Bioprocess Biosyst Eng (2015) 38:1447–1453
DOI 10.1007/s00449-015-1386-7

ORIGINAL PAPER

Desulfurization and denitrogenation of heavy gas oil by *Rhodococcus erythropolis* ATCC 4277

D. Maass[1] · D. Todescato[1] · D. E. Moritz[1] · J. Vladimir Oliveira[1] · D. Oliveira[1] · A. A. Ulson de Souza[1] · S. M. A. Guelli Souza[1]

Received: 6 January 2015 / Accepted: 2 March 2015 / Published online: 11 March 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract Some of the noxious atmospheric pollutants such as nitrogen and sulfur dioxides come from the fossil fuel combustion. Biodesulfurization and biodenitrogenation are processes which remove those pollutants through the action of microorganisms. The ability of sulfur and nitrogen removal by the strain *Rhodococcus erythropolis* ATCC 4277 was tested in a biphasic system containing different heavy gas oil concentrations in a batch reactor. Heavy gas oil is an important fraction of petroleum, because after passing through, the vacuum distillation is incorporated into diesel oil. This strain was able to remove about 40 % of the nitrogen and sulfur present in the gas heavy oil. Additionally, no growth inhibition occurred even when in the presence of pure heavy gas oil. Results present in this work are considered relevant for the development of biocatalytic processes for nitrogen and sulfur removal toward building feasible industrial applications.

Keywords Denitrogenation · Desulfurization · Heavy gas oil · *Rhodococcus erythropolis*

Introduction

The combustion of fossil fuels generates polluting compounds such as sulfur and nitrogen dioxides (SO_2, NO_2). These compounds are harmful to human health and to the environment. Therefore, environmental regulations around the world have been demanding a reduction in the sulfur and nitrogen contents, especially for sulfur, requiring concentrations lower than 15 ppm [1]. The attainment of fuels with ultra low polluting content is becoming a problem for the petrochemical industry, since the existing catalytic hydrogenation processes cannot achieve the required removal levels because it is not capable of removing completely the heterocyclic sulfur and nitrogen compounds [1–6]. Another issue is that the main part of the crude oil extracted worldwide is progressively exhibiting higher levels of sulfur content and heavier density values [2].

An alternative technology, called biodesulfurization (BDS), has been developed in the latest decades to replace and/or complement the conventional process. In BDS technique, degradation of sulfur occurs through the action of microorganisms that act as catalysts removing selectively the sulfur from heterocyclic compounds under mild temperatures and pressures [2, 7, 8].

The industrial application of BDS depends on several factors; one of them is the selection of microbial strains with the ability to remove sulfur from fossil fuels without decreasing the energetic capacity, while industrially and environmentally stable. Numerous microorganisms belonging to different genera have demonstrated the ability to remove the sulfur present in heterocyclic compounds such as *Brevibacterium* sp. [9], *Corynebacterium* sp. [10], *Rhodococcus* sp. [11], *Paenibacillus* sp. [12], *Pseudomonas* sp. [13], *Gordonia* sp. [14], and *Bacillus* sp. [15]. However, the products obtained at the end of the BDS process are not always the same and almost none being economically advantageous [16, 17].

The strain *Rhodococcus* sp. has been widely applied to BDS process because it is able to remove sulfur from heterocyclic compounds, turning it into sulfate, without

✉ S. M. A. Guelli Souza
selene.souza@ufsc.br

A. A. Ulson de Souza
augusto.souza@ufsc.br

[1] EQA/UFSC, Department of Chemical and Food Engineering, Federal University of Santa Catarina, C.P. 476, Florianópolis, SC CEP 88040-900, Brazil

breaking the aromatic rings, thus, keeping fuels energetic capacity [7, 18, 19]. Another great advantage of this bacterium is its hydrophobic characteristics which increases the contact between the cell and the sulfurous compounds in the fuels [20]. When compared with other strains, *Rhodococcus* sp. presents a greater specific degradation rate and stability during BDS process [11, 16, 21–27].

Similarly, the biodenitrogenation (BDN) is a process where nitrogen is removed from the heterocyclic compounds through the action of microorganisms. However, the development of this process did not advance because the microorganisms capable of nitrogen removal use a degradation pathway where only liberate nitrogen in the course of completely degrading the substrates [8].

For practical purposes, development of BDS and BDN requires the use of real products and byproducts from petroleum distillation such as diesel, gasoline, and gas oils [11, 22–24, 27–30]. The heavy gas oil (HGO) is an intermediate fraction obtained from the vacuum distillation used in the production of diesel and some lubricants. The desulfurization and denitrogenation of this fraction could result in the reduction of subsequent treatment processes, increasing the industrial and economic viability of the BDS and BDN [31]. Based on these aspects, the main objective of this work was to evaluate the desulfurization and denitrogenation ability of *Rhodococcus erythropolis* ATCC 4277 in a batch reactor with different HGO concentrations.

Materials and methods

Materials

The heavy gas oil used was kindly denoted by Petrobras S.A., from the Marlim Sul P-56 platform, Rio de Janeiro, Brazil.

Bacterial strain and maintenance medium

Rhodococcus erythropolis ATCC 4277 used in this study was acquired from Tropical Foundation of Research and Technology André Tosello, Campinas, São Paulo State, Brazil. Following the recommendation of the supplier, it was maintained on Petri dishes containing Yeast Malt Agar medium (YMA) comprised 3.0 $g \cdot L^{-1}$ of yeast extract, 3.0 $g \cdot L^{-1}$of malt extract, 5.0 $g \cdot L^{-1}$of bactopeptone, 10.0 $g \cdot L^{-1}$of glucose, 2.0 $g \cdot L^{-1}$ of calcium carbonate, and 20.0 $g \cdot L^{-1}$of agar, and stored at 4 °C for further use [31].

Minimum inhibitory concentration (MIC)

Diffusion method

In Petri plates containing YMA medium, previously inoculated with a microbial suspension of 4.0 (±0.5) $g \cdot L^{-1}$, wells of 1.0-cm diameter along the medium were made. The wells were filled with HGO and other compounds to be tested (diesel, crude oil, and atmospheric residue), and then the plates were left in the incubator for 48 h at 28 °C. The MIC was considered that concentration and/or pure compound are capable of developing an inhibition halo greater or equal to 1.4-cm diameter on the microbial growth [32, 33].

Broth dilution MIC—macrodilution

The broth macrodilution method considers the relationship between the growth of the microorganism in the liquid medium and the concentration of the tested substance. The evaluation is performed by comparing microbial growth for each concentration tested against a biological standard. The assays were conducted in flasks containing different concentrations of YMA medium and HGO, as shown in Table 1. Afterward, it was added 5 mL of a *Rhodococcus erythropolis* preculture with an absorbance of 0.75 at 600 nm (OD_{600}). The flasks remained in an orbital shaker for 24 h at 28 °C and 200 rpm.

The MIC was calculated using Eq. (1), where the final cell concentration in each flask was measured throw turbidimetry at 600 nm (OD_{600}) and compared to a standard cell concentration without addition of HGO [33].

$$\%\ \text{cells growth} = \frac{[X]_{\text{final}} - [X]_{\text{standard}}}{[X]_{\text{final}}} \times 100 \quad (1)$$

Culture conditions

The *R. erythropolis* cells were grown in 0.125-L flasks containing 0.05 L of YM medium for 18 h on an orbital shaker at 150 rpm and 28 °C. Then, 5 % (v/v) of this preculture, at an absorbance of 0.85 at 600 nm (OD_{600}),

Table 1 Test conditions for MIC determination by macrodilution method

HGO ratio % (v/v)	YMA (L)	HGO (L)
0 (standard)	1.0	0
10	0.9	0.1
20	0.8	0.2
30	0.7	0.3
40	0.6	0.4
50	0.5	0.5
60	0.4	0.6
70	0.3	0.7
80	0.2	0.8
90	0.1	0.9
100	0	1.0

was transferred to 0.5-L flasks containing 0.1 L of YM medium. Those flasks remained on an orbital shaker during 18 h at 150 rpm and 28 °C. The cells were recovered by centrifugation at 5000×*g* for 20 min at 4 °C, discarding the supernatant and washed with 0.1 M phosphate buffer (pH 7.0) [34].

Biodesulfurization and biodenitrogenation

The BDS and BDN assays were carried out in 0.5-L flasks containing 1.0 g·L^{-1} of cells with a total volume of reaction medium of 0.2 L. The reaction medium consisted of two phases: an aqueous (YM medium) and other organic (HGO) at different concentrations, as described in Table 2. The biocatalytic system remained on an orbital shaker for 18 h at 28 °C and 200 rpm [11]. All experiments were performed in triplicate so that the values presented in tables and figures are in fact average of three replicates.

Sulfur and nitrogen contents determination

After submitted to BDS and BDN processes, the content of the flasks was centrifuged at 5000×*g* at 4 °C for 20 min and then the sulfur and nitrogen contents in the HGO were determined by an elementary analysis CHNS. A flash elemental analyzer (C.E. Elantech, Inc., model 1112) is used for bulk CHNS weight percent determination of the HGO with triplicate samples employed.

Optical microscopy

At the end of BDN and BDS processes, samples were collected from the reaction media containing 40 and 100 % (v/v) of HGO and analyzed by microscopy (BIOVAL) by increasing 400 times the extent of the objective lens (Olympus, model EA40) and ocular (Olympus, model PL 100/1.25).

Table 2 Test conditions for desulfurization and denitrogenation of HGO by *Rhodococcus erythropolis* ATCC 4277 whole cells

HGO ratio % (v/v)	YMA (L)	HGO (L)
0 (standard)	0.2	0
10	0.18	0.02
20	0.16	0.04
30	0.14	0.06
40	0.12	0.08
50	0.10	0.10
60	0.08	0.12
70	0.06	0.14
80	0.04	0.16
90	0.02	0.18
100	0	0.20

Results and discussion

Minimum inhibitory concentration (MIC) of HGO

The MIC assays by diffusion method served to determine qualitatively the ability that *R. erythropolis* ATCC 4277 has to grow in the presence of HGO and other petroleum fractions. When tested by the diffusion method, inhibition halo was not observed for any of the compounds, as noted in Fig. 1.

The adaptability of *R. erythropolis* to high concentrations or pure HGO is due to the fact that these bacteria modulate the viscosity of cell membrane lipids to maintain or increase the fluidity of the same by reducing the degree of saturation, increasing the ratio cis/trans of fatty acids and by increasing the relative amount of branched fatty acids [35, 36].

Tests of MIC by macrodilution method aimed to determine quantitatively the concentration of HGO that inhibits the growth of *R. erythropolis*. Results shown in Fig. 2 confirm the data obtained by the diffusion method, hence demonstrating that the growth of *R. erythropolis* suffers no inhibition in the presence of HGO.

Rather, the presence of HGO in the culture medium promotes a higher cell growth, especially for HGO concentrations from 10 to 50 % (v/v) (Fig. 2). Other authors

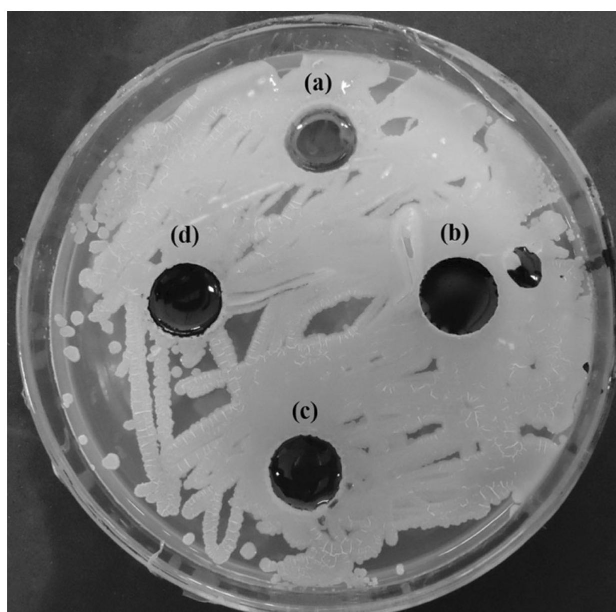

Fig. 1 Determination of MIC through diffusion method for **a** diesel; **b** atmospheric residue; **c** petroleum; **d** heavy gas oil

also had a good cell adaptation when using approximately 20 % (v/v) of organic phase [12, 15, 24, 25]. The presence of hydrocarbons in abundance, as well as nitrogen and sulfur compounds, contributes to a better nutrition of the microorganism acting as a substrate and a co-substrate. The slight decrease in cell concentration for higher concentrations of HGO is due to the resistance of the microorganism decreases with the increase of the toxicity of the system, thus, making it impossible to maintain the cell viability under more severe conditions [4, 37, 38].

Biodesulfurization

Heavy gas oil samples, after subjected to desulfurization process, were analyzed by CHNS, with results of this elementary analysis reported in Table 3.

The best results were achieved for HGO ratios of 40, 20, and 60 % (v/v) where the mass of sulfur removed per gram of $(266 \pm 9) \times 10$, $(246 \pm 3) \times 10$, and $(221 \pm 2) \times 10$ μg, desulfurization rate of 148, 137, and 123 mg sulfur kg^{-1} h^{-1}, and removal percentages of 42.7, 39.4, and 35.5 % (Fig. 3), respectively.

Since the HGO contains various easily desulfurizable compounds, such as thiols and sulfides, besides recalcitrant compounds as DBT [14], the mass of sulfur removed and the desulfurization rate obtained is superior to those reported by other authors for the same ratio of organic phase in the BDS process [11, 21–24, 27, 32]. In those researches, synthetic fuel was used containing only DBT or oils with low sulfur content decreasing this way the offer of sulfur. Others authors who worked with light gas oil and heavy gas oil also observed an increase in the desulfurization activity when compared with model oils [2, 14, 29].

Although *R. erythropolis* has a hydrophobic characteristic that increases the contact between cells and the sulfurous compounds, the high viscosity of HGO may have difficulted the access to this compounds leading this way to a minor removal percentages [15, 21, 39]. A solution to this problem has been developed employing surfactants to increase the contact between those two phases [2].

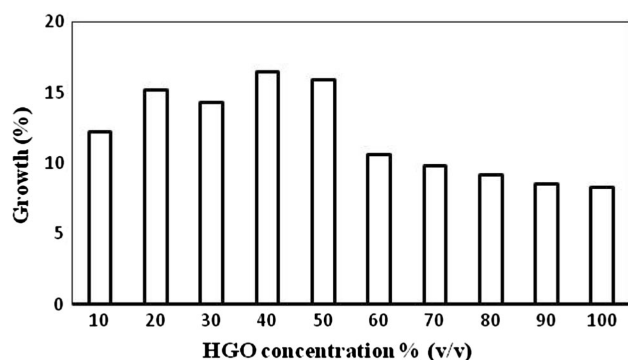

Fig. 2 Determination of MIC through macrodilution method for different HGO concentrations

Biodenitrogenation

Table 4 shows the results of CHNS analysis performed for HGO samples after being subjected to BDN process.

Similarly to what happened to the sulfur compounds, the best results for nitrogen removal were achieved in the biphasic systems containing 40, 20, and 60 % (v/v) HGO, where the masses of reduced nitrogen were $(162 \pm 1) \times 10$, $(151 \pm 2) \times 10$, and $(117 \pm 1) \times 10$ μg and the percentages of reduction 43.2, 40.2, and 31.2 % (Fig. 3), respectively.

These results are similar to those achieved by other nitrogen removal techniques such as oxidative denitrogenation [31]. However, the removal occurred simply by free cells of *R. erythropolis* without the need to add other chemical reagents or catalysts.

In order to understand what led the cells of *R. erythropolis* to remove more sulfur and nitrogen in the experiments containing 40 % (v/v) of HGO, and why they were not capable of removing any quantity of sulfur and nitrogen in the tests with 100 % (v/v) of HGO, both reactions media were analyzed by optical microscopy (Fig. 4).

From Fig. 4a, it can be noted the presence of a considerable amount of refringent cells, which is an indication that the cells of *R. erythropolis* ATCC 4277 were able to adapt well to the reaction medium containing 40 % (v/v). Moreover, cells are dispersed in the medium, which is also an indication that the medium provides favorable conditions for the microorganism. A good adaptation of the cells to this particular reaction medium is also observed in the result of MCI test by macrodilution method (Fig. 2) [35, 37–40].

A nutritional deprivation growth condition increases cell surface hydrophobicity, which acts as an ignition power for a cell–cell junction or biogranulation. Adhesion occurs when the electrostatic repulsion is overcome by van der Waals forces and hydrophobic interactions [40]. Furthermore, the cells form an aggregate to protect cell population against the toxicity of hydrophilic compounds, since this microorganism resistance decreases with the increase of the system toxicity, restraining the cells to maintain their viability under severe conditions. Thus, these factors have influenced the ability of desulfurization and denitrogenation of *R. erythropolis* ATCC 4277, since the cells are aggregated and there is no evidence of refringent cells, as shown in Fig. 4 [35, 37–40].

Conclusions

The strain *R. erythropolis* ATCC 4277 was able to grown in every concentration of heavy gas oil tested. Different concentrations of heavy gas oil in the biocatalytic process

Table 3 Sulfur content in HGO after submitted to BDS process with *Rhodococcus erythropolis* ATCC 4277

HGO ratio (% v/v)	S concentration ($\mu g.g^{-1}$) × 10^{-1}	Mass of S removed (μg) × 10^{-1}
Standard	625 ± 2	–
10	622 ± 3	0.2 ± 0[a]
20	378 ± 3	246 ± 3
30	522 ± 6	103 ± 9
40	358 ± 3	266 ± 9
50	444 ± 2	181 ± 9
60	403 ± 3	221 ± 2
70	461 ± 3	164 ± 3
80	542 ± 1	82 ± 1
90	478 ± 9	147 ± 1
100	625 ± 3	0

[a] Mass of sulfur removed per gram of HGO

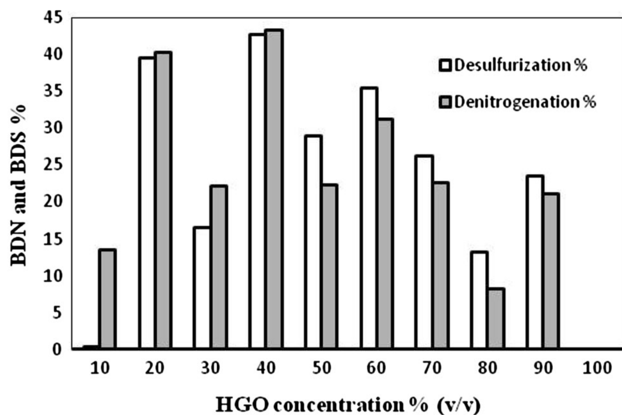

Fig. 3 Denitrogenation and desulfurization percentage for the different HGO concentrations tested in a batch reactor containing *Rhodococcus erythropolis* ATCC 4277 free cells

Table 4 Nitrogen content in HGO after submitted to BDN process with *Rhodococcus erythropolis* ATCC 4277

HGO ratio (% v/v)	N concentration ($\mu g.g^{-1}$) × 10^{-1}	Mass of N removed (μg) × 10^{-1}
Standard	376 ± 2	–
10	325 ± 3	50 ± 0[a]
20	225 ± 2	151 ± 2
30	293 ± 7	83 ± 1
40	213 ± 3	162 ± 1
50	292 ± 9	84 ± 3
60	259 ± 1	117 ± 1
70	291 ± 0	85 ± 1
80	345 ± 3	31 ± 3
90	297 ± 4	79 ± 9
100	426 ± 7	0

[a] Mass of nitrogen removed per gram of HGO

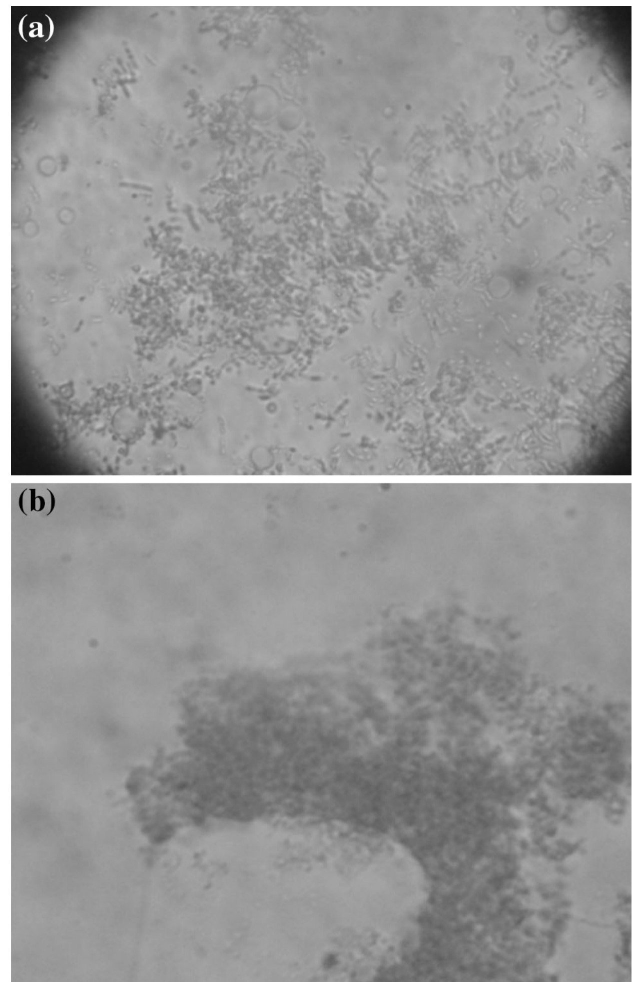

Fig. 4 Fluorescence microscopy of *Rhodococcus erythropolis* ATCC 4277 cells after 18 h in a batch reactor containing **a** 40 % (v/v) and **b** 100 % (v/v) HGO

affected the desulfurization rate, and mass of nitrogen and sulfur removed. The best desulfurization result achieved was with water–oil rate of 40 % (v/v) where the mass of sulfur removed per gram was of 266 (±9) × 10 μg, desulfurization rate of 148 mg sulfur kg^{-1} h^{-1}, and removal percentage of 42.7 %. Similarly the greatest results to BDN process were in the systems using 40 % (v/v) of HGO where 162 (±11) × 10 μg, and the percentages of reduction 43.2 %. This strain was able to remove this quantity of pollutants from a real fraction of petroleum in the presence of a high concentration thereof. This represents an advance in the development of BDN and BDS processes in order to make it economic, environmental, and industrially viable.

Acknowledgments The authors thank the Brazilian Petroleum Agency—ANP, through PRH-09/MECPETRO, and CNPq for the financial support and scholarships.

References

1. Oyama ST, Zhao H, Gott T, Zhao H, Lee Y (2008) Transition metal phosphide hydroprocessing catalysts: a review. Catal Today 143:94–107
2. Dinamarca MA, Rojas A, Baeza P, Espinoza G, Ibacache-Quiroga C, Ojeda J (2014) Optimizing the biodesulfurization of gas oil by adding surfactants to immobilized cell systems. Fuel 116:237–241
3. Onaka T, Konishi J, Ishii Y, Maruhashi K (2001) Desulfurization characteristics of termophilic *Paenibacillus* sp. strain A11-2 against asymmetrically alkylated dibenzothiophenes. J Biosci Bioeng 92:193–196
4. Alves L, Mesquita E, Gírio FM (1999) Dessulfurização bacteriana de combustíveis fósseis. Bol Biotech 62:3–8
5. Stanislaus A, Marafi A, Rana MS (2010) Recent advances in the science and technology of ultra low sulfur diesel (ULSD) production. Catal Today 153:1–68
6. Song C (2003) An overview of new approaches to deep desulfurization for ultra-clean gasoline, diesel fuel and jet fuel. Catal Today 88:211–263
7. Gupta N, Roychoudhury PK, Deb JK (2004) Biotechnology of desulfurization of diesel: prospects and challenges. Appl Environ Microb 66:356–366
8. Le Borgne S, Quintero R (2003) Biotechnological processes for the refining of petroleum. Fuel Process Technol 81:155–169
9. Afferden MV, Schacht S, Klein J, Trüper HG (1990) Degradation of dibenzothiophene by *Brevibacterium* sp. Arch Microbiol 153(4):324–328
10. Maghsoudi S, Kheirolomoom A, Vossoughi S, Tanaka E, Katoh S (1999) Selective desulfurization of dibenzothiophene by newly isolated *Corynebacterium* sp. strain P32C1. Biochem Eng J 5:11–16
11. Maghsoudi S, Vossoughi S, Kheirolomoom A, Tanaka E, Katoh S (2001) Biodesulfurization of hydrocarbons and diesel fuels by *Rhodococcus* sp. strain P32C1. Biochem Eng J 8:151–156
12. Izumi Y, Ohshiru T (2001) Purification and characterization of enzymes involved in desulfurization of dibenzotiophene in fossil fuels. J Mol Catal A Chem 11(1–4):1061–1064
13. Keulen FV, Correia CN, Da Fonseca MM (1997) Solvent selection for the biotransformation of terpenes by *Pseudomonas putida*. J Mol Catal A Chem 5(1–4):295–299
14. Chang JH, Chang YK, Ryu HW, Chang HN (2000) Desulfurization of light gas oil in immobilized-cell systems of *Gordona* sp. CYKS1 and *Nocardia* sp. CYKS2. FEMS Microbiol Lett 182:309–312
15. Kirimura K, Furuya T, Nishii Y, Ishii Y, Kino K, Usami S (2001) Biodesulfurization of dibenzothiophene and its derivatives through the selective cleavage of carbon-sulfur bonds by a moderately thermophilic bacterium *Bacillus subtilis* WU-S2B. J Biosci Bioeng 91(3):262–266
16. McFarland BL (1999) Biodesulfurization. Curr Opin Biotech 2(3):257–264
17. Setti L, Lanzarini G, Pifferi PG (1997) Whole cell biocatalysis for an oil desulfurization process. Fuel Process Technol 52:145–153
18. Kilbane JJ II (2006) Microbial biocatalyst developments to upgrade fossil fuels. Curr Opin Biotech 17:305–314
19. Gray KA, Rhee S, Chang JH, Chang YK, Chang HN (1996) Biochemical characterization of the biodesulfurization of dibenzothiophene by *Rhodococcus* sp. strain IGTS8—potential role in fossil fuel desulfurization. Abstr Pap Am Chem S 212:54
20. Monticello DJ (2000) Biodesulfurization and the upgrading of petroleum distillates. Curr Opin Biotech 11:540–546
21. Folsom BR, Schieche DR, DiGrazia PM, Werner J, Palmer S (1999) Microbial desulfurization of alkylated dibenzothiophenes from a hydrodesulfurized middle distillate by *Rhodococcus erythropolis* I-19. Appl Environ Microb 65(11):4967–4972
22. Guchhait S, Biswas D, Bhattacharya P, Chowdhury R (2005) Biodesulfurization of model organo-sulfur compounds and hydrotreated diesel—experiments and modeling. Chem Eng J 112:145–151
23. Maass D, Oliveira D, Souza AAU, Souza SMAGU (2014) Biodesulfurization of a system containing synthetic fuel using *Rhodococcus erythropolis* ATCC 4277. Appl Biochem Biotechnol 174:2079–2085
24. Li G, Li S, Qu S, Liu Q, Ma T, Zhu L, Liang F, Liu R (2008) Improved biodesulfurization of hydrodesulfurized diesel oil using *Rhodococcus erythropolis* and *Gordonia* sp. Biotechnol Lett 30(10):1759–1764
25. Naito M, Kawamoto T, Fujino K, Kobayashi M, Marushashi K, Tanaka A (2001) Long-term repeated biodesulfurization by immobilized *Rhodococcus erythropolis* KA2-5-1 cells. Appl Microbiol Biotechnol 55:374–378
26. Yu B, Xu P, Zhu S, Cai X, Wang Y, Li L, Li F, Liu X, Ma C (2006) Selective biodegradation of S and N heterocycles by a recombinant *Rhodococcus erythropolis* strain containing carbazole dioxygenase. Appl Environ Microb 72(3):2235–2238
27. Zhang Q, Tong MY, Li YS, Gao HJ, Fang XC (2007) Extensive desulfurization of diesel by *Rhodococcus erythropolis*. Biotechnol Lett 29:123–127
28. Castorena G, Suarez C, Valdez I, Amador G, Fernandez L (2002) Sulfur-selective desulfurization of dibenzothiophene and diesel oil by newly isolated *Rhodococcus* sp. strains. FEMS Microbiol Lett 215:157–161
29. Dinamarca MA, Ibacache-Quiroga C, Baeza P, Galvez S, Villarroel M, Olivero P, Ojeda J (2010) Biodesulfurization of gas oil using inorganic supports biomodified with metabolically active cells immobilized by adsorption. Bioresour Technol 101:2375–2378
30. Yang J, Hu Y, Zhao D, Wang S, Lau PCK, Marison IW (2007) Two-layer continuous-process design for the biodesulfurization of diesel oils under bacterial growth conditions. Biochem Eng J 37:212–218
31. Otsuki S, Nonaka T, Takashima N, Qian W, Ishihara A, Imai T, Kabe T (2000) Oxidative desulfurization of light gas oil and vacuum gas oil by oxidation and solvent extraction. Energy Fuel 14:1232–1239
32. Ma C, Feng J, Zeng Y, Cai X, Sun B, Zhang Z, Blankespoor HD, Xu P (2006) Methods for the preparation of a biodesulfurization biocatalyst using *Rhodococcus* sp. Chemosphere 65:165–169
33. Andrews JM (2001) Determination of minimum inhibitory concentrations. J Antimicrob Chemoth 48:5–16
34. Davoodi-Dehaghani F, Vosoughi M, Ziaee AA (2001) Biodesulfurization of dibenzothiophene by a newly isolated *Rhodococcus erythropolis* strain. Bioresour Technol 101:1102–1105
35. Heipieper HJ, Neumann G, Cornelissen S, Meinhardt F (2007) Solvent-tolerant bacteria for biotransformations in two-phase fermentation systems. Appl Environ Microb 71:961–973
36. Whyte LG, Slagman SJ, Pietrantonio F, Bourbonniere L, Koval SF, Lawrence JR, Inniss WE, Greer CW (1999) Physiological adaptations involved in alkane assimilation at a low temperature by *Rhodococcus* sp. strain Q15. Appl Environ Microb 65:2961–2968
37. Carvalho CCCR, Parreño-marchante B, Neumann G, Da Fonseca MMR (2005) Adaptation of *Rhodococcus erythropolis* DCL14 to growth on n-alkanes, alcohols and terpenes. Appl Environ Microb 67:383–388

38. Carvalho CCCR, Poretti A, Da Fonseca MMR (2004) Solvent toxicity in organic-aqueous systems analyzed by multivariate analysis. Appl Environ Microb 26:361–375
39. Carvalho CCCR, Da Fonseca MMR (2005) The remarkable *Rhodococcus erythropolis*. Appl Environ Microb 67:715–726
40. Carvalho CCCR, Wick LY, Heipieper HJ (2009) Cell wall adaptations of planktonic and biofilm *Rhodococcus erythropolis* cells to growth on C5 to C16 n-alkane hydrocarbons. Appl Microbiol Biotechnol 82:311–320