Efficiency of rhamnolipid colloidal gas aphrons to remove cadmium and zinc from metal-contaminated soil samples

Claudio Sabbatini Capella Lopes[1,2,*], Douglas Braga Teixeira[1], Bernardo Ferreira Braz[1], Ricardo Erthal Santelli[1], Livia Vieira Araújo de Castilho[1,3], José Gregório Cabrera Gomez[4], Rui de Paula Vieira de Castro[1], Lucy Seldin[2], Denise Maria Guimarães Freire[1*]

[1]Centro de Tecnologia, Universidade Federal do Rio de Janeiro – Instituto de Química, Rio de Janeiro-RJ, Brazil
[2]Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro – Instituto de Microbiologia Professor Paulo Góes, Rio de Janeiro-RJ, Brazil
[3]Instituto Alberto Luiz Coimbra de Pós-Graduação e Pesquisa em Engenharia (COPPE), Universidade Federal do Rio de Janeiro – Departamento de Engenharia Oceânica, Rio de Janeiro-RJ, Brazil
[4]Instituto Ciências Biomédicas, Universidade de São Paulo- SP, Brazil
*Corresponding authors: Lopes, C. S. C. Departamento de Bioquímica, Laboratório de Biotecnologia Microbiana – 549-1 & 2, Instituto de Química, Centro de Tecnologia, Universidade Federal do Rio de Janeiro, Av. Athos da Silveira Ramos, 149, Bloco A, Rio de Janeiro, RJ CEP 21941-909, Brazil

Abstract— *Soil contamination by metal wastes represents a threat to the environment and to human health. Rhamnolipids have been recognized for its metal removal properties and presenting high biodegradability, however their application is sometimes hampered due to downstream cost production. In contrast, colloidal gas aphrons (CGAs) can serve as a cost-effective alternative to reduce the consumption of biosurfactants while also being significantly efficient in flushing off heavy metals in remediation processes. However, data concerning rhamnolipid CGAs, such as bubble diameter, stability, and percolation through porous media under water-wet conditions, are scarce. Until now, only a few studies have demonstrated the possibility of using biosurfactant CGAs to remediate metal-contaminated soils, and even fewer studies have compared its efficiency to previous methods where regular foam is applied. Therefore, a rotational central composite design was developed considering several variables and stability and bubble diameter as response parameters. Rotation speed and agitation time had a significant effect on the stability of aphrons, reaching over 600 s of stable CGA. Alkaline pH has no significant effect on the stability of rhamnolipid CGA´s. Flow rate experiments showed a significant pressure drop when rhamnolipid CGAs were pumped through the remediation column. Soil column experiments demonstrated that rhamnolipid CGA exhibited a 38 % removal rate for cadmium and a 53 % removal rate for zinc after a percolation of 10 pore volumes at pH 9.0. Extraction of cadmium and zinc was highly influenced by the number of pore volumes percolated. Based on the present results, it can be demonstrated that colloidal gas aphrons can display a similar soil remediation efficiency when compared to previous studies that applied regular foam as a metal extraction agent. The application of rhamnolipid CGAs in metal-contaminated soils is imperative for a more efficient and environmentally friendly metal remediation process.*

I. INTRODUCTION

Soil contamination by metal wastes represents a threat not only to the environment but also to human health, especially through accumulation in food chains or contamination of water bodies [1]. As a result, metal contaminants have raised several concerns worldwide while also increasing the demand for new methods to remove toxic metals from contaminated soils in an environmentally friendly way while also diminishing time operation and financial resources to promote a satisfactory remediation of a metal-contaminated terrain [2].

Soil washing is an *ex-situ* remediation method frequently used to address this type of contamination, and it is best suited to completely remove heavy metals from solid matrices while also allowing the return of socioeconomic activities of the terrain [3] However, many metal extraction agents are considered harmful to the soil microbiota or affect its chemical properties [4].

Under economic aspects some soil remediation methods use synthetic surfactants with metal extracting properties in certain contaminated areas, but most synthetic surfactants have a low biodegradability [5], which represents a drawback considering the socio-environmental context of the remediation process.

Biosurfactants are natural compounds derived from bacteria, yeast, and plants, and these natural substances have been recognized to be more biodegradable than synthetic surfactants while also presenting a lower critical micellar concentration [6] . Rhamnolipids are one of the most promising biosurfactants for application in metal remediation steps due to several reports regarding their powerful tensioactive properties and interesting metal extraction properties [7].

However, the high costs related to its large-scale production represent a major downside for its application in field remediation steps. One way to increase the economic viability of a soil washing procedure with rhamnolipids is to test the efficiency of rhamnolipid extracts produced with agro-industrial wastes as raw materials and without any purification steps Considering the strong tensioactive properties of biosurfactants, it is possible to physically modify the composition of rhamnolipid micelles into a temporally colloidal state to increase their metal remediation properties.

Colloidal gas aphrons (CGAs) or microfoams are composed of highly stable microbubble dispersions, and the current literature has reported their effectiveness in remediating or mitigating oil spills in soils and sediments [8]. The application of CGAs in remediation methods is desirable due to their higher efficiency in removing contaminants from solid matrices while using less surfactant solution [9]. However, there are only a few studies regarding the stability and bubble diameter of rhamnolipid CGA and even fewer reports about percolation of rhamnolipid CGA through porous media under water-wet conditions.

In the present study, several parameters were evaluated to test the best stability and bubble size diameter for the generation of CGAs using a rhamnolipid extract solution produced with agro-industrial substrates. Afterwards, a series of soil column experiments were performed to better understand the percolation of rhamnolipid colloidal gas aphrons and extract solutions through porous media and the efficiency of rhamnolipid CGA to extract metal contaminants from soil samples.

II. MATERIALS AND METHODS

2.1. Rhamnolipid production

A genetically engineered *Pseudomonas aeruginosa* strain LFM 634 [10] was used for rhamnolipid production. This modified strain, denoted 1201, was inoculated on Luria Bertani medium (HiMedia, Mumbai®) and incubated at 30 °C under agitation (180 rpm) for 24 h. Afterwards 5 mL of the bacterial growth was inoculated into a 1-LErlenmeyer flask containing 300 mL of a pre-inoculum medium with the following composition (per liter): 1 g $NaNO_3$, 3 g KH_2PO4, 7 g K_2HPO4, 0.2 g $MgSO_4.7H_2O$, 5 g of yeast extract, 5 g of peptone, and 30 g of glycerol. Growth conditions consisted of incubation at 30 °C in a rotatory shaker at 180 rpm for 40 h. The absorbance of the bacterial growth was measured at 600 nm (Spectrophotometer MultiSpec – 1501; Shimadzu Corporation®, Japan), and the absorbance value was converted in cell concentrations (g/L) [11]. Subsequently, 30 mL of the pre-inoculum was added to a 1-L conical flask containing 270 mL of a minimal medium for rhamnolipid production. The minimal medium for the rhamnolipid production displayed the following composition (per liter): 30 g glycerol and 5.5 g $NaNO_3$, respectively (C/N ratio of 15). The medium was also supplemented with the following composition (per liter): 7 g K_2HPO_4, 3 g KH_2PO_4 and 0.2 g $MgSO_4.7H_2O$. The fermentation was carried out for 168 h at 180 rpm and 30 °C. After the removal of bacterial cells by centrifugation (8.000 rpm/ 15 min.) the crude broth was stored at -22 °C prior to use in the remediation tests.

2.2. Rhamnolipid characterization

2.2.1. Rhamnolipid quantification by High-Performance Liquid Chromatography

Rhamnolipid (RL) in the crude extract was quantified indirectly by HPLC using rhamnose as the reference. The RL molecules were hydrolyzed with 10 mmol L^{-1} H$_2$SO$_4$ and heated at 100 °C for 4 h to release the rhamnose molecules; the reaction was stopped by adding NaOH (10 mmol L^{-1}). The samples were filtered with 0.22 µm syringe filters and analyzed by HPLC. L-rhamnose standards (Sigma-Aldrich) were also filtered and injected at concentrations of 100 to 5000 mg L^{-1} to prepare a standard curve. The rhamnose concentration was converted to RL using a factor calculated from the data for RL congeners quantified by means of mass spectrometry (MS). Rhamnose was analyzed via HPLC using an Agilent Technologies 1260 Infinity system with a Bio-Rad HPX-87H Ion Exclusion Column, 300 mm × 7.8 mm at 45 °C, with the mobile phase consisting of an aqueous solution of H$_2$SO$_4$ (5 mmol L^{-1}) at a flow rate of 6 mL/min and a refractive index detector (RID).

2.2.2. *Determination of rhamnolipid congeners by mass spectrometry*

Total RLs were extracted as described previously [12], with minor modifications. Briefly, cells were removed from the medium by centrifugation (10,000 rpm/10 min), and the supernatant was acidified to pH 3–4 with concentrated HCl. The RLs were extracted with a 1/3 volume of ethyl acetate. The organic extract was dried with anhydrous sodium sulfate (Na$_2$SO$_4$) and evaporated using a rotatory evaporator. The evaporation residue was dissolved in methanol/chloroform/2-propanol at a ratio of 1:2:4 containing 7.5 mmol L^{-1} ammonium acetate (CH$_3$COONH$_4$). Finally, the mixture was centrifuged at 10,000 rpm for 5 min to separate the impurities, and the supernatant was filtered through 0.22-µm syringe filters and stored. The extracted biosurfactant samples were analyzed by direct injection in a Linear Trap Quadrupole (LTQ) Orbitrap XL mass spectrometer (Thermo Fisher Scientific) equipped with a nanoelectrospray ion source using capillaries of a PicoTip Emitter (Glass Tip, 1P-4P, New Objective), with an ionization voltage of 3.5 kV and capillary transfer temperature of 180 °C. Each independent data-acquisition cycle consisted of a scan in Orbitrap under a mass-energy resolution of 60,000

(FWHM, m/z 400) with an m/z range of 100–2000 Da, followed by fragmentation by tandem mass spectrometry (MS–MS) of the most abundant precursor ions by induced collision dissociation (ICD) in the linear trap or in the high-energy collision-induced dissociation cell (HCD). All samples were prepared in methanol at a ratio of 1:1 (v/v) containing 0.1 % glacial acetic acid. The chemical formula calculation from the measured values of m/z and extracted ion current data (EIC) was performed using Xcalibur 2.0 (Thermo Fisher Scientific). High-resolution EIC was obtained by processing the MS complete scanning data using potential rhamnolipid ions with 10 ppm mass tolerance [13].

2.2.3. *Surface tension and critical micellar concentration determination*

The following parameters were analysed to compare the respective tensioactive properties of the products: surface tension (ST) and critical micellar concentration (CMC). The ST was determined using the pendant-drop technique [14], all measurements were carried out in a DSA 100S Goniometer model OF 3210. The results were expressed as the mean value of at least ten pendant drops at 23 °C and 55 % relative humidity. Based on the ST values of serially diluted crude rhamnolipid extract solutions, the CMC was determined by plotting the surface tension versus the concentration of RL in the solution, [15].

2.3. *Production of rhamnolipid CGA*

2.3.1. *Statistical Experimental Design (DOE) of the CGA parameters*

Based on previous studies regarding the stability and average bubble size diameter (Bd) of CGAs [16], a rotational central composite design (RCCD) with five central points (c) was established based on the following variables: rotation (6000-12000 rpm), pH (6-9), and agitation time (30-120 s). The response parameters were stability and average bubble size diameter (table 1). Statistica software, version 7.0 (StatSoft, USA)., was used to develop and analyse the experimental designs.

Table 1. Rotational central composite design for determination of the optimum stability and bubble size diameter.

Assays	Rotation (rpm)	pH	Agitation time (s)
1	7214	5.8	48
2	7214	5.8	102
3	7214	8.2	48
4	7214	8.2	102
5	10786	5.8	48
6	10786	5.8	102
7	10786	8.2	48
8	10786	8.2	102
9	6000	7.0	75
10	12000	7.0	75
11	9000	5.0	75
12	9000	9.0	75
13	9000	7.0	30
14	9000	7.0	120
15 (c)	9000	7.0	75
16 (c)	9000	7.0	75
17 (c)	9000	7.0	75
18 (c)	9000	7.0	75
19 (c)	9000	7.0	75

(c) Central points of the RCCD

2.3.2. Generation of rhamnolipid CGAs

The initial volume of the rhamnolipid extract was 150 mL. All experiments were conducted at room temperature (25 ± 0.2 °C). An automatic high-speed disperser was used to generate the microbubble dispersion (Ultra Turrax IKA T25 Digital Staufen, Germany) with a maximum speed of 24000 rpm. The disperser was maintained at a fixed height based on the lab shelf during all the CGA experiments [17].

2.3.3. Stability and bubble size measurements

After the rhamnolipid was stirred for a specific amount of time, the dispersion was transferred to a 150 mL graduated cylinder for measurement of the drainage liquid of the CGA. The stability of the microbubble system was quantified as the time taken for half of the liquid to drain from the dispersion, i.e., the half-life (L1/2). For stability measurements each RCCD assay was performed six times [18]. (Fig.1)

For bubble size measurements, after generation of a microbubble dispersion, a 3 mL aliquot was placed in a cavity glass slide under a light microscope (Axioscopo, model-40, Zeiss, Germany) operating under standard mode with 4 X magnification and coupled to a photographic camera (Axiocam ICC-1, Zeiss). A total of 5 photographs were taken for each RCCD assay and a total of 300 bubbles were counted per assays. The respective diameter was measured using Microscope software axiovision® version 4.6.3 (Zeiss). Afterwards, the average bubble size diameter was recorded for later analysis [16]. (Fig. 1).
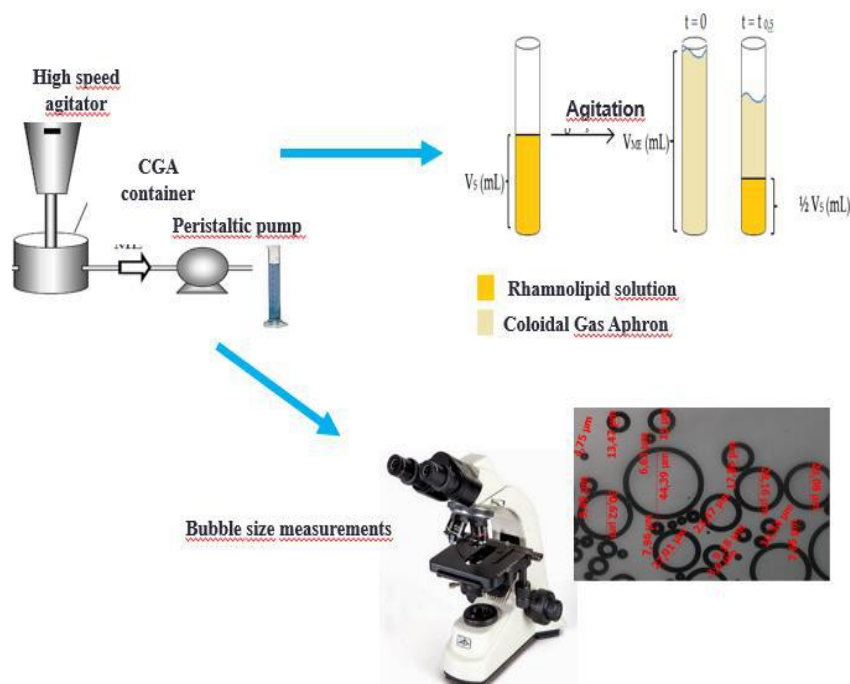
Fig 1. Schematic design of the stability and bubble size measurements of rhamnolipid CGA.

2.4. Physicochemical properties of the soil samples

To establish an artificial contamination protocol simulating a short-term contamination site, a sandy soil sample, classified according to the USDA soil classification [19], was kindly provided by Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA AGROBIOLOGIA). The sample was air-dried for 24 h and sieved with a 2 mm-mesh sieve to separate the coarse sand and stone.

Additionally, the physicochemical properties of the soil sample were characterized using standard methods [20] and are listed in Table 2. The amount of Cd and Zn in the sandy soil was extracted following the United States Environmental Protection Agency method 3051A [21] and analysed by inductively coupled plasma optical emission spectrometry (ICP OES) (Table 2).

Table 2. Physicochemical characteristics of the soil samples

Soil type	Sandy
pH	5.1
C $^{(\%)}$	1.2
Porosity (%)	44
CEC (cmolcdm^{-3})	6.3
Al (mg kg^{-1})	7.3
K (mg kg^{-1})	34
Ca (mg kg^{-1})	330
Mg (mg kg^{-1})	106
Cd (mg kg^{-1})	1.2
Zn (mg kg^{-1})	22

C $^{(\%)}$: Total soil organic carbon content; CEC: Cation exchange capacity

2.4.1. Soil artificial contamination

To establish a contamination protocol simulating a short-term contamination site, salts of Cd $(NO_3)_2.4H_2O$ and Zn $(NO_3)_2.6H_2O$ were used to prepare two separate solutions of cadmium (40 mg L^{-1}) and zinc (1000 mg L^{-1}). Then, 4 kg of sandy soil was weighed in a plastic container and mixed with all three metal solutions. The mixture was stirred for 3 weeks and then allowed to stand for 3 months.

To simulate field conditions, the contaminated soil was percolated by artificial rain in a column washing system [22], with modifications. The artificial rain was based on a variety of inorganic salts [23]. The contaminated soil was packed in a 20-cm plexiglass cylinder. For every 3 cm of sandy soil that was loaded into the column, nylon nets 5 cm in diameter with a pore size of 3 mm were inserted to prevent flow channelling when the artificial rain was percolated through the column. Based on previous assays, the contaminated soil was percolated with exactly four times its pore volume (4 PV) with artificial rain to mimic natural soil leaching conditions (fig.2).
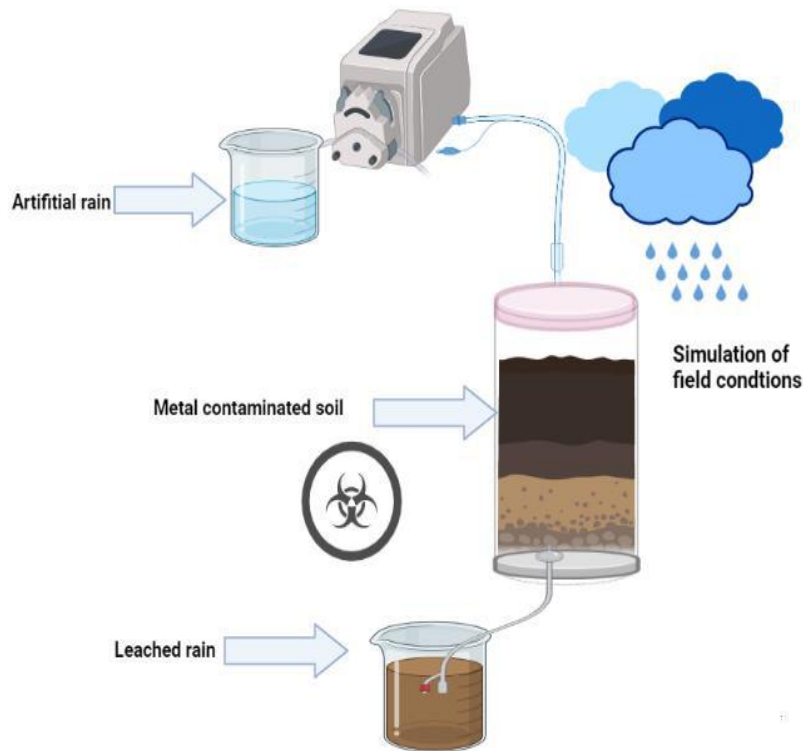

Fig 2. Schematic design of the percolation of metal contaminated soil by artificial rain.

The calculated pore volume was 135 mL for every 574 g of soil. After percolation by artificial rain, the contaminated soil was mixed once again and allowed to stand for 96 h at 25 °C. The soil samples were then dried at 40 °C for 24 h and extracted following the USEPA 3051A method [21]. The concentrations of Cd and Zn in the contaminated soil samples were analysed by ICP OES.

2.5. Percolation assays

2.5.1. Flow rate assays

Percolation assays were performed by packing another remediation column with sandy soil (soil mass: 125 g/L PV=38 mL) and then percolating it with either rhamnolipid CGA or rhamnolipid extract solution at different flow rates (10, 15, 18, 30, 40, 45, 49, 58, 64, 78, 115 and 130 mL min^{-1}). A pressure gauge was installed at the entry of the soil column, and once the desired flow rate was selected, the peristaltic pump was turned on. After the pressure inside the column was stabilized (3-5 s), the pressure build-up was recorded. Each flow rate was tested in triplicate.

2.5.2. Soil remediation assays

Two separate soil column experiments were performed to evaluate the metal remediation efficiency of rhamnolipid CGA and rhamnolipid solution in its liquid state, considering the starting pH 9.0 and number of pore volumes (PV: 4-10) (fig 3). In the first set of experiments based on previous experiments regarding the stability and bubble size diameter of rhamnolipid CGAs, the rotation speed and agitation time were maintained constant (12000 rpm/120 s).
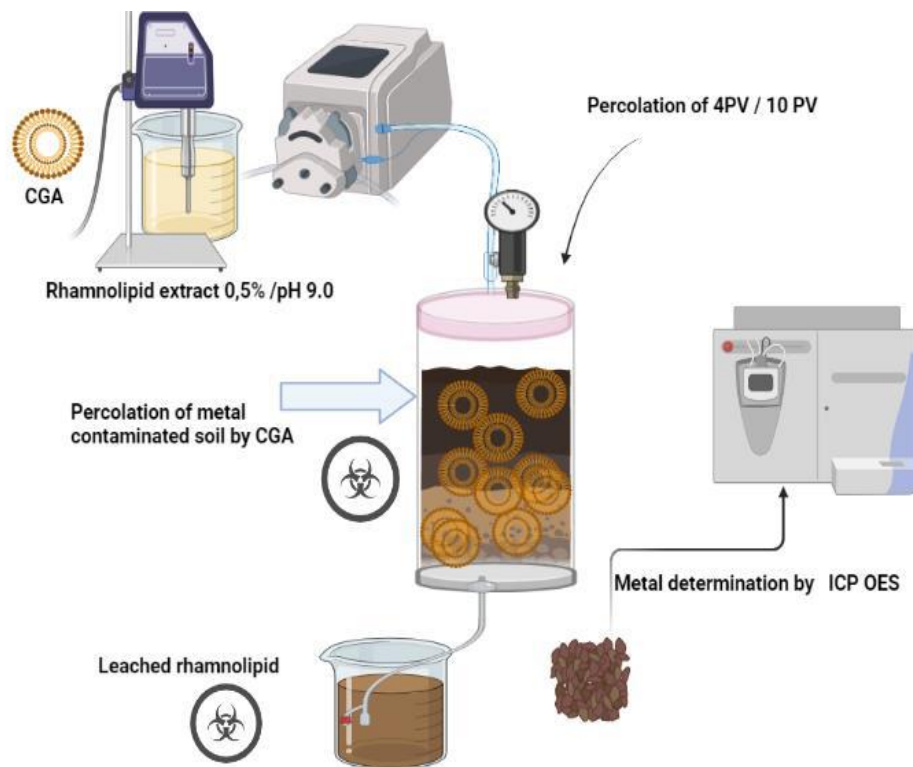
Fig.3. Schematic design of the percolation of metal contaminate soil by rhamnolipid CGA.

In the second set of experiments, the rhamnolipid extract in its liquid state was tested for its efficiency in removing metal contaminants from short-term contaminated sites. Finally, the contaminated soil was percolated by deionized water adjusted to pH 9.0 at the exact corresponding number of pore volumes of the rhamnolipid solution and CGA (negative control). The amount of contaminated soil submitted to remediation assays was 125 grams, the column height was 5 cm, and the calculated pore volume was 38 mL.

Then, all remediated soil samples were homogenized separately and dried at 40 °C for 24 hours. All remediated soil samples were submitted to extraction procedures according to the USEPA 3051A method [21]. The concentrations of Cd and Zn in the soil samples were analysed by ICPOES. The results and standard deviation are expressed as the mean average of triplicate assays. The following data were statistically analysed using ANOVA and averages *post hoc* compared using HSD-Tukey test (5 % of probability), using *Statistica*7.0 software. Finally, the results were converted to percentage values, and removal rates were defined as the percentage of metal removal from the rhamnolipid CGA or rhamnolipid solution and deduced from the removal rate of water at pH 9.0 (negative control).

III. RESULTS AND DISCUSSION

3.1 Characterization of the rhamnolipid extract

Quantification of rhamnolipid in the crude extract by HPLC demonstrated a concentration of 5.0 g L^{-1} (0.5 %) of the biosurfactant and a CMC of 72 mg L^{-1}. MS analysis revealed the presence of several congeners within the rhamnolipid extract, with fatty acid chain lengths ranging from C_8-C_{10} to C_{12}-C_{10}. The most abundant di-rhamnolipid congener was Rha-Rha$C_{10}C_{10}$, while the most abundant mono-rhamnolipid congener was Rha$C_{10}C_{10}$. The proportions of mono- and di-rhamnolipid congeners were 15 and 85%, respectively (Table 3). There have been reports of different rhamnolipid congeners with application in the remediation of metal contaminants. Until now, few works have demonstrated the characterization of rhamnolipid extracts with metal binding properties. which opens the perspective for new insights regarding the remediation of metal wastes from soil samples. In a previous study by our group, several microscale assays demonstrated that a rhamnolipid extract produced using agro-industrial wastes without any purification steps, presenting 85% di-rhamnolipid congener and 15% mono-rhamnolipid congener exhibited metal binding activity towards cadmium and zinc [24].

Table 3. Identification of rhamnolipid congeners produced by P. aeruginosa. 1201 through mass-spectrometry analysis.

Molecular ion m/z [M-H]	Relative quantitative (%) [a]	Rhamnolipid congener		
393.2	14.02	Rha-$C_{12:2}$Cl		
394.2	3.01	Rha-$C_{12:1}$Cl	Rha-$C_{12:2}$K	
475.3	20.17	Rha-C_8C_{10}	Rha-$C_{10}C_8$	
503.3	90.55	Rha-$C_{10}C_{10}$	Rha-C_8C_{12}	Rha-$C_{12}C_8$
539.3	67.71	Rha-C_8C_{10}Cl	Rha-C_8C_{12}Cl	Rha-$C_{12}C_8$Cl
541.3	22.91	Rha-$C_{10}C_{10}$K	Rha-C_8C_{12}K	Rha-$C_{12}C_8$K
621.3	18.02	RhaRha-C_8C_{10}	RhaRha-$C_{10}C_8$	
649.4	100.00	RhaRha-$C_{10}C_{10}$		
685.4	39.74	RhaRha-$C_{10:1}C_{10}$K	RhaRha-$C_8C_{12:1}$K	RhaRha-$C_{10}C_{10}$Cl
687.4	14.68	RhaRha-$C_{10}C_{10}$K		

Rha: Rhamnose. [a]: Calculated by addition of [M-H]$^-$ and fragments produced by *in-source* fragmentation.

3.2 Stability and bubble size diameter of rhamnolipid CGAs

Colloidal gas aphrons are influenced by several parameters, and even a subtle change in certain conditions can promote different outcomes regarding the stability and bubble size diameter of the macrofoam dispersion. In the present study, an RCCD was developed considering the parameters that could play a role in CGA stability and bubble size diameter. Rotation speed had a significant effect on the stability of aphrons, as well as agitation time. Rhamnolipid CGAs reached its maximum half-life (600 s) when 10786-12000 rpm was applied over a period of 102-120 s. Increasing the rotation speed and agitation time specially between 75 and 120 seconds, resulted in higher stability values (fig. 3a). Additionally, between 5000 and 8500 rpm within a stirring time of 30-120 s, an increase in the half-life of rhamnolipid CGA was observed with an average half-life of 400 s (Fig. 3a).

Several parameters may influence the stability and average bubble size of microfoams and ultimately affect their field applications. Previous studies have focused on observations based on fixed conditions of rotation speed, stirring time and pH. However, the application of an RCCD is useful to understand how different variables may simultaneously affect important CGA properties. To date, there has been scarce information regarding the behaviour of rhamnolipid CGA when submitted to binominal stirring time and stirring speed. Both parameters are of special interest to reduce time and energy to promote microfoams with satisfactory stability. According to the stability results presented by [16], a rhamnolipid CGA generated under fixed conditions of 8000 rpm for 180 s at an initial concentration of 0.4 % reported an average stability of 400-500 s. These stability values are in accordance with the present results; however, based on the data presented, it is also possible to obtain microfoams with a satisfactory half-life even under stirring times of less than 180 seconds, evidencing the possibility of optimizing the generation of stable colloidal dispersions using shorter time intervals, which is desirable for field applications.

According to [25] stirring time does not have an influence on the half-life of aphron dispersions. However, based on stability assays performed with a synthetic surfactant (cetyltrimetyl ammonium bromide 0.4 %), the authors reported a significant increase in the stability of the CGA as the stirring speed was elevated from 5000 to

5500 rpm, within the same time stirring of the present study (30-120s).

In the present study, considering the higher stirring speed range tested in the present study (5000-8500 rpm) and the same stirring time tested, the stability of the CGA dispersions was similar (~300 s); however, when the biosurfactant was submitted to higher velocities under 30-120 s, a significant improvement in the half-life of the CGAs was observed (Fig. 3a). Thus, it can be inferred that stirring time influences the half-life of CGAs under stirring velocities between 9000 and 12000 rpm. Moreover, the binomial stirring speed and stirring time may play a role in the stability of a rhamnolipid CGA, especially under higher stirring velocities and stirring times between 30-120 s.

Recently, [26], reported the influence of stirring speed on the stability of CGAs generated from synthetic cationic and anionic surfactants. It was demonstrated that optimum half-life values were obtained between 11000 and 12000 rpm, and by further agitation at 13000 rpm, there was a significant reduction in the stability of the CGA suspensions. Until now, there has been scarce information regarding the influence of the maximum stirring speed and rotation time on the half-life of CGAs; however, it can be inferred that rotation speeds above 12000 rpm may play a role in the stability of microfoams, especially under stirring conditions above 102 seconds.

Regarding the influence of pH (5.8-9.0), no positive or negative influence was observed on the stability of the rhamnolipid colloidal gas aphrons (Fig. 3b). This was also observed by previous authors when experimenting with synthetic anionic surfactants [27,28]. However, other studies have demonstrated that the stability of rhamnolipid CGA and reported that alkaline conditions could have a negative influence on the stability of rhamnolipid CGA [16]. The authors discussed the negative influence of alkaline pH on the stability of CGAs based on the previous study [29], who reported that the dissociation of rhamnolipid at pH 8.0 promotes electrostatic repulsion between adjacent ionized carboxyl groups of the molecule. The authors further stated that when rhamnolipid CGAs were generated under alkaline conditions, these electrostatic repulsions promoted a significant reduction in the concentration of rhamnolipid at the bubble surface and decreased the viscosity and elasticity of the bubble surface, which ultimately led to bubble coalescence.

However, [16] used NaOH to prepare alkaline solutions of rhamnolipids and generate rhamnolipid microfoams under alkaline conditions, whereas in the present study, NH₄OH was used to subject the rhamnolipid extract to these same conditions. Thus, having not observed any significant changes in the stability of the rhamnolipid microfoams, it is possible that the partial dissociation of NH_4OH may have played a role in the stability of rhamnolipid microfoams, as the hydrophilic region of the rhamnolipid molecule was not fully dissociated under the alkaline conditions tested, resulting in less electrostatic repulsion between the hydrophilic portions and thus not promoting any significant modifications in the viscosity and elasticity of the microbubble surface. Additionally, there has been previous studies demonstrating the influence of sodium chloride (NaCl) upon the micellization process of rhamnolipids at pH 6.8. The presence of electrolytes with a total dissociation property, promotes an increase of size and number of rhamnolipid micelles due to reduction of the electrostatic repulsion between hydrophilic heads of the biosurfactant molecule [30]. But again, its not confirmed if the same effect is possible to occur under strong alkaline conditions, and if it could imply in a reduction the stability of rhamnolipid CGA.

Regarding average bubble size diameter of the rhamnolipid CGA dispersion, it wa observed that the higher and lower bubble size of the rhamnolipid CGAs ranged from 60-220 µm (Fig. 3c). The average bubble diameter was affected by the rotation speed and agitation time. The average bubble size diameter of rhamnolipid CGA fluctuated between 100 and 60 µm within a agitation time of 30 to 75 s and rotation speeds of 6000 and 12000 (Fig. 3c). These average sizes for a rhamnolipid CGAs are in accordance with results presented previously [16], however, within the agitation time of 102 and 120 s as more rotational kinetic energy was applied towards the rhamnolipid extract bubble size average was affected, producing larger rhamnolipid CGAs, and reaching over 220 µm. However, between 10786 and 12000 rpm, the size of the rhamnolipid CGA decreased by 120 µm, demonstrating a saturation tendency towards the amount of rotation speed, which can influence the average size of rhamnolipid CGAs. Other researchers also reported that higher velocities and agitation time could affect bubble average diameter, as more shear forces could influence the average bubble diameter within the microfoam dispersions [26]. The authors reported that at 13000 rpm, there was a reduction in the bubble size average of the CGAs produced using the synthetic surfactants SDS and Triton-X, which is in accordance with the results in the present study.
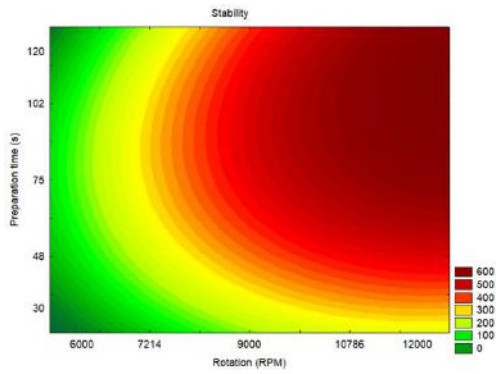
Fig. 3a. Response surfaces for stability of rhamnolipid CGA. Rotation speed versus rotation time.
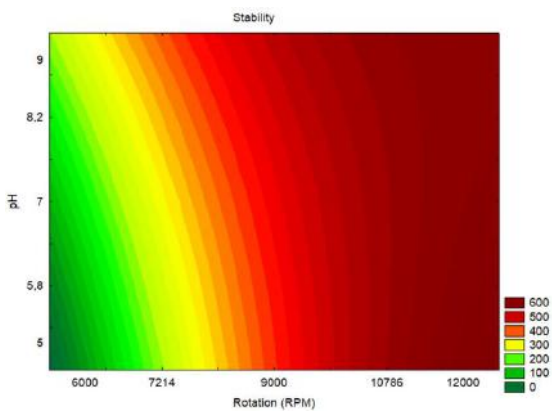

Fig. 3b. Response surfaces for stability of rhamnolipid CGA. Rotation versus pH.
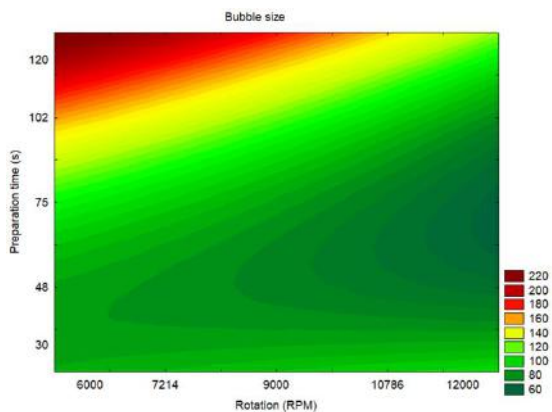

Fig. 3c. Response surface for bubble size diameter rhamnolipid CGA dispersion. Rotation versus agitation time.

3.3 Soil washing experiments with the rhamnolipid CGA dispersion and rhamnolipid extract solution.

3.3.1 Flow rate experiments

In the present study, several flow rate assays were conducted to evaluate the performance of a rhamnolipid CGA and a rhamnolipid extract in a liquid state when submitted to metal-contaminated soil. After selecting the best conditions for CGA stability and average bubble diameter, a series of percolation experiments were conducted under different flow rates, and the respective pressure build-up was recorded to better evaluate the suitable flow rate for percolation of the rhamnolipid extract and rhamnolipid CGA through the remediation column. Between the flow rates of 30 and 130 mL min^{-1}, a significant pressure drop was always observed when rhamnolipid CGA was pumped through the remediation column (Fig. 4) compared to the rhamnolipid extract solution. However, this was not observed under a flow rate of 10-12 mL min^{-1}; thus, this was the flow rate throughout the rest of the remediation assays. Additionally, the remediation assays, when performed under a low flow rate (10-12 mL min^{-1}), led to a significant amount of time dispended and were significantly laborious, while increasing the flow rate to 20-30 mL min^{-1} generated pressure build-up, channelling, and obstruction points inside the remediation column. The effect of pressure build-up of a nonaqueous GCA when percolated trough porous media was reported elsewhere [31]. The authors also reported that as the volume of CGA fluid increased, the pressure drop across the porous media also increased.
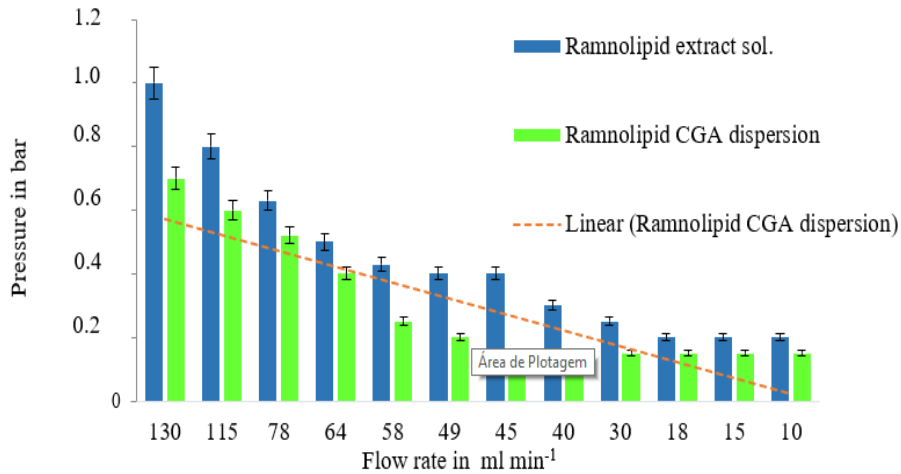
Fig.4. Pressure drop of rhamnolipid CGA in comparison to rhamnolipid extract. Down flow mode.

Also there has been reports of the influence of channelling and pressure build-up when flow rates above 18 ml min^{-1} were applied to a packed remediation column containing a soil sample contaminated by arsenic [32].

3.3.2 Metal remediation efficiency of rhamnolipid CGA dispersions

A sandy soil sample was artificially contaminated to simulate a short-term contamination site and percolated by artificial rain to simulate field conditions. The final concentrations of cadmium and zinc were 39 and 511 mg kg^{-1}, respectively. Percolation of 4 PV of rhamnolipid CGA dispersion resulted in a removal rate of 13 %, which was considered inferior in comparison to the negative control and thus considered a insignificant remediation, however after percolation of 10PV a remediation of 38% was observed (fig. 5a). The removal rate of zinc after 4PV was 42% and after 10PV 53 % (fig. 5a). The rhamnolipid solution at pH 9.0 exhibited the following removal rates for cadmium: 39 and 46 % and for zinc: 62 and 67 % (fig. 5b). Throughout the experiment, the average time to percolate 4 pore volumes of CGA dispersion or rhamnolipid extract solution was 3-4 hours, and percolation assays of 10 pore volumes took an average of 6-8 hours.
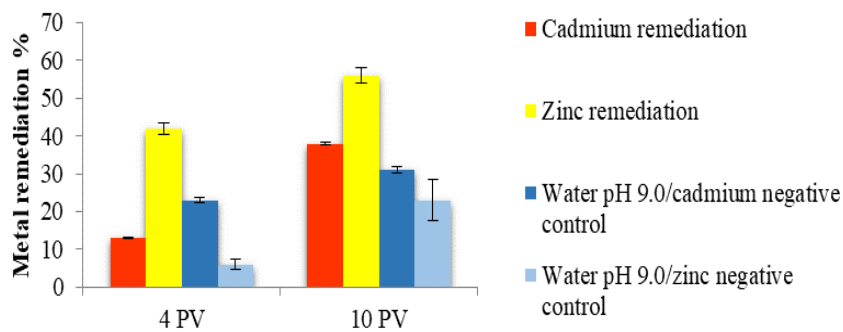

Fig. 5a. Metal remediation percentage of Rhamnolipid CGA (0.5%) pH 9.0; PV: Pore volume.
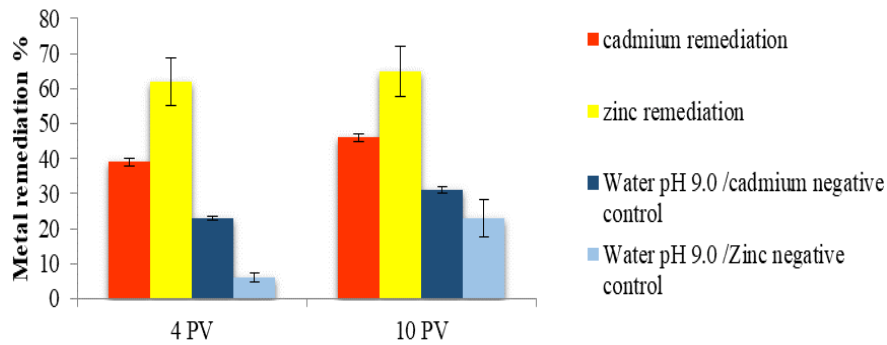
Fig. 5b. Metal remediation percentage of Rhamnolipid solution (0.5%) pH 9.0; PV: Pore volume.

Colloidal gas aphrons are composed of 40-70 % gas content, high stability, and resistance towards shear forces [16]. Regular foams, which are formed by the expansion of gas content involved in a thin and continuous surfactant liquid phase, have been successfully applied in the soil remediation process [33]. However, their low stability is a drawback for field applications since foams are a thermodynamically metastable system [34].

CGAs have been reported as a more stable foam structure due to their triple shell conformation, where the internal film containing a surfactant monolayer displays its hydrophobic tail oriented towards the gas phases and the external film consists of a double-layered hydrophilic shell facing the aqueous medium [35]. Until now, few studies have demonstrated the possibility of using biosurfactant microfoams to remove metal from soil samples, and even fewer studies have compared its efficiency to previous methods in which regular foam was applied.

In a soil flushing experiment with metal-contaminated sand [36], demonstrated the efficiency of regular foam produced with biosurfactants to remove cadmium from the contaminated site. The authors reported a removal rate of cadmium of 20% when the biosurfactant surfactin was applied, while rhamnolipid foams removed 40% of cadmium from contaminated sand samples after percolation of 24 pore volumes. Based on the present results, it can be demonstrated that colloidal gas aphrons can display a similar soil remediation efficiency when compared to previous studies that applied regular foam as a metal extraction agent, however spending less surfactant solution to achieve almost the same results in terms of soil remediation. This is due to its unstable characteristics and higher tendency towards coalescence of regular foams when compared to colloidal gas aphrons.

Maity et al. [37] reported a metal remediation efficiency of 56 % towards zinc after 48 hours of contact of saponin foams with metal-contaminated soil samples. In the present study, 10 pore volumes were percolated in approximately 6-8 hours, demonstrating that while both methods could result in significant remediation percentages of Zn, rhamnolipid CGA dispersion are less time-consuming, which can be inferred in a more practical approach for metal removal in large scale remediation methods. Mukhopady et al. [32] compared the remediation efficiency of colloidal gas aprons and a saponin extract to remove arsenic from soil samples, when percolated in down flow mode. According to the authors in both situations percolation in a descendent mode was favored by gravity and thus the water flow properties of colloidal gas aphrons implicated in a similar remediation between the saponin extract and the saponin colloidal gas aphrons. This was also demonstrated in the present study. The rhamnolipid extract and the rhamnolipid colloidal gas aphrons when percolated in down flow mode exhibited a similar remediation efficiency for cadmium and zinc after the percolation of 10 PV.

Based on the present results, it can be demonstrated that water flow conditions also played a role in the remediation of rhamnolipid solution and rhamnolipid CGA. Column remediation assays demonstrated a similar percolation pattern; hence, remediation of cadmium and zinc was considered similar. Recently, Pasdar et al. [38], used schematic micromodel patterns to study the role of sodium dodecyl sulfate and sodium dodecyl benzene sulfonate CGAs in controlling fluid invasion under water and oil-wet conditions. Microscopic observations revealed that CGA dispersions perform better under oil wet conditions than under water wet conditions, which is a condition more suited for metal-contaminated terrains.

3.3.3 Influence of number pore-volume

The number pore volume had a significant influence on the extraction of metal contaminants. The continuous washing procedure by a biosurfactant with metal binding properties such as the rhamnolipid molecule affects metal mobilization by permitting continuous contact of the biosurfactant with the soil samples, which contributes to metal desorption In the rhamnolipid extract solution

experiment and the CGA rhamnolipid experiment, the number of pores progressively increased, and the percentage of cadmium and zinc was removed from the soil (Fig. 5). Recently in a metal contaminated sandy loam sample, Ugwu et al. [38] demonstrated the extraction of 63% of Cd after percolation of 10 PV of a rhamnolipid solution. In a column flushing experiment, Wang and Mulligan [39], reported a significant amount of arsenic, copper, lead, and zinc removal from a mine tail sample after percolation of a 70-pore volume of a 0.1 % rhamnolipid solution with an initial pH of 11.0. Tang et al. [40], also observed that after multiple washes with two biosurfactants, considerable amounts of copper, zinc, chromium, lead, nickel, and manganese were removed from metal-contaminated sludge.

IV.   CONCLUSIONS

The increasing extraction of metal ores has led to a worldwide demand for new methods to remove or mitigate the hazards of metal contaminants from soils. Such methods need to be in line with future strategies aimed at reforestation or resumption of existing socioeconomic activities. The development of natural substances with significant biodegradability and metal extraction properties has become extremely interesting for these strategies.

In the present study, a rhamnolipid extract produced with agro-industrial waste was submitted to modification of its physical structure into CGA dispersion, and several parameters were evaluated to determine the best condition for application in an *ex-situ* soil washing method. Based on the present results, high rotation speeds during a short period of time can influence the stability and bubble diameter of rhamnolipid CGA. Additionally, alkaline conditions did not play an important role in the stability and bubble diameter of the CGAs. The binomial rotation speed and agitation time increases the average size of the rhamnolipid CGA to a point were afterwards the rhamnolipid microbubbles start to decrease its average size, demonstrating a saturation tendency towards the amount of rotation speed and agitation time which can produce large rhamnolipid CGAs with an the average size of 200 μm.

Soil column experiments regarding the pressure build-up behaviour of the rhamnolipid CGA and the rhamnolipid extract demonstrated that during percolation under several flow rates, a significant pressure drop was observed when flow rates between 30- and 130-ml min were applied for the rhamnolipid CGA. The rhamnolipid microfoam exhibited a superior efficiency for metal remediation of metallic contaminants (cadmium and zinc) when compared to previous studies regarding metal

remediation of regular foams. Likewise, soil column experiments demonstrated that the rhamnolipid extract was also efficient in removing cadmium and zinc from a short-term contaminated site.

Ultimately, this study opens the prospect of applying a rhamnolipid extract as microfoams in remediation processes of metal-contaminated soils. In addition, there is increasing knowledge about parameters related to the remediation efficiency of rhamnolipid microfoams and features related to microfoam percolation through porous media, such as low-pressure accumulation and efficient and rapid remediation of metals from contaminated soils.

REFERENCES

[1] Sall, M. L., Diaw, A. K. D., Gningue-Sall, D., Efremova Aaron, S., Aaron, J. J.,2020. Toxic heavy metals: impact on the environment and human health, and treatment with conducting organic polymers, a review. Environ. Sci.Pollut.Res. 27, 29927-29942.

[2] Azevedo, D. C. B. D., Toledo, G. D. A., Cohen, S. C., Kligerman, D. C.,Cardoso, T. A. D. O., 2020. Brumadinho disaster: contributions to public policies and sanitation management in emergency periods. Saúde Debate. 44, 221-233.

[3] Hou, D., Al-Tabbaa, A., Hellings, J.,2015. Sustainable site clean-up from megaprojects: lessons from London 2012. In Proceedings of the Institution of Civil Engineers-engineering sustainability.ICE Virtual Library, London. 168, pp.61-70.

[4] Udovic, M., Lestan, D., 2012. EDTA and HCl leaching of calcareous and acidic soils polluted with potentially toxic metals: remediation efficiency and soil impact. Chemosphere, 88, 718-724.

[5] Tiehm, A., 1994. Degradation of polycyclic aromatic hydrocarbons in the presence of synthetic surfactants. Appl. Environ. Microbiol. 60,258–263

[6] Sobrinho, H. B., Luna, J. M., Rufino, R. D., Porto, A. L. F., Sarubbo, L. A.,2013. Biosurfactants: classification,

properties and environmental applications. Recent. Dev. Biotechnol. 11,1-29.

[7] Liu, G., Zhong, H., Yang, X., Liu, Y., Shao, B., Liu, Z., 2018. Advances in applications of rhamnolipids biosurfactant in environmental remediation: A review. Biotechnol. Bioeng. 115,796-814.

[8] Zhang, M., Feng, Y., Zhang, K., Wang, Y., Pan, X., 2020. Impact of salinity on colloidal ozone aphrons in removing phenanthrene from sediments. J. Hazard. Mater. 384, 121436.

[9] Boonamnuayvitaya, V., Jutaporn, P., Sae-ung, S., Jarudilokkul, S., 2009. Removal of pyrene by colloidal gas aphrons of a biodegradable surfactant. Sep. Purif. Technol. 68,411-416.

[10] Honna CY (2013) Obtenção e análise de mutantes de Pseudomonas aeruginosa afetados na biossíntese de ramnolipídeos. Dissertation, Universidade de São Paulo. doi: 10.11606/D.42.2013.tde-26062014-112650.

[11] Kronemberg, F. A., 2007. Produção de ramnolipídios por Pseudomonas aeruginosa PA1 em biorreator com oxigenação por contactor de membranas. Thesis. Universidade Federal do Rio de Janeiro.

[12] Déziel,E., Lepine,F., Milot, S., Villemur, R.,2003. rhlA is required for the production of a novel biosurfactant promoting swarming motility in Pseudomonas aeruginosa: 3-(3-hydroxyalkanoyloxy) alkanoic acids (HAAs), the precursors of rhamnolipids. Microbiol.149, 2005–2013.

[13] Strohalm, M., Hassman, M., Košata, B., Kodíček, M., 2008. Mass data miner: an open-source alternative for mass spectrometric data analysis. Rapid Commun. Mass Spectrom. 22, 905-908.

[14] Song, B.,Springer, J.,1997. Surface phenomena of liquid crystalline substances: Temperature-dependence of surface tension. Mol.Cryst. Liq. Cryst.Sci.Technol. Sect. A. 307,69-88.

[15] Sheppard, J.D., Mulligan, C. N., 1987. The production of surfactin by Bacillus subtilis grown on peat hydrolysate. Appl. Microbiol. Biotechnol.27,110–116 doi:10.1007/BF0025193

[16] Feng,W., SInghal, N., Swift, S., 2009. Drainage mechanism of microbubble dispersion and factors influencing its stability. J. Colloid. Interface Sci.337,548-554

[17] Sebba, F., 1985. Improved generator for micron-sized bubbles. Chem. Ind. 91-92.

[18] Sebba, F.,1987. Foams and Biliquid Foams. Chichester, UKWiley

[19] United States Department of Agriculture – USDA Soil Survey Staff (1999) Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys. 2nd edition. Natural Resources Conservation Service. U.S. Department of Agriculture Handbook 436. U. S. Government Printing Office, Washington, DC.

[20] Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA, 1997. Manual de métodos de análise de solo,2nd ed. Centro Nacional de Pesquisas de Solos, Rio de Janeiro, Brazil.

[21] United States Environmental Protection Agency – USEPA (2007) Method 3051A – Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils.

[22] Zhen, M., Tang, J., Li, C., Sun, H., 2021. Rhamnolipid-modified biochar-enhanced bioremediation of crude oil-contaminated soil and mediated regulation of greenhouse gas emission in soil. J. Soils Sedim.21, 123-133.

[23] Oorts,K., Ghesquiere,U., Smolders,E., 2007. Leaching and aging decrease nickel toxicity to soil microbial processes in soils freshly spiked with nickel chloride. Environ. Toxicol. Chem.26,1130–1138.

[24] Lopes, C. S. C., Teixeira, D. B., Braz, B. F., Santelli, R. E., de Castilho, L. V. A., Gomez, J. G. C., Castro, R.P.V., Seldin, L., Freire, D. M. G., 2020. Application of rhamnolipid surfactant for remediation of toxic metals of long-and short-term contamination sites. Int. J. Environ. Sci. Technol. 1-14.

[25] Matsushita, K., Mollah, A.H., Stuckey, D.C., Del Cerro C., Bailey, A.I, 1992. Predispersed solvent extraction of dilute products using colloidal gas aphrons and colloidal liquid aphrons: aphron preparation, stability and size. Colloids Surf.69,65–72.

[26] Keshavarzi, B., Javadi, A., Bahramian, A., Miller, R., 2019. Formation and stability of colloidal gas aphron based drilling fluid considering dynamic surface properties. J. Petrol. Sci. Eng.n174, 468-475.

[27] Save, S. V., Pangarkar, V. G., 1994. Characterization of colloidal gas aphrons. Chem. Eng.Commun.127, 35-54.

[28] Jauregi, P., Gilmour, S., Varley, J., 1997. Characterization of colloidal gas aphrons for subsequent use for protein recovery. Chem. Eng. J. 65,1-11.

[29] Champion, J. T., Gilkey, J. C., Lamparski, H., Retterer, J., Miller, R. M.,1995. Electron microscopy of rhamnolipid (biosurfactant) morphology: effects of pH, cadmium, and octadecane. J. Colloid. Interface Sci. 170,569-574.

[30] Helvaci, Ş. Ş., Peker, S., Özdemir, G., 2004 Effect of electrolytes on the surface behavior of rhamnolipids R1 and R2. Colloid. Surf. Biointerface. 35, 225-233.

[31] Shivhare, S., Kuru, E., 2014. A study of the pore-blocking ability and formation damage characteristics of oil-based colloidal gas aphron drilling fluids. J. Pet. Sci. Eng. 122, 257-265.

[32] Mukhopadhyay, S., Mukherjee, S., Hashim, M. A., Gupta, B. S.,2015. Application of colloidal gas aphron suspensions produced from Sapindus mukorossi for arsenic removal from contaminated soil. Chemosphere. 119,355-362.

[33] Mulligan, C. N., Wang, S., 2006. Remediation of a heavy metal-contaminated soil by a rhamnolipid foam. Eng. Geol. 85, 75-81.

[34] Vignes-Adler, M., Weaire, D.,2008. New foams: Fresh challenges and opportunities. Curr. Opin. Colloid Interface Sci.13, 141-149.

[35] Jauregi, P., Mitchell, G. R., Varley, J., 2000.Colloidal gas aphrons (CGA): dispersion and structural features. AIChE J. 46, 24-36.

[36] Haryanto, B., Chang, C. H., 2014. Foam-enhanced removal of adsorbed metal ions from packed sands with

biosurfactant solution flushing. J. Taiwan Inst. Chem. Eng. 45, 2170-2175.

[37] Maity, J. P., Huang, Y. M., Hsu, C. M., Wu, C. I., Chen, C. C., Li, C. Y., Jean, J., Chang, Y., Chen, C. Y. (2013). Removal of Cu, Pb and Zn by foam fractionation and a soil washing process from contaminated industrial soils using soapberry-derived saponin: a comparative effectiveness assessment. Chemosphere. 92, 1286-1293.

[38] Pasdar, M., Kamari, E., Kazemzadeh, E., Ghazanfari, M. H., Soleymani, M.,2019. Investigating fluid invasion control by Colloidal Gas Aphron (CGA) based fluids in micromodel systems. J. Nat. Gas Sci. Eng.66, 1-10.

[39] Ugwu, E. C., Gupta, B. S., Adebayo, A., Martínez-Villegas, N.,2021. Column Experiment for the Removal of Cadmium, Copper, Lead and Zinc from Artificially Contaminated Soil using EDTA, Rhamnolipids, and Soapnut. Eur. J. Environ. Earth Sci. 2, 1-7.

[40] Wang, S., Mulligan, C. N.,2009. Rhamnolipid biosurfactant-enhanced soil flushing for the removal of arsenic and heavy metals from mine tailings. Process. Biochem.44, 296-301.

[41] Tang, J., He, J., Qiu, Z., Xin, X., 2019. Metal removal effectiveness, fractions, and binding intensity in the sludge during the multiple washing steps using the combined rhamnolipid and saponin. J. Soils Sediments., 19, 1286-1296.