

1 **Biosurfactants-facilitated leaching of metals from spent**  
2 **hydrodesulfurization catalyst**

3 Running title: Bioleaching of spent catalysts

4 Shareefah ALSaquer<sup>1</sup>, Meena Marafi<sup>2</sup>, Ibrahim M. Banat<sup>3</sup>, Wael Ismail<sup>1\*</sup>

5 <sup>1</sup>Environmental Biotechnology Program, Life Sciences Department, College of Graduate  
6 Studies, Arabian Gulf University, Manama, Bahrain

7 <sup>2</sup>Petroleum Refining Department, Petroleum Research and Studies Center, Kuwait  
8 Institute for Scientific Research, Safat, Kuwait

9 <sup>3</sup>School of Biomedical Sciences, University of Ulster, Coleraine, County Londonderry  
10 BT52 1SA, Northern Ireland, UK

11 \*Corresponding author: Environmental Biotechnology Program, Life Sciences

12 Department, College of Graduate Studies, Arabian Gulf University, Manama, Bahrain,

13 Tel: +97336146948; Fax: +97317239664, E-mail: [waelame@agu.edu.bh](mailto:waelame@agu.edu.bh)

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24 **Abstract**

25 Aims: To investigate the capabilities of different types of biosurfactants (rhamnolipids,  
26 lipopeptides, sophorolipids) to remove metals and carbon from the hazardous spent  
27 hydrodesulfurization (HDS) catalyst generated by petroleum refineries.

28 Methods and Results: Biosurfactants were prepared and used to treat spent HDS catalyst.  
29 Metal and carbon contents were analyzed and compared with those from no-biosurfactant  
30 control treatments. All biosurfactant treatments increased carbon loss percentage from the  
31 spent HDS catalyst. The lipopeptide treatment LI, containing 17.34 mg/mL of crude  
32 biosurfactants, caused the highest carbon loss percentage (44.5%). Rhamnolipids were, in  
33 general, better than sophorolipids and lipopeptides as metal-removing agents. The metal  
34 content decreased as the concentration of rhamnolipids decreased. The R5 treatment,  
35 which contained 0.4 mg/L of crude rhamnolipids, caused the highest reduction in metal  
36 content. Molybdenum, Nickle and Vanadium contents were reduced by 90%, 30%, and  
37 70%, respectively.

38 Conclusions: Biosurfactants might have potential application for metals and coke  
39 removal from spent HDS catalysts. The bioleaching capability depends on the type and  
40 concentration of the biosurfactant.

41 Significance and Impact of the Study: This study, after further in-depth investigations,  
42 might lead to the development of an eco-friendly and economic technology to treat or  
43 even regenerate the environmentally hazardous spent HDS catalysts, which are generated  
44 in huge amounts by the petroleum refineries.

45 **Keywords:** biosurfactants, spent HDS catalyst, bioleaching, coke deposition, rhamnolipids,  
46 sophorolipids, molybdenum

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## 48 **Introduction**

49           The petroleum industry depends heavily on thermochemical catalytic processes  
50 known as hydrotreatment (HDT) and hydroprocessing for different oil refining  
51 operations. These processes utilize huge amounts of solid inorganic catalysts to speed up  
52 different chemical reactions (Akcil *et al.* 2015). Hydroprocessing catalysts usually consist  
53 of molybdenum (Mo) or tungsten (W) supported on an alumina carrier with the aid of  
54 cobalt or nickel as promoters that encourage the removal of sulfur, nitrogen, and metals  
55 from the treated oil by means of hydrodesulfurization (HDS), hydrodenitrogenation  
56 (HDN), and hydrodemetallation (HDM) reactions, respectively (Marafi *et al.* 2003)

57           The fresh catalysts are poisoned and deactivated during the different catalytic  
58 processes due to the deposition of hazardous metals (Ni and V) and coke originating from  
59 the treated feedstock (Marafi and Stanislaus 2008a; 2008b). The amount of spent  
60 catalysts generated by the petroleum industry worldwide was estimated at 150,000-  
61 170,000 tons/year (Dufresne 2007). At the current rate of consumption, ca 178,000  
62 tons/year of hydrotreatment catalyst and 358,000 tons/year of fluid catalytic cracking  
63 catalyst is required (Ahmed and Menoufy 2012; Srichandan *et al.* 2012). This amount is  
64 steadily increasing due to the increase in the processing of heavier feedstocks and the  
65 growing demand for cleaner fuels (Shahrabi-Farahani *et al.* 2014).

66           The spent catalyst generated by the petroleum refining industry is designated by  
67 United States Environmental Protection Agency as a toxic and environmentally  
68 hazardous waste (Akcil *et al.* 2015). Although spent refining catalysts constitute only ca  
69 4% (weight) of the overall refinery wastes, they are classified as one of the most

70 hazardous wastes generated by petroleum refineries (Liu *et al.* 2005; Akcil *et al.* 2015).  
71 Therefore, it requires proper handling and disposal. Heavy metals such as V, Ni, Mo, and  
72 Co present on the spent catalysts can be leached by water after disposal and therefore  
73 exacerbate environmental pollution. Furthermore, spent hydroprocessing catalysts can  
74 liberate toxic gases upon exposure to water. Coke deposition on the hydroprocessing  
75 catalysts that contain a substantial amount of nitrogen can lead to the formation of the  
76 hazardous hydrogen cyanide (HCN) gas. Accordingly, environmental regulations  
77 regarding the handling of the spent refining catalysts are becoming increasingly stricter  
78 (Marafi and Stanislaus 2003).

79 Different strategies have been applied to treat or handle spent refining catalysts,  
80 such as disposal in landfills, rejuvenation or regeneration for reuse, and recovery of  
81 valuable metals via physicochemical treatments (Asghari *et al.* 2013). Landfill disposal is  
82 environmentally constrained, energy intensive, requires high cost and liable dumpsite,  
83 thus making it less preferable. Moreover, in some cases, the pretreatment of spent  
84 catalysts before landfilling is essential, which in turn increases the cost (Marafi and  
85 Stanislaus 2008a; Macaskie *et al.* 2010). Spent catalyst rejuvenation is an appealing  
86 option for reactivation and reuse of the spent catalysts (Marafi and Stanislaus 2011).  
87 Nonetheless, the spent catalysts rejuvenation technology is not available to oil refineries  
88 and can only be carried out for a limited number of cycles. Eventually, the spent catalyst  
89 is irreversibly deactivated and must be discarded and replaced with a fresh batch  
90 (Pradhan and Kumar 2012). It is also not possible to reactivate spent catalysts that are  
91 deactivated by thermal degradation or phase separation (Marafi *et al.* 2003). Furthermore,

92 conventional rejuvenation processes are facilitated by physicochemical treatments that  
93 are associated with environmental and economic constraints.

94 Metal recovery from spent refining catalysts has been investigated to reduce the  
95 environmental hazard, minimize landfill usage, and meet current market demand for  
96 metals. This is based on the fact that spent refining catalysts represent a significant  
97 secondary ore/source of valuable metals such as Pt, Re, V, Ni, Mo, Co, Cu, Al, and Fe  
98 (Srichandan *et al.* 2012; Motaghed *et al.* 2014). Furthermore, metal removal can help  
99 regenerate spent catalysts that are poisoned with metal deposition (Marafi and Stanislaus  
100 2003). Conventional techniques for metal extraction from various sources include  
101 hydrometallurgy and pyrometallurgy. Despite reasonable extraction efficiencies, the  
102 application of these two techniques is restricted due to the use of high strength acids and  
103 alkalies (secondary pollutants), high energy consumption (reflected as high cost), and  
104 emission of toxic gases that require downstream treatment (Srichandan *et al.* 2012;  
105 Asghari *et al.* 2013).

106 Biotechnology-based approaches for metal recovery, such as bioleaching  
107 (biohydrometallurgy), offer several advantages as compared to conventional  
108 physicochemical methods. Bioleaching is simpler to operate, economic, environmentally  
109 compatible, and even more efficient (Santhiya and Ting 2005; Mishra *et al.* 2007;  
110 Asghari *et al.* 2013; Shahrabi-Farahani *et al.* 2014). Bioleaching is carried out using  
111 whole microbial cells or microbial products such as chelating agents, acids,  
112 polysaccharides, siderophores as well as biosurfactants (Franzetti *et al.* 2015). Microbial  
113 bioleaching of spent refining catalysts has been reported widely using fungi (*Penicillium*  
114 *simplicissimum*, *Aspergillus niger*) and iron-oxidizing and sulfur-oxidizing bacteria

115 (*Acidithiobacillus ferrooxidans*; *Acidithiobacillus thiooxidans*) (Srichandan *et al.* 2012;  
116 Motaghed *et al.* 2014; Shahrabi-Farahani *et al.* 2014).

117 Biosurfactants are surface-active microbial products that are gaining increasing  
118 interest due to their superior physicochemical characteristics and environmental  
119 compatibility as compared to synthetic (petroleum-based) surfactants (Banat *et al.* 2014).  
120 Biosurfactants can be applied in diverse fields including environmental protection, soil  
121 washing, bioremediation, upgrading of heavy oils, enhanced oil recovery, oil spill  
122 cleaning, tanker cleanup, viscosity control, emulsification, formulation of petrochemicals,  
123 etc (Vijayakumar and Saravanan 2015; De Almeida *et al.* 2016). Moreover, different  
124 kinds of biosurfactants have been applied for metals removal from industrial effluents  
125 and contaminated soil (Franzetti *et al.* 2015; Sarubbo *et al.* 2015). El Zeftawy and  
126 Mulligan (2011) reported that rhamnolipid biosurfactants in micellar-enhanced  
127 ultrafiltration is effective in leaching numerous metals such as Cd, Pb, Cu, Zn, and Ni  
128 from industrial wastewater. A mixture of rhamnolipid biosurfactants leached Zn, Pb, Cu,  
129 and Cd from polluted soil (Slizovskiy *et al.* 2011). Moreover, *Bacillus subtilis* A21  
130 produced surfactin and fengycin that were highly efficient in chelating metals such as Cd,  
131 Co, Pb, Ni, Cu, and Zn from petroleum resulting in low phytotoxicity of soils (Singh and  
132 Cameotra 2013). Nonetheless, to our knowledge, the application of biosurfactants for  
133 bioleaching or regeneration of spent refining catalysts has not been previously explored.  
134 Therefore, in this study we investigated the applicability of different types and  
135 concentrations of biosurfactants for bioleaching of metals from spent HDS catalysts.  
136 Surface area and pore volume of the treated catalyst were also analyzed.

137 **Materials and methods**

138 Bacteria

139 *Candida bombicola* ATCC 2221 was used for sophorolipid production (Smyth *et*  
140 *al.* 2014). *Pseudomonas aeruginosa* AK6U was used for rhamnolipid biosurfactants  
141 production. This strain was isolated and characterized in previous investigations at the  
142 laboratories of the Environmental Biotechnology Program-Arabian Gulf University  
143 (Ismail *et al.* 2014; 2015; 2017). It produces rhamnolipid biosurfactants using glucose or  
144 heavy vacuum gas oil (HVGO) as a carbon source (Ismail *et al.* 2017). The NCE3 strain  
145 was used to produce lipopeptide biosurfactants (Ismail *et al.* 2013). The NCE3 strain is a  
146 *Bacillus megaterium* strain, which grows on and emulsifies crude oil (Ismail *et al.* 2013).

147 Culture media and growth conditions

148 Luria-Bertani (LB) agar and broth media were prepared according to the  
149 manufacturer's instructions (Sigma-Aldrich, Germany). The LB broth was used for the  
150 preparation of starter cultures. LB agar plates were used for bacterial growth and  
151 preservation for short time. The AK6U strain was streaked on LB agar plates and  
152 incubated for 48 hours, while NCE3 was incubated for 24 hours. To produce  
153 biosurfactants, bacteria were grown on HVGO in mineral salts medium whose  
154 composition was described (Ismail *et al.* 2017). All cultures were incubated at 30°C.

155 Production of rhamnolipid biosurfactants by AK6U strain

156 Rhamnolipid biosurfactants were produced by the AK6U strain in mineral salts  
157 medium complemented with 10% (v/v) of autoclaved HVGO (Provided by Bahrain

158 Petroleum Company-Bahrain) as a sole carbon source and incubated for 11 days under  
159 shaking (180 rpm) at 30°C (Ismail *et al.* 2017).

#### 160 Production of lipopeptide biosurfactants by NCE3 strain

161 Starting with a streak plate of the NCE3 strain, a single colony was inoculated  
162 into a 100 mL Erlenmeyer flask containing 20 mL LB broth. The flask was incubated in  
163 an orbital shaker for 13 hours at 30°C and 180 rpm. Then, 10 mL from the culture were  
164 transferred into a 1-L Erlenmeyer flask containing 400 mL LB broth and incubated in an  
165 orbital shaker at 30°C for 21 hours. The cells were harvested and washed with phosphate  
166 buffer (0.1M, pH 7). The washed cell pellet was resuspended in 20 mL of phosphate  
167 buffer and the cell suspension was used to inoculate three 2-L Erlenmeyer flasks. Each  
168 flask contained 600 mL of mineral salts medium and 400 mL of autoclaved HVGO (as a  
169 carbon and sulfur source). Each flask was inoculated with 5 mL of the cell suspension,  
170 which contained 0.21 g dry cell weight. All flasks were incubated for 27 days in an  
171 orbital shaker at 180 rpm and 30°C.

#### 172 Production of sophorolipid biosurfactants

173 Sophorolipids were produced using *C. bombicola* ATCC 2221, which was  
174 inoculated in a bioreactor containing glucose yeast extract and urea medium and operated  
175 in fed-batch conditions at 28°C (feeding glucose and rapeseed oil over 7 days). Crude  
176 extract mixture was obtained as the settled product from fed-batch cultivation operated  
177 without the use of antifoam and extracted as described (Smyth *et al.* 2014).



178 Extraction and quantification of the crude biosurfactants

179 At the end of the incubation period, all the contents of the flasks were transferred  
180 into clean separating funnels and allowed to settle for 30 minutes. After the oil and  
181 aqueous phase (growth medium) were resolved, the aqueous phase was drained into clean  
182 centrifuge tubes and subjected to centrifugation (10,000 rpm, 10 min). The supernatants  
183 were pooled in clean glass bottles and stored at 4°C. This is the cell-free and oil-free  
184 culture supernatants from which the crude biosurfactants were extracted. Crude  
185 biosurfactants were extracted from cell-free supernatants of AK6U cultures and crude  
186 lipopeptide biosurfactants were extracted from cell-free supernatant of the NCE3 culture  
187 and quantified as described (Ismail *et al.* 2014; 2015). The oil displacement assay and  
188 surface tension measurement were performed to detect biosurfactants in culture samples  
189 and extracts (Ismail *et al.* 2014; 2015).

190 Treatment of the spent HDS catalyst with biosurfactants

191 The spent HDS catalyst (designated here as the as-received catalyst) was provided  
192 by Kuwait Institute for Scientific Research (KISR)-Petroleum Research Center-Kuwait.  
193 The spent catalyst composition was (wt%): 45.3% support (oxide), 30% carbon, 8.7%  
194 MoO<sub>3</sub> (Mo 5.8%), 5.3% NiO (Ni 4.5%), and 10.7% V<sub>2</sub>O<sub>5</sub> (V 6%). Samples of the as-  
195 received spent HDS catalyst were treated with different concentrations of lipopeptide,  
196 rhamnolipid, and sophorolipid biosurfactants. All the treatments were carried out with 3  
197 grams of the spent catalyst mixed with 25 mL of the treatment solution in 100 mL glass  
198 flasks (Table 1). Treatments were performed with cell-free culture supernatants  
199 containing rhamnolipids (from the AK6U cultures) or lipopeptides (from the NCE3  
200 cultures). The basal buffer, which was used for the dilution of the culture supernatants

201 consists of phosphate buffer, ammonium chloride, and water as described for the  
202 composition of the mineral salts medium (Ismail *et al.* 2017). In case of treatment assays  
203 with sophorolipid biosurfactants, deionized water was used for dilution (Table 2). The  
204 negative (no-biosurfactants) control assays were carried out by incubating the as-received  
205 spent HDS catalyst with deionized water or growth medium basal buffer. At the end of  
206 the treatment period (3 hours at 30°C with shaking at 180 rpm), the whole content of the  
207 assays was centrifuged in clean plastic tubes at 3500 rpm for 10 minutes. The  
208 supernatants were decanted, leaving the treated spent catalyst at the bottom of the tubes.  
209 The catalyst was washed once with 25 mL of deionized water, and the washed catalyst  
210 was subsequently dried in an oven at 95°C for 14 hours.

211

#### 212 Physicochemical analysis of the spent HDS catalyst

213         Following the biosurfactant treatments, the physicochemical properties of the  
214 spent HDS catalyst were analyzed including pore volume, surface area, metal content,  
215 and coke content. ICP spectrometer (Teledyne-Leeman Labs-Prodigy-High Dispersion  
216 ICP) was used to measure the concentration of different metals (Mo, V, Ni) in the spent  
217 catalyst. This method involves atomizing the sample in a high-temperature plasma and  
218 resolving the atomic spectra into the lines of each element by optical grading in an optical  
219 spectrometer. The surface area of the spent HDS catalyst was determined by the  
220 Brunauer-Emmet-Teller (BET) method using Tri-Star surface area analyzer  
221 (Micrometrics Corporation). The nitrogen adsorption-desorption measurements for  
222 specific surface area (SSA) and total pore volume (TPV) were carried out at -196°C  
223 (liquid nitrogen) in the relative pressure (P/P<sub>0</sub>) range of 0.05 to 0.3 with BET method.

224 Carbon loss was measured by loss on ignition (LOI) in presence of air, determination of  
225 volatile matter, and carbon oxidation behavior of the catalyst. Typically, about 100 mg of  
226 sample is heated from ambient to 650°C at the rate of 4°C per minute in air using normal  
227 furnace for decoking.

228 Statistical analysis

229 Results of the spent catalyst treatments are presented as the average of duplicate  
230 treatments  $\pm$  standard deviation. The significance of the differences was tested via one  
231 way analysis of variance (ANOVA) using the Tukey test with  $P$  set to 0.05 with the JMP  
232 statistical software (version 10.0.2, SAS Corporation, Chicago, Illinois, USA).

## 233 **Results**

234 Production of rhamnolipid biosurfactants

235 To produce rhamnolipid biosurfactants, the *P. aeruginosa* AK6U strain was  
236 cultured in mineral salts medium with HVGO as a sole carbon source. Cultures were  
237 monitored visually throughout the incubation period for growth and biosurfactants  
238 production. The cultures' turbidity increased with time, which is a direct indication for  
239 growth. Furthermore, the dispersion and emulsification of the oil increased with time as  
240 compared to uninoculated controls (Fig. S1). These changes in the consistency of the oil  
241 provide a preliminary indication for biosurfactants production. At the end of the  
242 incubation period, the oil and biomass were separated from the culture to obtain cell-free  
243 culture supernatants. The presence of biosurfactants in the cell-free culture supernatants  
244 was confirmed via the oil displacement assay (Fig. S2). This was obvious from the

245 larger clearing zone in the oil displacement assay. Measurement of surface tension  
246 confirmed production of biosurfactants in the cell-free culture supernatants.

247 The surface tension of the HVGO culture was reduced to 30.6 mN/m, while that of  
248 the uninoculated control was 52.8 mN/m. The reduction in surface tension of the growth  
249 medium in growing cultures as compared to the uninoculated medium provided a direct  
250 evidence for biosurfactants production. Extraction of the crude biosurfactants from cell-  
251 free culture supernatants produced crude biosurfactants yield of 10 g/L.

#### 252 Production of lipopeptide biosurfactants

253 To produce lipopeptide biosurfactants, the NCE3 strain was cultured in mineral  
254 salts medium containing 40% HVGO as both carbon and sulfur source. The culture  
255 turbidity increased with time, which indicates growth of the NCE3 strain. There was also  
256 temporal changes in the consistency of the added HVGO in terms of dispersion and  
257 emulsification (Fig. S3). At the end of the incubation period, the cell-free culture  
258 supernatants were collected and tested by the oil displacement assay. As shown in Fig.  
259 S2, the oil layer was completely cleared, which is a strong evidence for the presence of  
260 biosurfactants. The production of biosurfactants in the NCE3 cultures was further  
261 confirmed by the reduction of culture surface tension from 69.71 mN/m to 29.8 mN/m.  
262 The crude biosurfactants were extracted from the cell-free culture supernatants to yield  
263 17.34 g/L.

#### 264 Physicochemical characteristics of the biosurfactants-treated spent HDS catalyst

265 Samples of spent HDS catalyst (as-received) were treated with different types and  
266 concentrations of crude biosurfactants as described in Tables 1 and 2. The biosurfactants

267 used were sophorolipids (produced by *C. bombicola* ATCC 22214), lipopeptides  
268 (produced by the NCF3 strain), and rhamnolipids (produced by the AK6U strain).  
269 Catalyst samples from all treatments and the controls were analyzed for surface area, pore  
270 volume, coke (carbon), and metals (Mo, V, Ni) content.

271 Results of surface area analysis are shown in Fig. 1. As compared to the untreated  
272 catalyst (as-received), all treatments including the negative controls (no biosurfactants)  
273 caused changes in the surface area. Some treatments lead to increase, while others lead to  
274 decrease in the surface area as compared to the untreated catalyst. The surface area of the  
275 spent catalyst from the no-biosurfactant controls was significantly higher than that of the  
276 as-received catalyst ( $P < 0.0005$ ). All biosurfactant treatments exhibited concentration-  
277 dependent profiles or patterns.

278 For the sophorolipid treatments, increasing the biosurfactants concentration  
279 decreased the surface area. Spent catalyst from all sophorolipid treatments had lower  
280 surface area than that of the corresponding control treatment, except the S1 treatment  
281 (lowest sophorolipid concentration). The S1 treatment had the highest surface area among  
282 all biosurfactants treatments. The surface area of the spent catalyst from the S1 treatment  
283 was significantly higher than that of the untreated catalyst ( $P < 0.0001$ ). However, there  
284 was no significant difference in surface area of spent catalyst from the S1 treatment as  
285 compared to the spent catalyst from the corresponding control treatment (ContS) ( $P >$   
286  $0.05$ ). The general trend for the lipopeptide and rhamnolipid treatments was similar to  
287 that of the sophorolipid treatments. In summary, the biosurfactants treatments did not  
288 cause significant increase in surface area of the spent HDS catalyst when compared to the  
289 corresponding control treatments.

290 As it was the case with the surface area, all treatments, including the negative  
291 controls, caused changes in pore volume as compared to the untreated catalyst (Fig. 2).  
292 Some treatments increased, others decreased the pore volume. Both no-biosurfactant  
293 controls caused an increase in the pore volume. All biosurfactants-treated catalyst  
294 samples had lower pore volume than that of the negative control catalyst samples.  
295 However, as compared to the as-received (untreated) catalyst, all biosurfactants-treated  
296 samples had higher pore volume, except the L1 treatment. Differences between the  
297 treatments were statistically insignificant ( $P > 0.05$ ).

298 All biosurfactant treatments caused significantly higher percentage of carbon loss  
299 from the spent HDS catalyst as compared to the untreated catalyst ( $P < 0.01$ ) (Fig. 3). In  
300 addition, the two negative controls increased the carbon loss as compared to the untreated  
301 catalyst. However, all carbon loss values were very similar. There was no significant  
302 difference among the biosurfactant treatments and between the different concentrations of  
303 the same biosurfactant ( $P > 0.05$ ). There were no clear concentration-dependent patterns.  
304 The L1 treatment caused the highest carbon loss value, which was significantly higher  
305 than that caused by the negative controls and all the S (sophorolipid) and R (rhamnolipid)  
306 treatments ( $P < 0.03$ ).

307 All treatments, including the no-biosurfactant controls, caused changes in the Mo  
308 content of the spent catalyst, most of which were statistically insignificant (Fig. 4). The  
309 sophorolipid treatments caused an apparent increase in Mo content as compared to the  
310 untreated catalyst and the corresponding negative control treatments. This increase in Mo  
311 content and the increase caused by some other treatments is statistically insignificant ( $P >$   
312  $0.05$ ). Moreover, there was no significant difference in Mo content among the lipopeptide

313 and the sophorolipid treatments ( $P > 0.05$ ). All lipopeptide and rhamnolipid treatments  
314 had lower Mo content as compared to the corresponding negative controls. However, this  
315 decrease in Mo content was also statistically insignificant ( $P > 0.05$ ). The most striking  
316 result is the reduction in Mo content caused by the rhamnolipid treatment R5. This  
317 treatment significantly decreased the Mo content ( $P < 0.03$ ) of the spent HDS catalyst by  
318 85% and that of the negative control treatment by 90%. To summarize, the biosurfactants  
319 and negative control treatments did not cause significant change in Mo content, with the  
320 exception of the R5 treatment, which drastically reduced the Mo content.

321 Fig. 5 shows the results of Ni content analysis. All treatments, even the negative  
322 control, decreased the Ni content of the spent HDS catalyst as compared to the untreated  
323 catalyst. However, only the water control treatment (ContS) and the rhamnolipid  
324 treatments R3, R4, and R5 caused significant decrease in Ni content ( $P < 0.03$ ). The R5  
325 treatment caused a removal rate of 30% as compared to the corresponding control  
326 treatment. All sophorolipid and lipopeptide treatments had Ni content higher than that of  
327 the corresponding control treatments. However, the differences in Ni content were  
328 insignificant ( $P > 0.05$ ), except for the S3 treatment. In contrast, the rhamnolipid  
329 treatments followed a concentration-dependent pattern, where decreasing the  
330 biosurfactants concentration decreased the Ni content. Apparently, the results for the Ni  
331 content indicate that there is no significant difference between most of the treatments.  
332 The best results in terms of Ni removal/leaching were attributed to the rhamnolipid  
333 treatments R3, R4, and R5, which significantly decreased the Ni content.

334

335 Most of the treatments caused changes in V content compared to the untreated  
336 catalyst (Fig. 6). However, the changes in V content were mostly insignificant except for  
337 the water control (ContS) and some rhamnolipid treatments. The strongest reduction in V  
338 content was brought about by the water-treatment (negative control) ( $P = 0.015$ ). The  
339 rhamnolipid treatment R5 also caused a significant decrease in V content. It caused a V  
340 removal efficiency of 70% as compared to the corresponding negative control treatment.  
341 None of the sophorolipid treatments caused significant change in V content. As compared  
342 to the untreated catalyst and the control treatment, the lipopeptide-treated catalyst  
343 samples appeared to have higher V content. However, this apparent increase in V content  
344 was insignificant ( $P > 0.05$ ). All rhamnolipid-treated catalyst samples had lower V  
345 content than the negative control samples and the untreated catalyst. There was no  
346 significant difference between treatments having various concentrations of the same  
347 biosurfactants.

## 348 **Discussion**

349 Regeneration of spent hydroprocessing catalysts via biological processes has  
350 attracted an increasing interest. Bioprocesses can be applied to remove metals from spent  
351 refinery catalysts. This is achieved via bioleaching or biohydrometallurgy (Asghari *et al.*  
352 2013; Akcil *et al.* 2015). Bioleaching may implement microbial cells or some microbial  
353 products. In this study, we investigated the effect of different types and concentrations of  
354 microbial biosurfactants on metals and coke content of spent HDS catalyst. Surface area  
355 and pore volume of the treated catalyst were also analyzed.



356           The observed changes in the spent HDS catalyst criteria were dependent on the  
357 type and concentration of the biosurfactants. The changes in surface area were  
358 concentration-dependent for the three biosurfactants. The observed decrease in the  
359 surface area with the increase in biosurfactants concentration may be attributed to  
360 blocking of the catalyst pores with high concentration of biosurfactants. The increase in  
361 surface area at low biosurfactants concentrations may be due to removal of metals and  
362 coke, which were deposited on the catalyst during refining. Changes in pore volume  
363 followed a similar trend. However, it is difficult to conclude the effect of biosurfactants  
364 on the pore volume. This is because all the biosurfactant treatments gave pore volumes  
365 values lower than those of the corresponding no-biosurfactant controls. However, some  
366 biosurfactant treatments caused an increase in surface area and pore volume as compared  
367 to the untreated (as-received) catalyst.

368           All biosurfactant treatments had a positive impact on coke or carbon content of  
369 the spent HDS catalyst. The lipopeptide treatment L1 (the highest concentration of  
370 lipopeptides) caused the highest and most significant carbon loss percentage. This is  
371 probably due to the oil displacement activity of the lipopeptide biosurfactants. It appears  
372 that the lipopeptide biosurfactants enhanced or facilitated carbon loss from the spent  
373 catalyst. Many biosurfactants are known for their oil-displacement capabilities, and that  
374 is why they can be used in washing of soil polluted with oil/hydrocarbons, cleaning of oil  
375 storage tanks, and bioremediation oil-impacted environments (Walter *et al.* 2010; De  
376 Almeida *et al.* 2016).

377           The changes in metals (Mo, Ni, and V) content were dependent on the type and  
378 concentration of the biosurfactants. In this context, rhamnolipids were much better than

379 sophorolipids and lipopeptide biosurfactants. However, for reliable comparison of the  
380 bioleaching efficiency of different biosurfactants, it is important to use equal  
381 concentrations in the corresponding treatments.

382 Rhamnolipid treatments significantly decreased metal content of the spent HDS  
383 catalyst when compared to the as-received (untreated) and control (no-biosurfactant)  
384 treatments. Interestingly, the lowest concentration of rhamnolipids (the R5 treatment)  
385 caused the strongest decrease in metals content. Mulligan *et al.* (1999) reported a similar  
386 case in their study of soil and sediment washing using the lipopeptide biosurfactant  
387 surfactin. The authors found that surfactin at a concentration of 0.25% had metal removal  
388 efficiency higher than that performed by a 1% surfactin solution.

389 For Mo, there was no significant change in Mo content in all treatments except  
390 the rhamnolipid treatment R5. This could be due to the fact that Mo is a main constituent  
391 of the catalyst matrix, which makes its removal a difficult task for the bioleaching  
392 treatments. In this context, the apparent decrease in Mo content due to water treatment is  
393 statistically insignificant and falls within experimental error range. Nonetheless, it  
394 appears that the concentration of rhamnolipids used in the treatment R5 was sufficiently  
395 powerful to extract Mo from the spent catalyst matrix to cause significant decrease.

396 Analysis of the Ni content revealed a pattern similar to that observed for Mo.  
397 Most interestingly, among the biosurfactant treatments, those containing rhamnolipids  
398 (R3, R4, and R5) caused significant decrease in Ni content in a concentration-dependent  
399 manner. For V content, also the rhamnolipid treatments caused the highest reduction in V  
400 content as compared to other biosurfactant treatments and the corresponding negative

401 control treatment. However, the water treatment also caused significant decrease in Ni  
402 and V content as compared to the untreated catalyst and most of the biosurfactant  
403 treatments. This suggests that Ni and V were more easily accessible than Mo for removal  
404 just by water. In summary, the rhamnolipid biosurfactants appear to have better potential  
405 than lipopeptides and sophorolipids for metals removal from the spent HDS catalyst  
406 (Mulligan *et al.* 2001).

407         The low metal removal rates observed for most of the treatments could be due to  
408 blocking the pore mouth on the spent HDS catalyst surface by carbon deposition. This  
409 might reduce the accessibility of the entrapped metals to leaching solutions containing  
410 biosurfactants. This also could be the reason for the observed low surface area and pore  
411 volume. We analyzed the metal content using the treated solid catalyst, which could be  
412 the reason for the large error bars observed in some treatments. This could be  
413 circumvented in future studies by measuring the metal content in the bioleaching solution  
414 instead.

415         The ability of water to leach metals from spent refining catalysts has been reported  
416 (Marafi and Stanislaus, 2003). However, this raises the question; why and how water  
417 leached more metals from the spent catalyst as compared to most biosurfactant  
418 treatments? Although the data reported in this study do not allow direct and clear answer  
419 to this question, potential causes could be proposed. First, perhaps the biosurfactants used  
420 in the study were not the best choice for metal leaching from the spent HDS catalyst.  
421 Second, biosurfactants activity depends on several parameters such as pH, temperature,  
422 salinity, the nature of the substrate, presence of co-contaminants, etc (Sriram *et al.* 2011;  
423 Franzetti *et al.* 2015). These factors need to be optimized to harness the best possible

424 activity. These conditions have not been optimized in the current study. That is why the  
425 metal leaching capabilities did not reveal the best, which biosurfactants could do.

426 It is, nonetheless, interesting that the strong metal removal mediated by the  
427 rhamnolipid treatment R5 did not require any pretreatment (de-coking or de-oiling) of the  
428 spent HDS catalyst. Although metal recovery is known to be more efficient with de-  
429 coked catalyst, we performed our bioleaching experiments without de-oiling or de-  
430 coking, while depending on the known oil displacement capabilities of biosurfactants.  
431 This can have beneficial environmental and economic consequences. It further indicates  
432 that there is a room for improvement of the metal leaching capability.

433 Several studies have demonstrated the capability of some microorganisms to  
434 remove metals from spent refinery catalysts via bioleaching. For instance, Amiri *et al.*  
435 (2011) studied bioleaching of tungsten-rich spent hydrocracking catalyst using  
436 *Penicillium simplicissimum*. The authors reported maximum extraction rate at 3% (w/v)  
437 spent catalyst. The recovery efficiency was 100% for W, 92% for Mo, and 66% for Ni.  
438 The bioleaching agents (lixivants) were gluconic acid and red pigments produced by the  
439 fungus. Recently, Shahrabi-Farahani *et al.* (2014) used *Acidithiobacillus thiooxidans* for  
440 bioleaching of metals from a hydrocracking spent catalyst. At optimal conditions, the  
441 maximum extraction efficiency was 87% of Mo, 37% of Ni, and 15% of Al.

442 Various studies have also demonstrated the applicability of biosurfactants to  
443 remove metals from industrial effluents and contaminated sites. However, to our  
444 knowledge, the deployment of biosurfactants for metal removal from or regeneration of  
445 spent refining catalysts has not been reported. Bodagh *et al.* (2013) used rhamnolipids

446 produced by *P. aeruginosa* MA01 to remove Cd, Zn, and Cu from wastewater. Moreover,  
447 El Zeftawy and Mulligan (2011) used rhamnolipid biosurfactants in micellar-enhanced  
448 ultrafiltration application to remove Pb, Cd, Zn, Cu, and Ni from contaminated water.  
449 The lipopeptide biosurfactants surfactin and lichensin were investigated for removal of  
450 Zn and Cr ions from aqueous solutions (Zouboulis *et al.* 2003). Altogether, these studies  
451 clearly show the bioleaching capabilities of biosurfactants. This is in agreement with the  
452 data presented in this study, showing the ability of biosurfactants to remove metals from  
453 the spent HDS catalyst.

454         The simultaneous removal of metals and organic pollutants from co-contaminated  
455 soil was also demonstrated. Singh and Cameotra (2013) showed the ability of the  
456 lipopeptide biosurfactants surfactin and fengycin to remove petroleum hydrocarbons and  
457 metals (Cd, Co, Ni, Zn, and Pb) from co-contaminated soil. This is also in accordance  
458 with the data presented in the current study, showing the simultaneous removal of metals  
459 and coke (carbon) from spent HDS catalyst. Several investigations showed the  
460 dependence of the bioleaching capacity of biosurfactants on many factors, including pH,  
461 soil type, the nature and concentration of contaminants, the biosurfactants concentration,  
462 the congener composition (for rhamnolipids), etc (Franzetti *et al.* 2015). This might  
463 explain the variations and trends of changes in the spent HDS catalyst characteristics  
464 observed in the current study.

465         The data presented here do not indicate how biosurfactants interacted with the spent  
466 HDS catalyst to remove metals. However, there are reports in the literature that discussed  
467 possible mechanisms for metals removal from other polluted matrices. Interaction of  
468 biosurfactants with metals include ion exchange, precipitation-dissolution, counter-ion

469 association, and electrostatic interactions depending on the charge of the applied  
470 biosurfactants (Rufino *et al.* 2012). An ionic biosurfactant form nonionic metal  
471 complexes that are more stable compared to those formed by binding of the metals to soil  
472 particles. This is followed by dissociation of the biosurfactant-metal complexes from the  
473 soil matrix into solution and sequestration of the metals into micelles. Cationic  
474 biosurfactants can replace charged metal ions on the surface of soil particles via  
475 competition for some of the negatively charged surfaces (ion exchange). It is worth  
476 noting that mono-rhamnolipid biosurfactants have a strong affinity for metals such as  
477  $\text{Cd}^{+2}$ ,  $\text{Zn}^{+2}$ , and  $\text{Pb}^{+2}$ , through its carboxyl groups (Juwarkar *et al.* 2007). This can lead to  
478 the removal of metal ions from soil surfaces even in the absence of biosurfactant  
479 micelles.

480         Biosurfactants-mediated rejuvenation of and metal removal from spent refining  
481 catalysts deserves further in-depth investigations. Further studies should focus on the  
482 optimization of bioprocess conditions. Several factors could be studied such as pH,  
483 temperature, contact time between the catalyst and the biosurfactants solution, use of  
484 mixtures of biosurfactants, use of other types of biosurfactants, different congeners'  
485 profiles of rhamnolipids, etc. Moreover, the bioleaching configuration or strategy (direct  
486 vs. indirect, one-stage vs two-stage, treatment in aqueous solutions vs column systems)  
487 could be investigated. It is also important to apply the approach to different kinds of spent  
488 hydroprocessing and hydrotreatment catalysts. Moreover, it remains to test whether the  
489 changes made in the spent catalyst characteristics can lead to at least partial regeneration  
490 of the catalytic activity.

491 This study shows the potential of biosurfactants for metals and coke removal from  
492 spent HDS catalysts commonly used in the petroleum refining industry. The effect of  
493 biosurfactants varied depending on the type and concentration of the applied  
494 biosurfactant. In general, rhamnolipids showed better metal-removing capabilities as  
495 compared to sophorolipids and lipopeptides. The results also showed that biosurfactants  
496 could be applied for the treatment of spent refining catalysts in a crude form or even in  
497 spent culture supernatants without further purification.

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#### 503 **Conflict of Interest**

504 The authors declare that they have no competing interests.

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- 628

629 **Table 1** Treatment of the spent HDS catalyst with rhamnolipid and lipopeptide  
 630 biosurfactants

| Type of Biosurfactant    | Treatment Volume (mL) |              | Concentration of the Biosurfactant (mg/mL) | Treatment Code |
|--------------------------|-----------------------|--------------|--|----------------|
|                          | Culture Supernatant   | Basal Buffer |  |                |
| Lipopeptide<br>(L)       | 25                    | -            | 17.34                                      | L1             |
|                          | 20                    | 5            | 13.9                                       | L2             |
|                          | 10                    | 15           | 7  | L3             |
|                          | 5                     | 20           | 3.5  | L4             |
|                          | 1                     | 24           | 0.7  | L5             |
| Rhamnolipids<br>(R)      | 25                    | -            | 10   | R1             |
|                          | 20                    | 5            | 8  | R2             |
|                          | 10                    | 15           | 4  | R3             |
|                          | 5                     | 20           | 2  | R4             |
|                          | 1                     | 24           | 0.4  | R5             |
| No-Biosurfactant Control | -                     | 25           | -  | Cont           |

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633 **Table 2** Treatment of the spent HDS catalyst with sophorolipid biosurfactants

| Treatment                 | Treatment Volume   |                      | Concentration of the Biosurfactant (v%) | Treatment Code |
|---------------------------|--------------------|----------------------|---|----------------|
|                           | Biosurfactant (μL) | Deionized Water (mL) |   |                |
| Sophorolipids<br>(S)      | 5                  | 25                   | 0.02%                                   | S1             |
|                           | 10                 | 24.99                | 0.04%                                   | S2             |
|                           | 50                 | 24.95                | 0.2%                                    | S3             |
|                           | 100                | 24.9                 | 0.4%                                    | S4             |
|                           | 500                | 24.5                 | 2%                                      | S5             |
| No-Biosurfactant Controls | -                  | 25                   | -                                       | ContS          |

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649 **Figure Legends**

650 **Figure 1** Surface area measurements for spent HDS catalyst samples treated with  
651 different types and concentrations of biosurfactants. As-received, untreated catalyst;  
652 ContS, negative control treatment with water (no biosurfactants); Cont, negative control  
653 treatment with mineral salts medium basal buffer (no biosurfactants); S, treatments with  
654 sophorolipids in water; L, treatments with lipopeptide biosurfactants in cell-free culture  
655 supernatant; R, treatments with rhamnolipids in cell-free culture supernatant. Details of  
656 the treatments are shown in Tables 1 and 2. Error bars represent standard deviation ( $n$   
657 =2).

658 **Figure 2** Pore volume measurements for spent HDS catalyst samples treated with  
659 different types and concentrations of biosurfactants.

660 **Figure 3** Carbon loss measurements for spent HDS catalyst samples treated with  
661 different types and concentrations of biosurfactants.

662 **Figure 4** Molybdenum (Mo) content measurements for spent HDS catalyst samples  
663 treated with different types and concentrations of biosurfactants.

664 **Figure 5** Nickel (Ni) content measurements for spent HDS catalyst samples treated with  
665 different types and concentrations of biosurfactants.

666 **Figure 6** Vanadium (V) content measurements for spent HDS catalyst samples treated  
667 with different types and concentrations of biosurfactants.

668 **Figure S1** Growth of the AK6U strain in mineral salts medium containing 10% (v/v)  
669 HVGO as a sole carbon source. Control: uninoculated medium + HVGO.

670 **Figure S2** Oil displacement assay for detection of biosurfactants in cell-free culture  
671 supernatants from (A) AK6U cultures on HVGO and (B) NCE3 cultures on HVGO. (C)  
672 Negative control (uninoculated growth medium + HVGO).

673 **Figure S3** Growth of the NCE3 strain in mineral salts medium containing 40% (v/v)  
674 HVGO as a sole carbon and sulfur source. Control: uninoculated medium + HVGO.

Figure 1

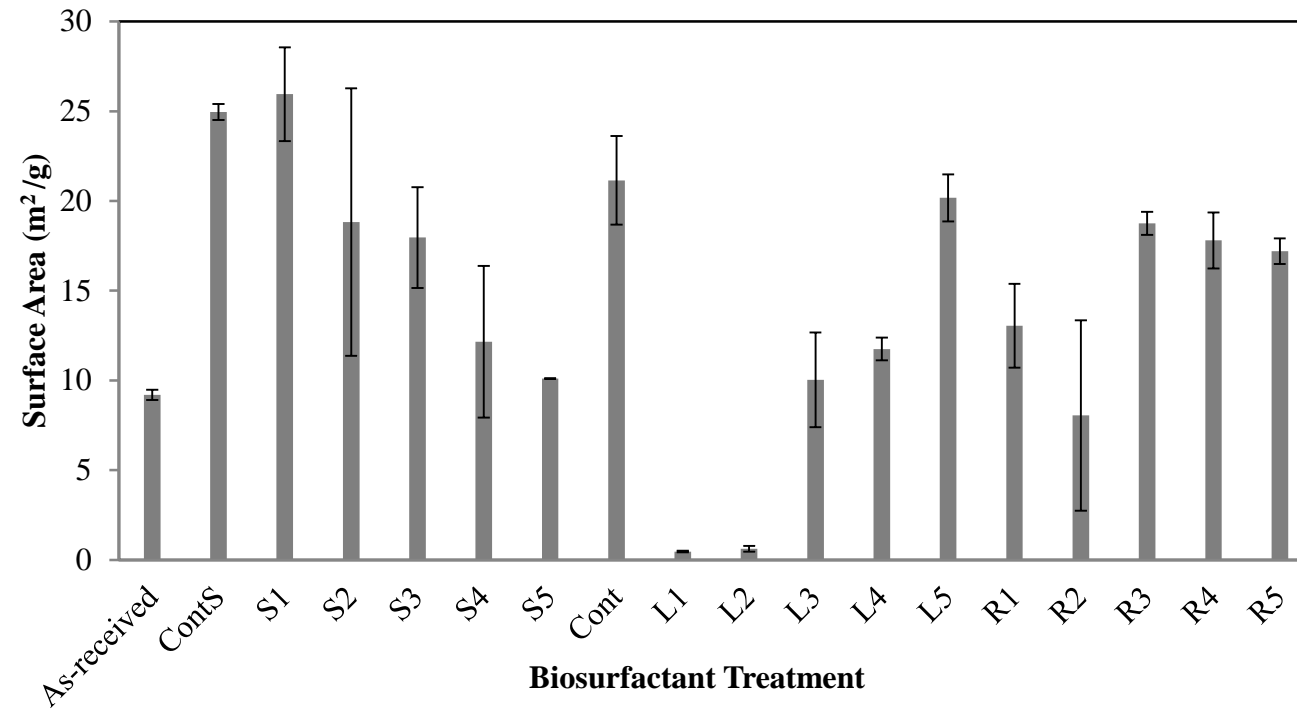




Figure 2

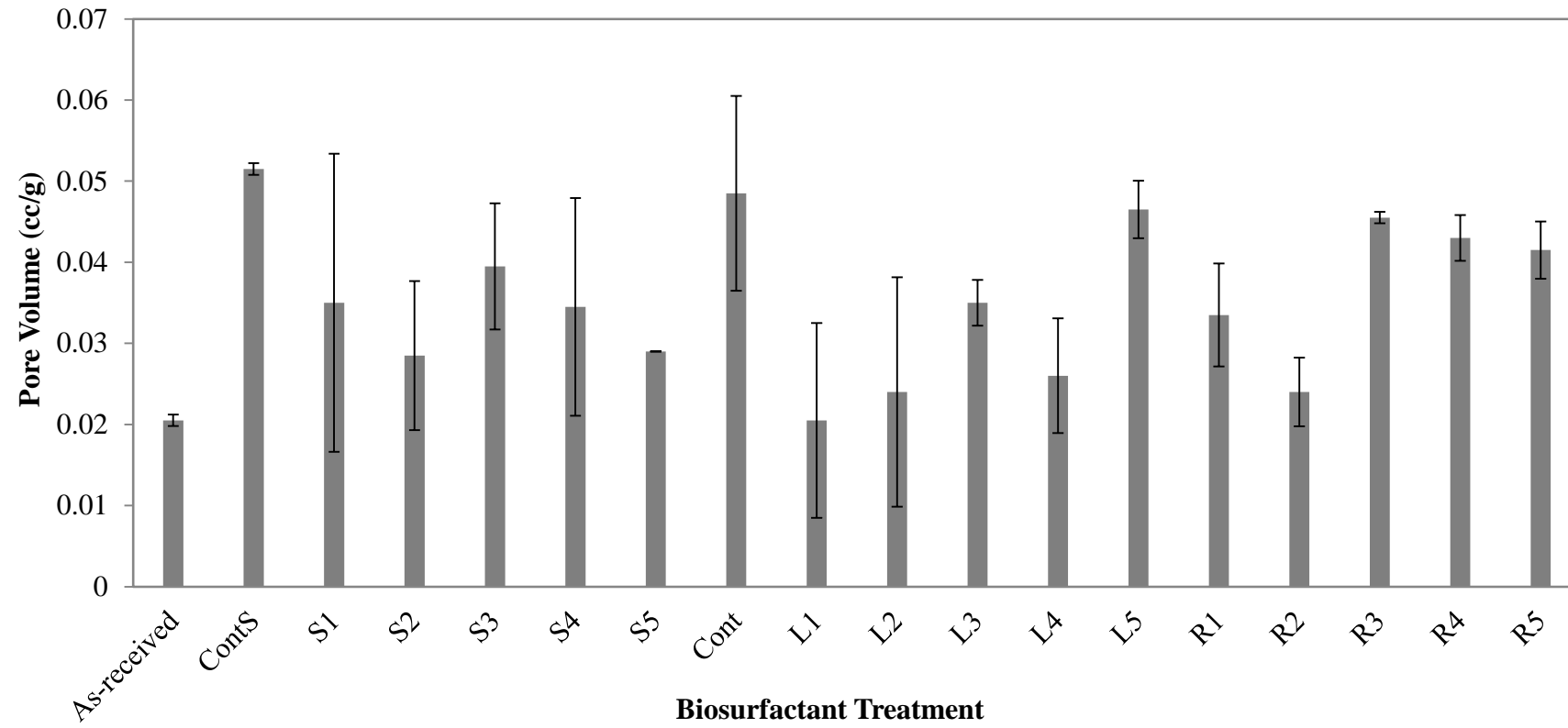


Figure 3

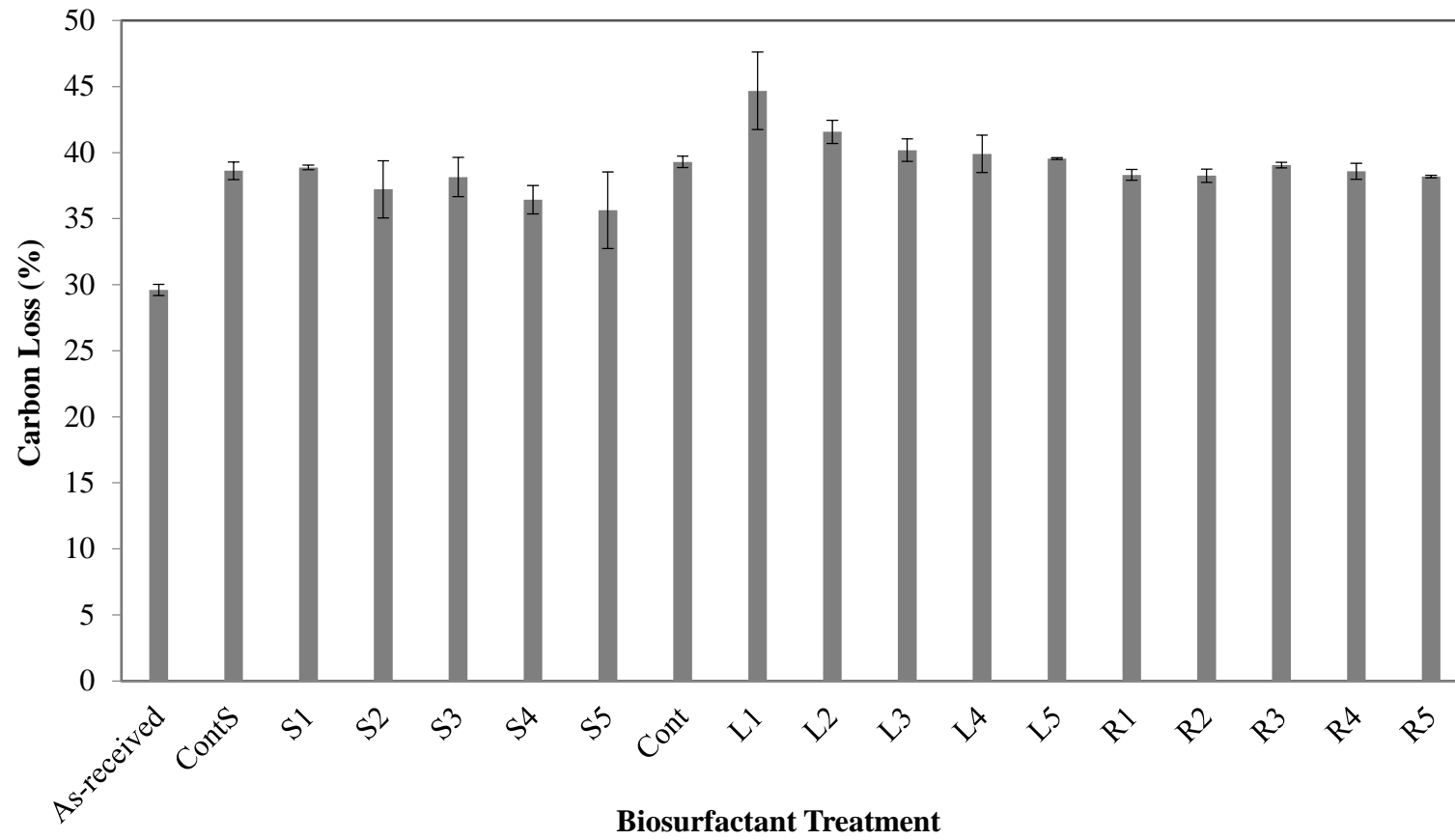


Figure 4

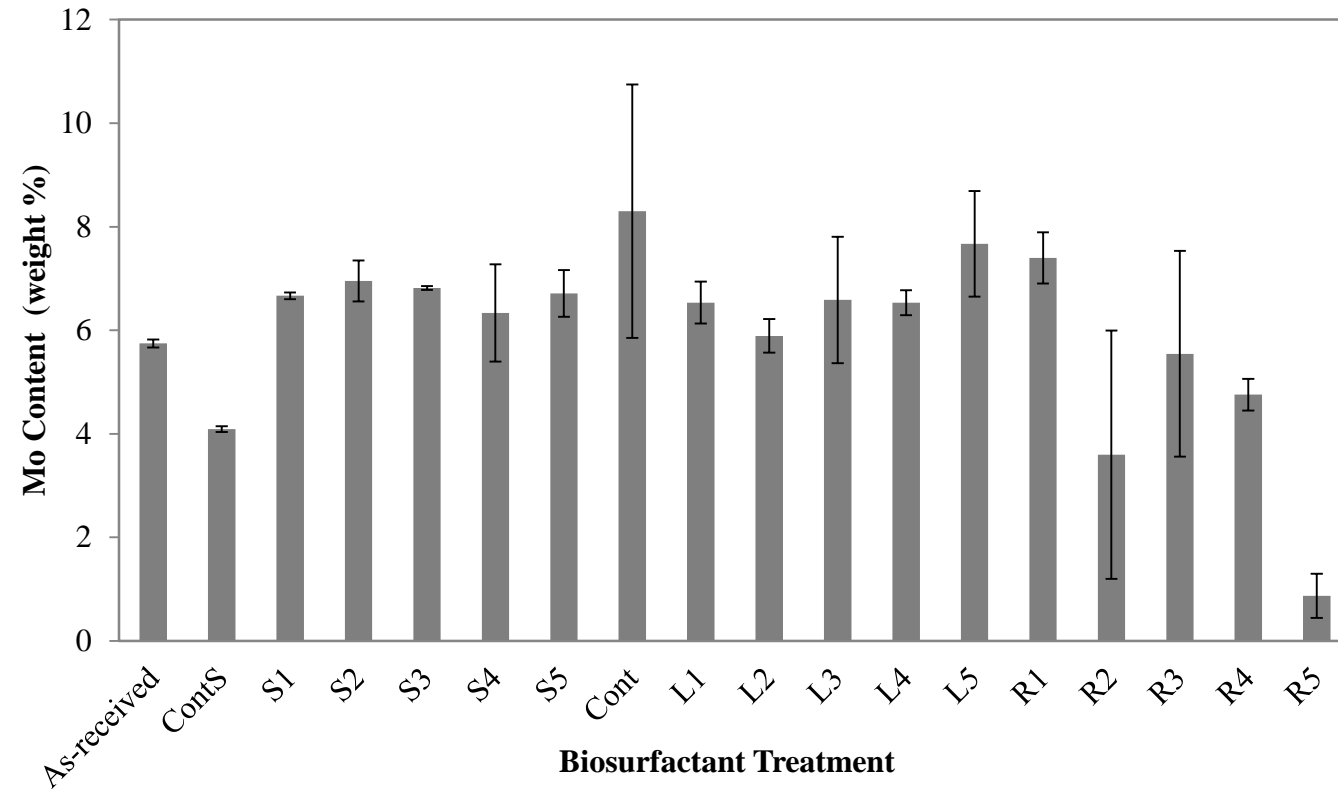


Figure 5

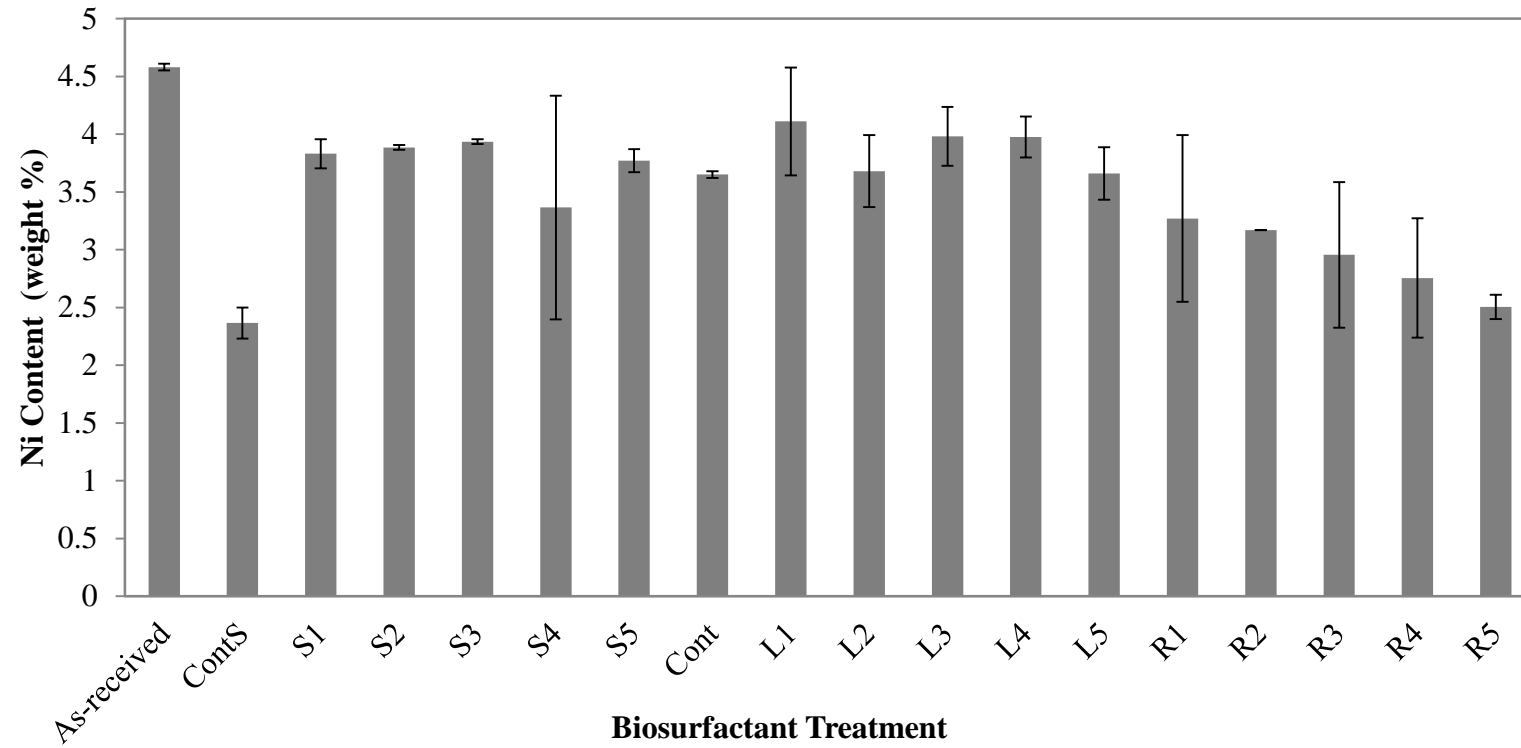
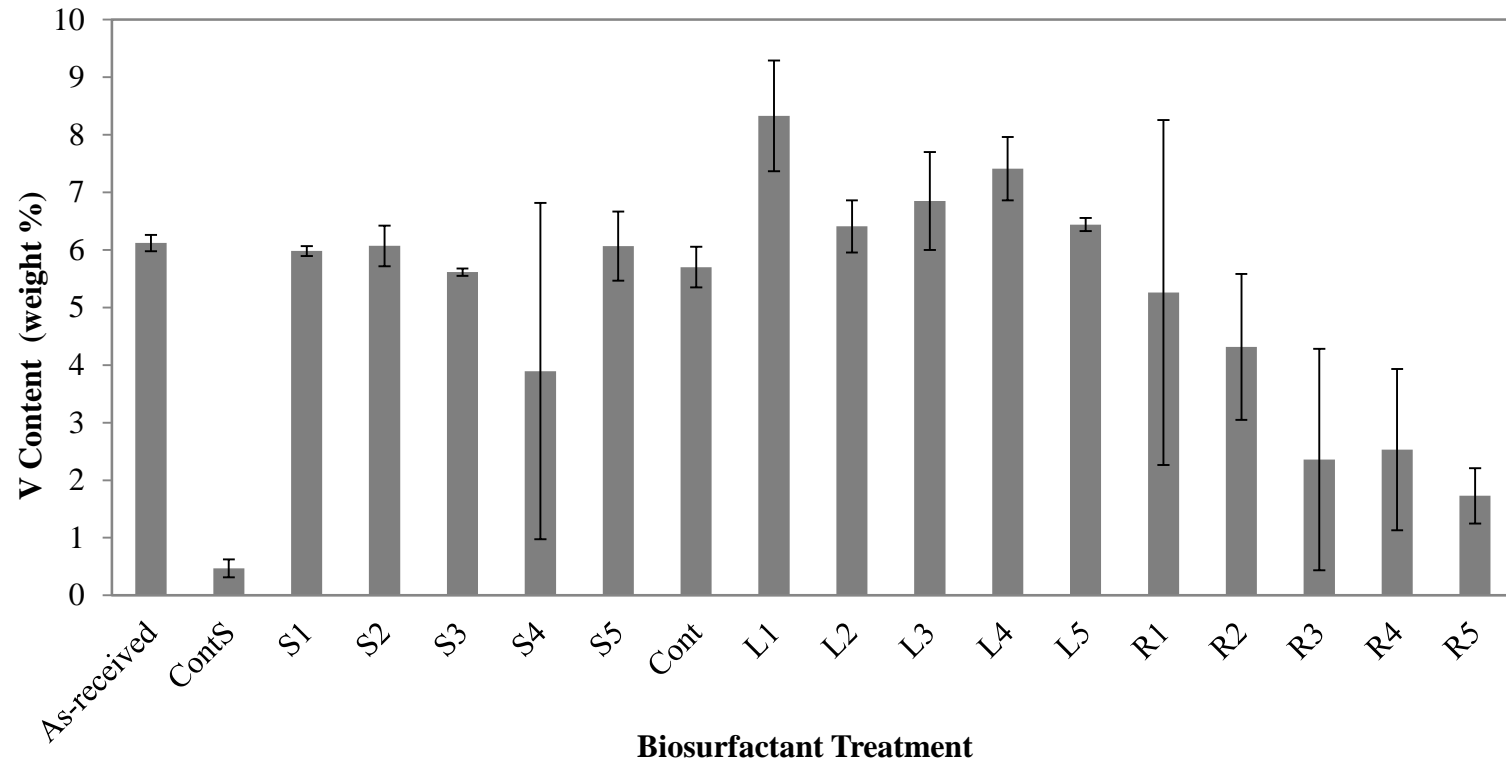


Figure 6



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## Biosurfactants-facilitated leaching of metals from spent hydrodesulfurization catalyst

Shareefah ALsaqer<sup>1</sup>, Meena Marafi<sup>2</sup>, Ibrahim M. Banat<sup>3</sup>, Wael Ismail<sup>1\*</sup>

<sup>1</sup>Environmental Biotechnology Program, Life Sciences Department, College of Graduate Studies, Arabian Gulf University, Manama, Bahrain

<sup>2</sup>Petroleum Refining Department, Petroleum Research and Studies Center, Kuwait Institute for Scientific Research, Safat, Kuwait

<sup>3</sup>School of Biomedical Sciences, University of Ulster, Coleraine, County Londonderry BT52 1SA, Northern Ireland, UK

\*Corresponding author: Environmental Biotechnology Program, Life Sciences Department, College of Graduate Studies, Arabian Gulf University, Manama, Bahrain, Tel: +97336146948; Fax: +97317239664, E-mail: [waelame@agu.edu.bh](mailto:waelame@agu.edu.bh)

Figure S1

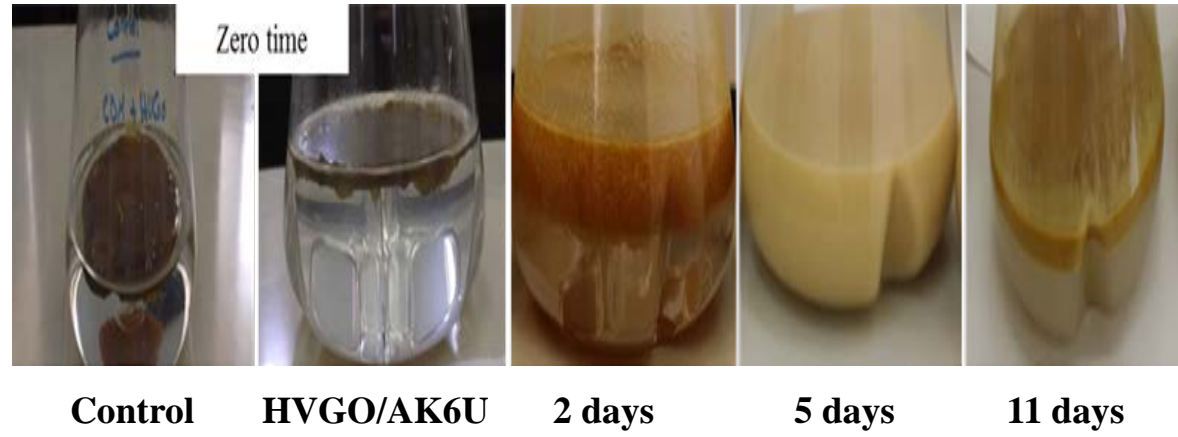


Figure S2



**Negative control**

**A**

**B**



Figure S3

