NIGERIAN JOURNAL OF BIOTECHNOLOGY

Nig. J. Biotech. Vol. 36 (1): 54-61 (June 2019)
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org
DOI: https://dx.doi.org/10.4314/njb.v36i1.8

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Integrated co-cultured bacterial strains capabilities to aqueous sulfide and chemical oxygen demand mitigation from high-strength petroleum refinery wastewater

Mani, M[1, 2*] and Abd.Aziz M. A[1]

[1]Faculty of Chemical and Natural Resources Engineering, University Malaysia Pahang (UMP), Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia
[2]Faculty of Science, Department of Biological Sciences, Kano University of Science and Technology (KUST), Wudil, 3244, Kano, Nigeria

Abstract

The heterogeneous nature of petroleum refinery based wastewater (PRW) couple with the documented paucity of the classical physicochemical mitigation approaches were behind the quest for a cost-effective and ecofriendly alternative with minimum negative effects. The toxic and inhibitory substances contained in PRW have made its treatment strenuous using a simple pure culture. A novel integrated bacterial mixed culture (BMC), *Pseudomonas putida* (ATCC 49128) and *Bacillus cereus* (ATCC 14579) with traceable imprints in biodegradation of high-strength PRW was proposed as a suitable alternative for sulfide oxidation and COD reduction with a potential alternative to classical physicochemical mitigation approaches. Comparative biodegradation potential of two acclaimed bacteria mixed-culture regimen was evaluated. The degree of inhibition cast was assessed based on the effectiveness of BMC to remove the targeted compounds (aqueous hydrogen sulfide and COD) within the assigned period of 8 hours hydraulic retention time (HRT). The PRW was found to contained initial concentration of 8,155 ppm COD, and 500 ppm sulfide, coupled with other refractory substances. The experiment was repeated batch-wise under defined optimal conditions of 0.25 L/min O_2, the temperature of 30 °C, agitation of 140 rpm at 8 hours retention time. The results obtained indicated 97.47±1.7 % (mg/L) COD reduction and 99.75±1.8 (ppm) sulfide removal efficiency (RE). In addition, the SEM analysis revealed further the potential of BMC to degrade sulfide from PRW to economically feasible elemental sulfur species, while EDXS demonstrated a proportionate elemental composition revealing appreciable sulfur content and high organic carbon accumulation. Despite the expected toxicity and inhibitory effect of the medium, an overwhelming biodegradation was achieved disproportionately, hence the model can be suggested for further research of repulsive wastewaters.

Keywords: Biodegradation, petroleum refinery wastewater, mixed-culture, Batch culture

Corresponding author: mmahmadu@gmail.com; Tel +601136560301

Introduction

Based on the existing documents, oil and gas industries were the leading anthropogenic sources of several environmental contaminations, specifically hydrogen sulfide (H_2S) by up to 90%. The petroleum refinery wastewater (PRW) remains the most hazardous owning to its refractory nature comprising of PAHs, oil and grease, BTX, phenol among (Chen et. al., 2015; Liu, et. al., 2014). These pollutants were products of various crude processing units, such as desalting, distillation, fluid catalytic cracking, coking, visbreaking, hydrogenation and hydrodesulfurization, and many more (Azizan, et. al., 2016; IPIECA, 2010; Ishak, Malakahmad, & Isa, 2012). The classical physicochemical treatment approaches (electrocoagulation, adsorption, thermal and catalytic oxidation, ozonation, membrane separation) have been quite promising in terms of faster conversion rate and huge turnover, except for its high cost of maintenance (Azizan et. al., 2016), energy consumption and consequential production of secondary pollutants (Watsuntorn, et. al., 2017; Zytoon, et. al., 2014).

The biological process of sulfide attenuations (biofilters, biotrickling filters, bioscrubber, activated sludge) from the aqueous medium on the other hand, could handle some of the aforementioned limitations, because of its ease of operation and management, cheap and environmental friendly (Jamaly et. al., 2015; Guerreroa et. al., 2016; Pang et. al., 2017). Moreover, its application is tailored to suit the different forms of wastewater, the targeted outputs and potential to prevail unfriendly physical and environmental conditions. Additionally, most of the reported texts related to H_2S mitigation were from the gas phase, while those in dissolved form were scarcely analyzed (Guerreroa et. al., 2016). The complexity of PRW made it less likely to be successfully treated by a simple biodegrading pure culture. As such an integration of co-cultured isolates with traceable imprints in high-strengthen industrial wastewater biodegradation was found quite promising. A novel *Pseudomonas putida* (ATCC 49128) and *Bacillus cereus* (ATCC 14579) with uncontested records for inhibitory compounds treatment from industrial wastewaters (Ahmad et. al., 2017; Cerqueira et. al., 2012; Reshma & Mathew, 2014) were proposed. However, their integration as mixed culture for treatment of aqueous H_2S from PRW was not reported elsewhere thus could not be accessed prior to this work, except a few reports as pure culture form for H_2S mitigation. In this study, the potential of the *P. putida* and *B. cereus* as a bacterial mixed culture (BMC) was proposed as sulfide and chemical oxygen demand (COD) degrading model for PRW. As such the model could be suggested for further application to treating high-strengthen contaminants from both domestic and industrial sludge.

Materials and Methods

Bacterial culture and cultivation

Two referenced BMC isolates, *P. putida* (ATCC 49128)/B1 and *B. cereus* (ATCC 14579)/B2 were used for this study. The initial growth media was source from certified local agent in Malaysia and utilized based on the manufacturer's instruction. Typically, 8 g of nutrient broth (NB) was dissolved in a liter of deionized water and agitated on a hot plate to homogenize. Thereafter, it was sterilized at 121 °C temperature and 15 psi pressure for 15 min, and cooled to 47 °C and later dispensed in 20 mL sampling bottles. Preparation of pre-cultures was done by transferring a loopful of the cell from a stock of actively growing culture on a nutrient agar plate with the aid of sterilized wire loop into the freshly prepared NB (10% w/v) bottles. This was separately done for each isolate and incubated for 24 hours at 37 °C. The viability of the stock cultures was maintained throughout the experimental process using a periodic sub-culture at least fortnightly on nutrient agar (NA) and refrigerated at 4 °C until use (Ahmad et. al., 2017).

Characterization and quality analysis of petroleum refinery based wastewater (PRW)

Prior to the experimental studies, the wastewater composition analysis was done in according to the standard APHA 2540 protocol (ICPCB, 2011). A reported analysis by (Azoddein, 2013) on the same sample showed that it contained multiple pollutants such as hydrocarbon, polycyclic and aromatic hydrocarbon, phenols, mercaptans, oil and grease, sulfides, ammonium, metals derivatives such as mercury, cyanides and other chemicals. The analyzed result was compared with standard A and B of the Malaysian industrial wastewater quality analysis to ascertain the level of contamination in the PRW.

Experimental set up

The experiment was conducted in a 2 L laboratory-scale batch reactor type BIOTRON (LiFlus GX, Intran, Korea). The method adopted herewith was as described elsewhere (Ahmad et. al., 2017) as modified. Prior to start up, the fermenter was fed with 500 mL acclimatizing media for the isolates and sterilized along with other accessories at 121 ºC temperature and 15 psi pressure for 20 min. After cooling, the calibrated reactor was inoculated with the two isolates, B1 and B2 (10% vv^{-1}) of the total reactor volume) in the ratio of 60:40. The variation in the strain volume was to compensate for the complementary impact of helper bacteria effect (Ahmad et. el., 2017). The efficiency of the process (aerobic) depends on influent sulfide concentration and aeration rate, thus need more of the aerobic isolate (B1). While B2 being a spore-forming and facultative anaerobe was needed as a supportive isolate against the inhibitory effect of repulsive components of the PRW and toxic metabolites as reported (Villahermosa et. al., 2016). To fully explore the capabilities of the BMC as well as reflect the high-strength sulfide concentration reported elsewhere (Lohwacharin and Annachhatre, 2010), the PRW was augmented with exogenous sulfide source ($Na_2S.9H_2O$) to balance the concentration at 500 ppm. The temperature of the medium was maintained at required of 30 °C using a cooling system (thermostat water jacket) from water bath as described (Moghanloo et. al., 2010). Complete homogeneity of the vessel content was maintained with double Rushton mechanical impeller and one foam breaker. Aeration was achieved using an air compressor (HIBLOW HP-80, Japan) with an inbuilt air sparging tube from the reactor base.

The reactor was started and operated batch-wise, and the optimum operating conditions of aeration rate, agitation, the temperature of 0.25 L min^{-1}, 140 rpm and 30 °C in 8 hours hydraulic retention time (HRT). that yield highest sulfide removal efficiency (RE) were adopted based on the previous findings (Lohwacharin and Annachhatre, 2010; Moghanloo et al., 2010). The medium pH was sustained at 8.5 using 0.5 M buffer solutions based on the reported literatures and preliminary experimental works to ascertain the best suitable condition for the isolates' growth and effective RE. After proposed acclimatization of the BMC, the reactor was fed with PRW to balance the final working volume of the reactor at 1.5 L. To further explore the PRW biodegradation potential of BMC, the effect proposed optimum process parameters of temperature, aeration, and agitation on the RE were analyzed. This was to create headspace for collected gases and condensing vapor that could result splashing effect or foaming. Furthermore, dissolved oxygen (DO) was maintained within the range of 5 to 20 mg/L as the least value to the end of the experimental cycle. The oxidation rate of the system was estimated using the Eq. 1.0.

$$\text{RE } (\%) = \frac{C_0 - C_t}{C_0} \text{ x } 100 \qquad (1.0)$$

where RE is the removal efficiency, C_0 is the initial sulfide concentration; C_t is the recorded concentration at time t.

Analytical Methods

The samples were analyzed for concentrations of COD, and sulfide removal efficiency according to standard methods for the examination of water and wastewater and using standard methods (APHA, 2005). For COD, a colorimetric method using a standard dichromate closed reflux method (reacting sulfuric acid and potassium dichromate), was adopted. A spectrophotometer (DR 2800, Hach, Jenway, USA) was used to measure the absorbance of COD at 600 nm wavelength. While total dissolved sulfide (TDS) depletion was measured using methylene blue method (reaction between hydrogen sulfide with N, N-dimethyl-p-phenylenediamine (DMPPDA) sulfate salt) at a photometric range of 5-800 µg/L S^{2-} and wavelength of 665 nm.

SEM-EDXS

The morphology of the surface image of the bacterial biofilm was assessed using Scanning Electron Microscopy (SEM) for the presence of cells-sulfur particles aggregate, while the collective elemental identification and composition spectrum was analyzed using Energy Dispersive X-ray Spectroscopy (EDXS). The treated sample was analyzed using a high-resolution SEM (Hitachi, TM3030Plus, Japan). Initially, aliquots samples were withdrawn terminally in 35 ml sampling tubes and transferred to Karnovsky's fixative solution for primary fixation as described previously (Patel et. al., 2016;Villahermosa et. al., 2016) as modified in (Reshma, & Mathew, 2014). Hydration was achieved in thrice distilled water for 15 min with centrifugation at 5000 rpm for 5 min each. This was followed by dehydration in a graded ethanol solution of (30%, 50%, 70%, and 90 %) for 10 min each and absolute ethanol (100%) for 15 min. The samples were pipetted to 1 cm diameter aluminum foil paper and air-dried at room temperature in an enclosed bio-safety cabinet overnight. The sample specimens were stuck onto a stub using double-sided colloidal silver for mounting. Finally, the prepared samples were subjected to SEM analyses and observed at accelerating voltage of 15 keV.

Results and Discussion

Biodegradation capabilities of COD at the optimum operational process

The oxygen consumption of the oxidizable substances (COD) for the hydrocarbon oil wastewater at the microaerobic condition of 0.25 L/min was found to be enhanced by the moderate temperature of 30 °C, while homogeneity of the medium content was sustained at 140 rpm impeller speed. Table 1 showed the rate of COD consumption over 8 hours HRT under medium aeration and mesophilic (30 °C) temperature condition. It can be seen that an overwhelming performance occurred between the periods of 4-6 hours with 90.53±1.5 % COD reduction. This trend of highest COD consumption could probably be traced to active substrate utilization during the active metabolic period of exponential growth phase of the growing isolates. In addition, the performance was probably due to the potential of BMC to withstand the effect of non-biodegradable components of PRW. Asadi and Ziantizadeh (2011) reported that presence of non-biodegradable COD in an industrial wastewater was a stumbling block due to its inhibition of microbial community. The BMC model was able to register the highest COD reduction of 97.47±1.7 % at the end of 8 hours HRT. This finding was higher than what was reported of 73.89 % in 24 hours by (Liang, et. al., 2015) and 83.9±1.7 under optimum conditions of HRT (24 hours) and MLVSS (4000 mg/L) by (Elmolla et. al., 2012).

A similar reduction rate by (Muhamad, et. al., 2013) who reported at 97.2 % under low aeration rate within 3 day and 750 mg/L influent COD concentration using attached growth with granular activated carbon. It can, therefore, be suggested that the complementary impact of mixed culture further strengthened the earlier reports regarding their highest performance over the pure culture isolates for recalcitrant wastewaters treatment.

Table 1: Biodegradation capability of COD from Petroleum Refinery Based Wastewater

Time	COD consumption (ppm)	% COD Removal	Discharge standard* Standard A	Standard B
0 hr.	8,155	0.00	-	-
2 hr.	7,554	7.37± 1.1	Above limit	Acceptable
4 hr.	4,381	42.11±1.4	Above limit	Acceptable
6 hr.	415	90.53±1.5	Above limit	Acceptable
8 hr.	11	97.47±1.7	Above limit	Acceptable

***Metcalf and Eddy, 2003**

Potential and effect of operational parameters on sulfide biodegradation

The sulfide biodegradation of the PRW was carried out based on the model established optimum process variables of aeration (0.25 L/min), temperature (30 °C) and agitation (140 rpm), at a residence time of 8 hours. The removal efficiency of $H_2S_{(aq)}$ in a submerged growth pattern was tagged to these significant process parameters of initial sulfide concentration, aeration rate, temperature, and mixing. The parameters were also fingered to not only affect the rate of removal and growth of the biodegrading isolates but even the nature of product species. Figure 2 and Table 2 showed the trend of sulfide reduction termed as RE over the period of the experimental process. The low RE of 15.43±0.9 % (Table. 2) in the first two hours of the startup indicated the usual acclimatization of these isolates to its new environment which is required to prepare the organism to next phases of metabolic activities as pointed previously (Schultz and Kishony, 2013). The next period of between 4-6 hours corresponded to exponential growth phase, which was characterized by highest cellular activities of substrate (sulfide biodegradation) utilization and cell biomass synthesis. However, it is worth noting that in almost all biodegradation studies reported especially involving inhibitory substances, the late exponential phase is marred by a slowed growth and declined in degradation rate (Ahmad et. al., 2017). It was reported by other researchers that the inhibitory concentration of aqueous dissociated sulfides ions is in the range of 100 – 800 ppm, while for the undissociated form (H_2S) it ranges between 50 – 430 ppm (McDonald, 2009; Schultz and Kishony, 2013). This reports further supported the tolerance capabilities of this model (BMC) to high-strength PRW with aqueous sulfide concentration and COD of up to 500 ppm and 8,155 mg/L. This may be due to the accumulation of metabolic residue or filling of undissociated dissolved H_2S that inhibit the bacterial growth due to impairment of cell proteins, coenzyme activities, and sulfide assimilation (Guerreroa et. al., 2016).

Nevertheless, it can be seen that the pattern of H_2S and COD removal rate was quite smooth and consistent in this study. This was due to the mutual interactions effects of BMC that enable the consortium avoid or reduce the impact of hard and recalcitrant pollutants. It was reported elsewhere that mixed culture of bacteria and other microorganisms' consortium were able to escape the inhibition of constituents in its medium due to the mechanism of complementary bacteria-interactions-helper effect (Garcia, 2016). The micro-oxygenated medium of the reactor, coupled with medium temperature and mixing that ensure homogeneity of the content, facilitated RE of 100 % or reduction of the sulfide content to 0 ppm.

The previous report affirmed the capabilities of mixed culture bacteria to biodegrade complex PRW due to the presence of rich and appropriate enzymatic drive (Das and Chandran, 2011). It is therefore suggested that the model could propose for biodegradation of stiff-necked wastewaters such as petroleum refinery based wastewater. Moreover, it can be seen that both COD and sulfide reduction to permissible discharge level of 50/100 COD mg/L and 0.5 ppm was attainable within the experimental period of 8 hours HRT. Generally, BMC degraded many compounds from hydrocarbon oil wastewater with corresponding cell growth and biomass synthesis (EDXS analytes), indicating multiple biodegradation potentials of these isolates with disproportionate efficiencies.

Table 2: Sulfide removal efficiency of BMC from PRW under optimum operation

Time (hours)	Sulfide reduction (ppm)	RE (%)
0	500	0.00
2	422.85	15.43±0.9
4	246.70	50.66±1.1
6	107.20	78.56±1.4
8	1.25	99.75±1.8

SEM-EDXS of Samples Biofilm

The SEM-EDXS offer an insight on the mechanism of isolates upon introduction into the polluted medium, in which they responded by the formation of resistance layer (biofilm) that allows tolerance to toxic and inhibitory medium. The resistant hydrogels contained an upshot of oxidized soluble sulfide to probably organic elemental sulfur. The SEM micrographs and the EDXS analysis of the extra polymeric substances (EPS) from the biofilm surface of the oxidized PRW samples were evaluated to further figure out biodegradation efficiency of the BMC. It has been established that the nature of the product species depends on the sulfide concentration and aeration rate, with a tendency for S^0 selectivity at a low O_2/S^{-2} ratio of 0.2-0.5 DO mg/L. Figure 1 shows the SEM micrograph of BMC biofilm from the blank sample prior to degradation while the oxidized sulfide samples with an overwhelming sulfur deposition coagulates is represented in figure 2. The EDXS analysis (Figure 3) revealed a corresponding elemental product percentage. Sample from the dispersed active sludge of the batch reactor where sulfur sediment was presumably retained was withdrawn through the sampling port. It can be seen that the sulfur excretes from the image was quite visible in form of deposited cells-sulfur granules of the biofilm (Fig. 2), while a freely disperse rod-shaped bacterial cells without noticeable sulfur granules was observed from the SEM micrograph of the wastewater sample prior to biodegradation process (Fig. 1). It was reported that high influent sulfide concentration and low aeration rate favored elemental sulfur formation. Additionally, some patches of rod-shaped bacterial cells can be seen with extracellularly deposited sulfur granules, while the major portion revealed coagulated biofilms with less visible outer sulfur excretes (probably intracellular). Although the cells-sulfur deposition aggregate was less than what was reported previously by (Jensen, et. al., 2016) and (Lohwacharin and Annachhatre, 2010), however, the aggregates were relatively formed with that a large SOB cluster of sulfur excretes coagulates. It can be seen that micrographs had some hollow pore spaces further affirming the incorporation of air bubbles in the biofilm that enables further metabolism of membrane bond sulfur.

Moreover, the EDXS of the sample reveals the detail elemental composition with their corresponding weights. Figure 2 showed the elemental composition and relative weight or proportion. From the elemental spectrum analysis, it can be seen that sulfur proportion of 0.32 % was at the lowest range in the treated sample. Additionally, growth indicated by organic carbon accumulation (63.72 %) was significant, indicating overwhelming substrates utilization from the PRW. The proportion of phosphate groups which serve as acceptors of the electron donated by reduced sulfur (S^{-2}), enhance significant electron transfer equilibrium. Consequently, this facilitated efficient energy production and growth, signified by biomass accumulation.

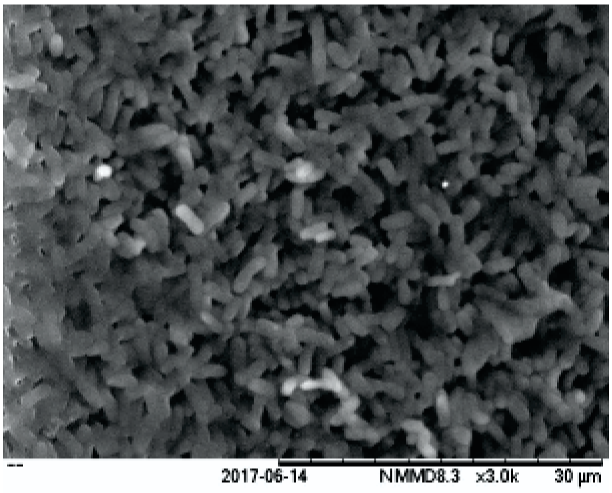

Figure 1: Micrographic image of the sample before degradation sample

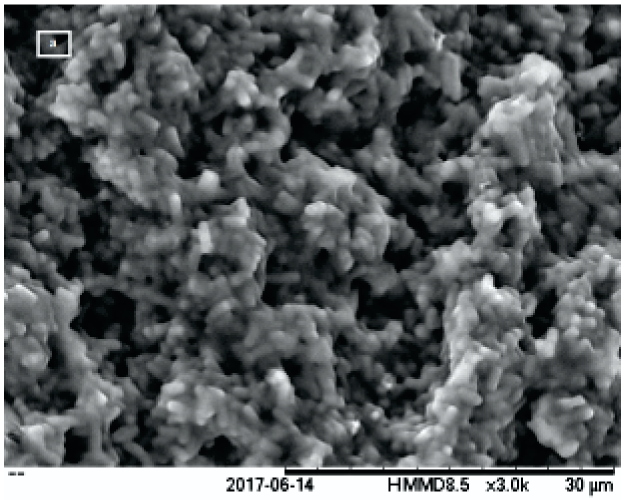

Figure 2: Micrographic image of the degraded sample SEM image

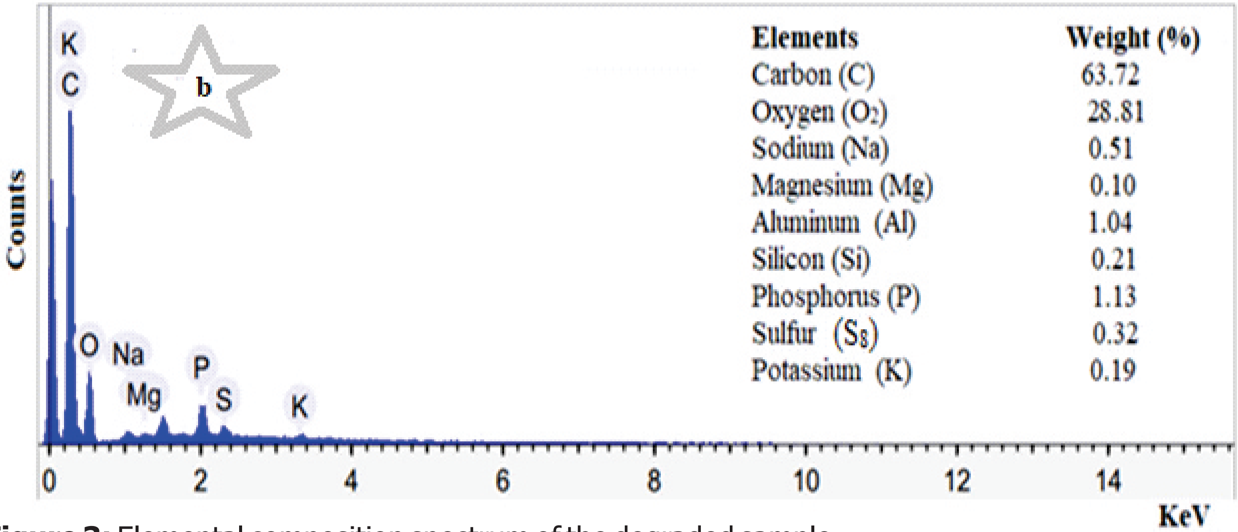

Figure 3: Elemental composition spectrum of the degraded sample

Conclusion

The potential capabilities of an integrated co-cultured (BMC) *P. putida* (ATCC 49128) and B. cereus (ATCC 14579) model for aqueous sulfide oxidation and COD reduction from heterogeneous and repulsive petroleum refinery based wastewater was significantly achieved. This was affirmed under the earlier established optimum process parameters of influent sulfide concentration, aeration rate, agitation, and temperature of 500 ppm, 0.25 L/min, 140 rpm and 30 °C, respectively. The mutual complementary helper effect of the individual strain to the consortium was believed to take care of much-anticipated constraints of the $H_2S_{(aq)}$ and other PRW components' inhibitory effects. Despite the repulsive nature of this wastewater, the consortium was able to biodegrade the two pollution indicators of COD and sulfide with RE of 97.47±1.7 % and 99.75±1.8 %, respectively. Furthermore, the adopted optimum process parameters condition was believed to favor elemental sulfur species formation over sulfate as confirmed by the SEM-EDXS analyses which reveal relatively high orthorhombic elemental sulfur (S_8). Based on this finding it can be suggested that the BMC offer a perfect option for biodegradation of the targeted pollutants from petroleum refinery based wastewater to permissible discharge limit.

Acknowledgment

The authors acknowledged the financial support of University Malaysia Pahang (UMP) through Postgraduate Research Scheme (PGRS 170366).

References

Azoddein, A. M. (2013). Mercury Removal From Petroleum Based Industries Wastewater by Pseudomonas putida (ATCC 49128) in Membrane Bioreactor. A Ph.D. Thesis, University Malaysia Pahang (UMP).

Ahmad, M.M., Azoddein, A.M., Zahari, M.A.K.M., Seman, M. N., Saedi, M. J., Olalere, O. A., and Alara, O. R. (2017). Optimization of Process Parameters in Mixed Sulfide Oxidation Bacterial Culture Using Response Surface Methodology as a Tool. J. King Saud Univ. - Sci. 1–8. https://doi.org/10.1016/j.jksus.2017.11.001

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, 21 st Edit. ed. American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC.

Asadi, A., and Ziantizadeh, A.A.L. (2011). Statistical Process Analysis and Optimization of an Aerobic SBR Treating an Industrial Estate Wastewater Using Response Surface Methodology (RSM). Iran. J. Energy Environ. 2: 356–365. https://doi.org/10.5829/idosi.ijee.2011.02.04.3424

Azizan, N. H., Apun, K. A., Bilung, L. M., Vincent, M., Roslan, H. A. and Husaini, A. S. A. (2016). Crude Oil Bioremediation by Indigenous Bacteria Isolated from Oily Sludge. J. Teknol. 2: 61–66.

Cerqueira, V. S., Hollenbach, E. B., Maboni, F., Camargo, F. A. O., Peralba, M. do C.R. and Bento, F. M. (2012). Bioprospection and selection of bacteria isolated from environments contaminated with petrochemical residues for application in bioremediation. World J. Microbiol. Biotechnol. 28: 1203–1222. https://doi.org/10.1007/s11274-011-0923-z

Chen, M., Xu, P., Zeng, G., Yang, C., Huang, D. and Zhang, J. (2015). Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting: Applications, microbes and future research needs. Biotechnol. Adv. 33: 745–755. https://doi.org/10.1016/j.biotechadv.2015.05.003

Das, N., and Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnol. Res. Int. 2011, 941810. https://doi.org/10.4061/2011/941810

Elmolla, E. S., Ramdass, N. and Chaudhuri, M. (2012). Optimization of Sequencing Batch reactor Operating Conditions for Treatment of High-stength Pharmaceutical Wastewater. J. Environ. Sci. Technol. 5: 452–459.

Garcia, S. L. (2016). Mixed cultures as model communities: Hunting for ubiquitous microorganisms, their partners, and interactions. Aquat. Microb. Ecol. 77: 79–85. https://doi.org/10.3354/ame01789

India Central Pollution Control Board. P R Division, (2011). Guide Manual: Water and WasteWater Analysis 1–195.

IPIECA Operations Best Practice, (2010). Petroleum refining water / wastewater, Report:The global oil and gas industry association for environmental and social issues.

Ishak, S., Malakahmad, A. and Isa, M. H. (2012. Refinery wastewater biological treatment: A short review. J. Sci. Ind. Res. 71: 251–256.

Jamaly, S., Giwa, A. and Hasan, S. W. (2015). Recent improvements in oily wastewater treatment: Progress , challenges , and future opportunities. J. Environ. Sci. 37: 15–30. https://doi.org/10.1016/j.jes.2015.04.011

Jensen, H., Biggs, C. A., Karunakaran, E. (2016). The importance of sewer biofilms. Wiley Interdiscip. Rev. Water 3: 487–494. https://doi.org/10.1002/wat2.1144

Jukic, A. (2011). Petroleum Refining: Introduction, Products Refinery's Configuration, Hydrogen Technologies.

Liu, S., Ma, Q., Wang, B., Wang, J. and Zhang, Y. (2014). Advanced treatment of refractory organic pollutants in petrochemical industrial wastewater by bioactive enhanced ponds and wetland system. Ecotoxicology 23: 689–698. https://doi.org/10.1007/s10646-014-1215-9

Lohwacharin, J. and Annachhatre, A. P. (2010). Biological sulfide oxidation in an airlift bioreactor. Bioresour. Technol. 101: 2114–2120. https://doi.org/10.1016/j.biortech.2009.10.093

Guerreroa, L., Montalvo, S., Huilinir, C., Campos, J. L., Barahona, A. and Borja, R. (2016). Advances in the biological removal of sulphides from aqueous phase in anaerobic processes: A review. Environ. Rev. 24: 84–100. https://doi.org/10.1139/er-2015-0046

Ahamd, M. M., Azoddein, A. M., Zahari, A. K.M., Seman, M. N., and Jami, M. S. (2017). Microbial interactions in response to sulfide effect on mesophilic bacterial mixed culture (BMC) growth. J. Adv. Res. Mater. Sci. 1: 10–20.

McDonald, H.B. (2009). Effect of sulfide inhibition and organic shock loading on anaerobic biofilm reactors treating a low-temperature, high-sulfate wastewater. Ph.D Thesis. https://doi.org/10.2175/106143008X325656

Moghanloo, G. M. M., Fatehifar, E., Saedy, S., Aghaeifa, Z. and Abbasnezhad, H. (2010). Biological oxidation of hydrogen sulfide in mineral media using a biofilm airlift suspension reactor. Bioresour. Technol. 101: 8330–8335. https://doi.org/10.1016/j.biortech.2010.05.093

Muhammad, M. H., Sheikh Abdullah, S. R., Mohammad, A. B., Abdul Rahman, R. and Hasan Kadhum, A. A. (2013). Application of response surface methodology (RSM) for optimisation of COD, NH3-N and 2,4-DCP removal from recycled paper wastewater in a pilot-scale granular activated carbon sequencing batch biofilm reactor (GAC-SBBR). J. Environ. Manage. 121: 179–190. https://doi.org/10.1016/j.jenvman.2013.02.016

Pang, B. W., Jiang, C. H., Yeung, M., Ouyang, Y. and Xi, J. (2017). Removal of dissolved sulfides in aqueous solution by activated sludge: mechanism and characteristics. J. Hazard. Mater. 324: 732–738. https://doi.org/10.1016/j.jhazmat.2016.11.048

Parkin, G. F., Lynch, N. A., Kuo, W., Van Keuren, E. L., Bhattacharya, S. K., Parkin, F., Van Keuren, L. and Lynch, A. (1990). Interaction between sulfate reducers and methanogens fed acetate and propionate. Res. J. Water Pollut. Control Fed. 62: 780–788.

Patel, S. K. S., Kumar, P., Singh, M., Lee, J. K. and Kalia, V. C. (2015). Integrative approach to produce hydrogen and polyhydroxybutyrate from biowaste using defined bacterial cultures. Bioresour. Technol. 176: 136–141. https://doi.org/10.1016/j.biortech.2014.11.029

Patel, S. K. S., Selvaraj, C., Mardina, P., Jeong, J. H., Kalia, V. C., Kang, Y. C. and Lee, J. K. (2016). Enhancement of methanol production from synthetic gas mixture by Methylosinus sporium through covalent immobilization. Appl. Energy 171: 383–391. https://doi.org/10.1016/j.apenergy.2016.03.022

Reshma, J. K. and Mathew, A. (2014). Biodegradation of Phenol-Aerobic and Anaerobic Pathways. Int. J. Sci. Nat. 5: 366–387.

Schultz, D.and Kishony, R. (2013). Optimization and control in bacterial lag phase. BMC Biol. 11: 120. https://doi.org/10.1186/1741-7007-11-120

Villahermosa, D., Corzo, A., Garcia-Robledo, E., González, J. M. and Papaspyrou, S. (2016). Kinetics of Indigenous Nitrate Reducing Sulfide Oxidizing Activity in Microaerophilic Wastewater Biofilms. PLoS One 11, e0149096. https://doi.org/10.1371/journal.pone.0149096

Watsuntorn, W., Ruangchainikom, C., Rene, E. R., Lens, P. N. L.and Chulalaksananukul, W. (2017). Hydrogen sulfide oxidation under anoxic conditions by a nitrate-reducing, sulfide-oxidizing bacterium isolated from the Mae Um Long Luang hot spring, Thailand. Int. Biodeterior. Biodegrad. 124: 196–205. https://doi.org/10.1016/j.ibiod.2017.06.013

Liang, Z., An, T., Li, G. and Zhang, Z. (2015). Aerobic biodegradation of odorous dimethyl disulfide in aqueous medium by isolated Bacillus cereus GIGAN2 and identification of transformation intermediates. Bioresour. Technol. 175: 563–568. https://doi.org/10.1016/j.biortech.2014.11.002

Zytoon, M. A. M., AlZahrani, A. A., Noweir, M. H. and El-Marakby, F. A. (2014). Bioconversion of high concentrations of hydrogen sulfide to elemental sulfur in airlift bioreactor. Hindawi Publ. Corp. Sci. World J. 2014: 675673. https://doi.org/10.1155/2014/675673