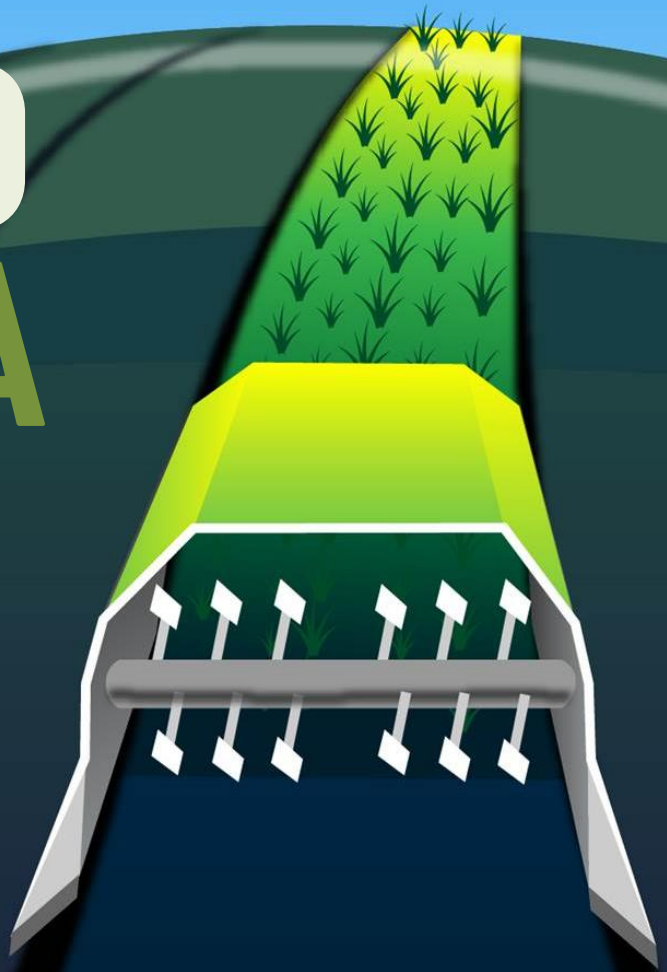
ROBO NOVA

METAGENOMICS AND ENGINEERING OF BIOREMEDIATION PROCESSES



INTERSOL
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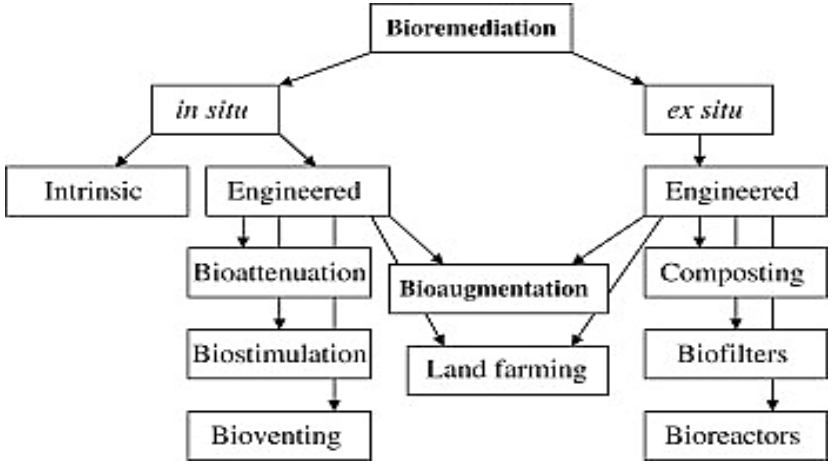
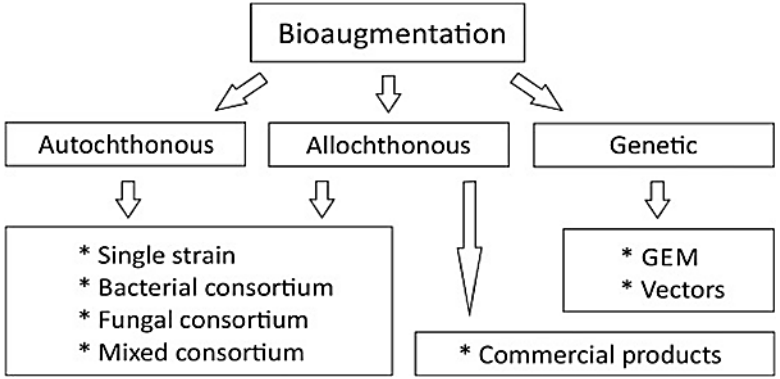
Discover New Directions

- Biological processes for the remediation of contaminated environmental matrices are based on the ability of microorganisms such as fungi and bacteria to transform/degrade, partially or completely, organic contaminants.

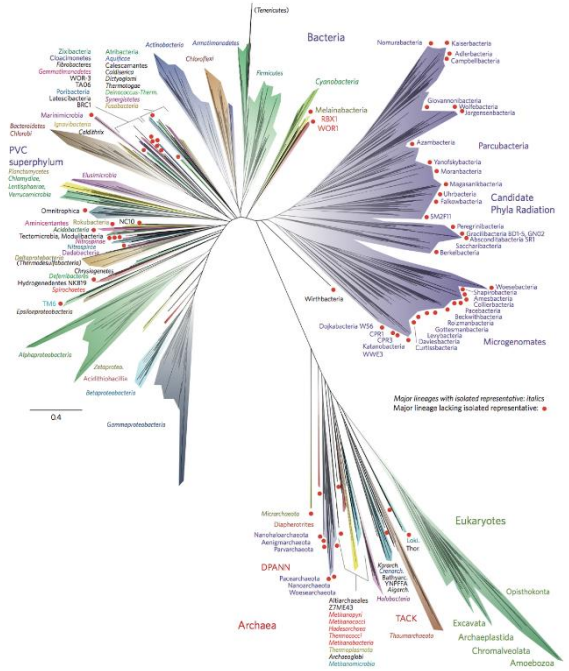
- Microorganisms can promote the recovery of contaminated environments through numerous biochemical mechanisms that can lead to the **complete degradation of the pollutant or its conversion to intermediate products that can persist in the matrix subjected to remediation, bind to constituents of the matrix, or move away from the matrix by volatilization**.





- The considerable interest in the possibility of exploiting these processes in the remediation of contaminated sites derives from the **potential greater sustainability of biotechnologies**, from an environmental and economic point of view, compared to chemical-physical technologies.

- Current *best practices* are often insufficient to achieve decontamination objectives in **fixed times**: biological remediation strategies are site-specific, their effectiveness is linked to complex ecological factors and the mechanisms that control the growth, coexistence and functions of the microorganisms that inhabit contaminated matrices.



In addition to this, the processes are affected by a very large number of parameters:

- **Chemical and physical parameters of the soil;**
- **Chemical parameters of contamination,**
- **Weather conditions.**

Physical soil parameters

Texture
Structure
Stratigraphy
Density

Porosity
Permeability
Humidity

Soil chemical parameters

Geochemical and mineralogical composition
pH
Cation exchange capacity
Buffering capacity
Organic matter content (TOC)
Redox potential

Contamination characteristics

Concentration
Distribution
Adsorption capacity on specific soil fractions

Solubility
Solid-liquid partition coefficient
Chromatographic delay factors
Vapour pressure and volatility
Solubility products
Reduction potential
Stability constants of reaction complexes
Speciation (chemical form)

$$\begin{cases} I_{xx}\ddot{\phi} &= \dot{\theta}\dot{\psi}\,(I_{yy}-I_{zz}) - J\,\dot{\theta}\,\Omega_r + \tau_x \\ I_{yy}\ddot{\theta} &= \dot{\phi}\dot{\psi}\,(I_{zz}-I_{xx}) + J\,\dot{\phi}\,\Omega_r + \tau_y \\ I_{zz}\ddot{\psi} &= \dot{\phi}\dot{\theta}\,(I_{xx}-I_{yy}) + \tau_z \end{cases}$$

Many variables create

1) the **impossibility of creating models with complete similarity** (effective theories that include all parameters)
2) the **impossibility of replicating processes and methods** on different environmental remediation scenarios,

making the design of the interventions complex.

Other critical aspects:

1) difficulty in **establishing beforehand limits of admissibility** for biological treatment (limit concentration of inorganic and organic components of contamination);
2) **limited efficacy of commercial preparations** (enzymes, alien microorganisms) for bioaugmentation;
3) **difficulties in the practical management** of biomass cultivation and inoculation of native microorganisms
4) the need for strict control of **biological risk factors.**

- The complex of the results obtained with the application of innovative protocols and a methodological approach based on the study of soil metagenome have opened encouraging paths towards overcoming some of the critical issues of the industrial use of Bio-remediation

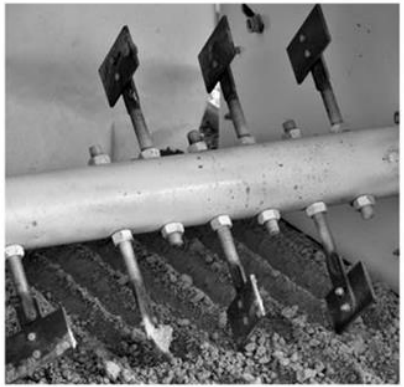
HOWEVER

- **The pilot test, on a significant scale, remains the only real support for the site-specific design of a bio-remediation intervention.**

- **RoboNova is a pilot plant, in which:**

• on-site interventions are modeled, working on the specific matrix;
• the processes are monitored in terms of degradation kinetics and matrix toxicity
• monitoring of the metabolic activity of microorganisms and the evolution of the matrix ecosystem is carried out;
• the environmental impacts (consumption, emissions, waste produced) of the technology are measured.



The use of the pilot plant allows to develop an executive design of the interventions on a real scale in terms of:

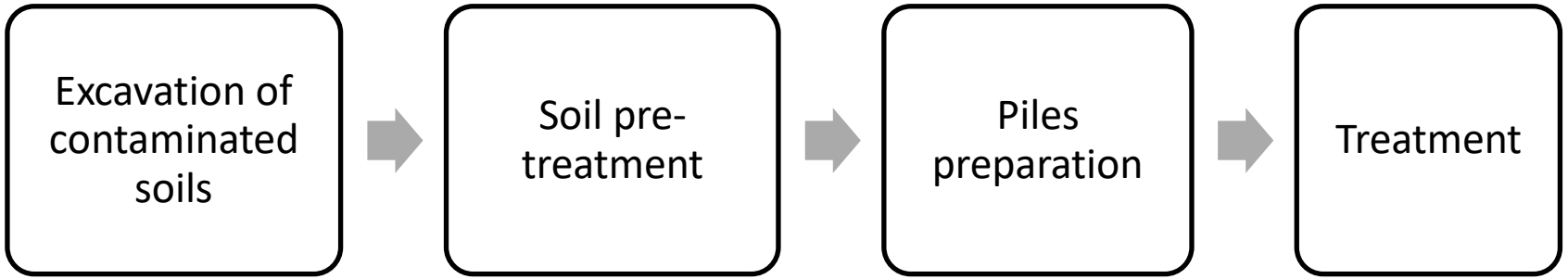
- **methods and tools** (e.g. structures and machinery suitable for site-specific applications);
- **treatment procedures,**
- **kinetics of the processes and duration of remediation,**
- **monitoring practices;**
- **support protocols** for a correct management of the environmental and health impacts of the technology (e.g. soil metagenomics, chemical and ecotoxicological assessments).

- RoboNova is the **model** of an on-site technology (on the reclamation site) ex situ (treatment on a matrix removed from the natural site), which exploits **oxidative-aerobic** processes against heterotrophic **microorganisms native** to the matrix being treated (soil, sediment) using the organic contaminant as a source of carbon and energy and oxygen as an electron acceptor.

- These processes determine the **reduction of organic contaminants** by:

1. mineralization;
2. transformation by partial oxidation;

and promote the **humification of the matrix**, returning an ecologically active techno-soil.



Bioremediation optimization study

Soil pre-treatment

- Soil characterization
- Addition of bulking agent
- Optimization of NPK ratio and pH

Biostimulation

- Aeration by tilling
- CPN ratio
- Water irrigation strategy
- Temperature

Bioaugmentation

- Hyperactive and concentrated native culture

RoboNova 1.0: an automated testing facility

RoboNova 2.0 – mobile pilot plant and lab facility

Bio-Flushing

modeling of biodegradation kinetics

Bio-Flushing

modeling of biodegradation kinetics

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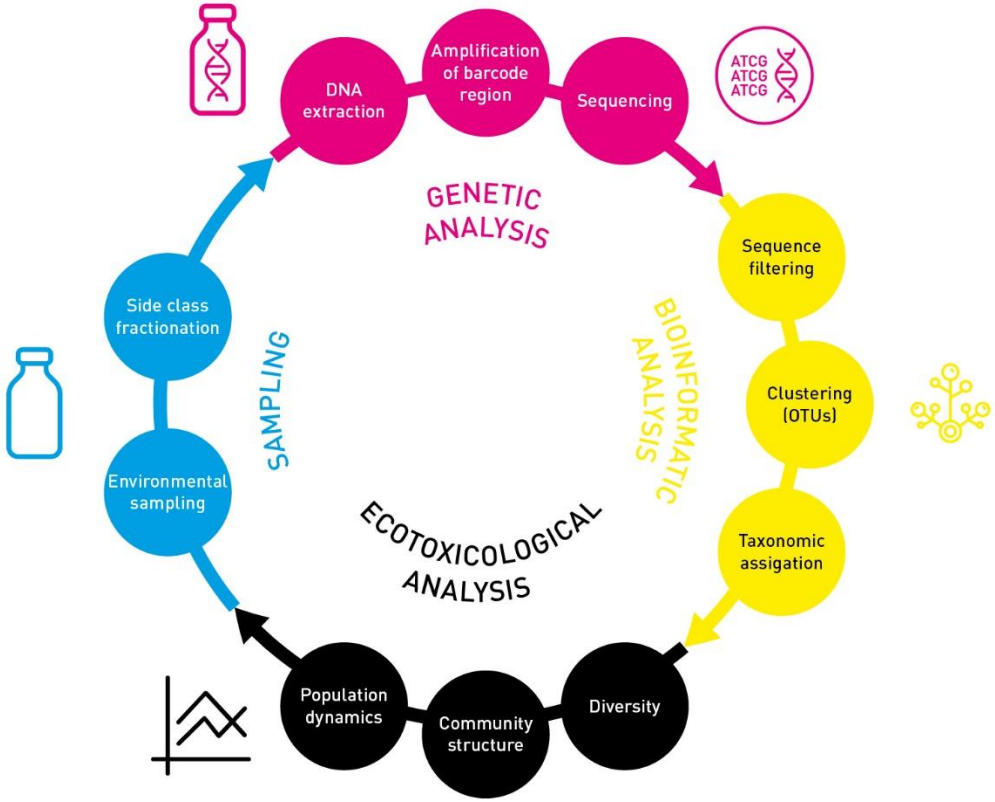
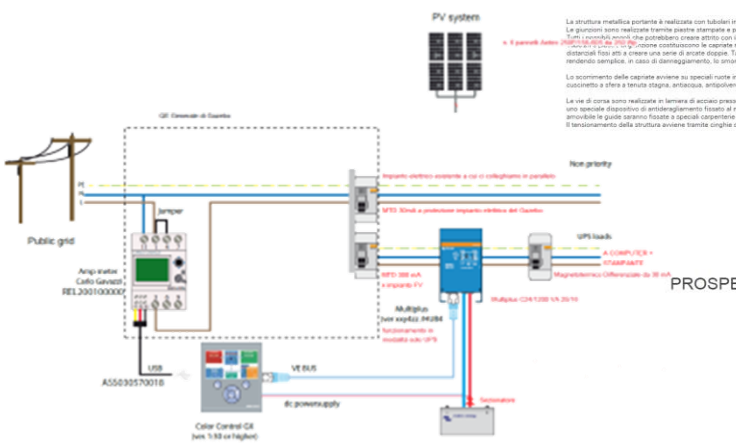
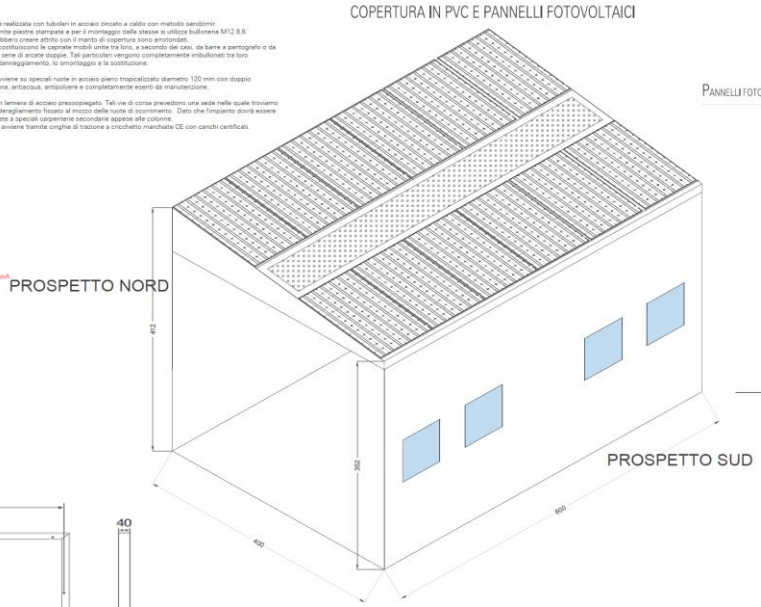
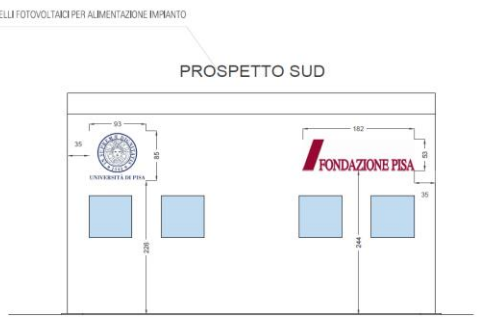
Microbiome characterization and treatment test cycles





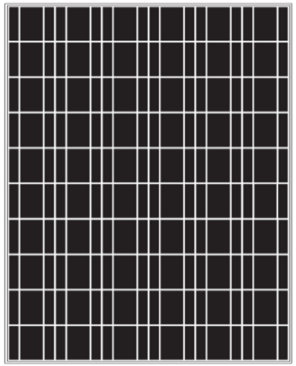


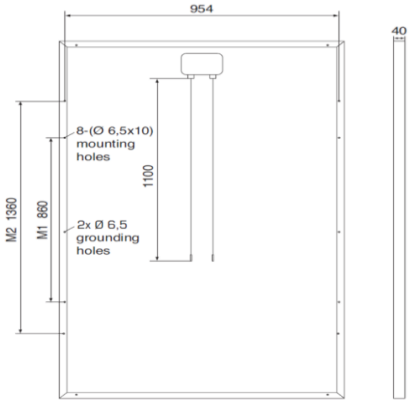


L'involucro è realizzato con manto in PVC, bispalmato con un trattamento ignifugo EN13501-1 , del peso in opera di grammi 900 al mq (vedi scheda tecnica del tessuto).
Il manto è dotato di speciali placche termosaldate che ospitano delle mezze anelle metalliche che ne permettono il fissaggio in modo solido alla struttura in carpenteria metallica per mezzo di speciali cinghie ad alta resistenza .Il manto di copertura risulta rinforzato con un doppio strato di tela antiusura in tutte le zone a rischio lacerazione che potrebbe essere causata dall'attrito con i componenti metallici dovuto alla continua azione dei fenomeni atmosferici

Il manto di copertura garantisce la massima impermeabilità agli agenti atmosferici, in quanto viene fornito in un unico manufatto che incorpora copertura superiore e fianchi, formato da più pezzi saldati tra loro.

Le testate sono dotate dei frontali triangolari realizzati con manti PVC, ancorati alla struttura in carpenteria mediante speciali profili in alluminio estruso
Le testate anteriore e/o posteriore saranno chiuse con tende scorrevoli apribili in duplice partita complete di paletti antivento, chiavistelli a terra, cinghie tendifrici, maniglie e ganci di chiusura.





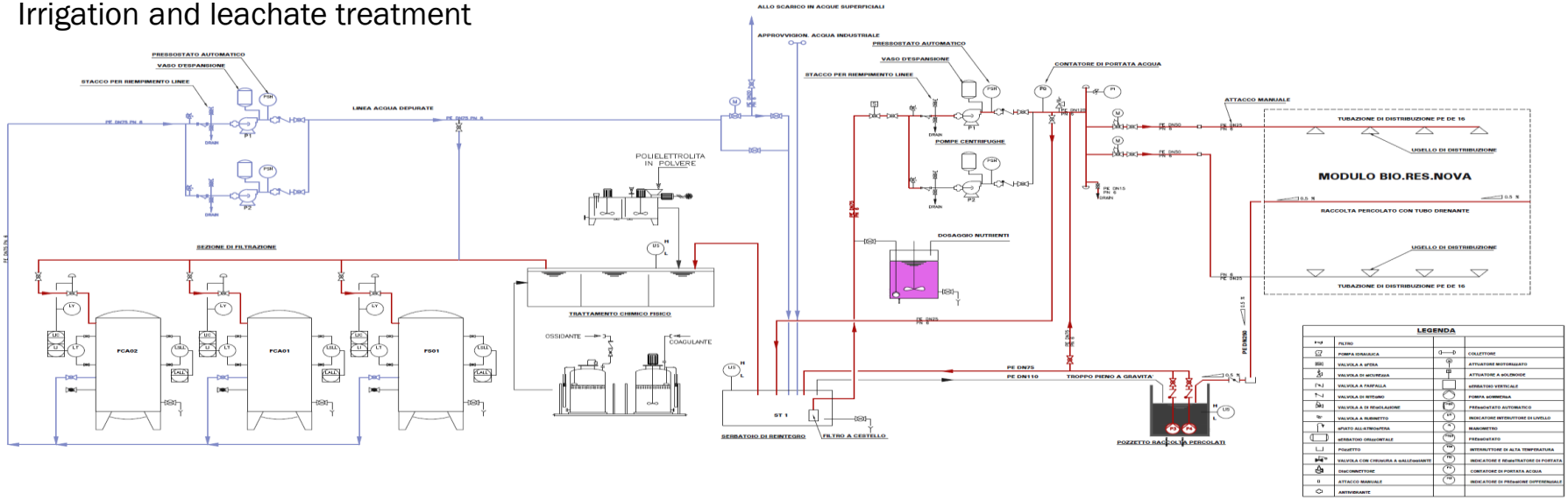
Energy

Photovoltaic

- The pilot is a mobile system, with partial energy autonomy conferred by the use of single-layer structure with photovoltaic panels

Water management

Irrigation and leachate treatment



- The water system provides for the humidification of the matrices through a misting system mounted in the intrados of the RoboNova frame. Irrigation water not retained by the soil being treated is collected from the tank below the paving deck and conveyed to a leachate collection well. From the cockpit the water is raised back to the distribution tank and can be sent 1) again to the nebulization2) to a chemical-physical purification treatment.

Gaseous emissions

Monitoring and treatment of VOC emissions

- The bioreactor is equipped with an air intake and treatment system designed with the aim of maintaining, inside the casing, an **air quality compatible with the possible presence of operators** inside it, although the movement of the soils takes place in an automated way (**relevant aspect for the scale up**).

- The atmosphere inside the bioreactor is typically characterized by the presence of **dust and volatile organic contaminants** contained in the polluted matrix and /or produced by the treatment. The system has been designed for the filtration of these types of contaminants and has been sized to guarantee up to 25 spare parts of the volume of the tent per hour.

- The treatment plant shall be prepared for **the sampling necessary for monitoring emissions.**

Unità di adsorbimento su carboni attivi

Filtro meccanico

Soil improvers

Bulking agents and humification substrates

- The bulking agent used is a **green compost** or a fine **wood chip** that, in addition to increasing the microbial load of the soil, provides organic carbon with high molecular weight that can be used as a skeleton for the progress of the humification processes of the organic substance.

- As a bulking agent, the **growth substrate** used by the mushroom production food industry (abundant in ligninolytic fungal enzymes) is also used.

- Mixing of the bulking agent and fertilizer can be carried out even during the formation of heaps. Bulking is laid out on the bed of the pile together with the pellet fertilizer (the vault-heaps mixes them together).
-

Nutrients and correctors

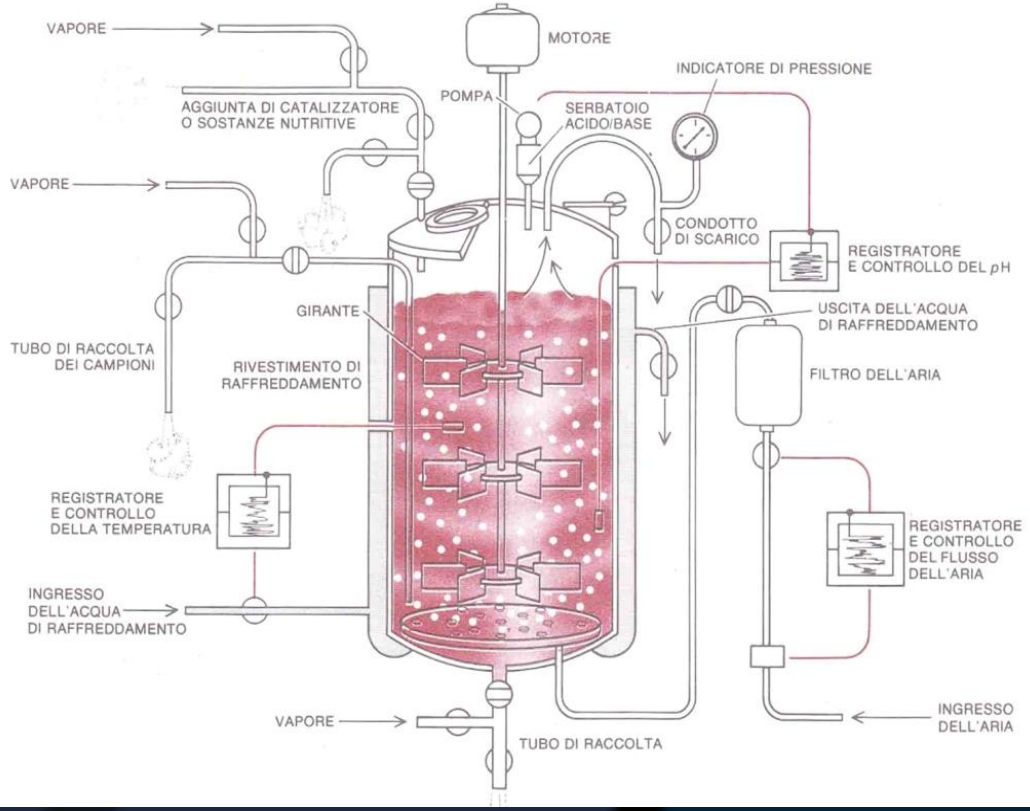
- **Nitrogen and phosphate fertilizations** will be administered as granular palatable compositions in the preparation phase, but also during treatment, when the C/N/P ratio returns to excessive carbon favor, as a result of the degradation processes themselves.

- Commercially, mixtures called superphosphates or superphosphates are used, with a P_2O5 content of 16-22%, formed by calcium phosphate monoacid, CaHPO4, insoluble in water but soluble in organic acids, by bicide calcium phosphate, $Ca(H_2PO4)_2$, soluble in water, and by calcium sulphate.

- A soil improver/corrector being tested is zeolite.

Bioaugmentation - Mycoaugmentation

Growth of the selected microbial candidates



Batch reactor with a volume of 0.5 m3 equipped with a heating jacket that keeps the biomass suspended in the temperature range 24-28 °C

Air compressor

Nutrient dosing via peristaltic pumps

Monitoring

Matrix to monitor	Purpose	Sampling frequency	Parameters to be analyzed
Solid matrix: soil	Verify the minimum conditions for the activation of the oxidative processes of interest	Weekly	1. C/N/P 2. Water content 3. Soil pH 4. Temperature 5. Eubacteria counter 6. Contamination 7. Microbial ecology
Interstitial gases and surface emissions	Verify the activation and constancy of the oxidative processes of interest Monitor the quality of any emissions	Continuously between one turn and another with dedicated probes. Weekly respirometric tests	1. CO2 2. O2 (molecular oxygen) 3. CH4 (methane) 4. VOC
Surrounding atmosphere	Assess the risks to the health of workers on site and the surrounding population	Weekly during the first month. Monthly in the following months	1. VOC 2. microdust 3.
Leachate water	Ecotoxicology and genotoxicity of any elutriates	In case of production of elutriates weekly control	-Ecotoxicology with *Vibrio fischeri* -Genotoxicity on apexes of *Vicia faba*

Case Study - Engineering of a 15,000 m³ landfarming treatment plant

- Location: South of Italy
- Implemented technology: Landfarming with biostimulation and bioaugmentation treatment protocols
- Level of contamination: [C>12] < 2500 mg/kg for soil from industrial areas, [C>12] < 1500 mg/kg for soil from residential areas

Phase 1: Pilot lab tests on soil samples from contaminated site

Pilot test treatment protocol:

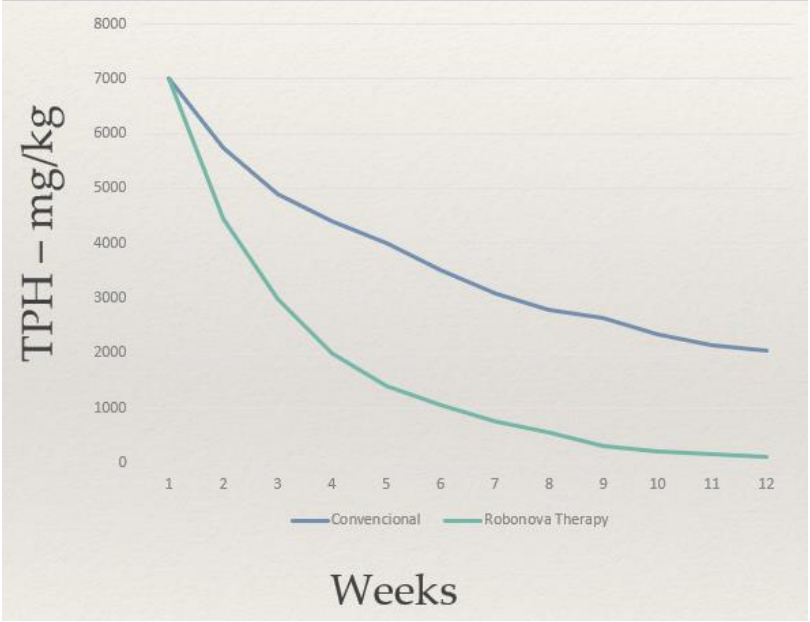
- 1st step: biostimulation treatment to correct the C/N to 10/1
- 2nd step: bioaugmentation treatment with autochthonous fungine inoculum

Case Study - Engineering of a 15,000 m³ landfarming treatment plant

- Isolation of autochthonous fungal strains by enrichment with diesel fuel (1% v/v) as only C-source
- Taxonomic characterization by 18s rDNA sequencing
- Evaluation of metabolic capabilities by degradation tests (discoloration of recalcitrant dye)
- Mesocosms trials

Faster reclamation rate

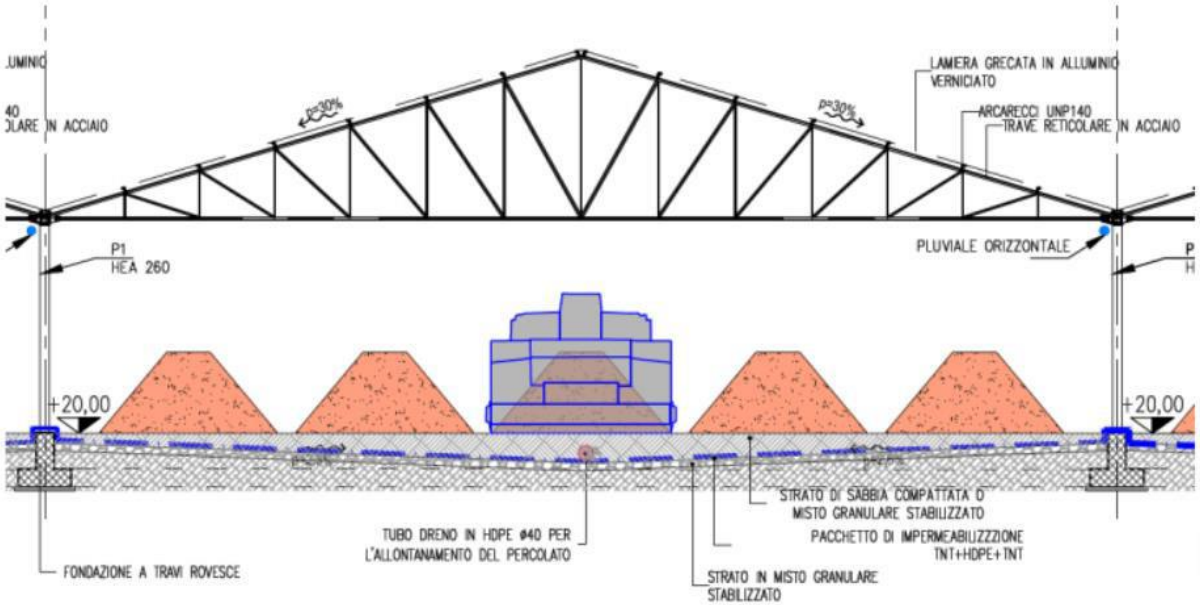
Lower cost



Case Study - Engineering of a 15,000 m³ landfarming treatment plant

Phase 2: Engineering of full-scale remediation works

- Soil piles undergoing treatment, for a total of 15,000 m3
- Soil pre-treatment station & storage facilities for amendments
- Metallic structure to protect the soil piles from atmospheric agents & facilitate temperature and humidity control
- Aeration of the matrix by regular soil mixing (windrow turner)
- Correction of the ratio of macronutrients C/N/P



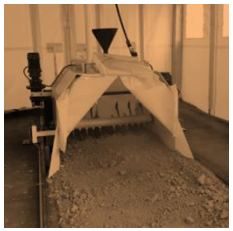
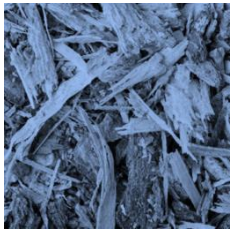
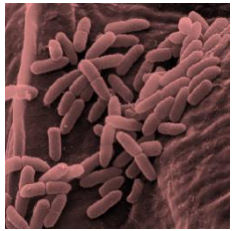
Case Study - Engineering of a 15,000 m³ landfarming treatment plant

Phase 2: Engineering of full-scale remediation works

- Monitoring plan to:
  - optimize the biodegradation rates
  - determine the kinetics of contamination abatement
  - monitor vapor emissions
  - monitor migration of contaminants in properly collected rain/leaching water

- Remediation target of TPH concentration under 750 mg/kg (for soil for industrial use) and 50 mg/kg (for soil for residential use) scheduled to be achieved within a maximum of 16 weeks

“The Earth we live on, we did not inherit from our fathers, we borrowed it from our children”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