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Table of contents

- 1. Introduction and technology description
- 2. Applicability of the EiCLaR technologies
- 3. Technical performance and benefits of the EiCLaR technologies
- 4. Conclusions and recommendation for Service Providers
- 5. Additionnal information and contacts

1. Introduction and technology description

EiCLaR developed scientific and technical innovations for in-situ bioremediation technologies.

Four in-situ bioremediation technologies were developed into industrial processes for the rapid efficient costeffective treatment of a range of environmental pollutants, such as chlorinated solvents, heavy metals, hydrocarbons. These technologies show great promise in addressing environmental pollution challenges while minimizing the need for disruptive excavation or costly conventional remediation methods.

The aim of the "White papers" is to provide a technical briefing for each of the EiCLaR technologies, targeted to the different practitioner audiences:

- « White paper » for site owners/ managers (including real estate developers)
- « White paper » for regulators
- « White paper » for service providers
- « White paper » for environmental service procurement personnel



Enhanced Innovative *In Situ* Biotechnologies for Contaminated Land Remediation

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page 2



This "White paper" provides the following key informational content:

- Identification of the most likely EiCLaR application niches in the near, medium and long term in consultation with a range of market opinion formers, technology users, site managers and regulatory interests;
- Guidance on most appropriate use of EiCLaR technologies, matching technologies to problems and site characteristics;
- Synthesis across EiCLaR technologies that identifies the most significant technology development
 opportunities to TRL9 on the basis of likely cost competiveness, time to completion, usage of space, risk
 management performance and sustainability, benchmarked against currently available solutions;
- · Conclusion and recommendations.

The EiCLaR technologies are summarized below:

Electro-Nano bioremediation (ENB)

Electro-nano bioremediation is an innovative and advanced remediation technology that combines three key processes to efficiently clean up contaminated environments. ENB integrates Electro-kinetics, nanotechnology, and bioremediation processes to tackle complex and persistent pollutants in soil and groundwater. The combined use of electrokinetics, nanotechnology, and bioremediation in Electro-nano bioremediation provides a synergistic effect, leading to faster and more efficient cleanup of contaminated sites. This technology shows great promise in addressing contaminant source treatment while minimizing the need for disruptive excavation or costly conventional remediation methods.

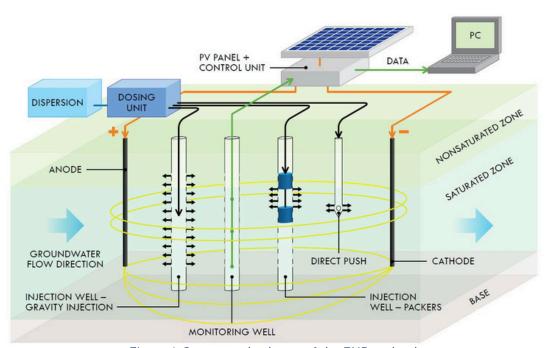


Figure 1: Conceptual scheme of the ENB technology



page 3



Monitored Bioaugmentation Remediation (MBR)

The aerobic metabolic degradation represents a new and promising process to remove chloroethenes from the subsurface environment. Aerobic chloroethene biodegradation can occur under natural conditions and after addition of oxygen in engineered systems in-situ or on-site. The aerobic degradation without the need for auxiliary substrates has a high potential for practical application. While the bacteria do not seem to be present in sufficient numbers at many polluted sites, bioaugmentation in combination with electrode application and specific qPCR monitoring has been developed in EiCLaR. This aerobic technology targets chloroethene contaminated groundwater and provides an important alternative to anaerobic approaches.

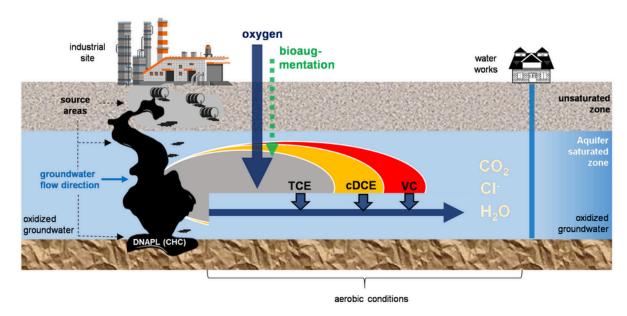


Figure 2: Process schematic for biodegradation of chloroethenes through bioaugmentation with aerobic metabolic chloroethene degraders and oxygen infiltration

Bioelectrochemical Remediation (BER)

BER can simultaneously remove pollutants and recover energy from the contaminant. Up to now, most studies of bioelectrochemical systems have targeted treating wastewater or novel compound synthesis. In EiCLaR, the bioelectrochemical system approach has been developed for industrial sites polluted with mixtures of pollutants. With BER, we have targeted typical soil and groundwater contaminants, including aromatic hydrocarbons (e.g. PAH) and chlorinated solvents. In addition to straight-forward degradation at both electrodes, we have also included pollutant and substrate monitoring at the anode and microbial electrolysis to fuel chosen reactions at the cathode. A wide range of redox reactions catalysed by the microbial population present at/or near the electrodes includes anaerobic oxidations of reduced contaminants at the anode and the reduction of chlorinated compounds at the cathode. This inexpensive technology enhances natural attenuation and actively degrades groundwater contaminants.



page 4



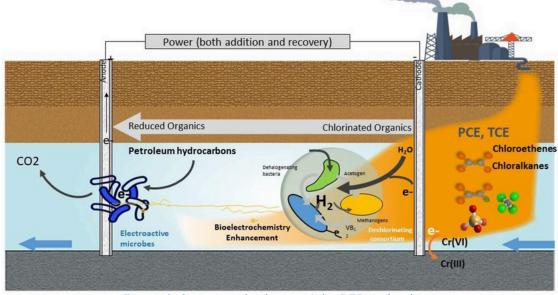


Figure 3: Conceptual scheme of the BER technology

 Numerical reactive transport model for aerobic chloroethene bioaugmentation (within the MBR) and bioelectrochemical systems (within the ENB)

The aim of this software is to describe the aerobic chloroethene degradation including electrobioaugmentation and electrokinetic transport and finally, to optimize the in-situ bioremediation for the ENB and MBR technologies. The numerical model will be able to simulate the recently discovered aerobic TCE degradation. The model can accompany microcosm testing, medium-scale pilots and full field applications.

Enhanced Phytoremediation (EPR)

Phytoremediation is a cost-effective and environmentally friendly remediation technology, however, for plants to thrive, soil toxicity must first be reduced and the treatment is traditionally applied for surface soils. EiCLaR has developed an approach that combines two processes – phytoremediation for shallow contamination and electrochemical oxidation for deeper soil contamination. Low-voltage electricity is applied to iron electrodes inserted into the contaminated soil and helps immobilize metals, while also stimulating soil bacteria to aid in the degradation of organic contaminants. The injection of mycorrhizal inoculum further enhances the synergistic interactions between plants, microorganisms, and mycorrhizae, facilitating the degradation or immobilization of contaminants in the topsoil. This technique is intended to reduce metal toxicity through immobilization, promote the phytodegradation of organic pollutants in shallow soil layers, and induce bioelectrochemical oxidation in deeper soil layers. The treatment of mixed heavy metal and organic pollutants are targeted by this approach.



page 5



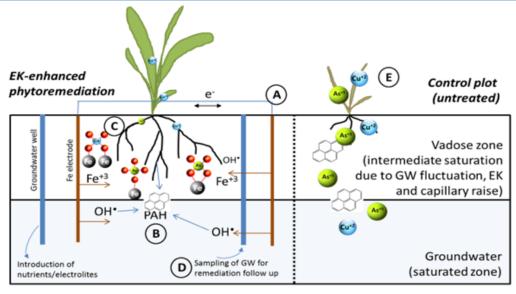


Figure 4: Principles of the enhanced phytoremediation approach - Integrated Contaminant Stabilization and Degradation Technology

EiCLaR Decision Support Tool (DST)

The Decision Support Tool (DST) can be used to determine whether a potential treatment technology (in total 24 technologies including the four EiCLaR technologies (ENB, MBR, BER, EPR) is a viable candidate to remediate a given contaminated site. The DST is a simple online resource to identify likely technology fitness for purpose for specific site characteristics, particularly for new users and smaller organisations. The tool provides users and organisations with a limited remediation know-how. The DST is organised in a comprehensible and user-friendly way to provide easy access to understanding operational performance in real-world applications. The DST supports this service with unique remediation option appraisal engine and support for document drafting.

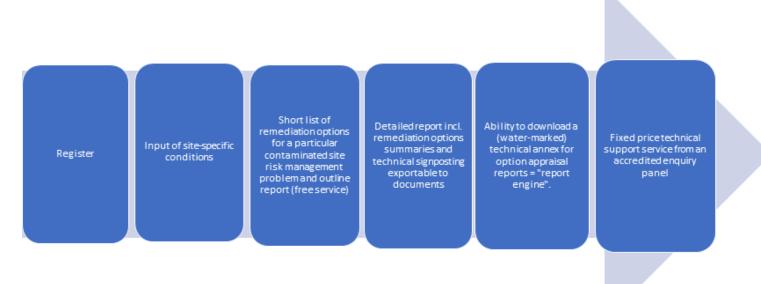


Figure 5: Front-end workflow of the Decision Support Tool.



page 6



2. Applicability of the EiCLaR technologies

Introducing the applicability of the EiCLaR technologies involves outlining how each technology can address specific contaminants and their associated treatment feasibility. They are summarised in the tables below:

Table 1: Target conta	minants by the 4 EiC	Electro-nano- bioremediation (ENB)	Monitored Bioaugmentatio n (MBR)	Bioelectrochemical Remediation (BER)	Enhanced Phytoremediation (EPR)
Treatable contaminants	Halogenated organic compounds	Prime targets: Cl-ethenes, Cl- methanes, brominated aliphatics, HCH, lindane Possible targets: Cl- ethanes, Cl- benzenes	Prime target: Chloroethenes (TCE/cDCE/VC)	Chlorinated solvents and microbial monitoring at the cathode	
	Non- halogenated organic compounds			Petroleum hydrocarbons and compound concentration monitoring at the anode	Petroleum hydrocarbons
	PAH = non halogenated	No		Petroleum hydrocarbons and compound concentration monitoring at the anode	РАН
	PFAS	Yes, with adopted design and operation		To be determined	No
	PCBs (halogenated organic compounds)	Efficient for some congeners, must be tested in laboratory in advance		Chlorinated compounds at the cathode	
	Cationic Trace Elements	Cr, As, Cu, durability/ stability/ permanency of metal stabilisation must be evaluated.		Possible metal redox reactions at the cathode	Cu, Cr





Treatable contaminants	Oxyanionic Trace Elements	U, Zn			As
	Others	Nitrates, suplhates, phosphates			
	Max. concentration	Not limited	~50mg/L TCE	Dissolved phase within or outside the source zone	Unlimited for layers below root zone

Table 2: Feasibility protocol for the 4 EiCLaR technologies.

Technology acronym		Electro-nano- bioremediation (ENB)	Monitored Bioaugmentatio n (MBR)	Bioelectrochemical Remediation (BER)	Enhanced Phytoremediation (EPR)
Site requirements/ Limitations	Saturated zone (below water table, no soil air)	Suitable	Suitable	Suitable	Suitable
	Unsaturated zone (includes root zone)	Not suitable	Suitable	Not suitable	Potentially suitable
	Plume (dissolved phase)	Suitable	Suitable	Suitable	Suitable
	Residual phase NAPL (discontinuous phase)	Suitable	Suitable	Suitable	Suitable
	NAPL pool (continuous phase)	Potentially suitable	Not suitable	Not suitable	Suitable
	Sorbed	Suitable	Potentially suitable	Suitable	Not suitable



page 8



3. Technical performance and benefits of the EiCLaR technologies

The EiCLaR technologies offer innovative, cost-effective solutions for soil and groundwater remediation by combining electrochemical, biological, and nanotechnological processes, enhancing treatment efficiency and reducing chemical usage. These technologies provide adaptable, environmentally friendly methods that improve contaminant degradation, minimize side effects, and lower operational costs compared to traditional approaches.

The main **benefits** of using the four EiCLaR technologies are summarised below:

Electro-nano bioremediation (ENB):

- The combination of electrochemical processes, nanotechnology and bioremediation leads to quicker remediation compared to traditional methods.
- Environmentally friendly process using small doses of environmentally friendly materials ZVI and carbon substrate, chemical reducing agents enhanced electrokinetically and biologically using synergistic effects of both biological and electrochemical processes, leading to increased reactivity of zvi reagents, improved efficiency (also in lower permeable soils and more persistent contaminants) and reduced costs and lower chemical usage;
- No contaminant concentration limits chemical reduction suitable and cost effective for higher contaminant loads, bioreduction for lower contaminant concentrations; the system is easily adaptable to various contaminant compositions;
- Compared to conventional pure ZVI/ biodegradable system ENB represent "engineered solution" as DC system can optimize and control in-situ reduction process in real-time depending on monitoring results measured in real-time. This allows to adjust and control remotely conditions for nanoremediation and biostimulation without further addition of chemical additives, reagents, and buffers and to make important savings of costs (material and O&M).
- The method stimulates microbial activity, promoting natural bioremediation processes in conjunction with electrochemical treatment.

• Monitored Bioaugmentation (MBR):

- No need for auxiliary substrates
- Less oxygen needed for site remediation (since oxygen is used more efficiently compared to cometabolic processes)
- Complete mineralization of contaminants
 - CO2, H2O and Cl- as product of contaminant degradation
 - No risk of accumulation of cDCE or VC
- Aerobic process
- No unfavourable anaerobic side reactions such as sulphide and methane formation



page 9



Bioelectrochemical Remediation (BER):

- Wide range of compounds to be degraded
- Setup costs are low compared to other technologies
- Maintenance and energy costs are minimum (monitoring requires input less than 9V battery)
- Natural attenuation is enhanced by the presence of the electrodes
- Simple installation (does not requires specific knowledge)
- Extremely low overall cost

• Enhanced Phytoremediation (EPR):

- Simultaneously targeting mixed contaminants to completely degrade organic molecules and immobilise inorganic contaminants in situ/on site
- Innovative use of stimulated phyto(bio)remediation via and electric field and additional amendments,
 e.g. arbuscular mycorrhizal fungi (AMF), for degradation/immobilisation of contaminants
- Facilitated distribution of nutrients and immobilising agents through the soil profile

For a more detailed description of each of the EiCLaR technologies, please refer to the Technical Bulletins: claire.co.uk/eiclar.

To enhance the selection process for remediation technologies, the Decision Support Tool (DST), developed in EiCLaR project, provides a powerful resource that combines expert analysis with practical usability. This tool is designed to assist users in evaluating and ranking remediation technologies based on site-specific conditions, promoting sustainable decision-making.

• Key features of the DST include:

- Evaluation of conditions and rules, and determination of a suitability score using a fuzzy logic approach.
- Ranking of the EiCLaR technologies by their estimated degree of sustainability and provision of a ranked shortlist of remediation technologies and either a generic or custom report.
- Web interface with the ability to save, return and modify technical inputs
- Extensible for both technologies and parameters.
- Free to use after registration.
- Generic downloadable PDFs will be free.

The DST is available on the following website: <u>contaminatedland.info</u>.





4. Conclusions and recommendations for Service Providers

The EiCLaR technologies represent advanced in-situ sustainable solutions designed to address a variety of pollution challenges efficiently, sustainably, and cost-effectively. For service providers, the following recommendations outline best practices to leverage the specific benefits of each technology, enhance their implementation, and achieve optimal remediation outcomes.

· Site assessment and characterization

- **ENB**: Conduct a comprehensive site assessment to identify the extent of contamination, types of contaminants, and hydrogeological conditions. Use tracer tests and well-bore geophysics to characterize groundwater flow velocity and directions. Analyse hydrochemical and hydrogeological properties of the aquifer, utilize geophysical methods and sampling to characterize aquifer structure and properties.
- MBR: Conduct a comprehensive site assessment to identify the extent of contamination, types of
 contaminants, and hydrogeological conditions. Analyse hydrochemical and hydrogeological properties of
 the aquifer. Analyse the potential for microbial biostimulation with PCR-based methods that have been
 developed for aerobic metabolic TCE degrading bacteria. If appropriate, perform laboratory microbial
 degradation tests with groundwater and/or soil to better evaluate microbial degradation potential and
 need for bioaugmentation.
- BER: Assess soil conductivity and groundwater chemistry and flow to inform electrode placement, ensure efficient electrochemical interactions and determine target contaminant identity and concentrations.
- EPR: Analyse soil conditions, especially in the root zone, and determine contaminant types to select compatible plant species and fungi for effective contaminant degradation (e.g. PAH) or stabilisation (e.g. As). Perform a thorough site analysis to determine contaminant types and concentrations, hydrogeological conditions, soil texture, groundwater table and flow, essential for targeting pollutants like arsenic and PAHs effectively at various depths.

· Selection of appropriate reagents

- **ENB**: Choose appropriate nano/ micro materials and biosubstrate based on the specific contaminants, and aquifer properties. Assess the stability, reactivity, and environmental impact of selected reagents.
- MBR: Choose appropriate oxygen delivery based on contaminant concentration and aquifer properties.
 Assess conditions for providing biostimulation: are intrinsic organisms available or will bioaugmentation with enrichment cultures be an option?
- BER: No reagents to select.
- EPR: Explore amendments or nutrients that support root growth and mycorrhizal colonization, enhancing
 the interaction between plants, fungi, bacteria and contaminants. Use iron amendments for arsenic
 stabilization and reactive oxygen species (ROS) for PAH degradation. Iron electrodes provide both
 continuous amendment and might contribute to ROS generation, while arsenic-tolerant plants like reed
 canary grass aid in treatment.

page 11



EiCLaR White Paper for Service Providers



Concept design for on-site implementation

- ENB: Design a well-designed injection strategy to ensure even distribution of reagents within the
 contaminated aquifer. Consider the use of multiple injection points and techniques such as direct push or
 well injection. Perform laboratory reactor tests for defining of reagent doses and reaction times. Perform
 electrokinetic tests to define DC system dimensions (current, spacing and position of electrodes). Perform
 operational strategy and plan.
- MBR: Plan for potential oxygen or nutrient injections as needed to support microbial degradation. Develop
 a well-designed injection strategy to ensure even distribution of reagents within the contaminated
 aquifer. Consider the use of multiple injection points and techniques such as direct push or well injection.
 Perform operational strategy and plan.
- BER: Ensure effective distribution of electrodes to facilitate uniform microbial activity across the contamination zone, keeping electrode spacing optimal for low-voltage application. Use existing or install new groundwater sampling wells.
- EPR: Configure electrodes in a grid for uniform electrochemical oxidation. Adjust grid spacing for optimal coverage and efficient energy use, ensuring thorough contaminant stabilization in situ. Design the layout of plants and fungal inoculation to promote even root-zone coverage and maximize nutrient distribution across the treatment area.

Optimization of nanomaterial and biosubstrate properties

- ENB: Define reagent concentrations and injection schedules that enhance nanomaterial reactivity and microbial stimulation. Optimize conditions for biofunctionalization, aiming to improve contaminant interaction and extend reagent activity.
- MBR: Not applicable
- BER: Not applicable
- EPR: Not applicable

Monitoring and evaluation

- **ENB**: Implement a comprehensive monitoring plan to assess real-time contaminant reduction and groundwater quality. Use in-situ sensors to remotely monitor and adjust the DC system, reducing the need for additional chemical inputs.
- MBR: Establish a robust monitoring plan to assess the effectiveness, including pre- and post-injection contaminant levels. Include sampling wells from the source zone, as well as up- and downstream the contaminant source. Apply qPCR methods on groundwater samples to easily detect a growth of stimulated/added microorganisms.
- BER: Monitor low-voltage inputs and microbial response, observing contaminant concentrations and groundwater quality to validate system efficiency and adjust as necessary.
- EPR: Set up monitoring to assess plant and fungi health, microbiological activity, contaminant uptake, groundwater and soil quality, verifying that degradation and immobilization of contaminants occur as expected.



page 12



 EPR: Set up monitoring to assess plant and fungi health, microbiological activity, contaminant uptake, groundwater and soil quality, verifying that degradation and immobilization of contaminants occur as expected.

Regulatory compliance

- For ENB: Obtain permits for using reactive substances (e.g., elemental iron nanoparticles), constructing boreholes, and handling water treatments associated with the process. Conduct an environmental impact assessment to address potential risks, ensuring the materials and reaction products align with natural mineralogy. Adhere to safe voltage limits (0–60 V) for DC application, which typically avoids special permit requirements itself.
- For MBR: Verify that the injected inoculum culture used aligns with environmental regulations
- For BER: Ensure that the in-situ remediation processes and low-voltage applications comply with regulatory guidelines for subsurface interventions.
- For EPR: Confirm that chosen plant and fungal species meet local environmental standards, especially
 when using non-native varieties for phytoremediation. The system's in situ approach minimizes land
 disturbance, aligning with environmental standards and regulatory requirements.

· Sustainability and long-term monitoring

- **For ENB**: Use environmentally benign nanomaterials whenever possible and prioritize remote adjustments to minimize operational costs and reduce reagent consumption.
- For MBR: Incorporate sustainability by using existing microbial communities and avoiding additional substrates, ensuring the treated site remains stable over time.
- For BER: Monitor the longevity and maintenance of electrodes to keep replacement needs low, ensuring an economically viable and sustainable process.
- For EPR: Plan for ongoing monitoring to ensure plants and fungi continue to perform well in stabilizing contaminants, reducing the risk of recontamination, while stabilised elements in deeper soil remain stable over time.

Conclusion: Service providers adopting EiCLaR technologies can expect significant benefits in terms of cost, efficiency, and sustainability by following these recommendations. Each technology has unique strengths tailored to various contamination and site conditions, providing versatile options for effective, in-situ remediation across a broad spectrum of environmental challenges.



page 13



5. Additional information and contacts

• Electro-Nano Bioremediation (ENB):

Simon Kleinknecht - Research Facility for Subsurface Remediation (VEGAS)

Photon Water

Petr Kvapil - Photon Water

Jaroslav Nosek - <u>Institute for Nanomaterials, Advanced Technologies and Innovation</u>



Monitored Bioaugmentation Remediation (MBR):

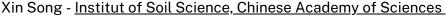
Andreas Tiehm and Steffen Hertle - Technologiezentrum Wasser



Bioelectrochemical Remediation (BER):

Timothy Vogel - Microbiome Engineering

Azariel Ruiz-Valencia - Centre National de la Recherche Scientifique







Matthias Loschko and Luca Trevisan - BoSS Consult



Jurate Kumpiene, Ivan Carabante and Kim Johansson - <u>Lulea University of Technology</u>



Erkki Lindberg - Ekogrid

• Decision Support Tool (DST):

Paul Bardos, Matthias Loschko, Jonas Allgeier, Helen McLennan https://contaminatedland.info/



Marta Popova - SPAQUE



















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