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BIODEGRADATION OF EFFLUENT CONTAMINATED WITH DIESEL OIL AND GASOLINE USING CHITOSAN AS A NATURAL COAGULANT IN A CONTINUOUS PROCESS

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Abstract - This study evaluated the effects of aeration (constant aeration, intermittent aeration and a lack of aeration) and hydraulic retention time (HRT) (2, 3 and 4 days) on a continuous process with cell recycling, using chitosan as a natural coagulant for the sedimentation of a C_1 mixed culture. This culture was used for the biodegradation of hydrocarbons present in the effluent contaminated with diesel oil and gasoline. The responses monitored included the turbidity removal (TR), total petroleum hydrocarbon (TPH) removal and volatile suspended solids (VSS). Constant aeration and an HRT of 4 days produced the best results for the continuous process, resulting in the highest TPH removals (94% and 75% reductions in the supernatant and reaction tank, respectively) and TR (95%).

Keywords: Continuous process; Biodegradation; Chitosan; Mixed culture.

INTRODUCTION

The effluent generated in terrestrial fuel distribution terminals usually has a high organic load that is the consequence of the presence of the hydrocarbon constituents of gasoline and diesel oil (complex mixture consisting basically of paraffinic, olefinic, aliphatic hydrocarbons and aromatic compounds and, in smaller amounts, molecules containing sulfur, nitrogen, metals, oxygen, etc). Therefore, biodegradation is impaired by recalcitrance of some of these compounds present in gasoline and diesel oil and the high organic load values occasioned by these hydrocarbons.

The use of pure cultures (bacteria, yeasts or filamentous fungi) and mixed cultures for the decontamination of sites contaminated with petroleum and petroleum derivatives is often a promising technological solution, which significantly reduces or eliminates the pollution load that may be present in the soil or in liquid media (Bielecka *et al*., 2002; Díaz *et al*., 2000; Lakha *et al*., 2005; Townsend *et al*., 2004; Gogoi *et al*., 2003; Grishchenkov *et al*., 2000).

The activated sludge process is one of the most widely used technologies for biological treatment of wastewater. This process offers great advantages, including a decreased susceptibility to the inhibition of aerobic bacteria due to the short chemical adaptation time of the mud, a lower propensity of the effluents to generate unwanted features and the lack of a

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*To whom correspondence should be addressed need for a post-treatment. Additionally, the biochemistry and microbiology of the aerobic processes are well understood, and the process provides operational flexibility and a high removal efficiency of the compounds (Rosa *et al*., 2006).

The use of a process tank with vigorous mechanical stirring for biological treatment of effluents contaminated with gasoline and oil diesel may impair the sedimentation of the mixed culture (Vieira *et al*., 2012). To enable the application of an activated sludge continuous process, mechanical agitation of the effluent is necessary, as well as a coagulant that produces sedimentation of the mixed culture. Chitosan, due to its biodegradability and power, has proven polymer coagulant potential in the substitution of synthetic materials widely used in sewage treatment (Wibowo *et al*., 2007).

In studies on the biodegradation of effluent from fuel distribution terminals, Vieira *et al*. (2009a) observed that the treatment of wastewater containing hydrocarbons in a batch process gave a better result with intermittent aeration. Vieira *et al*. (2012) noted that, in treating this type of wastewater, sequential batch processes with natural coagulant were better in the cases having intermittent aeration. However, in a continuous process, it is important to evaluate the biodegradation using continuous aeration, intermittent aeration and the absence of aeration.

Studies by Vieira *et al*. (2012) showed that the use of coagulants, such as chitosan, showed better results in terms of biodegradability and settling in sequential batch reaction tanks when compared with *Moringa oleifera* (natural coagulant), ferric chloride and aluminum sulfate (Vieira *et al*., 2010).

Thus, the objective of this study was to evaluate the HRT (2, 3 and 4 days) and the level of aeration in the reaction tank (continuous aeration, intermittent aeration for 30 minutes at 6 hour intervals and the absence of aeration), by monitoring the turbidity removal (TR), total petroleum hydrocarbon (TPH) removal and volatile suspended solids (VSS), employing chitosan for the settling of the mixed culture, aiming at the cell recycle in a continuous process. The initial and final COD of the continuous process were also monitored.

MATERIALS AND METHODS

Effluent

The effluent used was obtained from an inland fuel distribution terminal where spills of fuels (gasoline and diesel) occur occasionally during loading of the fuel onto transport trucks. Due to the low C:N:P in this effluent, 0.40 K_2HPO_4; 1.124 KH_2PO_4; 3.4 NH_4NO_3; 0.10 $MgSO_4.7H_2O$; 0.03 $CaCl_2$. 2 H_2O; 0.03 $MnSO_4.H_2O$ and 4.00 autolyzed residual brewery yeast were added (all in g/L). With the addition of these nutrients, a C:N:P ratio of 100:20:2.7 was obtained (Vieira *et al*., 2009b). Effluent samples were collected according to the procedures recommended by the Associação Brasileira de Normas Técnicas in document ABNT/NBR 9898 (ABNT/NBR, 1987) entitled "Preservation and sampling techniques of liquid effluents in recipient containers (Vieira *et al*., 2007). The samples were stored at 4 °C after their collection.

Microorganisms

The C_1 mixed culture employed in this study was identical to the mixed culture used by Vieira *et al*. (2007). The microorganisms in the mixed culture have been previously isolated and identified (Vieira *et al*., 2007). In this study for acclimation, the fuel concentrations were progressively increased in the medium since the TPH removal remained greater than 70% on average. The time to achieve this level of removal was 15 days for each increase in fuel concentration. The microbial density was increased due to successive cultivations with increasing concentrations of combustible performed every 48 hours for 12 days at 30 ± 1 °C and 150 rpm.

Biodegradation Effluent

Continuous System

Figure 1 shows the continuous system specifically configured for this study that was constructed with acrylic plastic and sealed with rubber stoppers.

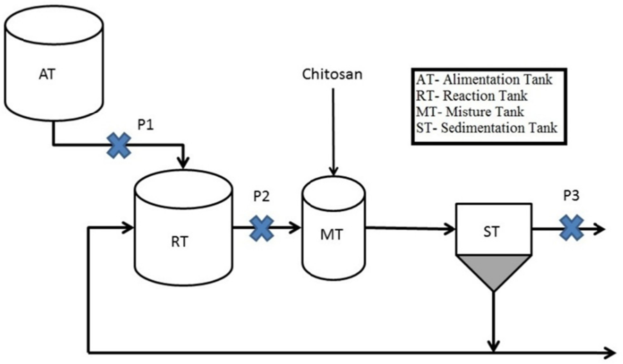

Figure 1: Scheme of the continuous process used in biodegradation of wastewater contamined with diesel oil and gasoline.

The experimental system employed in this study consisted of a reaction tank (with a working volume of 9 L and containing six ports connected to a mechanical stirrer, a thermometer, a dissolved oxygen probe, a gas outlet and a pH meter), an alimentation tank (working volume of 4 L), a mixture tank (working volume of 4 L), which provided the mixture of chitosan to the effluent and a sedimentation tank (working volume of 2 L). Reaction tanks operated continuously with an HRT of 2, 3 and 4 days at room temperature (29 ± 2 °C).

For the analysis of TPH and turbidity removals, we adopted the following criteria.

a) Equation (1) was considered for the TPH removal in the reaction tank:

$$RTPHc(\%) = \frac{S_o - S}{S_o} \tag{1}$$

b) Equation (2) was considered for the TPH removal in the supernatant:

$$RTPHc(\%) = \frac{S_o - S_e}{S_o} \tag{2}$$

c) Equation (3) was considered for the turbidity removal:

$$RT(\%) = \frac{T_R - T_C}{T_R} \tag{3}$$

where:

S_o = TPH Concentration in the alimentation tank (P1)
S = TPH Concentration in the reaction tank output (P2)
S_e = TPH Concentration in the supernatant (P3)
Tr = Turbidity in the reaction tank (P2)
Tc = Turbidity in the supernatant (P3).

Reaction Tank Operating Conditions

Constant aeration using a bubble diffuser was performed to obtain a concentration of 1.5 mgO$_2$/L. Intermittent aeration consisted of air injections every 6 h at a flow rate of 150 L/h for 30 min. The air flow rate was measured with a rotameter and controlled by an in-line valve.

The system was maintained at 29 ± 2 °C and was agitated continuously at 200 rpm/min. At the beginning of the process, the pH was adjusted to 7.0 (Vieira *et al*., 2009a). The reaction tank was operated for 20, 30 and 30 days with HRTs of 2, 3 and 4 days, respectively.

Nine different aeration regimes (Table 1) were tested by varying the aeration time for each HRT condition. The aeration time for the intermittent aeration shown in Table 1 represents the sum of all air injection times.

Table 1: Results of Regime, HRT and Aeration Frequency under different experimental conditions.

Exp.	Regime (total aeration time /HRT) (hours/days)	HRT in the reaction tank (days)	Aeration Frequency
1	0/2	2	n/a
2	24/2	2	Intermittent
3	48/2	2	Constant
4	0/3	3	n/a
5	36/3	3	Intermittent
6	72/3	3	Constant
7	0/4	4	n/a
8	48/4	4	Intermittent
9	96/4	4	Constant

Conditions with constant aeration (experiments 3, 6 and 9), without aeration (experiments 1, 4 and 7) and with intermittent aeration (experiments 2, 5 and 8).

The produced sludge was continuously removed from the sedimentation tank to maintain the concentration of volatile suspended solids (VSS) in the reaction tank.

Our target VSS and solid residence time (SRT) were 1.8 gVSS/L and 10 days, respectively, to maintain a Food/Microorganism (F/M) ratio of around 0.6 for HRT of 2, 3 and 4 days. If the solids in the aeration tanks increased above 1.8 g/L, the operator released more sludge until the VSS level was again 1.8 g/L. If the VSS dropped below 1.8 g/L, the operator released less sludge and allowed the solids concentration to increase. The SRT was also controlled through these sludge withdrawals.

Natural Coagulants

Chitosan (CH)

One gram of chitosan powder (Sigma Aldrich Inc.) was dissolved in 100 mL of 0.25 mol/L hydrochloric acid while stirring with a magnetic stirrer at room temperature until a standard solution of 1% had been obtained.

Analytical Techniques

The concentration of TPH was quantified according to the method described by Vieira *et al.* (2007). To measure TPH, hydrochloric acid was added to 10 ml of effluent to achieve a pH of 2 or less. TPH was then extracted from the acidic mixture using the solvent S-316 (dimer/trimer chlorotrifluoroethylene). Quantification of the extracted material was completed using an infrared spectrophotometer (Horiba, USA model OCMA-350). This spectrophotometes is adapted to measure aliphatic and aromatic hydrocarbons, independent of the number of carbon atoms in the compound. During the analysis, absorbances associated with CH, CH_2 and CH_3 groups are measured at wavelengths close to 3.4 μm, specifically CH (3.8 μm), CH_2 (3.42 μm) and CH_3 (3.50 μm). Associated software then converts the absorbance into total hydrocarbon content (U.S EPA, 2001). The volatile, total and fixed suspended solids were quantified along with SVI, COD (chemical oxygen demand) and SOUR according to the Standard methods for the examination of water and wastewater (Standard Methods for the Examination of Water and Wastewater, 1998). The turbidity was measured with a turbidity meter (model 2100N, Hach).

RESULTS AND DISCUSSION

Table 2 shows the average TR and TPH removal in the reaction tank and in the supernatant obtained with different aeration regimes.

Table 2: Results of Regime, TPH removals and TR under different experimental conditions.

Exp	Regime (total aeration time/HRT) (hours/days)	TPH removal in the reaction tank (%)	TPH removal in the supernatant (%)	TR (%)
1	0/2	50	73	84
2	24/2	50	72	80
3	48/2	55	82	87
4	0/3	60	80	88
5	36/3	58	80	83
6	72/3	65	87	92
7	0/4	70	85	90
8	48/4	70	83	85
9	96/4	75	94	95

TPH Removal in the Reaction Tank

The effluent showed an initial TPH of 7000 ± 400 mg/L. Table 2 shows that the process of constant aeration generally produced the best results for removal, with a removal of over 75% for aeration time/HRT of 96/4 (exp.9), followed by values of 65% and 55% for aeration time/HRT of 72/3 (exp.6) and 48/2 (exp.3), respectively.

The experiments without aeration and intermittent aeration demonstrate that, for the same HRT (experiments 1-2, 4-5 and 7-8), the system has a similar behavior, and the results were similar. This is most likely the result of the short period of aeration, which occurred at 6-hour intervals for 30 minutes. However, for this situation (experiments 7 and 8) is interesting to note that the amount removed in the reaction tank was also 70%. This suggests that low HRT enhances the biodegradation of the effluent by C_1 mixed culture.

Vieira *et al.* (2010, 2012) studied the biodegradability of the same effluent presented in this work in a sequential batch reactor (SBR) tank of 5 L using chitosan, *Moringa oleifera* and ferric chloride with polyacrylamide as coagulant. In these experiments, the TOC and TPH removals for 4 cycles of operation were: *Moringa oleifera* (31±1.5% and 28±1.4%), chitosan (82% ± 0.5 and 80 ± 1.0%) and for the combination of ferric chloride and polyacrylamide (78.4± 1.0% and 77.1±1.2%), respectively. When compared with the best results obtained in this present study for the aeration regime of 96/4 (Experiment 9, TPH removal in the reaction tank was 75% and COD removal was 77%, while in the supernatant the TPH and COD removals were 94% and 95%, respectively. These values demonstrate that the continuous process is more efficient because the working volume of the reaction tank was 9 L. The initial COD was 6330 ±520 mg/L. The results also show that the biodegradability of the mixed culture was not impaired by adding chitosan during the experiments. Moreover, this system was operated continuously thereby simulating the actual conditions of a possible treatment of contaminated effluent with diesel oil and gasoline. This demonstrates the possibility of operating a pilot scale system to treat large volumes of effluents.

Figure 2 shows the behavior of responses for TPH removal (in the reaction tank and supernatant) and TR as a function of aeration (constant aeration, intermittent aeration and a lack of aeration) and HRT of each experiment.

TPH Removal in the Supernatant

Figure 2 shows the TPH removal in the reaction tank and supernatant. By analyzing these figures, it can be observed that the difference between the TPH removal in the reaction tank and in the supernatant was approximately 30% for the HRT of 2 days, 25% for the HRT of 3 days and 20% for the HRT of 4 days. This difference was due to the greater influence of the HRT and not to significant changes in aeration frequency. Preliminary tests showed that chitosan is not efficient for hydrocarbon adsorption.

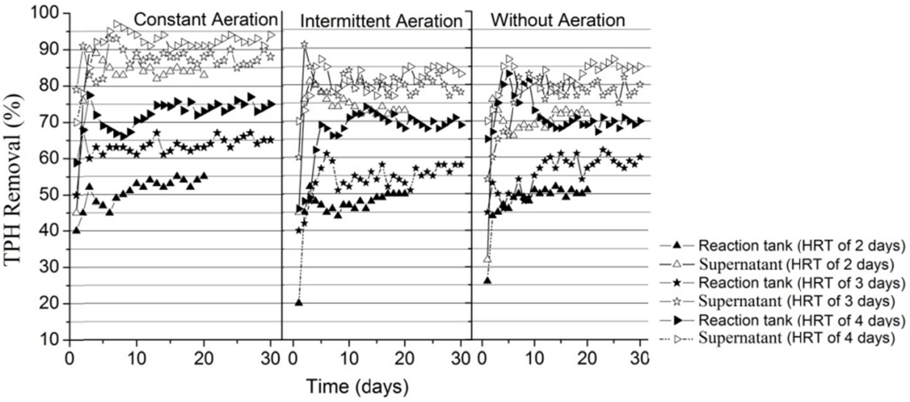

Figure 2: TPH removal as a function of HRT and aeration regime.

Crisafully *et al*. (2008) also studied the adsorption of polycyclic aromatic hydrocarbons (pyrene, anthracene, acenaphthene, naphthalene) present in petrochemical wastewater. Results revealed that the adsorption efficiencies followed the order: green coconut shells > sugar cane bagasse > chitin > chitosan. The literature indicates that chitosan may serve as a chelating agent for negatively charged molecules, such as vitamins, proteins, fatty acids, polysaccharides and mineral salts (Bossi and Prasher, 2000; Lee *et al*., 2001; Guibal, 2004). Some of these substances are produced by microorganisms and are released in the fermentation. This may explain the increase in TPH removal in the sedimentation tank.

It is observed that the lower HRT rate correlated with a higher TPH removal in the reaction tank and supernatant and that the smallest difference in TPH removal of these two conditions increases because of higher removal in the reaction tank. This fact shows that the largest HRT provided greater bioremediation. Some of this behavior can also be explained by the influence of pH on the supernatant, and the more acidic condition (pH = 6) found for the HRT of 2 days that gradually increased for the HRT of 3 days and 4 days with values of 6.4 and 6.6, respectively.

Turbidity Removal

The turbidity removal exhibited a behavior similar to that observed for the TPH removal in the supernatant (Figure 3). The best value of turbidity removal (95%) was found for the aeration regime of 96/4. Table 2 shows that the decrease of the HRT results in a decreased TR. This decrease in turbidity can be related to decreased VSS present in the supernatant with decreasing HRTs. The values of VSS were of 0.5 g/L in the experiments at an HRT of 2 days, 0.4 g/L at an HRT of 3 days and, finally, 0.25 g/L at an HRT of 4 days, regardless of aeration. This decrease in turbidity is possibly due to a decrease in the cell concentration observed in the supernatant. This also justifies the reduction of TPH with increasing HRT.

It may also be noted that the experiments with constant aeration presented the best results compared with experiments with intermittent aeration and without aeration. However, according to Widdel and Rabus (2001), for aromatic compounds like benzene, toluene, xylene and ethylbenzene that are degradable without oxygen and are more water-soluble and thus more easily dispersed, degradation may be facilitated in reaction tanks without aeration.

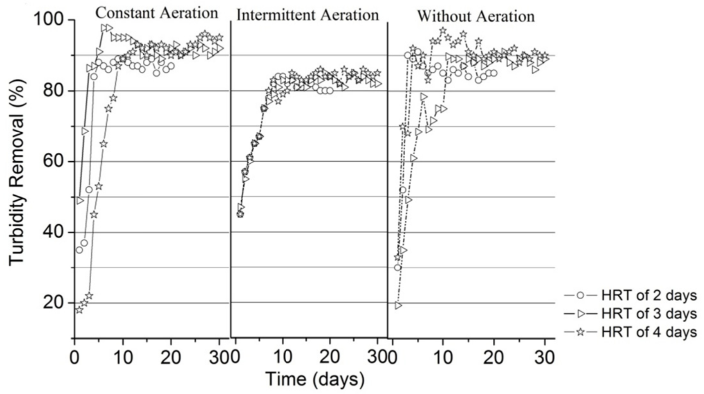

Figure 3: Turbidity removal as a function of HRT and aeration regime.

This behavior was different from that observed by Vieira *et al*. (2012), where a batch reaction tank with intermittent aeration showed better results. It was noted in this study that, in the continuous process without aeration, the dissolved oxygen concentration was 0.3 mgO_2/L. This fact correlates with the vigorous agitation of the medium and the recycle cell, which may also have contributed to the better results with constant aeration for the continuous process under study. Moreover, the continuous process, using a system with activated sludge coagulating agent and chitosan, proved to be very promising for the removal of hydrocarbons from wastewater generated in the fuel terminals.

The use of a natural coagulant for the settling had the purpose of obtaining a biodegradable sludge without any other toxic product or heavy metal, in order to ensure the treatment of effluent with hydrocarbons in an environmentally friendly manner.

CONCLUSIONS

The use of chitosan solubilized in HCl proved to be effective in continuous processes, because after 30 days of the process there was no change in the average efficiency of COD and TPH removals. In the continuous process, the best results for removal of TPH and turbidity came from the continuous aeration system with a feed flow rate of 1.6 mL / min, which corresponds to the hydraulic residence time of 4 days. In this condition, we obtained turbidity removal of 95%, TPH removals of 75% and 94% in the reaction tank and supernatant, respectively, and a VSS concentration of 1.8 g/L in the reaction tank. These results are of interest from an environmental perspective because this process allows the use of biodegradable and natural coagulants for the treatment of effluents containing diesel fuel and gasoline, making the treatment sustainable and environmentally friendly.

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NOMENCLATURE

CH	chitosan (mg/L)
TPH	total petroleum hydrocarbon
TR	turbidity removal
HCl	hydrochloric acid (mol/L)
VSS	volatile suspended solids (g/L)
HRT	hydraulic retention time (days)

REFERENCES

ABNT/NBR 9898, Preservação e técnicas de amostragem de efluentes líquidos e corpos receptores, Rio de Janeiro-RJ (1987). (In Portuguese).

Bassi, R., Prasher, S. O. and Simpson, B. K., Removal of selected metal ions from aqueous solutions using chitosan flakes. Separation Science and Technology, 35, 547-560 (2000).

Bielicka, K., Kaczorek, E., Olszanowski, A. and Voelkel, A., Examination of biodegradation of hydrocarbons in emulsified systems. Polish Journal of Environmental Studies, 11(1), 11-16 (2002).

Crisafully, R., Milhome, M. A. L., Cavalcante, R. M., Silveira, E. R., De Keukeleire, D. and Nascimento, R. F., Removal of some polycyclic aromatic hydrocarbons from petrochemical wastewater using low-cost adsorbents of natural origin. Bioresource Technology, 99(10), 4515-4519 (2008).

Gogoi, B. K., Dutta, N. N., Goswami, P. and Mohan, T. K., A case study of bioremediation of petroleum-hydrocarbon contaminated soil at a crude oil spill site. Advances in Environmental Research, 7(4), 767-782 (2003).

Grishchenkov, V. G., Townsend, R. T., McDonald, T. J., Autenrieth, R. L., Bonner, J. S. and Boronin, A. M., Degradation of petroleum hydrocarbons by facultative anaerobic bacteria under aerobic and anaerobic conditions. Process Biochemistry, 35(9), 889-896 (2000).

Guibal, E., Interactions of metal ions with chitosan-based sorbents: A review. Separation and Purification Technology, 38(1), 43-74 (2004).

Saleh-Lakha, S., Miller, M., Campbell, R. G., Schneider, K., Elahimanesh, P., Hart, M. M. and Trevors, J. T., Microbial gene expression in soil: methods, applications and challenges. Journal of Microbiological Methods, 63(1), 1-19 (2005).

Lee, S. T., Mi, F. L., Shen, Y. J. and Shyu, S. S., Equilibrium and kinetic studies of copper (II) ion uptake by chitosan-tripolyphosphate chelating resin. Polymer, 42(5), 1879-1892 (2001).

Díaz, M. P., Grigson, S. J., Peppiatt, C. J. and Burgess, J. G., Isolation and characterization of novel hydrocarbon-degrading euryhaline consortia from crude oil and mangrove sediments. Marine Biotechnology, 2(6), 522-532 (2000).

Rosa, D. R., Cammarota, M. C. and Freire, D. M. G., Production and utilization of a novel solid enzymatic preparation produced by *Penicillium restrictum* in activated sludge systems treating wastewater with high levels of oil and grease. Environmental Engineering Science, 23(5), 814-823 (2006).

Standard Methods for the Examination of Water and Wastewater. 20th Ed. (1998).

Townsend, G. T., Prince, R. C. and Suflita, J. M., Anaerobic biodegradation of alicyclic constituents of gasoline and natural gas condensate by bacteria from an anoxic aquifer. FEMS Microbiology Ecology, 49(1), 129-135 (2004).

Vieira, R. B., Vieira, P. A., Cardoso, S. L., Ribeiro, E. J. and Cardoso, V. L., Sedimentation of mixed cultures using natural coagulants for the treatment of effluents generated in terrestrial fuel distribution terminals. Journal of Hazardous Materials, 231, 98-104 (2012).

Vieira, R. B., Vieira, P. A., Ribeiro, E. J. and Cardoso, V. L., The sedimentation of mixed cultures used in the treatment of effluents generated from terrestrial fuel distribution terminals. Journal of Hazardous Materials, 184(1), 177-183 (2010).

Vieira, P. A., Faria, S., Vieira, R. B., De Franca, F. P. and Cardoso, V. L., Statistical analysis and optimization of nitrogen, phosphorus, and inoculum concentrations for the biodegradation of petroleum hydrocarbons by response surface methodology. World Journal of Microbiology and Biotechnology, 25(3), 427-438 (2009).

Vieira, P. A., Vieira, R. B., Faria, S., Ribeiro, E. J. and Cardoso, V. L., Biodegradation of diesel oil and gasoline contaminated effluent employing intermittent aeration. Journal of Hazardous Materials, 168(2), 1366-1372 (2009).

Vieira, P. A., Vieira, R. B., De França, F. P. and Cardoso, V. L., Biodegradation of effluent contaminated with diesel fuel and gasoline. Journal of Hazardous Materials, 140(1), 52-59 (2007).

Wibowo, S., Velazquez, G., Savant, V. and Torres, J. A., Effect of chitosan type on protein and water recovery efficiency from surimi wash water treated with chitosan–alginate complexes. Bioresource Technology, 98(3), 539-545 (2007).

Widdel, F. and Rabus, R., Anaerobic biodegradation of saturated and aromatic hydrocarbons. Current Opinion in Biotechnology, 12(3), 259-276 (2001).