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Petrochemical effluent treatment using natural coagulants and an aerobic biofilter

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Abstract. Coagulation-flocculation (CF) was tested coupled with an aerobic biofilter to reduce total petroleum hydrocarbon (TPHs) concentration and toxicity from petrochemical wastewater. The efficiency of the process was followed using turbidity and chemical oxygen demand (COD). The biofilter was packed with a basaltic waste (*tezontle*) and inoculated with a bacterial consortium. Toxicity test were carried out using *Lactuca sativa* var. capitata seeds. Best results for turbidity removal were obtained using alum. Considerable turbidity removal was obtained when using *Opuntia spp*. COD removal with alum was 25%, for *Opuntia* powder it was 36%. The application of the biofilter allowed the removal of 70% of the remaining TPHs after 30 days with a biodegradation rate (BDR) value 47 $mgL^{-1}d^{-1}$. COD removal was slightly higher with BDR value 63 $mgL^{-1}d^{-1}$. TPH kinetics allowed a degradation rate constant equal to $4.05 \times 10^{-2}d^{-1}$. COD removal showed similar trend with $k = 4.23 \times 10^{-2}d^{-1}$. Toxicity reduction was also successfully achieved by the combined treatment process.

Keywords: petrochemical wastewater; oil biodegradation; TPHs degradation submerged biofilters; design parameters; toxicity reduction

1. Introduction

Petrochemical industry generates important amounts of contaminants eventually released into the atmosphere, soil and, importantly, into natural water courses. In the late case, total petroleum hydrocarbons (specifically aliphatic hydrocarbons) are among the most common pollutants found in these effluents resulting from the different stages involved in the process and other incidences related with the oil industry operation such as accidental spills, leakages from storage of crude oil, wash down operations or vessel clean-outs (Chavan and Mukherji 2008). Several different conventional technologies have been reported for the treatment of such type of wastewater (Torres *et al*. 2009), among these phase change technologies, suspended and immobilized biological treatment have been identified as a suitable, cost-effective treatment method for generated wastewater at field application in reduced space and without use of expensive equipment (Torres *et*

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al. 2009). These conventional processes used alone are often related to many different operational problems such as inhibition due to relatively high concentration of toxic chemicals, long retention time and/or start-up periods and the generation of large amounts of sludge (Schmit *et al*. 2009).

Several different authors have reported the application of coagulation-flocculation (CF) process to wastewater effluents contaminated with surfactants and/or oil derivatives using a wide variety of synthetic coagulants and flocculants agents such as ferric chloride (Torres *et al*. 2009, Aboulhassan *et al*. 2006), ionic polyelectrolytes (Sarika *et al*. 2006), aluminum sulfate (Ahmad *et al*. 2006), alum polychloride and lime (Torres *et al*. 2009), among others. One possibility to avoid many of the previously described inconveniences is by the use coupled treatment systems capable to enhance microbial growth, improve nutrients removal efficiency, allow continuous process operation and increase the system's capability for handling toxic pollutants (Wang *et al*. 2011, Annadurai *et al*. 2007, Torres *et al*. 2010).

Our research group have reported the successful application of aerobic submerged biofilters for the treatment of wastewater contaminated with high concentrations of different pollutants such as pesticides (Bandala *et al*. 2006, Santacruz *et al*. 2005), phenol and chlorophenols (Torres *et al*. 2010) as well as domestic wastewater and the wastewaters generated in the surfactant enhanced soil washing process using a single bacteria culture (Mijaylova-Nacheva and Moeller-Chavez 2010, Zamudio-Perez *et al*. 2013). However, several other authors have found that symbiotic association among different bacteria genera yields higher treatment efficiencies when compared with single bacterial systems (Gargouri *et al*. 2011, Owsianiak *et al*. 2009).

In recent works, natural coagulants have been identified as interesting possibilities with potential application in high-load chemical industry effluents producing similar performance comparable to alum sulfate, ferric chloride and other commercial coagulant agents (Torres *et al*. 2009). Natural coagulants such as *Moringa oleifera* seeds, (Joshua and Vasu 2013), guar gum and its derivatives (Zhang *et al*. 2013), tara gum, locust bean gum and *Prosopis laevigata* seed gum (Torres *et al*. 2012) have been tested in the past for the treatment of surface and wastewater through the coagulation-flocculation process for the improvement of water quality. Relatively few works dealing with the use of these natural coagulants jointly with biological processes for the treatment of wastewater containing high concentration of hydrocarbons have been reported in the past. The aim of this work is demonstrate the feasibility of application of coupled processes, coagulation-flocculation using natural coagulants followed by immobilized cell processes using a submerged aerobic biofilter inoculated with a bacterial consortium for the effective treatment of hydrocarbon-rich wastewater effluents from the petrochemical industry.

2. Experimental

2.1 Reagents

All the chemical reagents used in this work, the locust bean gum (technical grade, Drogueria Cosmopolita Mexico), guar gum (technical grade, Drogueria Cosmopolita Mexico) and aluminum sulfate (Kemira Water, Mexico) were used as received without any further purification. *Opuntia* spp. powder was generated in our laboratory. *Opuntia spp*., commonly called nopal (Mexico), prickly pear or cactus leaf is a cactacean growing in Mexico, southern USA and many other arid and semiarid regions (Miller *et al*. 2008). In a recent work, Miller *et al*. (2008) reported the coagulant properties of *Opuntia spp*. They found that this material was able to reduce turbidity up to 98% in synthetic water samples. The coagulant preparation procedure from *Opuntia* spp. was as

described in Miller *et al*. (2008). Briefly, *Opuntia* spp. pads were purchased from local markets in Puebla, Mexico. Fresh pads were cut into strips (1 cm width) and dried at ~ 60°C for 24 h. Dry *Opuntia* spp. was ground in a coffee grinder and the resulting particles were sieved into mesh 100 (pore size 0.147 mm in diameter). The resulting *Opuntia spp*. powder was stored in the refrigerator at 4°C until use and no product was stored for more than two weeks under these conditions.

2.2 Wastewater sample characterization

Hydrocarbon-rich wastewater (HRWW) from a petrochemical industry was obtained from a refinery effluent at eastern Mexico. The sample was analyzed for determination of pH, electric conductivity (EC), total petroleum hydrocarbon (TPHs), chemical oxygen demand (COD), turbidity, biochemical oxygen demand (BOD), total solids, hardness as $CaCO_3$, Cd, Co, Hg, Ni, Pb, Zn, Fe and Al using standard methods procedures (APHA, AWWA, WPFC, 1995).

2.3 Coagulation-Flocculation procedure

Three natural coagulants (guar gum, locust bean gum and *Opuntia spp*. powder) were tested at different concentrations. Table 1 show the different experimental runs performed and the specific experimental conditions used in every case. All the experimental runs were carried out in a jar test apparatus under the following conditions: (i) rapid mixing at 100 rpm for 3 minutes, (ii) low mixing at 20 rpm for 15 min and (iii) sedimentation for 20 minutes. Samples of the wastewater were taken after and before the CF process for analysis of COD, color and turbidity. Assessments were carried out under controlled conditions at room temperature (20 ± 2°C).

2.4 Bacterial strain isolation

Different bacterial strains were isolated from the raw wastewater sample in Petri dishes using a mixture of the HRWW and agar which was previously sterilized by autoclave. The raw HRWW was used as source of the bacterial strains which can growth on those plates using the TPHs as the only carbon source. The colonies isolated from the plates with different morphology were re-streaked on diverse selective and no selective media (i.e., McConkey, salt and mannitol and nutrient agar). Then the isolated colonies were identified by biochemical analysis using the biomeriux® biochemical tests and the semi-automatic miniAPI reader. *Bacillus subtilis* and *Bacillus cereus* strains were also included from our strain collection and cultured on HRWW agar to confirm their capacity to grown under these conditions. All the strains were grown in a nutrient broth to have enough cells and then mixed in equal amounts (3×10^8 $CFUg^{-1}$) to seed the biofilter.

Table 1 Coagulant doses used in the experimental assessments carried out in this work

Reagent	Tested doses ($g\ L^{-1}$)	Initial pH
$Al_2(SO)_4$	0, 2, 3, 4, 5, 6	7.8
Locust bean gum	0,0.0625, 0.125, 0.25, 0.375, 0.5, 1.0	7.8
Guar gum	0, 0.125,0.25, 0.5, 1.0	7.8
Opuntia spp.	0, 0.02, 0.03, 0.04, 0.05	7.8

2.5 Biodegradation assessments

Two 0.066 L (0.4 m long, 0.046 m I.D.) glass columns similar to those reported elsewhere (Torres *et al*. 2010) were employed in series for the experimental assessments. The columns were packed with c.a. 284 g of *tezontle*, a red basaltic scoria (US mesh between 4 and 8) very abundant in Central Mexico. This material is commonly used with decorating purposes in yards and walls. It has also been used as a filtering material because of its low cost, high porosity and strength, low density and because it has demonstrated interesting performance as the solid matrix for immobilized cell biodegradation processes (Torres *et al*. 2010, Santacruz *et al*. 2005, Tamari *et al*. 2005, Di Carlo *et al*. 2010). The physical characteristics of the packaging material used were: void space 55%; porosity 76% and true particle density 2.79 gcm^{-3} (Vargas-Tapia *et al*. 2008, Tamari *et al*. 2005). Fig. 1 shows the configuration of the aerobic biofilter used.

Once packed, the system was fed with the wastewater after the coagulation-flocculation process at 30 $mLmin^{-1}$ during 24 h and then it was inoculated with a mixture the previously isolated strains (*Pseudomonas* spp, *B. subtilis*, *B. cereus*, *Klebsiella* and *E. coli*) as described previously at a final biomass load of 3×10^8 $CFUg^{-1}$. The system was kept at 36 ± 0.1°C using a constant temperature water flux jacket and the detention time in the biofilter was adjusted to 2.2 min. In order to assure the presence of dissolved oxygen in the system, the water was collected in an open reservoir after the biofilter and recycled to the top of the biofilter using a peristaltic pump (see Fig. 1). Samples were obtained from the columns set every 60 hours and submitted for determination of COD, EC and TPHs as described in Section 2.2. In the same way, initial raw wastewater (influent) and treated wastewater (effluent) samples were submitted for toxicity test carried out as described in Section 2.6. All the analyses were carried out by triplicate and the data shown are the average of the tree assessments and the error bars are the standard deviation estimated for the data.

2.6 Toxicity test

In order to determine the toxicity of the wastewater before and after the treatment process,

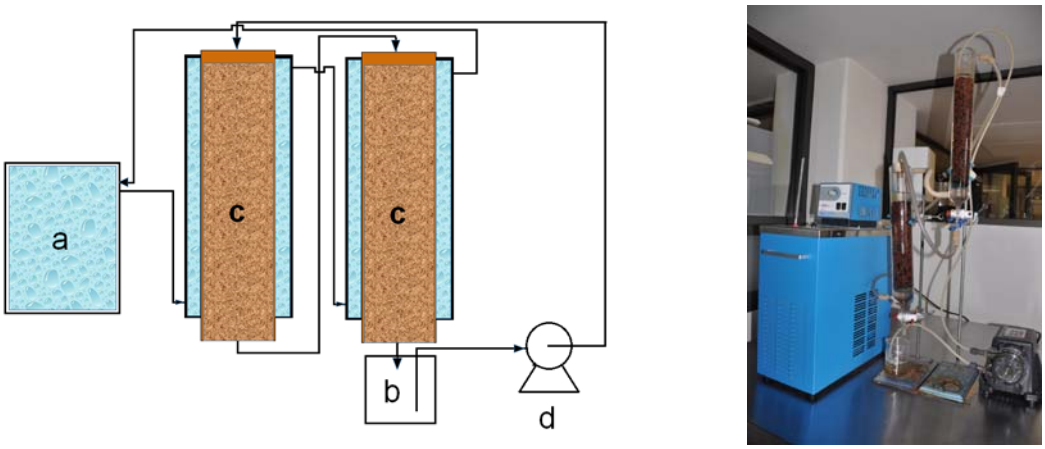

Fig. 1 Experimental setup of the aerobic biofilter used in this work: (a) is the constant temperature water bath; (b) is the water reservoir; (c) is the packed biofilter; and (d) is the peristaltic pump used for recycling the water through the biofilter

toxicity tests were performed in the influent and effluent of the treatment process using *Lactuca sativa* L. *var*. *capitata* seeds (lettuce seeds) Germinal®. Up to 135 seeds were placed in a tray filled with a substrate prepared with 1.5% agar-agar and the raw or treated water. A blank control was prepared in the same fashion, replacing the raw or treated water with deionized water; additionally a phenol solution (25 mgL^{-1}) was also used as positive control. The system was maintained under a 12 hours photoperiod at 25 ± 0.5°C during 168 hours. Finally, after 120 hours, the number of seeds that had germinated was counted, and the roots elongation (RE) of the germinated seeds (GS) was measured as proposed by Bagur *et al*. (2010). The data were used to establish two toxicity indices: GS, calculated by Eq. (1)

$$GS = \frac{G_s - G_c}{G_c} \tag{1}$$

where G_s is the average number of germinated seeds in the samples (%), G_c is the average number of germinated seeds in the blank control (%). In statistical terms, this index represents the normalized residual percentage of germinated seeds after the experiment. RE, was calculated by Eq. (2)

$$RE = \frac{El_s - El_c}{El_c} \tag{2}$$

where El_s is the average length of the seed roots in the samples (cm), El_c is the average length of the seed roots in the blank control (cm). In statistical terms, this value represents the normalized residual elongation of the root of the germinated seeds per treatment.

These indexes are designed in such a way that their values can vary from -1 (maximum phytotoxicity) to >0. This enabled us to establish the following scale: (a) 0 to -0.25 low toxicity, (b) -0.25 to -0.5 moderate, (c) -0.5 to -0.75 toxic and (d) -0.75 to -1 very toxic. RE values > 0 would indicate stimulation of the growth of the seed (hormesis) (Bagur *et al*. 2010).

3. Results and discussion

3.1 Wastewater characterization

Data obtained from the initial characterization of the wastewater sample obtained are show in Table 2. As shown, the raw wastewater presented almost neutral pH value (7.8), relatively high conductivity (1,185 $mScm^{-1}$) and extremely high amounts of TPHs and COD (10, 200 and 19,440 mgL^{-1} respectively). It is interesting noticing that TPH concentration found in the wastewater is quite high when compare to levels previously reported in effluents from petrochemical industry (i.e., 1,440 mgL^{-1}, Ben Hamed *et al*. 2010) and other results for the analysis of oil industry wastewater in the past, where HTPs concentration up to 40 mgL^{-1} were reported (Galil *et al*. 1988, Rebhun and Galil 1988). In the same way, COD was also higher that previous reports (Ben-Hamed *et al*. 2010). COD and BOD values are 19,440 and 298 mgL^{-1}, respectively giving a BOD/COD ratio of 0.015, considered non biodegradable, probably associated with the high amount of oil hydrocarbons. Total suspended solids concentration is 14,900 mgL^{-1} and hardness 1,065 mgL^{-1} as $CaCO_3$. Finally, metal concentrations were determined significantly low if compared with, for example, Mexican legislation for wastewater effluents.

Table 2 Results of the chemical characterization of the raw wastewater water

Parameter	Units	Concentration	Parameter	Units	Concentration
pH	Unitless	7.8	Cd	mg/L	0.005
Conductivity	mS/cm	1,185	Cu	mg/L	0.047
Turbidity	FTU	680	Cr	mg/L	0.047
COD	mg/L	19,440	Hg	mg/L	0.0008
BOD	mg/L	298	Ni	mg/L	0.052
TSS	mg/L	14,900	Pb	mg/L	0.033
Hardness, as $CaCO_3$	mg/L	1,065	Zn	mg/L	0.75
TPH's	mg/L	10,200	Fe	mg/L	5.13
			Al	mg/L	0.25

3.2 Coagulation-Flocculation (CF) assessments

3.2.1 Turbidity and COD removal

Fig. 2 show the turbidity removal reached using the coagulant tested in this work at different doses for every material. As showed in Table 1, very different coagulant doses were used depending on its water solubility. The best results were obtained using alum, for this coagulant turbidity removal as high as 88% was reached for coagulant dose from 2 to 4 gL^{-1}. Guar gum and locus bean gum has the lowest removal values, in both cases turbidity removal was lower than 30% independently of the coagulant concentration used. It is noticeable that considerable turbidity removal was obtained for the use of *Opuntia spp*. as coagulant. Turbidity removal up to 70% was reached by the use of only 0.03 gL^{-1} of this coagulant. Further increase in the coagulant concentration did not show any improvement in turbidity removal.

Chemical oxygen demand (COD) concentration in the raw water was 19,440 mgL^{-1}, as stated earlier in Table 2. After obtaining the best conditions for turbidity removal, assessment of COD removal were performed under the same experimental conditions. Results for these experiments are depicted in Table 3. From Table 3 it is interesting to compare the different COD removal achieved depending on the dose of coagulant used. Note that, in parentheses, the optimum doses are included. The best performance for alum was achieved at 4 gL^{-1}, while for locust bean and guar gums, optimum dose was of 0.5 gL^{-1}. Finally, for *Opuntia spp*. powder, only 0.03 gL^{-1} resulted in the best performance.

Alum sulfate, despite having the highest turbidity removal efficiencies, was not the coagulant showing the highest COD removal efficiencies. For this case, only 25% of the initial COD was removed. Locust bean gum and guar gum produced very similar results for COD removal for experimental runs (34.0 and 34.7% for locust bean gum and guar gum, respectively) and comparatively higher than the results obtained with alum sulfate. It is interesting to observe the results generated in the experiments using *Opuntia spp*. as the coagulant. *Opuntia spp*. demonstrated competitive results when compared with any of the other coagulants tested generating up to 36% of COD removal.

Because of the complexity of the process, it is very common to mix the effect of coagulation with flocculation or to talk of the process such as coagulation-flocculation unit. At this point, it would be very useful to remember the actual definitions of coagulation and flocculation. The first

Table 3 COD remaining (in mgL^{-1}) in raw and treated water using the different coagulants. Initial raw water COD = 19,440 mgL^{-1}

Test conditions (dose in gL^{-1})	Alum sulfate (4)	Locust bean gum (0.5)	Guar gum (0.5)	*Opuntia spp.* (0.03)
Remaining COD (mgL^{-1})	14,600	12,820	12,680	12,440
COD removal (%)	24.8	34.0	34.7	36.0

term refers to the process in which the suspended particles in a liquid are maintained through repulsive forces among themselves. When a coagulant (very commonly a salt) is added, the repulsive forces are weakened and the particles can get closer and tend to form bigger particles, which tend to settle due to the Stokes law. In contrast, flocculation comprehends the use of polymers which can be branched or not, to agglomerate those big particles forming big clusters knew as flocs, which will settle at higher velocities than those observed for initial particles. That is why the traditional process comprehends the use of a Fe^{3+} or Al^{3+} salt, together with a synthetic polymer to achieve the best coagulation-flocculation-sedimentation rates. When using only a polymer (natural or synthetic in nature), this product can be used first as a coagulant (reducing the repulsive forces among the particles by charge neutralization) and at the same time, due to its long and branched structure is capable of agglomerating the big particles produced in the coagulation process. Many authors, distingue three types of action for the polymers: (a) polymer bridging among particles; (b) charge neutralization including electrostatic patch effects; and (c) depletion flocculation (Bolto and Gregory 2007). Guar and locust bean gums are polysaccharides but they do not poses charges distributed in their structures. Nevertheless, they act as coagulant and flocculant in the process. The main difference among guar and locust bean gum is the ratio between the manuronic (M) and galacturonic (G) units. For guar gum the M/G ratio is 1.7, while for locust bean the M/G value is of 3.7 (Wu *et al*. 2009, Torres *et al*. 2012).

Opuntia spp. powder contains mucilage, which is essentially poly-galacturonic acid (a sort of pectin, but it has not been reported if the methylation grade for this kind of pectine is high or low), plus some neutral sugars. It has been reported that this pectin-like molecule can interact with particles, and this behavior is very related to the following aspects: (1) It is very important to take into account the pH of the media, since the poly-galacturonic acid is more spread or packed depending on the pH; and (2) It is very important the amount of Ca^{2+} present in the media, since polygalacturonic acid structure can form a packed structure with calcium ions, called the egg-box model (not far from the structure formed between alginic acid and Ca^{2+} ions) (Torres *et al*. 2012, 2013).

In relation with sludge generation, estimation by gravimetric measurements performed in the treatment effluent showed that sludge production increased as the amount of the coagulant dose increased in agreement with previous reports by Torres *et al*. (2012, 2013). Under similar dose conditions (i.e., 0.2 g L^{-1}, data not shown) the highest amount of sludge was generated by the *Opuntia spp*. powder (c.a. 5.3 mL L^{-1}) compared with the other coagulant tested with sludge production in the range between 2.1 and 2.5 mL L^{-1}. Nevertheless, under the best experimental conditions identified for the highest turbidity and COD removal efficiencies, *Opuntia spp*. powder required to achieve the best performance was 0.03 g L^{-1}, over two orders of magnitude lower than the aluminum sulfate dose required (4 g L^{-1}) and slightly higher than one order of magnitude lower than the guar gum and locust bean gum coagulant dose (0.5 g L^{-1}), identified as the best treatment

conditions. When the best experimental conditions were tested for wastewater treatment by coagulation-flocculation using the different coagulants, no significant sludge production difference was observed between them suggesting that the application of natural gums as coagulant agent will not increase the costs related with sludge treatment. Moreover, it would be expected that sludge produced in the coagulation-flocculation treatment process using the natural gums will show higher biodegradability than these produced in the process using aluminum sulfate. This thesis, however, requires additional study to be demonstrated.

3.3 Bacterial strain isolation

Table 4 shows the main characteristics of all bacterial strains used for inoculation of the biofilter tested in this work jointly with some references to their reported oil-related biodegradation capabilities. Except for *E. coli*, with no previous reports on its use for oil-related hydrocarbon biodegradation, all the stains used in the biofilter have been reported with interesting biodegradative capabilities in the past. It is noteworthy that, from the three strains isolated from the HRWW, two are related with widely reported bacteria genus (*Klebsiella* and *Pseudomonas*) used for crude oil biodegradation isolated mainly from oil spills (Das and Mukherjee 2007). In the case of *E. coli*, it was not surprising find it in the raw wastewater sample since this is a bacteria widely spread in environmental samples. Addition of two different species belonging to the *Bacillus* genus was done in order to enhance the biodegradative capability of the inoculum related with the demonstrated ability of many different species belonging to this genus to biodegrade hydrocarbons (Pijanowska *et al*. 2007).

3.4 Bacterial growth

Fig. 3 depicts cell counts (as $CFUg^{-1}_{tez}$) during the entire degradation process in the biofilter. The behavior found was quite similar to the widely reported for microbial processes with an initial poor bacterial growth (lag phase, data not shown) related with an acclimation process, followed by the usual log phase increasing the amount of bacterial attached *tezontle*. The maximum growth rate occurs after 8 days when the amount of bacteria reached about 10^5 $CFUg^{-1}_{tez}$. These results are comparable to finding reported by Zamudio-Perez *et al*. (2013), these authors found biomass loads for the aerobic submerged filter that employed to treat wastewaters arising from oil-contaminated soil washing, values around $2-4\times10^8$ heterotrofic $CFUg^{-1}_{tez}$ depending on the wastewater type.

Table 4 Characteristics of the bacterial strains used in this work

Strain	Characteristic	Source	Biodegradation capability
Bacillus subtilis 167	Aerobic, G (+) rod	UCHC*	Crude oil (Das and Mukherjee 2007)
Bacillus cereus	Aerobic G (+) rod	P&P wastewater	Hydrocarbons (Pijanowska *et al*. 2007)
Escherichia coli	Aerobic/facultative G (-) rod	HRWW	
Klebsiella oxytoca	Aerobic /facultative G (-) rod	HRWW	Crude oil (Chamka *et al*. 2011)
Pseudomonas aeruginosa	Aerobic G (-) rod	HRWW	Crude oil (Das and Mukherjee 2007)

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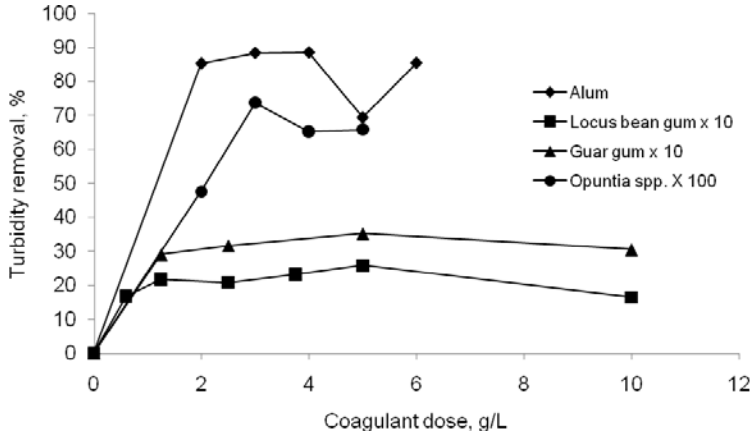

Fig. 2 Turbidity removals as a function of coagulant dose

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The cell count showed a plateau around the value reached at day 8 during the next 24 days, then the death phase began. As expected, the different stages described earlier for bacterial growth are related with the behavior observed for both, TPHs and COD. The highest decrease on contaminants concentration occurs during the log phase growth of microorganisms, the decrease of TPHs and COD keep constant during the plateau in cell counts and the lowest removal was observed during the last 6 days of the process. As shown also in Fig. 3, this behavior is the same for both, gram positive (bacteria growing in nutrient agar media) and gram negative bacterial cells (bacteria growing in McConkey media) and it is consistent with the degradation of contaminants

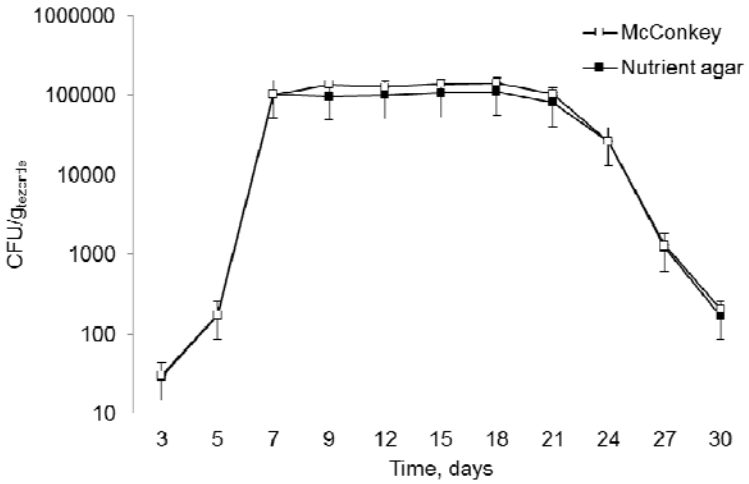

Fig. 3 Cells counts during the degradation process in the biofilter

(i.e., TPHs and COD). These results suggest that all the different strains inoculated in the biofilter where present until the end of the process since no difference was shown by counting cells growing in selective and non selective media. *Klebsiella* and *Pseudomonas* were consistently isolated all the time during the experimental assessments and the amount of gram positive populations (i.e., *Bacillus cereus* and *B. subtilis*) was close similar during TPHs degradation by comparing the total counts against gram negative counts on Fig. 3.

When the bacteria growth chart (Fig. 3) is compared with TPHs decrease in the sample during the biodegradation process (Fig. 4) it is clear that the main removal of the pollutants occurs during the first days of the process and coincide with the bacteria maximum growth rate as it has been widely reported in the past (Chamka *et al*. 2011). Similar trend is observed when compare the behavior of the COD concentration against bacterial growth.

3.5 Hydrocarbon degradation

Fig. 4 depicts a typical degradation curve for TPHs removal from the wastewater during the treatment using the aerobic biofilter after coagulation-flocculation (CF) process using *Opuntia spp*. Final TPHs concentration achieved was as low as 600 mgL^{-1} after 30 d (about 95% removal). These results are quite higher than some previously reported for the degradation of total hydrocarbons (Chang *et al*. 2011) where biodegradation as high as 55% of TPHs was achieved after 60 d. Considering the lineal decay of hydrocarbon concentration showed in Fig. 4, it was possible to estimate the biodegradation rate (BDR), an important time scale value for bioremediation in oil-contaminated environments, of the process as 47 $mgL^{-1}d^{-1}$. It is noteworthy that the BDR value obtained is not as high as expected. In previous works, our research group have found quite higher BDR values (up to one order of magnitude higher) for the degradation of pollutants with higher toxicity (i.e., chlorophenols derivatives or pesticides) using the same type of biofilter but using specific bacterial strains (Santacruz *et al*. 2005, Torres *et al*. 2010). However, in this work we have deal with higher pollutant concentrations and also TPHs may include a complex mixture

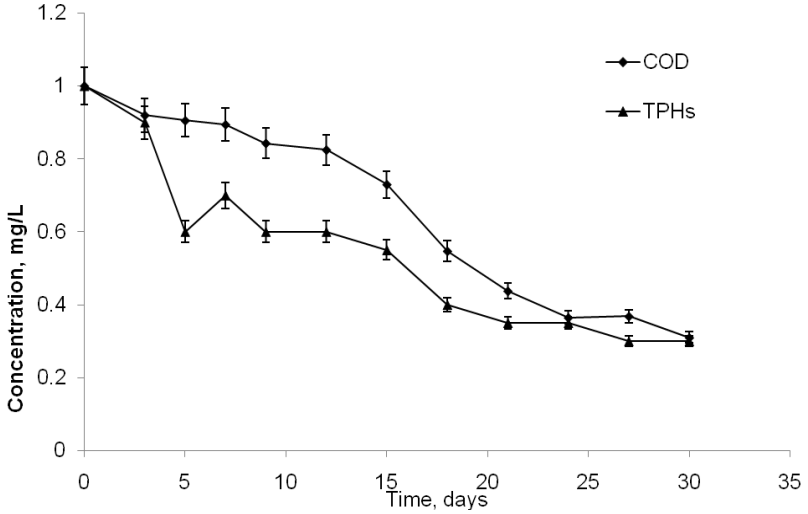

Fig. 4 Typical COD and TPHs removal trends along the wastewater treatment in the tested biofilter

of contaminants that may result on the lower bacterial adaptation. When TPH data in Fig. 4 where used to fixing a first order kinetic, the reaction rate value determined was 4.05×10^{-2} d^{-1} which agrees with previously reported results on the reaction rate values for other specific contaminants (Torres *et al*. 2010).

Considering the behavior of the COD values during the biodegradation process, it is easy to note that COD shows a trend very similar to the TPHs. In this case, overall COD removal was 69% (meaning a final COD value in the treated water of 3,856 mgL^{-1}) and BDR value achieved is slightly higher (63 $mgL^{-1}d^{-1}$) to the observed for the TPHs (i.e., 47 $mgL^{-1}d^{-1}$). It is noteworthy that the overall COD removal achieved using both, coagulation-flocculation and biodegradation processes sequentially, was as high as 95% of the total COD amount determined in the raw wastewater, significant higher than the results previously reported for the best conditions using natural (or even conventional) coagulants in single treatment processes for wastewater with COD loads over 23 times lower (Torres *et al*. 2012, 2013). Despite the COD load remains high after the proposed treatment, these results are particularly interesting considering that the final effluent produced after the coupled treatment process is now suitable to be submitted for conventional wastewater treatment processes (i.e., activated sludges, oxidation ponds or artificial wetlands) with high possibility of being successfully treated before its release to the environment.

It is possible that the presence of other contaminants besides the TPHs in the wastewater may provide to the bacterial consortium with some additional feed that they may use jointly with the TPHs in the biodegradation procedure. In the same way, the biodegradation rate constant determined for COD measurements considering a first order kinetics was quite similar, slightly higher, to the previously reported for TPHs biodegradation (4.23×10^{-2} d^{-1}). Zamudio-Perez *et al*. (2013) have reported that the treated wastewaters were reduced up to 63 (for the TW80 wastewater) and 68% (for the LBG wastewater) of TPH removal, respectively when using an aerobic submerged biofilter batch wise. Degradation rates in terms of TPHs were up to 22 $mgL^{-1}d^{-1}$ for TW80 wastewater and 7.1 $mgL^{-1}d^{-1}$ for the locust bean gum wastewater.

3.6 Design parameters estimation

Since refinery wastewater effluents may possess high volumes, estimation of design parameters for the process result in an interesting exercise in order to achieve an easier way for the layout of the wastewater process by application of the tested methodology. From data collected in this work, the *n* coefficient proposed by Eckenfelder (1961) was estimated as proposed in Eq. (3)

$$\frac{S_e}{S_O} = e^{-kD/Q^n} \tag{3}$$

where S_e is the effluent substrate concentration (mgL^{-1}); S_0 is the influent substrate concentration (mgL^{-1}); D is the depth of the medium (m); Q is the hydraulic loading rate ($m^3m^{-2}min^{-1}$); k is the treatability constant (min^{-1}) and n is the coefficient related to the medium characteristics. In order to determine n value, in this work we have assumed that most of the organic matter in the effluent is biodegradable and estimated the value of the hydraulic loading rate as 0.018 $m^3m^{-2}min^{-1}$. For these conditions n was determined as 0.502 quite similar to the value previously reported for modular plastic media, widely known as 0.5 (Benefield and Randall 1980).

Other interesting design parameters determined for the system are the volumetric loading rate (V_L) estimated as 1.79 gd^{-1}, bacteria load (1.029×10^5 $CFU/g_{tezontle}$) and the hydraulic detention time of the process (0.366 h). Finally, the cell mass yield of the process was estimated from the

Monod model as proposed by El-Nass *et al*. (2009) using Eq. (4)

$$Q_S = \frac{\mu}{Y} \tag{4}$$

where Q_s is the specific consumption rate ($kgkg^{-1}d^{-1}$); μ is the specific growth rate (d^{-1}) and Y is the cell mass yield ($kg\ kg^{-1}$). In agreement with the Monod model, when the system is plenty of nutrients and bacteria, the growth rate constant is equal to the half saturation constant (i.e., one-half the maximum). Assuming stationary state, the specific growth rate may be determined as 1.72 d^{-1} and the specific consumption rate computed as 16.61 $kg\ kg^{-1}d^{-1}$. From these values, the cell mass yield for the process is 0.1 $kg\ kg^{-1}$ about one fourth of typical Y values for aerobic suspended processes (Peavy *et al*. 1985). However, the obtained *Y* value is reasonable considering that attached-culture systems are well known to generate lower amount of biomass than suspended processes.

3.7 Toxicity reduction

Table 5 depicts the results from the toxicity test performed using *Lactuca sativa L*. var. capitata seeds. As shown, the negative control showed higher average of germinated seeds (> 90%) and the highest average root elongation (1.918 cm). In this case GS index was unable to estimate and the RE index was zero which was related with low to no toxicity at all. On the opposite, the positive control (phenol) did not allowed seed germination and the GS and RE index were -1 one in both cases meaning very toxic. These values obtained for the control experiments allowed us to determine the validity of the process and the confidence related with the toxicity test procedure.

When the raw and treated water was tested for toxicity, interesting results were observed as shown also in Table 5. GS index value in the wastewater before any treatment was as low as -0.89% identified as very toxic with a RE index value of -0.30 cm stated as moderate toxicity. These results mean that the raw wastewater is capable to generate up to 10% inhibition of the seed germination process and agree with previous reports (Banks and Schultz 2005) where the high sensitivity of lettuce seeds to petroleum hydrocarbon is consigned. However, rood elongation in germinated seeds seems to be less sensitive to the presence of TPHs as shown in the RE column, Table 5. Raw water showed low decrease in rood elongation (about 30% less) than seed germinated without adding TPHs. As reported by An *et al*. (2002) this behavior showed by *L. sativa* is related with the solubility of TPHs in water. These authors proposed that highly soluble pollutants (i.e., MTBE) affects more extensively to plant growth than seed germination due to its absorption in plant tissues. From our results seems that HTPs works in the opposite because its low water solubility getting seed germination as the main endpoint instead of plant growth.

Table 5 Toxicity test results obtained before and after wastewater treatment

Sample	Seed number (*n*)	Germinated seeds (%)	Average root elongation (cm)	GS (%)	RE (cm)
Blank	135	93.33	1.918	-	0.00
Phenol	135	0.00	0.00	-1.00	-1.00
Effluent	135	88.89	1.443	-0.07	-0.04
Influent	135	83.70	1.107	-0.89	-0.30

Considerable improvement in toxicity values were obtained for the water after treatment. In such case GS index value went from -0.89 (very toxic) to -0.07 (low toxicity) as shown in Table 5. That means quite important increase in the residual percentage of germinated seeds after the experiments.

Since, as proposed earlier, seed germination seems to be the most sensitive parameter to the presence of hydrocarbons it is not surprising that root's residual elongation measured also shows an improvement when treated water was tested. In such case, root length decreased only 0.04 cm (in average) when treated water was tested against 0.3 cm (again, almost one magnitude order) when the raw water was used for lettuce growth.

4. Conclusions

Industrial refinery wastewater was treated by combining non-conventional coagulation-flocculation (CF) and biological processes. The main design parameters of both processes were estimated. It was shown that it is possible to substitute the aluminum salts by only natural polysaccharides. The effects of this substitution would be: (1) turbidity and COD removals are very similar to those obtained with Al salts; (2) the produced sludge will be more biodegradable; and (3) produced water will contain less amounts of metals, aluminum in particular.

The best turbidity removals were obtained with alum (90%) and *Opuntia spp*. powder (70%). In contrast, turbidity removals reached using guar and locust bean gums were of 30 and 20%, respectively. COD removals obtained with alum by itself were as high as 25%, while for *Opuntia spp*. powder were up 36%. In the case of guar and locust bean gums, COD removals were of 35 and 34%, respectively.

After CF process, the remaining TPHs were successfully removed from a petrochemical wastewater using the propose process coupling CF with an aerobic biofilter inoculated with a bacterial mixture using gram positive (*Bacillus subtilis* and *Bacillus cereus*) and gram negative cells (*Escherichia coli*, *Klebsiella oxytoca*, and *Pseudomonas aeruginosa*) the process occurred jointly with an important decrease in the COD concentration and toxicity in the effluent. Design parameters (i.e., *n* coefficient from Eckenfelder model, volumetric loading rate, bacteria load and hydraulic detention time) of the process were estimated.

Toxicity reduction in the effluent was achieved by the application of the biofilter going from rood elongation (RE) index value of -0.30%, considered as low toxic up to -0.04%, assumed as non toxic. The germinated seeds (GS) index went from -0.89 (very toxic) to -0.07 cm, assumed as non toxic. Lettuce seeds were confirmed as an accurate model for toxicity determination in hydrocarbon-polluted samples as previously reported.

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