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To meet stringent emission standards stipulated by regulatory agencies, the oil industry is required to bring down the sulfur content of fuels. Oil supplies 38% of the worldwide energy, and as the light oil is limited and meanwhile the energy demand is increasing, it is a must to use heavy crude oil and therefore desulfurize it to meet environmental standards. As it is not feasible to desulfurize all the sulfur containing compounds of heavy crude oil by the existing methods (such as hydro-desulfurization) we have focused on biodesulfurization of heavy crude oil. We have isolated the first native fungus which has been identified as Stachybotrys sp. and is able to remove sulfur and nitrogen from heavy crude oil selectively at 30 °C. This fungus is able to desulfurize 76% and 64.8% of the sulfur content of heavy crude oil of Soroush oil field and Kuhemond oil field (with the initial sulfur contents of 5 wt % and 7.6 wt %, respectively) in 72 and 144 h, respectively. We have studied the impacts of several parameters on heavy crude oil biodesulfurization efficiency of our fungus strain such as initial pH of the medium, water—oil ratio, and number of spores in the suspension used for inoculation. This fungus strain has been isolated as a part of the heavy crude oil biodesulfurization project initiated by Petroleum Engineering Development Company (PEDEC), a subsidiary of National Iranian Oil Company.