



**BIOremediation systems exploiting SYnergies
for improved removal of Mixed pOllutants**

Deliverable D6.1

Preliminary report on LCA

Deliverable information

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Executive Summary

The objective of BIOSYSMO is to remove contaminants from soils, sediments, and groundwater through the application of NBS, offering a more biodiversity/ecosystem-friendly alternative to ex-situ (conventional physical-chemical) treatments.

To achieve this, BIOSYSMO focuses on identifying and isolating the most promising microorganisms and plants from contaminated sites, followed by their conditioning for use in the following systems:

- **Soil pollution management through phytoremediation assisted by microorganisms.** Fungus/bacteria have been proved to create synergies with plants (for instance, poplars for BIOSYSMO) to enhance phytoremediation effectiveness.
- **Sediment pollution management through estuarine phytoremediation.** Microbial consortia can be enriched from rhizosediments, and their subsequent interaction with *Juncus maritimus* or *Phragmites australis* supports the removal of both organic and inorganic contaminants in estuaries.
- **Groundwater and wastewater pollution management using BES.** BES technologies have been proven effective in removing pollutants from both groundwater and wastewater. BES technologies can be implemented both in-situ and ex-situ.
- **Integrated water phytoremediation and BES for groundwater and wastewater.** Certain aquatic plants are capable of removing metals, organochlorines, organophosphates, carbamates, and pyrethroid pesticide compounds due to their uptake and accumulation capacities. These plants can operate independently or can be combining with BES in a hybrid system boosting pollutant removal.
- **Soil pollutant management through microbial biostimulation and bioaugmentation:** The use of various experimental strategies for biostimulation and bioaugmentation promotes the degradation and removal of soil contaminants.

BIOSYSMO is composed of eight WPs, covering the following areas: i) contaminated site characterization; ii) microbial and plant isolation; iii) technical development; iv) the use of computational tools for biosystems design and implementation; v) microbial bioaugmentation and biostimulation; vi) risk assessment; vii) technology scaling-up; viii) sustainability assessment; ix) legislation assessment; x) roadmap for wider implementation, xi) communication, dissemination, and exploitation; and xii) project coordination.

In this context, the sustainability assessment is addressed through tasks 6.1 (environmental LCA), 6.2 (LCC and NPV), and 6.3 (social LCA and SIA). In accordance with the Grant Agreement, the specific goal of task 6.1 is to achieve higher positive environmental impacts of the proposed solutions than similar commercial remediation methods. To achieve the consecution of this specific objective, two deliverables are scheduled: i) D6.1 — “Preliminary report on LCA” (M32) and D6.5 — “Final report on LCA and social impact” (M44). This document corresponds to D6.1, which is submitted in parallel with D6.2, with the aim of identifying the environmental and economic hotspots within BIOSYSMO. More specifically, BSY presents the preliminary results for task 6.1 in the current intermediate deliverable. In summary, these initial findings will form the foundation for the full execution of the task, the results of which will be reported in D6.5.

The first section of the current deliverable provides an introduction, outlining the literature assessment conducted by BSY at the beginning of the task to support subsequent work. Specifically, this deliverable

highlights the use of LCA as a tool for environmental evaluation (see the information provided at the front of section 1 — “Introduction”). Additionally, the types of ecosystems are presented, emphasizing their importance from an environmental perspective and the threats they face due to the presence of pollutants (Section 1.1 — “Ecosystem types”). In this connection, the environmental importance of the systems treated in BIOSYSMO is shown (Section 1.2 — “Importance of the systems treated in BIOSYSMO”). Accordingly, the application of pollutants removal treatments is needed providing the distinctive attributes that differentiate each type of remediation. Specifically, these treatments may involve conventional physico-chemical methods or various biotreatments. Notably, biotreatments can differ in nature and this variability influences how the treatments are implemented. For instance, innovative NBS, currently under development within BIOSYSMO, offer in-situ pollutant removal while minimizing disruption to the affected sites (Section 1.3 — “Remediation of pollutants in soil, sediments and liquid ecosystems”). Finally, BSY has also carried out a comprehensive literature review of LCA case studies relevant to BIOSYSMO-related technologies as well as potential benchmarks, in line with the task description outlined in the Grant Agreement (Section 1.4 — “LCAs applied to remediation technologies”).

The LCAs have been carried out in accordance with ISO standards, considering the four standard stages:

- I. Goal and scope definition.
- II. LCI (data collection).
- III. LCIA (environmental impact assessment).
- IV. Results interpretation and optimization.

Accordingly, the sections of the current deliverable are structured around these stages:

- Stage 1 corresponds to sections 2.1 (“Goal definition”), 2.2 (“Scope definition”), 2.3 (“Functional Unit”), 3.1 (“Assumptions”) and 3.3 (“System description”).
- Stage 2 is based on content from sections 3.1 (“Assumptions”), 3.2 (“Data compiled for the environmental assessment”), 3.3 (“System description”) and 3.4 (“Data gathered from partners and literature”).
- Stage 3 is covered in the section 3.5 (“Environmental impact assessment”) and all subsections of section 4 (“Alignment with the biodiversity assessment”), which outlines a roadmap for incorporating the study of biodiversity and ecosystem services into task 6.1.
- Stage 4 is addressed across all subsections of the section 5 (“Results and discussion”).

All outcomes from the LCA are summarized in the last section.

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Table of Abbreviations

Abbreviation	Definition
AER	Anion exchange resin
AEM	Anion exchange membrane
AXIA	Axia Innovation UG (partner 9 in the Grant Agreement)
BES	Bioelectrochemical system
BES-CE	BES for removal of organochlorides
BES-HC	BES for removal of hydrocarbons
BES-M	BES for removal of metals
BTEX	Presence of benzene, toluene, ethylbenzene and xylene compounds
BSY	Blue Synergy (partner 6 in the Grant Agreement)
CEM	Cation exchange membrane
CIIMAR	Centro Interdisciplinar de Investigacao Marinha e Ambiental (partner 7 in the Grant Agreement)
COD	Chemical oxygen demand
EXE	Exelisis Ike (partner 12 in the Grant Agreement)
GRLCA	Generalised remediation Life Cycle Assessment
IDE	Idener Research & Development Agrupacion de Interes Economico (BIOSYSMO's coordinator)
ISCO	In-situ chemical oxidation
ISO	International Standard Organization
JSI	Institut Jozef Stefan (partner 3 in the Grant Agreement)
LCA	Life Cycle Assessment
LCI	Life Cycle Inventory
LCIA	Life Cycle Impact Assessment
LEITAT	Acondicionamiento Tarrasense Asociacion (partner 4 in the Grant Agreement)
MFC	Microbial Fuel Cell
NBS	Natural Based System
NZVI	Nano-scale zero-valent iron
NPV	Net Present Value
PAHs	Polycyclic aromatic hydrocarbons
PFAS	Polyfluoroalkyl substances
P&T	Pump and Treat
S-S	Solidification and stabilization of wastes

SEAR	Surfactant enhanced aquifer remediation
Social LCA	Social Life Cycle Assessment
SIA	Social Impact Assessment
TAUW	Tauw GMBH (partner 11 in the Grant Agreement)
TKN	Total Kjeldahl nitrogen
TPH	Total Petroleum Hydrocarbons
TRL	Technology Readiness Level
TSS	Total suspended solids
UBU	Universidad de Burgos (partner 2 in the Grant Agreement)
UMLP	Université Marie et Louis Pasteur (previously specified as Communauté d'Universités et Établissements Université Bourgogne - Franche – Comte — partner 5 in the Grant Agreement)
UPM	Universidad Politécnica de Madrid (partner 8 in the Grant Agreement)
WP	Work Package

1 Introduction

LCA is a tool used to identify, assess, and measure the potential environmental impacts of an established system¹. In this regard, LCA examines the full life cycle of a product, process or service, and may include data from the following stages: i) resource extraction, ii) raw material processing, iii) product manufacturing, iv) product distribution, v) product usage, and vi) waste management².

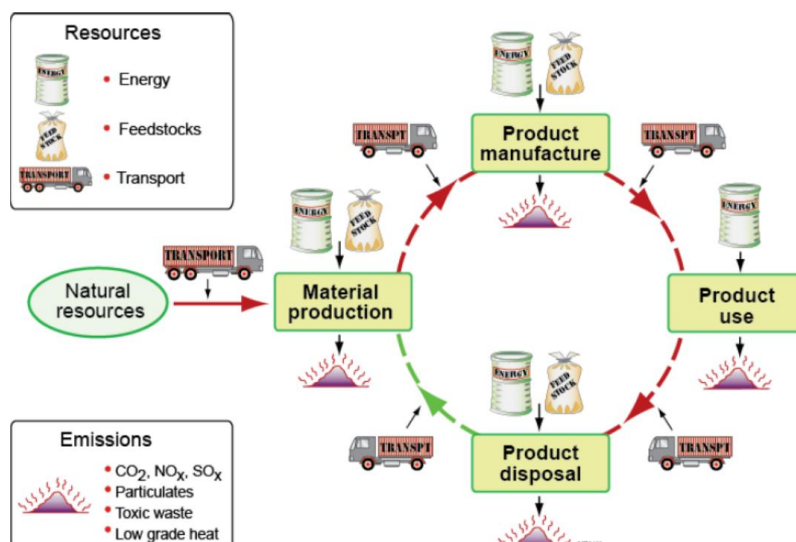


Figure 1. Stages involved in the life cycle of any system. Source: ³.

As defined by SETAC, LCA is a methodology for assessing the environmental impacts of a product, process, or activity across the stages outlined in Figure 1. It considers all impacts from raw material extraction to final disposal, identifying energy and materials used, waste released, and opportunities for environmental improvements⁴. LCA, together with LCC, can aid decision-making by promoting sustainability⁵.

It is recommended to conduct an LCA according to ISO standards, as it provides a common global reference for implementation, which is valuable for comparing different assessments⁶. However, it is essential to ensure that the system boundaries must be identical and consistent when comparing

¹ G. Finnveden, J. Potting, 2014. **Life Cycle Assessment**. In: P. Wexler (Ed.), **Encyclopaedia of Toxicology**. Academic Press. Massachusetts, United States of America.

² G. Sonnemann, F. Castells, M. Schuhmacher, 2004. **Integrated Life-Cycle and Risk Assessment for Industrial Processes**. Lewis Publishers. London, United Kingdom.

³ A.C. Marques, 2014. **Teaching Sustainability Design of Products to Engineering Students**. International Journal of Performability Engineering 10, 589-604.

⁴ S. Toniolo, R.C. Tosato, F. Gambaro, J. Ren, 2020. **Life cycle thinking tools: Life cycle assessment, life cycle costing and social life cycle assessment**. In: J. Ren, S. Toniolo (Eds.), **Life Cycle Sustainability Assessment for Decision-Making — Methodologies and Case Studies**. Elsevier. Amsterdam, The Netherlands.

⁵ D. Cohan, D. Gess, 1994. **Integrated Life-Cycle Management**. Proceedings of 1994 IEEE International Symposium on Electronics and The Environment, 149-154.

⁶ International Standard Organization, 2006. **Environmental management — Life cycle assessment — Principles and framework**. <https://www.iso.org/standard/37456.html> (accessed on 21/02/2025).

systems⁷. ISO standards also define the implementation of LCA in four phases: i) definition of the goal and scope, ii) data collection, iii) environmental impact assessment and iv) results interpretation².

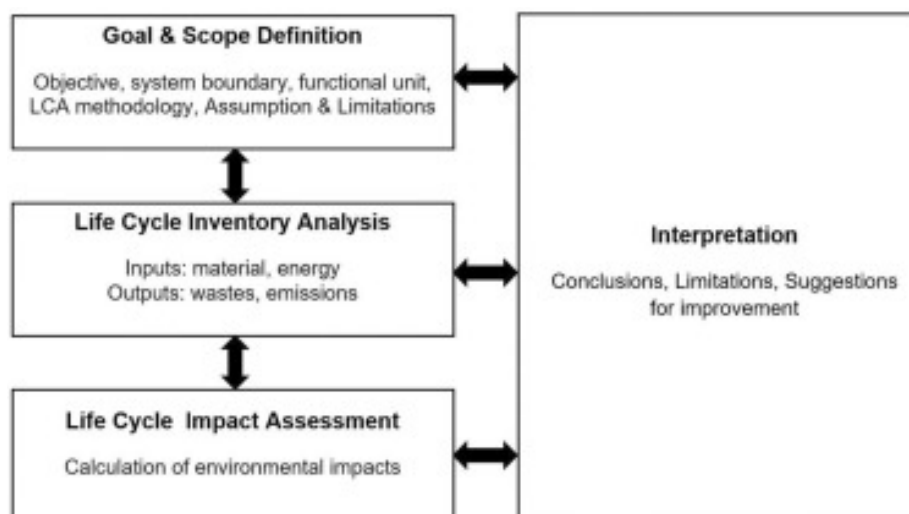


Figure 2. 4 steps for LCA execution. Source: ⁸.

In short, the goal and scope definition phase of LCA establishes the purpose and method for incorporating environmental information into decision-making, with the primary aim of selecting the system to assess. Within this system, various alternatives can be evaluated to select the one with the least environmental impact. This phase also determines the necessary data quality and resources, which can vary depending on the scope, such as comparing competing products or identifying opportunities for reducing resource use and emissions⁹. Therefore, the scope definition is a critical stage. In this connection, the scope of the assessment depends on the specific stages (among those in Figure 1) selected for the LCA execution¹⁰. Accordingly, different approaches can be considered, including: i) “Cradle-to-Grave”, ii) “Cradle-to-Gate (2)”, “Gate (1)-to-Gate (2)” as shown in Figure 3. Additionally, an ideal approach, known as “Cradle-to-Cradle”, can be considered, where no waste is produced, and all materials are fully recovered for reuse as raw materials¹¹. This “Cradle-to-Cradle” approach is fully in line with the principles of the circular economy, although it is very challenging to achieve in practice.

⁷ A.C. Hetherington, A.L. Borrión, O.G. Griffiths, M.C. McManus, 2013. **Use of LCA as a development tool within early research: challenges and issues across different sectors**. International Journal of Life Cycle Assessment 19, 130-143.

⁸ S. Kokare, J.P. Oliveira, R. Godina, 2023. **Life cycle assessment of additive manufacturing processes: A review**. Journal of Manufacturing Systems 68, 536-559.

⁹ E. Nieuwlaar, 2013. **Life Cycle Assessment and Energy Systems**. In: E. Nieuwlaar (Ed.), **Reference Module in Earth Systems and Environmental Sciences**. Elsevier. Amsterdam, The Netherlands.

¹⁰ Rochester Institute of Technology, 2020. **What is life cycle assessment (LCA)?** <https://www.rit.edu/sustainabilityinstitute/blog/what-life-cycle-assessment-lca#:~:text=An%20LCA%20is%20a%20systematic,that%20make%20up%20industrial%20production> (accessed on 21/02/2025).

¹¹ A. Bjørn, M.Z. Hauschild, 2018. **Cradle to Cradle and LCA**. In: M.Z. Hauschild, R.K. Rosenbaum, S.I. Olsen (Eds.), **Life Cycle Assessment — Theory and Practice**. Springer Cham. Cham, Switzerland.

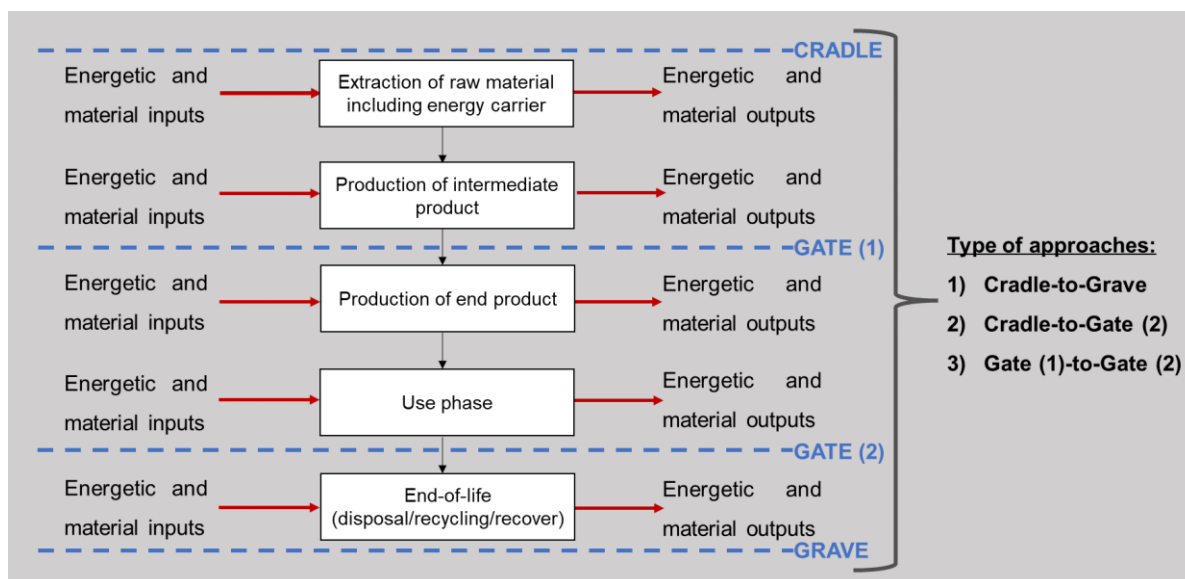


Figure 3. Potential scopes for the LCA execution.

According to the stages defined by ISO above, stage 2 is the LCI analysis. In this phase, all energy and material requirements, along with emissions to air, water, and soil, are quantified for the scenarios to be evaluated. More specifically, a flow diagram must be created to identify the different stages involved in each scenario. This stage aims to identify opportunities for reducing environmental impacts and guide further analysis on potential environmental effects⁹.

In phase 3, LCIA evaluates the potential impacts of the flow diagrams identified in the previous stage (LCI analysis). Specifically, the phase 3 involves selecting impact categories, as well as classifying and characterizing the results. Additionally, the results may optionally be normalized, grouped, or weighted to aid in their interpretation⁹.

Finally, the last phase is the interpretation of the results, during which the significant findings of the environmental assessment are identified, verified, and evaluated. It is crucial to ensure the completeness and consistency of the data, and sensitivity analysis may be necessary to formulate accurate environmental conclusions and recommendations. These conclusions should provide relevant and actionable outcomes⁹. In this sense, it is important to balance the identified environmental and economic outcomes (from Task 6.2). Specifically, these outcomes should help partners avoid inappropriate investments and minimize environmental impacts¹².

¹² P. Gluch, H. Baumann, 2004. **The life cycle costing (LCC) approach: a conceptual discussion of its usefulness for environmental decision-making.** Building and Environment 39, 571-580.

1.1 Ecosystem types and pollution

Groundwater, the world's largest freshwater reservoir, is crucial for ecosystem sustainability and human adaptation to climate change¹³. Maintaining optimal groundwater health is essential for both human well-being and environmental quality¹⁴.

Furthermore, soil serves as a natural filter for groundwater, and when the soil becomes excessively contaminated, it can result in increased groundwater pollution¹⁵. Although soil pollution has historically received less attention than air and water pollution, it is equally responsible for significant environmental damage¹⁶. Soil supports diverse ecosystems, and contaminants within these ecosystems negatively impact biodiversity, making pollutant removal vital for the preservation of these systems¹⁷. Moreover, soil ecosystems are integral to nutrient cycling, and disturbances in these systems can cause significant environmental damage by disrupting balance¹⁸.

In addition, estuaries are highly productive ecosystems that play a key role in preserving biodiversity and providing essential ecosystem services. These services include: (i) protecting coastlines, (ii) capturing and storing carbon, (iii) purifying water, and (iv) supporting fishery resources. Despite their importance, estuaries face growing threats from human activities and climate change, making their conservation a critical priority¹⁹.

In summary, the presence of pollutants (in all these environments) poses a serious risk to both human health and ecosystems. For instance, herbicides and pesticides in the environment have been linked

¹³ B.A. Aderemi, T.O. Olwal, J.M. Ndambuki, S.S. Rwanga, 2022. **A Review of Groundwater Management Models with a Focus on IoT-Based Systems**. Sustainability, 14, 148.

¹⁴ M. Sophocleous, 2002. **Environmental implications of intensive groundwater use with special regard to streams and wetlands**. In: R. Llamas, E. Custodio (Eds.), **Intensive Use of Groundwater — Challenges and Opportunities**. A.A. Balkema Publishers. Lisse, Netherlands.

¹⁵ S.D. Keesstra, V. Geissen, K. Mosse, S. Piirainen, E. Scudiero, M. Leistra, L. van Schaik, 2012. **Soil as a filter for groundwater quality**. Current Opinion in Environmental Sustainability 4, 507-516.

¹⁶ M. Romantschuk, K. Lahti-Leikas, M. Kontro, P. Galitskaya, H. Talvenmäki, S. Simpanen, J.A. Allen, A. Sinkkonen, 2023. **Bioremediation of contaminated soil and groundwater by in situ biostimulation**. Frontiers in Microbiology 14, 1258148.

¹⁷ R.G. Morgado, S. Loureiro, M.N. González-Alcaraz, 2018. **Changes in Soil Ecosystem Structure and Functions Due to Soil Contamination**. In: A.C. Duarte, A. Cachada, T. Rocha-Santos (Eds.), **Soil Pollution — From Monitoring to Remediation**. Academic Press. Massachusetts, United States of America.

¹⁸ A. Cachada, T. Rocha-Santos, A.C. Duarte, 2018. **Soil and Pollution: An Introduction to the Main Issues**. In: A.C. Duarte, A. Cachada, T. Rocha-Santos, **Soil Pollution — From Monitoring to Remediation**. Academic Press. Massachusetts, United States of America.

¹⁹ E.B. Barbier, S.D. Hacker, C. Kennedy, E.W. Koch, A.C. Stier, B.R. Silliman, 2011. **The value of estuarine and coastal ecosystem services**. Ecological Monographs — Ecological Society of America 81, 169-193.

to the development of cancers and birth defects²⁰. Furthermore, pollutants can disrupt ecosystem services; for example, species richness may decline due to the presence of pollutants²¹.

Besides, the types of contaminants in soil and groundwater vary widely, depending on the processes and production histories of each site, while sediments are primarily contaminated by heavy metals²². Anyway, pollutants remediation is necessary for all types of ecosystems to mitigate environmental impacts and promote their development²³.

1.2 Importance of the systems treated in BIOSYSMO

As noted above in the Executive Summary, BIOSYSMO technologies can be applied to restore a variety of contamination scenarios across diverse ecosystems, including: i) groundwater, ii) surface water contaminated by wastewater, iii) soils, and iv) estuarine sediments. In this respect, the following table presents all contaminated sites assessed in BIOSYSMO, along with the potential NBS applicable for their restoration.

Table 1. Polluted sites evaluated in BIOSYSMO.

Site	Short description	Site manager	Potential NBS applicable for restoration
1	Soil contaminated with metals and organic pollutants	UMLP	Phytoremediation assisted by microorganisms
2	Soil contaminated with Hg and chlorinated ethenes	UMLP	Phytoremediation assisted by microorganisms
3	River sediments contaminated with heavy metals and PAHs	TAUW	Integrated water phytoremediation and BES (water phytoremediation can be also applied independently)
4	Estuarine sediments contaminated with pharmaceuticals and heavy metals	CIIMAR	Estuarine phytoremediation
5	Estuarine sediments contaminated with pharmaceuticals and heavy metals	CIIMAR	Estuarine phytoremediation
6	Groundwater contaminated with metal(loid)s	TAUW	Integrated water phytoremediation and BES (Both technologies can also be applied independently)

²⁰ J.W. Kang, 2014. **Removing environmental organic pollutants with bioremediation and phytoremediation.** Biotechnology Letters 36, 1129-1139.

²¹ Biodiversity Information System for Europe, 2023. **Indicators of ecosystem condition.** <https://biodiversity.europa.eu/europes-biodiversity/ecosystems/indicators-of-ecosystem-condition> (accessed on 20/02/2025).

²² J. Deng, N. Wei, X. Yang, M. Xiao, X. Li, N. Wang, C. Zhang, H. Zhang, J. Dong, 2025. **Life cycle inventory dataset for systematic environmental remediation of soil, groundwater and sediment.** Resources, Conservation and Recycling 212, 107960.

²³ D. Hou, D. O'Connor, 2020. **Green and sustainable remediation: concepts, principles, and pertaining research.** In: D. Hou (Ed.), **Sustainable Remediation of Contaminated Soil and Groundwater — Materials, Processes, and Assessment.** Butterworth-Heinemann. Oxford, United Kingdom.

7	Groundwater contaminated with volatile hydrocarbons (C ₅ -C ₁₂), BTEX and naphthalene	TAUW	BES-CE
8	Groundwater contaminated with chlorinated hydrocarbons	TAUW	BES-CE
9	Groundwater contaminated with chlorinated ethenes, methanes and hexachlorbutadiene	TAUW	BES-CE and BES-HC
10	Soil contaminated with metals, TPHs and PAHs	UMLP	Phytoremediation assisted by microorganisms
11	Soil and groundwater contaminated with TPHs (heating oil, lubricating, and cooling oil), organochlorides and metal(loid)s	TAUW	BES-CE, BES-HC and BES-M
12	Soil and groundwater contaminated with TPHs (motor oil)	TAUW	Soil bioaugmentation-biostimulation and BES-CE
13	Soil contaminated with Lindane	UBU	Soil bioaugmentation-biostimulation
14	Groundwater contaminated with chlorinated hydrocarbons	TAUW	BES-CE

These sites were evaluated and characterized by the technical partners involved in WP1, and the resulting data has been considered in the development of the current deliverable.

Regarding the potential NBS to be applied, BIOSYSMO technologies are advancing from an initial TRL of 3–4 toward a final TRL of 5–6 across BIOSYSMO's case studies. Further details on these case studies are provided in the following section ("Remediation of pollutants in soil, sediments and liquid ecosystems").

The contaminants identified by the technical partners (in accordance with D1.1) are presented in Table 1 for each polluted site. The presence of these compounds is important for the execution of the current deliverable.

1.3 Remediation of pollutants in soil, sediments and liquid ecosystems

Soil, sediment and liquid remediation can be ex-situ or in-situ. Ex-situ methods are the removal and transfer of the pollutants from contaminated areas to a separate location for treatment. In contrast, in-situ methods treat the contaminated material directly at the site of contamination, eliminating the need for transportation. This approach integrates the treatment process into the existing environment, causing minimal disturbance²⁴. Both types of treatments have their advantages and disadvantages, as shown in the table below.

²⁴ A. Hussain, F. Rehman, H. Rafeeq, M. Waqas, A. Asghar, N. Afsheen, A. Rahdar, M. Bilal, H.M.N. Iqbal, 2022. **In-situ, Ex-situ, and nano-remediation strategies to treat polluted soil, water, and air – A review.** Chemosphere 289, 133252.

Table 2. Advantages and disadvantages of in-situ and ex-situ remediation treatments from an environmental perspective.
Source: ²⁵.

Result	In-situ treatments	Ex-situ treatments
Advantages	Soil structure and biodiversity rehabilitates more quickly.	Remediation processes are quicker. Easier to control. Easier to monitor.
Disadvantages	Longer time to reach remediation objective. More difficult to control remediation processes. More difficult to monitor.	Significant habitat disruption. Soil structure and biodiversity harder to re-establish, which is less aligned with biodiversity protection.

When designing treatment units, it is important to consider both the technical aspects for treatment and the operating time. The operating time may extend over several years, which should also be factored into the design process²⁶.

More specifically, remediation is typically classified into biological (also known as bioremediation) and physical-chemical treatments. Biological treatment involves the design and operation of facilities that use a system based on bacteria/plants/fungi/other living organism to degrade or transform the organic and inorganic components of waste flows^{27,28}. In contrast, physical-chemical treatments, often referred to as conventional treatments, address pollutants removal through: i) physical technologies, ii) chemical technologies, or iii) a combination of both physical and chemical operations. In particular, physical treatments focus on removing pollutants without altering the biochemical properties of the system. These treatments generally aim to generate two flows, each enriched with the desired compound, and are typically applied before chemical and biological treatments. Examples of physical-chemical treatments include: i) screening, ii) sedimentation, iii) skimming, iv) aeration without biochemical reactions, v) thermal treatment (for instance, thermal desorption), vi) adsorption, vii) membrane-based technologies, viii) gravity separation, ix) magnetic separation, x) sieving or xi) mechanical treatments. Regarding chemical treatments, they address the pollutant removal through the addition of inducing a series of chemical reactions. For instance, they include: i) ion exchange, disinfecting through chlorination, UV light or ozone addition, iii) neutralization, iv) precipitation, vii) soil washing, viii)

²⁵ R. Thompson, 2021. **Introduction to tackle soil pollution**. In: FAO, UNEP (Eds.), **Global Assessment of Soil Pollution**. FAO Report. Rome, Italy.

²⁶ N.P. Cheremisinoff, 1998. **Treating Contaminated Groundwater and Leachate**. In: N.P. Cheremisinoff (Ed.), **Groundwater Remediation and Treatment Technologies**. William Andrew Press. New York, United States of America.

²⁷ P. Bhunia, 2014. **Fundamentals of Biological Treatment**. In: S. Ahuja (Ed.), **Comprehensive Water Quality and Purification — Reference Work — Volume 3 — Wastewater Treatment and Reuse**. Elsevier. Amsterdam, The Netherlands.

²⁸ M.S. Ayilara, O.O. Babalola, 2023. **Bioremediation of environmental wastes: the role of microorganisms**. *Frontiers in Agronomy* 5, 1183691.

electrokinetic processes, ix) S-S technology, x) chemical extraction, xi) chemical reduction/oxidation, xii) dehalogenation or xiii) incineration^{25,29}.

There are different mechanisms involved in microbial bioremediation, including: i) enzymatic oxidation, ii) enzymatic reduction, iii) bioaugmentation, iv) bio-stimulation, v) bioleaching, vi) biosorption, vii) bioaccumulation or viii) precipitation²⁸. In this regard, bioaugmentation refers to the microbial promotion through the addition of a significant dose of microorganisms at the contaminated site. It is important to note that bioaugmentation may introduce non-native microorganism when native populations are insufficient, which can conflict with biodiversity protection by altering the indigenous ecosystem^{28,30}. Additionally, involves the promotion of microbial activity through the addition of nutrients (such as nitrogen, potassium or phosphorus), metabolites, electron donors, electron acceptors, enzymes, biosurfactants, etc.²⁸.

Bioremediation treatments offer several key benefits: **i) Natural growth enhancement** – Pollutants can stimulate the growth of necessary microorganisms, reducing dependence on external inputs; **ii) Eco-friendly waste management** – Bioremediation breaks down pollutants, lowering overall toxicity and enabling safer waste disposal; **iii) Minimal disruption** – It can be conducted on-site with minimal interference to regular activities, aligning with the supply of ecosystem services and reducing transportation-related health risks; **iv) Cost-efficiency** – As a biological process, bioremediation is relatively low-cost and thus economically attractive; and **v) In-situ treatment** – Contaminated soil, sediment or water can be treated without excavation or pumping, minimizing ecological disturbance³¹.

When considering the advantages and disadvantages of physical-chemical treatments for soil remediation, each technology has its strengths and limitations, as outlined below with some examples. Soil/sediment S-S is effective in immobilizing heavy metals in contaminated soil/sediment. However, it may render the soil unsuitable for agricultural or other productive uses due to alterations in its structure. Leaching technology is a fast and efficient method for removing pollutants from soil, but it carries a significant risk of groundwater contamination. Oxidation-reduction technology can reduce the toxicity of pollutants, but the introduction of chemicals into the environment may cause unintended effects on ecosystems. Photocatalytic degradation is known for its high efficiency, although its effectiveness is limited by the need for catalysts and the presence of light. Electrodynamics repair technology is associated with minimal environmental impact throughout the system's life cycle; however, its high equipment costs limit its widespread application. Finally, organic matter improvement promotes the natural degradation of pollutants but is constrained by the specific conditions of the soil³².

²⁹ S.F. Ahmed, M. Mofijur, S. Nuzhat, A.T. Chowdhury, N. Rafa, M.A. Uddin, A. Inayat, T.M.I. Mahlia, H. Chyuan Ong, W.Y. Chia, P.L. Show, 2021. **Recent developments in physical, biological, chemical, and hybrid treatment techniques for removing emerging contaminants from wastewater**. Journal of Hazardous 416, 125912.

³⁰ N. Kuppan, M. Padman, M. Mahadeva, S. Srinivasan, R. Devarajan, 2024. **A comprehensive review of sustainable bioremediation techniques: Eco friendly solutions for waste and pollution management**. Waste Management Bulletin 2, 154-171.

³¹ M. Sanjana, R. Prajna, S.K. Urvi, R.V. Kavitha, 2024. **Bioremediation – The recent drift towards a sustainable environment**. Environmental Science Advances 3, 1097.

³² L. Ma, 2024. **Study on Soil Pollution Control and Land Ecological Restoration Technology**. International Journal of Natural Resources and Environmental Studies 3, 36-44.

Among in-situ bioremediation technologies, NBS represent a biological approach that aims to maximize nature's benefits while supporting ecosystem protection and restoration³³. In this regard, they stand out as a promising alternative, as pollutants serve as substrates for microorganisms that transform them into non-toxic products in the environment. Therefore, the integration between microorganisms and the ecosystem is complete, as no external intervention is required³⁴.

The extracted material in all ex-situ treatments must be replaced leading to a disruption of the system. Accordingly, the in-situ treatments (including NBS) are always considered as most effective from an environmental/ecological perspective³⁵.

The five case studies for BIOSYSMO are NBS. These technologies are:

- Case study 1 — Poplar phytoremediation assisted by microbial.
- Case study 2 — Estuarine phytoremediation assisted by microbial.
- Case study 3 — Water phytoremediation plus BES water wastewater.
- Case study 4 — BES for groundwater/wastewater.
- Case study 5 — Soil bio-stimulation-bioaugmentation.

Case study 1:

Phytoremediation is increasingly recognized as an eco-friendly approach to pollution mitigation, offering a range of ecological benefits such as: i) carbon dioxide sequestration, ii) erosion control, iii) improved water infiltration, iv) dust suppression, v) pollutant degradation, and vi) restoration of native flora and fauna. Social benefits also emerge, including enhanced aesthetic value and the creation of new recreational areas³⁶.

In this regard, poplars, particularly when supported by appropriate microbial consortia, are strong candidates for phytoremediation due to: i) their rapid growth, ii) high biomass yield, and iii) effectiveness in remediating metal-contaminated soils. Beyond reducing contamination risks, their use also supports sustainable remediation and enables the valorisation of biomass on marginal lands³⁷.

³³ B. Sowińska-Świerkosz, J. García, 2022. **What are Nature-based solutions (NBS)? Setting core ideas for concept clarification.** Nature-Based Solutions 2, 100009.

³⁴ K.E. Enerijiofi, 2020. **Bioremediation of environmental contaminants: a sustainable alternative to environmental management.** In: G. Saxena, V. Kumar, M.P. Shah (Eds.), **Bioremediation for Environmental Sustainability — Toxicity, Mechanisms of Contaminants Degradation, Detoxification, and Challenges.** Elsevier. Amsterdam, The Netherlands.

³⁵ A.M. Pranjić, P. Oprčkal, A. Mladenović, P. Zapussek, M. Urleb, J. Turk, 2018. **Comparative Life Cycle Assessment of possible methods for the treatment of contaminated soil at an environmentally degraded site.** Journal of Environmental Management 218, 497-508.

³⁶ V.C. Pandey, O. Bajpai, 2018. **Phytoremediation: From Theory Toward Practice.** In: V.C. Pandey, K. Baudh (Eds.), **Phytomanagement of Polluted Sites — Market Opportunities in Sustainable Phytoremediation.** Elsevier. Amsterdam, The Netherlands.

³⁷ L. Ciadamidaro, D. Blaudez, M. Chalot, 2024. **Poplar as a woody model for the phytomanagement of trace element contaminated soils.** Advances in Botanical Research 109, 63-103.

Nevertheless, phytoremediation is still under development, as further research is needed to better understand how plants accumulate and tolerate heavy metals³⁸. In this connection, different types of phytoremediation have already been identified, including: i) phytostabilization, ii) phytoextraction, iii) phytovolatilization, iv) phytodegradation, v) rhizodegradation, vi) phytodesalinization and vii) rhizofiltration (see figure below)^{39,40}.

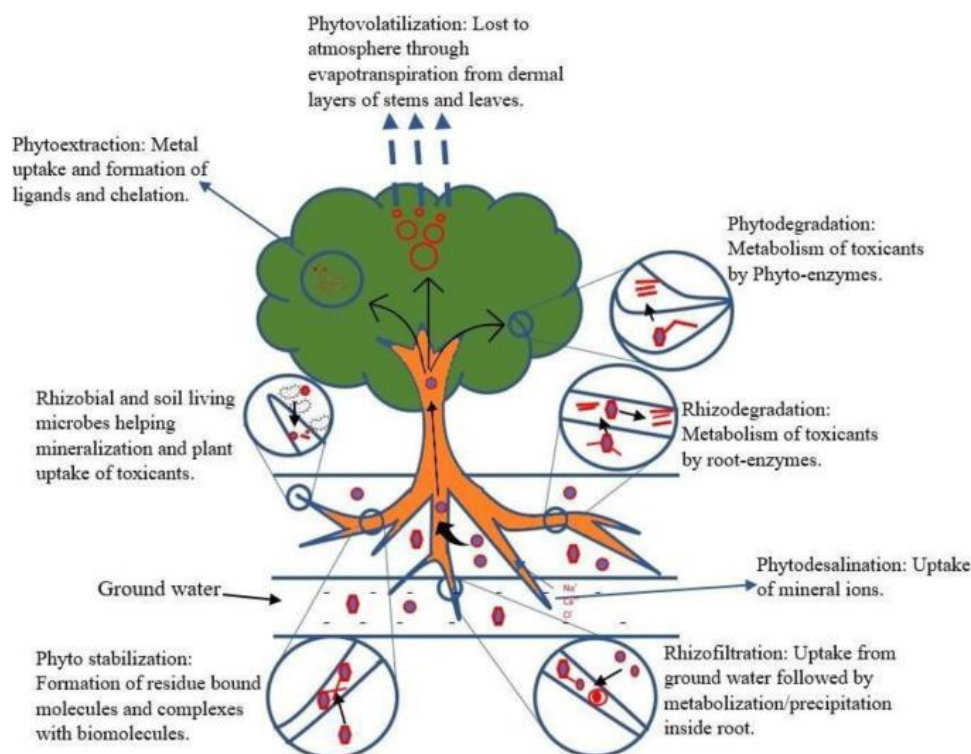


Figure 4. Types of phytoremediation. Source: ⁴⁰.

As previously mentioned, phytoremediation can be enhanced by adding microorganisms (bacteria or fungi) that support plant growth. These microorganisms promote growth through, alleviate stress and facilitate degradation. Specifically, they stimulate plant growth through mechanisms such as producing phytohormones, siderophores, and chelating substances, which indirectly contribute to bioremediation and the microbe-mediated removal of pollutants. Additionally, microorganisms in the rhizosphere also help mobilize pollutants, making them available for uptake by plants. Besides, they also assist plants in tolerating various environmental stresses. In turn, plants release exudates and enzymes that

³⁸ A. Yan, Y. Wang, S.N. Tan, M.L.M. Yusof, S. Ghosh, Z. Chen, 2020. **Phytoremediation: A Promising Approach for Revegetation of Heavy Metal-Polluted Land**. *Frontiers in Plant Biotechnology* 11, 359.

³⁹ T. Phillips, 2019. **6 Types of Phytoremediation**. <https://www.thoughtco.com/six-types-of-phytoremediation-375529> (accessed 20/02/2025)

⁴⁰ A. Kafle, A. Timilsina, A. Gautam, K. Adhikari, A. Bhattarai, N. Aryal, 2022. **Phytoremediation: Mechanisms, plant selection and enhancement by natural and synthetic agents**. *Environmental Advances* 8, 100203.

enhance biochemical and microbial activities in the surrounding soil, further supporting bioremediation⁴¹.

Case study 2:

An estuary/salt marshes is a semi-enclosed coastal water body where freshwater from land mixes with seawater⁴². As a result, estuaries are unique ecosystems with rich biodiversity^{43,44}. However, the organisms living in these valuable ecosystems are negatively impacted by the inflow of inadequately treated wastewater and its discharges⁴⁵. For instance, the presence of pharmaceuticals/emerging compounds, along with metals, may be appeared in this system as a consequence of uncontrolled discharges. Given this situation, Fernandes et al. (2015)⁴⁶ demonstrated the removal of pharmaceutical pollutants by salt marsh plants associated with microorganisms. For this, it is possible to isolate microorganisms from estuarine sediments, followed by microbial bioaugmentation, before integrating the plant/microbial biosystem for estuarine revitalization. In this context, the microorganisms can colonize *Juncus maritimus* and *Phragmites australis*⁴⁷. This type of phytoremediation for contaminated estuaries has been shown to be more environmentally friendly than landfill treatment⁴⁸.

Case study 3:

The integration of phytoremediation with BESs offers a promising approach for enhancing pollutant removal from soil and groundwater from a sustainability perspective. This combination improves

⁴¹ M.S. Rabani, I. Hameed, T.A. Mir, B.A. Wani, M.K. Gupta, A. Habib, M. Jan, H. Hussain, S. Tripathi, A. Pathak, M.B. Ahad, C. Gupta, 2022. **Microbial-assisted phytoremediation**. In: R.A. Bhat, F.M.P. Tonelli, G.H. Dar, K. Hakeem (Eds.), **Phytoremediation — Biotechnological Strategies for Promoting Invigorating Environs**. Academic Press. Massachusetts, United States of America.

⁴² W.L. Filho, A.M. Azul, L. Brandli, A.L. Salvia, T. Wall, 2020. **Estuaries: Dynamics, Biodiversity, and Impacts**. In W.L. Filho, A.M. Azul, L. Brandli, A.L. Salvia, T. Wall (Eds.), **Life Below Water**. Springer Cham. Cham, Switzerland

⁴³ S. Pasquaud, R.P. Vasconcelos, S. França, S. Henriques, M.J. Costa, H. Cabra, 2015. **Worldwide patterns of fish biodiversity in estuaries: Effect of global vs. local factors**. Estuarine, Coastal and Shelf Science 154, 122-128.

⁴⁴ A. Valle-Levinson, 2010. **Definition and classification of stuaries**. In: A. Valle-Levinson (Ed.), **Contemporary Issues in Estuarine Physics**. Cambridge University Press. Cambridge, United Kingdom.

⁴⁵ J.P. Fernandes, C.M.R. Almeida, F. Andreotti, L. Barros, T. Almeida, A.P. Mucha, 2017. **Response of microbial communities colonizing salt marsh plants rhizosphere to copper oxide nanoparticles contamination and its implications for phytoremediation processes**. Science of The Total Environment 581-582, 801-810.

⁴⁶ J.P. Fernandes, C.M.R. Almeida, M.C.P. Basto, A.P. Mucha, 2015. **Response of a salt marsh microbial community to antibiotic contamination**. Science of The Total Environment 532, 301-308.

⁴⁷ C. Teixeira, C.M. R. Almeida, M.N. da Silva, A.A. Bordalo, A.P. Mucha, 2014. **Development of autochthonous microbial consortia for enhanced phytoremediation of salt-marsh sediments contaminated with cadmium**. Science of The Total Environment 493, 757-765.

⁴⁸ T. Soleimani, F. Sordes, I. Techer, G. Junqua, M. Hayek, M. Salgues, J.-C. Souche, 2023. **Comparative environmental and economic life cycle assessment of phytoremediation of dredged sediment using *Arundo Donax*, integrated with biomass to bioenergy valorization chain**. Science of The Total Environment 903, 166160.

treatment efficiency by harnessing the complementary roles of vegetation and microbial activity. Moreover, it supports resource recovery while promoting energy-efficient contaminant removal⁴⁹.

Case study 4:

In a BES, microorganisms are exposed to an electric current to capture electrons, which are then utilized to treat pollutants in the liquid medium⁵⁰. The rationale for using BES is based on several key factors: (i) electrodes serve as electron acceptors/donors without requiring external intervention, (ii) microorganisms grow on the electrode surface, (iii) the electric field is likely to stimulate natural microbial degradation activity, and (iv) the system requires minimal energy consumption.

Currently, there is limited information available on the environmental impacts and economic evaluations of BES technologies^{51,52,53}. This gap underscores the importance of conducting assessments to identify the key environmental and economic hotspots of the novel BES systems⁵⁴.

Case study 5:

Bioaugmentation is the process of introducing microorganisms into contaminated sites to accelerate the degradation of pollutants, particularly when existing microbes are insufficient or ineffective. Alternatively, biostimulation promotes the activity of indigenous microorganisms by optimizing environmental conditions. This promotion may be conducted by: i) the addition of nutrients (for instance, nitrogen or phosphorus), ii) the supply of oxygen or iii) the enrichment of carbon sources. In short, both techniques improve the effectiveness of bioremediation, either by enriching microbial populations or by enhancing nutrients conditions to support microbial activity⁵⁵.

⁴⁹ L.K. Bahrdwaj, D. Upadhyay, H. Jain, P. Rath, D. Kumar, S. Bajpai, 2024. **Recent trends of phytoremediation for the wastewater treatment and resource recovery**. In: A.L. Srivastav, I. Zinicovscaia, L. Cepoi (Eds.), **Biotechnologies for Wastewater Treatment and Resource Recovery — Current Trends and Future Scope**. Elsevier. Amsterdam, The Netherlands.

⁵⁰ K. Rabaey, J. Rodríguez, L.L. Blackall, J. Keller, P. Gross, D. Batstone, W. Verstraete, K.H. Nealson, 2007. **Microbial ecology meets electrochemistry: electricity-driven and driving communities**. The ISME Journal 1, 9-18.

⁵¹ H. Wang, H. Luo, P.H. Fallgren, S. Jin, Z.J. Ren, 2015. **Bioelectrochemical system platform for sustainable environmental remediation and energy generation**. Biotechnology Advances 33, 317-334.

⁵² J. Zhang, H. Yuan, I.M. Abu-Reesh, Z. He, C. Yuan, 2019. **Life Cycle Environmental Impact Comparison of Bioelectrochemical Systems for Wastewater Treatment**. Procedia CIRP 80, 382-388.

⁵³ S. Jung, J. Lee, Y.-K. Park, E.E. Kwon, 2020. **Bioelectrochemical systems for a circular bioeconomy**. Bioresource Technology 300, 122748.

⁵⁴ D.A. Corona-Martínez, S.Y. Martínez-Amador, J.A. Rodríguez-De la Garza, E.I. Laredo-Alcalá, P. Pérez-Rodríguez, 2025. **Recent Advances in Scaling up Bioelectrochemical Systems: A Review**. BioTech 14, 8. Departamento de Ciencias del Suelo, Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Buenavista, Saltillo 253

⁵⁵ G.O. Adams, P.T. Fufeyin, S.E. Okoro, I. Ehinomen, 2015. **Bioremediation, Biostimulation and Bioaugmentation: A Review**. International Journal of Environmental Bioremediation & Biodegradation 3, 28-39.

1.4 LCAs applied to remediation technologies

Traditional remediation has primarily focused on the contaminated site and the potential health risks to people in its vicinity. However, this traditional approach does not account for environmental impacts arising from: i) raw materials acquisition, ii) needed transport or iii) waste management. Today, the application of LCA in remediation decision-making is bringing attention to these aspects, specifically the environmental impacts throughout the life cycle of remediation technologies⁵⁶.

LCAs have been previously used for the environmental assessment of soil, groundwater or wastewater remediation^{57,58,59}. For instance, Ambaye et al. (2023)⁵⁷ found that electricity consumption (along with biosurfactant production) is the main contributor to the environmental impact of BES systems used for remediating soil contaminated with PAHs. A more detailed discussion of BES environmental impacts is presented later in the current section.

Other physical-chemical treatments can also be used for groundwater remediation. For example, Goyal and Mondal (2022)⁶⁰ performed an LCA for arsenic and fluoride removal from groundwater, considering two potential scenarios: i) aluminium/hydroxide/oxide nanoparticles adsorption (scenario 1) and ii) electrocoagulation technology (scenario 2). Adsorption technology, with a Global Warming Potential of 35.25 kg CO₂ (eq) for the treatment of 720 L of groundwater contaminated with arsenic (0.5 mg L⁻¹) and fluoride (10 mg L⁻¹) has environmental impacts nearly eight times greater than electrocoagulation (with a Global Warming Potential of 4.51 kg CO₂ (eq) for the same groundwater treatment).

In addition, Boyer et al. (2021)⁶¹ conducted a LCA to compare various regeneration strategies for an AER system used in the remediation of PFAS-contaminated groundwater. The evaluated scenarios: i) included full waste incineration (scenario 1a), ii) methanol recovery (scenario 1b), iii) combined methanol and brine recovery (scenario 1c), iv) the use of alternative salts with methanol (scenario 2a), and v) aqueous-only regeneration for a weak-base AER (scenario 2b). In this connection, the study found that incineration resulted in more severe effects on human toxicity, ecotoxicity, and eutrophication. In contrast, recycling methanol and brine led to reduced environmental impacts by decreasing the amount of waste sent for incineration. However, future research is needed to confirm

⁵⁶ D. Hou, D. O'Connor, 2020. **Green and sustainable remediation: concepts, principles, and pertaining research**. In: D. Hou (Ed.), **Sustainable Remediation of Contaminated Soil and Groundwater — Materials, Processes, and Assessment**. Butterworth-Heinemann. Oxford, United Kingdom.

⁵⁷ T.G. Ambaye, F. Formicola, S. Scaffoni, A. Franzetti, M. Vaccari, 2023. **Life cycle assessment of bioslurry and bioelectrochemical processes for sustainable remediation of soil polluted with petroleum hydrocarbons: An experimental study**. Sustainable Production and Consumption 36, 416-424.

⁵⁸ P. Bayer, M. Finkel, 2006. **Life cycle assessment of active and passive groundwater remediation technologies**. Journal of Contaminant Hydrology 83, 171-199.

⁵⁹ C. Corbella, J. Puigagut, M. Garfí, 2017. **Life cycle assessment of constructed wetland systems for wastewater treatment coupled with microbial fuel cells**. Science of The Total Environment 584-585. 355-362.

⁶⁰ H. Goyal, P. Mondal, 2022. **Life cycle assessment (LCA) of the arsenic and fluoride removal from groundwater through adsorption and electrocoagulation: A comparative study**. Chemosphere 304, 135243.

⁶¹ T.H. Boyer, A. Ellis, Y. Fang, C.E. Schaefer, C.P. Higgins, T.J. Strathmann, 2021. **Life cycle environmental impacts of regeneration options for anion exchange resin remediation of PFAS impacted water**. Water Research 207, 117798.

the feasibility of effectively reusing these materials. Additionally, the highest impacts from regeneration were observed in human toxicity, ecotoxicity, and eutrophication. Moreover, using alternative salts instead of NaCl during regeneration increased environmental impacts, while natural salts caused less environmental change.

For soil remediation, Song et al. (2018)⁶² conducted an environmental assessment using an environmental LCA in addition to a socio-economic assessment. The environmental assessment considered two scenarios: i) scenario 1 — soil excavation followed by soil washing, thermal desorption, and S-S technology, with the blocks derived from S-S technology used as backfill material and ii) scenario 2 — soil excavation followed S-S technology, with the blocks refused in a landfill site. Scenario 2 exhibited significantly higher environmental impacts, with its global result being four times higher than that of Scenario 1. Therefore, Scenario 1 is preferable from an environmental perspective. In this scenario 1, soil excavation and backfill material generation were identified as the main environmental drivers.

Specifically for the thermal desorption treatment, Liang et al. (2023)⁶³ have implemented an LCA for this ex-situ technology in Jiangsu Province (China). They found that decisions related to plant's location could reduce the impacts, as a greater distance from the site resulted in higher impacts. In this connection, optimizing remediation decisions could have led to an 81.6% reduction in the environmental impacts.

NZVI can be used for pollutant removal in groundwater or soil^{64,65}. Visentin et al. (2019)⁶⁵ evaluated the environmental impacts of three different synthesis processes: i) milling, ii) hydrogen gas-based reduction, and iii) sodium borohydride-based reduction. Their findings indicate that hydrogen gas-based reduction results in the highest global environmental impact due to its complexity and high energy demand, whereas sodium borohydride-based reduction has the lowest impact. Milling also contributes significantly to environmental burdens, primarily due to its high electricity consumption. Regarding specific impact categories, sodium borohydride-based reduction has the greatest effect on ecosystem quality due to reagent production, while hydrogen gas-based reduction has the most significant impact on climate change and resource depletion. Sensitivity analysis highlights the critical role of energy sources, with the highest impacts observed in the United States of America due to reliance on fossil fuels, whereas Brazil's renewable energy mix leads to the lowest environmental burdens.

⁶² Y. Song, D. Hou, J. Zhang, D. O'Connor, G. Li, Q. Gu, S. Li, P. Liu, 2018. **Environmental and socio-economic sustainability appraisal of contaminated land remediation strategies: A case study at a mega-site in China**. Science of The Total Environment 610-611, 391-401.

⁶³ T. Liang, M. Huo, L. Yu, P. Wang, J. Zheng, C. Zhang, D. Wang, A. Ding, F. Li, 2023. **Life cycle assessment-based decision-making for thermal remediation of contaminated soil in a regional perspective**. Journal of Cleaner production 392, 136260.

⁶⁴ A.M. Zafar, M.A. Javed, A.A. Hassan, M.M. Mohamed, 2021. **Groundwater remediation using zero-valent iron nanoparticles (nZVI)**. Groundwater for Sustainable Development 15, 100694.

⁶⁵ C. Visentin, A.W.d.S. Trentin, A.B. Braun, A. Thomé, 2019. **Lifecycle assessment of environmental and economic impacts of nano-iron synthesis process for application in contaminated site remediation**. Journal of Cleaner Production 231, 307-319.

Hou et al. (2014)⁶⁶ conducted a GRLCA to evaluate the environmental sustainability of four remediation technologies: (i) P&T, (ii) enhanced in situ bioremediation (an NBS remediation fully aligned with BIOSYSMO treatments), (iii) permeable reactive barriers, and (iv) in situ chemical reduction. The study highlighted the critical influence of site-specific conditions on environmental performance. In general, in situ treatments were associated with lower life-cycle impacts, although containment methods such as P&T and permeable reactive barriers could outperform them under certain hydrogeological scenarios. Key contributors to environmental impacts included: i) energy consumption, ii) use of reactive materials, iii) and iv) the requirements for long-term monitoring. Additionally, each technology exhibited distinct environmental hotspots, suggesting that optimization strategies should be based on the specific nature of the treatment. The GRLCA model: i) provides practical guidance for strategic remediation planning, particularly when full LCAs are not feasible, and (ii) emphasizes the importance of accounting for secondary impacts in environmental assessments.

In this regard, the traditional LCIA approach indicates that NBS scenarios typically result in lower environmental impacts compared to resource-intensive remediation technologies. Concerning this, the harvesting phase is identified as a key hotspot, significantly contributing to the environmental impact of NBS⁶⁷.

In the LCA literature, several common types of NBS remain unassessed, partly due to the complexity of adapting LCA to urban planning and large-scale environmental projects. The concept of ecosystem services provides a useful framework for classifying the functional units of NBS, distinguishing between: i) provisioning, ii) regulating, iii) cultural, and iv) supporting services. This concept is further explored in section 4 — Alignment with the biodiversity assessment⁶⁸.

The conventional LCA approach for phytoremediation focuses on stages such as: i) site preparation, ii) biomass cultivation, and iii) harvesting, using treated surface area as the functional unit. In this context, Simla et al. (2025)⁶⁹ suggested extending the boundaries to include the production of energy recovery and by-products, as well as their distribution to end users. Their findings indicated that fuel consumption significantly affects environmental impacts, while contaminant removal helps reduce ecotoxicity and human toxicity. In short, scaling up operations and using renewable energy could offer additional environmental benefits.

⁶⁶ D. Hou, A. Al-Tabbaa, J. Luo, 2014. **Assessing effects of site characteristics on remediation secondary life cycle impact with a generalised framework.** Journal of Environmental Planning and Management 57, 1083-1100.

⁶⁷ K. Alshehri, M. Harbottle, D. Sapsford, A. Beames, P. Cleall, 2023. **Integration of ecosystem services and life cycle assessment allows improved accounting of sustainability benefits of nature-based solutions for brownfield redevelopment.** Journal of Cleaner Production 413, 137352.

⁶⁸ P. Larrey-Lassalle, S.A. Decker, D. Perfido, S. Naneci, B. Rugani, 2022. **Life Cycle Assessment Applied to Nature-Based Solutions: Learnings, Methodological Challenges, and Perspectives from a Critical Analysis of the Literature.** Land 11, 649.

⁶⁹ T. Simla, A. Korus, K. Petela, W. Stanek, M. Ortner, A. Szlęk, 2025. **Phy2Climate: Life Cycle Assessment of phytoremediation combined with biofuel production.** Renewable Energy, <https://doi.org/10.1016/j.renene.2025.122979>.

Specifically, LCIA of phytoremediation aims to identify environmental concerns, including: i) acidification, ii) climate change effect, iii) eutrophication potential, iv) risks to public health from soil pollution and v) risks to ecosystems⁷⁰.

Phytoremediation efficiency can be boosted by incorporating specific microorganisms into tree systems, a process known as phytoremediation assisted by microorganisms⁷¹. This plant/microorganism integration promotes contaminant removal, leading to improved environmental outcomes. Nevertheless, it is essential to consider the energy demands of microbial applications and the impacts of post-remediation biomass management. Future LCA studies should address these factors to provide a comprehensive environmental assessment of all inputs and outputs within the system⁷².

Regarding the application of LCA for phytoremediation assisted by microorganisms, sediment phytoremediation using *A. donax* at laboratory scale has proven to be environmentally competitive. Several environmental categories show positive impacts, including: i) fossil depletion, ii) water depletion, iii) climate change, iv) ozone depletion, and v) natural land transformation. Additionally, the environmental performance of this remediation treatment is further enhanced when biomass is utilized (for instance for bioenergy production). In this regard, a sensitivity analyses identified: i) biomass yield, ii) energy conversion efficiency, and iii) sediment depth as key factors affecting environmental performance. Finally, compared to sediment landfills, phytoremediation scenarios (particularly those incorporating biomass valorisation) offered significantly lower environmental impacts and potentially reduced costs⁴⁸.

A recent publication by Dufossé et al. (2024)⁷³ evaluated the environmental impacts of a vertical flow wetland system for wastewater treatment in France. This system consists of two treatment stages with gravel and sand filters, designed to serve small communities by effectively removing pollutants such as COD, TSS, and TKN. Unlike conventional systems, it operates without aeration (an energy-intensive process) relying instead on natural filtration and microbial activity. However, it requires significant land use and emits greenhouse gases such as nitrous oxide. The system also produces sludge, which is applied as fertilizer, reducing the need for mineral fertilizers and their associated emissions. A comparison with conventional activated sludge technology highlights the vertical flow wetland's advantages in resource efficiency and certain environmental impact categories, despite trade-offs in land use and climate change potential.

⁷⁰ S. Aghili, A. Golzary, 2023. **Greening the earth, healing the soil: A comprehensive life cycle assessment of phytoremediation for heavy metal contamination.** Environmental Technology & Innovation 32, 103241.

⁷¹ C.A. Syamlal, A. George, D. Sayantan, 2024. **Phytoremediation: An Eco-Friendly Solution for Environmental Contamination.** International Journal of Advanced Research in Science, Communication and Technology (IJARSCT) 4, 553-577.

⁷² K. Alshehri, Z. Gao, M. Harbottle, D. Sapsford, P. Cleall, 2023. **Life cycle assessment and cost-benefit analysis of nature-based solutions for contaminated land remediation: A mini-review.** Heliyon 9, e20632.

⁷³ K. Dufossé, N. Forquet, P. Molle, M. Pradel, E. Loiseau, 2024. **The importance of mass balance in life cycle assessment of nature-based solutions for wastewater treatment: key learning points from a case study.** Blue-Green Systems 7, 1-14.

In assessing the sustainability of BES, especially in groundwater remediation, energy consumption (primarily electricity consumption, as previously mentioned) is a critical factor⁷⁴. Zou and He (2018)⁷⁵ noted that current BES technologies exhibit low energy yields, making energy production an unviable benefit and highlighting the need for LCAs to identify opportunities for efficiency improvements^{74,76}. In alignment with this, Chin et al. (2022)⁷⁷ performed a comparative LCA of fifteen BES configurations spanning seven system types, including various MFCs, microbial electrolysis cells, microbial desalination cells, and MFCs integrated with: (i) anaerobic digestion, (ii) constructed wetlands, (iii) microalgae, and (iv) membrane distillation. Regarding this integration with complementary technologies, this was shown to enhance both pollutant removal and resource recovery. Among all configurations, the double-chamber air-cathode MEC had the lowest overall environmental impact across all endpoint categories, while the MFC integrated with membrane distillation exhibited the least impact on human health and ecosystems. In line with the previous mentioned literature^{74,75,76}, electricity consumption during operation was identified as the dominant environmental hotspot, contributing over 90% of global warming potential. In short, the study underscores the need to optimize BES configurations and recommends that future LCAs incorporate inoculum cultivation, given its potential environmental significance.

While the study by Chen et al. (2022)⁷⁷ presents results for several systems, the most relevant for the case study developed in UBU for BIOSYSMO is the constructed wetland integrated with a BES. For this specific configuration, the authors report a significant reduction in the oxygen demand of the wastewater, which translates into a lower environmental impact. This demonstrates the potential of the integrated system to enhance sustainability performance compared to other configurations analyzed in the study. In addition, it is important to highlight a very recent publication⁷⁸, released few weeks ago, which emphasizes that ongoing advancements in this type of technology together with extended system lifespans are expected to further improve sustainability outcomes from environmental, economic, and social perspectives⁷⁸.

2 Goal and scope definition

The goal and scope definition are crucial, since this specifies how results will contribute to environmental improvement⁷. More specifically, goal definition should focus on the future improvement

⁷⁴ D. Cecconet, F. Sabba, M. Devecseri, A. Callegari, A.G. Capodaglio, 2020. ***In situ* groundwater remediation with bioelectrochemical systems: A critical review and future perspectives**. Environment International 137, 105550.

⁷⁵ S. Zou, Z. He, 2018. **Efficiently “pumping out” value-added resources from wastewater by bioelectrochemical systems: A review from energy perspectives**. Water Research 131, 62-73.

⁷⁶ Z. Ge, L. Wu, F. Zhang, Z. He, 2015. **Energy extraction from a large-scale microbial fuel cell system treating municipal wastewater**. Journal of Power Sources 297, 260-264.

⁷⁷ M.Y. Chin, Z.X. Phuang, K.S. Woon, M.M. Hanafiah, Z. Zhang, X. Liu, 2022. **Life cycle assessment of bioelectrochemical and integrated microbial fuel cell systems for sustainable wastewater treatment and resource recovery**. Journal of Environmental Management 320, 115778.

⁷⁸ P. Parab, M. Kumar, N.K. Singh, S. Pillai (2025), **Life Cycle Assessment of Constructed Wetlands Integrated with Bioelectrochemical Systems for Wastewater Treatment: A Sustainable Approach**. In: M. Kumar, N.K. Singh, A.S. Mathuriya (Eds.), **Integrated Bioelectrochemical–Constructed Wetland System for Future Sustainable Wastewater Treatment**. Springer Nature. London, United Kingdom.

of the technology from an environmental perspective⁷⁹. In contrast, the scope definition outlines the size and level of detail of the study required to achieve the objective. Depending on the selected scope, the following features may be defined^{4,79}:

- I. System elements to be included (specified in “**section 3.3 — System description**”).
- II. Functional unit (specified in “**section 2.3 — Functional Unit**” and “**section 3.4 — Data gathered from partners and literature**”).
- III. System boundaries (specified in “**section 3.3 — System description**” and “**section 3.4 — Data gathered from partners and literature**”).
- IV. Allocation procedures for material and energy consumption (specified in “**section 3.3 — System description**” and “**section 3.4 — Data gathered from partners and literature**”).
- V. Environmental impact assessment (specified in “**section 3.5 — Environmental impact assessment**”).
- VI. Key environmental impacts to consider in the results report (specified in “**section 3.5 — Environmental impact assessment**”).
- VII. Assumptions (specified in “**section 3.1 — Assumptions**”, “**section 3.3 — System description**” and “**section 3.4 — Data gathered from partners and literature**”).
- VIII. Data (specified in “**section 3.2 — Data compiled for the environmental assessment**”, “**section 3.3 — System description**” and “**section 3.4 — Data gathered from partners and literature**”).
- IX. System limitations (specified in “**section 3.3 — System description**” and “**section 3.4 — Data gathered from partners and literature**”).
- X. Considerations for initial data (specified in “**section 3.1 — Assumptions**”, “**section 3.3 — System description**” and “**section 3.4 — Data gathered from partners and literature**”).
- XI. Information to be included in the results report (specified in “**section 5 — Results and discussion**”).

To define the goal and scope, it may be useful to answer the following questions: i) what, ii) how much, iii) how well and iv) how long⁸⁰.

2.1 Goal definition

The main goal of this environmental assessment is to identify key environmental hotspots associated with: (i) the NBS technologies developed in BIOSYSMO and (ii) the most effective and mature remediation technologies currently in use (also called as conventional soil, sediments and liquid

⁷⁹ C. van der Giesen, S. Cucurachi, J. Guinée, G.J. Kramer, A. Tukker, 2020. **A critical view of the current application of LCA for new technologies and recommendations for improved practice**. Journal of Cleaner Production 259, 120904.

⁸⁰ European Commission — Joint Research Centre, 2010. **International Reference Life Cycle Data System (ILCD) Handbook — General guide for Life Cycle Assessment — Detailed guidance**. Publications Office of the European Union. Luxembourg, Luxembourg.

remediation treatments). This analysis will help define environmental specifications for BIOSYSMO's technologies while also identifying limitations in conventional approaches.

Comparing emerging technologies with established commercial products can be problematic due to issues such as unclear system boundaries, undefined product functions, and incomplete process details. This comparison may lead to incorrect conclusions⁸¹. Therefore, the primary goal of the current deliverable has been to identify environmental hotspots for BIOSYSMO's technologies and benchmarks, which can be applied to support the scaling-up of these BIOSYSMO's technologies.

The findings will be shared with technical partners to guide environmental improvements during the scaling-up process.

In summary, this study assesses the potential environmental impacts of BIOSYSMO's remediation technologies together with widely used conventional methods. By identifying the most critical environmental hotspots, this approach will support targeted optimizations in the next phase of the project.

2.2 Scope definition

To ensure consistency, it is essential to align the system boundaries for both the LCA and LCC analyses. Accordingly, the same scenarios have been assessed in both analyses, as described in section 3.3 — “System description”, with baseline data provided in section 3.4 — “Data gathered from partners and literature”.

Currently, BIOSYSMO's technologies are at the laboratory scale, with technical partners planning to scale them up soon. As a result, the environmental evaluation has been conducted at this (laboratory) scale. In contrast, benchmark scenarios have been assessed at a mature scale, as relevant literature provides data at this level.

The environmental assessment for BIOSYSMO's and benchmark's technologies includes: i) pollutant removal, ii) required infrastructure for this process and iii) end-of-life management associated with each individual scenario.

At the current scale of BIOSYSMO's technologies (TRL 3-4), results from the biodiversity assessment will not contribute to technological improvements, as BIOSYSMO's have not yet been tested in the field⁸². Consequently, biodiversity impact from BIOSYSMO's technologies is not included in the current deliverable. Additionally, the assessment of ecosystem services provided by NBS depends on the TRL, so that these measurements are not currently feasible⁸³.

⁸¹ A.C. Hetherington, A.L. Borrión, O.G. Griffiths, M.C. McManus, 2013. **Use of LCA as a development tool within early research: Challenges and issues across different sectors**. International Journal of Life Cycle Assessment 19, 130-143.

⁸² S. Harris, G. Tsalidis, J. Berzosa-Corbera, J.J. Espi-Gallart, F. Tegstedt, 2021. **Application of LCA and LCC in the early stages of wastewater treatment design: A multiple case study of brine effluents**. Journal of Cleaner Production 307, 127298.

⁸³ N. Van Cauwenbergh, P.A. Dourojeanni, P. van der Zaag, M. Brugnach, K. Dartee, R. Giordano, E. Lopez-Gunn, 2015. **Beyond TRL – Understanding institutional readiness for implementation of nature-based solutions**. Environmental Science & Policy 127, 293-302.

In D6.5, the LCA will be updated for BIOSYSMO's technologies once they are scaled to TRL 5. Additionally, BSY will include an assessment of biodiversity and ecosystem services, as the scale will then be significant. Furthermore, BSY will adjust the LCAs for conventional physical-chemical treatments, selected as benchmarks for BIOSYSMO's technologies (listed in in Table 2), using the particular specifications of the contaminated site for D6.5. The resulting LCAs will then be compared to validate the environmental sustainability of NBS treatments.

2.3 Functional Unit

The definition of the functional unit forms the foundation of the environmental assessment⁸⁴. In this connection, data related to material and energy requirements along with pollution (to soil, water, and air ecosystems) are compiled according to the functional unit, as they are specified in relation to the function of the product or system established⁸⁵.

Due to the different nature of the systems evaluated in BIOSYSMO, each system (described below) has its own functional unit. The corresponding functional units are specified in the system descriptions below.

Due to the current variation in TRL between BIOSYSMO's and benchmark's technologies, it was not possible to assign a common functional unit across all of them. In D6.5, functional units for comparing BIOSYSMO's technologies with benchmarks will be harmonized, taking into account the specific conditions of the polluted sites. This harmonization will be carried out once BIOSYSMO technologies have been scaled up, their performance at higher TRLs has been established, and their comparability to mature processes has been determining.

3 LCA Methodology

3.1 Assumptions

As mentioned above, BIOSYSMO's scenarios are assumed to be at laboratory scale, which is the current stage of the BIOSYSMO project.

The LCA study used SimaPro 9.6.0.1 software, primarily supported by the Ecoinvent Database (Ecoinvent 3), with the Recipe Midpoint (H) methodology for the LCIA. This approach was selected because it harmonizes two widely used impact assessment methods, CML and Eco-indicator 99, into a single framework⁸⁶. Additionally, the following databases supported the implementation of the LCAs:

⁸⁴ T. Nemecek, X. Bengoa, J. Lansche, A. Roesch, M. Faist-Emmenegger, V. Rossi, S. Humbert, 2020. **World Food LCA Database — Methodological Guidelines for the Life Cycle Inventory of Agricultural Products**. World Food LCA Database (WFLDB). Quantis and Agroscope. Lausanne and Zurich, Switzerland.

⁸⁵ O. Jolliet, M. Saadé-Sbeih, S. Shaked, A. Jolliet, P. Crettaz, 2016. **Environmental Life Cycle Assessment**. CRC Press. London, United Kingdom.

⁸⁶ M.J. Goedkoop, R. Heijungs, M.A.J. Huijbregts, A. De Schryver, J. Struijs, R. Van Zelm, 2013. **ReCiPE 2008: A life cycle impact assessment method which comprises harmonised category indicators at the midpoint and the endpoint level**. https://www.rivm.nl/sites/default/files/2018-11/ReCiPe%202008_A%20lcia%20method%20which%20comprises%20harmonised%20category%20indicator%20at%20the%20midpoint%20and%20the%20endpoint%20level_First%20edition%20Characterisation.pdf (accessed on 26/02/2025).

i) Agri-footprint - economic - system Database, ii) Agri-footprint - economic - unit Database, iii) EU & DK Input Output Database, iv) Industry data 2.0 and v) USLCI Database.

Regarding the data sources, they have been obtained from: i) the partner's background (by means of surveys), ii) literature, iii) relevant reports, iv) the databases included in the SimaPro software (e.g., the Ecoinvent database, as mentioned above) and v) information disseminated by key players. In any case, the highest priority is given to the partners' data, as they are more accurate for our LCA implementation.

The LCA methodology is well-established, with its fundamental principles being scientifically supported. However, no universally accepted method exists for addressing changes in biogenic carbon stocks in biomass, as well as the production and removal of carbon dioxide by living organisms⁸⁷. In this regard, we have reviewed previous cases reported in the literature. Specifically, the recent publication by Favaretto et al. (2025)⁸⁸ have analysed 13 publications of sustainability LCA studies for

⁸⁷ I. Leinonen, 2022. **A general framework for including biogenic carbon emissions and removals in the life cycle assessments for forestry products.** The International Journal of Life Cycle Assessment 27, 1038-1043.

⁸⁸ L. Favaretto, B. Rugani, C. Calfapietra, M. Labra, W.G. Nissim, 2025. **Modelling the environmental implications of phytoremediation in urban settings: A review of the integration of life cycle assessment and ecosystem services approaches.** Urban Forestry & Urban Greening 107, 128788.

phytoremediation treatment between 2010 and 2024^{67,89,90,91,92,93,94,95,96,97,98,99,100,101}. In this connection, they found no homogeneous approach regarding the removal of carbon dioxide due to plant

⁸⁹ P. Suer, Y. Andersson-Sköld, 2011. **Biofuel or excavation? - Life cycle assessment (LCA) of soil remediation options.** Biomass and Bioenergy 35, 969-981.

⁹⁰ N. Witters, R.O. Mendelsohn, S. Van Slycken, N. Weyens, E. Schreurs, E. Meers, F. Tack, R. Carleer, J. Vangronsveld, 2012. **Phytoremediation, a sustainable remediation technology? Conclusions from a case study. I: Energy production and carbon dioxide abatement.** Biomass and Bioenergy 39, 454-469.

⁹¹ N. Witters, R. Mendelsohn, S. Van Passel, S. Van Slycken, N. Weyens, E. Schreurs, E. Meers, F. Tack, B. Vanheusden, J. Vangronsveld, 2012. **Phytoremediation, a sustainable remediation technology? II: Economic assessment of CO₂ abatement through the use of phytoremediation crops for renewable energy production.** Biomass and Bioenergy 39, 470-477.

⁹² M. Vigil, M.F. Marey-Pérez, G. Martinez Huerta, V. Álvarez Cabal, 2015. **Is phytoremediation without biomass valorization sustainable? — Comparative LCA of landfilling vs. anaerobic co-digestion.** Science of The Total Environment 505, 844-850.

⁹³ D. O'Connor, X. Zheng, D. Hou, Z. Shen, G. Li, G. Miao, S. O'Connell, M. Guo, 2019. **Phytoremediation: Climate change resilience and sustainability assessment at a coastal brownfield redevelopment.** Environment International 130, 104945.

⁹⁴ M. Vocciante, A. Caretta, L. Bua, R. Bagatin, E. Franchi, G. Petruzzelli, S. Ferro, 2019. **Enhancements in phytoremediation technology: Environmental assessment including different options of biomass disposal and comparison with a consolidated approach.** Journal of Environmental Management 237, 560-568.

⁹⁵ M. Vocciante, A. de Folly D'Auris, E. Franchi, G. Petruzzelli, S. Ferro, 2021. **CO₂ footprint analysis of consolidated and innovative technologies in remediation activities.** Journal of Cleaner Production 297, 126723.

⁹⁶ J.J. Espada, R. Rodríguez, V. Gari, P. Salcedo-Abraira, L.F. Bautista, 2022. **Coupling phytoremediation of Pb-contaminated soil and biomass energy production: A comparative Life Cycle Assessment.** Science of The Total Environment 840, 156675.

⁹⁷ L.-D. Lin, J.-R. Ho, B.-Y. Yang, C.-H. Ko, F.-C. Chang, 2021. **Life cycle assessment of heavy metal contaminated sites: phytoremediation and soil excavation.** International Journal of Phytoremediation 24, 334-341.

⁹⁸ M.N. Lynch, J.A. Satrio, 2022. **Utilization of Grassy Biomass Grown in Heavy-Metal Contaminated Soil as Feedstock for Bioenergy Production - An LCA Study.** IOP Conference Series: Earth and Environmental Science 1034, 012022.

⁹⁹ G. Todde, G. Carboni, S. Marras, M. Caria, C. Sirca, 2022. **Industrial hemp (Cannabis sativa L.) for phytoremediation: Energy and environmental life cycle assessment of using contaminated biomass as an energy resource.** Sustainable Energy Technologies and Assessments 52, 102081.

¹⁰⁰ T. Soleimani, F. Sordes, I. Techer, G. Junqua, M. Hayek, M. Salgues, J.-C. Souche, 2023. **Comparative environmental and economic life cycle assessment of phytoremediation of dredged sediment using *Arundo Donax*, integrated with biomass to bioenergy valorization chain.** Science of The Total Environment 903, 166160.

¹⁰¹ J.J. Espada, R. Rodríguez, A. Delgado, G. Vicente, L.F. Bautista, 2024. **Assessing Environmental Sustainability of Phytoremediation to Remove Copper from Contaminated Soils.** Sustainability 16, 2441.

metabolism. Furthermore, most publications did not include this carbon dioxide removal in the scope of their LCAs, and thus, we have not included it in our LCA execution.

Estimating material transport data is complex due to uncertainties regarding vehicle type, fuel, distance, and factors like road conditions, terrain, and infrastructure, all of which affect the environmental performance. As a result, environmental impacts associated with transportation has been excluded from our assessments. Nevertheless, transportation impacts are included when transport is an integral part of a scenario (for instance, soil transport in ex-situ treatments). In such cases, the relevant transport details are provided in the specific case study within section 3.3 — System description.

The electricity grid is not homogeneous across Europe, meaning that environmental impacts vary depending on the location. Furthermore, a comparison with benchmarks has not been conducted in the current stage due to the different scale among BIOSYSMO's scenarios and benchmarks. Therefore, the main objective has been the identification of environmental hotspots. As a result, for all scenarios, no regional allocation for electricity has been made, and the general European electricity grid has been used.

3.2 Data compiled for the environmental assessment

The LCI is developed in a comprehensive manner in order to identify and assess the associated environmental impact throughout the entire life cycle, based on the defined functional unit².

When data for minor stages in the LCI is difficult to obtain, it is preferable to exclude these unknown data to minimize the errors resulting from the lack of information. The data excluded contributes no more than $\pm 1\%$ to any environmental impact category, making its impact minimal and insignificant across the entire LCA. Therefore, the resulting error is negligible and virtually non-existent. As a result, this cut-off methodology has been implemented¹⁰².

As mentioned earlier, the data has mostly been collected from partners. In this connection, the table below provides details about the questions the partners answered in the surveys used for data collection.

Table 3. Example of questions for data collection.

Question	Information brought by this question
Description of the system	Key details necessary for defining the scenarios assessed in the LCA
Defining the time required for one trial	An estimate of the time required for the remediation of pollutants
Defining the volume needed for one set of experiments	Establishing the remediation capacity
Diagram outlining the system	A flowchart illustrating the sequence of all steps involved in the scenario

¹⁰² V. Tascione, A. Simboli, R. Taddeo, M. Del Grosso, A. Raggi, 2024. **A comparative analysis of recent life cycle assessment guidelines and frameworks: Methodological evidence from the packaging industry.** Environmental Impact Assessment Review 108, 107590.

List of materials needed specifying their consumption	Quantification of the consumption of each material required for the scenario
Expected lifetime for the system	An estimate of the system's expected lifetime to allocate its derived impact throughout BIOSYSMO's service
List of operations needed for the system construction	Mapping out all the steps involved in the manufacturing of BIOSYSMO's system
List of pollutant specifying the initial and final concentration	Specifying each pollutant degradation as a result of BIOSYSMO's treatment
List of auxiliary materials needed for the remediation	Determining each auxiliary material and its consumption for the pollutants treatment
Determining power consumption	An estimate of power consumption in BIOSYSMOs to allocate its derived impact
List of the materials required for BIOSYSMO's reactors	Determining each material needed for BIOSYSMO's reactor

3.3 System description

All scenarios to be evaluated in the current deliverable are described below, and they are in line with: i) the experimental research performed by the technical partners, ii) the Grant Agreement and iii) the five case study for BIOSYSMO (previously specified). Specifically, all these scenarios are presented in the following table.

Table 4. Cases of study evaluated in Deliverable 6.1.

Cases of study	Name	Data source
Case study 1a	Management of soil pollutants using forests phytoremediation assisted by microorganisms	UPM and UMLP
Case study 1b	Management of estuarine pollution through bacteria-assisted sediment-based phytoremediation	CIIMAR, JSI and IDE
Case study 1c	Management of soil pollutants using S-S technology	Literature
Cases study 2a	Management of groundwater pollutants using BES technology	LEITAT
Case study 2b	Management of heavy metals in groundwater by means of the use of zeolites adsorption	Literature
Case study 2c	Management of organic compounds in groundwater by means of the use of the photo Fenton technology	Literature
Case study 3a	Management of heavy metals in groundwater using a hybrid system combining water phytoremediation and BES	UBU and LEITAT
Case study 3b	Management of heavy metals in groundwater using "P&T technology"	Literature
Case study 4	Microorganisms' inoculation strategy	Literature

3.3.1 Management of soil pollutants using forests phytoremediation assisted by microorganisms – Case study 1a

This synergy between plants and microorganisms creates an optimal environment for pollutant removal, which has been considered in the case study 1a. This case of study has considered a previous fungal inoculum from UMLP to be inoculated in poplars cultivated by UPM for the removal of cadmium, zinc, lead and PAH.

Specifically, for research on poplar growth, BSY evaluated UPM's operations at the laboratory level. In this sense, UPM analysed poplar genotypes and their growth potential along with their pollutant uptake capacity. To this end, they cultivated thirty-six poplar trees, divided as follows: i) six wild-type trees as a control group, ii) six transgenic trees as a control group, iii) six wild-type trees for zinc uptake, iv) six transgenic trees for zinc uptake, v) six wild-type trees for cadmium uptake, and vi) six transgenic trees for cadmium uptake. Under these conditions, the experiment lasted 2016 hours (12 weeks), during which the concentrations of soil pollutants (zinc and cadmium) were maintained constant at $7.85 \cdot 10^{-5}$ g/mL, and $5.62 \cdot 10^{-5}$ g/mL, respectively. As a result, the pollutant removal capacities were determined as follows:

- For wild-type trees: $4.41 \cdot 10^{-7}$ (zinc), and $4.00 \cdot 10^{-8}$ g/mg (cadmium).
- For transgenic trees: $8.39 \cdot 10^{-7}$ g/mg (zinc), and $5.80 \cdot 10^{-8}$ g/mg (cadmium).

Based on information provided by UPM, BSY has identified 5 phases for the execution of the LCA, as shown in the figure below.

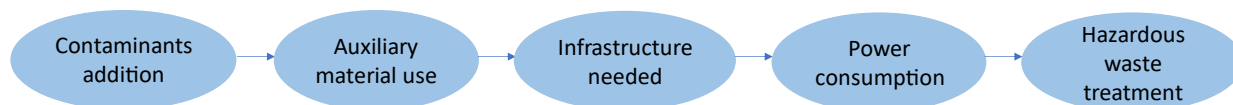


Figure 5. Phases included in the LCA execution for poplars cultivation – Case study 1a.

In addition, for fungus supporting the phytoremediation, BSY has evaluated the UMLP's operation at laboratory level. In this connection, UMLP has researched the microbial support by means of using an existing microbial collection. This collection has been growth using potato glucose agar in Petri dishes. Moreover, the spores have been quantified by means of Malassez cells. Additionally, the fungus growth was supported by the use of the following equipment: i) a microbial incubator, ii) an autoclave, iii) a laminar flow cabinet, iv) a pH meter, v) a balance and vi) a freezer. Based on information provided by UMLP, BSY has identified two phases for the execution of the LCA, as shown in the figure below.

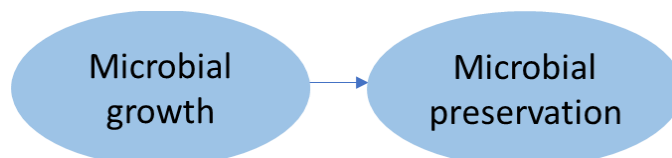


Figure 6. Phases included in the LCA execution for microbial cultivation – Case study 1a.

At the current stage of the research, full integration of the poplar with the microorganisms has not yet been achieved. Consequently, they have been studied separately. The environmental impacts connected to the integration will be examined in D6.5.

3.3.2 Management of estuarine pollution through bacteria-assisted sediment-based phytoremediation – Case study 1b

The situation for case study 1b is analogous to that of case study 1a, where the interaction between plants and microorganisms significantly enhances pollutant removal. In short, this symbiotic relationship accelerates the removal of pollutants.

Specifically, CIIMAR has contributed to LCA by providing data on: i) the research conducted to determine the bioremediation potential of *Juncus maritimus* and ii) the preparation of the microbial inoculum.

The bioremediation potential of *Juncus maritimus* was evaluated through a seven-day experiment focused on the removal of cadmium and ketoprofen. These contaminants were introduced at the beginning of the experiment, with additional doses of ketoprofen added throughout the week. Under these conditions, the phytoremediation process demonstrated removal efficiencies of 94% for cadmium and 67% for ketoprofen. Based on information provided by CIIMAR, BSY has identified two phases for the execution of the LCA, as shown in the figure below.

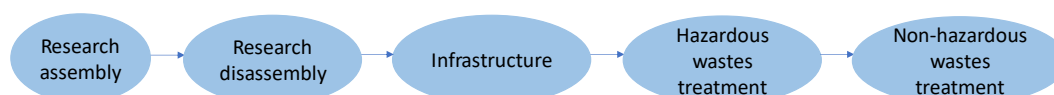


Figure 7. Phases included in the LCA execution for *Juncus maritimus* phytoremediation – Case study 1b.

The microbial inoculum has already been cryopreserved in CIIMAR laboratory. Consequently, microbial isolation and purification have not been accounted for our assessment. Based on information provided by CIIMAR, BSY has identified four phases for the execution of the LCA, as shown in the figure below.

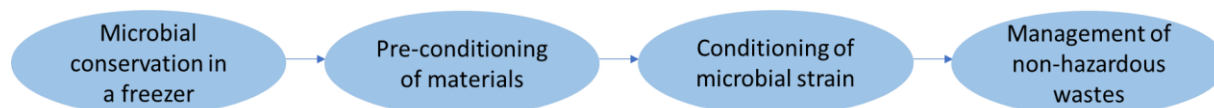


Figure 8. Phases included in the LCA execution for microbial growth – Case study 1b.

This data from CIIMAR has been further supplemented with information from the literature to provide a comprehensive understanding of the material and energy consumption for the *Juncus maritimus* phytoremediation supported by a microbial inoculum^{103,104,105}.

At the current stage of the research, full integration of the *Juncus maritimus* with the microorganisms isolated from estuary sediments has not yet been achieved. Consequently, they have been studied separately. The environmental impacts connected to the integration will be examined in D6.5.

¹⁰³ C.S.C. Calheiros, J. Carecho, M.P. Tomasino, M.R. Almeida, A.P. Mucha, 2020. **Floating Wetland Islands Implementation and Biodiversity Assessment in a Port Marina.** Water 12, 3273.

¹⁰⁴ R.S. Matias, S. Gomes, L.G.A. Barboza, C.M.R. Almeida, A. Marques, L. Guilhermino, L.M.P. Valente, 2024. **Occurrence of microplastics and metals in European seabass produced in different aquaculture systems: Implications for human exposure, risk, and food safety.** Science of The Total Environment 929, 172535.

¹⁰⁵ S. Cavenati, P.N. Carvalho, C.M.R. Almeida, M.C.P. Basto, M.T.S.D. Vasconcelos, 2012. **Simultaneous determination of several veterinary pharmaceuticals in effluents from urban, livestock and slaughterhouse wastewater treatment plants using a simple chromatographic method.** Water Science & Technology 66, 603-611.

3.3.3 Management of soil pollutants using S-S technology — Case study 1c

S/S technology is a two-step ex-situ process: i) stabilization and ii) solidification. Stabilization encapsulates soil pollutants into a monolithic solid while maintaining adequate structural integrity. On the other hand, solidification reduces leachate movement through adsorption, precipitation, or the incorporation of pollutants into the monolithic solid¹⁰⁶. In this connection, S-S technology is a conventional waste management technology focused on soil treatment with a long history of implementation that has become increasingly attractive in recent years, as it is able to isolate contaminants (i.e., heavy metals) in soils and sediments^{107,108}. The binder for the current assessment is Portland cement, as this material exhibits low permeability (which helps avoid the generation of harmful leachates) along with strength and resistance in the presence of heavy metals¹⁰⁸.

In defining the pollutants to be treated by S-S-technology, the information provided in the Grant Agreement, Deliverable 1.1, and by the US Government¹⁰⁹ has been considered. In this context, the LCA is applied to the full treatment of 6 hectares of soil contaminated by heavy metals (1,002 mg heavy

¹⁰⁶ F. Jin, 2020. **Long-term effectiveness of in situ solidification/stabilization**. In: D. Hou (Ed.), **Sustainable Remediation of Contaminated Soil and Groundwater — Materials, Processes, and Assessment**. Butterworth-Heinemann. Oxford, United Kingdom.

¹⁰⁷ W.S. Adaska, S.W. Tresouthick, P.B. West, 1991. **Solidification and Stabilization of Wastes Using Portland Cement**. Portland Cement Association. Minnesota, United States of America.

¹⁰⁸ V.R. Ouhadi, R.N. Yong, M. Deiranlou, 2021. **Enhancement of cement-based solidification/stabilization of a lead-contaminated smectite clay**. Journal of Hazardous Materials 403, 123969.

¹⁰⁹ United States Government, 2025. **Solidification and Stabilization**. <https://www.frtr.gov/matrix/Solidification-and-Stabilization/> (Accessed on 03/03/2025).

metals per kg of soil) using S-S technology. For this assessment, BSY has utilized data from the literature, which is specified below^{110,111,112,113,114,115,116,117,118,119,120,121,122,123,124}.

¹¹⁰ C. Turco, A.C.P. Junior, E.R. Teixeira, R. Mateus, 2021. **Optimisation of Compressed Earth Blocks (CEBs) using natural origin materials: A systematic literature review.** Construction and Building Materials 309, 125140.

¹¹¹ J.G. Speight, 2011. **Production, properties and environmental impact of hydrocarbon fuel conversion.** In: M.R. Khan (Ed.), **Advances in Clean Hydrocarbon Fuel Processing — Science and Technology — A volume in Woodhead Publishing Series in Energy.** Woodhead Publishing. Cambridge, United Kingdom.

¹¹² G.E. Voglar, D. Leštan, 2011. **Efficiency modeling of solidification/stabilization of multi-metal contaminated industrial soil using cement and additives.** Journal of Hazardous Materials 192, 753-762.

¹¹³ M.A. Helsel, C.F. Ferraris, D. Bentz, 2016. **Comparative Study of Methods to Measure the Density of Cementitious Powders.** Journal of Testing and Evaluation 44, 2147-2154.

¹¹⁴ A. Atmaca, N. Atmaca, 2016. **Determination of correlation between specific energy consumption and vibration of a raw mill in cement industry.** Anadolu University Journal of Science and Technology A - Applied Sciences and Engineering 17, 209-219.

¹¹⁵ A.A. Emin, S. Hasan, S. John, B. Ebru, P. Mehmet, 2017. **Evaluating the Effects of Improving Forest Road Standards on Economic Value of Forest Products.** Croatian Journal of Forest Engineering 42, 245-258.

¹¹⁶ M.J. Harbottle, A. Al-Tabbaa, C.W. Evans, 2007. **A comparison of the technical sustainability of in situ stabilization/solidification with disposal to landfill.** Journal of Hazardous Materials 141, 430-440.

¹¹⁷ British Columbia Government, 2010. **Productivity Estimating Guide.** https://www2.gov.bc.ca/assets/gov/farming-natural-resources-and-industry/forestry/timber-pricing/interior-timber-pricing/north_area_ece_productivity_estimating_guide.pdf (accessed on 03/03/2025).

¹¹⁸ Sino Heavy Vehicles, 2025. **HOWO 14m³ to 18m³ Concrete Mixer Truck.** https://www.sinoheavyvehicles.com/e_productshow/?19-HOWO-14m3-to-18m3-Concrete-Mixer-Truck-19.html (accessed on 03/03/2024).

¹¹⁹ SinoWay, 2025. **Specification Sheet for Concrete Truck Mixers.** https://sinoway-sh.com/concrete_truck_mixer/672.html (accessed on 03/03/2025).

¹²⁰ C. Bouma, 2025. **Average Weight (kg) of a Farm Tractor (11 examples).** <https://tractoraddict.com/average-weight-kg-of-a-farm-tractor-30-examples/#:~:text=Most%20standard%20utility%20farm%20tractors,%2C%20on%20average%2C%202%2C388%20kilograms>. (Accessed on 03/03/2025).

¹²¹ Rydell Chevrolet, 2021. **How long do trucks last?** <https://www.rydellchev.com/blogs/740/rydell-chevrolet-buick-gmc-cadillac-news/how-long-do-trucks-last/> (Accessed on 03/03/2025).

¹²² Volvo Trucks, 2025. **Volvo FH specifications — Everything you need to know about the Volvo FH, in detail.** <https://www.volvotrucks.com.au/en-au/trucks/models/volvo-fh/specifications.html#accordion-c704afa2e8-item-c8cb16cfb7> (Accessed on 03/03/2025).

¹²³ M. Sörengård, D.B. Kleja, L. Ahrens, 2019. **Stabilization and solidification remediation of soil contaminated with poly- and perfluoroalkyl substances (PFASs).** Journal of Hazardous Materials 367, 639-646.

¹²⁴ I.T. Mercante, M.D. Bovea, V. Ibáñez-Forés, A.P. Arena, 2012. **Life cycle assessment of construction and demolition waste management systems: a Spanish case study.** The International Journal of Life Cycle Assessment 17, 232-241.

Based on all the information gathered, BSY has identified four phases for the execution of the LCA, as shown in the figure below.

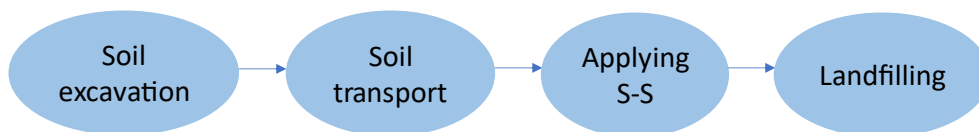


Figure 9. Phases included in the LCA execution — Case study 1c.

The estimated duration of the treatment is approximately 3.29 years in accordance with BSY's estimation. During this time, contaminated soil is excavated for pollutant removal and transported by heavy-duty trucks to a treatment facility located 50 km away. At the facility, activated carbon is applied as an additive, followed by encapsulation using Portland cement. The resulting treated material is then disposed of in a conventional landfill located 25 km from the facility, according to reference ¹²⁴.

3.3.4 Management of groundwater pollutants using BES technology — Case of study 2a

Within the BIOSYSMO project, three base BES scenarios are under technical development: (i) BES for hydrocarbon removal (BES-HC), (ii) BES for metal removal (BES-M) and BES for removal of organochloride compounds (BES-CE).

BES-HC is being applied for treating groundwater at Site 7, which is contaminated with TPHs and BTEX compounds. Treatment takes place in flat-plate BES reactors and results in decontaminated groundwater. A peristaltic pump is used to continuously circulate the groundwater through the reactors and buffer tanks, while a potentiostat is needed to control and monitor both the system and reactor operation. Additionally, auxiliary equipment (such as tubing) is required.

On the other, BES-M is used for treating groundwater at Site 11, which is contaminated with metalloids compounds. This treatment is conducted in a BES-M reactor built by LEITAT, which is composed of: i) endplates, ii) current collectors, iii) reactors chambers, iv) anode, v) cathode, vi) AEM (FAB-pk-130 Fumasep), vii) CEM (FKB-pk-130 Fumasep), viii) Tyvek membrane, ix) spacers and x) auxiliary components (sealing gaskets, screws & nuts and tubing).

LEITAT has provided data on the construction of both BES-HC and BES-M systems. This information has been incorporated into the environmental assessment to ensure alignment with sustainability

goals. To strengthen this assessment, additional data from the literature has been used to provide a comprehensive overview of the material consumption associated with BES construction^{125,126,127,128,129}.

Based on information provided by LEITAT, BSY has identified three phases for the execution of the LCA related to BES-HC, as shown in the figure below.

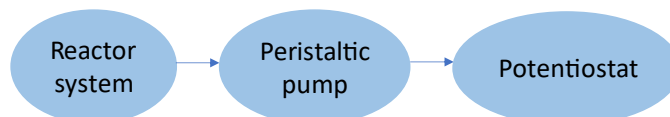


Figure 10. Phases included in the LCA execution related to BES-HC construction — Case study 2a.

Based on information provided by LEITAT, BSY has identified four phases for the execution of the LCA related to BES-M, as shown in the figure below.

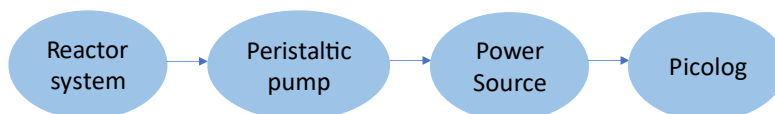


Figure 11. Phases included in the LCA execution related to BES-M construction — Case study 2a.

At present, LEITAT is still developing the BES-CE system and working on the operational characterization of all BES configurations. The environmental impacts associated with these developments will be evaluated in D6.5.

3.3.5 Management of heavy metals in groundwater by means of the use of zeolites adsorption — Case of study 2b

Adsorption may be the most suitable alternative for removing heavy metals from groundwater due to several advantages: i) cost-effectiveness, ii) ease of operation, iii) no additional costs for sludge removal, iv) quick processing time, and v) versatility in various applications¹³⁰. In this context,

¹²⁵ V. Koskue, V.-P. Pyrhönen, S. Freguia, P. Ledezma, M. Kokko, 2022. **Modelling and techno-economic assessment of (bio)electrochemical nitrogen removal and recovery from reject water at full WWTP scale.** Journal of Environmental Management 319, 115747.

¹²⁶ Media Mark, 2025. **Portátil gaming - HP Victus 15-fa0052ns, 15.6" Full HD, Intel® Core™ i5-12450H, 16GB RAM, 512GB SSD, RTX™ 3050, Sin sistema operativo, Plata mica.** <https://www.mediamarkt.es/es/product/portatil-gaming-hp-victus-15-fa0052ns-156-full-hd-intel%C2%AE-corei5-12450h-16gb-ram-512gb-ssd-rtxtm-3050-sin-sistema-operativo-plata-mica-1564063.html> (accessed on 08/04/2025).

¹²⁷ HP, 2024. **What is the Average Lifespan of a Computer in the UK?** <https://www.hp.com/gb-en/shop/tech-takes/average-computer-lifespan#:~:text=While%20a%20well%2Dmaintained%20computer,from%20three%20to%20eight%20years> (accessed on 08/04/2025).

¹²⁸ Ossila, 2025. **Potentiostat — Electrochemistry Supplies, Lab Equipment.** <https://www.ossila.com/products/potentiostat> (accessed on 08/04/2025).

¹²⁹ Biorad, 2025. **Mini-PROTEAN® Spacer Plates with 1.0 mm Integrated Spacers #1653311.** <https://www.biorad.com/es-es/sku/1653311-mini-protean-spacer-plates-with-1-0-mm-integrated-spacers?ID=1653311> (accessed on 08/04/2025).

¹³⁰ L. Velarde, M.S. Nabavi, E. Escalera, M.-L. Antti, F. Akhtar, 2023. **Adsorption of heavy metals on natural zeolites: A review.** Chemosphere 328, 138508.

adsorption using zeolites demonstrates very high selectivity for removing heavy metals, as well as the ability to treat nitrified liquids¹³⁰. Furthermore, the use of zeolites in adsorption is: i) widely adopted, ii) highly efficient and iii) commercially sustainable alternative for heavy metals removal. Concerning this, the alkali alteration of zeolites further enhances their ability to remove these pollutants (heavy metals). As a result, BSY has selected this technology as the benchmark for groundwater treatment using BES for heavy metal management. The treatment has been evaluated at an industrial scale¹³¹.

In this connection, BSY has calculated the energy and material consumption together with the emissions associated with treating 1 litter of groundwater, as outlined in ¹³². Additional literature information has been incorporated to further support these calculations^{133,134,135,136}.

Based on all the information gathered, BSY has identified three phases for the execution of the LCA, as shown in the figure below.

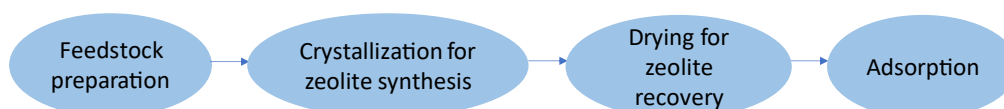


Figure 12. Phases included in the LCA execution — Case study 2b.

3.3.6 Management of organic compounds in groundwater by means of the use of the photo Fenton technology — Case of study 2c

The presence of organic compounds in groundwater can have negative ecological impacts, making it challenging to define a minimum threshold value for their concentration. Additionally, these compounds affect the overall ecological health of water bodies, making their removal imperative¹³⁷. Specifically,

¹³¹ B.S. Rathi, P.S. Kumar, J.N.I. Susana, J.F. Virgin, R. Dharani, S. Sanjay, G. Rangasamy, 2024. **Recent research progress on the removal of heavy metals from wastewater using modified zeolites: A critical review.** Desalination and Water Treatment 319, 100573.

¹³² J. Yu, 2007. **Synthesis of Zeolites.** In J. Čejka, H. van Bekkum, A. Corma, F. Schüth (Eds.), **Studies in Surface Science and Catalysis — Studies in Surface Science and Catalysis — Volume 168.** Elsevier. Amsterdam, The Netherlands

¹³³ M.R. Mirbaloochzehi, A. Rezvani, A. Samimi, M. Shayesteh, 2020. **Application of a Novel Surfactant-Modified Natural Nano- Zeolite for Removal of Heavy Metals from Drinking Water.** Advanced Journal of Chemistry-Section A, S612-S620.

¹³⁴ A. Mojiri, E. Razmi, B.K. Dermani, S. Rezania, N. Kasmuri, M. Vakili, H. Farraji, 2024. **Adsorption methods for arsenic removal in water bodies: a critical evaluation of effectiveness and limitations.** Frontiers in Water 6, 1301648.

¹³⁵ A. Khaleque, M.M. Alam, M. Hoque, S. Mondal, J.B. Haider, B. Xu, M.A.H. Johir, A.K. Karmakar, J.L. Zhou, M.B. Ahmed, M.A. Moni, 2020. **Zeolite synthesis from low-cost materials and environmental applications: A review.** Environmental Advances 2, 100019.

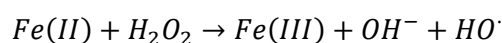
¹³⁶ M.F. Mubarak, A.M.G. Mohamed, M. Keshawy, T.A. el-Moghny, N. Shehata, 2022. **Adsorption of heavy metals and hardness ions from groundwater onto modified zeolite: Batch and column studies.** Alexandria Engineering Journal 61, 4189-4207.

¹³⁷ J.W. Roy, L. Grapentine, G. Bickerton, 2018. **Ecological effects from groundwater contaminated by volatile organic compounds on an urban stream's benthic ecosystem.** Limnologica 68, 115-129.

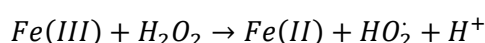
organic compounds in water can exist in three forms: i) floating on the liquid surface, ii) emulsified in water and iii) dissolved in water^{137,138}.

The two first forms are typically removed using gravity-based methods, often combined with chemical additives to promote the formation of flocculants or coagulants. In contrast, the removal of dissolved organic compounds requires chemical or biological reactions, or the application of adsorption techniques¹³⁸.

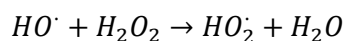
In this context, photo-Fenton technology is an advanced treatment method used to remove recalcitrant organic compounds. It employs hydroxyl radicals generated from hydrogen peroxide and iron to degrade these substances. The following equations describe the radical formation process involved¹³⁹:



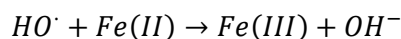
Equation 1



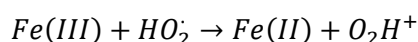
Equation 2



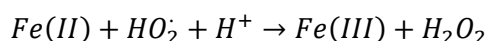
Equation 3



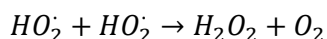
Equation 4



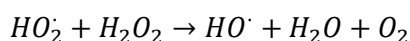
Equation 5



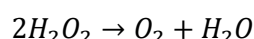
Equation 6



Equation 7



Equation 8



¹³⁸ S. Jafarinejad, 2017. **Treatment of Oily Wastewater**. In: S. Jafarinejad (Ed.), **Petroleum Waste Treatment and Pollution Control**. Elsevier. Amsterdam, The Netherlands.

¹³⁹ J.J. Pignatello, E. Oliveros, A. MacKay, 2006. **Advanced Oxidation Processes for Organic Contaminant Destruction Based on the Fenton Reaction and Related Chemistry**. Critical Reviews in Environmental Science and Technology 36, 1-84.

Equation 9

BSY has integrated literature information from references^{26,139,140,141,142}. Based on this, energy and material consumption, along with emissions, were calculated for a treatment of 2 years at Site 7.

Based on all the information gathered, BSY has identified three phases for the execution of the LCA, as shown in the figure below.

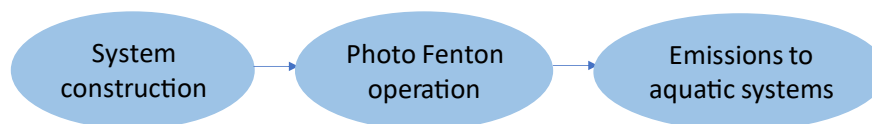


Figure 13. Phases included in the LCA execution — Case study 2c.

3.3.7 Management of heavy metals in groundwater using a hybrid system combining water phytoremediation and BES — Case of study 3a

The integration of water phytoremediation with BES allows for effective water treatment by combining: (i) the uptake capacity of wetland plants and (ii) the microbial activity in BES stimulated by electrical currents. This synergistic approach enhances the removal of organic matter, nutrients, heavy metals, and other micropollutants, while promoting sustainability through low energy requirements and ecological compatibility^{49,143}.

This integrated system will be implemented in the coming months as part of the BIOSYSMO project. In this context, LEITAT will characterize the operation of BES in line with case study 2a. Meanwhile, UBU is currently assessing the performance of water phytoremediation. Based on UBU results, BSY has evaluated the LCA related to this process. In D6.5, BSY will adapt the LCA scenario based on experimental results obtained by technical partners, including the performance of the BES technology.

Specifically, UBU has evaluated the water phytoremediation process of polluted groundwater in a greenhouse experiment conducted at laboratory scale. Approximately 197 liters of polluted groundwater were treated in wetland mesocosms over eight months. During the first four months, polluted water was recirculated at a flow rate of 1.54 L per week. In the following four months, only clean water was added to compensate for evapotranspiration. A total of eight mesocosms were interconnected, each with a maximum operational volume of 70 liters and a void space of 38%

Based on the data collected from the phytoremediation study, BSY has identified two phases for LCA execution, as shown in the figure below. The final products of the water phytoremediation process are treated materials free of contaminants: (i) gravel, (ii) treated groundwater, and (iii) biomass. Consequently, BSY incorporated an additional phase considering the potential exploitation of these materials. However, this scenario is currently limited by the low TRL and is not yet feasible.

¹⁴⁰ M. Magdy, M.G. Alalm, H.K. El-Etriby, 2021. **Comparative life cycle assessment of five chemical methods for removal of phenol and its transformation products.** Journal of Cleaner Production 291, 125923.

¹⁴¹ S. Sumardi, F.R Mufakhir, Y.I Supriyatna, W. Astuti, Sudibyo, A.S. Handoko, A. Febrisiantosa, W. Agustina, D.N. Surahman, 2022. **Processing industrial iron waste into ferrous sulfate as an iron fortification: A preliminary study.** IOP Conference Series: Earth and Environmental Science 1017, 012029.

¹⁴² C. Corbella, J. Puigagut, M. Garfí, 2017. **Life cycle assessment of constructed wetland systems for wastewater treatment coupled with microbial fuel cells.** Science of The Total Environment 584-585, 355-362.

¹⁴³ B. Saba, M. Khan, A.D. Christy, B.V. Kjellerup, 2019. **Microbial phyto-power systems – A sustainable integration of phytoremediation and microbial fuel cells.** Bioelectrochemistry 127, 1-11.

Nevertheless, the potential for system expansion presents an interesting opportunity for future development.

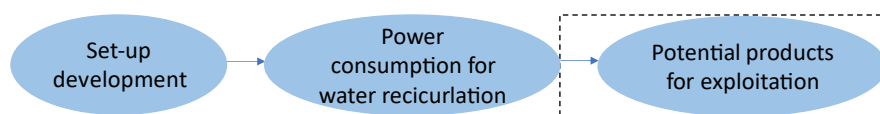


Figure 14. Phases included in the LCA execution — Case study 3a (including the system expansion).

3.3.8 Management of heavy metals in groundwater using “P&T technology” — Case of study 3b

P&T is a well-established and mature technology that has been widely used for decades to remediate groundwater contaminated with dissolved-phase pollutants, such as solvents, metals, and fuel oils^{144,145,146}. The core principle of this treatment involves extracting contaminated groundwater to the surface, where it undergoes purification to remove harmful contaminants. This process not only helps eliminate pollutants but also prevents their migration into clean ecosystems and drinking water sources^{147,148}.

While P&T is highly effective in the initial stages, it faces challenges in long-term performance, with residual contamination often remaining and treatment efficiency decreasing over time. However, it continues to be an essential method for free product recovery and plume containment. In recent years, P&T technology has evolved, incorporating more sustainable and efficient techniques to enhance pollutant removal. Some of the advanced methods currently applied during the pollutant removal stage include: i) ISCO, ii) in-situ surfactant/co-solvent flushing, iii) SEAR, iv) in-situ thermal management treatment, v) in-situ bioremediation, vi) in-situ chemical reduction, vii) in-situ contaminant desorption, viii) in-situ chemical precipitation of metals, ix) in-situ ion exchange, x) in-situ air stripping, xi) in-situ adsorption using activated carbon, xii) in-situ reverse osmosis, xiii) in-situ chemically assisted clarification, xiv) in-situ filtration, and xv) in-situ ultraviolet radiation oxidation^{149,150}.

¹⁴⁴ P. Ciampi, C. Esposito, M.P. Papini, 2024. **Review on groundwater circulation wells (GCWs) for aquifer remediation: State of the art, challenges, and future prospects.** Groundwater for Sustainable Development 24, 101068.

¹⁴⁵ G. Bartow, C. Davenport, 1995. **Pump-and-Treat Accomplishments: A review of the Effectiveness of Ground Water Remediation in Santa Clara Valley, California.** Groundwater Monitoring & Remediation 15, 140-146.

¹⁴⁶ M.L. Brusseau, 2013. **Use of Historical Pump-and-Treat Data to Enhance Site Characterization and Remediation Performance Assessment.** Water, Air and Soil Pollution, 224, 1741.

¹⁴⁷ J.G. Speight, 2020. **Remediation technologies.** In: J.G. Speight (Ed.), **Natural Water Remediation — Chemistry and Technology.** Butterworth-Heinemann. Oxford, United Kingdom.

¹⁴⁸ K.C. Carroll, M.L. Brusseau, G.R. Tick, M.R. Soltanian, 2024. **Rethinking pump-and-treat remediation as maximizing contaminated groundwater.** Science of The Total Environment 918, 170600.

¹⁴⁹ A.T. Besha, D.N. Bekele, R. Naidu, S. Chadalavada, 2018. **Recent advances in surfactant-enhanced In-Situ Chemical Oxidation for the remediation of non-aqueous phase liquid contaminated soils and aquifers.** Environmental Technology & Innovation 9, 303-322.

¹⁵⁰ Environmental Protection Agency, 1996. **Pump-and-Treat Ground-Water Remediation — A Guide for Decision Makers and Practitioners.** <https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockkey=30004PC8.TXT> (accessed on 27/02/2025).

The treated water from the P&T system is either released into adjacent sewage systems or re-injected into the aquifer, depending on the specific site and regulatory requirements. One of the main advantages of P&T technology is its hydraulic control, as the pumping rate can be adjusted according to the needs of subsequent remediation treatments^{147,148}.

For the effective design and implementation of a P&T facility, several factors must be considered, including: i) extraction rates, ii) the duration of operations, iii) influent water quality, iv) alternatives for primary treatment technologies, v) selection of chemical and process materials, vi) collection and disposal of treatment waste, vii) effluent management and associated standards, viii) electricity¹⁵¹.

¹⁵¹ US Environmental Protection Agency, 2009. **Green Remediation Best Management Practices: Pump and Treat Technologies.** https://19january2017snapshot.epa.gov/sites/production/files/2015-04/documents/gr_fact_sheet_pt_12-31-2009.pdf (accessed on 27/02/2025).

For this assessment, BSY has utilized data from the literature, which is specified below
152,153,154,155,156,157,158,159,160,161,162,163,164,165,166,167,168,169,170.

Based on all the information gathered, BSY has identified five phases for the execution of the LCA, as shown in the figure below.

¹⁵² A. Casasso, T. Tosco, C. Bianco, A. Bucci, R. Sethi, 2020. **How Can We Make Pump and Treat Systems More Energetically Sustainable?** Water 12, 67.

¹⁵³ P.Y. Baneh, B. Ahmadi, H. Salehzadeh, H. Mohammadi, B. Shahmoradi, B. Ghaderi, 2024. **Assessment of heavy metal contamination in groundwater of rural areas of Kurdistan Province Iran: A comprehensive study.** Heliyon 10, e39833.

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¹⁵⁹ US Environmental Protection Agency, 2025. **Community Guide to Pump and Treat.** <https://semspub.epa.gov/work/HQ/401617.pdf> (accessed on 10/04/2025).

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¹⁶¹ GAP Group, 2025. **4" Sewage Pump.** <https://www.gap-group.co.uk/catalogue/4-sewage-pump> (accessed on 10/04/2025).

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¹⁶⁴ C. Yu, C. Loureiro, J.-J. Cheng, L.G. Jones, Y.Y. Wang, Y.P. Chia, E. Faillace, 1993. **Data Collection Handbook to Support Modeling the Impacts of Radioactive Material in Soil.** <https://www.osti.gov/servlets/purl/10162250> (accessed on 10/04/2025).

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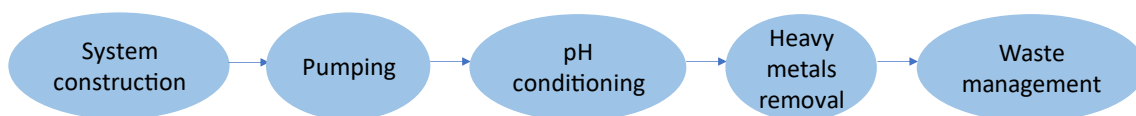


Figure 15. Phases included in the LCA execution — Case study 3b.

The effect of re-injecting or exploiting groundwater will be assessed in D6.5.

3.3.9 Microorganisms' inoculation strategy — Case of study 4

The microbial inoculation is a critical step for effective removal, as living organisms must exhibit the proper concentration and activity¹⁷¹. In this regard, the process should not be treated as a 'black-box,' as the conditions and proper strain selection are crucial¹⁷². In this way, BSY has collected the data reported by Zhao et al. (2017)¹⁷³ for the environmental assessment of the contamination for a constructed wetland using bioaugmentation.

Based on this information, three phases are included in the execution of the LCA, as shown in the figure below.

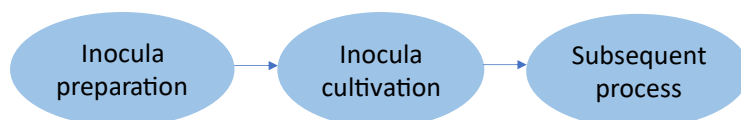


Figure 16. Phases included in the LCA execution — Case study 4.

3.4 Data gathered from partners and literature

As mentioned above, the LCI phase is a crucial phase for the execution of the LCA. For this, data from partners and literature have been compiled.

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¹⁶⁷ Aqion, 2025. **Acidification with Strong Acids to pH 3.** <https://www.aqion.de/site/118> (accessed on 10/04/2025).

¹⁶⁸ Immersion Heaters UK Ltd, 2025. **Water Heating Calculator.** <https://immersionheaters.uk/heating-calculators/calculate-the-time-required-to-heat-a-volume-of-water/> (accessed on 10/04/2025).

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3.4.1 Case of study 1a

The data compiled for the LCI related to poplars cultivation is presented in the table below, specified for the functional unit of this case of study (12 weeks of research cultivating poplars).

Table 5. LCI for Case study 1a — Poplar information.

Input/output	Element	Amount
Phase 1 — Contaminants addition		
Input	ZnCl₂	28.55 g
Input	CdCl₂	15.30 g
Phase 2 — Auxiliary material use		
Input	Pots	36 units
Input	Perlite	6,480 g
Input	Water	302.4 L
Phase 3 — Infrastructure needed		
Input	One Aralab Fitoclima 20000 PL growth chamber	0.71% of total service
Input	Two Sanyo Fitotron SGC097 growth chambers	0.09% of total service
Phase 4 — Power consumption		
Input	Power consumption	1,154.16 kWh
Phase 5 — Hazardous waste treatment		
Output	Management of non-hazardous waste	6,480 g of perlite

The SimaPro software databases have been supplemented with ^{174,175,176,177,178,179,180}.

The data compiled for the LCI related to fungus cultivation is presented in the table below, specified for the functional unit of this case of study (3 months of research cultivating microorganisms).

Table 6. LCI for Case study 1a — Microorganisms information.

Input/output	Element	Amount
Phase 1 — Microbial growth		
Input	Potato glucose agar	1 kg
Input	Petri dishes	200 units
Input	Malassez cells for counting spores	4 units
Input	Microbial incubator	0.33% of total service
Input	Autoclave	0.20% of total service
Input	Laminar flow cabinet	0.15% of total service
Input	pH meter	0.42% of total service
Input	Laboratory balance	0.25% of total service

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¹⁸⁰ FDM, 2025. **Compra tu cámara climática FDM — Optimiza tu investigación con cámaras climáticas — FDM, garantizando precisión y fiabilidad.** https://fdm.enviromakers.it/es?cid=search-chambers-es&gad_source=1&gbraid=0AAAAADu3dvuiR33esod4Ejj2Vny66fXQr&gclid=Cj0KCQjw2ZfABhDBARIsAHFTxGyOnCAX1BBYtxemqBIJlev6ScRQ5v9vdSlrve0phxM8opHWxrHuwV4aAuDVEALw_wcB (accessed on 21/04/2025).

Input	Power consumption for laboratory equipment (microbial incubator autoclave, laminar flow cabinet and pH meter)	8,400 kWh
Phase 2 — Microbial preservation		
Input	Laboratory freezer	0.84% of total service
Input	Power consumption for laboratory freezer	900 kWh

The SimaPro software databases have been supplemented with
181,182,183,184,185,186,187,188,189,190,191,192,193,194,195,196,197,198,199,200,201,202,203.

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¹⁸² VWR, 2025. **Laboratory freezers, MediLine, LGPv series**. <https://es.vwr.com/store/product/9092483/laboratory-freezers-medi-line-lgpv-series> (accessed on 08/04/2025).

¹⁸³ Eppendorf, 2025. **Freezer Racks - Ultra Low Temperature Freezer**. <https://www.eppendorf.com/ca-en/Products/Cold-Storage/Freezer-Racks/Freezer-Racks-p-PF-229186#:~:text=Standard%20freezer%20racks%20are%20available,each%20with%20its%20own%20benefits> (accessed on 08/04/2025).

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¹⁸⁶ Novolab, 2025. **Counting chamber Malassez**. <https://www.novolab-labware.com/counting-chamber-malassez.html> (accessed on 08/04/2025).

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¹⁸⁸ Microbe Notes, 2024. **Incubator Uncovered: Types, Uses & Precautions**. <https://microbenotes.com/incubator/#:~:text=The%20outer%20wall%20is%20made,smooth> (accessed on 08/04/2025).

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¹⁹¹ Wikipedia, 2025. **pH meter**. [https://en.wikipedia.org/wiki/PH_meter#:~:text=It%20usually%20has%20a%20glass.conditions\)%20or%20the%20quinhydrone%20electrode](https://en.wikipedia.org/wiki/PH_meter#:~:text=It%20usually%20has%20a%20glass.conditions)%20or%20the%20quinhydrone%20electrode) (accessed on 08/04/2025).

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¹⁹³ BioSan, 2025. **Magnetic Stirrer with hot plate**. <https://biosan.lv/products/general-lab-equipment/-magnetic-mechanical-stirrers/-msh-300-magnetic-stirrer-with-hot-plate/> (accessed on 08/04/2025).

¹⁹⁴ Jackery, 2024. **How Many Watts Does A Blender Use**. <https://www.jackery.com/blogs/knowledge/how-many-watts-does-a-blender-use#:~:text=A%20blender's%20power%20consumption%20generally,blender%20uses%20is%20its%20motor> (accessed on 08/04/2025).

3.4.2 Case of study 1b

The data compiled for the LCI related to *Juncus Maritimus* phytoremediation is presented in the table below, specified for the functional unit of this case of study (1 week of research conducting *Juncus Maritimus* phytoremediation).

Table 7. LCI for Case study 1b— Plant (*Juncus Maritimus*) information.

Input/output	Element	Amount
Phase 1 — Assembling the experiment		
Input	Glass fibre filters	10 units
Input	Nitrate cellulose filters	10 units
Input	Metal doping	3 mg per L to treat

¹⁹⁵ C.V. Vidhya, 2022. **Microalgae — The ideal source of biofuel.** In: B. Gurunathan, R. Sahadevan, Z.A. Zakaria (Eds.), **Biofuels and Bioenergy — Opportunities and Challenges.** Elsevier. Amsterdam, The Netherlands.

¹⁹⁶ Bluetti Power, 2025. **How Much Energy Does A Kettle Consume?** <https://www.bluettipower.com/blogs/news/kettle-energy-consumption#:~:text=However%2C%20a%20typical%20electric%20kettle,it%20takes%20to%20boil%20water.&text=Therefore%2C%20boiling%20a%20kettle%20for,approximately%200.075%20kWh%20of%20energy> (accessed on 08/04/2025).

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²⁰⁰ Daigger Scientific, 2025. **Your pH meters don't last forever but here's what you can do to make the best of it!** <https://www.daigger.com/your-ph-meters-don-t-last-forever-but-here-s-what-you-can-do-to-make-the-best-of-it#:~:text=Even%20with%20proper%20care%20and,last%20just%201%2D3%20years> (accessed on 08/04/2025).

²⁰¹ American Scale Company LLC, 2023. **Maintaining Analytical Balances: A Comprehensive Guide.** <https://www.americanscaleus.com/knowledge-center/lab-balance-maintenance> (accessed on 08/04/2025).

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²⁰³ Z. Walker, 2024. **Heavy Copper Weights: How They Work in Flexible Printed Circuit Boards.** <https://blog.epectec.com/heavy-copper-weights-how-they-work-in-flexible-printed-circuit-boards#:~:text=Typical%20rigid%20PCBs%20have%20a,%2C%20flexibility%2C%20and%20overall%20cost> (accessed on 21/04/2025).

Input	Pharmaceutical doping	0.6 mg per L to treat
Input	Deionised water	7 L
Phase 2 — Disassembling the experiment		
Input	Deionised water	1.3 L
Input	Nitric acid solution	20% of 1 L
Input	Methanol/formic acid mixture	100 mL (96% of methanol and 4% of formic acid)
Input	Falcon and Eppendorf plastic tubes	10 units
Input	Glass vials	10 units
Input	Use of solid-phase extraction cartridges material	1 week of operation
Input	Aluminium foil Material	10% of 1 unit of 30 meters
Input	Pipette tip volumes	10 units
Input	Nitrogen gas at 35°C for drying the samples	10-100 L (to be evaluated its influence)
Phase 3 — Infrastructure		
Input	Weighing precision balance	0.02% of total service
Input	Filtration system: i) pump + ii) apparatus	0.01% of total service
Input	Shaker	0.01% of total service
Input	Manifold for SPE + Pump	0.01% of total service
Phase 4 — Hazardous wastes treatment		
Output	Treatment of metals	1.26 mg
Output	Treatment of hydrocarbons	1.39 mg
Output	Treatment of wastewater	7 L
Phase 4 — Hazardous wastes treatment		
Output	Treatment of wastewater	1.5 L

The data compiled for the LCI related to microbial growth is presented in the table below, specified for the functional unit of this case of study (2 days of microbial cultivation).

Table 8. LCI for Case study 1b— Microbial cultivation information.

Input/output	Element	Amount
Phase 1 — Microbial conservation in a freezer		
Input	Power consumption for microbial conservation in a freezer	10 kWh
Phase 2 — Pre-conditioning of materials		
Input	Agar	0.10 kg
Input	Malassez cells for counting spores	4.00 units
Input	Sodium chloride	1.00 g
Input	Falcons and eppendorf plastic tubes	10.00 units
Input	Sterile Loops	5.00·10 ⁻³ units
Input	Petri dishes	200.00 units
Input	Pipette tips	10.00 units
Input	Power consumption for autoclave	2.60 kWh
Phase 3 — Conditioning of microbial strains		
Input	Power consumption for biohazard safety cabinet use	1.20 kWh
Input	Power consumption for microbial incubator use	15.84 kWh
Input	Power consumption for spectrophotometer use	0.24 kWh
Input	Power consumption for vortex use	0.03 kWh
Input	Power consumption for orbital incubator use	57.60 kWh
Phase 4 — Non-hazardous waste management		
Output	Management of non-hazardous waste	1 L

The SimaPro software databases have been supplemented with 201,204,205,206,207,208,209,210,211,212,213,214,215,216,217,218,219,220,221,222,223,224,225,226,227.

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- 205 Sterlitech, 2022. **Glass Fiber Filters.** <https://www.sterlitech.com/glass-fiber-filter.html#:~:text=Q%3A%20What%20is%20the%20function,are%20resistant%20to%20fiber%20shedding> (accessed on 09/04/2025).
- 206 Hawach Scientific, 2025. **Cellulose Nitrate Membrane Filter.** <https://www.hawach.com/filter-membranes/cellulose-nitrate-membrane-filter.html> (accessed on 09/04/2025).
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- 210 Lab Comercial — Material de Laboratorio, 2025. **50ml Complete Clear Glass Vial, 20mm.** <https://www.labcomercial.com/en/bottles-and-glass-containers/668-frasco-vial-50-ml-transparente-completo.html> (accessed on 09/04/2025).
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3.4.3 Case of study 1c

The data compiled for the LCI is presented in the table below, specified for the functional unit of this case of study (1 kg of heavy metal treated).

Table 9. LCI for Case study 1c.

Input/output	Element	Amount
Phase 1 — Excavation		
Input	Diesel for excavation	56.31 mg of diesel
Input	Excavator for soil removal	$4.14 \cdot 10^{-10}$ amount
Phase 2 — Soil transport		
Input	Diesel for transport	1.52 g of diesel

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²¹⁹ Chegg, 2025. **Weight of empty 125 ml Erlenmeyer flask = 88.9 g Weight of Erlenmeyer.** <https://www.chegg.com/homework-help/questions-and-answers/weight-empty-125-ml-erlenmeyer-flask-889-g-weight-erlenmeyer-flask-water-filled-neck-237-g-q87926281> (accessed on 21/04/2025).

²²⁰ Laboratories Humeau, 2025. **MEZ-Stream® Vacuum Filtration Pump.** https://www.humeau.com/media/blfa_files/TC_EZ-streamMerck_20110000100_EN_140220.pdf?srltid=AfmBOop8IES4pxA-zbDI8cGKcDk9TE6DUfa6bgV_um1-U3erU0T8ww55 (accessed on 21/04/2025).

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²²² Particles Plus, 2025. **Vacuum Pump Life Management.** <https://particlesplus.com/pump-management/> (accessed on 21/04/2025).

²²³ Sun Hydraulics, 2025. **Manifolds: Materials of Construction.** <https://www.sunhydraulics.com/tech-resources/manifolds-materials-construction> (accessed on 21/04/2025).

²²⁴ Exacta Labcenter, 2025. **Laboratory manifold.** [https://shop.exactalabcenter.it/\\$B\\$einfo842018_lang_uk.htm](https://shop.exactalabcenter.it/Beinfo842018_lang_uk.htm) (accessed on 21/04/2025).

²²⁵ BenchTop Lab Systems, 2025. **Solid Phase Extraction (SPE) Manifold.** https://www.btlabsystems.com/SPE_Vacuum_Manifold?srltid=AfmBOoqk2xLWoGYmPbSCbJKO_uGNYgZG6_uUuKY1dwW6F5U6ceY9oOT8 (accessed on 21/04/2025).

²²⁶ KNF, 2025. **Mini Diaphragm Vacuum Pump — Laboport® N 816.3 KT.18.** <https://knf.com/en/global/solutions/laboratory-equipment/details/laboport-n-8163-kt18> (accessed on 21/04/2025).

²²⁷ <https://profilab24.com/en/laboratory/shaker-vortexers/phoenix-instrument-laboratory-shaker-rs-os-5-rs-ls-5> (accessed on 21/04/2025).

Input	Truck for soil transport	$1.55 \cdot 10^{-8}$ amount
Phase 3 — Stabilization-solidification process		
Input	Activated carbon	2.94 g of activated carbon
Input	Diesel for concrete mixer truck	1.28 g of diesel
Input	Portland cement	0.15 kg of Portland cement
Input	Water	0.32 kg of water
Input	Concrete mixer truck for encapsulation	$9.87 \cdot 10^{-9}$ amount
Phase 4 — Landfilling		
Output	Blocks refused in a landfill site	1.15 kg

The SimaPro software databases have been supplemented with ¹²⁴.

3.4.4 Case of study 2a

The data compiled for the LCI for the construction of the BES-M reactor is presented in the table below, specified for the functional unit of this case of study (construction of 1 system for 2 years of operation).

Table 10. LCI for Case study 2a — Construction of BES-M reactor.

Input/output	Element	Amount
Phase 1 — Reactor unit		
Input	Endplates	20% of total service
Input	Screws and nuts	20% of total service
Input	Current collectors	20% of total service
Input	Chamber	20% of total service
Input	Tubing	20% of total service
Input	Anode	20% of total service
Input	Cathode	20% of total service
Input	AEM (FAB-pk-130 Fumasep)	20% of total service
Input	CEM (FKB-pk-130 Fumasep)	20% of total service
Input	Tyvek membrane	20% of total service
Input	Sealing gasket	20% of total service

Input	Spacers	20% of total service
Phase 2 — Peristaltic pump		
Input	Peristaltic pump	20% of total service
Phase 3 — Picolog for monitoring and control		
Input	Picolog	20% of total service
Phase 4 — Power source		
Input	Tenma 72-2715 (Farnell)	20% of total service

The SimaPro software databases have been supplemented with ^{228,229,230,231,232,233,234,235,236,237,238,239}.

The data compiled for the LCI for the construction of the BES-HC reactor is presented in the table below, specified for the functional unit of this case of study (construction of 1 system for 2 years of operation).

Table 11. LCI for Case study 2a — Construction of BES-HC reactor.

Input/output	Element	Amount
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²²⁸ C. Minke, U. Kunz, T. Turek, 2017. **Carbon felt and carbon fiber - A techno-economic assessment of felt electrodes for redox flow battery applications.** Journal of Power Sources 2017, 342. 116-124.

²²⁹ V. Koskue, V.-P. Pyrhönen, S. Freguia, P. Ledezma, M. Kokko, 2022. **Modelling and techno-economic assessment of (bio)electrochemical nitrogen removal and recovery from reject water at full WWTP scale.** Journal of Environmental Management 319, 115747.

²³⁰ Media Markt, 2025. **Portátil gaming - HP Victus 15-fa0052ns, 15.6" Full HD, Intel® Core™ i5-12450H, 16GB RAM, 512GB SSD, RTX™ 3050, Sin sistema operativo, Plata mica.** <https://www.medimarkt.es/es/product/portatil-gaming-hp-victus-15-fa0052ns-156-full-hd-intel%C2%AE-coretm-i5-12450h-16gb-ram-512gb-ssd-rtxtm-3050-sin-sistema-operativo-plata-mica-1564063.html> (accessed on 08/04/2025).

²³¹ HP, 2024. **What is the Average Lifespan of a Computer in the UK?** <https://www.hp.com/gb-en/shop/tech-takes/average-computer-lifespan#:~:text=While%20a%20well%2Dmaintained%20computer,from%20three%20to%20eight%20years> (accessed on 08/04/2025).

²³² Ossila, 2025. **Potentiostat — Electrochemistry Supplies, Lab Equipment.** <https://www.ossila.com/products/potentiostat> (accessed on 08/04/2025).

²³³ Biorad, 2025. **Mini-PROTEAN® Spacer Plates with 1.0 mm Integrated Spacers #1653311.** <https://www.biorad.com/es-es/sku/1653311-mini-protean-spacer-plates-with-1-0-mm-integrated-spacers?ID=1653311> (accessed on 08/04/2025).

²³⁴ Wikipedia, 2025. **Glutaraldehyde.** <https://en.wikipedia.org/wiki/Glutaraldehyde#:~:text=Glutaraldehyde%20is%20produced%20industrially%20by,This%20reaction%20essentially%20mimics%20ozonolysis> (accessed on 08/04/2025).

²³⁵ H. Marczak, 2022. **Energy Inputs on the Production of Plastic Products.** Journal of Ecological Engineering 23, 146-156.

²³⁶ CS, 2004. **How to Choose Computer Power Supply?** <https://www.cs.miami.edu/home/burt/learning/Csc521.061/notes/psu.html#:~:text=There%20is%20a%20large%20variation,lb%2C%20a%201.4lb%20gap> (accessed on 08/04/2025).

²³⁷ Farnell, 2025. **Fuente de Alimentación de Banco, Lineal, Programable, 1 Salida, 0 V, 60 V, 0 A, 2 A.** https://es.farnell.com/tenma/72-2715/alimentaci-n-banco-1-canal-60v/dp/2563988?srltid=AfmBOor8FhVN9-oKjclBv_32_Bj4csf6tBVh59RrTL5pk9mUEIsteD5i (accessed on 08/04/2025).

²³⁸ Dupont, 2025. **DuPont™ Tyvek® Supro.** <https://www.dupont.es/products/tyvek-supro.html> (accessed on 08/04/2025).

²³⁹ Müller Alhorn, 2025. **Tyvek: Manufacturing, Applications, Processing.** <https://www.mueller-ahlhorn.com/en/tyvek-manufacturing-applications-processing/#:~:text=Production%20of%20Tyvek%3A%20The%20production,together%20using> (accessed on 08/04/2025).

Phase 1 — Reactor unit		
Input	Endplates	20% of total service
Input	Screws and nuts	20% of total service
Input	Chamber	20% of total service
Input	Tubing	20% of total service
Input	Nets	20% of total service
Input	Anode	20% of total service
Input	Anode connectors	20% of total service
Input	Cathode	20% of total service
Input	Cathode current collector	20% of total service
Input	Sealing gaskets	20% of total service
Phase 2 — Peristaltic pump		
Input	Peristaltic pump	20% of total service
Phase 3 — Potentiostat system for monitoring and control		
Input	Potentiostat system	20% of total service

The SimaPro software databases have been supplemented with ^{229, 230, 231,232, 236,240,241}.

3.4.5 Case of study 2b

The data compiled for the LCI is presented in the table below, specified for the functional unit of this case of study (1 L of groundwater treated).

Table 12. LCI for Case study 2b.

Input/output	Element	Amount
Phase 1 — Preparation of the feedstocks (zeolite synthesis)		

²⁴⁰ Aurora Pro Scientific, 2025. **Peristaltic Pump, Industrial, MaxFlow Rate 500 mL/min, Max 4 Channels - G100-1J**. <https://www.auroraprosci.com/peristaltic-pump--industrial-maxflow-rate-500-ml-min-max-4-channels-g100-1j?srltid=AfmBOopsIIYNUsbyzJZ0szaPvsbO5T9WI8jcZGqbwhRIs5TZUW-5cR2> (accessed on 09/04/2025).

²⁴¹ Valco, 2013. **Components and raw materials for pumps and motors — Design and manufacturing with innovative materials to increase companies competitiveness**. https://www.valco.eu/sitemanager/file/Valco_components_raw_materials_pumps_motors_technical_catalogue_jan2013.pdf (accessed on 09/04/2025).

Input	Deionised water	295.68 mL
Input	Sodium hydroxide	2.67 g
Input	Sodium aluminate	3.06 g
Input	Sodium metasilicate	57.27 g
Input	Energy consumption	0.40 Wh
Phase 2 – Crystallization for zeolite synthesis		
Input	Energy consumption	3.48 kWh
Phase 3 – Drying for product recovery (zeolite synthesis)		
Input	Adsorbent's washing using water	3.70 L
Input	Energy consumption	10.44 kWh
Output	Wastewater from the process	3.69 L
Phase 4 – Adsorption process		
Input	Heat	125.52 kJ
Input	Energy consumption	0.15 Wh
Input	Phosphoric acid	0.16 mL

The SimaPro software databases have been supplemented with ^{176,242,243, 244,245,246}.

²⁴² E. Arkhangelsky, I. Levitsky, V. Gitis, 2017. **Considering energy efficiency in filtration of engineering nanoparticles.** Water Supply, 1212-1218.

²⁴³ Z. Berk, 2009. **Mixing.** In: Z. Berk (Ed.), **Food Process Engineering and Technology — A volume in Food Science and Technology.** Academic Press. Massachusetts, United States of America.

²⁴⁴ Labstac, 2025. **Drying Oven.** <https://labstac.com/Oven-Incubator/Drying-Oven/c/2409> (accessed on 09/04/2025).

²⁴⁵ Minnesota Flower Growers Bulletin, 1991. **How to calculate the amount of phosphoric acid needed to neutralize water of a known alkalinity.** https://hortscans.ces.ncsu.edu/uploads/h/o/how_to_c_5257034cd985f.pdf? (accessed on 14/04/2025).

²⁴⁶ Z. Yin, L. Shin, J. Luo, S. Xu, Y. Yuang, X. Tan, J. Zhu, 2023. **Pump Feature Construction and Electrical Energy Consumption Prediction Based on Feature Engineering and LightGBM Algorithm.** Sustainability 15, 789.

3.4.6 Case of study 2c

The data compiled for the LCI is presented in the table below, specified for the functional unit of this case of study ($21 \text{ m}^3 \text{ h}^{-1}$ of groundwater treated during two years of treatment).

Table 13. LCI for Case study 2c.

Input/output	Element	Amount
Phase 1 – System construction		
Input	Borosilicate tubes with CPC reflectors	92.40 g
Input	Concrete for photo Fenton facility	1.58 L
Input	Aluminium for photo Fenton facility	10.50 g
Phase 2 – Photo Fenton operation		
Input	Hydrogen peroxide	0.43 kg
Input	Sulphuric acid	12.60 g
Input	Ferrous sulphate	0.14 kg
Input	Sodium hydroxide	11.45 g
Input	Power consumption derived from mixing application	0.04 kWh
Input	Power consumption derived from pump utilization	0.63 kWh
Phase 3 – Emissions to aquatic systems		
Output	Emissions of Fe^{2+} to water	2.00 g Fe^{2+}
Output	Emissions of sludge to water	4.30 g sludge
Output	Emissions of carbon dioxide to water	21.74 g CO_2
Output	Emissions of catechol to water	2.41 g catechol
Output	Emissions of hydroquinone to water	0.66 g hydroquinone
Output	Emissions of formic acid to water	12.20 g formic acid
Output	Emissions of benzoquinone to water	1.14 g benzoquinone

The SimaPro software databases have been supplemented with ^{26,140,141,247}.

²⁴⁷ Aropha, 2025. **ThOD calculation based on formula.** <https://www.aropha.com/resources/tools/thod-calculator/> (accessed on 09/04/2025).

3.4.7 Case of study 3a

The data compiled for the LCI related to water phytoremediation is presented in the table below, specified for the functional unit of this case of study (8 months of research).

Table 14. LCI for Case study 3a.

Input/output	Element	Amount
Phase 1 — System included in our assessment		
Input	Containers (90 L)	8 units
Input	Bulkhead nozzle	16 units
Input	Rubber pipe	10 meters
Input	Timer switch	1 unit
Input	Pipe Clips	16 units
Input	Pump	$3.20 \cdot 10^{-3}$ units
Input	Power source	0.2 units
Phase 2 — Power consumption for water recirculation in the set up		
Input	Power consumption for water recirculation in the set up	0.03 kWh
Phase 3 — Potential products for exploitation		
Input	Gravel	347.20 kg
Input	Treated groundwater	197 liters
Input	Total biomass	57 grams

The SimaPro software databases have been supplemented with ^{248,249,250,251,252,253,254,255,256,257,258,259,260}

3.4.8 Case of study 3b

The data compiled for the LCI related to P&T is presented in the table below, specified for the functional unit of this case of study (4 years of operation).

Table 15. LCI for Case study 3b.

Input/output	Element	Amount
²⁴⁸	Termo Fisher Scientific, 2025. Laboratory Bottles. https://www.thermofisher.com/es/es/home/life-science/lab-plasticware-supplies/reusable-plasticware/laboratory-bottles.html (accessed on 10/04/2025).	
²⁴⁹	Axel, 2025. SUIKO — 1-2416-01 ML Type Container 90L ML-90. https://www.axel-gl.com/en/asone/d/1-2416-01/?print=true (accessed on 10/04/2025).	
²⁵⁰	Apso parts, 2025. LEGRIS™ Bulkhead straight screw-in nozzle LF 3000 3136. https://www.apsoparts.com/en-INT/14313605-legristm-bulkhead-straight-screw-in-nozzle-lf-3000-3136.html (accessed on 10/04/2025).	
²⁵¹	IQS, 2025. Rubber Tubing: Types, Materials and Production https://www.iqsdirectory.com/articles/rubber-tubing.html (accessed in 10/04/2025).	
²⁵²	Woodside Fusion LTD, 2025. Polyethylene Pipe Weight Chart. https://www.woodside.uk/free-flange-tables/polyethylene-pipe-weight-chart/ (accessed on 10/04/2025).	
²⁵³	Halomedicals, 2025. Temporizador de laboratorio. https://halomedicals.com/product/lab-timer/?srsltid=AfmBOoovyFHaEOqJNCdCogySuglxdD7RqnVtzDEZsL1_eZcuvbcti5fG (accessed on 10/04/2025).	
²⁵⁴	Essentra Components, 2024. A guide to pipe clamps. https://www.essentracomponents.com/en-us/news/solutions/wire-cable/a-guide-to-pipe-clamps?srsltid=AfmBOoqxftT66Ds-pUcvg2C1Y17MSI_QINfry4D8YXN67GMCoytYjZrn (accessed on 10/04/2025).	
²⁵⁵	Hken, 2025. Unistrut Steel Pipe Clamp 32mm x 150mm, 14mm. https://hken.rs-online.com/web/p/pipe-clips/2577619?srsltid=AfmBOoqFucRUkzZ2T53Zyl7nSUhzCHvP-Fp8RTuVpL5ttfo7wILQAc4g (accessed on 10/04/2025).	
²⁵⁶	Savino Barbera, 2025. Corrosion resistant pumps and mixers. https://savinobarbera.com/?lang=en&gad_source=1&gbraid=0AAAAADmgQWW8IBjKZb69XI8esXBG0muZZ&gclid=Cj0KCQjwnui_BhDIARIsAEo9GuthSLjZ18XREPhsNfWmybVN5c8zGO6KN6Jlxi4ZNd4jJiJG8d-eDEsaAh6KEALw_wcB (accessed on 10/04/2025).	
²⁵⁷	Lead Fluid, 2025. How Long Does Peristaltic Pump Tubing Last? https://leadfluidpump.com/how-long-does-peristaltic-pump-tubing-last/#:~:text=As%20a%20peristaltic%20pump%2C%20ordinary.tube%20can%20reach%20400%20hours (accessed on 10/04/2025).	
²⁵⁸	Drifton, 2025. LabN3 Peristaltic Pump. https://www.drifton.eu/shop/9-flow-rate-peristaltic-pumps/2450-labn3-peristaltic-pump/ (accessed on 10/04/2025).	
²⁵⁹	ET System, 2025. Programmable DC power supplies. https://www.et-system.de/en/programmable-dc-power-supplies/dc-power-supplies (accessed on 10/04/2025).	
²⁶⁰	Amazon, 2025. DC Power Supply Variable, Adjustable Switching DC Lab Power Supply Laboratory for Electroplate 60V 48V 36V 30V 24V 12V 3A 5A 1A 10A for Laboratory, School, Repair, Electrolyzation(30V 5A). https://www.amazon.com/-/es/179967715/dp/B0DNMF7P8D (accessed on 10/04/2025).	

Phase 1 — Infrastructure		
Input	Needed material for the construction of unit for containing the activated carbon	48,339.62 kg
Input	Needed material for the construction of unit for conditioning the pH	30,452.05 kg
Input	Pump system	1,133.33 kg
Phase 2 — Pumping operation		
Input	Power for pumping	2,966,822.58 kWh
Phase 3 — pH conditioning		
Input	Nitric acid consumption	28.68 kg
Phase 4 — Heavy metals removal		
Input	Activated carbon consumption	1,070,815.45 tonnes
Input	Power for heating	53,479.65 kWh
Phase 5 — Waste management		
Input	Energy consumption for the management of the adsorption system	13,776.79 MJ
Input	Diesel consumption for the management of the adsorption system	314.21 L
Input	Energy consumption for the management of the pH conditioning tank	8,678.83 MJ
Input	Diesel consumption for the management of the pH conditioning tank	197.94 L
Input	Energy consumption for the management of the activated carbon wastes	185,143,991.42 MJ
Input	Diesel consumption for the management of the activated carbon wastes	6,960,300.43 L
Input	Energy consumption for the management of the metal wastes arising from the pump	1.70 GJ
Input	Diesel consumption for the management of the metal wastes arising from the pump	7.37 L

The SimaPro software databases have been supplemented with ^{261,262}.

3.4.9 Case of study 4

The data compiled for the LCI related to microbial inoculation is presented in the table below, specified for the functional unit of this case of study (100 L of inoculum).

Table 16. LCI for Case study 4.

Input/output	Element	Amount
Phase 1 — Inoculum preparation		
Input	Heat	0.01 kWh
Input	Electricity	5 Wh
Phase 2 — Inoculum cultivation		
Input	Heat	0.03 kWh
Input	Electricity	12.00 Wh
Input	Na ₃ C ₆ H ₅ O ₇	1·10 ⁻³ kg
Input	NH ₄ Cl	2·10 ⁻⁴ kg
Input	KH ₂ PO ₄	2·10 ⁻⁴ kg
Input	K ₂ HPO ₄	3.5·10 ⁻⁴ kg
Input	NaCl	1·10 ⁻⁴ kg
Input	CaCO ₃	1·10 ⁻³ kg
Input	(NH ₄) ₂ SO ₄	4·10 ⁻⁴ kg
Input	MgSO ₄	5·10 ⁻⁵ kg
Input	NaH ₂ PO ₄	5·10 ⁻⁵ kg
Input	MnSO ₄	2·10 ⁻⁶ kg
Phase 3 — Subsequent process		
Input	Heat	9.01·10 ⁻³ kWh

²⁶¹ National Library of Medicine, 2025. **Nitric Acid**. <https://pubchem.ncbi.nlm.nih.gov/compound/Nitric-Acid> (accessed on 10/04/2025).

²⁶² LGC, 2025. **Density, Diesel (Nominal: 0.8375 g/mL @ 15°C)**. <https://www.lgcstandards.com/ES/en/Density-Diesel-Nominal-0-8375-g-mL-15-C-/p/ALK-CRM-DEGO> (accessed on 10/04/2025).

Input	Electricity	3.00 Wh
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The SimaPro software databases have been supplemented with ^{263,264,265,266}.

3.5 Environmental impact assessment

The environmental impact assessment is a core element of LCA implementation, as its main objective is to identify and quantify environmental impacts²⁶⁷. Specifically, the environmental impact assessment evaluates and quantifies the effects of emissions and resources extractions within the defined boundaries²⁶⁸.

Based on previous works, the environmental impact assessment is conducted in three steps²⁶⁹:

- **Characterization:** This step measures the quantities of substances released from the defined system (case study) and assesses their contribution to each environmental impact category. This is done using equivalent factors, such as carbon dioxide equivalents for the climate change category²⁶⁹.
- **Normalization:** In this step, each environmental impact estimated in the previous stage is adjusted based on the system's service life and the functional unit. This helps estimate a specific environmental impact. Additionally, the contributors to each impact (such as material/energy consumption or emissions to soil, liquid, or air systems) are compared internally to identify the most critical ones. These critical contributors are reported and discussed in the last phase of the LCA (results interpretation)²⁶⁹.
- **Weighting:** In the final step, the most important environmental impact categories are selected in line with the goals and scope defined at the start of the LCA process, adhering to ISO principles²⁶⁹.

²⁶³ Guizhou Kexin Chemical, Metallurgical Co Ltd, 2014. **Production method of dipotassium phosphate.** <https://patents.google.com/patent/CN104003362A/en> (accessed on 10/04/2025).

²⁶⁴ I. Alexander, Menachem Bar-on, 1986. **Process for the manufacture of monopotassium phosphate.** <https://patents.google.com/patent/US4678649A/en> (accessed on 10/04/2025).

²⁶⁵ D.P. Minh, A. Nzihou, P. Sharrock, 2015. **Production of Sodium Dihydrogen Phosphate Using Sodium Chloride and Orthophosphoric Acid.** Industrial and engineering chemistry research 54, 12467-12473.

²⁶⁶ PCE Instruments, 2025. **Environmental measurement.** <https://www.pce-instruments.com> (accessed on 10/04/2025).

²⁶⁷ S. Yang, K. Ma, Z. Liu, J. Ren, Y. Man, 2020. **Development and applicability of life cycle impact assessment methodologies.** In: J. Ren, S. Toniolo (Eds.), **Life Cycle Sustainability Assessment for Decision-Making — Methodologies and Case Studies.** Elsevier. Amsterdam, The Netherlands.

²⁶⁸ M.A.J. Huijbregts, Z.J.N. Steinmann, P.M.F. Elshout, G. Stam, F. Verones, M. Vieira, M. Zijp, A. Hollander, R. van Zelm, 2017. **ReCiPe2016: a harmonised life cycle impact assessment method at midpoint and endpoint level.** International Journal of Life Cycle Assessment 22, 138-147.

²⁶⁹ J. Liu, X. Ma, 2019. **The analysis on energy and environmental impacts of microalgae-based fuel methanol in China.** Energy Policy 37, 1479-1488.

From a general approach, the environmental impact assessments for BIOSYSMO were evaluated using ReCiPe Midpoint, as mentioned above. It includes the impacts on human health, ecosystem quality and resource consumption²⁶⁸.

This impact assessment measures seventeen environmental categories. Eight of these categories are related to human health impacts: i) global warming, ii) fine particulate matter formation, iii) ozone formation (related to human health), iv) ionizing radiation, v) stratospheric ozone depletion, vi) human toxicity (related to cancer effect), vii) human toxicity (related to non-cancer effect) and viii) water consumption. Global warming and water use are also considered when measuring ecosystem quality effects. Additionally, ReCiPe Midpoint method assesses ozone formation from an ecosystem quality perspective. Other categories measuring ecosystem quality include: i) freshwater ecotoxicity, ii) freshwater eutrophication, iii) terrestrial ecotoxicity, iv) terrestrial acidification, v) land use vi) marine eutrophication and vii) marine ecotoxicity. Finally, mineral resource scarcity and fossil resource scarcity are the categories related to resource consumption²⁶⁸.

Specifically, fine particulate matter formation and ozone formation (related to human health) measures the effect on the increase of the respiratory diseases. The increase in various types of cancer is connected to the following categories: i) human toxicity (related to non-cancer effect), ii) ionizing radiation and iii) stratospheric ozone depletion. Both ionizing radiation and stratospheric ozone depletion are used to measure the effects on increase in other diseases/causes. Moreover, human toxicity (related to non-cancer effect) and global warming are linked with the increase in other diseases/causes. From a human health perspective, global warming together with water consumption also associated with an increase in malnutrition. Both global warming and water consumption affect the quality of the ecosystem, as they are related to damage to freshwater and terrestrial species. Regarding this damage to freshwater species, freshwater ecotoxicity and freshwater eutrophication categories are involved in these damages. The damage to terrestrial species is correlated with: i) ozone formation from an ecosystem perspective, ii) terrestrial ecotoxicity, iii) terrestrial acidification and iv) land use. Moreover, marine ecotoxicity category is involved in damage to marine species. Finally, both mineral resource scarcity and fossil resource scarcity affect the availability of resources²⁶⁸.

4 Alignment with the biodiversity assessment

BSY has worked closely with: i) the definition of biodiversity and ecosystem services terms for BIOSYSMO and ii) a literature review of previous cases of measurement of biodiversity and ecosystem services.

4.1 Defining “biodiversity” and “ecosystem services” for BIOSYSMO

Biodiversity and ecosystem services are interlinked factors of ecosystems, each with distinct values and implications. Biodiversity refers to the variety of life forms within a given ecosystem, representing existence value. In short, biodiversity is the inherent value of species and ecosystems, independent of their utility to humans²⁷⁰. This concept coincides with the notion of intrinsic value in environmental ethics, which stresses the importance of preserving species for their own sake²⁷¹. On the other hand,

²⁷⁰ I. Callesen, 2016. **Biodiversity and ecosystem services in life cycle impact assessment – Inventory objects or impact categories?** Ecosystem Services 22, 94-103.

²⁷¹ R. Attfield, 1998. **Existence value and intrinsic value.** Ecological economics 24, 163-168.

ecosystem services refer to the benefits that humans derive from ecosystems and reflect functional values. These services are often categorised into: i) provisioning (for instance food, and water and supply), ii) regulating (for instance, climate regulation or water purification), iii) supporting (for instance, nutrient cycling or pollination) and iv) cultural (for instance, recreational and spiritual benefits) services²⁷⁰.

4.2 Previous cases of measuring “biodiversity” and “ecosystem services” in LCA

An important consideration is the classification of environmental impacts into: i) midpoint methodology and ii) endpoint methodology²⁷². The endpoint methodology primarily focusses on human effect, while the midpoint methodology address effects on natural resources and a range of organisms, including humans and other living beings. Endpoint methodologies typically involve the fate factor, the impact factor, and the characterization factor²⁷³. This fate factor considers the spatial distribution and intensity of pressures resulting from human activities. The impact factor refers to the potential damage or effect, where, for example, biodiversity loss can be correlated with the intensity of a specific pressure. Finally, the characterization factor is calculated by multiplying the impact factor by the fate factor²⁷³.

4.3 Application to BIOSYSMO

The multifunctionality of NBS presents a challenge in LCA due to the difficulty of quantifying multiple benefits within a single functional unit. Emerging methods, such as territorial LCA and ecosystem service-based allocation, aim to address these challenges, while future research should focus on optimizing NBS combinations and refining LCA methodologies to better assess environmental, economic, and social trade-offs⁶⁸.

Regarding biodiversity assessment, the “Ecological Scarcity Method” provides a framework for quantifying environmental pressures by converting them into eco-factors. This is accomplished through a comparison between actual emissions or resource consumption and predefined critical limits, capturing the relative scarcity of environmental capacity. The method is particularly well-suited for application in biodiversity assessments within LCA, as it integrates regionalised environmental thresholds and policy-based targets. Such contextualisation enables a more accurate evaluation of environmentally relevant impacts, including: i) land use, ii) eutrophication, and iii) the emission of toxic substances. All these relevant impacts significantly contribute to biodiversity loss. In this connection, the existing scientific literature, notably the reference²⁷⁴, substantiates the relevance of the Ecological Scarcity Method in LCA due to its capacity to reflect geographically specific environmental constraints and pressures. Given the spatial heterogeneity of biodiversity impacts, the regional focus of this method

²⁷² H.U. de Haes, E. Lindeijer, 2002. **The conceptual structure of life-cycle impact assessment**. In: H.U. de Haes, G. Finnveden, M. Goedkoop (Eds.). **Life-Cycle Impact Assessment: Striving towards Best Practice**. SETAC Press. Pensacola, United States of America.

²⁷³ R. de Groot, B. Fisher, M. Christie, 2010. **Integrating the ecological and economic dimensions in biodiversity and ecosystem service valuation**. In: P. Kumar (Ed), **The Economics of Ecosystems and Biodiversity: The Ecological and Economic Foundations**. Taylor and Francis Group. London, United Kingdom.

²⁷⁴ R. Frischknecht, S.B. Knöpfel, 2013. **Swiss Eco-Factors 2013 according to the Ecological Scarcity Method**. Federal Office for the Environment FOEN, Bern.

enhances its effectiveness in identifying ecological hotspots and informing robust environmental decision-making. In summary, the Ecological Scarcity Method represents a valuable tool for aligning LCA outcomes with broader ecological sustainability objectives²⁷⁴.

5 Results and discussion

The significant results from all case studies are outlined below, followed by a discussion to identify the environmental hotspots.

5.1 Environmental results and discussion for case of study 1a

The breakdown of each contributor to the poplar's cultivation at UPM's facility is provided the in the table below.

Table 17. LCA results for case study 1a — Poplar's cultivation.

Environmental impact category	Unit	Total	Contaminants addition	Auxiliary material use
Global warming	kg CO ₂ (eq)	566.95	2.01	12.53
Terrestrial acidification	kg SO ₂ (eq)	2.29	0.01	0.05
Freshwater eutrophication	kg P (eq)	0.25	4.84·10 ⁻⁴	0.01
Terrestrial ecotoxicity	kg 1,4-DCB	4,846.55	24.96	161.50
Land use	m ² _a crop (eq)	20.21	0.08	1.06
Mineral resource scarcity	kg Cu (eq)	9.05	0.02	0.49
Fossil resource scarcity	kg oil (eq)	155.85	0.64	3.62
Water consumption	m ³	10.45	0.05	0.41

Table 17. LCA results for case study 1a — Poplar's cultivation (continue from the table above).

Environmental impact category	Unit	Infrastructure need	Power consumption	Hazardous waste treatment
Global warming	kg CO ₂ (eq)	308.63	237.49	6.29
Terrestrial acidification	kg SO ₂ (eq)	1.57	0.65	0.02
Freshwater eutrophication	kg P (eq)	0.20	0.04	1.08·10 ⁻³
Terrestrial ecotoxicity	kg 1,4-DCB	2,835.13	1,777.84	47.12

Land use	m ² _a crop (eq)	8.40	10.40	0.28
Mineral resource scarcity	kg Cu (eq)	7.77	0.75	0.02
Fossil resource scarcity	kg oil (eq)	76.55	73.10	1.94
Water consumption	m ³	6.51	3.39	0.09

Based on the results in the table above, BSY has identified the two main drivers of UPM's research from an environmental perspective: i) the use of the chambers (infrastructure needed phase) and ii) the associated power consumption (power consumption phase), which is mainly due to operating these chambers for poplar cultivation. Regarding the importance of the chambers, it is derived from their nature, specifically: i) the metal contained in the equipment's chassis and ii) the electronics modules needed for their operation. Concerning power consumption, this parameter is critical as it depends on the location. Specifically, for the current scenario, Spain's electricity mix has been considered, given that UPM is located in Madrid. However, the associated environmental impacts could be higher if the activity were carried out in other EU countries with a different electricity mix, as shown in the figure below.

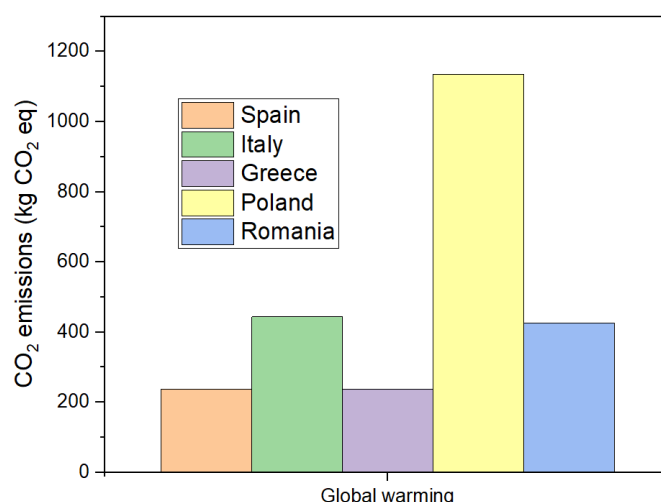


Figure 17. Sensitive analysis for poplars cultivation in function of the location — Case study 1a.

The breakdown of each contributor to microbial growth at UMLP's facility is provided in the table below.

Table 18. LCA results for case study 1a — Microbial assistance — Microbial growth.

Environmental impact category	Unit	Use of potato glucose agar	Use of Petri dishes	Use of Malassez cells for counting spores	Manufacturing of the microbial incubator	Manufacturing of the autoclave
Global warming	kg CO ₂ (eq)	1.56	5.25	$7.73 \cdot 10^{-4}$	0.08	0.88
Terrestrial acidification	kg SO ₂ (eq)	0.01	0.03	$4.12 \cdot 10^{-6}$	$4.39 \cdot 10^{-4}$	$6.50 \cdot 10^{-4}$

Freshwater eutrophication	kg P (eq)	$4.84 \cdot 10^{-4}$	$2.24 \cdot 10^{-3}$	$2.31 \cdot 10^{-7}$	$6.15 \cdot 10^{-5}$	$8.08 \cdot 10^{-4}$
Terrestrial ecotoxicity	kg 1,4-DCB	10.35	64.39	$5.55 \cdot 10^{-3}$	0.56	60.00
Land use	m ² _a crop (eq)	1.35	0.53	$2.22 \cdot 10^{-5}$	$2.03 \cdot 10^{-3}$	0.06
Mineral resource scarcity	kg Cu (eq)	$3.59 \cdot 10^{-3}$	0.04	$6.05 \cdot 10^{-6}$	$1.34 \cdot 10^{-3}$	0.03
Fossil resource scarcity	kg oil (eq)	0.37	1.39	$1.91 \cdot 10^{-4}$	0.02	0.22
Water consumption	m ³	0.27	0.03	$5.23 \cdot 10^{-6}$	$4.47 \cdot 10^{-4}$	0.01

Table 18. LCA results for case study 1a — Microbial assistance — Microbial growth (continue from the table above).

Environmental impact category	Unit	Manufacturing of the laminar flow cabinet	Manufacturing of the pH meter	Manufacturing of laboratory balancer	Power consumption for microbial incubator
Global warming	kg CO ₂ (eq)	1.33	1.40	0.87	33.03
Terrestrial acidification	kg SO ₂ (eq)	$2.13 \cdot 10^{-3}$	$5.81 \cdot 10^{-4}$	$3.61 \cdot 10^{-4}$	0.11
Freshwater eutrophication	kg P (eq)	$8.97 \cdot 10^{-4}$	$1.49 \cdot 10^{-3}$	$9.21 \cdot 10^{-4}$	0.03
Terrestrial ecotoxicity	kg 1,4-DCB	180.11	38.50	21.91	161.66
Land use	m ² _a crop (eq)	0.06	0.11	0.07	1.25
Mineral resource scarcity	kg Cu (eq)	0.05	0.05	0.03	0.06
Fossil resource scarcity	kg oil (eq)	0.32	0.37	0.23	9.27
Water consumption	m ³	0.01	0.02	0.01	0.64

Table 18. LCA results for case study 1a — Microbial assistance — Microbial growth (continue from the table above).

Environmental impact category	Unit	Power consumption for autoclave operation	Power consumption for laminar flow cabinet	Power consumption for pH meter	Power consumption for balancer operation
Global warming	kg CO ₂ (eq)	2,477.44	132.13	23.12	46.25
Terrestrial acidification	kg SO ₂ (eq)	8.57	0.46	0.08	0.16

Freshwater eutrophication	kg P (eq)	2.28	0.12	0.02	0.04
Terrestrial ecotoxicity	kg 1,4-DCB	1,212.44	646.64	113.16	226.32
Land use	m ² _a crop (eq)	94.03	5.02	0.88	1.76
Mineral resource scarcity	kg Cu (eq)	4.45	0.24	0.04	0.08
Fossil resource scarcity	kg oil (eq)	694.95	37.06	6.49	12.97
Water consumption	m ³	47.77	2.55	0.45	0.89

In this regard, the resulting network for the Global Warming Potential category during the microbial growth phase, as modelled in SimaPro software, is presented below.

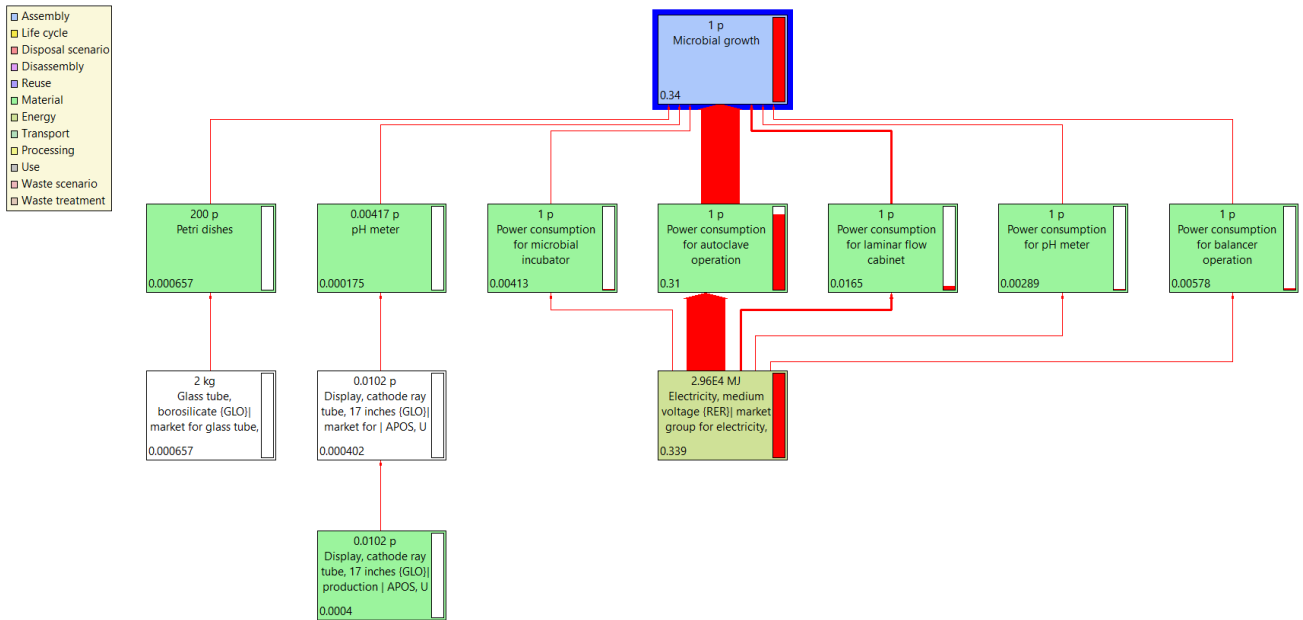


Figure 18. Network for the global warming category from SimaPro software for the microbial growth phase.

Below is a breakdown of the environmental impact results for the microbial preservation phase.

Table 19. LCA results for case study 1a — Microbial assistance — Microbial preservation.

Environmental impact category	Unit	Manufacturing of the freezer	Power consumption for freezer
Global warming	kg CO ₂ (eq)	26.38	297.29
Terrestrial acidification	kg SO ₂ (eq)	0.13	1.03
Freshwater eutrophication	kg P (eq)	0.02	0.27

Terrestrial ecotoxicity	kg 1,4-DCB	244.81	1,454.93
Land use	m ² _a crop (eq)	1.28	11.28
Mineral resource scarcity	kg Cu (eq)	0.86	0.53
Fossil resource scarcity	kg oil (eq)	5.99	83.39
Water consumption	m ³	0.25	5.73

Based on the results in the tables above, it is found that the microbial growth phase contributes more significantly to environmental impacts than the microbial preservation phase. This highlights the importance of both the cultivation period and the preservation duration in assessing environmental impact. In this regard, BSY's results indicate that the microbial growth is the most critical phase, primarily due to the power consumption for the autoclave operation.

Additionally, it is noteworthy that new inventory items were created for the equipment used by UMLP, as they were not available in any of SimaPro's databases. These newly created items include: i) autoclave, ii) laminar flow cabinet, iii) microbial incubator, iv) pH meter, v) laboratory balance, and vi) laboratory freezer. The development of these items has proven useful for the environmental assessment of other case studies within the BIOSYSMO project.

The associated environmental impacts of this equipment were calculated based exclusively on their use for BIOSYSMO-related services. As shown in the table below, these impacts are significant. Therefore, proper maintenance of the equipment is essential to extend their operational lifespan and, consequently, reduce their overall environmental footprint. The high environmental impacts resulted from the use of electronic components and metals in the manufacturing of this equipment.

Table 20. LCA results for newly introduced items in the environmental assessment of the equipment used by UMLP.

Environmental impact category	Unit	Autoclave	Laminar flow cabinet	Microbial incubator	pH meter	Laboratory balance	Laboratory freezer
Global warming	kg CO ₂ (eq)	440.16	902.77	24.32	336.89	347.36	3,123.89
Terrestrial acidification	kg SO ₂ (eq)	0.32	1.45	0.13	0.14	0.14	15.68
Freshwater eutrophication	kg P (eq)	0.40	0.61	0.02	0.36	0.37	2.68
Terrestrial ecotoxicity	kg 1,4-DCB	30,001.18	122,477.05	167.61	9,239.48	8,762.24	28,990.81
Land use	m ² _a crop (eq)	28.30	38.37	0.61	26.65	26.61	151.65
Mineral resource scarcity	kg Cu (eq)	15.76	31.83	0.40	11.85	12.50	101.66

Fossil resource scarcity	kg oil (eq)	111.99	21.57	5.44	88.94	91.17	708.97
Water consumption	m ³	5.05	9.07	0.13	4.18	4.26	301.21

5.2 Environmental results and discussion for case of study 1b

The results for Case Study 1b are presented below, outlining the environmental impacts associated with each phase of the assessment for the estuaries phytoremediation along with the microbial growth.

Table 21. LCA results for case study 1b — Estuaries phytoremediation.

Environmental impact category	Unit	Total	Assembling the experiment	Disassembling the experiment
Global warming	kg CO ₂ (eq)	4.64	0.28	4.30
Terrestrial acidification	kg SO ₂ (eq)	0.02	$1.36 \cdot 10^{-3}$	0.02
Freshwater eutrophication	kg P (eq)	$1.76 \cdot 10^{-3}$	$1.42 \cdot 10^{-4}$	$1.54 \cdot 10^{-3}$
Terrestrial ecotoxicity	kg 1,4-DCB	25.25	3.48	20.28
Land use	m ² _a crop (eq)	0.09	0.02	0.07
Mineral resource scarcity	kg Cu (eq)	0.04	$3.61 \cdot 10^{-3}$	0.03
Fossil resource scarcity	kg oil (eq)	1.26	0.07	1.17
Water consumption	m ³	0.04	0.01	$6.68 \cdot 10^{-4}$

Table 21. LCA results for case study 1b — Estuaries phytoremediation (continue from the table above).

Environmental impact category	Unit	Infrastructure	Hazardous waste management	Non-hazardous waste management
Global warming	kg CO ₂ (eq)	0.07	0.00	0.00
Terrestrial acidification	kg SO ₂ (eq)	$4.02 \cdot 10^{-4}$	0.00	0.00
Freshwater eutrophication	kg P (eq)	$7.55 \cdot 10^{-5}$	0.00	0.00
Terrestrial ecotoxicity	kg 1,4-DCB	1.49	0.00	0.00
Land use	m ² _a crop (eq)	$4.48 \cdot 10^{-3}$	0.00	0.00
Mineral resource scarcity	kg Cu (eq)	0.01	0.00	0.00

Fossil resource scarcity	kg oil (eq)	0.02	0.00	0.00
Water consumption	m ³	0.04	-0.01	-1.50·10 ⁻³

From these results, it is found that the two phases connected to wastes treatments showed an almost negligible contribution to the overall environmental impact. This outcome is primarily attributed to the short duration allocated for the growth of *Juncus maritimus* (functional unit — 1 week of research). In this connection, significant values were only observed for water consumption category. Therefore, extending the time period considered for the functional unit could potentially influence these findings. Moreover, the two most significant contributors to the environmental impacts were: i) the assembling the experiment phase and ii) the disassembling the experiment phase. In this connection, the disassembling phase showed a greater impact due to material consumption, which included: i) deionised water, ii) nitric acid, iii) methanol, iv) formic acid, solid-phase extraction cartridges material, vii) aluminium foil, viii) pipette tips and ix) nitrogen gas. Regarding the nitrogen gas consumption, it can vary significantly depending on the control system used. More specifically, the environmental impact may increase by 10%, as shown in the figure below.

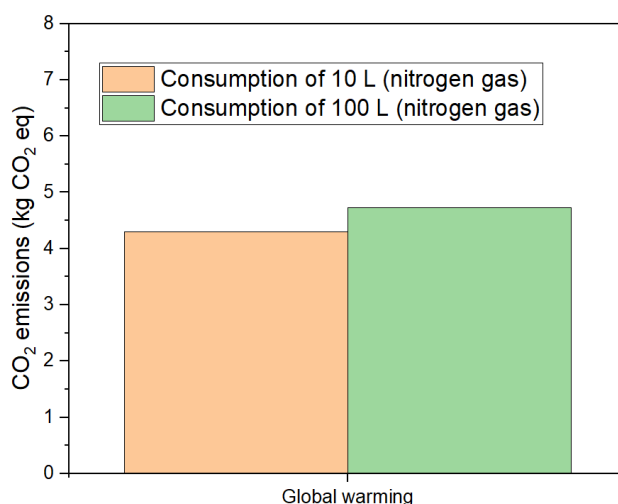


Figure 19. Variation in environmental impact between 10 L and 100 L consumption of nitrogen for case study 1b.

5.3 Environmental results and discussion for case study 1c

The results for Case Study 1c are presented below, outlining the environmental impacts associated with each phase of the assessment: (i) soil excavation, (ii) soil transport, (iii) S-S treatment, and (iv) disposal of the treated block in a conventional landfill. All values are reported per functional unit (1 kg of heavy metal treated).

Table 22. LCA results for case study 1c.

Environmental impact category	Unit	Total	Excavation	Soil transport	S-S	Landfilling
Global warming	kg CO ₂ (eq)	19,454.04	0.06	3.75	12.06	19,438.16
Terrestrial acidification	kg SO ₂ (eq)	53.87	1.72·10 ⁻⁴	0.01	0.05	53.80

Freshwater eutrophication	kg P (eq)	0.52	$8.28 \cdot 10^{-6}$	$1.32 \cdot 10^{-3}$	0.01	0.51
Terrestrial ecotoxicity	kg 1,4-DCB	84,422.43	0.63	42.94	54.37	84,324.49
Land use	m ² _a crop (eq)	192.48	$1.77 \cdot 10^{-3}$	0.12	0.22	192.15
Mineral resource scarcity	kg Cu (eq)	14.18	$2.46 \cdot 10^{-4}$	0.04	0.02	14.11
Fossil resource scarcity	kg oil (eq)	5,724.40	0.07	2.49	4.34	5,717.49
Water consumption	m ³	13.66	$1.59 \cdot 10^{-4}$	0.02	0.35	13.29

The landfilling phase stands out as the primary contributor to most impact categories. It has the highest influence on: i) global warming potential, ii) terrestrial ecotoxicity, iii) fossil resource scarcity, and iv) land use, underscoring its dominant role in the overall environmental burden. According to the reference ¹²⁴, this phase involves: i) diesel consumption for equipment operation — 0.20 kg of diesel tonne⁻¹ of concrete managed, and ii) additional diesel for transporting the treated concrete blocks from the S-S facility to a landfill designated for construction and demolition waste. Among these, transportation emerges as the main contributor to the impacts observed in the landfilling phase, as shown in the figure below.

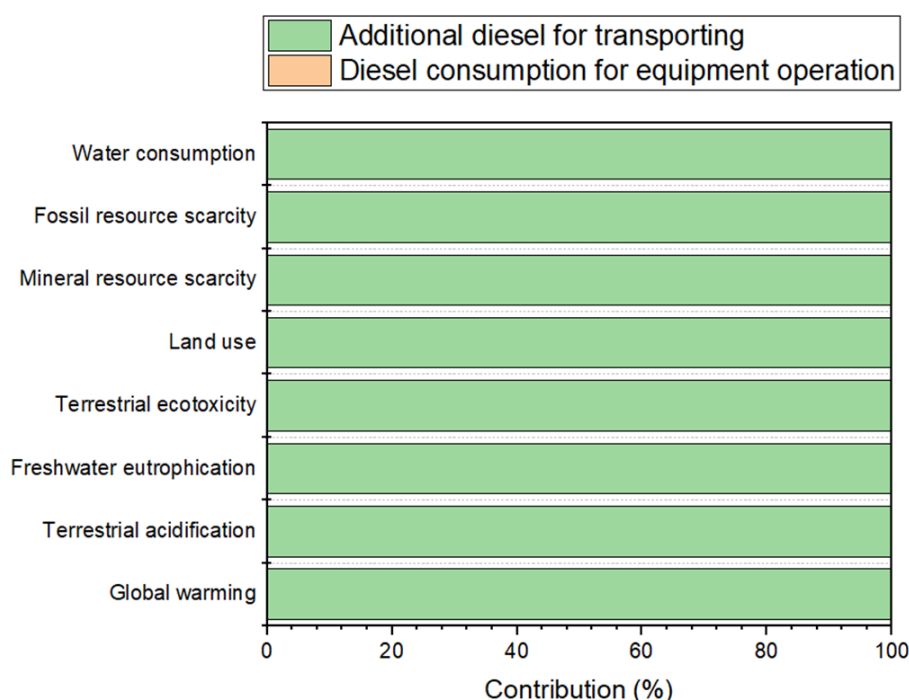


Figure 20. Contributors in the landfilling phase.

The current case study has also been analysed using the Ecological Scarcity 2021 (a methodology related Ecological Scarcity Method), with the results presented in the following table.

Table 23. LCA results for case study 1c — A biodiversity approach.

Environmental impact category	Unit	Total	Excavation	Soil transport
Water resources	UBP	712,058,089.10	-3,643.12	-242,244.18
Energy resources	UBP	34,011,123,763.72	24,353.52	4,759,440.20
Mineral resources	UBP	511.667.743.32	497,203.32	93,015,547.00
Land use	UBP	360,096,495.30	9,164.00	735,656.20
Global warming	UBP	54,836,560,485.30	1,087,531.30	67,224,084.00
Ozone layer depletion	UBP	71,189,124.62	1,296.88	93,512.41
Main air pollutants and PM	UBP	11,700,924,875.29	670,175.29	51,167,080.00
Carcinogenic substances into air	UBP	65,094,7137.64	78,236.64	15,114,172.00
Heavy metals into air	UBP	2,259,585,680.84	234,763.84	45,836,332.00
Water pollutants	UBP	6,203,840,678.10	253323,30	8,326,260.90
POP into water	UBP	826,427,783.35	26,944.25	1,454,180.90
Heavy metals into water	UBP	134,925,275.63	111,380.13	15,666,352.00
Pesticides into soil	UBP	54,621,585.08	333.82	36,109.58
Heavy metals into soil	UBP	38,586,437.59	2,384.53	255,671.16
Radioactive substances into air	UBP	706,769.01	0.01	0.66
Radioactive substances into water	UBP	707,253,485.21	318.57	30,953.05
Noise	UBP	0.00	0.00	0.00
Non-radioactive waste to deposit	UBP	3,325,640,528.68	47,877.68	4,272,932.00
Radioactive waste to deposit	UBP	34,537,531,279.53	28,429.73	2,777,912.00

Table 23. LCA results for case study 1c — A biodiversity approach (continue from the table above).

Environmental impact category	Unit	S-S	Landfilling
Water resources	UBP	3,279,336.40	709,024,640.00
Energy resources	UBP	14,376,970.00	33,991,963,000.00

Mineral resources	UBP	37,844,343.00	380,310,650.00
Land use	UBP	1,684,175.10	357,667,500.00
Global warming	UBP	219,487,870.00	54,548,761,000.00
Ozone layer depletion	UBP	59,486.34	71,034,829.00
Main air pollutants and PM	UBP	136,009,620.00	11,513,078,000.00
Carcinogenic substances into air	UBP	12,544,169.00	62,3210,560.00
Heavy metals into air	UBP	27,911,585.00	2,185,603,000.00
Water pollutants	UBP	7,517,493.90	6,187,743,600.00
POP into water	UBP	1,319,448.20	823,627,210.00
Heavy metals into water	UBP	6,999,613.50	112,147,930.00
Pesticides into soil	UBP	151,939.68	54,433,202.00
Heavy metals into soil	UBP	765,395.90	37,562,986.00
Radioactive substances into air	UBP	1.65	706,766.69
Radioactive substances into water	UBP	94,743.59	707,127,470.00
Noise	UBP	0.00	0.00
Non-radioactive waste to deposit	UBP	1,0431,219.00	3,310,888,500.00
Radioactive waste to deposit	UBP	8,519,937.80	34,526,205,000.00

The results in the table above show that the Ecological Scarcity 2021 Methodology and the ReCiPe Midpoint Methodology are correlated, as the same drivers affect the environmental burdens in both cases. Therefore, the environmental optimization outcomes from the ReCiPe Midpoint Methodology are also applicable to the Ecological Scarcity 2021 Methodology. However, this must be confirmed for all scenarios in further analysis.

5.4 Environmental results and discussion for case of study 2a

The LCA results for constructing the two novel BES developed in BIOSYSMO are presented in the tables below based on our estimations. These calculations assume a 2-year service period dedicated to BIOSYSMO and a general equipment lifetime of 10 years. However, certain components, including the cathode, anode, spacers, anion exchange membrane (AEM, FAB-pk-130 Fumasep), cation exchange membrane (CEM, FKB-pk-130 Fumasep), and the computer, are assumed to have a lifespan of 5 years. This adjustment is due to the need for more frequent updates in line with technological advancements.

Table 24. LCA results for the construction of BES-HC.

Environmental impact category	Unit	Total	Reactor system	Peristaltic pump	Potentiostat
Global warming	kg CO ₂ (eq)	20.72	1.91	0.75	18.05
Terrestrial acidification	kg SO ₂ (eq)	0.09	0.01	$2.78 \cdot 10^{-3}$	0.08
Freshwater eutrophication	kg P (eq)	0.02	$6.00 \cdot 10^{-4}$	$2.98 \cdot 10^{-4}$	0.02
Terrestrial ecotoxicity	kg 1,4-DCB	391.74	165.06	21.78	204.89
Land use	m ² _a crop (eq)	74.40	0.03	$-2.09 \cdot 10^{-3}$	74.37
Mineral resource scarcity	kg Cu (eq)	0.43	0.03	0.01	0.38
Fossil resource scarcity	kg oil (eq)	5.18	0.53	0.23	4.42
Water consumption	m ³	0.19	0.01	0.01	0.16

Table 25. LCA results for the construction of BES-M.

Environmental impact category	Unit	Total	Reactor system	Peristaltic pump	Power source	Picolog
Global warming	kg CO ₂ (eq)	10.64	2.54	0.75	7.35	18.05
Terrestrial acidification	kg SO ₂ (eq)	0.05	0.01	$2.78 \cdot 10^{-3}$	0.04	0.08
Freshwater eutrophication	kg P (eq)	0.01	$9.71 \cdot 10^{-4}$	$2.98 \cdot 10^{-4}$	0.01	0.02
Terrestrial ecotoxicity	kg 1,4-DCB	575.91	368.71	21.78	185.41	204.89
Land use	m ² _a crop (eq)	0.49	0.05	$-2.09 \cdot 10^{-3}$	0.45	74.37
Mineral resource scarcity	kg Cu (eq)	0.42	0.06	0.01	0.34	0.38
Fossil resource scarcity	kg oil (eq)	2.87	0.67	0.23	1.97	4.42
Water consumption	m ³	0.11	0.02	0.01	0.07	0.16

The endplates and chamber are the primary contributors to the reactor system, as shown in the figure below (extracted from the SimaPro software).

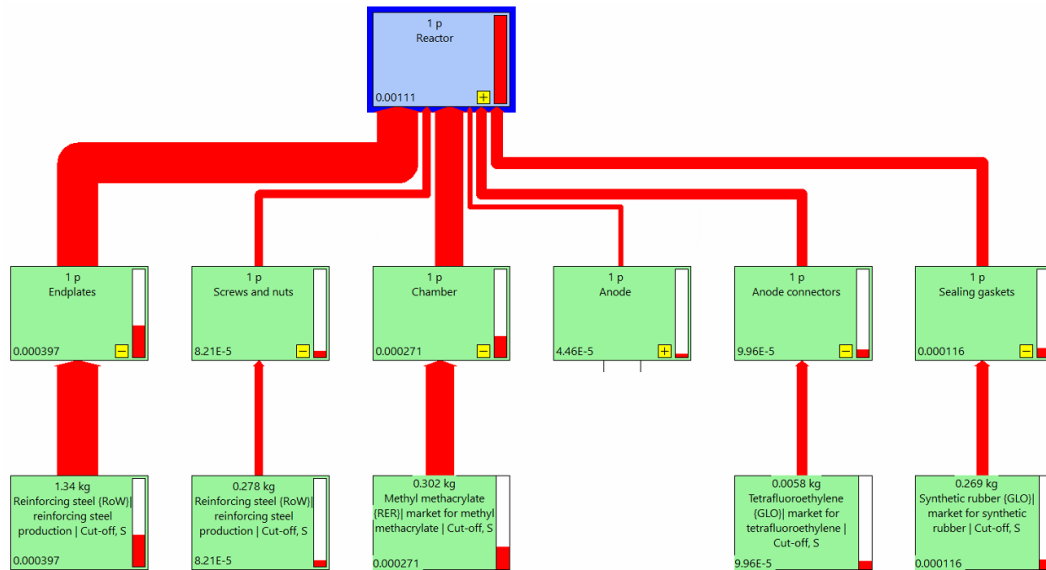


Figure 21. Network for the global warming category from SimaPro software for the construction of the reactor system for BES-HC.

The spacers are the main contributor to the reactor system, as shown in the figure below (extracted from the SimaPro software).

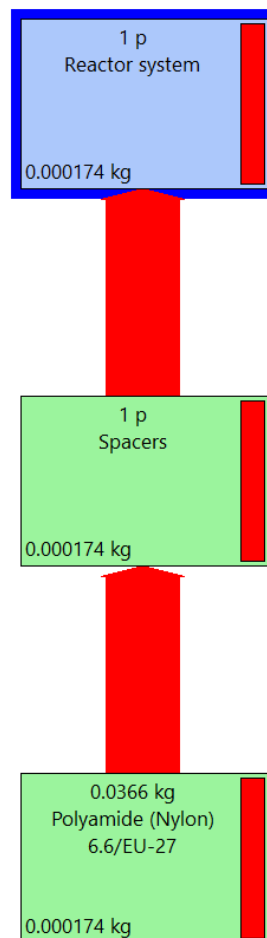


Figure 22. Network for the global warming category from SimaPro software for the construction of the reactor system for BES-M.

5.5 Environmental results and discussion for case of study 2b

The results for Case Study 2b are presented below, outlining the environmental impacts associated with each phase of the assessment: i) preparation of the feedstocks, ii) crystallization for zeolite synthesis, iii) drying for product recovery and iv) adsorption process. All values are reported per functional unit (1 L of heavy metal treated).

Table 26. LCA results for case study 2b.

Environmental impact category	Unit	Total	Preparation of the feedstocks	Crystallization for zeolite synthesis	Drying for product recovery	Adsorption process
Global warming	kg CO ₂ (eq)	4.69	0.08	1.15	3.45	0.01
Terrestrial acidification	g SO ₂ (eq)	16.21	0.25	3.98	11.94	0.04
Freshwater eutrophication	g P (eq)	4.28	0.05	1.06	3.18	1.86·10 ⁻³
Terrestrial ecotoxicity	kg 1,4-DCB	23.66	1.03	5.62	16.89	0.11
Land use	cm ² _a crop (eq)	1,778.16	31.28	436.09	1,308.78	2.02
Mineral resource scarcity	g Cu (eq)	9.03	0.75	2.07	6.21	0.01
Fossil resource scarcity	g oil (eq)	1,316.51	22.16	322.30	967.32	4.74
Water consumption	L	89.65	0.85	22.16	66.62	0.03

Based on the results in the table below, the drying recovery phase is identified as having the most significant environmental impacts in this case study. In contrast, the adsorption process contributes minimally to the environmental impacts, with the overall processes involved in zeolite synthesis being the main drivers of the environmental burden.

The environmental impact associated with the drying recovery phase arises mainly from its high electricity consumption (10.44 kWh per liter of groundwater treated), as illustrated in the figure below.

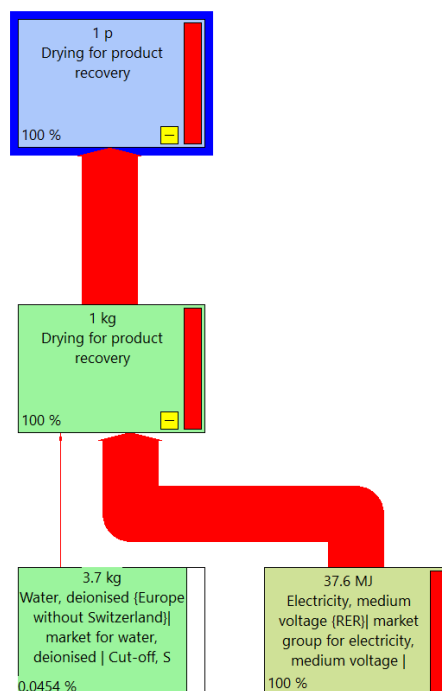


Figure 23. Network for the global warming category from SimaPro software for the environmental assessment of Case study 2b — Drying for zeolite recovery phase.

A similar finding was observed for the crystallization phase, as shown in the figure below. According to the Table 12, the energy consumption for this phase is equal to 3.48 kWh.

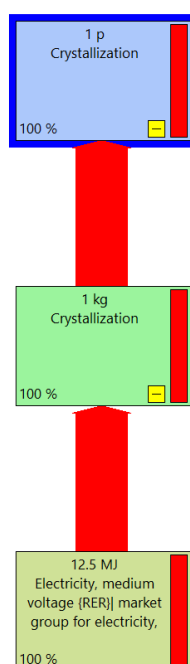


Figure 24. Network for the global warming category from SimaPro software for the environmental assessment of Case study 2b — Crystallization phase.

5.6 Environmental results and discussion for case study 2c

The results for Case Study 2c are presented below, outlining the environmental impacts associated with each phase of the assessment: (i) reactor construction, ii) photo Fenton operation and iii)

emissions to aquatic systems. All values are reported per functional unit ($21 \text{ m}^3 \text{ h}^{-1}$ of groundwater treated during two years of treatment).

Table 27. LCA results for case study 2c.

Environmental impact category	Unit	Total	Reactor construction	Photo Fenton operation	Emissions to aquatic systems
Global warming	kg CO ₂ (eq)	3,647.00	1,674.16	1,972.84	0.00
Terrestrial acidification	kg SO ₂ (eq)	109.49	56.87	52.62	0.00
Freshwater eutrophication	kg P (eq)	14.12	4.95	9.02	0.14
Terrestrial ecotoxicity	kg 1,4-DCB	243,627.09	103,338.96	140,286.77	1.36
Land use	m ² _a crop (eq)	1,226.21	787.32	438.89	0.00
Mineral resource scarcity	kg Cu (eq)	251.17	114.02	137.15	0.00
Fossil resource scarcity	kg oil (eq)	9,309.84	3,034.75	6,275.09	0.00
Water consumption	m ³	827.39	126.02	701.37	0.00

From the results in the table above, the main sources of environmental pressure are identified as (i) the construction of the system, particularly the use of concrete, and (ii) the Photo-Fenton process. The environmental impact of the Photo-Fenton operation is largely driven by the consumption of chemicals, especially hydrogen peroxide, as illustrated below. In contrast, emissions to aquatic systems contribute minimally to the overall environmental impact. Therefore, finding a balance between pollutant removal and aquatic emissions could be a promising strategy for reducing the environmental footprint.

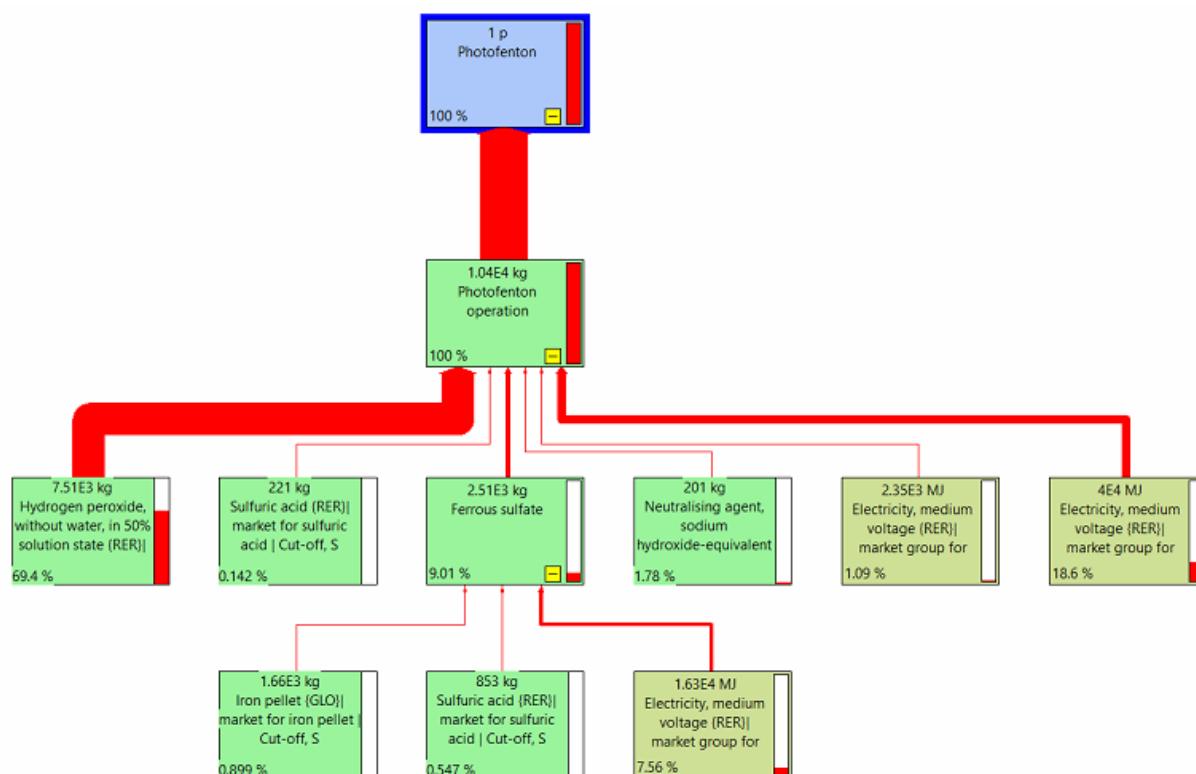


Figure 25. Network for the global warming category from SimaPro software for the environmental assessment of Case study 2c — Photo Fenton phase.

5.7 Environmental results and discussion for case study 3a

The results for Case Study 3a are presented below, outlining the environmental impacts associated with each phase of the assessment: (i) system included in our assessment. All values are reported per functional unit (8 months of operation).

Table 28. LCA results for case study 3a — Water phytoremediation.

Environmental impact category	Unit	Total	System included in our assessment	Power consumption for water recirculation in the set up	Potential products for exploitation
Global warming	kg CO ₂ (eq)	19.03	23.64	0.01	-4.62
Terrestrial acidification	kg SO ₂ (eq)	0.05	0.07	$3.43 \cdot 10^{-5}$	-0.02
Freshwater eutrophication	kg P (eq)	0.01	0.01	$9.13 \cdot 10^{-6}$	$-1.02 \cdot 10^{-3}$
Terrestrial ecotoxicity	kg 1,4-DCB	383.18	478.12	0.05	-94.99
Land use	m ² _a crop (eq)	-0.32	0.43	$3.76 \cdot 10^{-4}$	-0.75
Mineral resource scarcity	kg Cu (eq)	0.29	0.31	$1.78 \cdot 10^{-5}$	-0.02

Fossil resource scarcity	kg oil (eq)	20.27	21.58	$2.78 \cdot 10^{-3}$	-1.31
Water consumption	m ³	-0.13	0.57	$1.91 \cdot 10^{-4}$	-0.69

In UBU scenario, the environmental impact is mainly originated from the construction of the system. These impacts can be mitigated by reusing the installation for future research, which extends the operational lifetime of the facility. Additionally, the power consumption associated with liquid recirculation has had a negligible effect on the overall environmental impact. Besides, although the study was conducted at a laboratory scale, the potential exploitation of products from Case study 3a, such as biomass or treated groundwater, could support a reduction of the environmental impact by avoiding the need for alternative resource use.

5.8 Environmental results and discussion for case study 3b

The results for Case Study 3b are presented below, outlining the environmental impacts associated with each phase of the assessment: (i) infrastructure, ii) pumping, iii) pH conditioning, iv) heavy metals removal and v) waste management. All values are reported per functional unit (4 years of operation).

Table 29. LCA results for case study 3b.

Environmental impact category	Unit	Total	Infrastructure	Pumping	pH conditioning
Global warming	kg CO ₂ (eq)	5,203,697.32	7,679.42	980,016.80	106.30
Terrestrial acidification	kg SO ₂ (eq)	22,455.43	18.95	3,391.64	0.26
Freshwater eutrophication	kg P (eq)	3,205.12	1.40	902.61	0.01
Terrestrial ecotoxicity	kg 1,4-DCB	17,442,126.52	71,725.28	4,796,134.80	5,694.34
Land use	m ² _a crop (eq)	115,764.84	463.22	37,196.61	0.86
Mineral resource scarcity	kg Cu (eq)	6,130.32	1,571.27	1,761.40	0.12
Fossil resource scarcity	kg oil (eq)	1,375,737.10	3,326.86	274,905.79	8.49
Water consumption	m ³	50,588.53	12,518.18	18,897.62	0.49

Table 29. LCA results for case study 3b (continue from the table above).

Environmental impact category	Unit	Heavy metals removal	Waste management
Global warming	kg CO ₂ (eq)	3,677,894.40	538,000.40
Terrestrial acidification	kg SO ₂ (eq)	17,183.14	1,861.45
Freshwater eutrophication	kg P (eq)	1,805.96	495.13
Terrestrial ecotoxicity	kg 1,4-DCB	9,933,661.90	2,634,910.20

Land use	m ² _a crop (eq)	57,689.41	20,414.74
Mineral resource scarcity	kg Cu (eq)	1,830.58	966.95
Fossil resource scarcity	kg oil (eq)	946,174.73	151,321.23
Water consumption	m ³	8,805.71	10,366.54

From these results, it is observed that the P&T technology is connected to significant environmental impacts across all phases included in the current assessment: i) infrastructure development, ii) water pumping, iii) pH conditioning, iv) heavy metals removal, and v) waste management. Among these, the removal of heavy metals emerges as the primary contributor to environmental burden, mainly due to the use of activated carbon (as illustrated in the figure below).

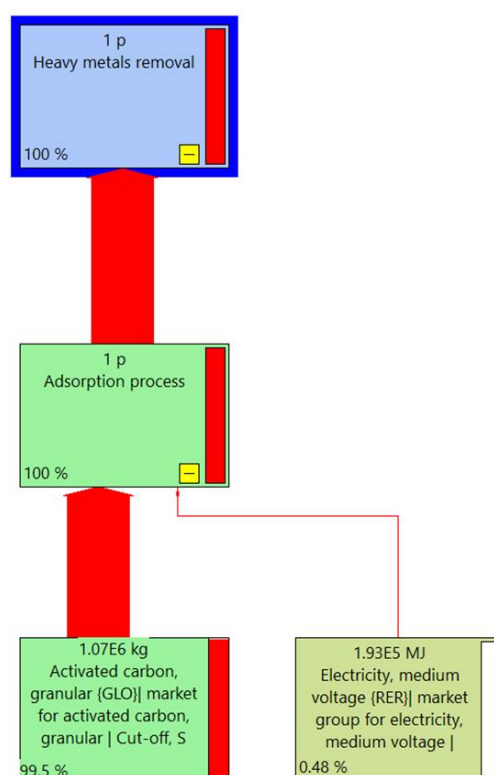


Figure 26. Network for the global warming category from SimaPro software for the environmental assessment of Case study 3b — Heavy metals removal.

Regarding the pumping phase, power consumption could be optimized based on the scale required for the removal of contaminants. This parameter should be considered in further analyses from an environmental perspective.

5.9 Environmental results and discussion for case study 4

The results for Case Study 4 are presented below. All values are reported per functional unit (100 L of inoculum).

Table 30. LCA results for case study 4.

Environmental impact category	Unit	Total
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Global warming	kg CO ₂ (eq)	$1.37 \cdot 10^{-3}$
Terrestrial acidification	kg SO ₂ (eq)	$8.27 \cdot 10^{-5}$
Freshwater eutrophication	kg P (eq)	$4.87 \cdot 10^{-7}$
Terrestrial ecotoxicity	kg 1,4-DCB	0.03
Land use	m ² _a crop (eq)	$4.16 \cdot 10^{-4}$
Mineral resource scarcity	kg Cu (eq)	$5.91 \cdot 10^{-5}$
Fossil resource scarcity	kg oil (eq)	$3.69 \cdot 10^{-4}$
Water consumption	m ³	$8.09 \cdot 10^{-5}$

For the current case study, the possibility of diluting the media starting from a lower concentration was evaluated, with the expectation of a lower associated environmental impact. However, this assumption was not confirmed for a 10% dilution, as the environmental impact associated with the water used for dilution outweighed the benefits of the dilution itself (as illustrated in the figures below).

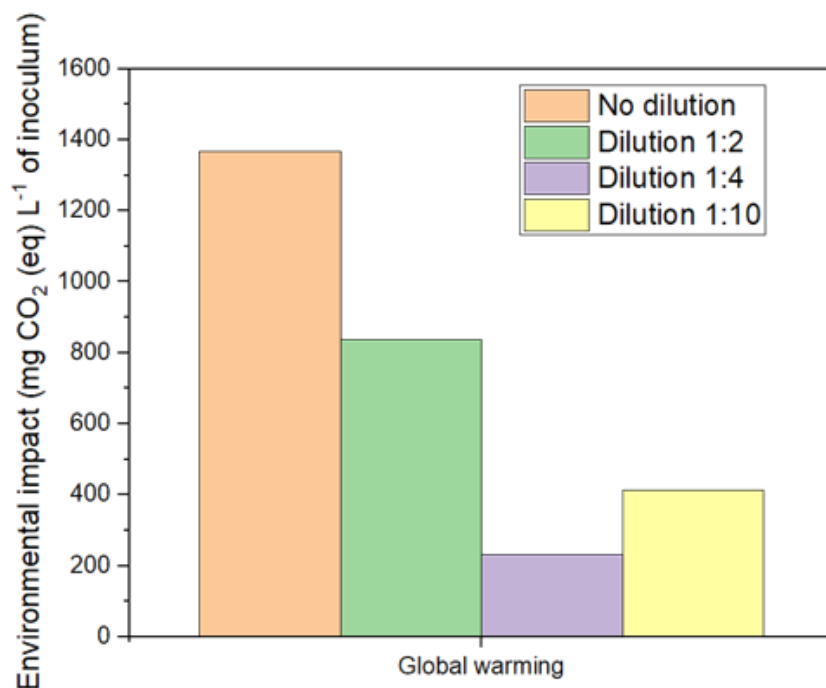


Figure 27. Environmental impact connected to the inoculum dilution — Global warming.

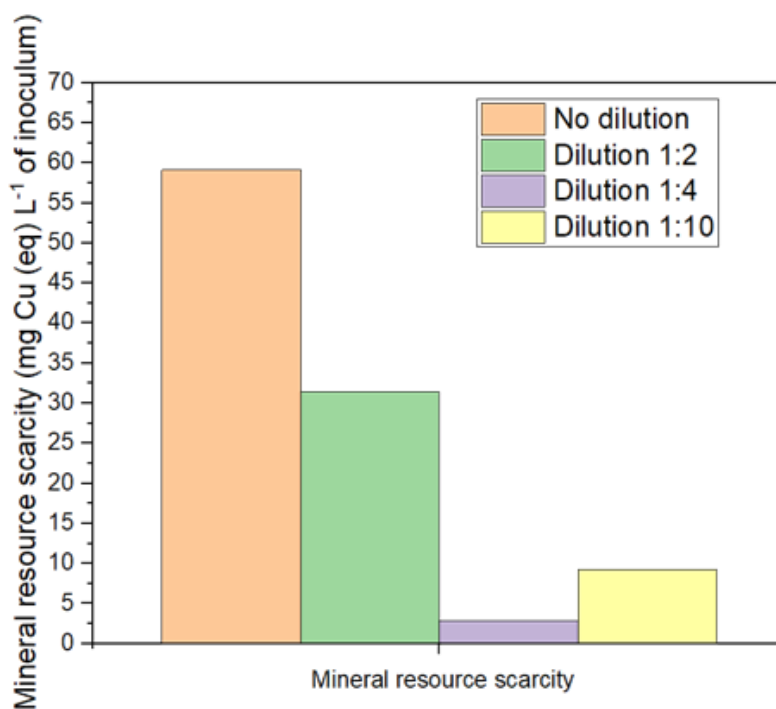


Figure 28. Environmental impact connected to the inoculum dilution — Mineral resource scarcity.

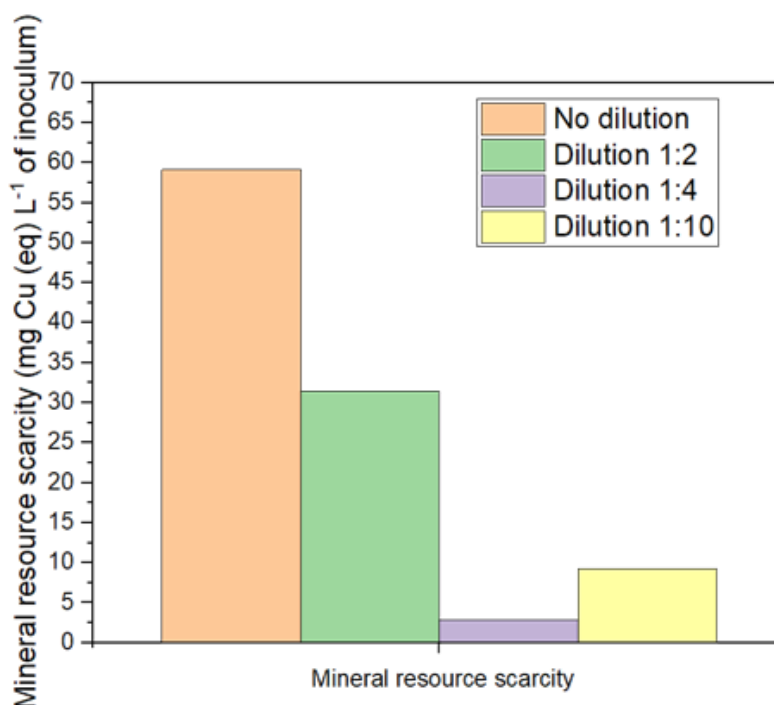


Figure 29. Environmental impact connected to the inoculum dilution — Land use.

6 Conclusions, recommendations and hotspots identification

Given the results available at this stage, the LCA execution has found the following:

- The environmental impacts associated with poplar cultivation at UPM's facility are largely driven by infrastructure requirements and power consumption, with the use of chambers and the electricity needed for their operation being the main contributors to global warming and other environmental categories.
- In the microbial growth phase of Case study 1a, power consumption for the autoclave and other laboratory equipment significantly contributes to environmental impacts. The use of electronic equipment and metals in the manufacturing of these devices is a major source of these impacts, highlighting the importance of optimizing energy use and extending equipment lifespan.
- The microbial preservation phase of Case study 1a shows lower environmental impacts compared to microbial growth of the same case study, primarily because the power consumption of freezers and other equipment in this phase is relatively less impactful.
- The primary contributors to environmental impacts in Case study 1b were the assembling and disassembling phases. The disassembling phase, in particular, had the highest impact due to material consumption, including: i) deionised water, ii) acids, iii) methanol, and iv) nitrogen gas.
- The environmental impact from waste treatment in Case study 1b was minimal, mainly because of the short duration of the experiment. Waste management processes had an almost negligible contribution to the overall environmental footprint.
- It is recommended to minimize material usage in Case study 1b, particularly: i) deionised water, ii) acids, and iii) nitrogen gas. Special attention should be given to controlling nitrogen gas

consumption, as it can significantly increase the impact. For this case study, it is recommended to evaluate the environmental impacts of the research from a long-term approach.

- The landfilling phase in Case study 1c is the main contributor to environmental impacts, as observed in both the ReCiPe Midpoint and Ecological Scarcity 2021 approaches.
- The Ecological Scarcity 2021 and ReCiPe Midpoint Methodologies demonstrate a clear correlation, with both identifying the same environmental drivers responsible for the observed impacts.
- In Case study 2a, components such as the cathode, anode, and membranes, which have a shorter lifespan, contribute significantly to environmental impacts. Extending the lifespan of these components through improvements in durability and technology could help reduce long-term environmental burdens.
- To reduce environmental impacts in Case study 2b, it is recommended to focus on enhancing energy efficiency during the drying and crystallization phases. This could be achieved by exploring alternative energy sources or optimizing the processes to minimize overall energy consumption.
- The construction of the reactor system and the Photo-Fenton operation are the main contributors to the environmental impacts in Case study 2c. Optimizing both phases would be essential for reducing the overall environmental footprint.
- In Case study 2c, emissions to aquatic systems have a minimal environmental contribution in comparison to other phases of the process. A key strategy for reducing the overall environmental footprint could involve focusing on finding a balance between the Photo Fenton operation and the releases to aquatic system.
- In Case study 2c, emissions to aquatic systems contribute minimally to the overall environmental impact. A potential strategy to reduce the environmental footprint could involve intentionally lowering the removal efficiency of pollutants, leading to the release of higher contaminant levels into the aquatic system, but with reduced material and energy consumption for the Photo-Fenton operation.
- In Case study 3a, the environmental impacts primarily arise from the construction of the system. Mitigating these impacts can be achieved by reusing the installation for future research, thereby extending the operational lifetime of the facility.
- In Case study 3b, heavy metals removal is the primary contributor to environmental burdens. A potential strategy to reduce impacts could involve optimizing the use of activated carbon or exploring alternative materials for this phase.
- In Case study 4, diluting the media starting from a lower concentration did not result in a reduced environmental impact for extreme dilutions (10%). The environmental impact connected to the water used for dilution outweighed any potential benefits. It is recommended to reconsider the dilution strategies used in bioaugmentation applications to minimize environmental impacts.