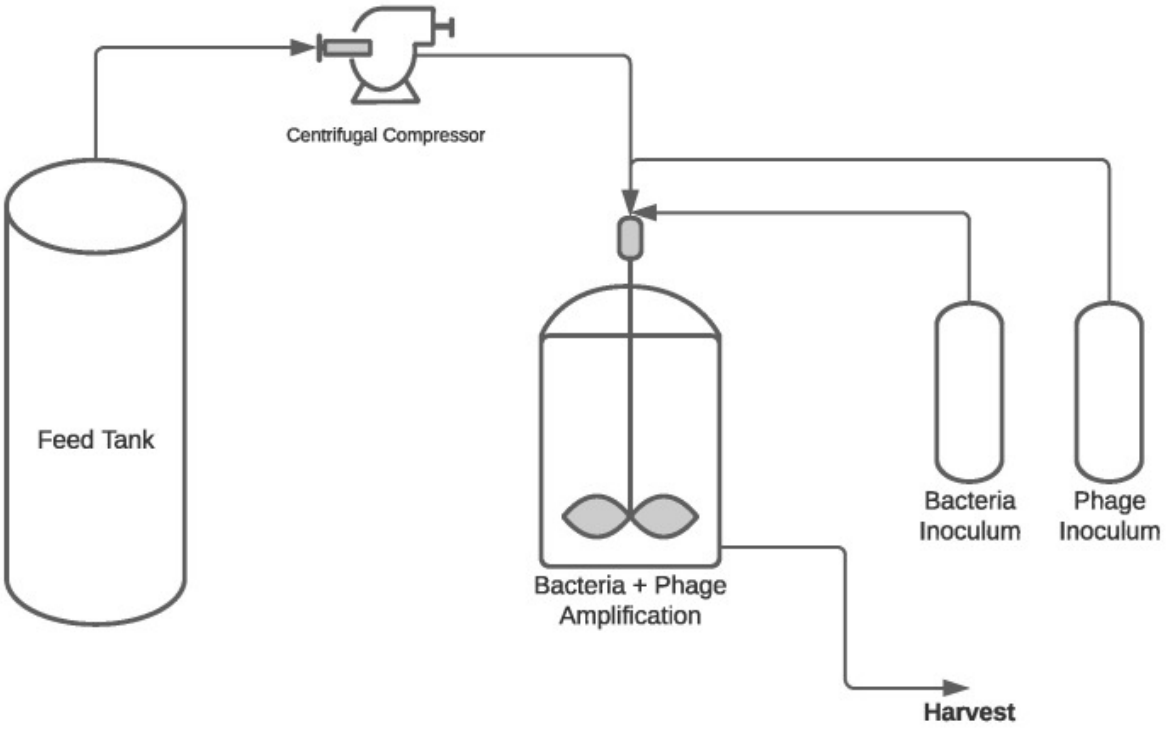
Cell line development (CLD)

It's the development of a bank of phage and bacteria, as well as knowing their properties, to create the optimised combination (what type of phage, what type of bacteria, what ratio, in what medium, how many in one batch..) to optimise the production while still having a efficient clinical dose at the end.

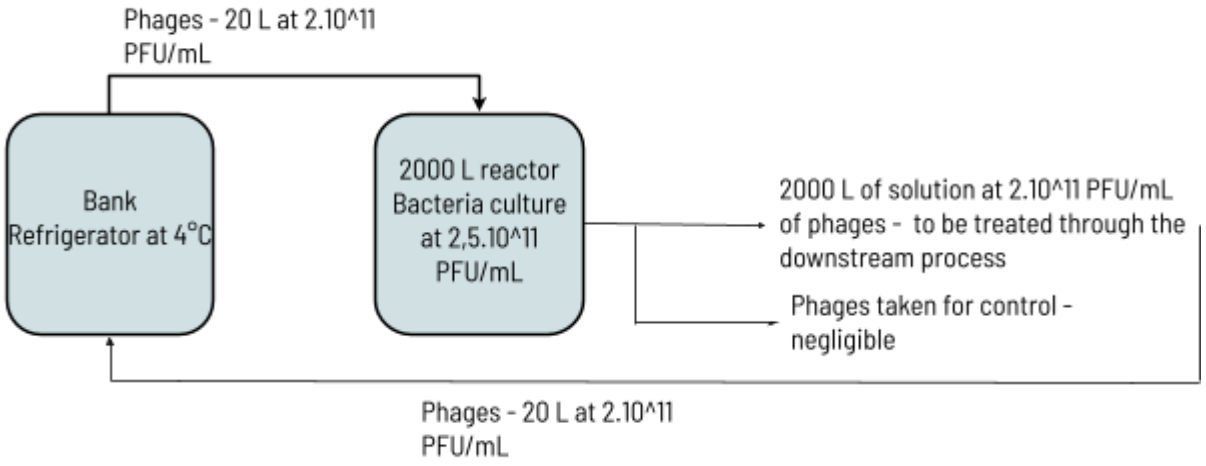
Our hypothesis is that the production line is already operational, so that the cell line development is complete and approved. Then the only thing that we consider in this part is the assessment of the storage and conservation of the phage's bank.

Upstream processing (USP)

Upstream processing refers to the initial stages in the production of the bacteriophages. It consists of the reproduction of the phages and the harvesting of the product, as well as the stock of phages and bacterias for future amplifications.



We consider a batch process. The medium is firstly fed into the fermenter, then the bacteria is amplified until reaching the optimal population. At that time the optimised MOI (multiplicity of infection: phage per host) of phage is added and is harvested after lysis occurs. The batch time is then: BT = Feeding Time + Bacteria amplification time + Phage amplification time + Cleaning Time.
The feeding time and cleaning time are approximately 2 hours.
The bacteria growth is made during 9h at 37°C under agitation.
The phage amplification time is about 2 to 3 hours.
As this sums up to 14 hours, we consider that we can only produce one batch per day.



With the amount of product needed per year calculated earlier: 630 * 10^3 L and the hypothesis that batches are produced 365 day/year, the production system would require a reactor of 2000 L (1726 L/day needed).

We want to obtain 2000 L of lysate at 10^11 PFU/mL

The need is 2000L at 10^11 PFU per mL per day.
The replication factor is about 10^2
Then it is needed to start the reaction at 10^9 PFU per mL.
So the volume needed to be collected is

$$2000L \times \frac{10^9}{10^{11}} = 20L$$

Amount of PFU at the start of the reaction:

$$2000L \times 10^9 PFU\ per\ mL = 2.10^{12} PFU$$

Amount of bacteria needed, knowing that the MOI is 10 bacteria/PFU:

$$10\ bacteria/PFU \times 2,5.10^{12}\ PFU = 2,5 \times 10^{13}\ bacteria$$

Volume of solution of bacteria at 10^9 bacteria per mL:

$$\frac{2,5.10^{13}\ bacteria}{10^9\ bacteria/mL} = 2,5.10^4\ mL = 25\ L$$

We consider that the volume of the medium used to grow the bacteria is the final volume of solution (we neglect the 0.5 L of bacteriophages added to the reactor), which is 2000 L

	Culture medium for host bacteria	Quantities needed for a batch (2000 L)	Quantities needed for a dose (10 mL)
NaCl	1.0%	20 L	0.10 mL
Glucose	0.1%	2.0 L	1.0 mL
Tryptone L.42	1.0%	20 L	0.10 mL
Yeast extract	0.5%	10 L	0.05 mL

(growth conditions from [5])

Energy consumed by the agitation

We consider that the total energy consumed is equal to power consumed by the reactor: 2,2 kW*working time [19]

1 batch corresponds to a working time of 2 to 3h of contact between bacterias and phages plus ~9h culture of bacterias

As a result, the total energy consumption is:

$$E = 2,2\ kW \times (3 + 9) = 26,4\ kWh$$

This needs to be divided by the number of doses produced in one batch (cf full chart of product consumption)

Energy consumed by the heating

Heating is made during the bacterial growth for 9h at 37°C

$$Q = m \times C_p \times \Delta t$$

Numerical application:

$$Q = 2000\ kg \times 4186\ J/kg \times (37 - 20) = 39,46\ kWh$$

This value will obviously change at an industrial scale because of energy losses over time but, as a first approximation, we are forced to suppose that the reactor is perfectly thermally isolated.

Overall, the flux inventory gives us the following informations:

			Per batch	Per dose
UPS process	Electricity used by the reactor for mixing (kWh)		6,6	0,000033
	Electricity used by the reactor for heating (kWh) (37°C)		39,46	0,0001974
	phages from storage (L of solution)		20	0,0001
	Bacteria from storage (L of solution)		25	0,000125
	Medium for bacteria amplification			
		NaCl	20L	1e-1 mL
		glucose	2L	1 mL
		tryptone	20L	1e-1 mL
		yeast extract	10L	5e-2 mL
		water (L)	1947,25	0,00973625

Downstream processing (DSP)

This part of the process aims at transforming the crude lysate obtained at the end of the USP into the drug substance. In order to do so, the product needs to be purified through various separation processes.

Process suggested by Karine Gloux:
After discussion with our tutor, she sent us some latest studies on phage purification protocols. We chose the purification using iodixanol gradient but we kept a preliminary PEG precipitation to be sure that it will not clog the equipment.

The latest studies shows that the Iodixanol commercialised by Optiprep has a lower toxicity than the gradient of CsCl, which can be toxic on phages and prevent further infection.

Full process

Chloroform treatment

To eliminate any remaining bacteria and avoid any contamination, it is needed to treat the lysate with 2L of chloroform by simply adding it to the tank and stirring.

PEG precipitation

(polyethylene glycol precipitation)

Protocol:
adding 10% w/v polyethylene glycol (6000 g/mol) (densité 1,11) (9% en volume/volume) and let precipitate at 4°C for (15 min to overnight depending the documentation)
*Then centrifuged into pellet at 4500*g for 30 min*
diluting in 80 % water + 20 % glycerol

This is the most common and easy way to isolate phages.
This step serves to eliminate the medium used to grow bacteria, most bacteria cells and proteins and the chloroform.

Consumption:
Centrifugation: Capacity of 2000L then one batch, 2,2 kW for 30min -> 1,1 kWh

Ultracentrifugation

Protocol:
Medium: Water 40%, iodixanol 60% (Optiprep)
Diluted for gradient in PBS-MK (buffer 1mM MgCl2, 2,5 mM KCl), 4 solutions at 15%, 25%, 40%, 58%.
In each batch, 20% of solution at 15%, 15% of solution at 25%, 20% of solution at 40%, 12% of solution at 58%, 33% of lysate.
Ultracentrifugation for 2H (semi-continuous process in Industrial-scale Wasserman KII)
35% recovery in volume.

Consumption:
[26] A ultracentrifuge of laboratory size usually works at 230 V and 16 to 20 A, which corresponds to 4.6 kW for a single-phase current:

$$P = 230 * 20 = 4600\ W$$

Or **8 kW** for a three-phase current . We will take the highest value in our calculations.

Max volume in semi-continuous process: 200L
Volume of the reactor: 5L
Time needed in the reactor: 2h
The flow rate is:

$$Q = \frac{5L}{2h} = 2,5\ L/h$$

Total volume to be centrifuged: 2000L
Max. time: 24h
So, for one ultracentrifuge: 2.5 * 24 = 60L of solution go through each reactor in a day

Centrifuges needed to function:

$$n = \frac{6000}{60} \simeq 100$$

Total theoretical energy consumption:

$$100\ reactors \times 24h \times 8kW = 19200\ kWh/day$$

(Ultrafiltration)

After consulting Pierrette Guichardon on the subject of ultrafiltration, we learned that this method, even though it is advised in our sources and by our tutor, is not used at an industrial scale.

PEG precipitation

Another way to finalise the purification of our drug is to reuse a PEG precipitation, with the same protocol as above. The problem with this is that it probably doesn't reach the sanitary and health goals for a drug to be put on the market. As there is no further documentation on the subject we decided to keep this protocol.

Storage

Pure phage can be stored at 4°C for a year without consequences. If deep freezed at -80°C, they can be kept in good condition for 10 years, but that would be useless in our case.
However, bacteria need to be kept at -80°C or else they could grow and mutate in the medium.

Mixing with glycerol helps with conservation.[1]

We will consider the following storage conditions:

To avoid any contamination, stored phages are only taken at the end of the treatment process
Phages: 4°C, in a solution 80% water 20% glycerol

Bacterias: -80°C, in a solution 80% water 20% glycerol

Consumption:

			Per batch	Per dose
Storage	Electricity consumption from fridges for phages (4°C)		n.a	4.0e-6 kWh
	20L bottle		1	0,000005
	Electricity consumption from fridges for bacterias (-80°C)		n.a	7,23e-5 kWh
	25 L bottle		1	0,000005

Encapsulation

In order for the comparison to be as meaningful as possible, we chose to keep the same inoculation medium: capsules. For that we first and foremost need to freeze dry the solution.

Ref.	Host Bacteria										Formulation					Excipients															Method						Result / PFU Log reduction ● good ◐ moderate ○ poor / ③ value				
	B. cepacia	C. ulcerans	E. coli	Mycobacteriophage s	P. aeruginosa	S. aureus	S. enelerica	V. cholerae	Others		Broth / lysate;	Buffer	Saline	Water		Gelatine	Glycerol	Proteins. (casein, whey etc.)	Amino acids or peptides	Trehalose	Mannitol	Sucrose	Polyvinylpyrrolidone	Lactose	Leucine	Others					( ) -encapsulated					Before Storage	Storage conditions brown/blue – low/high relative humidity			
																														Freeze drying.	Air drying	Drying on filter paper	Spray drying	Spray freeze drying	Stored as liquid		Storage duration, months (LT – long term)	ambient temperature -25 C	refrigerated -4 C	sub-zero temperatures
[107]			Ec	M	Pa	Sa			12	B																	L		24	◐	●		
										B							Gl										L											
										B								P										FD						◐		◐	●	
										B								P												FP				○		○	○	

Comparative study of conservation ways of phages ; it shows that liquid and freeze drying conservation at 4°C is possible without losing PFU

Freeze drying process

Pretreatment

Protocol:
Diluting the bacteriophage solution in 1:1v, water based solution with 22% skim milk
*Centrifugation at 4500*g for 1h*

Skim milk is used as an excipient for the pills

Consumption:
Centrifugation: capacity of 2000L then one batch, 2,2kW for one hour so 2,2 kWh.

Freeze drying

Freeze drying to obtain powder [14] 1778x20mL per batch (one day)

Consumption:
25 m^3 of water per cycle, 150 KWh per cycle

Overall, the flux inventory gives us the following informations:

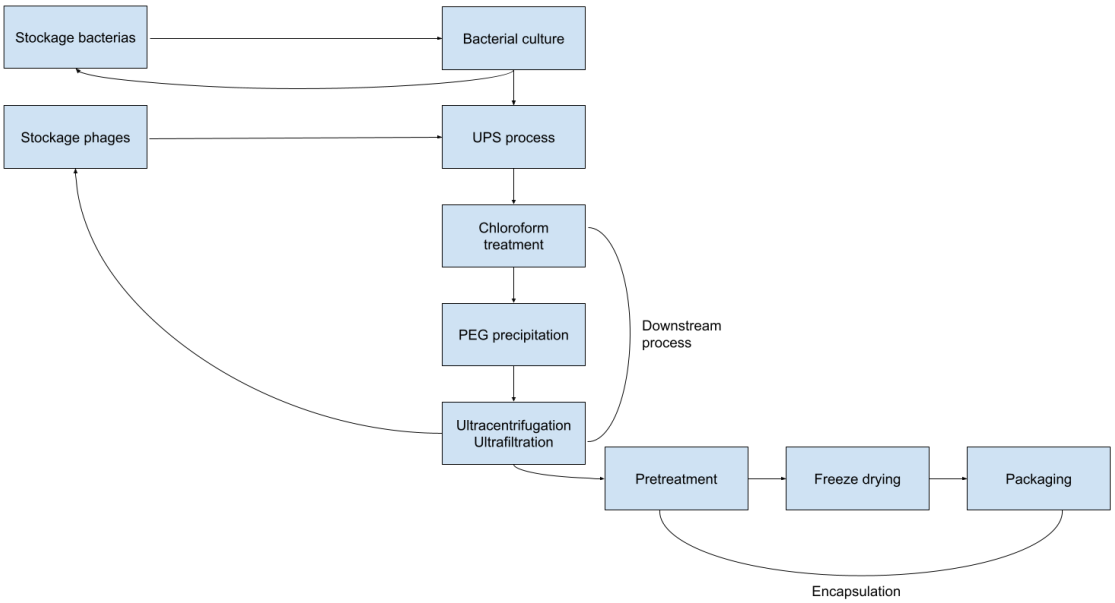
		Per batch	Per dose
Lyophilization	Electricity used by the centrifugation	2,2 kWh	1,1e-6 kWh
	Electricity used by the freeze-drying		8,4e-2 kWh
	Water consumption for the freeze-drying		14e-3 m^3
	22% Skim milk		10 mL
	20 mL phial		1

Packaging

The packaging is similar to the one of the amoxicillin except for the fact that, for one treatment, we only have to package one dose and not a full box. This means that the packaging for the bacteriophages is roughly equivalent to 1/6th of an amoxicillin box
[29]

Overall process

The process for producing bacteriophages is described in the following diagram.



Overall, the process uses up approximately:

2 e-1 kWh / dose
14 L of water / dose

This gives us the following carbon emissions for one dose :

$12{,}25\ g_{eq}CO_2$

The full table of flux is provided in Appendix n°1

Use

The use of the products (phages and antibiotics) does not generate any side product apart from the used packaging.

We consider that we function with lean manufacturing. Given the fact that the dose is to be taken one dose at a time and that the lifespan is more than one year, we can admit that the waste is negligible.

However, while estimating the impact of the use is, we will take into account the likelihood of resistive bacterias developing, which is much less in the case of phages for multiple reasons:

- Narrow host range: Bacteriophages typically have a narrow host range, meaning they can only infect and replicate within specific bacterial strains or species. They have evolved to recognize and bind to specific receptors on the bacterial cell surface. This specificity makes it less likely for phages to encounter and infect bacteria that are not susceptible to them. Consequently, the development of resistance is limited to the targeted bacteria.

- Co-evolution: Bacteriophages have a long-standing evolutionary relationship with bacteria. As bacteria evolve, phages also co-evolve to effectively infect and replicate within their specific hosts. This ongoing arms race between phages and bacteria leads to a constant adaptation and counter-adaptation process. As a result, the bacteria may develop resistance mechanisms against specific phages, but the phages also evolve to overcome these defences. This co-evolutionary dynamic helps maintain the effectiveness of phages as antimicrobial agents.

- Multiple mechanisms of action: Bacteriophages employ various mechanisms to kill bacteria, and these mechanisms can act synergistically. For example, some phages can directly kill bacteria by injecting their genetic material into the host cell, leading to the production of more phages and eventual lysis of the bacterium. Additionally, phages can carry enzymes called endolysins that degrade the bacterial cell wall, leading to bacterial cell death. This multifaceted approach makes it challenging for bacteria to develop resistance to phages, as they would need to simultaneously overcome multiple mechanisms.

- Phage diversity: Bacteriophages exhibit an immense diversity in terms of their genetic makeup and infectivity. This diversity ensures that even if bacteria develop resistance to one phage, there are often alternative phages that can effectively target and control the

resistant strains. This natural reservoir of phages provides a built-in defence against the emergence of resistant bacteria.

- Convenience and simplicity: bacteriophages only need to be absorbed once since the population autoregulates inside the body. It makes it less likely for anyone to not take the treatment according to the prescription. Stopping an antibiotics treatment before its full end can cause the development of resistant bacteria, which in terms makes it more dangerous for the population.

Because of this, the impact on human health of bacteriophages is estimated much lower than the impact of an equivalent use of antibiotics, especially on a larger scale. Deploying bacteriophages in a whole country would contribute to diminishing the mortality rate of bacterial infections.

Distribution

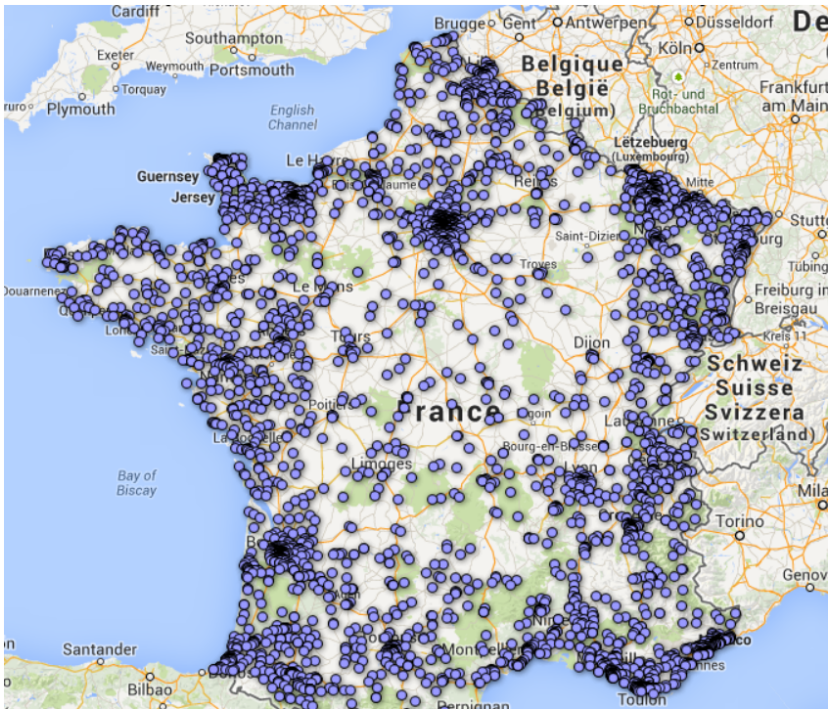
We consider every step of distribution from the plant to the consumer (pharmacy).

When it comes to pharmaceutical products, it is essential to have a reliable system in place for their traceability. This is crucial to ensure that the products are safe for consumption and to be able to locate them at every stage of the supply chain, from their production to their delivery to the end user.

There isn't much available information concerning the distribution of pharmaceuticals in France: in most cases, it is not advantageous for a company to share this type of information since it is linked to CO_2 emissions and could lower the popularity of their products. However, our goal is only to compare two products, and in this case we can easily give an estimation of the ratio by extrapolating the ratio of doses taken for one single treatment to the weight, and thus to the fuel consumed.
For one treatment, we can consider that distributing the antibiotics will cost less than half the amount of fuel distributing phages will.

In order to evaluate the importance of the distribution criteria compared to the manufacturing, use and disposal of both products, we tried to give an order of magnitude of the fuel consumed to distribute a product throughout the country.

First we identified hubs of pharmacies in France using the government's data.



Pharmacies in France

Identification of the major hubs involved:

Paris, Nice, Toulon, Marseille, Montpellier, Toulouse, Lourdes, Biarritz, Bordeaux, Limoges, La Rochelle, Poitiers, Tours, Nantes, Rennes, Angers, Le Mans, Quimper, Brest, Cherbourg, Caen, Le Havre, Rouen, Amiens, Calais, Lille, Valenciennes,Metz, Nancy, Strasbourg, Mulhouse, Besançon, Lyon, Annecy, Grenoble.

According to Google Maps, the shortest route through those hubs would be approximately 4500 km long which, with a truck, would correspond to 1485L of fuel and 92 $g_{eq}CO_2$ / t.km * 15e-6 t* 4500km = 6,21 $g_{eq}CO_2$ per dose for the antibiotics, and thus approximately 3 $g_{eq}CO_2$ per dose for the bacteriophages (if we consider that the trucks are full when circulating).

This is obviously a major contributor to the pollution emitted by our process. Nonetheless, this representation is highly simplified. Here is an example of the real distribution operated by Boiron in France:
The Laboratoires BOIRON are a French company selling homoeopathic products. Their main plant is located in Lyon and distributes the products to the different laboratories of the company. The laboratories then process the orders of the surrounding drug stores and ensure a local distribution via wholesale distributors. According to this process, the average distance travelled to distribute the products and the pollution associated with it are highly reliant on the location of the hubs, the amount of orders and the frequency of delivery of the products. We therefore cannot make any further hypothesis on the distribution process of our products.

Comparative study

Results

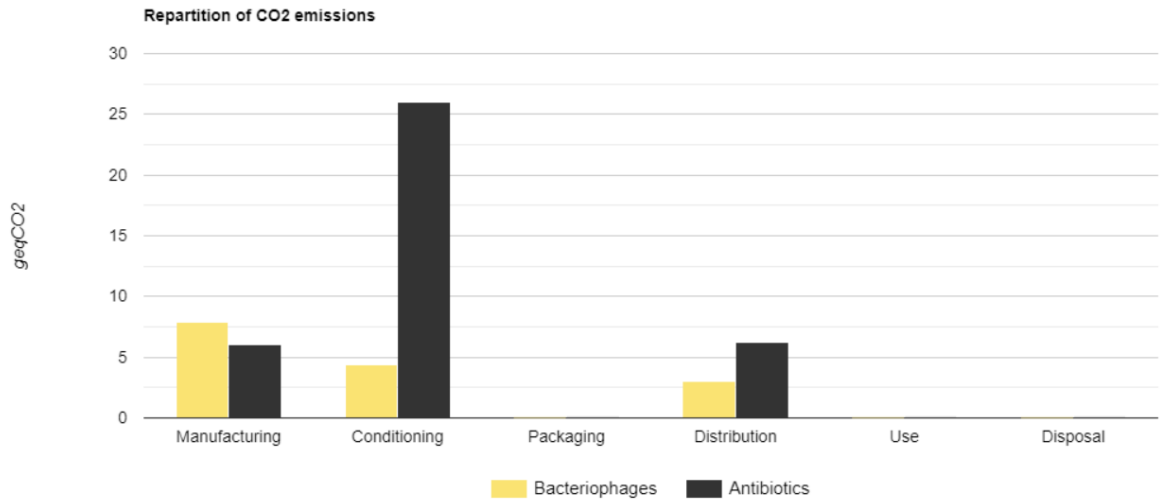
Based on our data, we can provide a comparison of the environmental impact of antibiotics and bacteriophages.

Using the ADEME database, we obtain the following CO_2 emissions table for one dose in g of eqCO2:

	Bacteriophages	Antibiotics
Manufacturing	7.88	6.52
Conditioning	4.368	26.208
Packaging	0.032	0.064
Distribution	3	6.21
Use	0.0132	0.1056
Disposal	0.0064	0.0064

Hypothesis:

- Conditioning of antibiotics is equivalent to 6 times the conditioning of bacteriophages
- Packaging of bacteriophages requires a smaller box and, after weighting experimentally, would be a bit less than a half of the packaging of antibiotics in terms of weight. We computed the data by pro rata.
- In a similar manner, we can assume that the emissions linked to the distribution of antibiotics is approximately two times those of the bacteriophages
- We assumed that taking a pill requires 10 cL water
- Since the disposal is clearly negligible, we did not bother to precisely compute the value for bacteriophages.



Limits and discussions

We did not have the ambition of presenting a full LCA or Carbon footprint assessment. In order to do so, we would require the time and funds necessary to test our protocols at least at pilot-scale. Our different hypothesis and our scope explains why our numbers are lower than the ones provided by the ADEME database.

Some equipment or processes don't even exist yet at an industrial scale : we had to adapt the ultrafiltration for example. Bacteriophages are also not distributed, and not produced in specifically designed buildings, which makes it impossible to extend the scope. Others are not necessarily well-documented since they have been perfected by companies who do not necessarily wish to see their work diffused publicly. If we were to work hand in hand with a company for a full carbon footprint assessment, we would obtain precise data, especially when it comes to the functioning of the company. As a result, this study cannot be used to assess the impact of antibiotics or phages individually : its point is only to provide a humble comparison.

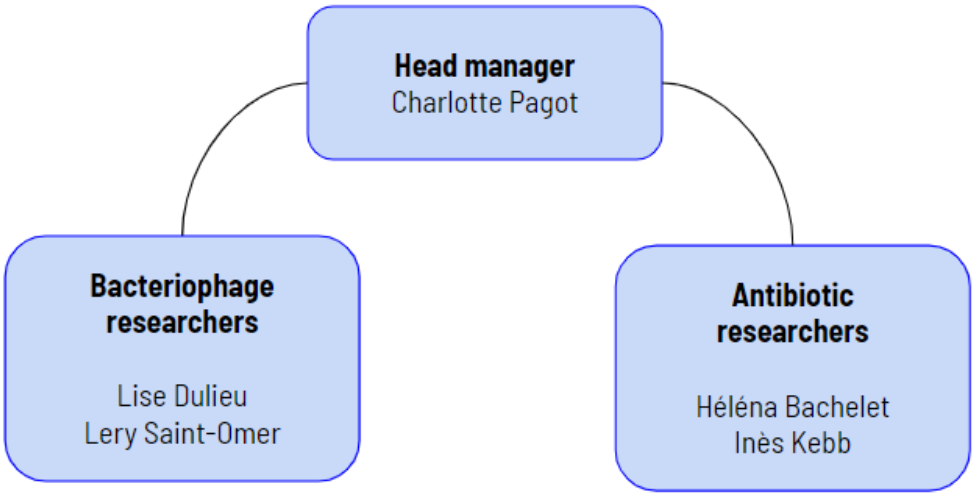
Furthermore, in order to simplify the study, we chose to only study one product : amoxicillin for antibiotics and type 4 phages produced to kill one specific type of *E. Coli*. We then extrapolated this result to other types of antibiotics and bacteriophages : it gives us an approximated value, but in order for example to create a good emission factor, we would need to study a good range of molecules and products, and only then compute the mean value. We would also need a precise uncertainty on this value, which we could not compute from the data we obtained from other publications.

Despite all these possible improvements, our study still highlights the fact that transitioning to bacteriophages would not only diminish the number of multi-drug resistant bacterias but also benefit the environment.

Project Management

During the project, our group used some tools and methods of management.

At the start of our project, we decided to organise our group.



In order to maintain a constant workload, we chose to organize our project around a precise schedule with specified milestones for each team member (see appendix n° 2 for full detail).

Since we were dispatched in two teams, we also needed to provide enough accessible information for the other group to make informed choices. As a result, we decided to keep a log up to date after every project session (see appendix n°3)

Appendix

Some of the documents constitute less formal project management support and, as such, are given in their original form and redacted in French.

1. Full flux table for bacteriophages

					Units
Storage	Elec from fridges for phages (4°C)		n.a	4,00E-06	kWh
	20L bottle		1	0,000005	
	Elec from fridges for bacterias (-80°C)		n.a	7,23E-05	kWh
	25 L bottle		1	0,000005	
UPS process	Electricity used by the reactor for mixing (kWh)		6,6	0,000033	kWh
	Electricity used by the reactor for heating (kWh) (37°C)		39,46	0,0001974	kWh
	phages from storage (L of solution)		20	0,0001	L
	Bacterias from storage (L of solution)		25	0,000125	L
	Medium for bacteria amplification				
		NaCl	20	0,0001	L
		glucose	2	0,00001	L
		tryptone	20	0,0001	L
		yeast extract	10	0,00005	L
		water (L)	1947,25	0,00973625	L
DS process	Chloroform		2	0,00002	L
	PEG		360	0,0036	L
	Water for dilution		3200	0,016	L
	Glycerol for dilution		800	0,004	L
	Energy from centrifugation		2,2kWh	0,000011	kWh

	Optiprep		1303	0,006515	L
	PBS for ultracentrifugation		2645	0,013225	L
	Energy from ultracentrifugation (kWh)			0,111314 2857	kWh
Refrigeration	Elec from fridges for phages (4°C) (kWh)			0,000060 2952381	kWh
Lyophilization	Electricity from centrifugation		2,2kWh	0,000011	kWh
	Electricity from freeze-drying			8,40E-02	kWh
	Water consumption from freeze-drying (m3)			1,40E+01	L
	22% Skim milk			10	mL
	20 mL phial			1	
Total	Energy			1,96E-01	kWh
	Water			1,40E+01	L

2. Work planner

Date jalon	Nature du jalon	Responsables
11/04/23	Cadre d'étude/Définition des livrables	Tout le monde
21/04/23	Inventory analysis: materials & manufacturing, Antibitotics	Inès, Héléna
21/04/23	Inventory analysis: materials & manufacturing, Bacteriophages	Léry, Lise, Charlotte
12/05/23	Inventory analysis: use, antibiotics	Inès, Héléna
12/05/23	Inventory analysis: use, bacteriophages	Lise, Charlotte
12/05/23	Inventory analysis: distribution, antibiotics	Charlotte, Lise
12/05/23	Inventory analysis: distribution, bacteriophages	Charlotte, Lise
12/05/23	Inventory analysis: disposal	Lise, Héléna, Inès
17/05/23	Impact assessment	
30/05/23	Création des livrables et rendu	Tout le monde

3. Full log

Carnet de bord

Séance du 04/04/2023
Ordre du jour

- ☑ ~~Brainstorming sur les aspects du cycle de vie des traitements~~
- ☑ ~~Répartition en groupes~~
- ☑ ~~Etablissement du planning et des jalons, livrables~~

Brainstorming:
https://miro.com/welcomeonboard/eFlSR3lDNVNna3ZGYXIwRUtLbXZaalJieEd2dVdoMFFQaWFzVGZ4YzFxTHVTWWc1SnRid0NmWkZEYlJ0NG51NXwzNDU4NzY0NTUwNzQ2MzEyMjk4fDI=?share_link_id=565407172202

Livrables:
Dans le rapport:

- tableau comparatif (chiffres)
- organigramme/map du cadre (cf scope sur les bilans carbone par exemple)
- fiches comparatives destinées aux entreprises ? pour les aider dans leur choix

Répartition des groupes:

Uniquement pour la fabrication, sont amenés à changer

Antibiotiques	Bactériophages
Inès Héléna	Léry Charlotte Lise

Planning:

https://docs.google.com/spreadsheets/d/15D6Z-LW9cuuDDB4oh4oCLIWT59QBA9Dz0JsuaxTFUJw/edit?usp=share_link

Ressources:

https://en.wikipedia.org/wiki/Life-cycle_assessment
https://fr.wikipedia.org/wiki/Antibiotique

Vidéo de vulgarisation sur les phages et leur utilisation:
https://www.youtube.com/watch?v=28RXYI4jSaw

https://research.pasteur.fr/fr/team/bacteriophage-bacterium-host/

LCA of antibiotics:
https://link.springer.com/article/10.1007/s11367-021-01908-y

French energy mix:
https://www.iea.org/countries/france
https://www.statista.com/statistics/1290216/carbon-intensity-power-sector-france/#:~:text=Power%20sector%20carbon%20intensity%20in%20France%202000%2D2021&text=In%202021%2C%20France's%20power%20sector,estimated%20at%2058%20gCO%E2%82%82%2FKWh.
2021 -> 58 gCO2eq/kWh

Séance du 11/04/2023

Bactériophages:

- Recherche et bibliographie sur les matières premières et les méthodes de productions des bactériophages
- Première estimation des doses prescrites par personne et de la production nécessaire annuelle pour traiter les cas de E. choli en France: décision de prendre ce point de comparaison avec les antibiotiques.
- Choix de la méthode batch et estimation du volume du réacteur
- Identification des besoins de stockage
- Vérification des sources et des informations auprès de K.Gloux

To-do séance prochaine:

- [ ] Calcul définitif des volumes de matériaux utilisés
- [ ] rapporter les données à une dose de traitement
- [x] ~~confirmer le dosage en chloroforme~~
- [x] ~~détailler le downstream processing~~
- [ ] estimer la recyclabilité des tubes plastiques employés
- [ ] estimer la dépense énergétique liée au refroidissement
- [x] ~~estimer la dépense énergétique liée à l'upstream process~~
- [ ] estimer les pertes de matériaux liées à la surveillance qualité

Général:

Choix de l'antibiotique le plus couramment vendu en France: l'amoxicilline (63M de boîtes par an) -> 63M de traitements par an

Antibiotiques

- Recherches sur la fabrication des antibiotiques
    - Choix d'un antibiotique
    - Choix d'un processus de fabrication
    - Première estimation des matières premières

Séance du 21/04/2023

Bactériophages:

- Rédaction du tableau des flux
- Mise au propre des calculs de la semaine dernière entamée
- Calcul de dépenses énergétiques
- Clarification de l'autosuffisance en bactéries
- Schéma données process
- Début de calcul des quantités pour le downstream process

To-do séance prochaine:

- ☑ ~~Calcul définitif des volumes de matériaux utilisés~~
- ☑ ~~Finir de clarifier le downstream process auprès de K.Gloux~~
- ☐ contacter Pierrette pour l'ultrafiltration (et le nettoyage du réacteur ?)
- ☐ Faire un choix de centrifugeuse pour chaque centrifugation (DP, Lyophilisation…)
- ☑ ~~Choisir entre encapsulation et pilule~~
- ☐ estimer la recyclabilité des tubes plastiques employés
- ☑ ~~estimer la dépense énergétique liée au refroidissement~~
- ☑ ~~estimer les pertes de matériaux liées à la surveillance qualité~~

Antibiotiques

- Analyse du processus de fabrication choisi
  - Matières premières
  - Mécanisme et conditions de réaction
  - Matériel
  - Déchets et solvants
- Enrichissement de la bibliographie
  - Posologie, Mode d'administration
  - Conditionnement (mise en forme)
  - Packaging

Séance du 03/05/2023

Bactériophages:

- RDV visio Karine Gloux et obtention de sources pour le DS process
- Rédaction de la partie utilisation du produit
- Rédaction de la partie distribution du produit
- Données sur le recyclage de l'emballage

To-do séance prochaine:

- ☐ Finir le DS process

- [ ] Mise en page

Antibiotiques:

cf Inès, j'étais absente…

Séance du 9/05/2023

Bactériophages:

- DS process: volumes de produits nécessaires, énergie consommée par l'ultracentrifugation (protocole Karine Gloux)
- Contact C.Jalain pour l'utilisation de SimaPro pour le packaging
- Recherche d'un appareil d'ultrafiltration

To-do séance prochaine:

- [ ] Finir l'ultrafiltration
- [ ] Emballage
- [ ] End of life, recyclage
- [x] ~~Commencer la rédaction~~
- [x] ~~Identifier les forces et les faiblesses du process~~
  -> facteur de qualité des données (environ 3 partout)

Antibiotiques:

- quantification des besoins en matière et en énergie pour la fabrication
- étude de la fin de vie de l'amoxicilline et son utilisation

Séance du 12/05/2023

Rédaction

- introduction en français
- tri manufacturing (bactériophages)
- incorporation raw materials dans manufacturing (bactériophages)

To-do séance prochaine:

- [ ] Finir l'ultrafiltration
- [ ] finir de mettre raw materials dans le reste
- [ ]
- [ ] Emballage

- ☐ End of life, recyclage

Général

- Entretien avec M. Jalain sur le sujet du cycle de vie et de l'utilisation du logiciel Simapro

Antibiotiques

- Entretien avec Me Guichardon au sujet des procédés en pharmaceutique
- Prises de contact avec un employé de Sanofi et Cyclamed

Mise au propre des travaux déjà accomplis

4. Reference for power consumption calculations

Ampère	Puissance en 230 V monophasé (en kVA)	Puissance en 230 V triphasé (en kVA)
16	3,7	6,4
20	**4,6**	**8**
25	5,8	10
32	7,4	12,7

5. Full tables of mass balances for antibiotics

	Yield (molar, massic)	0,9	1,5			Duration (h)	Reactor temperature (K)	DeltaT (K)	Cp solvent (KJ/kg/K)	Power heating (kW)	Energy heating (kWh)
	Nb of boxes sold yearly	63000000			Step 1 (in dichloromethane)	1	243,15	55	5,5	302,5	302,5
	Amoxicillin per pill	1	g		Step 2 (in water)	2,5	273,15	25	4,22	105,5	263,75
	pills per boxes	6									
	Mass of amoxicillin yearly	378000	kg		Volume de réacteur (/jour)	31	m3				
	Mass of amoxicillin weekly	7269,2	kg		Concentration en 6-APA	0,1	mol/L		Amoxicillin solubility	3,44	g/L in water
	Mass of amoxicillin daily	1035,6	kg		Volume total nécessaire	11 315	m3				
							DAILY				
	Species	Formula	Molar mass (g/mol)	Density (g/L)	Quantity introduced (kmol)	Mass introduced (kg)	Final qty (kmol)	Final Mass (kg)	Balance (kg)		
Reactant	6-APA	C8H12N2O3S	216,25	?	3,1E+00	6,8E+02	3,1E-01	6,8E+01	-6,1E+02		
Reactant	Dane salt	C13H14O5NK	303	?	3,1E+00	9,5E+02	3,1E-01	9,5E+01	-8,6E+02		
Reactant	Pivaloyl chloride	C5H9ClO	120,58	985	3,1E+00	3,8E+02	3,1E-01	3,8E+01	-3,4E+02		
Catalyst	triethylamine	C6H15N	101,19	726	3,1E+01	3,2E+03	3,1E+00	3,2E+02	-2,9E+03		
Solvent	dichloromethane	CH2Cl2	84,93	1330	73,5 m3	4,1E+04	-	4,1E+04	0,0E+00		
Reactant	chlorhydric acid	HCl	36,458	?					0,0E+00		
Solvent & rinsin	water	H2O	18,015	1000		3,0E+05			-3,0E+05		
Rinsing	acetone	C3H6O	58,08	784					0,0E+00		
Waste	Pivalic acid	C5H10O2	102,13	905	0,0E+00	0,0E+00	2,8E+00	2,9E+02	2,9E+02		
Waste	potassium chlorid	KCl	74,555	1984	0,0E+00	0,0E+00	2,8E+00	2,1E+02	2,1E+02		
Product	Amoxicillin	C16H19N3O5S	365,404	?	0,0E+00	0,0E+00	**2,8E+00**	**1,0E+03**	1,0E+03		

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