Biodegradation assessment of biological oil sludge from a petroleum refinery

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Abstract

Sludge with high concentration of hydrocarbons 470 mg/g was studied for 14 months of storage. The biodegradation was evaluated during the decomposition time by physicochemical analyses, hydrocarbons content, humic substances and phytotoxicity test. The final products showed a high degree of biodegradation as illustrated by a decrease of C/N and \( \text{NH}_4^+ / \text{NO}_3^- \) ratios, from 18.05 to 15.6 and from 26 to 2.25, respectively. Organic matter and total petroleum hydrocarbons exhibited a significant abatement rate of about 50.5% and 62% respectively. The evolution of humic substances showed a high degree of polymerization, 1.2%, which provides information on maturity of final product. The storage process led to reduction of phytotoxicity by the partial removal of toxic compounds such as low molecular-weight hydrocarbons and polycyclic aromatic hydrocarbons. This was also supported by the germination index, which exceeded 124% for alfalfa seeds after 14 months.

Keywords: Biological sludge, Hydrocarbons, C/N and \( \text{NH}_4^+ / \text{NO}_3^- \) ratios, Humification/Mineralization, Phytotoxicity.

1. Introduction

Petroleum refining and processes for cleaned installation generate a series of liquid effluents. Oil sludge is the main solid waste generated from petroleum industry during crude oil exploration, production, transportation, storage and refining processes [1, 2]. It has been estimated that for every 500 tonnes of refined crude oil, one tonne of waste of oil sludge is generated [3]. Sludge from oil refining has received increasing attention in the recent years. Petroleum-derived hydrocarbons (PHCs), mainly alkanes and paraffins with 1-40 carbon atoms, along with cycloalkanes and aromatic compounds, asphaltenes and resins [4, 5], are the most constituents of oil sludge making them potentially dangerous waste products.

Several methods for treating oil sludge have been developed, such as chemical treatment, pyrolysis, photocatalysis, incineration, ultrasonic treatment solidification/stabilization, solvent extraction, landfarming, and biodegradation [2, 6-12]. These methods are intended to remove a maximum of pollution contained in the sludge. However, because of the recalcitrant nature of oil sludge, few technologies can achieve a compromise between strict environmental regulations and reducing processing costs [13]. Biological processes can offer a combination of low cost simplicity and efficiency natural way of recycling oil refining sludge [14]. Landfarming, which consists of mixing hydrocarbon wastes with soil, being biodegradation with time has traditionally been the biological treatment method chosen to recycling the oil refinery sludge. The composting process is one of the biological tools for decomposing biodegradable organic waste, especially
sewage sludge [15]. All composting methods have similar features and processes that are close to the great natural cycles [15]. Epstein [16] and Marin et al., [17], have showed that the composting of petroleum wastes is a suitable bioremediation method to stabilize organic matter. For refinery sludge with hydrocarbons compounds, the intervening microorganisms must be able to use hydrocarbons as nutrients and energy necessary for their survival [18]. The enzymes synthetized by different microorganisms must catalyze the reactions by which the contaminants found in the oil sludge are degraded to simpler and less toxic compounds [19].

The main aim of this study is to assess the biotransformation during time of organic matter of stored biological oil refinery sludge which allowing to a better knowledge of the biological degradation process of organic waste without adding bulking agent.

2. Materials and methods

2.1. Sludge sample

Liquid effluents from a petroleum refinery located in Mohammedia (Morocco) are treated in a wastewater treatment plant. Two types of sludges are generated by this treatment, one from a primary sludge from the coagulation-flocculation process and the second one from biological tank. Sludge samples (1kg) taken at the internal discharge of the refinery, were obtained by careful mixing of several sub-samples taken at different points (height and depth) of the pile and quartering them. Different samples of stored stage were taken, such as, raw matter (fresh sludge), sludge stored for 4 and 14 months. The samples were stored at -20°C until analysis.

2.2. Physico-chemical analyses

The pH was measured on an aqueous extract of each sample at room temperature (1g/10ml of distilled water). Moisture contents were determined by drying a fresh sample of sludges at 105°C for 48 h [20]. Organic matter and ash content were calculated after calcination in a muffle furnace at 600°C for 6 h. Total Organic Carbon (TOC) was determined by titration after a reaction with a potassium dichromate solution [21]. Total Kjeldahl nitrogen (TKN) was assayed in 0.5g samples by using the classical Kjeldahl procedure, by steam distillation according to standard [22]. Likewise, ammonium ion content was assayed by alkaline distillation and nitrates after reduction by Dewarda alloying. All assays were carried out in triplicate.

2.3. Extraction of hydrocarbons

To determine Total Petroleum Hydrocarbons (TPH), two approaches were employed. Soxhlet extraction was applied. 4g of dried sludge were placed in a cartridge of cellulose, then the hydrocarbon extraction was carried out by 120 ml of a mixture of dichloromethane/diethyl ether (40ml/80ml). According to [23] 12 h of Soxhlet extraction can extract 95% of the hydrocarbons from a solid matter. Mechanical stirring extraction [24] was also applied. The same solvents as in the Soxhlet method were used. The mixture of solvents dichloromethane/diethyl ether, (15ml/30ml) was added to the different samples of sludge. A after stirring for 30 min, the mixtures were centrifuged for 15 min at 2500 rpm. The extraction cycle was repeated several times until a clear extract was obtained. For both methods, a few grams of silica gel were added to the extracts to remove polar materials, then the extracts were dried over a Na\textsubscript{2}SO\textsubscript{4} column [25]. The hydrocarbons were recovered after evaporation of the solvents mixture under partial vacuum (20 mm H\textsubscript{g}) in a Büchi Rotavapor. The quantity of TPH extracted is expressed in milligrams of TPH per gram of dry sample.

2.4. Extraction of humic substances

The humic substances were extracted from 30 g of fresh sample. The sample was treated three times with 40 ml of distilled water to extract the water-soluble non-humic substances (sugars, proteins, etc.) [26]. Then, the humic substances were extracted with 40 ml NaOH (0.1M). This was repeated several times until the obtained extract was colorless. The supernatant was centrifuged at 4000 g for 15 min. After filtration, the solutions were pooled and the humic acids were separated from the fulvic acids by precipitation with H\textsubscript{2}SO\textsubscript{4} at pH around 1 for 24 h at 4°C; the content of each fraction was determined by the MnO\textsubscript{4} oxidation method [27].
2.5. Phytotoxicity test

20 seeds of a significant plant species such as turnip and alfalfa were placed on filter paper in petri dishes, and tested with 5 ml of water-soluble extracts of raw and different stored sludges (from 10 g of fresh sample in 100 ml of distilled water). Three replicates were made. The petri dishes were then placed in darkness at room temperature (25°C). The germination index (GI) was computed as the product of the percentage of viable seeds. It was performed by monitoring the seedlings emergence, the number of germinated seeds (tests 24 h), and growth of roots (after 72 h), using the following equation [28]:

\[
\text{GI} \% = \frac{(\text{NGe} \times \text{LRe})}{(\text{NGw} \times \text{LRw})} \times 100
\]

NGe, NGw = number of seeds germinated in water soluble extracts and distilled water, respectively; and LRe, LRw = the length of rootlets in soluble extracts and distilled water, respectively.

2.6. Statistical analyses

The variations between the physico-chemical parameters, abatement rate of total petroleum hydrocarbons, organic matter and germination index during the storage, were studied using principal components analysis (PCA). The correlation between variables was carried out using SPSS Statistics version 20.

3. Results and discussion

3.1. pH and moisture evolution

During the storage of sludge the pH value tended to decrease from 8.02 to 6.8 after 4 months (Table 1), the decline recorded might be attributed to the formation of low molecular weight organic acids and dissolved CO₂ in the medium during the degradation of carbon compounds [29]. The presumable presence of alkanes as reported in many study of oil sludge [30-32] can also produce acids by their oxidation [5]. As storage time progressed (14 months) the pH value tends to stabilize around neutrality. The increase of pH from 4 to 14 months could be explained by the release of bases resulting from the degradation of macromolecular compounds [33]. Zenjari et al. [34] and El Fels et al. [15] have shown that the aerobic degradation with high concentration of oxygen gives a faster decomposition of the acids, and thus a faster rise in pH. However, the stabilization of pH is due to the buffering capacity of humus formed during organic matter decomposition. Otherwise, the pH range between 6 and 8 is found to be optimal to maintain the microbial degrading activity of hydrocarbons in oil refinery sludge [35].

The high level of moisture 81% (Table 1) for the raw sludge is due to non-availability of upstream drying treatment. The values of moisture for the two first stades (raw sludge and sludge stored for 4 months) are in agreement with the range consistent with biological remediation [36]. After 14 months of storage, moisture content decreased to 32.7%, as an evidence of biodegradation of organic matter by microorganisms. As a consequence the increase of temperature increased which allowed the evaporation of water.

<table>
<thead>
<tr>
<th>Table 1: Change in physicochemical parameters during storage of refinery sludge.</th>
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<tbody>
<tr>
<td>Storage times (months)</td>
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<tr>
<td>Raw matter</td>
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<tr>
<td>pH</td>
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<tr>
<td>Moisture %</td>
</tr>
<tr>
<td>OM %</td>
</tr>
<tr>
<td>TOC %</td>
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<tr>
<td>TKN %</td>
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<tr>
<td>C/N</td>
</tr>
<tr>
<td>NH₄⁺/NO₃⁻</td>
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3.2. Evolution of organic compounds

For the first 4 months of sludge storing we noted a low decrease of organic matter (OM) and total organic carbon (TOC) that of about 2.5% and 1.9%, respectively (Figure 1). This low decrease can be attributed to the presence of macromolecular and recalcitrant organic compounds. Biodegradation of oil refinery sludge can be affected by nutrients, temperature, microorganisms and characteristics of the sludge [13]. The type of treatment can also affect the biodegradation. In our case, sludge for the first 4 months stocked in drying bed without any turning process (for aeration) influences the bacterial activity and consequently OM degradation. Another study reported that many microorganisms (bacteria and fungi) can degrade PHCs, but there is no microbial strain which has the capacity to degrade all of the components found in oily sludge [37]. After 4 months of storage, sludge was transported to the internal discharge with turning process, which accelerates the process of degradation. The biodegradation rate reached 50.5% and 43.1% (Figure 1), respectively for OM and TOC after 14 months. That is due to the mineralization of the carbon brought about by microbial activity. Aeration during this second stage of storage favoured microorganisms activity as biooxidation which activates the organic matter degradation [38]. Said-Pullicino et Gigliotti [39] and El Fels et al. [15] showed that carbon losses have slowed down and that there is a resultant polymerization of carbon by humification mechanisms.

![Figure 1: Abatement rate of organic matter and Total Organic Carbon.](image)

The raw sludge has a low C/N ratio of about 18.05 (Table 1). After 14 months of storage the C/N ratio fell to 15.61 (Figure 2). This is directly related to the degradation of organic carbon by biological oxidation and microbial mineralization of OM and release of CO₂, which induces an augmentation of TKN concentration by reducing the mass of sludge [40]. The level of TKN content during oxidative biodegradation depends, on the one hand, on the nature of the nitrogen-having molecules and their ability to be mineralized, and on the other, on the nature of the carbon compounds present in the substrate. The recorded C/N ratio is consistent with several studies indicating that compost can be assimilated to maturity with a C/N ratio between 10 and 15 [15, 41, 42]. Roldan et al. [43] showed that the C/N ratio of about 30 found is an optimal value to enhance TPH degradation.

The value of the NH₄⁺/NO₃⁻ ratio fell from 26 to 2.25 (Figure 2) after 14 months of storage. This decrease is the result of nitrification of ammonium to nitrate by a specific bacteria like Nitrosomonas and Nitrobacter. Many studies reported that a ratio under 1 is considered as an indication of maturity [44-46]. The result indicates that storage allows the nitrification process to take place during time.

3.3. Degradation of Total Petroleum Hydrocarbons (TPH)

The two methods performed for TPH extraction show a high content of TPH in this type of oil sludge that exceeds 47% per dry mass (Figure 3). Mishra et al. [47] reported that degradation of TPH for 4 months can
only reach 16.8% in land treatment for control land without addition of nutrients and bacterial consortium. Other studies of composting reported a low decrease of TPH for control treatment between 7% and 32%, respectively for 1 and 12 months [17, 11].

![Figure 2: Evolution of C/N and NH\textsubscript{4}/NO\textsubscript{3} ratios.](image)

![Figure 3: TPH concentration determined by Soxhlet and mechanical stirring methods for raw sludge; sludge stored for 4 and 14 months.](image)

In our case, after 4 months of storage TPH decreased of about 30% and 32.8%, respectively for stirring and Soxhlet methods (Figure 4). This decline level in TPH level was found higher compared to other studies, such as Marin et al. [17] and Roldan-Carillo et al. [11]. Otherwise, the biodegradation rate of TPH was 32% (Figure 4) between 4 and 14 months of storage.

The same abatement rate for the two periods of storage (0-4 and 4-14 months) indicates that the kinetics of degradation at the first stage is very high and the biodegradation of TPH by microorganisms occurs progressively. First, while a group of microorganisms degrade the petroleum sludge constituents into intermediate compounds, other microorganisms used them for further degradation [37]. The higher decrease rate of TPH on the first phase can be related to the characteristics of the sludge compounds. As reported by many studies, Mishra et al. [30] and van Hamme et al. [31], TPH in oily sludge are composed by 40-52% alkanes, 28-31% aromatics, 8-10% asphaltenes, and 7-22.4% resins, which affect the biodegradation. Other study shows that degradation of TPH is higher for alkanes, followed by aromatics, polar compounds and asphaltenes [48, 49].
The final decrease in TPH in the refinery sludge after 14 months of storage is more than 60.8% and 63.6% with respect to the initial stage (raw matter), respectively for stirring and Soxhlet methods (Figure 4). This rate is similar to the reduction of TPH found by Kriipsalu et al. [50] after 373 days of treatment with sand. Marin et al. [17] showed that the greatest decrease in TPH occurring during the first 8 weeks of composting is linked to the positive effect of oxygenation. Britton [51] and Singer and Finnerty [52] reported that the hydrocarbons are used by the microorganisms as a carbon source for growth. And the higher degradation of the most labile hydrocarbons in petroleum refinery, sludge may be catalyzed by mono and dioxygenase enzymes synthesized by aerobic microorganisms. In such a reaction, the enzymes gradually oxidize the alkanes into alcohols and aldehydes in the presence of oxygen, producing acids that finally follow a metabolic pathway to produce CO₂ and water. Polynuclear aromatic hydrocarbons, alkanes and alkenes are initially degraded by the catalytic action of the oxygenase, which needs the presence of molecular oxygen [53]. As shown by Marin et al. [17] volatilization could also contribute to PHCs reduction.

3.4. Phytotoxicity testing
The germination index (GI) for the raw sludge shows a very low value, between 0.6% for turnip and 1% for alfalfa (Figure 5). This low level of GI is due to the high concentration of toxic compounds in sludge.
Toxic compounds are known by their inhibitory effect of the germination of the seeds and the growth of the roots, as reported by Al-Mutairi et al. [54] and El Fels et al. [15]. The oil sludge may create nutrient deficiency, which inhibits germination of seed and cause restricted growth of plants. Henner et al. [55] showed that germination and growth of plant are strongly inhibited by volatile, low molecular-weight hydrocarbons such as benzene, toluene, xylene and naphthalene. A recent study on the phytotoxicity of branched cyclohexanes found in the volatile fraction of diesel fuel has shown that a concentration in range of 0.1-5 mg/L had a significant impact on the germination of seeds and even reach no seed germination for ethylcyclohexane [56]. After 4 months of storage, we noted a slight increase of GI, about 26% and 34% for turnip and alfalfa, respectively (Figure 5). This increase can be related to the partial biodegradation of low molecular-weight hydrocarbons as alkanes, but the remaining phytotoxicity is due to the presence of other toxic compounds such as phenols, ammonia and poliaromatic hydrocarbons (PAH). The depressive effect of oil refinery sludge on seed germination depends on the sensitivity of the plant species. After 14 months of storage, the roots of alfalfa grew better and GI reached the level of 124%, compared to only 50% for turnip that showed higher sensitivity (Figure 5). Many studies reported that PAH contents in oily sludge inhibit physiological processes in plants, and once aromatic hydrocarbons are removed plants should be able to grow [55-57]. After 14 months of storage, the neutral pH and the decrease of ammonia while nitrate increased induced an increase of germination and growth of seed. On the other hand, degradation of hydrocarbons during time relieves the medium and makes it less toxic. The pores of the roots are no longer blocked by oil emulsion and allow the necessary nutrients for plant to move.

3.5. Humification and mineralization process
Biodegradation of organic matter includes humification and mineralization processes. After 4 months of storage we noted an increase in humic substances, about 17% (Table 2). This increase is the result of the degradation of easily biodegradable components such as proteins, lipids, carbohydrates and lignin [58]. However after 14 months humic substances decreased of about 56% (Table 2), suggesting that a big part of the OM was mineralized into inorganic compounds (CO₂, N₂…). Several theories on the formation of humic substances have been proposed, the most widely accepted theory by the scientific community involves polyphenols and quinones formation. This theory suggests that under the action of microorganisms the organic matter is converted into acids and polyphenolic aldehydes, which are then mineralized to CO₂ on the one hand, and on the other hand to polyphenols which are transformed into quinones for the formation of humic and fulvic acids [59]. A large increase in the polymerization degree (PD) from 0.53 to 1.26 (Table 2) was noted. The increase in this index can be explained by the formation of complex molecules, humic acids, in part through fulvic acids, that is adequate with the order of formation of humic substances [60]. Sanchez Mandero et al. [61], Veeken et al. [62], Huang et al. [63] and Vergnoux et al. [64] have shown that the increase of humic acid is an indicator of the humification degree and hence the degree of maturation of a compost.

<table>
<thead>
<tr>
<th>stored sludge (Months)</th>
<th>HS (g/kg)</th>
<th>HA (g/kg)</th>
<th>FA (g/kg)</th>
<th>F (FA/HS)</th>
<th>PD (HA/FA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw sludge</td>
<td>210.45</td>
<td>72.45</td>
<td>138</td>
<td>0.66</td>
<td>0.53</td>
</tr>
<tr>
<td>4</td>
<td>247.5</td>
<td>102.2</td>
<td>145.3</td>
<td>0.59</td>
<td>0.70</td>
</tr>
<tr>
<td>14</td>
<td>107.25</td>
<td>59.75</td>
<td>47.5</td>
<td>0.44</td>
<td>1.26</td>
</tr>
</tbody>
</table>

HS: humic substances; HA: humic acids; FA: fulvic acids.

3.6. Statistical Analyses
The results of PCA between the physico-chemical parameters, abatement rate of TPH, organic matter and germination index during the storage, are reported in Figure 6.
Figure 6: PCA component diagram of physico-chemical, abatement rate of TPH, OM and germination index for alfalfa and turnip.

The first component explains 77.4%, and the second component explains 63.3% of the variability between physico-chemical parameters. The projection on the plane of all these variables shows two inversely correlated domains: the first one gathers the abatement rate of TPH, OM, and germination index, which are closely correlated, as they increased during the storage. The second domain gathers the physico-chemical parameters (C/N, TOC, OM, TKN and NH$_4^+$/NO$_3^-$), which evolve similarly as they decreased during the storage.

Taken together, these results explain the relationship between these different physicochemical parameters during sludge storage.

Conclusion

The storage of refinery sludge from wastewater treatment plant of petroleum refinery of Mohammedia, exhibited a significant evolution during time. After 14 months of storage, the final product was evaluated to follow how pollution content in sludge was developed. In result of physicochemical analyzes, the C/N ratio is of about 15.6, NH$_4^+$/NO$_3^-$ ratio close to 2, a neutral pH, and a high organic matter degradation in the order of 50.5%. The biodegradation of organic compounds has been confirmed by the increase in polymerization degree with time.

The storage process undertaken led to the detoxification of the mass and the loss of toxic substances, as seen from the results of the petroleum hydrocarbons abatement rate of about 62% and phytotoxicity reduction determined by the GI rise by 50% and 124%, respectively for turnip and alfalfa.

The results indicate that these parameters and indicators give us information about the degree of change in the sludge from the petroleum refinery, show that the biotransformation resulting of intense microbial activity in this treatment can leads to a stable and mature final product. However, the time and the large quantity of waste stored make the treatment difficult to control, as shown by the decrease in humic substances at the final stage, and the NH$_4^+$/NO$_3^-$ ratio which remains higher than 1. In the present case the storage process allows the mineralization and nitrification processes to occur during time. However it is important to improve the operating effectiveness of the process, for example when composting with a bulking agent.
References