
	EU Environmental Technology Verification P 20: Statement of Verification	page: 1 number of pages: 8 version : 2 change #: 0 date: 2022/01/03	
Verification Body: Inspection body No.4055 CEMC ETVCZ 28. pluku 524/25 101 00 Praha 10	Technology: „ Bioleaching technology by heterotrophic bacteria “ Contract No.: 500 32	Proposer: ekolive, s.r.o., Americká trieda 3 040 13 Košice	

STATEMENT OF VERIFICATION



EU Environmental Technology
Verification pilot programme



České ekologické manažerské centrum
inspection body No. 4055, CEMC ETVCZ
(accreditation No. 49/2021)

Technology: „ Bioleaching technology by heterotrophic bacteria “

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Verification body:

Name: CEMC ETVCZ
inspection body No.4055
Contact: Ing. Jiří Študent

Address: 28. pluku 524/25
101 00 Praha 10
Czech Republic
Telefon: (+420) 274 771 353
E-mail: cemc@cemc.cz

Web: www.cemc.cz
ID: 45249741
VAT ID: CZ 45249741

Proposer:

Name: ekolive, s.r.o.
Contact: Ing. Darina Štyriaková,
Ph.D.

Address: Americká trieda 3
040 13 Košice
Slovakia
Phone: (+421) 948 299 345
E-mail: darina.styriakova@ekolive.eu

ID: 44 154 089
VAT ID: SK 2022617421

Signatures:

Verification Body

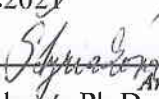
Prague, date 8.12.2021

Ing. Jiří Študent,
head of inspection body
Inspection body No.4055, CEMC ETVCZ

Proposer

Košice, date 8.12.2021

Ing. Darina Štyriaková, Ph.D.
CEO
ekolive, s.r.o.




ekolive s.r.o.
Americká trieda 2430/3
040 13 Košice
IČO: 44154089 IČ DPH: SK2022617421

Certificate number: A14518, A14526, A14477

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CEMC ETVCZ
inspekční orgán akreditovaný ČIA č. 4055
28. pluku 524/25, 101 00 Praha 10
tel.: 274 784 416-17
www.cemc.cz

	EU Environmental Technology Verification P 20: Statement of Verification	page: 2 number of pages: 8 verzion : 2 change č.: 0 
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Content:

1. Technology description	3
2. Application	3
2.1 Matrix	3
2.2 Purpose	3
2.3 Conditions of operation and use	3
2.4 Verification parameters definition summary	3
3. Test and analysis design	4
3.1 Existing and new test data	4
3.2 Laboratory or field conditions	4
3.3 Matrix compositions	4
3.4 Test and analysis parameters	4
3.5 Tests and analysis methods summary	5
3.6 Parameters measured	6
4. Verification results (performance, operational and environmental parameters)	6
5. Additional information, including <i>additional</i> parameters	6
6. Quality assurance and deviations	7
<i>Appendix No.1: Matrix compositions</i>	8



Verification Body:
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101 00 Praha 10

Technology: „**Bioleaching technology by heterotrophic bacteria**“
Contract No.: **500 32**

Proposer:
ekolive, s.r.o.,
Americká třída 3
040 13 Košice

1. Technology description

Comment: *The BIOLEACHING BY HETEROTROPHIC BACTERIA technology is the wasteless technology in fact. Compared to the alternatives, it is a technology without negative environmental impacts and also energy consumption is minimal. It allows the use of scarce sand sources by substituting inferior sand from local sources. The by-product of this technology is a fertiliser that can be used in agriculture.*

2. Application

2.1 Matrix

Comment: *Mineral grain as silica sand*

2.2 Purpose

Comment: *Removal of Fe₂O₃ from silica sand surface by bioleaching method*

2.3 Conditions of operation and use

Comment:
Silica sand with Fe₂O₃ content from 4,0 %w/w to 0,3 %w/w (reality 0.088 %w/w to 1.08 %w/w); starting pH 7.5 to 9.0; Final pH approx. 4.0; temperature 12 °C to 37 °C; One bioleaching cycle 4 days to 10 days; Whole bioleaching process 5 bioleaching cycles; Supply of substances from the proposer: Inoculum; heterotrophic substrate like sucrose; nutrient for bacteria in the form of ekocomplex®; Process is performed under non-sterile conditions; inoculation in the first round of leaching only; Dosage according to proposer's instructions.
Storage of samples: Inoculum was not stored because it was used immediately after delivery; heterotrophic substrate - sucrose: stored under normal laboratory conditions, protect from; moisture; Ekocomplex® stored under normal laboratory conditions.

2.4 Verification parameters definition summary

Comment:
Parameters defined from original claim: *“Removal of 40 % of the original amount of Fe₂O₃ from silica sand by bioleaching after five washing cycles, when starting concentration of Fe₂O₃ in silica sand is in the range 0.3 %w/w to 4.0 %w/w”.*
New parameter: *By-product leachate ekofertile for using in organic farming is proved as safe.*



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Performance parameters: *Reduction of the initial Fe₂O₃ concentration in silica sand by 40 % of the original amount after five washing cycles (iron oxide content (Fe₂O₃) before and after bioleaching).*

Operational parameters: *Anaerobic conditions (pH, redox potential, determination of the chemical oxygen demand concentration). Due to the non-sterile environment of the process, checking of safety parameters of the inoculum and leachate (bacterial count (aerobic heterotrophic bacteria & anaerobic heterotrophic bacteria), detection and enumeration of intestinal Enterococci & coliform bacteria & Salmonella spp.). Others also please see the par. 2.3.*

3. Test and analysis design

3.1 Existing and new test data

Comment:

Existing data could not be accepted (Proposal, pg.5): There was more bioleaching operations conducted (Slovenia, Slovakia, Croatia), however, not all testing and measurements were done by accredited testing bodies, i.e. ISO 17020. Thus new test will be conducted under direct supervision of accredited testing body ABITEC, s.r.o. and ALS CR – test body will be adjusted according to the expert evaluators requirements. Standard method of bioleaching process is defined.

3.2 Laboratory or field conditions

Comment:

Laboratory tests were chosen for verification, accredited tests under ISO 17020 required, the tests bioleaching were performed in a narrow temperature range of 19°C to 21° in anaerobic conditions. All tests will be performed in triplicate with two sand samples in the concentration range of Fe₂O₃ from 0.3 %w/w. to 4.0 %w/w (reality 0.088 %w/w to 1.08 %w/w). The leaching is carried out in five cycles, at the beginning of each cycle a freshly prepared leachate solution containing ekocomplex®, and heterotrophic substrate (molasses, saccharose) is supplied. The leaching solution was prepared according to the proposer's instructions. The pH was adjusted to 7.5 to 9.0. Inoculation of the sand was carried out with the supplied inoculum. Inoculum was homogenized on vortex. After homogenization, approximately 15 to 20 minutes, a leaching solution was poured onto the sand as directed by the proposer. The old leachate containing leached metals is withdrawn. The proposer requested necessity to perform the test under non-sterile conditions to decrease energy consumption in the spirit of ecology design.

3.3 Matrix compositions

Comment: *Please see Appendix I*

3.4 Test and analysis parameters

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Comment:

- *Determination of Fe_2O_3 in silica sand*
- *Removal of 40% of the original amount of Fe_2O_3*
- *Number of washing cycles*
- *pH* - length of leaching per cycle*
- *redox potential**
- *Determination of chemical oxygen demand (COD_{Cr} / $CHSK_{Cr}$) for each cycle - anaerobic conditions*
- *Temperature of the bioleaching**
- *Determination of heterotrophic bacterial counts and count of potentially pathogenic organismus present in the leachate (Salmonella genus bacteria, intestinal enterococci, faecal coliforms bacteria).*

* *The pH, redox potential with the temperature were measured in the leaching solution.*

3.5 Tests and analysis methods summary

Comment:

ISO 1770:1981/Amd 1:1983 – Solid-stem general purpose thermometers – Amendment 1

ČSN ISO 10523 – Water quality – Determination of pH. Determination of pH in leachates (SOP 2.3)

ČSN ISO 10390 – Soil quality – Determination of pH. Determination of pH of solid samples.

ISO 11271:2002 – Soil quality – Determination of redox potential of soil

ČSN 75 7837 – Detection and enumeration of coliform bacteria in non-disinfected waters (SOP 3.33)

ČSN EN ISO 7899-2 – Detection and enumeration of intestinal enterococci (SOP 3.35)

ČSN 75 7842 – Determination of aerobic heterotrophic bacteria at 20 °C (SOP 3.36)



ČSN 75 7841 – Enumeration of total anaerobic bacteria (Ambrozova J.: Microbiology in water technology, ISBN 80-7080-534-X, UCT Prague, 2004) – (SOP 3.38)

ČSN ISO 19250 – Detection of Salmonella spp. by spread plate method (SOP 3.42)

ČSN 75 7367 – Determination of oxidation-reduction potential (SOP 2.14)

ČSN ISO 15705 – Determination of the chemical oxygen demand index (ST-COD) - Small-scale sealed-tube (SOP 4.11)

SOP 28B – VZ lab, s.r.o. Determination of metals (Fe, Mn, Ni, Cu, Co, Cd, Zn, Pb, K, Na, Ca, Mg, Cr, Cr6+, Li, Ba, Sr, V, Al, Be, Ag, As, Ti) by flame atomic absorption spectrometry

	EU Environmental Technology Verification P 20: Statement of Verification	page: 6 number of pages: 8 version : 2 change č.: 0 
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3.6 Parameters measured

Comment:

- *Determination of Fe₂O₃ in silica sand is in the range 0.3 %w/w. to 4.0 %w/w (reality 0.088 %w/w to 1.08 %w/w *)*
- *Removal of 40 % of the original amount of Fe₂O₃ (result: removed 44 % and 76 % respectively)*
- *Number of washing cycles = 5 (result 5)*
- *pH approx. 4 at the end of the fifth cycle (result 3,8 – 4,3)*
- *Determination of COD_{Cr} in each leaching cycle (result, please see the Test report, par. 4.4.6-1 and 4.4.6-2)*
- *Temperature of the bioleaching: 12 °C to 37 °C (reality 19°C to 21°C)*
- *Determination of heterotrophic bacterial counts and count of potentially pathogenic organisms present in the leachate were at acceptable levels in relation to the treated effluent standard or not found at all (result and more details please see Test report, par. 4.4.6-1 and 4.4.6-2).*

4. Verification results (performance, operational and environmental parameters)

Comment:

Performance parameters (claim):

„Bioleaching technology by heterotrophic bacteria ensure removal of 40 % of the original amount of Fe₂O₃ from silica sand by bioleaching after five washing cycles, when starting concentration of Fe₂O₃ in silica sand is in the range from 0.3 %w/w. to 4.0 %w/w**.*

*By-product leachate ekofertile for using in organic farming is proved as safe by the result of the tests carried out *** and also on the basis of the registration of the bioleaching ekofertile in [Dutch Input List Product search](#).*

** Tests result: Fe₂O₃ removed from 44 % to 76 %,*

*** The initial Fe₂O₃ concentrations in silica sand (expressed in %w/w) were actually from 0.088 %w/w to 1.08 %w/w i.e. two orders of magnitude a difference in comparison to one order of magnitude difference requested (0.3 %w/w. to 4.0 %w/w), confirms the effectiveness of the bioleaching process,*



**** Colonies of pathogenic organisms such as Salmonella genus bacteria were not present; intestinal enterococci were found ≤ 5 CFU ml⁻¹; faecal coliform bacteria were found approx. 1,4x10³ CFU ml⁻¹. All bio-indicator were at acceptable levels in relation to the treated effluent standard or not found at all.*

Result and more details please see Test report, par. 4.4.6-1 and 4.4.6-2.

5. Additional information, including additional parameters¹

Comment:

¹ with comments or caveats where appropriate

	<p style="text-align: center;">EU Environmental Technology Verification P 20: Statement of Verification</p>	<p>page: 7 number of pages: 8 verzion : 2 change č.: 0</p> 
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Based on the results obtained for ecofertile and the proof of inclusion in the database, we reclassified the claim from additional parameters to claim (see par.4).

6. Quality assurance and deviations



Comment:

No deficiencies were found in the management of the test process according to the requirements of ISO 17020.

Deviations: Unplanned change in the extent of Fe₂O₃ contamination in the silica sand (please see par. 4) and extension of the declaration of safety of leachate as a nutrient for agricultural purposes (please see par. 4 and par.5). No others deviations from the Specific protocol and Test plan were made.

The change in the extent of the silica sand contamination is desirable as it further confirms the effectiveness of the bioleaching.

More detail information you can see in Verification report par. 4.2.1 and from witness report Appendix 8 – Test system assessment report.

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Appendix No.1: Matrix compositions

Matrix	<i>Mineral grain as silica sand</i>
Purpose	<i>Removal of Fe₂O₃ from silica sand surface by bioleaching method.</i>
Technology	<i>The leaching process takes place in impermeable basin/tank filled with polluted sand and with a batch change of the leaching solution in response to drop of pH below 4.0. The whole system is inoculated by bacterial suspension microlive and supplied by heterotrophic substrates as a source for bacteria producing organic acids.</i>
Performance parameters	<p>Verified parameters:</p> <ul style="list-style-type: none"> • <i>Detrmination of Fe₂O₃ in silica sand</i> • <i>Removal of 40% of the original amount of Fe₂O₃</i> • <i>Number of washing cycles</i> • <i>pH - length of leaching per cycle</i> • <i>redox potential</i> • <i>Determination of chemical oxygen demand (COD_{Cr}) for each cycle - anaerobic conditions</i> • <i>Temperature of the bioleaching</i> • <i>Determination of heterotrophic bacterial counts and count of potentially pathogenic organismus present in the leachate (Salmonella genus bacteria, intestinal enterococci, faecal coliforms bacteria).</i> <p>Conditions of test:</p> <ul style="list-style-type: none"> • <i>Starting pH 7.5 to 9.0</i> • <i>Final pH approx. 4.0</i> • <i>Temperature 12 °C to 37 °C</i> • <i>One bioleaching cycle from 4 days to 10 days</i> • <i>Whole bioleaching process 5 bioleaching cycles</i> • <i>Supplies from proposal:</i> <ul style="list-style-type: none"> ○ <i>Heterotrophic substrate like saccharose</i> ○ <i>Nutrient for bacteria in the form of ekocomplex®</i> ○ <i>Inoculum</i>