Biosurfactants-facilitated leaching of metals from spent hydrodesulfurization catalyst

Running title: Bioleaching of spent catalysts

Shareefah ALsaqer[1], Meena Marafi[2], Ibrahim M. Banat[3], Wael Ismail[1]*

[1]Environmental Biotechnology Program, Life Sciences Department, College of Graduate Studies, Arabian Gulf University, Manama, Bahrain

[2]Petroleum Refining Department, Petroleum Research and Studies Center, Kuwait Institute for Scientific Research, Safat, Kuwait

[3]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

Abstract

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

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

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

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

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

Introduction

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

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

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

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

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

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

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

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

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

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

Materials and methods

Bacteria

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

Culture media and growth conditions

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

Production of rhamnolipid biosurfactants by AK6U strain

Rhamnolipid biosurfactants were produced by the AK6U strain in mineral salts 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 30ºC (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 30ºC 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 30ºC 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 flaks. 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 30ºC.

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 28ºC (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_3 (Mo 5.8%), 5.3% NiO (Ni 4.5%), and 10.7% V_2O_5 (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

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

Physicochemical analysis of the spent HDS catalyst

Following the biosurfactant treatments, the physicochemical properties of the spent HDS catalyst were analyzed including pore volume, surface area, metal content, and coke content. ICP spectrometer (Teledyne-Leeman Labs-Prodigy-High Dispersion ICP) was used to measure the concentration of different metals (Mo, V, Ni) in the spent catalyst. This method involves atomizing the sample in a high-temperature plasma and resolving the atomic spectra into the lines of each element by optical grading in an optical spectrometer. The surface area of the spent HDS catalyst was determined by the Brunauer-Emmet-Teller (BET) method using Tri-Star surface area analyzer (Micrometrics Corporation). The nitrogen adsorption-desorption measurements for specific surface area (SSA) and total pore volume (TPV) were carried out at -196ºC (liquid nitrogen) in the relative pressure (P/P0) range of 0.05 to 0.3 with BET method.

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

Statistical analysis

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

Results

Production of rhamnolipid biosurfactants

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

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

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

Production of lipopeptide biosurfactants

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

Physicochemical characteristics of the biosurfactants-treated spent HDS catalyst

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

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

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

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

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

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

Various studies have also demonstrated the applicability of biosurfactants to remove metals from industrial effluents and contaminated sites. However, to our knowledge, the deployment of biosurfactants for metal removal from or regeneration of 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

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

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

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

Acknowledgements

The authors wish to acknowledge the technical support provided by Arabian Gulf University (Bahrain), Petroleum Research Center (KISR-Kuwait), and Ulster University (Northern Ireland-UK). We also thank Hanadi AlSheeha (Petroleum Research Center-KISR) for help with the physicochemical analysis of the spent catalyst.

Conflict of Interest

The authors declare that they have no competing interests.

References

Ahmed, H.S. and Menoufy, M.F. (2012) New trends in hydroprocessing spent catalysts utilization. In Petrochemicals ed. Patel, V. pp. 249-258. IntechOpen, doi: 10.5772/38595. Available from: http://www.intechopen.com/books/petrochemicals/reuse-and-treatment-of-hydrotreating-spent-catalyst.

Akcil, A., Vegliò, F., Ferella, F., Okudan, M.D. and Tuncuk, A. (2015) A review of metal recovery from spent petroleum catalysts and ash. *Waste Manag* **45**,420-433.

513 Amiri, F., Yaghmaei, S. and Mousavi, S.M. (2011) Bioleaching of tungsten-rich spent
514 hydrocracking catalyst using *Penicillium simplicissimum. Bioresour Technol*
515 **102**(2), 1567-1573.

516 Asghari, I., Mousavi, S., Amiri, F. and Tavassoli, S. (2013) Bioleaching of spent refinery
517 catalysts: a review. *J Ind Eng Chem* **19**(4), 1069-1081.

518 Banat, I.M., Satpute, S.K., Cameotra, S.S., Patil, R. and Nyayanit, N.V. (2014) Cost
519 effective technologies and renewable substrates for biosurfactants' production.
520 *Front Microbiol* **5**, 697. doi: 10.3389/fmicb.2014.00697.

521 Bodagh, A., Khoshdast, H., Sharafi, H., Zahiri, H.S. and Noghabi, K.A. (2013) Removal
522 of cadmium (II) from aqueous solution by ion flotation using rhamnolipid
523 biosurfactant as an ion collector. *Ind Eng Chem Res* **52**(10), 3910-3917.

524 De Almeida, D.G., Soares Da Silva, R.C.F., Luna, J.M., Rufino, R.D., Santos, V.A.,
525 Banat, I.M. and Sarubbo, L.A. (2016) Biosurfactants: promising molecules for
526 petroleum biotechnology advances. *Front Microbiol* **7**, 1718. doi:
527 10.3389/fmicb.2016.01718.

528 Dufresne, P. (2007) Hydroprocessing catalysts regeneration and recycling. *Appl Catal A-*
529 *Gen* **322**, 67-75.

530 El Zeftawy, M.M. and Mulligan, C.N. (2011) Use of rhamnolipid to remove heavy metals
531 from wastewater by micellar-enhanced ultrafiltration (MEUF). *Sep Purif Technol*
532 **77**(1), 120-127.

533 Franzetti, A., Gandolfi, I., Fracchia, L., Van Hamme, J., Gkorezis, P., Marchant, R. and
534 Banat, I.M. (2015) Biosurfactant use in heavy metal removal from industrial
535 effluents and contaminated sites. In Biosurfactants: Production and Utilization-
536 Processes, Technologies, and Economics, Surfactant Science Series 159 ed.
537 Kosaric, N. and Sukan, F.V. pp. 361-366. New York: CRC Press.

538 Ismail, W., Alhamad, N.A., El-Sayed, W.S., El Nayal, A.M., Chiang, Y-R. and Hamzah,
539 R.Y. (2013) Bacterial degradation of the saturate fraction of Arabian light crude

oil: biosurfactant production and the effect of ZnO nanoparticles. *J Pet Environ Biotechnol* **4**, 6. http://dx.doi.org/10.4172/2157-7463.1000163.

Ismail, W., El Nayal, A.M., Ramadan, A.R. and Abotalib, N. (2014) Sulfur source-mediated transcriptional regulation of the *rhlABC* genes involved in biosurfactants production by *Pseudomonas* sp. strain AK6U. *Front Microbiol* **5**, 432. doi: 10.3389/fmicb.2014.00423.

Ismail, W., Shammary, S.A., El-Sayed, W.S., Obuekwe, C., El Nayal, A.M., Abdul Raheem, A.S. and Al-Humam, A. (2015) Stimulation of rhamnolipid biosurfactants production in *Pseudomonas aeruginosa* AK6U by organosulfur compounds provided as sulfur sources. *Biotechnol Rep* **7**, 55-63.

Ismail, W., Mohamed, M.E., Awadh, M.N., Obuekwe, C. and El Nayal, A.M. (2017) Simultaneous valorization and biocatalytic upgrading of heavy vacuum gas oil by the biosurfactant-producing *Pseudomonas aeruginosa* AK6U. *Microb Biotechnol* **10**(6), 1628-1639.

Juwarkar, A.A., Nair, A., Dubey, K.V., Singh, S.K. and Devotta, S. (2007) Biosurfactant technology for remediation of cadmium and lead contaminated soils. *Chemosphere* **68**(10), 1996-2002.

Liu, C., Yu, Y. and Zhao, H. (2005) Hydrodenitrogenation of quinoline over Ni–Mo/Al_2O_3 catalyst modified with fluorine and phosphorus. *Fuel Process Technol* **86**(4), 449-460.

Macaskie, L.E., Mikheenko, I.P., Yong, P., Deplanche, K., Murray, A.J., Paterson-Beedle, M., Coker, V.S. *et al.* (2010) Today's wastes, tomorrow's materials for environmental protection. *Hydrometallurgy* **104**(3-4), 483-487.

Marafi, M. and Stanislaus, A. (2003) Options and processes for spent catalyst handling and utilization. *J Hazard Mater* **101**, 123-132.

565 Marafi, A., Fukase, S., Al-Marri, M. and Stanislaus, A. (2003) A comparative study of
566 the effect of catalyst type on hydrotreating kinetics of Kuwaiti atmospheric
567 residue. *Energy Fuels* **17**(3), 661-668.

568 Marafi, M. and Stanislaus, A. (2008a) Spent catalyst waste management: A review: Part
569 I- Developments in hydroprocessing catalyst waste reduction and use. *Resour*
570 *Conserv Recy* **52**(6), 859-873.

571 Marafi, M. and Stanislaus A, (2008b) Spent hydroprocessing catalyst management: A
572 review: Part II- Advances in metal recovery and safe disposal methods. *Resour*
573 *Conserv Recy* **53**(1-2), 1-26.

574 Marafi, M. and Stanislaus, A. (2011) Alumina from reprocessing of spent
575 hydroprocessing catalyst. *Catal Today* **178**(1), 117-123.

576 Mishra, D., Kim, D., Ralph, D.. Ahn, J. and Rhee, Y. (2007) Bioleaching of vanadium
577 rich spent refinery catalysts using sulfur oxidizing lithotrophs. *Hydrometallurgy*
578 **88**(1), 202-209.

579 Motaghed, M., Mousavi, S., Rastegar, S. and Shojaosadati, S. (2014) Platinum and
580 rhenium extraction from a spent refinery catalyst using *Bacillus megaterium* as a
581 cyanogenic bacterium: Statistical modeling and process optimization. *Bioresour*
582 *Technol* **171**, 401-409.

583 Mulligan, C.N., Yong, R.N., Gibbs, B.F., James, S. and Bennette, H.P.J. (1999) Metal
584 removal from contaminated soil and sediments by the biosurfactant surfactin.
585 *Environ Sci Technol* **33**(21), 3812-3820.

586 Mulligan, C.N., Yong, R.N. and Gibbs, B.F. (2001) Heavy metal removal from sediments
587 by biosurfactants. *J Hazard Mater* **85**(1-2), 111-125.

588 Pradhan, J.K. and Kumar, S. (2012) Metals bioleaching from electronic waste by
589 *Chromobacterium violaceum* and *Pseudomonads* sp. *Waste Manag Res* **30**(11),
590 1151-1159.

591 Rufino, R.D., Luna, J.M., Campos-Takaki, G.M., Ferreira, S.R. and Sarubbo, L.A. (2012)
592 Application of the biosurfactant produced by *Candida lipolytica* in the
593 remediation of heavy metals. *Chem Eng Trans* **27**, 61-66.

594 Santhiya, D. and Ting, Y-P. (2005) Bioleaching of spent refinery processing catalyst
595 using *Aspergillus niger* with high-yield oxalic acid. *J Biotechnol* **116**(2),171-184.

596 Sarubbo, L.A., Rocha, R.B., Luna, J.M., Rufino, R.D., Santos, V.A. and Banat, I.M.
597 (2015) Some aspects of heavy metals contamination remediation and role of
598 biosurfactants. *Chem Ecol* **31**(8),707-723.

599 Shahrabi-Farahani, M., Yaghmaei, S., Mousavi, S. and Amiri, F. (2014) Bioleaching of
600 heavy metals from a petroleum spent catalyst using *Acidithiobacillus thiooxidans*
601 in a slurry bubble column bioreactor. *Sep Purif Technol* **132**, 41-49.

602 Singh, A.K. and Cameotra, S.S. (2013) Efficiency of lipopeptide biosurfactants in
603 removal of petroleum hydrocarbons and heavy metals from contaminated soil.
604 *Environ Sci Pollut Res Int* **20**(10), 7367-7376.

605 Slizovskiy, I.B., Kelsey, J.W. and Hatzinger, P.B. (2011) Surfactant-facilitated
606 remediation of metal-contaminated soils: efficacy and toxicological consequences
607 to earthworms. *Environ Toxicol Chem* **30**(1), 112-123.

608 Smyth, T.J.P., Rudden, M., Tsaousi, K., Marchant, R. and Banat, I.M. (2014) Protocols
609 for the detection and chemical characterization of microbial glycolipids. In
610 Hydrocarbon and Lipid Microbiology Protocols ed. McGenity, T., Timmis, K.N.
611 and Nogales, B. Berlin: Springer, doi 10.1007/8623_2014_25.

612 Srichandan, H., Singh, S., Blight, K., Pathak, A., Kim, D.J., Lee, S. and Lee, S.W. (2012)
613 An integrated sequential biological leaching process for enhanced recovery of
614 metals from decoked spent petroleum refinery catalyst: A comparative study. *Int J*
615 *Miner Process* **134**, 66-73.

616 Sriram, M.I., Gayathiri, S., Gnanaselvi, U., Jenifer, P.S., Raj, S.M. and Gurunathan, S.
617 (2011) Novel lipopeptide biosurfactant produced by hydrocarbon degrading and

heavy metal tolerant bacterium *Escherichia fergusonii* KLU01 as a potential tool for bioremediation. *Bioresour Technol* **102**(19), 9291-9295.

Vijayakumar, S. and Saravanan, V. (2015) Biosurfactants-types, sources and applications. *Res J Microbiol* **10**(5), 181-192.

Walter, V., Syldatk, C. and Hausmann, R. (2010) Screening concepts for the isolation of biosurfactant producing microorganisms. In Biosurfactants, Advances in Experimental Medicine and Biology ed. Sen, R. pp. 1-13. New York: Springer.

Zouboulis, A., Matis, K., Lazaridis, N. and Golyshin, P. (2003) The use of biosurfactants in flotation: application for the removal of metal ions. *Miner Eng* **16**(11), 1231-1236.

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

Type of Biosurfactant	Treatment Volume (mL)		Concentration of the Biosurfactant (mg/mL)	Treatment Code
	Culture Supernatant	Basal Buffer		
Lipopeptide (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 (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

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

Treatment	Treatment Volume		Concentration of the Biosurfactant	Treatment Code
	Biosurfactant (µL)	Deionized Water (mL)	(v%)	
Sophorolipids (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

634

635

636

637

638

639

640

641

642

643

644

645

646

647

648

Figure Legends

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

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

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

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

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

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

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

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

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