

# NEO P1 WHITE PAPER



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# Background

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## VOCs and indoor air quality

Indoor air pollution can be categorized in two major groups: **Particulate Matter** (PM) and **Gaseous contaminants**, including **Volatile Organic Compounds** (VOCs).

Particulate matter are further categorized according to size. **Coarse particles**, between 10  $\mu\text{m}$  to 2.5  $\mu\text{m}$  in diameter are called  $\text{PM}_{10}$ , **fine particles** ( $\text{PM}_{2.5}$ ) are 2.5  $\mu\text{m}$  or smaller, and **ultrafine particles** ( $\text{PM}_1$ ) are 1  $\mu\text{m}$  or smaller. These are particles of various nature and origin that cause many adverse effects on human health. Fortunately, many studies have shown that fibrous media portable air purifiers, using technologies like High-Efficiency Particulate Air (HEPA) filters, can drastically reduce PM exposure, with efficiencies depending on particle size and filter type (Ref.).

Gas-phase pollutants can be divided into two groups: **Inorganic** like  $\text{SO}_2$ ,  $\text{NO}$ ,  $\text{NO}_2$ ,  $\text{CO}$ ,  $\text{O}_3$  and **organic**. These volatile organic compounds (VOCs) are among the most harmful indoor air pollutants, and include Formaldehyde (HCHO), Benzene, Toluene, Ethylbenzene, Xylene (BTX) and Polycyclic Aromatic Hydrocarbon (PAHs).

These VOCs can come from **outdoor pollution**, like car exhaust or industrial activities and are found in higher concentration in cities (Ref.). But their main sources are located **inside our homes**. They are continuously emitted by building materials, engineered wood products, architectural coatings, solvents, paints, household products like cleaning chemicals or deodorisers, and human activities like cooking or smoking (Ref.). **This causes indoor air to be on average 5 times more polluted than outdoor.**

Chronic exposure to these VOCs, even at low concentrations found typically indoors, can cause a variety of **adverse health effects**. They are known to cause allergies, eye and throat irritation (Ref.), aggravated asthma symptoms (Ref.), and even cancers in some cases (Ref. Ref.). These effects are especially pronounced in vulnerable groups like children (Ref.) and the elderly (Ref.).

The impact of indoor VOCs is not limited to physical health, but also impairs **cognitive function**, significantly reducing problem-solving abilities at work, with drastic effect of information usage and strategy (Ref.). This impacts both individuals and organizations and illustrates the extent of the negative effects of VOCs in our lives.

At the heart of the VOCs problem, in contrast with PM, is our inability to effectively purify them using traditional air purifiers. More on that later. The only two ways to partially mitigate them today is to **1) limit the presence** of VOC emitting substances, by buying low VOC versions of some products and **2) ventilate living spaces** by opening windows (Ref. Ref. Ref.). The first solution is rarely an option, as many VOC emitting products do not have low VOC alternatives widely available. The second solution remains unsatisfactory, as opening windows is not always practical in many climates and some buildings. It would also require a dozen ventilation sessions per day, as these VOCs are often continuously and constantly emitted from various indoor sources (Ref.).

## The limits of air purifiers

As knowledge and awareness of **the negative impact of low air quality** has increased over the past decades, so has the efforts to find practical solutions. One of these solutions is using portable air purifiers, evidenced by the drastic increase in public demand over the past few years and the expected market growth of this segment (Ref.).

The technologies behind portable air purifiers vary according to the type of pollutants addressed, with **HEPA filters** being the most successful at targeting PMs. But **this technology cannot filter out VOCs**, as

these compounds usually do not exceed 1 nanometer in diameter, a thousand times smaller than PM<sub>1</sub>, HEPA filters operating at the micrometer scale.

To address VOCs, many technologies have been developed, powering new generations of portable air purifiers. Some of these technologies have interesting properties, all have significant drawbacks, and in some cases can lead to more pollution being generated than remediated, as stated in the Environmental Protection Agency (EPA) [technical summary](#) (Table 1).

Air-cleaning technology	Targeted indoor air pollutant(s)	Mechanism(s) of action	Advantages	Disadvantages
Fibrous filter media	Particles	<p>Collection: Filter fibers capture particles</p> <ul style="list-style-type: none"> <li>- Mechanical filtration media rely on mechanical forces alone</li> <li>- Electrostatically-charged (i.e., «electret») media use mechanical fibers with an electrostatic charge applied to collect oppositely charged particles, enhancing removal efficiency</li> </ul>	<ul style="list-style-type: none"> <li>- If rated efficiency is high, they can have excellent removal capabilities for many particle sizes</li> <li>- Mechanical media filters see improved efficiency with loading</li> </ul>	<ul style="list-style-type: none"> <li>- Regular replacement is required</li> <li>- Used particle filters can be a source of sensory pollution/odors</li> <li>- High pressure drops on some fibrous media filters can negatively impact HVAC systems</li> <li>- Electret media filters see reduced efficiency with loading</li> <li>- Confusing number of test standards and rating metrics</li> </ul>
Electrostatic precipitation (ESP)	Particles	<p>Collection: Corona discharge wire charges incoming particles, which collect on oppositely charged plates</p>	<ul style="list-style-type: none"> <li>- Can have high removal efficiency for a wide range of particle sizes</li> <li>- Low pressure drop and minimal impacts on HVAC systems</li> <li>- Low maintenance requirements</li> </ul>	<ul style="list-style-type: none"> <li>- Sometimes ESPs have high ozone and nitrogen oxide generation rates</li> <li>- Efficiency typically decreases with loading and plates require cleaning</li> <li>- High electric power draw requirements</li> </ul>
Ionizers (i.e., ion generators)	Particles	<p>Collection: Similar to ESP, ionizers use a high-voltage wire or carbon fiber brush to electrically charge air molecules, which produces negative ions that attach to airborne particles; the charged particles are then collected either on oppositely charged plates in the air cleaner or become attracted to other surfaces in the room and deposited elsewhere</p>	<ul style="list-style-type: none"> <li>- Typically low power draw requirements</li> <li>- Quiet</li> <li>- Low maintenance</li> </ul>	<ul style="list-style-type: none"> <li>- Generates ozone</li> <li>- Typically low effectiveness because of very low airflow rates and clean air delivery rates (CADRs)</li> </ul>
Ultraviolet germicidal irradiation (UVGI)	Microbes	<p>Destruction: UV light kills/inactivates airborne microbes</p>	<ul style="list-style-type: none"> <li>- Can be effective at high intensity with sufficient contact time</li> <li>- Can be used to inactivate microbes on cooling coils and other surfaces</li> </ul>	<ul style="list-style-type: none"> <li>- Uncoated lamps can generate ozone Potential for eye injury</li> <li>- Effectiveness increases with lamp intensity, which is typically low in residential UVGI air cleaners</li> <li>- High electrical power draw requirements</li> <li>- Inactivates but does not remove microbes</li> </ul>

Adsorbent media	Gases	Collection: Gases physically adsorb onto high-surface-area media (typically activated carbon)	<ul style="list-style-type: none"> <li>- Potential for high removal efficiency for many gaseous pollutants in air cleaners with a sufficient amount of media for the application</li> <li>- No byproduct formation</li> </ul>	<ul style="list-style-type: none"> <li>- Regular replacement is required because its adsorption capacity is exhausted and physical adsorption is a reversible process, meaning pollutants may not be permanently captured</li> <li>- Effectiveness of many consumer-grade systems with small amounts of activated carbon is unknown</li> <li>- High pressure drops on some sorbent media filters can negatively impact HVAC systems</li> <li>- Different removal efficiency for different gases at different concentrations</li> <li>- Standard test methods are not widely used</li> </ul>
Chemisorbent media	Gases	Collection: Gases chemically adsorb onto media coated or impregnated with reactive compounds	Potential for high removal efficiency for many gaseous pollutants Chemisorption is an irreversible process, meaning pollutants are permanently captured	<ul style="list-style-type: none"> <li>- Regular replacement is required because its chemisorption capacity is exhausted</li> <li>- Effectiveness of many consumer-grade systems is unknown</li> <li>- High pressure drops on some sorbent media filters can negatively impact HVAC systems</li> <li>- Different removal efficiency for different gases at different concentrations</li> </ul>
Catalytic oxidation	Gases	Conversion: Most utilize photocatalytic oxidation (PCO) in which a high-surface-area medium is coated with titanium dioxide as a catalyst; incoming gases adsorb onto the media and UV lamps irradiate and activate the titanium dioxide, which reacts with the adsorbed gases to chemically transform them	<ul style="list-style-type: none"> <li>- Can degrade a wide array of gaseous pollutants (e.g., aldehydes, aromatics, alkanes, olefins, halogenated hydrocarbons)</li> <li>- Can be combined with adsorbent media to improve effectiveness</li> </ul>	<ul style="list-style-type: none"> <li>- Can generate harmful byproduct such as formaldehyde, and acetaldehyde, and ozone</li> <li>- No standard test methods</li> <li>- Often relatively low removal efficiency for many indoor gases, but high variability in removal for different gases</li> <li>- Lack of field studies to validate performance</li> <li>- Catalyst often has a finite lifespan</li> </ul>
Plasma	Gases	Conversion: Electric current is applied to create an electric arc; incoming gases are ionized and bonds are broken to chemically transform the gaseous pollutants	<ul style="list-style-type: none"> <li>- Can have high removal efficiency</li> <li>- Can be combined with other air-cleaning technologies (e.g., PCO) to improve performance and minimize byproduct formation</li> </ul>	<ul style="list-style-type: none"> <li>- Wide variety of plasma generation types yields confusion on how a product actually works</li> <li>- Byproducts are formed from many plasma technologies, including particles, ozone, formaldehyde, carbon monoxide, chloroform, nitrogen oxides, and a large number of other organic gases</li> <li>- Most studies have investigated gaseous removal while fewer have evaluated particle removal</li> </ul>

Intentional ozone generation	Gases	Conversion: Intentional generation of ozone using corona discharge, UV, or other method to oxidize odorous compounds and other gases	- Reacts with many indoor gases - Can be combined with other less- harmful technologies such as adsorbent media	- High ozone generation rates - High amounts of byproduct formation - Can cause degradation to indoor materials
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**Table 1:** Summary of air-cleaning technologies. Adapted from table 1 of the EPA residential air cleaners technical summary. EPA 402-F-09-002 | July 2018 | EPA Indoor Environments Division.

Air purifiers claiming to remediate VOCs use one, or more, of the 4 main technologies addressing gasses: Catalytic oxidation, plasma, ozone generation and adsorbent media.

### Catalytic oxidation

Also called **photocatalytic oxidation (PCO)**, this technology is used in many recent air purifiers and is based on the activation of compounds such as titanium dioxide by photons, usually in the UV range. These now activated catalysts are able to oxidize air molecules they come in contact with. The most concerning drawback of PCO is its lack of specificity: in some cases, **air cleaners based on these technologies can generate very harmful byproducts, including formaldehyde**. For example, the oxidation of ethanol produces acetaldehyde, a type 2B carcinogen (Ref.)

### Plasma

Plasma air purifiers apply high-voltage discharges to ionize incoming gasses, breaking their chemical bonds. They can have high removal efficiency, specifically against VOCs, but **may form a number of harmful byproducts**, such as carbon monoxide, ozone and formaldehyde.

### Ozone generation

**The generation of ozone indoors is harmful**, as it is a strong lung irritant. The EPA report states “Do not use ozone generators sold as air cleaners in occupied spaces. No federal agency has approved ozone generators for use in occupied spaces.”

### Adsorbent media

More often than not, the media in question is **activated carbon**, aka activated charcoal. This material has the interesting property of possessing a large amount of micropores or cavities, highly increasing its surface area. These cavities trap various chemical compounds, which makes it a popular solution for water and air purification for various pollutants. Its efficiency depends on many factors, including the type of VOC and the air cleaner design. But in all cases, the biggest drawback comes from the fact that **the VOCs are only adsorbed on the surface of activated carbon**. This leads to relatively quick saturation of the media, and can cause the release of these pollutants back into the indoor air in certain conditions, like heat, or if filters are not changed.

## Plants as natural air purifier

In 1989, **NASA** launched a research project, led by Wolverton, to study biological life support systems for space travel (Ref.). The results of this study suggests that various plants, and their associated microorganisms, have interesting removal properties for some VOCs. This sparked widespread public perception that potted plants positively impact indoor air quality, fueled by scores of articles about the **“best plants to**

**purify the air in your home”.** But, contrary to popular belief, while the plant’s ability to take up some VOCs is well documented in laboratory studies, the effect of regular potted plants on indoor air in complex environments, like a bedroom, is not as clear cut (Ref.). **Dozens of potted houseplants would be needed to have an impact in a single room, which is not practical.**

## Synthetic Biology and Neoplants

Faced with the inadequacies of traditional mechanical air purifiers, and the inefficiency of potted plants, people that would like to live in a healthy indoor environment do not have a real solution. At Neoplants, we believe in leveraging the power of Nature to create sustainable solutions to difficult problems. We do this through a groundbreaking type of technology, merging engineering and biology, called Synthetic Biology, or SynBio.

Using a combination of molecular biology, plant physiology, metabolism and microbiome engineering, we create bioengineered plants that can effectively fight air pollution. Giving Nature the ability to remediate the pollution caused by petro-chemical industries that ends up in our lungs in the form of VOCs. Our first product, Neo P1, is designed to fight against the most prevalent, harmful and difficult to target indoor air VOCs: Benzene, Toluene, Ethylbenzene and Xylene (BTX) and Formaldehyde.

BTX is a class of VOCs that have in common a very stable 6 carbon benzene ring. These compounds are constantly present in indoor air and the chronic exposure to them can cause a wide range of health issues, from fatigue, headaches, nausea and skin irritation, to more serious blood, central nervous system and upper respiratory tract problems (Ref. Ref. Ref. Ref.). Formaldehyde, a single carbon molecule, is a highly reactive indoor VOC that can cause irritation of the skin, eyes, nose and throat, and even cancer in some extreme cases (Ref.).

# Neo P1, the first bioengineered houseplant

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## General approach

### Nature as a depollution powerhouse

At its core, the problem we are tackling is simple: **how can we maximize the VOC depollution capacity of houseplants** so they have a positive impact on the indoor air quality of a room. However, not relying on electricity to power our technology is a major design constraint we have chosen to give ourselves. It’s an engineering problem, but one that should use biology and not traditional technology as a solution, turning it into a bioengineering problem.

We conceptualize it as a flux optimization issue: **optimizing the flux of VOC into the plant.** In normal plants, this VOC flux stops as soon as the plant reaches certain levels of accumulated intracellular VOC. To avoid this accumulation problem, Neo P1 metabolizes VOCs, just like it naturally does with CO2. We achieved this by **inserting synthetic metabolic pathways** that allow Neo P1 to use VOCs as carbon sources used in its normal cellular metabolism, creating more plant matter. This plant matter, in turn will absorb more VOCs, creating a solution that is sustainable by design.

# Metabolism

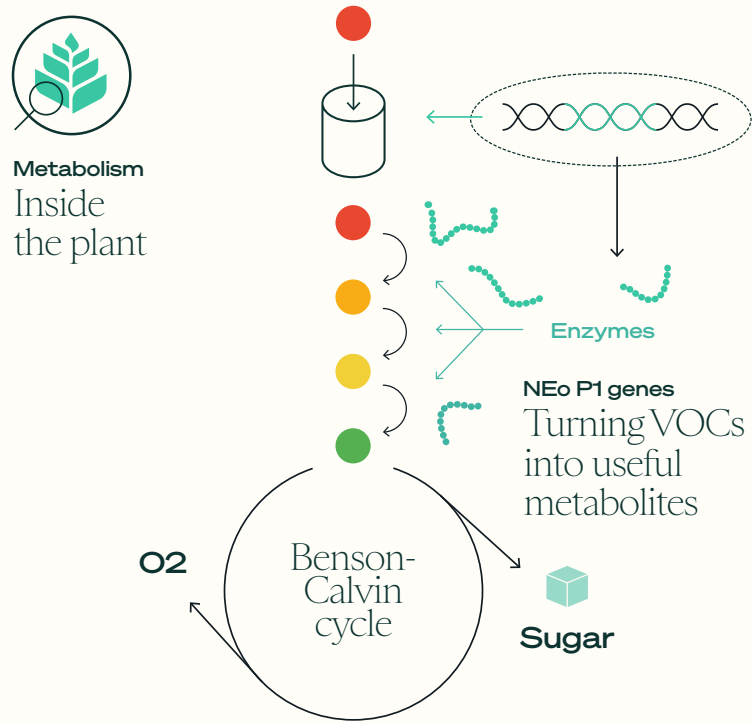


Figure 1: General scheme of the metabolism

The plant itself is only part of the solution, as no plant lives alone, but with a community of billions of beneficial fungi and bacteria, forming a microbiome that helps that plant thrive. This microbiome, mostly located in the soil near the plant root system, also has great potential to remediate VOCs. Therefore, **we engineered this community of bacteria**, using directed evolution, to maximize the depollution efficiency of the biological system we call a “neoplant”.

# Microbiome

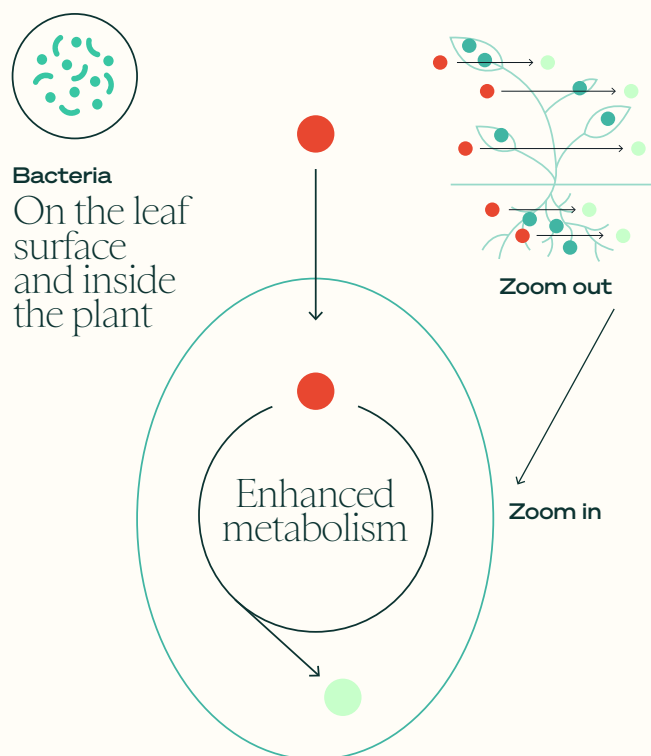


Figure 1: General scheme of the microbiome

The soil of Neo P1 is also customized to improve this depollution efficiency, using Powerdrops in our formulation. Finally, Neo P1 comes in a planter we call **“the shell”**, made with bioplastics and specifically designed to maximize passive air fluxes on the neoplant and its soil.

### Choosing a host plant

Choosing the right initial plant to host our bioengineering technologies in order to create Neo P1, was crucial. We chose **Epipremnum aureum**, commonly known as **pothos**, because of its physiological characteristics, making it a perfect candidate to be transformed into a neoplant.

- It has good **phytoremediation potential**. With its big waxy leaves and high growth rate, it absorbs a lot of VOC per unit of mass. It also naturally possesses genes coding for endogenous enzymes that have the potential of relediating some VOCs, although these genes are expressed at a low level.
- It's a **good houseplant**. Being very robust in various indoor conditions, requiring low maintenance, and being a perennial plant, can survive and grow continuously for decades. On top of this, the fact that it does not produce pollen or seeds ensures it will not spread in the wild.

### The “Shell” planter design

The planter of Neo P1, called the “Shell” is designed with two objectives in mind: **to maximize fluxes** coming in contact with Neo P1, as previously stated, but also **to drastically simplify the maintenance of the plant**. The Shell comes with its wooden stand and is constituted of four detachable modules: the outer layer, the water tank, the water level indicator and the inner basket. At the crossroads of organic and technical lines, the Shell is designed to maximize the air exchange between the soil and the room. Made of recyclable bio plastics, it will be a thoughtful, functional and elegant addition to interior spaces.



Figure 2 Various views of The Shell from different angles

The air flow between the outer layer and the inner basket is optimized thanks to openings located at the top and bottom of the planter. We also chose the height of the stand to maximize the impact of the neoplant in a room. The outer layer contains a water tank and a water level indicator, so that Neo P1 only needs to be watered once every 2 to 3 weeks. The basket holds the plant and keeps the soil hydrated thanks to capillary transfer of water through two small ropes running from the water tank to the soil. **The materials of the Shell are eco friendly bioplastics, and do not emit any harmful VOCs.** Finally, it does not contain any electronic compounds, does not consume electricity, does not require any filter or battery changes, all to minimize Neo P1's footprint on the planet, while maximizing its positive impact on the air we breathe.



# Microbiome engineering

## General concept

The objective of our **microbiome engineering efforts** is to customize the composition of the Neo P1's microbial ecosystem to maximize its VOC depollution efficiency. To choose the right microbial strains, we tested dozens of carefully selected bacteria and fungi that can use different VOCs as carbon sources. Two strains exhibited particularly interesting properties, the first can use formaldehyde as a sole carbon very efficiently, and the other does the same for the BTX pollutant class. These organisms are harmless to humans and animals, and are beneficial to the health of plants.

Their innate ability to use VOCs as carbon sources is encoded by genes, themselves translated into **enzymes** catalyzing biochemical reactions. These reactions form metabolic pathways that turn VOCs into harmless microbial metabolites such as sugar in the case of formaldehyde and amino acids in the case of BTX. These are used as carbon sources by the bacteria to grow, thrive and even help the plant to do the same.

**We then evolved these naturally occurring microbes to further increase the efficiency of their VOC metabolism through lab assisted artificial evolution, also called directed evolution.**

Lastly, we maximized the ability of these microbes to form long term interactions with the plant, thus creating a stable microbiome that keeps its VOC degrading efficiency at 100% for several weeks.

## Directed Evolution

Directed evolution is a step by step process that selects for, and enhances, the VOC degrading properties of microbes. A few milliliters of culture contains hundreds of billions of bacteria, some harboring tiny differences in genetic sequences, a subset of which will, by random chance, code for a slightly **increased efficiency in VOC metabolism**. We introduce in this culture a concentration of liquid VOC that is slightly higher than the initial tolerance level of these bacteria. The very few cells that survive are those that harbor the genetic code for this slightly increased VOC remediation efficiency. We then multiply those bacteria to recreate a population size of hundreds of billions of slightly enhanced organisms. We repeated this cycle dozens of times over 3 years to create organisms highly evolved for bioremediation of VOCs.

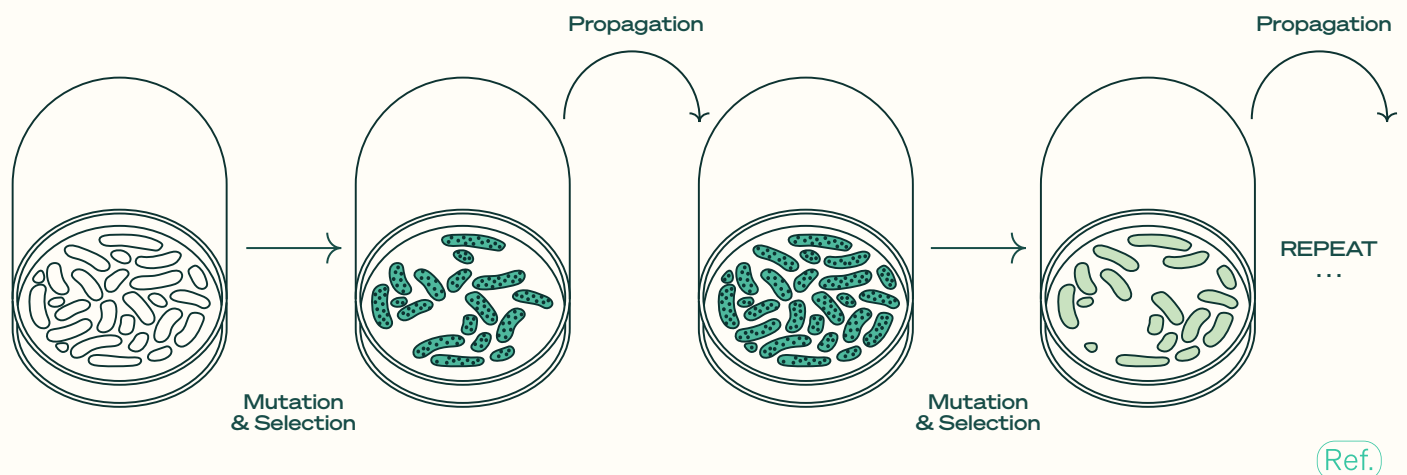
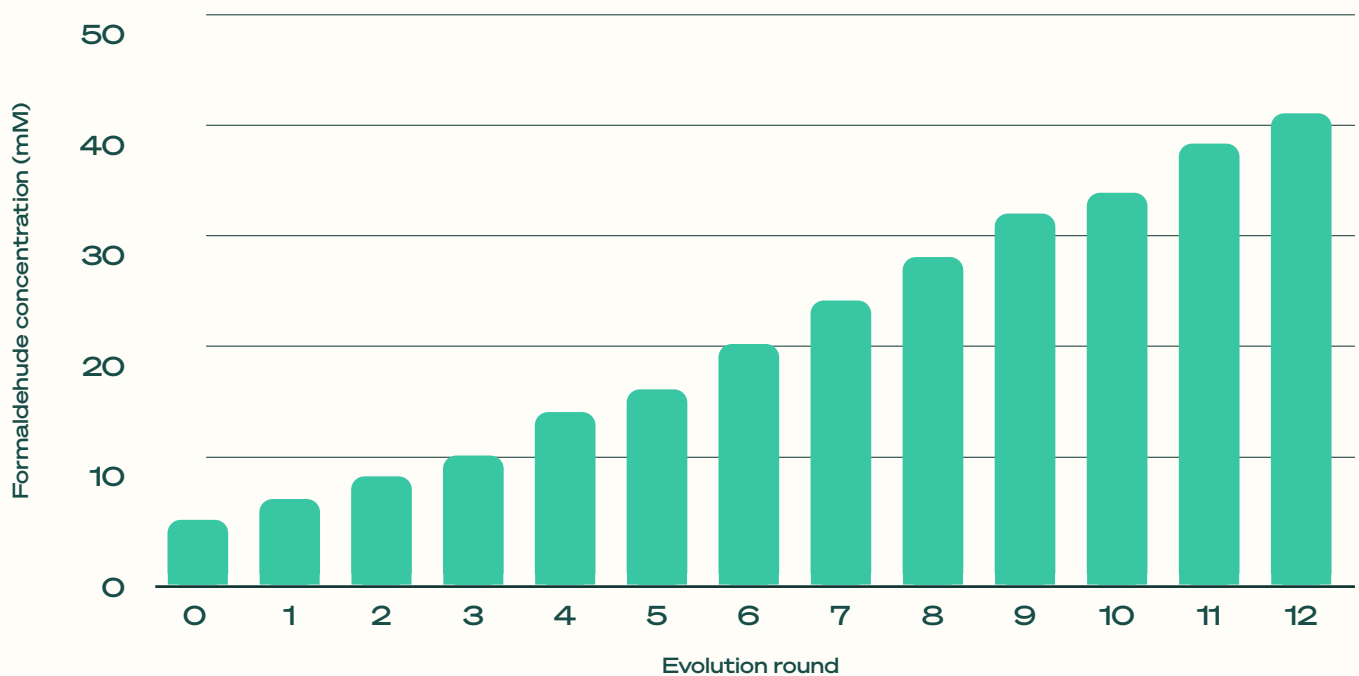


Figure 4: General directed evolution scheme

## Targeting Formaldehyde

To metabolize formaldehyde, we selected a strain of **Methylobacterium extorquens** bacteria that showed promising formaldehyde bioremediation capacities when inoculated on *Epipremnum aureum*. It's a rhizosphere and phyllosphere bacteria, meaning **it lives both in the soil and on the leaves of plants**. We drastically increased its ability to remediate formaldehyde in vitro, through directed evolution, by exposing it to many rounds of increasing formaldehyde concentration over a 3 year period. This leads to an increase in formaldehyde tolerance (figure 5).

### Increase of *Methylobacterium extorquens*' formaldehyde tolerance along rounds of directed evolution



**Figure 5:** Maximum tolerance level, in millimolar, of *Methylobacterium extorquens* to liquid formaldehyde across 12 rounds of directed evolution.

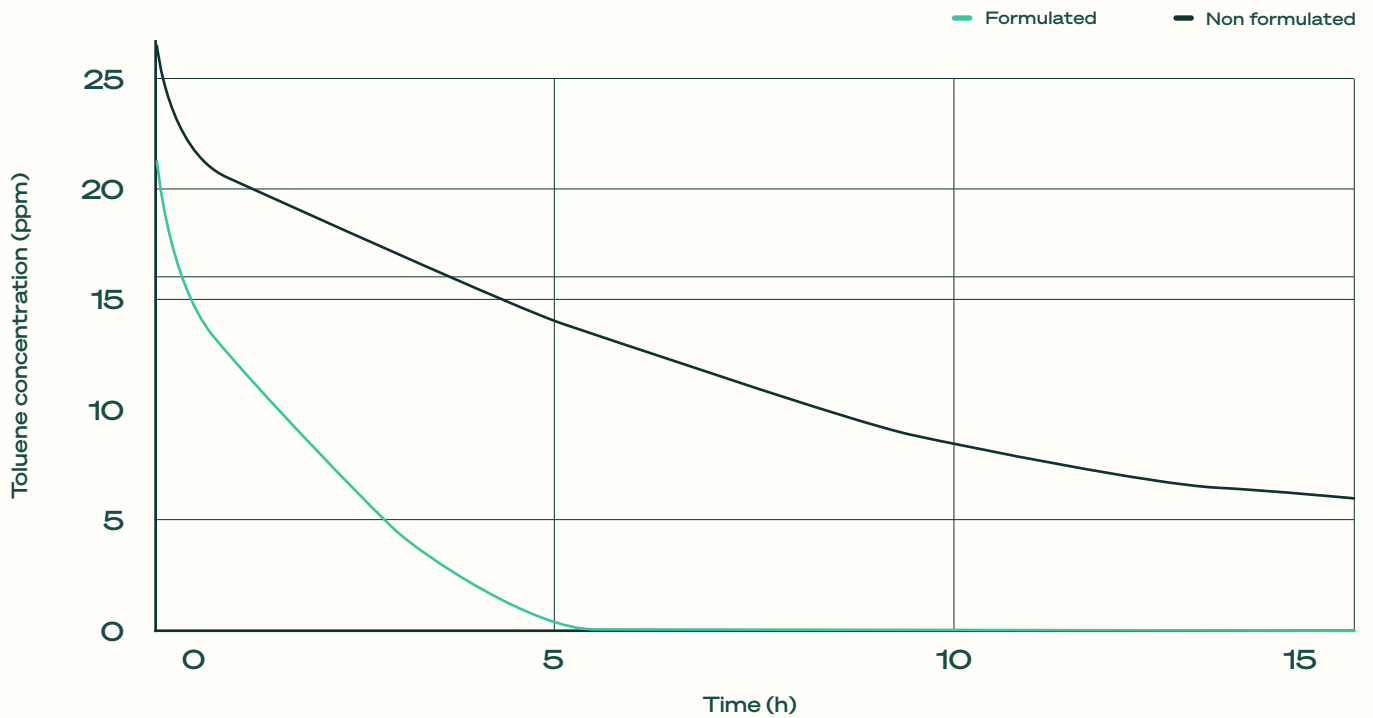
We then maximized its stability on/in the plant by optimizing the composition of its formulated media as well as the soil composition of Neo P1

## Targeting BTX

Using the same method to target BTX pollutants, out of the dozen microbes tested, **we selected a strain of *Pseudomonas putida* bacteria**. When tested on our plant in our lab, it showed the highest bioremediation efficiency against this class of pollutants. This BTX degrading strain is a rhizosphere bacteria, living in and around the roots of plants. During the same 3 year period, it underwent dozens of rounds of directed evolution with ever-increasing levels of toluene and benzene. This was done through automated processes in our lab, designed to further increase the strain's bioremediation potential when inoculated on *Epipremnum aureum*.

Similarly, this BTX-remediating strain's formulation was optimized to form a long lasting microbe-plant interaction in the soil and root system of our neoplant

## Degradation of toluene by *Epipremnum aureum* with or without optimised formulation, 4 Weeks Post-inoculation



**Figure 6:** Gaseous toluene degradation by *Epipremnum aureum* inoculated with *Pseudomonas putida* in formulated (green), and unformulated (gray) conditions, 4 weeks after inoculation.

### Enhancing VOCs degradation using the Powerdrops

Our Powerdrops contain lyophilized cells of our *Methylobacterium extorquens* and *Pseudomonas putida* engineered strains, as well as everything they need to thrive in the soil of Neo P1 and keep their populations high. These bacteria are also known to interact closely with the plant, mostly within its rhizosphere, where they exchange chemical compounds including sugars, minerals, nitrogen, and some pollutants. Hence, both the plant and its microbiome participate in the degradation of VOCs.

Each bacteria has different metabolic needs, which we addressed by creating two different formulations, tailored to (1) maintain the growth of the bacterial populations, (2) stimulate the metabolism of formaldehyde and BTX, and (3) maximize the shelf life of the Powerdrops.

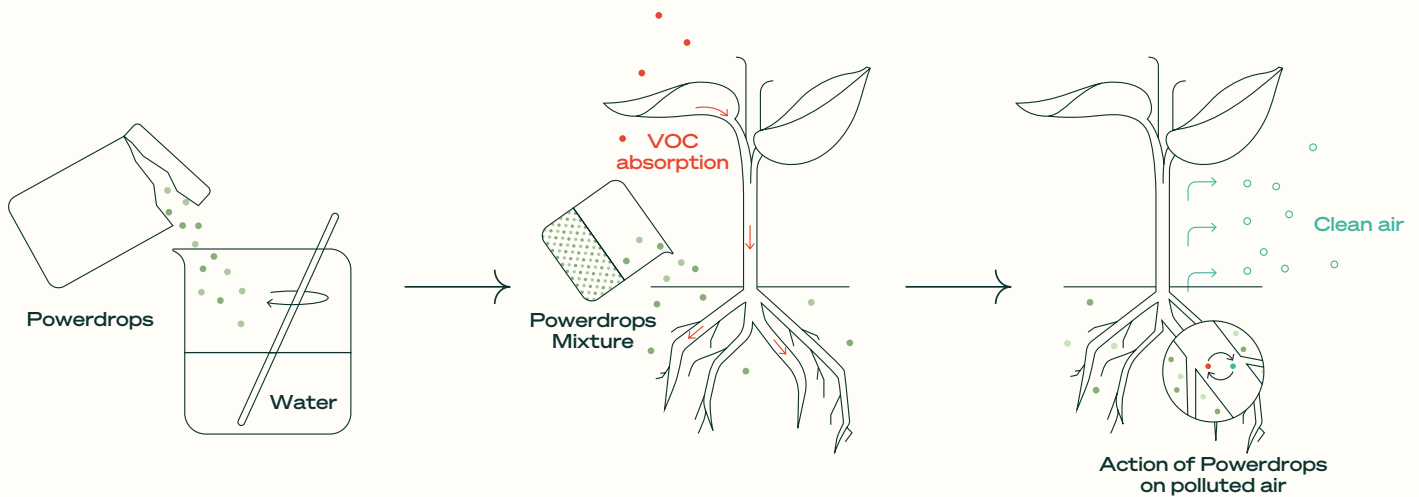


Figure 7: General representation of the symbiotic relationship between Neo P1 and its microbiome.

Ref.

## Metabolism engineering

### General concept

Microbiology is by no means the only way we bioengineered Neo P1. Our main technology pertains to the bioengineering of the plant itself. We increased the phytoremediation of *Epipremnum aureum* through metabolism engineering, by **inserting synthetic metabolic pathways** to enable the plant to use the VOCs it absorbs as carbon sources, drastically increasing its depollution efficiency. These pathways are constituted by enzymes catalyzing reactions turning VOCs into harmless plant metabolites that the plant naturally integrates into its endogenous metabolism, as it does with CO<sub>2</sub>.

To select the most performant system, **we created and tested hundreds of DNA constructs coding** for dozens of enzymes and several different pathways in plants. We then selected two distinct pathways: a formaldehyde assimilation pathway, turning this VOC into sugars; and a BTX assimilation pathway, turning them into muconic acid. We inserted each pathway into a separate line of *Epipremnum aureum*. Neo P1 actually contains two types of plants, one specialized for the phytoremediation of formaldehyde, and the other for BTX.

### Designing VOC-degrading DNA

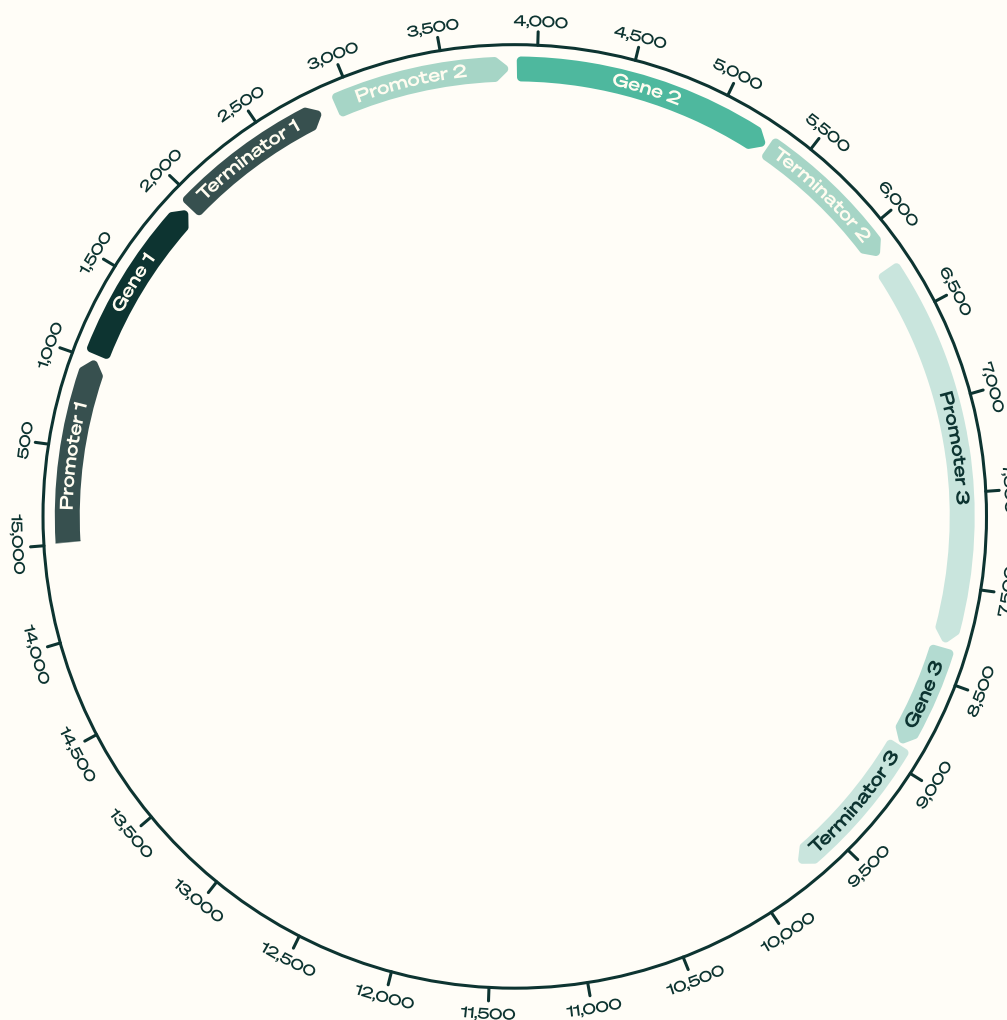
To create those DNA constructs, two classes of DNA elements are needed: genes, and non-coding regulatory elements. Both are sequences of DNA bases, of which there are 4: A, T, C and G; analogous to the 0-1 binary system used in computer softwares.

Genes are DNA sequences that, when expressed, can be transcribed into RNA, and then into proteins. The class of proteins that can catalyze biochemical reactions are called enzymes. Neo P1 contains enzymes that catalyze reactions turning VOCs into molecules that the plant can use to build whatever it needs to live and grow.

But these genes are only expressed if they are flanked by other specific DNA elements called non-coding regulatory elements. These are promoters, located upstream of, or “before”, the gene and terminators, located downstream of, or “after” the gene. These elements do not code for any protein, but they determine the level of expression of the gene flank. Having strong promoters and terminators flanking a gene

induce the transcription of high amounts of RNA and thus the production of high amounts of enzymes, leading to a highly active metabolic pathway.

Using molecular biology approaches in the lab, we stack many promoter-gene-terminator combinations together, forming what we call a DNA construct. We then circularized this construct to create a DNA plasmid (figure 8). This plasmid can, in turn, be incorporated into the genome of a plant by a process called transformation.



**Figure 8:** Visual representation of a 15,000 DNA base long plasmid containing a stack of 3 promoter-gene-terminators.

### Transforming *Epipremnum aureum*

Once the DNA plasmid is built, it is inserted into the plant's genome. This process is called "transformation" and is done through one of two methods: **biolistic DNA bombardment, or agrobacterium-mediated gene insertion**. The former is a procedure where we use a biolistic particle delivery system, or "gene gun" with DNA-coated gold particles to deliver the plasmid to the cells. The gold beads harboring our plasmid are literally shot into the plant tissue, penetrating the cell walls, plasma membranes, nuclear membranes and integrating the DNA inside the plant's genome. The latter is an indirect system where we first insert the plasmid into a bacteria called *Agrobacterium tumefaciens* by electroporation. This bacteria then infects *Epipremnum aureum* by inserting that DNA construct into the plant cells, allowing for relatively high transformation efficiencies. At Neoplants, we use both transformation methods.

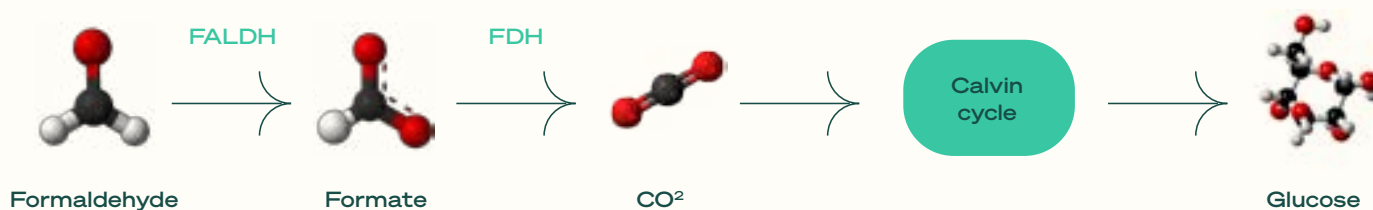
Once inserted into the genome of a plant, a DNA construct is read by the plant cell machinery as if it was its own, expressing whatever proteins the genes of that construct code for. In the case of Neo P1, we inserted constructs coding for VOC degrading enzymes forming synthetic metabolic pathways.

### Targeting Formaldehyde

To remediate formaldehyde, our metabolic engineering efforts were aimed at **oxidizing it into formate, and then into CO<sub>2</sub>**. This was done thanks to the combination of two enzymes: the formaldehyde dehydrogenase and the formate dehydrogenase.

Formaldehyde dehydrogenase (FALDH) is present in many organisms, but few plants. It does exist in *Epipremnum aureum*, explaining the natural phytoremediation properties of this plant, but is expressed at very low levels. To increase the expression level of that protein, we inserted a second copy of FALDH in our plant's genome, flanked with much stronger promoters and terminators. **This leads to much higher flux of formaldehyde into formate.**

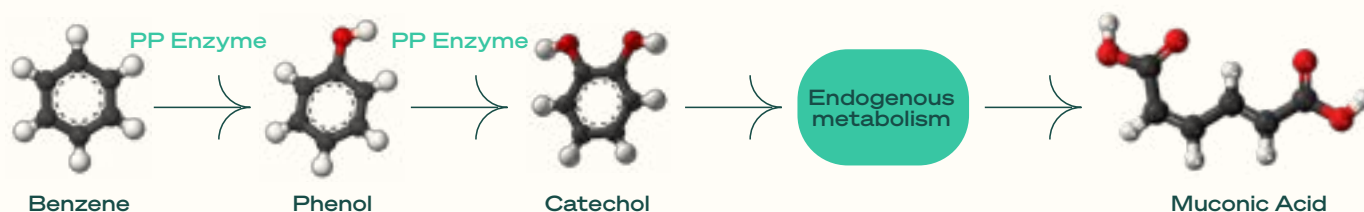
On the other hand, the formate dehydrogenase (FDH) coding gene that we inserted into Neo P1's genome does not come from *Epipremnum aureum*, but from an extremophile bacteria. That version of this enzyme is highly active, effectively turning formate into CO<sub>2</sub>, avoiding the accumulation of intracellular formate. That CO<sub>2</sub> molecule is then incorporated into the Calvin cycle, producing sugars (figure 9)



**Figure 9:** Simplified visual representation of the formaldehyde remediation pathway in Neo P1. The enzymes added are represented in green.

### Targeting BTX

To degrade BTX, a series of **two hydroxylation reactions** are needed, turning these VOCs into phenolic, then catecholic compounds. This pathway is composed of a single gene catalyzing these two reactions. This gene has been isolated from our own microbial strain of *Pseudomonas putida* that we use in our BTX degrading microbiome. By designing and inserting a DNA containing this gene in *Epipremnum aureum*, we gave our plant the completely new ability to turn these pollutants into compounds that can be integrated into its endogenous metabolism, creating harmless molecules, such as muconic acid (figure 10)

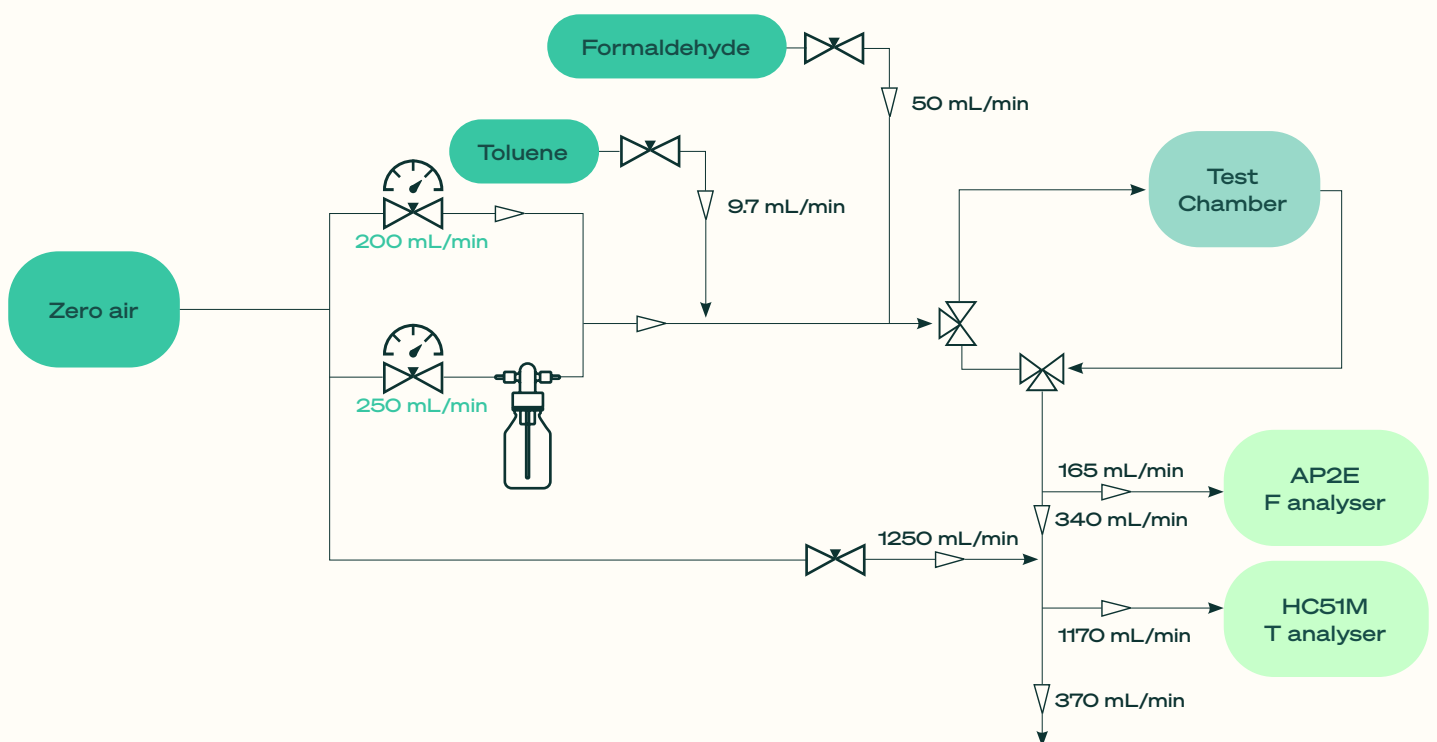


**Figure 10:** Simplified visual representation of the BTX remediation pathway in Neo P1 using benzene as an example. The enzyme added is represented in green.

# Phytoremediation capacity of Neo P1

## Experimental Procedure

In order to assess **the capacity of plants to capture VOCs** we designed an experiment where plants are placed in a 35L glass chamber under continuous flow of formaldehyde at around 500 ppb (parts per billion) and Toluene at around 750 ppb. The output gas is measured by a specific analyser per gas (**AP2E Ref.** for formaldehyde & **HC51M Ref.** for Toluene). The tests were designed and run in collaboration with Ecole des Mines-Télécom of Université de Lille, one of the leading institutes on indoor air quality in France. (figure 11).



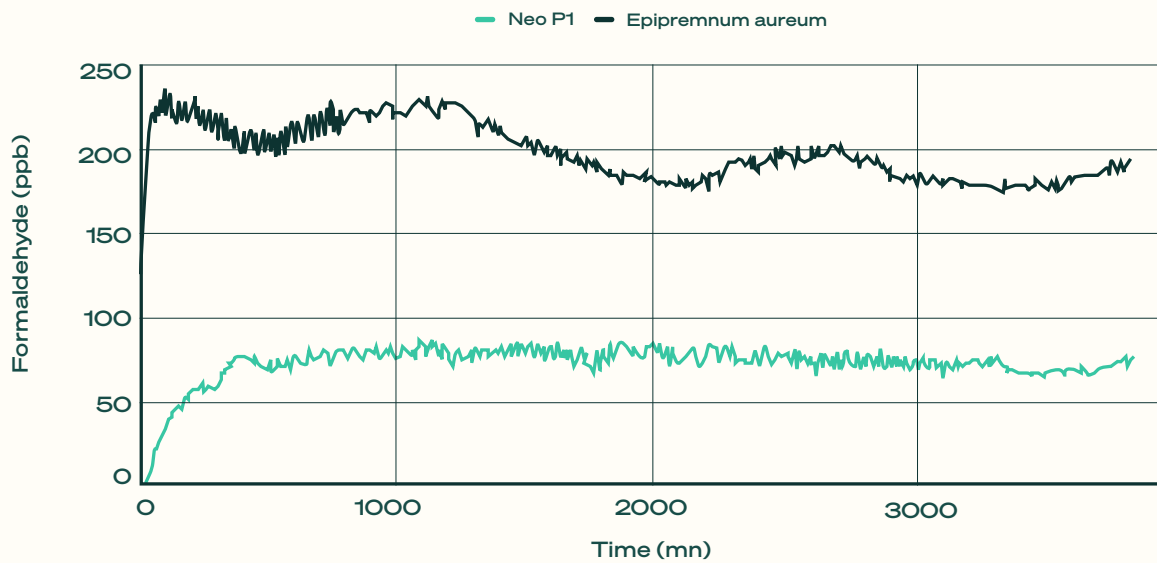
**Figure 11:** Visual representation of an experimental setup used to test the performance of Neo P1

The system was tested to be leakage proof, and the air exchange rate of the empty chamber is of 0,65L/H. The plants were tested over a period of 3 days for Toluene and 1 day for formaldehyde to **calculate the Clean Air Delivery Rate (CADR) normalized by plant weight**. CADR being a common metric used by traditional mechanical air purifiers.

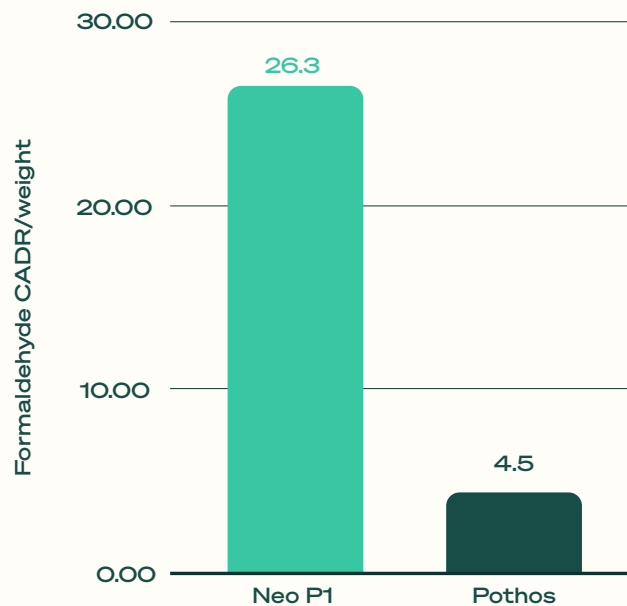
## Increased Phytoremediation of Neo P1:

By continuously measuring the concentration of formaldehyde of the outlet gas after a single passage through the test chamber, we observed a stabilization around 190ppb for the non-enhanced *Epipremnum aureum* and 75ppb for Neo P1. In this context, we calculated the CADR of both plants and normalized them by their weight to avoid bias due to the size of the plant. **CADR/weight of Neo P1 is 26.3 compared to 4.5 for the non-enhanced Epipremnum aureum (figure 12).**

## A) Formaldehyde degradation by Neo P1



## B) Neo P1 Formaldehyde Phytoremediation

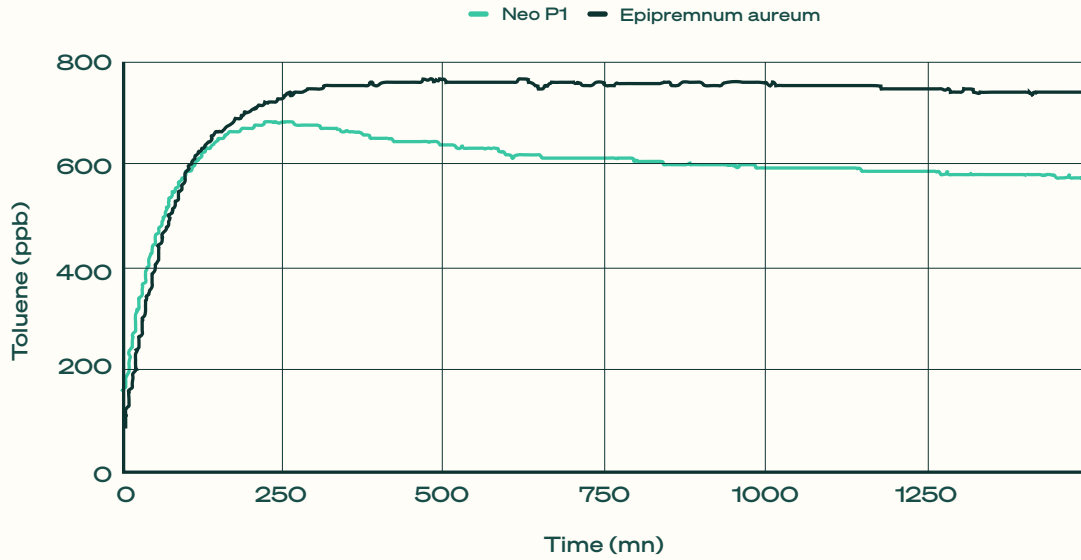


**Figure 12:** A) Formaldehyde outlet concentration after single passage through the test chamber containing either Neo P1 (green) or non-enhanced *Epipremnum aureum* (gray) B) Formaldehyde CADR (clean air delivery rate) of Neo P1 (green) or non-enhanced *Epipremnum aureum* (gray) normalized by plant weight

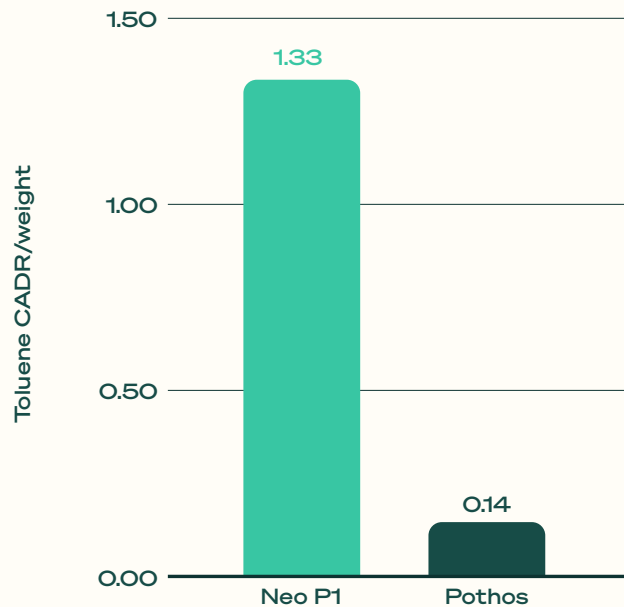
Using the same method, we measured the efficiency of Neo P1 at degrading the BTX class of pollutants, here represented by toluene. We observed a toluene concentration stabilized at 760ppb for the non-enhanced *Epipremnum aureum* and 600ppb for Neo P1. This leads to a toluene calculated CADR/weight of 3.6 for Neo P1 and 0 for *Epipremnum aureum* (figure 13). Even using extremely precise measurement, we could not detect any effect of *Epipremnum aureum* on toluene concentration.



## A) Toluene degradation by Neo P1



## B) Neo P1 Toluene Phytoremediation



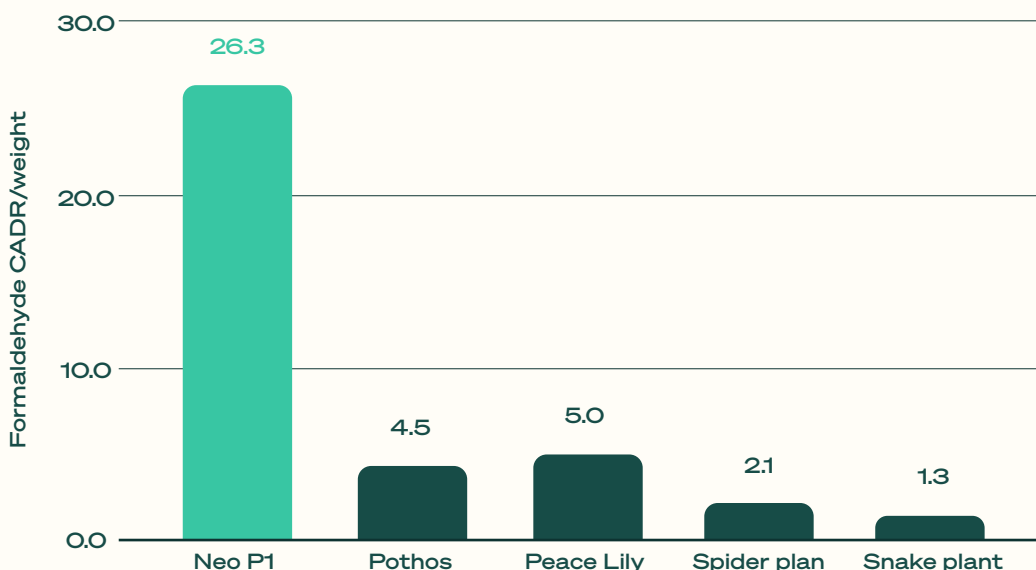
**Figure 13:** A) Toluene outlet concentration after single passage through the test chamber containing either Neo P1 (green) or non-enhanced *Epipremnum aureum* (gray) B) Toluene CADR (clean air delivery rate) of Neo P1 (green) or non-enhanced *Epipremnum aureum* (gray) normalized by plant weight

## Comparing Neo P1 to regular indoor houseplants

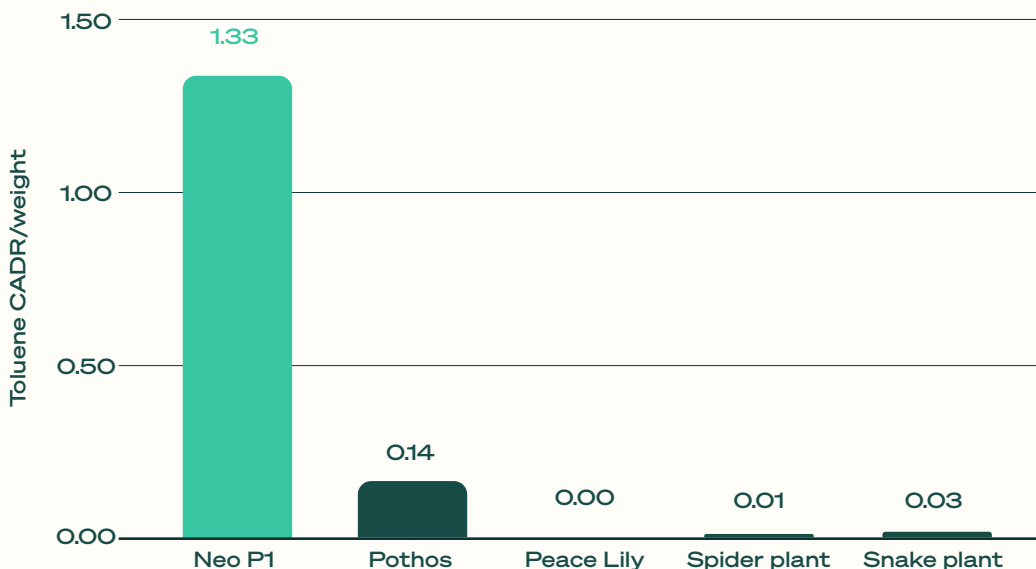
The ability of regular indoor houseplants to clean the air has been extensively talked about in popular articles as well as studied in the scientific literature. The most cited “air cleaning” houseplants are *Epipremnum aureum* (Pothos), *Spathiphyllum* (Peace Lily), *Chlorophytum comosum* (Spider Plant), and *Dra-*

caena trifasciata (Snake Plant). Using the same approach described above, we compared their phytoremediation efficiencies on both formaldehyde and toluene (figure 14).

### A) Formaldehyde Phytoremediation of a Selection of Indoor Plants



### B) Toluene Phytoremediation of a Selection of Indoor Plants



**Figure 14:** Comparison of the phytoremediation efficiencies of a selection of indoor houseplants for Formaldehyde (A) and Toluene (B)

Interestingly, none of the plants tested showed any significant phytoremediation efficiencies on toluene. Showing that, by bioengineering Neo P1, we developed a new phytoremediation ability to degrade the BTX class of pollutants that does not seem to be present in common houseplants.

# Conclusion

Neo P1 is the fruit of several years of synthetic biology efforts, using the latest technologies of microbiome and metabolism engineering, combined with custom designed hardware. It has drastically improved phytoremediation efficiencies on the most harmful and ubiquitous indoor VOCs compared to regular houseplants. It has the potential to improve the health and wellbeing of millions of people worldwide, while being a sustainable solution that has drastically less negative impact on the environment and the planet (figure 15).

	Traditionnal Air Purifiers	House plants	Neo P1
Formaldehyde	✗	⚠	✓
Benzene	✗	✗	✓
Toluene	✗	✗	✓
Xylene	✗	✗	✓
Particulate Matter	✓	✗	✗
Sustainability	✗	✓	✓

Figure 15: Value proposition of Neo P1

Neo P1 is the fruit of several years of synthetic biology efforts, using the latest technologies of microbiome and metabolism engineering, combined with custom designed hardware. It has drastically improved phytoremediation efficiencies on the most harmful and ubiquitous indoor VOCs compared to regular houseplants. It has the potential to improve the health and wellbeing of millions of people worldwide, while being a sustainable solution that has drastically less negative impact on the environment and the planet (figure 15).