Feather Waste As Petroleum Sorbent: A Study Of Its Structural Biodegradation

Chapter 6

FEATHER WASTE AS PETROLEUM SORBENT: A STUDY OF ITS STRUCTURAL BIODEGRADATION

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ABSTRACT

Using scanning electron microscopy (SEM), the present study evaluated the biodegradation of chicken feathers during a petroleum hydrocarbon removal process by a defined-mixed culture that pose the simultaneous abilities to remove petroleum hydrocarbons and produce keratinases in liquid culture. Biodegradation treatments were performed in Erlenmeyer flasks containing mineral media, 6% w/v of chicken feathers and 64,800 mg l^{-1} of petroleum hydrocarbons. Flasks were inoculated with the keratinolytic-mixed culture, which was previously obtained from a petroleum-polluted site, and then incubated at 28ºC, 180 rpm during 21 days. Every 7th day, a sample was collected and fractioned; one fraction was processed to be analyzed by SEM while the residual petroleum-hydrocarbons were extracted from the other fraction and quantified by gas chromatography. Controls without inocula were processed under same conditions. The photomicrographs illustrated the different stages of the feathers' biodegradation; they are first found intact without degradation while the microorganisms from the mixed culture appear only in the supernatants. After the 7th day a remarkable colonization of the feathers begins to be observed, along with a considerable degradation observed after the 14th day of incubation.

Keywords: chicken feathers, electron microscopy, sorbent, keratinases.

1. INTRODUCTION

In the Southeast of Mexico, it is common to encounter hydrocarbon-polluted sites located in areas of difficult access such as swamps, marshes, and mangles. This situation represents a challenge to any remediation effort due to the complicated entrance of appropriate machinery

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and personal activities. One of the strategies that have been implemented to clean up these sites includes the use of sorbents to recover pollutants and afterwards dispose of them. Synthetic compounds are the most used sorbents among them; polypropylene, polyurethane, polyethylene-tereftalate and teflon are the materials commonly employed (Ro et al., 1998). Despite the effectiveness of this strategy, the disposal of contaminated materials implies a complicated operation process. The cost related to the confinement and incineration of these polluted materials is high, without mention that the last alternative might cause even more environmental contamination, contributing to climate changes (Inagaki et al., 2002). Therefore the use of biodegradable sorbents to remove pollutants could be an "earth-friendly" and attractive alternative to eliminate definitively pollutants at these site conditions.

Different sorbents have been used for inorganic and organic contaminants, such as the wheat bran used for the removal of cadmium from wastewater (Singh et al., 2006). Choi and Cloud (1992) showed the high capacity of milkweed fiber (*Asclepias*) and cotton fiber to retain crude oil, and also the excellent oil sorption capacity and hydrophobic–oleophilic characteristics of the agricultural product, kapok (*Ceiba pentandra*) have been evaluated (Lim and Huang, 2007). The use of sorbents has been also appointed to enhance oil biodegradation (Biswas et al., 2005; Setti, et al., 1999).

As it was mentioned previously, different types of sorbents and other organic residues have been used both to remove pollutants in water, soil and sediments and to improve the remediation of polluted sites; however, there is scarce information about the effective degradation of these organic materials. Thus, although they are considered as biodegradable, it is necessary to determine if native microorganisms pose the abilities to degrade them at a great extent, in such a manner that their presence in the environment does not represent an additional pollution source. Then, the aim of the present work was to monitor the biodegradation of chicken feathers during a petroleum hydrocarbon removal process by a keratinolytic and petroleum-degrading mixed culture.

2. MATERIAL AND METHODS

2.1 Feather waste

Chicken feathers (CF) were used as feather waste and they were obtained from a poultry processing plant located in the neighborhood of Ecatepec, in the State of México. Previously to use, feathers were immersed overnight in a neutral detergent solution, then washed and rinsed thoroughly with tap water to remove the detergent. The sun-dried CF were ground in a Willey mill (A.H. Thomas, Philadelphia USA) using a 20 mesh sieve.

2.2 Treatment preparation

Treatments were carried out in batch format using Erlenmeyer flasks, containing 25 ml of liquid mineral media (MM), containing (g l^{-1}): KNO_3, 1; $FeCl_3$, 0.02; $MgSO_4$, 0.2; $CaCl_2$, 0.1 and

K_2HPO_4, 1, at pH 6.8; 6% w/v of chicken feathers and 64,800 mg l^{-1} of petroleum hydrocarbons. Flasks were inoculated with the keratinolytic-mixed culture obtained from a petroleum-polluted site (Cervantes et al., 2007), and incubated at 28ºC, 180 rpm during 21 days. Every 7th day, samples were collected and processed to be analyzed by SEM and residual petroleum-hydrocarbons were extracted and quantified by gas chromatography. Controls without inocula were processed at the same conditions.

2.3 Residual petroleum-hydrocarbons

The residual petroleum-hydrocarbons in culture media were extracted from eight successive additions (20 ml) of dichloromethane and then concentrated. One µl of each organic extract was analyzed by gas chromatography (Agilent Technologies, GC-FID system) with a HP capillary column (30 m x 320 µm x 0.25 µm), as follows: initial temperature 30°C; 30-100°C at 15°C min^{-1}; 100-200°C at 7°C min^{-1}; 200-250°C at 6°C min^{-1}. Injector and detector temperatures were 250 and 280°C, respectively. Helium was used as the carrier gas (1.5 ml min^{-1}). The petroleum-hydrocarbons concentration, expressed as total petroleum hydrocarbons (TPH), was calculated interpolating the total area in a calibration curve prepared with crude oil at concentrations of 0-300 mg l^{-1}. The petroleum-hydrocarbons absorbed into the waste were extracted in a Soxhlet apparatus during 8 h using dichloromethane as solvent. The content was concentrated and quantified as indicated above. This value was subtracted from the corresponding treatment, and then petroleum hydrocarbon removal is reported as a net loss value.

2.4 Scanning electron microscope (SEM) analysis

The samples of residual feather waste from each 7th day of the treatment and the inoculum added at initial time of the treatment were processed to be analyzed by SEM. The first processing step was an exhaustive wash with Luria-Bertoni (LB) broth, each sample was in a 1.5 ml polypropylene tube containing 1 ml of broth. It was agitated by inverting the tube and it was left to rest 15 min to sediment the sample. This procedure was repeated at least 6 more times. As soon as the sample was washed, the fixation process with glutaraldehyde at 25% (in LB broth, pH 6.8) was performed during 1.5 h by inversion agitation at room temperature and later it was incubated on ice during 1.5 h more. Afterwards, each sample was washed three times as mentioned previously with broth LB and then post-fixed adding 500 µl of OsO4 at 2% (in broth LB) during 1 h at 25°C in agitation by inversion in the dark. After this treatment, three washes were realized with broth LB in the same way and the tubes were kept overnight in refrigeration; later on, the sample was dehydrated using increasing concentrations of ethanol as follows. The first wash was with ethanol at 60% during 15 min, later another wash with ethanol at 70% during 15 min, the last wash was made using absolute ethanol and it was repeated three times. It is necessary to mention that in all washes the sample was mixed by inversion. Later the samples were dried out to critical point using a SAMDRI-780 TO dryer (Tousimis Research Corporation) and finally the samples were coated with gold using the Desk II (Denton vacuum) gold evaporator. Samples were visualized using a JSM 35C scanning electronic microscope (JEOL LTD, Tokyo, Japan).

3. RESULTS

The use of feather waste as petroleum sorbent has been reported by some authors (Breitenbeck and Grace, 1998). However none of these studies have shown the biodegradation of feathers during a hydrocarbon removal process. The present study analyzes by SEM the biodegradation of feathers during the process of petroleum removal. The Figure 1 shows the residual TPH content in the treatment during the incubation period, it illustrates that the petroleum removal presented maximum consumption (almost 60,000 mg l-1) until 9th day. This value was almost 90% of the initial petroleum-hydrocarbon content (64,800 mg l-1).

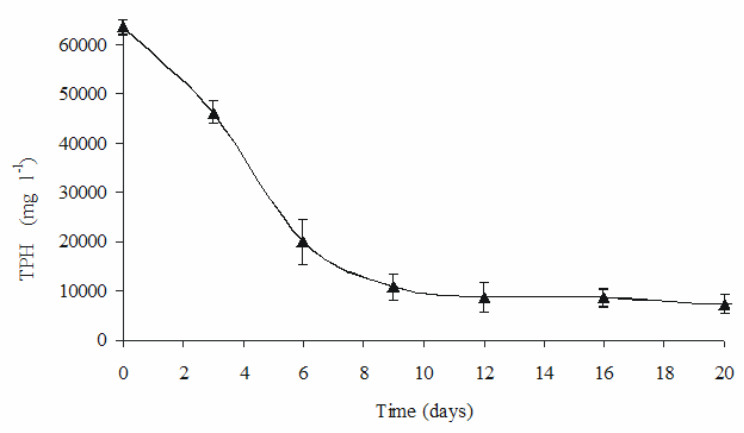

Figure 1. TPH consumption in the presence of chicken feathers by a keratinolytic and petroleum-degrading mixed culture.

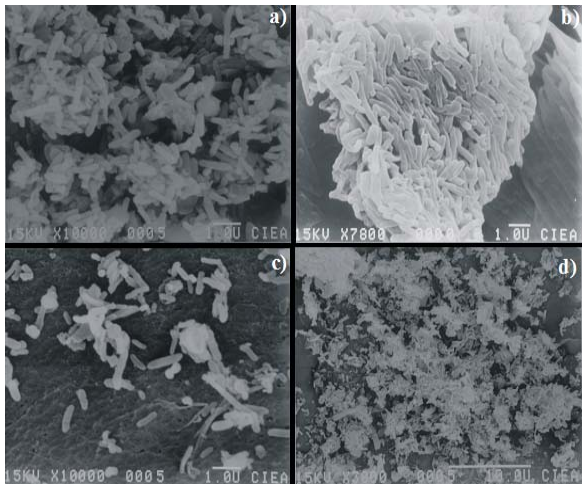

Figure 2. Representative SEM images of the inoculum used in the petroleum removal process. a) and c) 10,000X magnification, Bar 1.0 µm; b) 7,800X magnification, Bar 1.0 µm and c) 3,000X magnification, Bar 10.0 µm.

The micrographs corresponding to a sample of the inoculum added to the system are shown in Figure 2. The observed richness of the inoculum is the result of the presence of the eleven previously isolated bacteria (Cervantes et al., 2007) which exhibited capacity to degrade hydrocarbons and keratin. Each SEM image corresponds to a different area of the sample.

The monitoring of feather waste biodegradation was made every 7 days during the 21 days of treatment. The first sample corresponded to day 0 at the initial time of incubation; Figure 3 shows the keratin fibers from chicken feathers at this time.

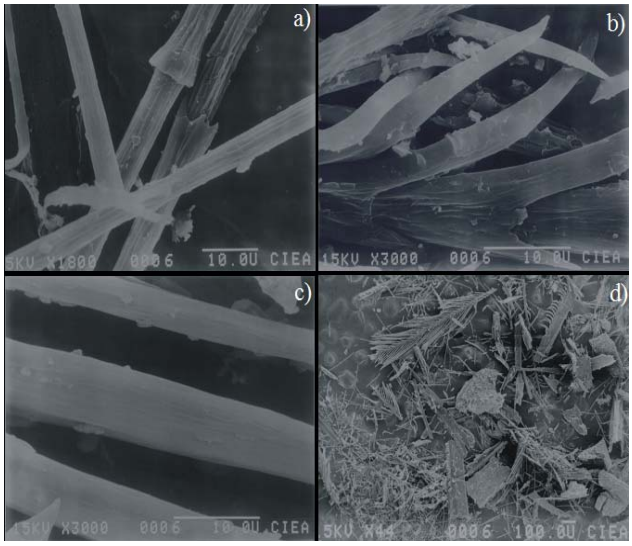

Figure 3. Scanning electron micrographs of feather waste at initial time of treatment. a) 1,800X magnification, Bar 10.0 μm; b) and c) 3,000X magnification, Bar 10.0 μm and c) 44X magnification, Bar 100.0 μm.

The degradation of the feather's structure was initially detected in the micrographs corresponding to the 7th day of incubation (Figure 4); the bacteria began to adhere on the feather's surface, and an apparent sporulation process of some bacillar structures could also be observed.

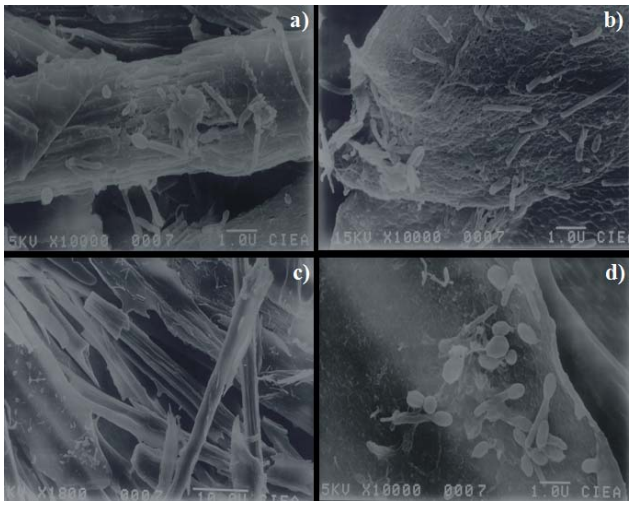

Figure 4. SEM images of feathers degradation by the mixed culture after a seven days period of petroleum removal. a), b) and d) 10,000X magnification, Bar 1.0 μm and c) 1,800X magnification, Bar 10.0 μm.

During the subsequent days of incubation, the adherence of microorganisms on the surface of chicken feathers continued (Figure 5). The bacterial colonization increased remarkably, and the sample corresponding to 14th day showed a massive growth in the majority of the fibers.

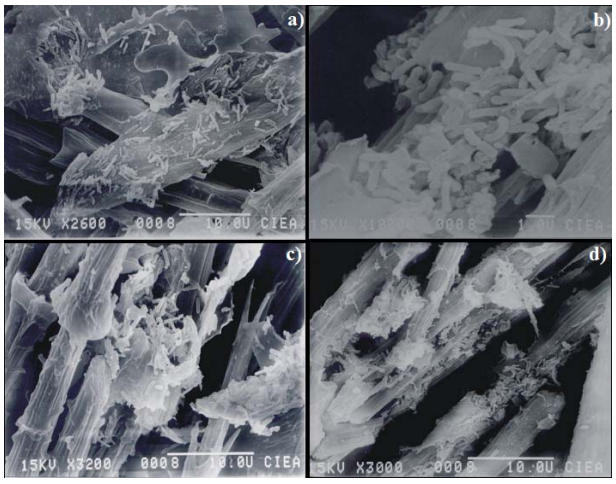

Figure 5. Scanning electron micrographs of feathers degradation by mixed culture at 14th day of petroleum removal. a) 2,600X magnification, Bar 10.0 μm b), e) and f) 10,000X magnification, Bar 1.0 μm; c) 3200X magnification, Bar 10.0 μm and d) 3,000X magnification, Bar 10.0 μm.

During the last 7 days of incubation, the scanning electron micrographs showed a total colonization on the keratin fibers which were attacked and in some zones the fiber was reduced to debris (Figure 6). However, there were some fibers which were not colonized and remained intact. It probably was due to the roughness of the same keratin fibers; however, there is not an exact description of this phenomenon.

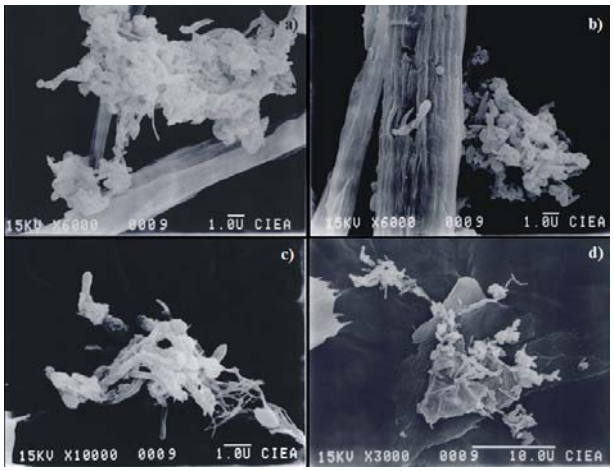

Figure 6. SEM images of feathers degradation by mixed culture at the 21th day of petroleum removal. a) and b) 6,000X magnification, Bar 1.0 μm; c) 10,000X magnification, Bar 1.0 μm and d) 3,000X magnification, Bar 10.0 μm.

4. DISCUSSION

The structural biodegradation of feather waste was studied using Scanning Electron Microscope analysis. According to the results it was possible to evidence the heterogeneous size of feathers at the initial time in spite of their milling. Additionally, it was possible to see apparent particles of different composition, which probably are originated in the rachis of feathers. These particles were in a minor proportion the most abundant the keratin fibers (Figure 2). The colonization on residual feathers was detected at day 7th (Figure 3), these micrographs showed that bacteria grew closely to the surface of keratin fibers and the fibers began to break down.

The surface of the residual keratin fibers were more severely attacked at day 14 of the incubation period; at this time the colonization was very evident and the keratin structure was significantly damaged. However, no bacterial cells were found attacking the rachis, suggesting that these particles were less biodegradable. Rammani et al., (2005) studied the structural and biochemical mechanism of feather degradation by Bacillus licheniformis strain RG1 and discovered a lack of bacterial cells on the rachis; but their structural studies reported that the bacteria closely adhere to the barbules, which produce keratinases that diffuse laterally, degrading the rachis and barbules. This phenomenon was not observed in the present work because the rachis of feathers was not degraded.

According to the gravimetrical quantification of the chicken feathers' degradation in the same treatment, the high degradation of this residue occurred during the first twelve days of treatment (Cervantes et al., 2007); however, according to the micrographs, the colonization was detected after the 7th day, which indicates two possible options.

First, the analysis by SEM was realized in residual feathers; therefore the chicken feathers degraded during the first 7 days of treatment were not evidenced by SEM, and it is not possible to assure that they were not colonized. Most likely, the feathers degraded during this period were

totally colonized and removed and therefore were not observed. This hypothesis can be sustained because the colonization was not homogeneous and only the particles colonized were removed.

On the other hand, in this treatment where the enhancement of petroleum removal was evidenced by the presence of chicken feathers, some interesting correlations were determined. During the first twelve days of treatment the exponential growth phase of bacteria and the simultaneous degradation of feathers and the petroleum were evident (Cervantes, et al., 2007); also the highest removal of TPH was detected (Figure 1). These events suggest the highest metabolism of involved bacteria during this period, and it is very important in the treatment. The enhancement of petroleum removal due to the presence of feathers could be attributed to the adherence of bacteria on the surface of keratin fibers because it could increase the contact between petroleum and bacteria.

However, the second option must also be considered: during the 12 first days of treatment the adherence of bacteria on keratin fibers was not very strong due to the petroleum sorbed into the fibers, and the biodegradation of chicken feathers may be attributable to extracellular enzymes released to the liquid media without contact between the bacteria and the surface of keratin, which could cause the visible colonization on keratin fibers after the petroleum removal process. At day 21, it was possible to evidence the total colonization of some keratin fibers but it was not possible to see the entire structure of these keratin fibers.

There are some SEM reports that shown the biodegradation of chicken feathers and it is correlated with keratinolytic activity, production of sulfhydryl groups and mid-exponential growth phase (Nam et al., 2002). The detection of colonization in the present study was not correlated with the loss of waste due to the complexity of treatment; however, the visual degradation of keratin fibers by the keratinolytic mixed culture was evident.

5. CONCLUSIONS

Scanning electron microscopy (SEM) showed the biodegradation of chicken feathers during a petroleum hydrocarbon removal process by a defined-mixed culture. Photomicrographs showed the different stages of feathers' biodegradation and also the colonization of bacteria on the surface of keratin fibers. However, additional studies are needed to evidence that chicken feathers increase the contact hydrocarbon-bacteria and then hydrocarbon removal.

6. ACKNOWLEDGEMENTS

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