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Anoxic biodegradation of petroleum hydrocarbons in saline media using denitrifier biogranules

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ABSTRACT

The total petroleum hydrocarbons (TPH) biodegradation was examined using biogranules at different initial TPH concentration and contact time under anoxic condition in saline media. The circular compact biogranules having the average diameter between 2 and 3 mm were composed of a dense population of *Bacillus* spp. capable of biodegrading TPH under anoxic condition in saline media were formed in first step of the study. The biogranules could biodegrade over 99% of the TPH at initial concentration up to 2 g/L at the contact time of 22 h under anoxic condition in saline media. The maximum TPH biodegradation rate of 2.6 $g_{TPH}/g_{biomass}$. d could be obtained at initial TPH concentration of 10 g/L. Accordingly, the anoxic biogranulation is a possible and promising technique for high-rate biodegradation of petroleum hydrocarbons in saline media.

1. Introduction

The extraction and processing the oil and gas fuels are resulted in generation a high volume of saline stream containing high concentration of hydrocarbons (Li et al., 2015; Sánchez-Vázquez et al., 2015). Various classes of toxic hydrocarbons are present in the oil-contaminated waters and thus their discharge into the receiving environment causes extensive environmental (soil, surface water, ground water, marine water) contamination (Dao et al., 2014; Shahi et al., 2016; Camus et al., 2015).Therefore, the hydrocarbons must to be eliminated before discharging the produced water to the environment or their recycling.

Due to its both effectiveness and cost-effectiveness, the biodegradation is the method of choice for the removal of hydrocarbons from the polluted streams (Moussavi and Ghorbanian, 2015; Ghorbanian et al., 2014; Wang et al., 2014). The main biological process used for treatment of wastewaters is the conventional activate sludge process or one of its modifications. In order to attain a high rate of TPH biodegradation in the activated sludge process, a high concentration of suspended biomass is usually required. The main challenges with biodegradation of high concentration of TPH in saline media (which is the case for produced waters) using activated sludge process are the limitation of the aeration (oxygen transfer) efficiency, the difficulty in sedimentation of the suspended flocs in order to clarify the treated effluent, and the stripping the hydrocarbons during the aeration, all of which make the process cost-intensive and unreliable for such cases. The aeration inefficacy and the bioflocs separation problem are more intense in the saline wastewater (Lefebvre and Moletta, 2006). An alternative process is the granulation of the flocs and aggregation of acclimatized microorganisms to form the dense biogranules. The granulation enables having a high concentration of biomass with the high settling velocity in the bioreactor thus attaining a high rate of biodegradation (due to high concentration of biomass) along with eliminating the clarification problems (due to the biomass aggregated as granule) (Gobi et al., 2011). In addition, the more favorable micro-environment maintained in the structure of granules provides a sustainable metabolism (Bhuvanesh et al., 2013) enable biomass to handle the high organic load rates and to resist to the toxic conditions (Chen et al., 2015; Erşan and Erguder, 2013). The main factors affecting the biomass granulation are source of inoculum, source and load of carbon, ingredients of water, organic to nitrate ratio, metabolic conditions and other operational parameters (Verawaty et al., 2013; Bhuvanesh et al., 2013; Chen et al., 2015; Erşan and Erguder, 2013). Although the biomass granulation in aerobic conditions is a well-investigated technology, vaporization/volatilization of hydrocarbons during the aeration (Ghorbanian et al., 2014; Moussavi and Ghorbanian, 2015) strongly restrict the application of aerobic granules in aerated bioreactors for biodegradation of petroleum hydrocarbons. Nonetheless, it has been shown that denitrifies can

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create the dense and stable granules with the good settling property (Bhuvanesh et al., 2013; Chen et al., 2015; Erşan and Erguder, 2013). Accordingly, there is a great potential in developing anoxic biogranules and thus in applying the biodegradation of petroleum hydrocarbons using granular biomass under the anoxic conditions. Despite reporting efficient biodegradation of petroleum hydrocarbon using suspended and fixed-film biomass in anoxic conditions (Moussavi and Ghorbanian, 2015; Ghorbanian et al., 2014), based on the reviewing the available literature, no study was found on the available literature on the biodegradation of petroleum hydrocarbons using anoxic granules.

Therefore, the present work was designed with the aim of exploring anoxic granulation for biodegradation of total petroleum hydrocarbons (TPH) in saline media under denitrifying process. The effect of TPH concentration, biodegradation time, denitrification inhibitor and solution temperature was examined on the TPH biodegradation using the anoxic biogranules. The specific TPH biodegradation rate was also calculated and compared with the literature.

2. Materials and methods

2.1. Granule formation and characterization

The suspended biomass acclimated for efficient biodegradation of TPH under anoxic conditions (Ghorbanian et al., 2014; Moussavi and Ghorbanian, 2015) was used as the source of inoculum for granule formation. The inoculum had a total suspended solid (TSS) of 4 g/L. At the beginning of the granulation, 100 mL of inoculum was transferred into a 1-L Erlenmeyer flask and 400 mL of nutrient solution (see below for the composition) was added to that. The pH of microbial suspension was regulated at 7.5 ± 0.2, which is in the optimum range of heterotrophic bacteria. Then the concentration of TPH in the prepared suspension was regulated at 1 g/L by adding aliquots of kerosene (as the sole source of TPH). The flask was finally put in a shaker incubator with the temperature of 35 °C and was started to shake at 150 rpm. The shaking was stopped after 24 h and the biomass was allowed to settle for 5 min. The supernatant was finally withdrawn. The operation was repeated for 8 weeks until the biomass was aggregated into circular granules. At this point the morphology and the microbial structure of the granules were observed using the field emission scanning electron microscopy (SEM) in a MIRA3 TESCAN microscope. Before SEM imaging, the granule was fixed with glutaraldehyde and the then it was coated with gold (Kashi et al., 2014).

2.2. Experimental procedure

After the formation of biogranules, the effect of biodegradation time, initial TPH concentration and denitrification inhibitors was evaluated on the TPH biodegradation using the developed biogranules under the anoxic conditions. All tests were conducted in 1-L Erlenmeyer flask in batch mode. For each test, the biogranules in flask were first rinsed with the fresh nutrient solution, aliquots of the stock nutrient solution was added to the flask, sufficient aliquots of nitrate solution was transferred to the flask, known aliquots of kerosene was added to the suspension, and the seawater was then added to the flask to bring the final volume of the suspension to 500 mL. The prepared suspension was shaken at 150 rpm for the target time period. After elapsing the predetermined shaking time (biodegradation time), the shaker was switched off and whole volume of the solution was withdrawn from the flask. The treated solution was analyzed for the target parameters. To confirm the biodegradation of TPH under the denitrifying conditions, the specific TPH biodegradation tests were conducted at the presence of different concentrations of 2, 4-dinitrophenol as the denitrification inhibitor (Kumar and Lin, 2010; Moussavi et al., 2011). All of the batch tests were conducted in triplicate and the mean of results was reported.

Kerosene was used as the source of TPH in the present study. To better resemble the produced water, seawater was used to prepare the TPH-laden wastewater. The stock nutrient solution was prepared by dissolving K_2HPO_4 and KH_2PO_4 as the source of phosphor and NH_4Cl as the source of nitrogen in seawater. The fresh nutrient solution was made by sufficient dilution of stock nutrient solution in seawater. The nitrate stock solution was prepared by dissolving the required amount of $NaNO_3$ salt in seawater to make a NO_3-N concentration of 100 g/L. The ratio of TPH to NO_3-N was kept constant at 2.5 during the whole course of study (Moussavi and Ghorbanian, 2015; Ghorbanian et al., 2014).

2.3. Analytical methods

The concentration of TPH in the feed and treated solution was determined using an InfraCal CVH/TPH analyzer calibrated using a GC/FID (Agilent 6890N, USA); measurements were taken according to the EPA method 1664 by employing a liquid-liquid extraction method and using perchloroethylene (PCE) as the extraction solvent, followed by infrared measurements. GC/FID analysis was performed with an Agilent HP-5 capillary column (30 m length, 0.32 mm i.d., and 0.25 μm film thickness) (Zhang et al., 2012) and an injection volume of 2 μL. Injector and detector temperatures were set at 295 °C and 300 °C, respectively. The initial oven temperature of 60 °C was held for 2 min and then heated to 300 °C at 8 °C/min and maintained for 7 min. N_2 was used as the carrier gas at a rate of 1.8 mL/min. The dried mass of biogranules in the flask was determined from weight of the wet biogranules and considering their humidity percentage. The humidity percentage of the wet biogranules was determined from the difference in wet and dried (putting the wet biogranules for 24 h at 105 °C) weight of one biogranules which was found to be 62.5%. All other analytical measurements were conducted according to standard methods.

The capability of the developed biogranules in TPH biodegradation was measured based on the biodegradation efficiency and the specific hydrocarbon (TPH) biodegradation rate (SHBR) as defined below:

$$\text{TPH biodegradation efficiency (\%)} = \frac{\text{TPH}_{initial} - \text{TPH}_{final}}{\text{TPH}_{initial}} \times 100 \quad (1)$$

$$\text{SHBR}(g_{\text{TPH}}/(g_{biomass}.\ d)) = \frac{(\text{TPH}_{initial} - \text{TPH}_{final}) \times V}{M_g \times t_b} \quad (2)$$

where $\text{TPH}_{initial}$ and TPH_{final} represent the initial and final concentration of TPH in the solution (g/L), respectively, V is the total volume of solution in the flask (0.5 L), M_g is the total dried mass of biogranules in the flask (3.3 g), and t_b is the shaking (biodegradation) time (d). It should be noted that the amount of dried biogranules at the end of biogranulation step was 3.3 g.

2.4. Isolation and bacteria identification

For identification of bacteria dominant in the granules contributed in the biodegradation of hydrocarbons, the microbial samples were taken from Erlenmeyer flasks. 10 mL of mixed liquor in the Erlenmeyer flasks and one of the typical granules transferred to 250 mL sterile Erlenmeyer flask that containing 100 mL minimal salt medium (MSM) and 1 mL of kerosene oil and then the granule was squished. The flasks were closed and incubated in