BIOMETAL DEMOnstration plant for the biological rehabilitation of metal bearing-wastewaters

Project number 619101

INFORMATION FOR FINAL PROJECT REPORT

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## 1. Final publishable summary report

### Executive summary (1 page)

Heavy metal pollution is one of the most important environmental problems today, even threatening human life. Due to industrialization of agriculture or abusive applications of agrochemicals like phosphate fertilisers, which have a high level of metals, the amounts of such metals deposited onto the surface of the Earth are many times greater than depositions from natural sources. Consequently, the presence of these pollutants in liquid wastes, due to their widespread use by modern society, is a matter of growing concern.

EU-funded BIOMETAL DEMO consortium worked to identify new options to improve the effectiveness of the way that wastewater treatment processes remove heavy metal substances from discharges. All of the team's efforts were focused on ensuring that actual metal treatment technologies comply with the reduction of metal concentration that is legally required by the EU for wastewater treatment.

In a general way, this project aimed to demonstrate the feasibility of applying novel biotechnologies for the treatment of metal polluted wastewaters through the design and development of three pilot plants to be implemented in three metal polluting industries:

- To treat the acid mine wastewater (AMD) using Sulphate Reducing Bacteria (SRB).
- To treat the wastewater of the electroplating plant and the acid mine drainages, both based on the biosorption processes (using biomasses and biopolymers).
- To treat wastewater coming from ceramic sector using the biosorption processes (using biopolymers).

This study provided important information regarding the performance of SRB treating real AMD and demonstrated that SRB bioreactor at a pilot scale attached with a neutralization step can efficiently remove metals and sulphate to levels below the recommended concentration for irrigation waters.

Pilot plants used to treat electroplating plant and the acid mine drainages, both based on biosorption, processes were able to carry out successfully the planned processes. They might be partially improved now that the procedure to optimize these processes is known. However, results obtained shows that these technologies can be implemented in order to achieve an acceptable biosorption of many dissolved metals.

Regarding wastewater coming from the ceramic industry, this study demonstrated that neither physical-chemical treatment nor biosorption processes are suitable for Boron elimination, which is the main metal to remove in this type of wastewater.

## Summary description of the project context and the main objectives (4 pages)

Heavy metal pollution is one of the most important environmental problems today, even threatening human life. Due to industrialization of agriculture or abusive applications of agrochemicals like phosphate fertilisers, which have a high level of metals, the amounts of such metals deposited onto the surface of the Earth are many times greater than depositions from natural sources. Consequently, the presence of these pollutants in liquid wastes, due to their widespread use by modern society, is a matter of growing concern.

A large number of industries produce and discharge wastes containing different heavy metals into the environment; among these industries, the following four appear as the main priority targets, particularly in the industrialized world:

1) acid mine drainage associated with mining operations. The lagoons of acid mine drainages presented in several abandoned mine areas around the world are an important environmental problem that is urgent to solve due to low pH and high metals concentration.
2) electroplating industry waste solutions (growth industry).
3) coal-based power generation (throughput of enormous quantities of coal).
4) nuclear power generation (uranium mining/processing and special waste generation).

EU-funded BIOMETAL DEMO consortium worked to identify new options to improve the effectiveness of the way that wastewater treatment processes remove heavy metal substances from discharges. All of the team's efforts were focused on ensuring that actual metal treatment technologies comply with the reduction of metal concentration that is legally required by the EU for wastewater treatment.

The BIOMETAL DEMO project aims to develop a hybrid bio-precipitation and biosorptive process for removal and recovery of metals from different industrial wastewaters, such as acid mine drainages, electroplating wastewaters and metal contaminated wastewater from frits and glaces in ceramic tile manufacture. These types of industrial wastewater share several common physicochemical characteristics, namely acid pH, metal cations and sulphate and other anions in solution, low presence or absence of organic matter, etc.

In contrast to conventional ion-exchange processes, the potential loading capacity of metal bioprecipitation processes would be high. With regards to conventional metal precipitation techniques, bioprecipitation by means of sulphate-reducing bacteria (SRB) and of immobilized phosphohydrolase biocatalysis leads to the formation of more insoluble and less voluminous metal sulphides and metal phosphates respectively, and do not require the addition of expensive chemicals since the precipitating agent is biologically produced by SRB from sulphate and by immobilized phosphohydrolase from cheap and waste phosphoesters, respectively. The possibility of selective metal sulphide precipitation can be an additional advantage in view of a subsequent metal recovery or in terms of an eventual utilization of the obtained precipitates. Moreover, some SRB and other anaerobic bacteria have shown ability for some metals reduction [Martins et al. 2009; Alexandrino et al. 2011]. This is the case of Cr(VI) and U(VI) that can be enigmatically reduced to the less toxic and less soluble Cr(III) and

U(IV), thus being removed as hydroxide and oxide precipitates, respectively. With regards to biosorption, the metal ion in solution is reduced and deposited in a chemically altered insoluble state (elemental metal) and/or as metal complex on the biosorbent and does not desorb spontaneously. If required, however, the metal can be recovered in a small volume or as concentrated slurry using a suitable desorbing agent and it is possible to re-use the biocatalytic and biosorptive reactor with retention of much of the original activity (Tsezos, 1986; Ramirez-Paredes et al. 2011).

In a general way, this project aimed to demonstrate the feasibility of applying novel biotechnologies for the treatment of metal polluted wastewaters through the design and development of three pilot plants to be implemented in three metal polluting industries - a pyritic mine, an electroplating and a ceramic manufacturing company. The Scientific and technical objectives of the project were

1. To explore, at laboratory scale, the feasibility of application of different bioprocesses for the biological rehabilitation of representative industrial wastewaters (acid mine drainage, electroplating effluents and ceramic tile manufacturing wastewaters) contaminated with heavy metals.
2. To evaluate the effect of environmental factors (sensitivity analysis) on the efficiency and kinetics of metal removal/recovery from such industrial wastewater by the different laboratory bioprocesses.
3. To evaluate at laboratory scale the potential for recycling of other industrial wastes as feedstock to support the metal biotreatment processes.
4. To carry out a comparative analysis of the performance and kinetics, energy inputs, technical and economic advantages and drawbacks of the different metal removal/recovery bioprocesses studied at laboratory scale.
5. To make the best decision about which bioprocess or a synergy of two integrated bioprocesses is the optimum choice to design and to build a demonstration pilot plant for scaling-up the corresponding metal removal biotreatment of such industrial wastewaters.
6. To explore, at pilot plant scale, the feasibility of application of selected bioprocess for the biological rehabilitation of the representative industrial wastewaters (acid mine drainage, electroplating and ceramic manufacture wastewaters) contaminated with heavy metals.
7. To evaluate the effect of environmental factors (sensitivity analysis) on the efficiency and kinetics of metal removal/recovery from such industrial wastewaters by the selected pilot plant bioprocess.
8. To monitor and to optimize the operation, the kinetics and the performance of the metal removal/recovery bioprocess integrated within the pilot demonstration plant.
9. To carry out an economic, social and technical analysis of the benefits of such tertiary biotreatment of metal polluted industrial wastewater could represent for the corresponding and related industrial sectors across the EU.

BIOMETAL DEMO project has been developed in two successive phases; the first one is a Research phase during the first two years and the second one a Demonstration phase during the last two years.

Preliminary research phase

BIOMETAL DEMO included four main research areas on active agents for metal uptake to be tested for their resistance to the main and most toxic metals present in the different industrial wastewater to be treated (acid mine drainages, electroplating effluents and wastewaters from the ceramic tile industry):

1) Research on metal bioprecipitation by means of sulphate-reducing bacteria (SRB), led by CCMAR.
2) Research on metal bioprecipitation using plant and microbial phytases and/or bacterial acid phosphatases, phosphohydrolase enzymes, which catalyse the hydrolysis of organic "phosphate donor" molecules, such as phytic acid or phytates and β-glycerophosphate. The immobilized phytase and/or bacteria were the active agents of thisbioprocess, led by USAL.
3) Research on metal biosorption by means of residual biomass provided by the agrifoodand textile industries (sugar beet pulp, brown algae and hemp woody core, respectively), led by UCM.
4) Research on biosorption using the biopolymers chitosan (derived from chitin, obtained from fungal biomass and at industrial scale from crustacean shells) and alginate (obtained from algal biomass) and related materials, led by ARMINES.

Demonstration phase

Demonstration was addressed to evaluate the economic feasibility of utilizing the previous approaches in real waste situations and to attempt to run the process continuously using readily available, cheap (waste) raw materials. In the middle of the project, the feasibility data and the modelling of the bioprocesses, already studied at laboratory scale, successfully led to the design and construction of demonstration pilot plants in the industrial factories. In this sense, a first demonstration pilot plant was designed and built by HLAB at IGO factory in Valladolid (Spain) for the treatment of its metal polluted wastewaters; a second one was designed and built by LCW at São Domingos mine (South Portugal) for the treatment of its acid drainages; a third one was designed and built by SER to treat wastewaters from a ceramic tile factory in Castellón (Spain); and a fourth one was designed and built by HLAB at its facilities in Albacete (Spain).

Designing and building of the demonstration pilot plants was carried out after:

a) a rigorous comparative analysis of the performance and kinetics, energy inputs, technical and economic advantages and drawbacks of the different metal removal/recovery bioprocesses studied at laboratory scale for each wastewater;

b) the best decision making about which bioprocess or a synergy of two integrated bioprocesses was the optimum choice for scaling-up regarding each wastewater to be treated.

In order to optimize the operation of each demonstration pilot plant, the industrial partners in close interaction with RTD partners, monitored and evaluated the effect of environmental factors (sensitivity analysis) on the efficiency and kinetics of metal removal/recovery from such industrial wastewaters by the corresponding pilot plant bioprocess. Finally, an economic, social and technical analysis of the benefits of such biotreatment for each metal polluted industrial wastewater was carried out for such industrial sectors across EU.

## Description of the main S & T results/foregrounds (25 pages)

The first work carried out by the BIOMETAL DEMO consortium was to agree on the most relevant parameters that would be evaluated throughout the project and to develop a list that can be used as reference.

The research partners, in order to agree on the guidelines on the common research framework and on the most relevant parameters that would be evaluated, met together to discuss on the procedures to follow in the research activities within the project and also to tackle diverse issues encountered with the effluents through communication with each of the SMEs involved.

The main concerns that rose to this effect were:

1. To identify the metals that were going to be taken into account during the research activities and monitored later on during the development of the project.
2. To determine the limitations to the technology: identify the range of concentrations that could be tackled in each process. The extremely high concentration of metals in some of the effluents to be treated (i.e. those from the galvanisation process) or relatively low concentration of contaminants (i.e. boron in the ceramics and glazes effluents and metals in the AMD from one of the mines selected) could be an issue.
3. The parameters as well as optimum operation conditions in each of the processes to be investigated are different. This way, some effluents may be less suitable for some bioprocess than for other.
4. Whether the presence of a physic-chemical treatment prior to the process is necessary. In some of the cases, there is a physic-chemical step currently in place in the final user. The necessity of this pre-treatment was evaluated, as in a way it may reduce the extreme concentration of metals so that biosorption processes can be operative but at the same time, it raises the pH to values far from the optimum for some bioprecipitation processes.

The most important parameters to be agreed upon were related to the typical concentration of metals and other pollutants found in the selected effluents. The most relevant metals to study were: zinc, lead, copper, cadmium, nickel, boron and uranium.

The chemical analysis of the ceramic industry effluents exhibited high concentrations of heavy metals such as zinc, aluminium, lead and boron (in the form of borosilicate). There was a physicochemical treatment in place which effectively removed metals and boron content present in the suspended solids, although it did not remove all dissolved boron, obtaining still boron in the treated effluent, which did not comply with regional emission limit values to water bodies (2 ppm).The bioprecipitation process by means of immobilised phytase enzyme proposed by USAL partner was, *a priori,* not considered to be effective to remove the borosilicate of these kind of effluents. Furthermore, the optimum pH for the bioprecipitation process was between 4.0 and 5.5-6. Then, an acidification step would be necessary to reduce the pH of the effluents from 8.5-8.9 to 4-6 and would mean that metals that could be present as residual suspended solids would be re-dissolved, increasing the concentration of metals to be treated.

With regards to the Portuguese acid mine drainages, it was proposed to combine the technologies that were being studied in BIOMETAL DEMO taking into account the efficiency of each one, i.e.: bioprecipitation processes by USAL and CCMAR could be suitable to treat AMD directly and sorption techniques by ARMINES and UCM could function as a polishing step. This way, RTD partners could report the characterization of their respective processes and their range of applicability depending on the concentration of effluents in order to propose different solutions to the SMEs depending on the type of wastewater.

The electroplating industry effluent would be the best candidate to be treated by means of biosorption, raising first the pH which could precipitate some of the Zn present, or also, combining biosorption after bioprecipitation processes.

For RTDs, it would be easier to treat the original acid waters and then, each group could adjust the pH to the optimum for their process, instead of starting from basic solutions and having to acidify them, which leads to the re-dissolution of metals.

- Bioprecipitation processes studied by USAL require effluents where pH is around 4-5.5.
- Bioprecipitation by means of SRB (CCMAR) need a neutralisation step to reach pH 6-7.
- Biosorption processes (ARMINES, UCM) need pH around 5-6.

In order to unify as much as possible the reporting of results among partners, the coordinator and later on the Commission, to facilitate further activities in the project, suggested the following parameters as basics for reporting:

1. Preparation method.
2. Operating conditions (pH, initial metal concentration, stirring performed, contact time, concentration of other relevant ions in solution, particle size if applicable, etc.).
3. Reporting of results: maximum metal uptake capacity, percentage of metal removed vs time (kinetics), kinetics and isotherms (batch sorption processes), kinetics and breakthrough curves (flow sorption processes), adsorption/desorption cycles (bioreactor reutilization), bioprecipitation reactor reutilization, metal selectivity in multicomponent solutions for each bioprocess, any additional parameters monitored within each biotechnology.

The RTD partners were working in the different research lines in which they had a great expertise. Besides, SME partners supported the RTD work sending wastewater samples, carrying out an extensive analysis of the effluents and explaining the processes in the pilot plant sites as well as the current treatment systems in them.

At the beginning of the project, partner CCMAR was focused on the selection of SRB resistant to metals and capable of removing them in batch screening tests. SRB communities were enriched from several environmental samples, such as soils from a thermal place, sediments from an estuary and a mining area and sludge from Waste Water Treatment Plants (WWTPs). They were re-inoculated in suitable nutrient media for SRB growth in the presence and absence of metals. The most efficient SRB community was chosen (community from WWTPs of Algarve, South Portugal) based on sulphate reduction and metal resistance. The selected consortium is able to reduce almost all (80%) of the sulphate present in the medium in less than 10 days in the absence of zinc

Other aspect that was tested was if the low sulphate concentration of some of the effluents to be treated in this project (e.g. effluents from IGO and from ENDEKA) was enough for the activity of the selected consortium. Results pointed out that implementation of SRB based processes to remove metals from wastewaters with relatively low sulphate concentrations seem to be feasible. In fact, in these experiments zinc concentrations achieved near-zero values at day four, after inoculation.

Then in a second phase, the samples of SRB selected, enriched from WWTPs of Algarve, South Portugal, were inoculated in suitable medium with different sub-products (wastes) from industries in order to select the best sub-products to be used as carbon source for efficient growth and bacterial activity. This task was very important taking into account that the economic viability of the treatment is highly dependent on the cost of the carbon source. The efficiency was seen in terms of concentration of produced sulphide, percentage of sulphate reduction and consuming time.

Finally, CCMAR was working on the set-up and optimization of 2 reactors Upflow Anaerobic Packed Bed Bioreactor (UAPB) type and operating in continuous flow to treat two Acid Mine Drainage (AMD) waters from two inactive mines. Simultaneously with the set-up of the reactors, preliminary tests in batch assays to assess the potential of SRB to treat metal-plating acidic wastewater rich in zinc and ceramics colours plant wastewater rich in boron were done. For metal-plating wastewater – "vertido de zincado", it was proved that SRB have potential in the treatment of such industrial acidic effluent with high zinc concentrations, if the pH is neutralized, if it has some sulphate (preferably above 150 mg/L) and if it has a small amount of organic(s) compound(s) that SRB can use as carbon and electron source, maintaining a COD/[sulphate] ratio favourable for these bacteria (<2).

Regarding boron, the concentration remained constant throughout the experiment in all the tests, which proves that no boron removal occurred through the activity of SRB.

Partner USAL started working in the analysis of the phytase activity of different cheap and abundant raw materials at different temperatures. The main sources of phytase studied were cereal (rye and wheat) brans and a series of commercial preparations of feed enzymes from DSM and BASF, available for huge amounts at low cost.

Then, an aqueous extraction of the best phytase was carried out, followed by the enzyme immobilization by different methods (two chemical and one physical) and supports. In this sense, keratin, main component of the locally abundant pig bristles wastes from slaughterhouses, and its chemical crosslinking with glutaraldehyde to the enzyme phytase, were selected as the optimized support and method for obtaining the immobilized phytase. Once USAL team selected the best immobilized phytase, laboratory scale packed-bed bioreactors were built for the bioprecipitation of metals from synthetic wastewaters, being studied the effects of essential variables such as pH and temperature on the kinetics and yields of metals removal. USAL carried out tests to removal metals from synthetic monocomponent and multicomponent wastewaters.

This metal bioprecipitation process was checked partially effective with monocomponent metal aqueous solutions. Besides such process, a synergic metal biosorption process onto keratin fibres of the support has been also found effective. However, with multicomponent metal aqueous solutions, the proven insolubility and stability of various phytate-metal complexes caused the chemical precipitation of metals and represents a competition and a potential risk for this bioprocess being postulated as a good candidate for metal removal from aqueous solutions.

As it was indicated at the significant risks and associated contingency plans of the project, immobilized bacteria (*Mycobacterium psychrotolerans* & *Amycolatopsis tucumanensis*), bacteria resistant and adapted to environments with high concentrations of metals and with acid phosphatase activity, were then postulated to be the new biological agents for bioprecipitating metals as metal phosphates from wastewaters. The substrate of the acid phosphatase enzyme was β-glycerophosphate that did not form insoluble metal complexes but was effectively hydrolyzed by the enzyme to inorganic phosphate, which, in the presence of metals, precipitated them as metal phosphates.

Consequently, it was decided to resume the research using immobilized acid phosphatase and bacteria (with acid phosphatase activity). Preliminary results were promising. In this sense, after checking the hydrolysis of 5 mM β-glycerophosphate by wheat acid phosphatase immobilized on the PVDF membranes, a synthetic aqueous solution of 6 metals Zn, U, Ni, Cu, Cd and Pb (20 $mgL^{-1}$ of each metal) in 5 mM acetic/acetate buffer, pH 4.5, to which was added 5mM β-GP, was dynamically incubated in batch with the immobilized acid phosphatase. The analysis of the remaining metals in solution during the operation time showed that only uranium was significantly removed from the aqueous solution (90% at 24 h). Furthermore, a multicomponent aqueous solution of 6 metals (Ni, Cd, Cu, Pb, Zn and U, at 20 $mgL^{-1}$ each one) in 5 mM acetic/acetate buffer pH 5.5 with added 50 mM β-glycerophosphatewas passed at 1.0 mL/min and room temperature through a bioreactor packed with small cubes of reticulated polyurethane foam loaded of those bacteria cells. Another identical metalized aqueous solution (+ 50 mM β-glycerophosphate) was passed through a reference bioreactor with empty cubes of polyurethane foam. The results for *Mycobacterium psychrotolerans* show a little removal of Pb (25 % at 2 h), Zn (20% at 2 h) and U (20% at 4 h) but they are much more promising for *Amycolatopsistucumanensis* with 80% elimination for Pb, 60% for Cu but only 20% for U. Scanned electronic photomicrographs and EDAX of the final crystalline precipitates of Pb, Cu and U phosphates were obtained.

Considering that these preliminary and positive results of metals bioprecipitation were obtained at the end of the second year of project and only with synthetic multicomponent metals aqueous solutions and that the next step should be to check such metal bioprecipitation with real industrial wastewaters and due to the fact that the following and decisive second half of the project, the building and starting up of the pilot demonstration plants with the best and more effective bioprocesses for metal removal from industrial wastewaters, should begin as soon as possible, it was decided to stop continuing the research of such acid phosphatase mediated metal removal by *Mycobacterium* and *Amycolatopsis* bacteria.

In the last phase of the project, with the aim of cooperating about the problem checked by all the partners regarding the failure of Boron removal from Endeka Ceramics wastewaters by all the bioprocesses studied by partners, USAL team iniciated the study of the microbiological biodiversity of industrial wastewaters from Goñabe and Endeka Ceramics and the growth, isolation and identification of bacterial strains resistant to the metals and boron and with the capacity of bioaccumulating metals and Boron from such industrial wastewaters.

After 18 days without antibiotics and carbon source, the growth in solid medium prepared with wastewaters from Goñabe and Endeka was abundant with high number of colonies of yeasts. With antibiotics and carbon source, just one small bacterial colony grew slowly with Goñabe wastewater. This study with Goñabe wastewaters was not continued but continued with Endeka wastewaters with high content of Boron. A diversity of microorganisms grew with antibiotics in Endeka Ceramics wastewater. They were Gram + (strains BFC1, BFC4), variable Gram (strain BFC8) and Gram - (strains BFC2, BFC3, BFC5, BFC6 and BFC7). The best grown strains were BFC1, BFC4 and BFC8 and they were identified by 16S ribosomal RNA (or 16S rRNA) gene sequencing as *Microbacterium profundi* Shh(49)T (BFC1), *Microbacterium kitamiensekitami* C2(T) (BFC4) and *Rhodococcus fascians* LMG 3623(T) (BFC8).

Once the three bacterial strains were isolated and identified, a growth test was carried out in M65 solid medium at different pHs, concluding that *Rhodococcus fascians* LMG 3623(T) (BFC8) grew the best in the whole range of pH (4.0-10.0) followed by *Microbacterium kitamiensekitami* C2(T) (BFC4) and *Microbacterium profundi* Shh(49)T (BFC1) which began to grow at pH between 5.5 and 6.5 and extended the pH interval of their growth as the days passed.

<u>Growth in solid medium of bacteria identified and isolated from ENDEKA wastewater</u>

After defrosting, *Microbacterium profundi* Shh(49)T (BFC1), *Microbacterium kitamiensekitami* C2(T) (BFC4) and *Rhodococcus fascians* LMG 3623(T) (BFC8) were re-seeded in series of standard M65 solid medium embedded with ultrapure water (as control) and with water enriched with increasing concentrations of Boron, Zn, Ni and Cu, at pH 7.0. These metals, main pollutants found in Endeka Ceramics wastewaters, were incubated individually with the growing cells. After 19 days of growth, it was concluded that *Microbacterium profundi* Shh(49)T (BFC1 strain) did not grow well in the presence of Ni, something at low concentrations of Zn and slowly at different concentrations of Cu. However, it grew better at low and medium Boron concentrations. *Microbacterium kitamiense* C2(T) (BFC4 strain) did not grow in the presence of Ni and Cu, it grew at low concentrations of Zn and better at low and medium concentrations of Boron. *Rhodococcus fascians* LMG 3623(T) (BFC8 strain) grew well in the presence of all Boron concentrations and more slowly at all Zn and Cu concentrations. In the presence of Ni, only grew at low and medium concentrations.

<u>Boron resistance of bacterial strains BFC1, BFC4 and BFC8</u>

With regards to Boron resistance, it was found that at low and medium Boron concentrations the three bacteria *Microbacterium profundi* Shh(49)T (BFC1 strain), *Microbacterium kitamiense kitami* C2(T) (BFC4 strain) and *Rhodococcus fascians* LMG 3623(T) (BFC8 strain) grew well but at high Boron concentration only *Rhodococcus fascians*LMG 3623(T) did. To further check the previous results, another test was carried out growing BFC1, BFC4 and BFC8 strains in standard M65 solid medium in the presence of 700 and 1000 mg/litre Boron at pH 7.0 for 21 days, checking how was their growth. At 700 mg/litre Boron: BFC1 grew after 12 days, BFC4 after 7 days and BFC8 after 3 days. At 1000 mg/litre Boron: BFC1 did not grow, BFC4 seemed to grow after 19 days and BFC8 grew after 3 days.

The conclusions of this study confirmed the previous ones: *Rhodococcus fascians* LMG 3623(T) (BFC8) grew the best on Boron (0,7 and 1.0 g/litre) followed of *Microbacterium kitamiense kitami* C2(T) (BFC4) and *Microbacterium profundi* Shh(49)T (BFC1).

<u>Bioaccumulation of Boron by bacterial strains BFC1, BFC4 and BFC8</u>

1. Growth of bacterial strains on M65 solid and liquid medium enriched with Boron

After defrosting and re-seeding in standard M65 solid and liquid medium (with ultrapure water as control and with water enriched with 50 and 100 mg/litre Boron) series of plates of the three bacteria were grown for 19 and 65 days. Afterwards, the biomass from 4 plates or flasks (150 ml) was collected to obtain the lyophilizate of each strain BFC1, BFC4 and BFC8 and its Boron bioaccumulation was calculated as mg Boron per g of lyophilized biomass by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) after measuring the decrease of Boron concentration in each growth medium. The three bacteria *Microbacterium profundi* Shh(49)T, *Microbacterium kitamiense kitami* C2(T) and *Rhodococcus fascians* LMG 3623(T) were able to bioaccumulate Boron (0,22 up to 2.0 mg Boron per gram of lyophilized biomass). To further demonstrate the bioaccumulation of boron inside *Microbacterium profundi* Shh(49)T (BFC1 strain), *Microbacterium kitamiense kitami* C2(T) (BFC4 strain) and *Rhodococcus fascians* LMG 3623(T) (BFC8 strain), the final bacterial biomasses were collected to perform TEM (Transmission Electron Microscopy) and STEM (Scanning Transmission Electron Microscopy).

<u>2. Dynamic test to check possible Boron bioaccumulation/biosorption by bacterial strains</u>

*Microbacterium profundi* Shh(49)T (BFC1 strain), *Microbacterium kitamiense kitami* C2(T) (BFC4 strain) and *Rhodococcus fascians* LMG 3623(T) (BFC8 strain) were immobilized as biofilms grown on the surface of a very porous polyurethane foam. The dynamic test to check Boron bioaccumulation/biosorption by bacterial strains was carried out as follows: a) growing in batch for 10 days in M65 liquid medium a portion of the solid biomass of each bacterial strain in contact with 0.5 g of cubes of commercial polyurethane foam, b) packing such polyurethane cubes (with bacterial biofilm) inside a 25.0 ml packed-bed bioreactor and c) recirculating through it 250mL of an aqueous solution with 50 mg/litre Boron for 4 days without pH control. The results showed that no bioaccumulation norbiosorption of Boron occurs.

*Microbacterium profundi* Shh(49)T, *Microbacterium kitamiense kitami* C2(T) and *Rhodococcus fascians* LMG 3623(T), isolated from Endeka Cermics wastewater, and with capacity for bioaccumulating Boron could have good performance for removing Boron from specific surface and underground waters with a little more concentration of Boron than that allowed by the European standards (1 mg/litre) and the World Health Organization (0.5 mg/litre). However, they would not have enough capacity to completely remove boron from industrial wastewater, such as EndekaCeramics´s, with 40-50 mg / litre of boron.

3. Boron removal from synthetic water by a consortium of bacteria from river Tormes (Salamanca, Spain) grown on a sand biofilter

After collecting and freezing the consortium of bacteria from the sand biofilter, a part was thawed and 3 fractions of 3 ml were taken and inoculated in 300 mL of culture medium YEPD (Yeast extract peptone dextrose) containing Boron at concentrations of 0, 50 and 100 mg/litre. These Boron concentrations are typical figures of Endeka Ceramics wastewater. Well above the allowed limit of 1.0 mg/litre of EU standards or the 0.5 mg/L required by the WHO for drinking water. Then, they were incubated in an oven at 28 ºC with shaking and aliquots were taken at different times (0, 2, 4, 7 days), centrifuged and measuring the concentration of Boron in the supernatants by means of ICP-OES. The preliminary results obtained showed a poor performance (10-20 % Boron removal) at the experimental conditions established (high Boron concentration and high ratio volume of water to treat / amount of biomass). Decreasing the ratio volume of water to treat/volume of wet biomass, decreasing the Boron concentration to treat and increasing the time of dynamic incubation, could permit to obtain better Boron removal results.

With regards to metals such as Pb, As, Hg, Cd, Cr, Ni, at concentrations somewhat higher than those allowed by European legislation for drinking wáter, they have been effectively removed from synthetic water by a consortium of microorganisms from river Tormes (Salamanca, Spain) grown on a sand biofilter.

Partner UCM worked in three lines. The first one is related to the modelling of the biosorption process from the plot of the sorption isotherms in mono- and multimetallic systems, using Matlab 5.1 software and a chemical speciation computer program PHREEQCI 6.2. The experimental biosorption data showed a good fit to the Langmuir model. The maximum sorption uptake and the affinity constant were calculated using Matlab 5.1 in mono- and multimetallic systems for both biosorbents (sugar beet pulp and *Fucus vesiculosus* (brown algae)) through the subsequent addition method. The software PHREEQCI 6.2 calculated both the amount of metal retained by the biomass and the number of unoccupied sites at equilibrium by entering the value of the metal concentration left in solution at equilibrium. To run the program, it was required to specify the physico-chemical parameters of the solution (pH, temperature, equilibrium metal concentration, ionic species in solution, etc.) and the characteristics of the biomass itself (q max, specific surface area and mass used).

In the second research line, partner UCM tested different feed flow rates. The bed height of the columns was studied by different amount of biomass and by different diameter of the columns. Main variables in continuous biosorption were determined. Low feed flow rate, high bed height and the use of serial columns considerably increase the metal uptake. In addition, the columns were scaled up to implement the process in the pilot plants. The experiments were performed using sugar better pulp and, in case of *Fucus vesiculosus*, a column with supports was used to reach a better percolation.

Later on, the columns were filled with 300 g of biomass and the composition of the pulp/alga mixture was varied: 1:2, 1:1, and 2:1. The columns percolated adequately in all the cases. A higher rate of brown alga leads to a longer saturation time. However, the breakthrough point was reached after 4 hours using 1:1 and 1:2 pulp/alga ratios. Consequently, the most cost effective option would be the use of 1:1 pulp/alga adsorbent.

In order to increase the efficiency of the contact between the bioadsorbent and the polluted solutions avoiding preferential channels, not only the drop-fed system was tested but also the reverse-fed. Another positive aspect of the reverse-fed was that the compaction of the brown seaweed bed in the glass column was avoided. Furthermore, series of two consecutive columns with reverse-fed were studied. The service time of the columns with sugar beet pulp columns considering the breakthrough point 10 mg/mL increased from 1 hour to 3 hours in the serial columns systems. The effect of the serial columns was very remarkable in the adsorption curves obtained using *F. vesiculosus*, increasing the service time from 1.5 to 5 hours.

The third research line of UCM was a desorption study using three eluents ($HNO_3$, HCl and $H_2SO_4$), as well as, evaluating the possibility of reusing the biomass in different sorption–desorption cycles. Nitric acid was the most effective eluent for both biomasses (sugar beet pulp and *Fucus vesiculosus*); however, the effect was more remarkable the case of sugar beet pulp. These results showed that the eluent choice depended not only on the metal, but also on the biomass. Cycles of sorption-desorption-sorption were performed using the chelating agent EDTA in the desorption step. The experiments could not be performed using *Fucus vesiculosus* as biosorbent due to the presence of a huge amount of alginate in brown algae. Alginate reacts with EDTA taking place a “swelling pressure” effect which leads to the obturation of channels. However, when these cycles were carried out with the sugar beet pulp, an increase of the metal uptake in the second and the third cycles was observed. That could be due to the reaction between the biomass bed and EDTA which increased the number of available sorption sites.

Afterwards, acetic acid was tested as eluent. Nevertheless, acetic acid was not able to desorb effectively the metals and a loss of sorption capacity was observed during the cycles of sorption-desorption. Cycles of sorption-desorption-sorption were also performed using a mixture of 0.1M $H_2SO_4$ and 0.1M HCl and a mixture of 0.1M $H_2SO_4$ and 0.1M HCl with 5%NaCl as eluents. There were problems during the regeneration of the biomass because it was necessary to neutralize the biosorbent and the bases degraded the biomass. Better results were obtained without than with NaCl, although the loss of binding capacity is similar to cycles performed using 0.1N HCl, $HNO_3$ and $H_2SO_4$.

On the first step of the project, ARMINES focused on the development of a series of polyethyleneimine (PEI) derivatives obtained by grafting different compounds on PEI. The targets of these sorbents were selected among base metals (Zn(II), Cu(II), Pb(II), Cd(II) and Ni(II)) and also boron (for ceramic industry). Preliminary tests showed that the best sorption properties for the recovery of heavy metals were obtained with PEI crosslinked with glutaraldehyde (PEI-GC), PEI-CS 2, PEI-histidine and PEI-tannic acid, while for boron the best results were obtained with PEI-tannic acid.

The materials were, in a second step, encapsulated in biopolymer beads (using renewable marine resources, such as alginate and chitosan). The sorption performances were compared between PEI-derivatives in powder, alginate and chitosan alone and the hybrid materials PEI-derivative/biopolymer. In addition to sorption properties the stability of the hybrid materials was considered as a critical parameter for selecting optimum sorbents.

A summary of these preliminary results is currently in press in “Separation Science & Technology” journal (Taylor & Francis Publications, Proceedings of the conference “Separation Science and Technology for Energy Applications” held at Oak Ridge National Laboratory, Oak Ridge, TN, USA in October 2014).

Higher stability is observed with alginate-based matrix and these hybrid materials were selected for investigating the effect of composition of the solution and for desorption. In simple solutions the PEI-derivatives do not increase sorption performance while in complex solutions hybrid materials are more efficient than alginate beads. Na(I) is exchanged with Ca(II) (used for the ionotropic gelation of alginate) causing the degradation of the hybrid material (similar phenomenon observed during metal desorption in acidic solutions); adding CaCl2 at 5 % of sodium content limits degradation and make possible using the composite material in complex solutions.

Regarding the technologies scale-up processes, partners LCW, HIDROLAB and SERVYECO worked in the design and built of the following pilot plants respectively:

- To treat the acid mine wastewater of São Domingos Mine using Sulphate Reducing Bacteria (SRB).
- To treat the wastewater of the electroplating plant (IGO) and the acid mine drainages, both based on the biosorption processes developed by UCM (using biomasses) and ARMINES (using biopolymers).

- To treat wastewater coming from ceramic sector using the biosorption processes developed by ARMINES (using biopolymers).

The first activity carried out by LCW was the acid mine drainages (AMD) characterization of Quinta do Bispo Mine and São Domingos Mine, which was necessary to developed latter tasks. Quinta do Bispo`s AMD presents low heavy metal concentration and high uranium concentration and the São Domingos Mine`s AMD presents high heavy metal concentration and no uranium. Both AMD have sulphates and the metal concentration change during the year due to rainfall.

Mina de São Domingos, Portugal, where the pilot plant with Sulphate Reducing Bacteria (SRB) was tested, is located in the south-west of the Iberian Peninsula, in which the intense mining activity has produced considerable amount of residues, causing the environmental deterioration of the zone, mainly due to acid mine drainage (AMD) - an extremely acid leachate with high contents of sulphate and metals. The system with (SRB) bioreactors optimized in this project is a low-cost alternative that allows to treat Acid Mine Drainage (AMD) water from the mining area in Mina de São Domingos, Portugal, lowering not only the metals but also sulphate to concentrations below the maximum recommended values for irrigation waters.

The construction of São Domingos Mine pilot plant was finished in December 2016. The pilot plant treatment process was based on the anaerobic sulfate reducing bacteria process developed at laboratorial scale by CCMAR and included the collection of the mine wastewater at mine lagoon, the acid mine neutralization, the mixture of the neutralized wastewater with the carbon source, the bioreaction and the separation of the sulphide produced in the process. The pilot plant started to operate in continuous mode on $11^{th}$ April 2017 and stopped on $5^{th}$ December 2017. During this period around 40 $m^3$ of acid mine drainages collected in a mine lagoon was treated in the pilot plant and tested three different carbon sources: winery wastes, municipal wastewater and a combination of winery wastes and municipal wastewater.

The pilot plant that HLAB built for the filtration of the AMD was planned to be sent to São Domingos mine, but finally it stayed at HLAB´s facility.

In the pilot plant the carbon sources selected according to the laboratorial tests (winery wastes and municipal wastewaters) were tested but a new carbon source (mix of winery wastes and municipal wastewater) that could be interested for a use at an industrial scale was also tested.

Data produced during the operation of São Domingos Mine pilot plant was collected and analyzed in order to validate the use of a sulphate reducing bacteria process to treat the acid mine drainages at an industrial scale. As expected, according to the results obtained at laboratory scale, the process tested at the pilot plant [AMD pH neutralization with limestones followed by bioremediation in a up-flow anaerobic packed bed bioreactor (UAPB) with SRB and then by a unit for hydrogen sulphide evaporation] was able to remove contaminants of AMD from Mina de São Domingos to values below the limits for irrigation water, except manganese when the raw AMD is highly contaminated. Nevertheless, an additional unit successfully tested at laboratory scale can be added to the process for the removal of manganese

During the operation of the pilot plant some issues were detected such as the saturation of the limestone in the neutralization tank, the damage of the pH and redox potential probes and the bioreactor "crash" when the concentration of metals was above a defined limit that required the study of potential solutions to implement at an industrial scale in order to solve these situations.

This study provided important information regarding the performance of SRB treating real AMD and demonstrated that SRB bioreactor at a pilot scale attached with a neutralization step can efficiently remove metals and sulphate to levels below the recommended concentration for irrigation waters.

At the beginning of the project, through collaboration between partner IGO and partner HLAB, the different effluents produced at IGO's facilities were analysed. Electroplating wastewater features milder conditions. COD is three times lower, which indicates less inorganic load. Zinc and chloride concentrations are ten times lower than in the galvanization effluent and iron is markedly decreased.

HLAB designed, built and installed the pilot plant for the biosorption operation in Valladolid, Spain. This pilot plant was operative since the 2$^{nd}$ of March, 2017. In this pilot plant, the metal bearing-water effluents of Industrial Goñabe's facilities was being filtrated and treated, which contain a lot of dissolved metals and other undesirable components, such as Chlorides.

This pilot plant included a double column equipment for the ARMINES filler (*Fucus*/ laminaria/ PEI beads) and a medium size filter for the UCM filler (a 1:1 mixture of *Fucus vesiculosus*, a type of algae, on the one hand, and sugar beet pulp, a derivative of the sugar industry, on the other hand). As foreseen, operation and monitoring of the pilot plant was made by partner IGO, as it was installed on its facilities. HLAB explained IGO how to operate properly the pilot plant.

Concerning this pilot plant, there were problems in the pretreatment of the Goñabe's effluents before entering the pilot plant. This was principally due to a very irregular outlet effluent of IGO. It was fully discussed between partners and this issue is not easy to solve, since this type of industry usually has effluents with a high irregularity. The main efforts were made to control dissolved metals concentration of wastewater entering the pilot plant. These levels can be controlled by modifying the inlet pH values: each dissolved metal starts to precipitate at a specific range of pH values. In addition, very low inlet pH values could sweep along the already trapped metals and very high inlet pH values could damage the fillers and exceed the pH emission limits. For this purpose, HLAB indicated IGO the required pH values in the wastewater that entered the pilot plant. This adjustment was made by dosing HCl or NaOH automatically, depending on the inlet pH values.

Once the pilot plant was operative, the main goal to accomplish was to complete a full biosorption cycle, in order to exhaust a portion of both the ARMINES filler and the UCM filler.

A "full biosorption cycle" includes as many biosorption and regeneration cycles as needed until the fillers are exhausted.

The best results using UCM's system correspond to Zn, Cu and Fe; and the best ones using the ARMINES' system correspond to Cu, Zn and Ni (these results were different at the beginning, during the first biosorptions). The worst obtained results correspond to dissolve Al. In conclusion, **UCM filler seems to be a good material for achieving a successful biosorption of, at least, dissolved Zn, Fe and Cu**, due to its physical properties and economic features. Big progress has been made on the research of the biosorption of these dissolved metals. Furthermore, carrying out a good pretreatment is very important.

Regarding to HLAB part of the project and after processing the results obtained, it can be said that good results were obtained in the pilot plants implemented by them. Pilot plants were able to carry out successfully planned processes. They might be partially improved now that the procedure to optimize these processes is known. However, results obtained shows that these technologies can be implemented in order to achieve an acceptable biosorption of many dissolved metals.

Through collaboration between partner SERYECO and Endeka Ceramics (client of SER), the wastewater from the ceramic tile industry were analyzed. Effluents from the ceramic production lines, has a pH slightly basic and the main contaminants present are suspended solids, heavy metals such as lead, zinc and iron, as well as chlorine ions. Boron presence is due to the use of borosilicate in the production of glazes and the concentration of this metal varies between 5-25 ppm in wastewater.

SERVYECO designed a prototype to treat wastewater from the ceramic tile industry. The technology proposed in BIOMETAL DEMO for the treatment of wastewater coming from Endeka Ceramics was focused on the elimination of heavy metals with special attention paid to Boron removal. The reason behind was that the target wastewater come from Endeka Glaze line in which high boron concentration was detected.

The first configuration proposed for this aim consisted of a physical-chemical step for the removal of solids followed by tertiary treatment. This treatment consisted in a column which was filled with a new bioadsorbent material. There were two different materials to carry out the demonstration phase. One of these materials was developed by Universidad Complutense de Madrid (UCM, Spain), and the other one was developed by ARMINES (France). Unfortunately, the material made in the UCM was unstable inside the column at industrial scale. For this reason, data obtained in the column outlet is only valid for the material developed by ARMINES.

The main outcomes from this configuration revealed that while Zinc and Aluminium were satisfactorily removed in the first step, the content of Boron in the effluent was similar to the inlet stream. It means that neither physical-chemical treatment nor Armines material is suitable for Boron elimination.

To overcome the mentioned drawbacks, Servyeco developed and proved an alternative treatment to the Armines material. The configuration consists of two steps: first, the physical-chemical system in which the reduction of solids, Zinc and Aluminium had been demonstrated and second, a batch process based on the addition of lime and ECOBOR 20 to deal with Boron removal.

After reviewing the collected data from the pilot plan in Endeka Ceramica, it can be concluded that:

- Physicochemical treatment works efficiently removing TDS, SS and metals like Zinc and Aluminium. However, it is inefficient removing Boron content. This is the expected behaviour because the aim of the project is the Boron elimination using a new tertiary fine tune treatment.
- Metals removal efficiency by bioadsorbents developed by ARMINES is quite high for Aluminium and Zinc, but decreasing the efficiency in specific samples, depending on the incoming metals concentrations. However, Boron removal efficiency is extremely low with this procedure. For that reason, an alternative treatment is necessary to reduce the Boron content from the wastewater.
- An alternative Boron removal procedure, based on a chemical treatment, it is proposed by SERVYECO. This process presents promising results with high efficiency values (> 94%).

CONTACTICA was responsible for carrying out the LCA and LCC for the different pilot plants implemented during this project. The impacts related to configurations tested were calculated for all the impact categories included in CML methodology.

According to results obtained with LCA carried out in the pilot plant that was installed in Goñabe's facilities, energy use and chemicals use were principally responsible for the impacts evaluated. Electricity leaded to shares up to 40% for abiotic potential and photochemical oxidant formation while the large amount of sodium hydroxide added to the acid drainage is the main reason behind the potential impacts. In fact, the contribution of NaOH represented more than 50% in most of categories, except for freshwater and terrestrial ecotoxicity and eutrophication in which case direct emissions were the main responsible for the impacts.

The SRB operated in São Domingos was analyzed using as carbon source domestic wastewater. Among inputs, transportation and energy are underlined as the main contributors to the impacts. The relevance of energy consumption is typically observed in studies performed at lab and pilot scale considering that equipment is generally overestimated. Regarding transport, carrying limestone from the provider to the pilot plant location is the main contributor to the impacts. The process requires 70 kg of limestone per FU from a distance of 50 km by road: 3.50 t·km. Sludge disposal in municipal landfills is harmful for freshwater ecotoxicity potential mainly due to the release of the leachate highly loaded with the adsorbed heavy metals. Finally, the alternative algae and sugar beet pulp filler was evaluated with LCA and LCC to be compared with the SRB bioreactor. According to environmental outcomes, in general the biological filler leads to higher burdens than the SRB bioreactor

Regarding the pilot plant at SERVYECO, the configuration studied demonstrated to be successful for Aluminum and Zinc removal in the first stage and for Boron elimination in the second step. However, Boron removal could not be supported within the environmental analysis due to the lack of characterisation factors for this metal. Even so, LCA allowed evaluating the burdens related to direct emissions of Zinc and all indirect impacts caused by chemicals, electricity and other inventory data. In this study, energy use represents more than 85% of the potential impacts in all categories except for abiotic depletion, in which case the production of ECOMIX® shows a share close to 55%. Main outcomes from the environmental assessment highlight the need of optimizing the process in terms of energy use.

Consequently, next steps could be focused on energetic optimization of the process, modelling and simulation for larger scales and improvement of characterisation models in LCA. To develop models to scale-up the technologies would be an effort positive and useful not only for LCA but also for technical purposes, considering a real scale-up one of the next steps of BIOMETAL DEMO project. Moreover, the consideration of Boron in toxicity related categories would be an advance in comparison with the current study. The improvement of characterisation models for inorganic substances is an interesting step to tackle specific studies dealing with metal-bearing wastewaters.

Throughout the entire project's life, several dissemination and communication activities have been carried out. These activities were done in order to achieve the following objectives:

- Practical transfer of knowledge from RTDs to SMEs consortium members and adequate skill training.
- Protection of the Intellectual Property (IP).
- Successful Exploitation of the project results with an appropriate marketing strategy.
- Dissemination of the innovative process and products benefits for replication to maximize environmental and socio-economical impacts.
- Communication of the project achievements and advantages of the innovation.

Communication between partners, and outreach activities outside the consortium with relevant stakeholders have been hold to establish partnerships, attract investors, secure IPR, solve regulatory issues, and define the key value proposition and market strategy.

Dissemination activities have been carried out inside and outside the consortium, so target of these actions is different.

**Dissemination inside the Consortium:** activities directed to project team level.

- Training activities and demonstration shows were organized during the project. These training activities included: training activities to SMEs from the project. meetings with trainings at a small scale in the pilot plants facilities, training activities to workers of the pilot plants.

- Web presence: The project website (www.biometaldemo.eu), which was developed at the beginning of the project's life, was the main communication platform among project partners. It was updated (at least every 3 months) with new generated documentation. It has a public area and a private one. The website is administrated by CTA.

- Mailing list and other telecommunication tools: Mailing lists, where all project partners were included, were a support of project website for dissemination among Consortium partners. Other telecommunications tools regularly used such as telephone, fax, as well as, videoconferences by Skype were also important to keep partners in touch in daily works and achieve objectives planned for this target group.
- Conferences and meetings: They were celebrated among Consortium partners in order to report about the project advances and dealing with all the emerging topics related to the project execution: Steering Committee, Project Coordination Committee, Kick-Off Meeting, Work packages Meetings and a Project Review Meeting.

**Dissemination outside the Consortium:** activities directed to managing authorities, scientists,-professional users and general public.

- Training activities and demonstration shows:
- Web presence: The project website (http://biometaldemo.eu/) was developed on month 3 of project life. As it was already explained, this web has a public area and a private one. News, documents and any other sort of information is available on this webpage. Project's partners also use their websites to disseminate the results of the project as well as news related to it.
- Newsletters: The newsletter of CONTACTICA contains information and news of the projects wherein the consulting participates in, including **BIOMETAL DEMO**. This report will be updated and sent regularly to the whole contact list of CONTACTICA S.L. as an effective dissemination tool.
- Publications in journals and magazines: Articles were published from the results obtained in the project by partners such as USAL, UCM, ARMINES.
- Face-to-face contacts: Dissemination of results was also done by personal contacts. They have been achieved in fairs and other public events.
- Conferences: One of the most useful way of dissemination was attending to Conferences, workshops and meetings both national and international. In many Conferences, oral presentations about BIOMETAL DEMO were done, presenting results obtained, advantages of this new technology and so on. So events with partners attending were SIGA International Trade Fair (28$^{th}$ February – 3$^{rd}$ March 2017), EIP WATER Conference (27$^{th}$-29$^{th}$ September 2017), Trade Fair SMAGUA 2016. Zaragoza (Spain), 8$^{th}$ – 11$^{th}$ March 2016.
- Networking: Contacts with other EU initiatives as well as with other research groups have been made throughout the duration of the project and may be done in the future. Some contacts made were with **COLUMBUS project** (in order to identify the results generated), **LIFE NEWEST, IMDEA ENERGÍA**, etc. Besides, other contacts were

made attending to different events such as **EXPOQUIMÍA** and **SUSCHEM 2017 BROKERAGE EVENT.**

## Potential impact (including the socio-economic impact and the wider societal implications of the project so far), main dissemination activities and exploitation of results (10 pages)

The expected final results of project BIOMETAL DEMO are:

- To design and building of the demonstration pilot plant at each industrial site.
- To develop novel technologies that will be applied to wastewater treatment facilities
- To provide greener bio-based technologies to metal contaminating industries

To achieve these objectives, the consortium carried out:

a) a rigorous comparative analysis of the performance and kinetics, energy inputs, technical and economic advantages and drawbacks of the different metal removal/recovery bioprocesses studied at laboratory scale for each wastewater;

b) the best decision making about which bioprocess or a synergy of two integrated bioprocesses is the optimum choice for scaling-up for each wastewater to be treated.

Expected final results are focused on the industrial validation and technical assessment of the wastewater treatment process. These results would be confirmed through several chemical analyses of the incoming/treated water samples monitored along the demonstration phase.

Technologies applied in **BIOMETAL DEMO** are based on biological treatments, which involve the destruction of waste materials by using living or non-living biomass. Microorganisms (including bacteria, fungi, and algae), plants and residual biomass such as bark, lignin, shell, etc are used. This biomass has proven capability to take up heavy metals from aqueous solutions. The bioprocess technologies that are being tested for heavy metal ion removal in the project are:

- Biosorption: property of certain types of biomass to bind and concentrate selected ions or other molecules from aqueous solutions. Biosorption is a metabolically passive process that uses chemical groups (carboxyl, sulfonate, phosphoryl, amido, amino, imidazole) present on the cell wall of dead biomass to bind ions of metals using mechanisms like ion exchange, complexation or physical adsorption. Algae, bacteria and fungi and yeasts have proved to be potential metal biosorbents.

- Bioprecipitation: use living microorganisms to promote chemical precipitation or redox reactions that can reduce the heavy metal ions to an insoluble state (as oxides, sulfides or free metals). Any microorganism that is resistant to superambient levels of metal ions is appropriate for bioprecipitation.

The main advantages of biological metal clean-up techniques are, as follows:

- Selectivity to remove only the desired metals

- Metal loading on biomass is often very high, leading to very efficient metal uptake.

- Cost-effectiveness. Especially using biosorption processes, that don't require nutrient costs for feeding the cells since non-living biomass is employed.
- The process is very rapid in the case of biosorption and takes place between few minutes to few hours. Furthermore, a wider range of operating conditions such as pH, temperature and metal concentration is possible because cells are non-living. No aseptic conditions are required for this process. Metal can be desorbed readily and then recovered, metal-loaded biomass can be incinerated, thereby eliminating further treatment.
- Biomass can be procured from the existing fermentation industries**,** which is essentially a waste after fermentation.
- Simple-operative process.
- The biological agent can be optimally designed to deal with the specific wastewater generated (e.g., using specific microorganisms for the pollutants present, or environmental conditions encountered, and to treat it directly at its source).
- Biological processes can be carried out in situ at the metal contaminated site.
- No chemicals agents required and there is isn't production of heavy metal sludge.

Henceforth, the final challenge is to design and build three demonstration pilot plants based on an optimized bioprocess or a synergy of some of them for the treatment of three metal polluted wastewaters of representative industries which are a mine, an electroplating and a ceramic tile company.

BIOMETAL DEMO results could have a great potential impact in an environmental context, because these systems can be set up on an industrial scale, which is the main objective. Furthermore, searching for a utility for the exhausted fillers and the regeneration acid solutions after their useful life is also a target of the project. Several solutions have been considered in this sense, as detailed in the related Deliverables.

**BIOMETAL DEMO** has a direct positive impact in wastewater treatment legislation at national and European Level. Industries and other potential contaminating facilities across the European Union will have to make adjustments so that they can comply with the new limits set (lower Environmental Quality Standards) for emissions by the respective River Basin Authorities. Thus, the application of technologies developed within project BIOMETAL DEMO could be geared towards facilitating industries to comply with these lowered limits in industrial facilities.

BIOMETAL DEMO has the involvement of five SMEs, which all have allocated human resources to R&D activities. Among these, IGO implemented the new technology developed within the project to treat their metal polluted wastewater, and, for this reason, focused its R&D in this issue. On the other hand, LCW, CTA, HLAB and SER partners were interested in the development of innovative solutions for wastewater treatment to implement in different industries among EU members.

Moreover, among these companies, there is a R&D performing SME, SER partner. This company dedicates a very high percentage of its human resources to R&D, and is composed by a high qualified group of technicians specialized in subjects as chemistry, pharmacy, biology, engineers with a wide experience in environmental matters related to water and their remediation. They deal with knowledge management in Engineering, Chemistry, Biology and Environmental Sciences in order to develop biotechnology, chemicals and innovative treatment facilities for the protection of the environment.

A direct market is already foreseen, as LCW has access to over 50 mine enclaves in Portugal for wastewater management. This ensures a great impact in terms of economic returns.

Furthermore, SER, HLAB and LCW partners have estimated their potential commercial expansion based on their current net of international agreements.

The planned human resources to increase the market uptake per partner are:

1. SERVYECO
    a. Business Unit manager: 1 (2018)
    b. National technical sales: 1 (2018), 1 (2019), 1 (2020), 1 (2021)
    c. Export technical sales: 2 (2018), 1 (2020), 1 (2021)
2. HIDROLAB
    a. National technical sales: 1 (2018), 1 (2019), 1 (2020), 1 (2021)

Due to the extensive potential market for SER, HLAB and LCW, and bearing in mind the complexity to evaluate it, we estimate a potential world market for **BIOMETAL DEMO** technology of 500 Million €/year; specifically, the estimated market potential for Spain is estimated to be 10Mll€/year (intern source).

The main countries in which the Project results may be commercialized should meet the following criteria:

- High industrialization.
- High environmental conscience and requirements with policies and regulations that favors sustainable development.
- Countries in which water is a valuable resource, of which efficient management and reuse is a priority.

The main markets foreseen for the project results are Spain, Portugal, France, Italy, Germany, UK, The Netherlands, Greece, Turkey, Morocco, China, USA, Argentina and Brazil, among others.

Taking into account the laboratory results already obtained in **BIOMETAL DEMO** Project, these are the main comments about the issues related to the estimated time to market:

<u>1. Investment in technology developed in **BIOMETAL DEMO**</u>

RTD partners are interested in continuing with laboratory research activities in order to optimize the operational processes of technologies developed in project **BIOMETAL DEMO**.

Regarding the SME partners:

- **LCW**: Since the design stage of the project, LCW intend to invest in the technology developed in the project. All LCW efforts have been to ensure that the research and development process is geared towards industrial use and is applicable to the treatment of water, particularly in abandoned or exploration mines.
- **SERVYECO**: Presented technology efficiency was tested during several months at semi-industrial level. However, there are still some limits that need to be overcame. For that reason, SERVYECO plans to invest resources in technology improvement, heading to overcome current technical limits.
- **HIDROLAB**: HLAB has found that the system based on UCM bed is now near to be competitive in the market. The bioabsorption was also successfully proven for treating AMD waters. The only remaining limitation is the short operative life of the beds: three bioabsorption cycles or services. This fact must be balanced by getting value from the exhausted bed and from regeneration rejects.

2. TRL achieved in **BIOMETAL DEMO**

**HIDROLAB** partner considers that both TR6 and TR7 have been achieved. Bioabsorption prototypes have achieved both for galvanic bath industries (Goñabe) and also for AMD Sao Domingos waters. UCM and ARMINES systems in both cases.

**SERVYECO**: TRL 6 has been successfully achieved. The technology is being tested at semi-industrial scale with acceptable results, in line with the previous lab scale tests. However, regarding the new technology tested by SERVYECO, it is still not able to substitute currently used technology, not reaching full operational conditions. That's whyTR7 is not reached yet.

3. Expected time to market for the technology developed in **BIOMETAL DEMO**

SME partners comments about this issue:

- **LCW**: LCW forecast is that the expiration time for the market for the technology will be about 1 year, which will correspond to 2018.
- **SERVYECO**: Based on the limits previously noticed, SERVYECO thinks the technology needs 1 – 2 years of development and fine tuning in order to reach the market.
- **HIDROLAB:** HLAB expects to quote for two bioabsorption systems by 2017-2018 period.

4. Future R&D project to minimize the risk of the call "death Valley" of the technology developed in **BIOMETAL DEMO**

Partners are willing to participate in research projects and assistance programs for process implementation and optimization and as well as for the transfer of knowledge aiming the most efficient possible implementation of the developed technology in full-scale operation.

However, Death Valley Risk for presented technology is already minimized for several reasons. On the one hand, as previously said, the market demand is currently very important because environmental reasons. On the other hand, currently used techniques lack on several environmental and economical aspects, being very necessary the introduction of a brand new technology, economically viable and environmentally friendly.

**HIDROLAB**: HLAB foresees a R&D project with public co-investment to minimize the risk of the call "Death Valley". According to HLAB, the limited life of the bioabsorption beds must be extended, and new research is needed.

**SERVYECO**: SERVYECO thinks Death Valley Risk for presented technology is already minimized for several reasons. On the one hand, as previously said, the market demand is currently very important because environmental reasons. On the other hand, currently used techniques lack on several environmental and economical aspects, being very necessary the introduction of a brand new technology, economically viable and environmentally friendly

5. Internal Business Plan and Exploitation Plan for the technology developed in **BIOMETAL DEMO**

- **LCW**: There is still no business plan for the technology and exploitation of the industrial application of technology.
- **SERVYECO**: SERVYECO tends to develop a complete Business Plan when introducing a new technology in the market. However, in this case the presented technology still needs to be improved in order to overcome current technical limits. When the technology was ready to go, SERVYECO will develop an internal Business and Exploitation Plan.
- **HIDROLAB**: HLAB will present proposals for two industrial cases: one for a galvanic bath industry and another one for AMD water.

POTENTIAL IMPACTS

- Growing global concerns over the use of chemicals in the water treatment as well as the difficulty in managing chemical sludge generated. Industries are shifting to cleaner production methods, demanding the development of environmental friendly, low-cost and efficient treatment technique for metal rich effluents.
- None of the funded European projects tackles the same water treatment processes for removal of heavy metal posed in **BIOMETAL DEMO**. Therefore, there are not overlaps.
- In the light of this patent research work, we can conclude that, so far, there does not exist any patent similar to biotechnologies proposed in **BIOMETAL DEMO**, not involving any barrier for the "freedom to operate".
- No competitors offer the same biotechnologies developed in **BIOMETAL DEMO**.
- Regarding the last publications in the scope of heavy metal-polluted wastewater treatment, it should be noted that several papers on biotreatments have been recently published; therefore it is expected that future trends in wastewater treatment technologies continue this line of research. This would provide an opportunity for the market uptake of **BIOMETAL DEMO** technologies.

- The heavy metals' polluted water cleaning allows an increased availability of water.
- Development of greener and innovative technologies for water cleaning adds value for this sector, increasing reputation of the company that establishes this kind of systems, generating employment and setting up new relationships.
- **BIOMETAL DEMO** has a direct positive impact in wastewater treatment legislation at national and European Level. Industries and other potential contaminating facilities across the European Union will have to make adjustments so that they can comply with the new limits set (lower Environmental Quality Standards) for emissions by the respective River Basin Authorities. Thus, the application of technologies developed within project **BIOMETAL DEMO** could be geared towards facilitating industries to comply with these lowered limits in industrial facilities.
- Regulation (EC) 166/2006 establishes an integrated pollutant release and transfer register at Community level (the European PRTR) in the form of a publicly accessible electronic database and lays down rules for its functioning, in order to implement the UNECE Protocol on Pollutant Release and Transfer Registers and facilitate public participation in environmental decision-making, as well as contributing to the prevention and reduction of pollution of the environment. Pollutant release data reported in the E-PRTR from industrial facilities throughout Europe will be useful for the dissemination of **BIOMETAL DEMO** in order to identify and target those European regions with higher concentration of heavy metal releases.
- The new biological-based technologies need not necessarily replace conventional treatment approaches but may complement them.
- Increasing of the knowledge, application and scaling up of biotreatments. Long-term, that model could be expanded to other sector, geographical areas, markets…

### Attached documents

Video: https://www.youtube.com/watch?v=lIuEYXlJL3A



## 2. Use and dissemination of foreground

Dissemination activities have been carried out inside and outside the consortium, so target of these actions is different. Carried out dissemination actions were detailed in D12.9.

**Dissemination inside the Consortium:** activities directed to project team level.

- <u>Training activities and demonstration shows</u> were organized during the project. These training activities included: training activities to SMEs from the project. meetings with trainings at a small scale in the pilot plants facilities, training activities to workers of the pilot plants.

- <u>Web presence:</u> The project website (www.biometaldemo.eu), which was developed at the beginning of the project's life, was the main communication platform among project partners. It was updated (at least every 3 months) with new generated documentation. It has a public area and a private one. The website is administrated by CTA.
- <u>Mailing list and other telecommunication tools:</u> Mailing lists, where all project partners were included, were a support of project website for dissemination among Consortium partners. Other telecommunications tools regularly used such as telephone, fax, as well as, videoconferences by Skype were also important to keep partners in touch in daily works and achieve objectives planned for this target group.

- Conferences and meetings: They were celebrated among Consortium partners in order to report about the project advances and dealing with all the emerging topics related to the project execution: Steering Committee, Project Coordination Committee, Kick-Off Meeting, Work packages Meetings and a Project Review Meeting.

**Dissemination outside the Consortium:** activities directed to managing authorities, scientists,-professional users and general public.

- Training activities and demonstration shows:
- Web presence: The project website (http://biometaldemo.eu/) was developed on month 3 of project life. As it was already explained, this web has a public area and a private one. News, documents and any other sort of information is available on this webpage. Project's partners also use their websites to disseminate the results of the project as well as news related to it.
- Newsletters: The newsletter of CONTACTICA contains information and news of the projects wherein the consulting participates in, including **BIOMETAL DEMO**. This report will be updated and sent regularly to the whole contact list of CONTACTICA S.L. as an effective dissemination tool.
- Publications in journals and magazines: Articles were published from the results obtained in the project by partners such as USAL, UCM, ARMINES.
- Face-to-face contacts: Dissemination of results was also done by personal contacts. They have been achieved in fairs and other public events.
- Conferences: One of the most useful way of dissemination was attending to Conferences, workshops and meetings both national and international. In many Conferences, oral presentations about BIOMETAL DEMO were done, presenting results obtained, advantages of this new technology and so on. So events with partners attending were SIGA International Trade Fair (28th February – 3rd March 2017), EIP WATER Conference (27th-29th September 2017), Trade Fair SMAGUA 2016. Zaragoza (Spain), 8th – 11th March 2016
- Networking: Contacts with other EU initiatives as well as with other research groups have been made throughout the duration of the project and may be done in the future. Some contacts made were with **COLUMBUS project** (in order to identify the results generated), **LIFE NEWEST, IMDEA ENERGÍA**, etc. Besides, other contacts were made attending to different events such as **EXPOQUIMÍA** and **SUSCHEM 2017 BROKERAGE EVENT.**

## 3. Workforce Statistics

***Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).***

| | Number of Women* | Number of Men* |
|---|---|---|
| Work package leader | 3 | 7 |
| Experienced researchers (i.e. PhD holders) | 4 | 8 |
| PhD student | 0 | 1 |
| Other | 11 | 21 |